

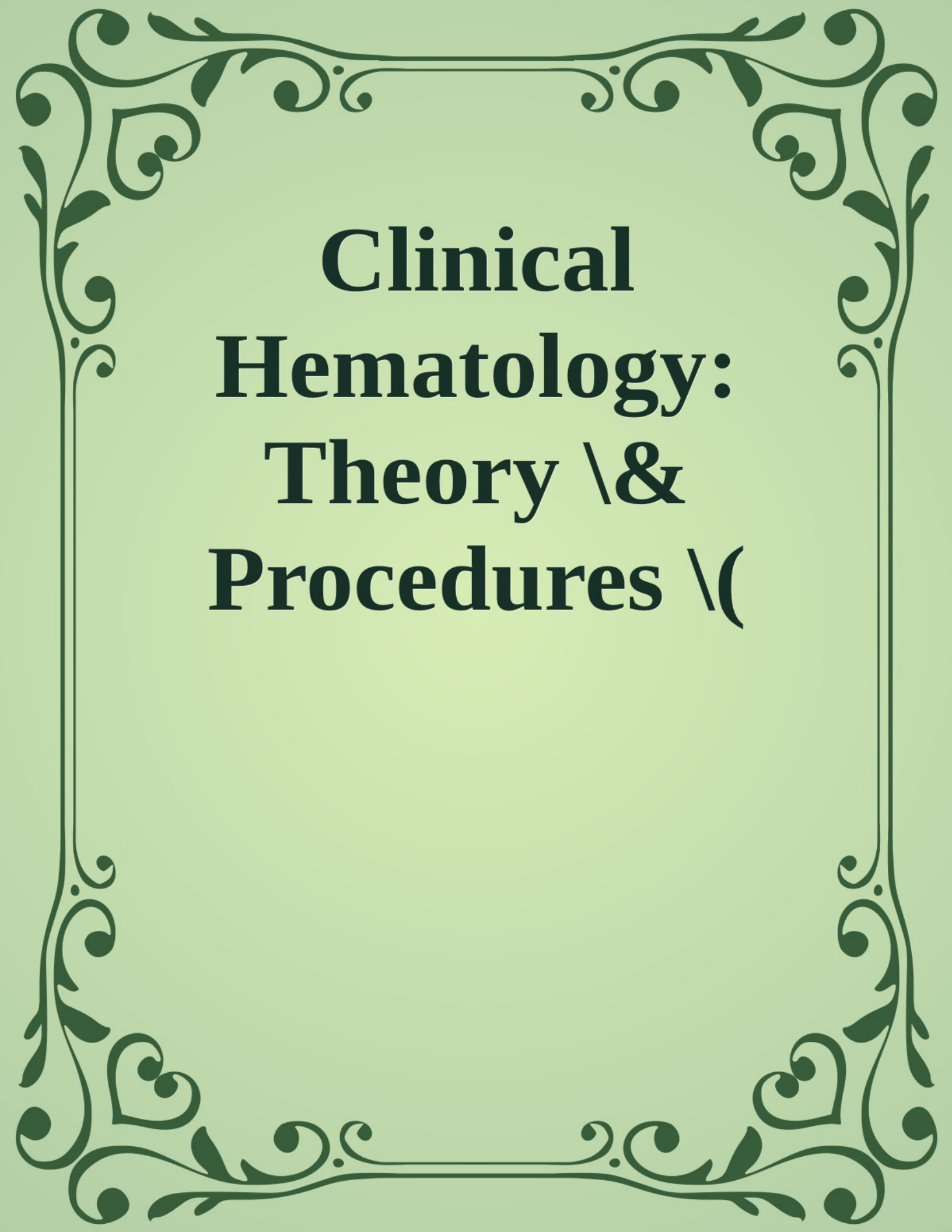
The background of the cover is a composite of microscopic images of blood cells. In the top right, there are purple-stained cells, likely platelets or small leukocytes. In the bottom left and center, there are larger, pink-stained cells, likely erythrocytes. A circular inset in the bottom right shows a magnified view of a blood smear with numerous small, round, pink cells and several larger, purple-stained cells.

MARY LOUISE TURGEON

CLINICAL HEMATOLOGY

THEORY AND PROCEDURES

SIXTH EDITION

A decorative border with intricate floral and scrollwork patterns in a dark green color, framing the central text.

Clinical Hematology: Theory \& Procedures \ (

The background of the book cover is a composite of microscopic images of blood cells. The top right shows a cluster of large, purple-stained cells, likely platelets or small lymphocytes. The bottom left and center show numerous red blood cells (erythrocytes) stained pink. The bottom right features a circular inset showing a dense field of red blood cells with several white blood cells (leukocytes) having dark, multi-lobed nuclei interspersed among them.

MARY LOUISE TURGEON

CLINICAL HEMATOLOGY

THEORY AND PRACTICE

SIXTH EDITION

 Wolters Kluwer

Clinical

Hematology

Theory and Procedures

S I X T H E D I T I O N

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Product Development Editor: J L k

Marketing Manager: L T m

Production Project Manager: D O z k

Design Coordinator: J W

Manufacturing Coordinator: M O z

Prepress Vendor: SP G

S x E

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Library o Congress Cataloging in Publication Data

N m : , M L , .

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D : S x . | P : W K , [] | I x.

I : LCCN | ISBN (k) S j : | MESH: H m D | H m —m C : LCC RB | NLM
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o my husband, Dick Mordaunt

May we continue to love exploring and

learning



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AB OUT THE AUTHOR

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Mary L. urgeon

St. Petersburg, Florida

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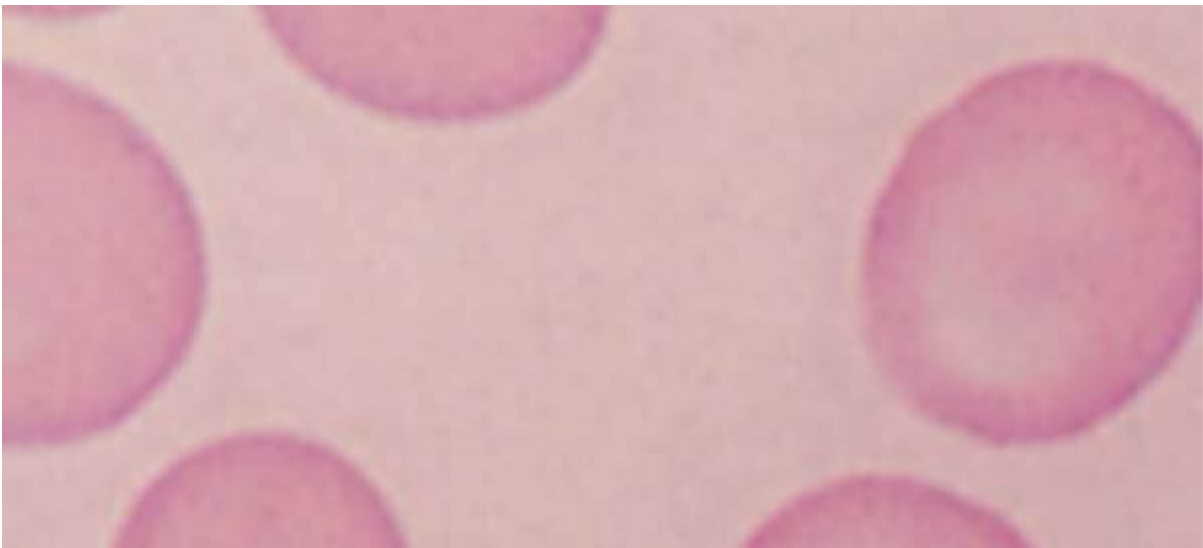
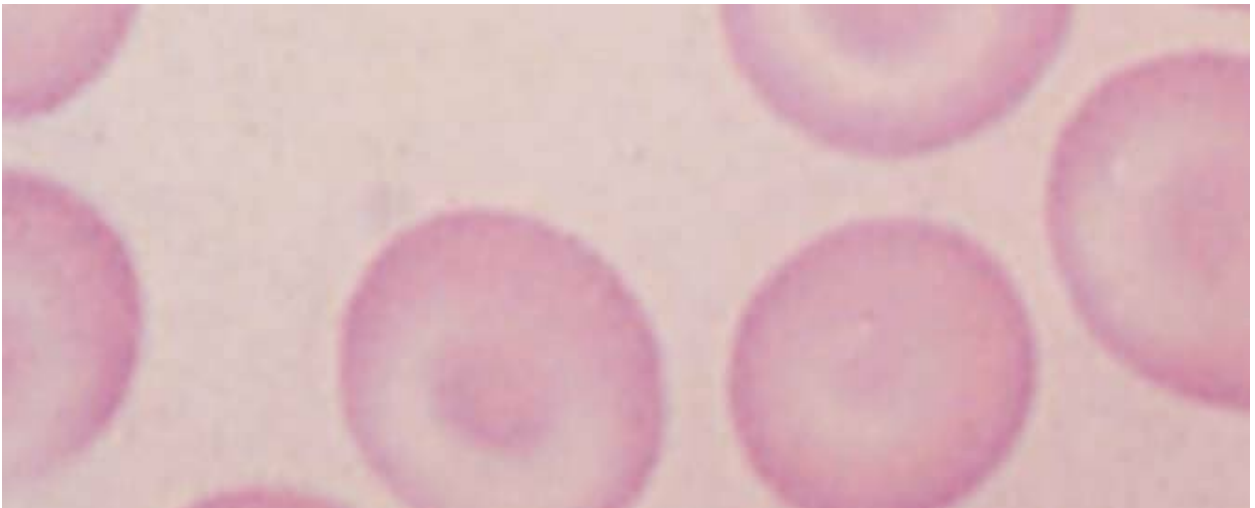
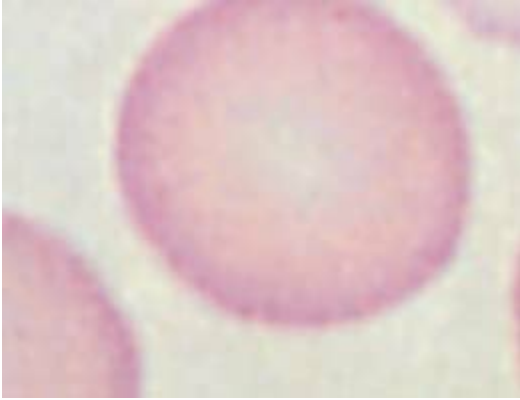
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PART ONE

Hematology Basics

Safety Practices and Quality in the

CHAPTER

Principles o Blood Collection

1 Hematology Laboratory

KEY TERMS

a ccura cy

d rift

p ro cie n cy te s tin g

b io h a za rd

Ha za rd Co m m u n ica tio n S ta n d a rd

q u a lity co n tro l (QC)

ca lib ra tio n

h u m a n im m u n o d e cie n cy viru s (HIV)

Sa fe ty Da ta S h e e ts

co e f cie n t o f va ria tio n (CV)

in fe ctio u s w a s te

s ta n d a rd d e via tio n

co n tro l s p e cim e n

Le ve y-J e n n in g s ch a rt

s ta n d a rd p re ca u tio n s

d e l t a c h e c k

n o s o c o m i a l i n f e c t i o n

s t a n d a r d s

d i s i n f e c t i o n

p a t h o g e n

t r e n d

d i s p e r s i o n

p r e c i s i o n

v a r i a n c e

LEARNING OUTCOMES

An ove rview of the hem ato logy laboratory

- Explain the purpose and correct procedure of handwashing.
- Explain the role of the hematology laboratory staff in providing qual-
- Describe the contents of the laboratory procedures manual.

ity patient care.

- Given a Safety Data Sheet, identify critical information.
- List ve basic functions of the hematology laboratory.

Quality as s e s s m e n t and quality control in the

Patient safe ty

hem atology labo ratory

- Name and describe the Institute of Medicine's six goals for health
- Summarize the essential nonanalytical factors in quality assessment. care delivery to improve the quality of American healthcare.
- Explain the delta check as a quality control method.
- Name and explain goal areas cited by the Joint Commission that
- Define terms used in quality control and basic statistical terms. have specific applications for the clinical laboratory.
- Define accuracy, precision, control material, mean, and standard deviation.

Safety for laboratory personnel

- Given the appropriate data, calculate the mean and standard deviation
- Explain the basic techniques for safety in the hematology laboratory. and create a quality control chart.

Describe the basic terms and state the formulas for the standard

- Explain the basic techniques in the prevention of disease deviation, coefficient of variation, and z score. transmission.

Describe the use of a Levey-Jennings quality control chart.

- Compare the features of general safety regulations governing the

Compare three types of changes that can be observed in a quality clinical laboratory, including components of the Occupational Safety control chart.

and Health Administration (OSHA)—mandated plans for chemical

Assess the most frequent application of a histogram.

hygiene and for occupational exposure to bloodborne pathogens,

Appraise the Westgard Rules and use of the Multirule Procedure

and the importance of the laboratory safety manual.

■ List and describe the basic aspects of infection control policies and

NOTE:

practices, including how and when to use personal protective equip-

■ indicates MLT and MLS core content

ment or devices (e.g., gowns, gloves, goggles), and the reasons for

indicates MLT (optional) and MLS advanced content

using standard precautions.

1

2

PART 1 ■ Hematology Basics

AN OVERVIEW OF THE HEMATOLOGY

LABORATORY

BOX 1.1

Health and Safety Agencies and Organizations, 1997

, 1997

■ U.S. Department of Labor

1997

Health and Safety (OSHA)

1997

■ Clinical Laboratory Standards Institute (CLSI)

1997

■ Centers for Disease Control and Prevention (CDC), Department of Health and Human Services (DHHS) **The Study of Hematology**

■ Clinical Chemistry (CAP)

Technical Manual

1997

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Complete Blood Count (CBC),

Maximum (-

), (k), (Technical Manual 'NPSG

), (ESR),

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Function s o f the Hem atolo gy Labo ratory

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T x m m

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(ICU), ICU,

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ICU. R k

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SAFE Y IN HE HEMA OLOGY

LABORA ORY

NOTE: Now is a good time to review key term definitions in

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the Glossary and ash cards on

.

k m .

A j ; , PA IEN SAFE Y

k , k mm .

M x

S , , q , , mm .

m -

S /

z (B x .).

, , .

CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

3

. U.S. D m L ' O S

Che m ica l Hyg ie ne Pla n

H A m (OSHA)

I , OSHA m m

. C L S I (CLSI)

m m m (CHP) x

. C D C P (CDC), U.S.

.

D m H H m S (DHHS)

T Hazard Communication Standard (HCS) (CFR

. C Am P (CAP)

. ()), , q m L OSHA CDC

m , , m Safety Data Sheets (SDS) (m M S D S) m z . E

z m m mm -

m z . T m -

m :

SDS m MSDS, x

SDS q

■ A m m

- , - m .

■ S m (. . , m ,

)

S D S m -

■ I z (. . , , m m . T m m ,)

k z

m k . T SDS

The Safe ty Of cer

z , , ,

z m . T m

A f

m m mm. T m

m m m .

m x

, x m , m

N , , O

S H A m ' (OSHA) H z

z m .

C mm S U S

m G H m z S m

C L C m (GHS),

Occupational S afety and He alth

m Am ' k-Adm inis tratio n Acts and S tandards . OSHA' H z C mm S

L O S

x j , , H A m (OSHA) CDC

m m m . T

-

H z C mm S k-m z .

“ k ,” G H m z

kkSmk “. ”

, USmm -

OS

Occupational Exposure to Bloodborne Pathogens

HA . I , AxHz

TOSHA-mm, OEx

CmmS . T

BP , mM . T -

mm -

q , mm , m

mm -

mm .

pathogen , B

OSHA mk

(HBV), C (HCV), mmm

z , qm , x -

(HIV). Tm

, mm

mm .

m . T m OSHA -

T CDC mm

k

m , k standard pre—

US k .

cautions. T CLSI -

OSHA CDC m

k m

-

m . I ,

. E

CDC mm m

m :

x m .

■ A m m

Avoiding Trans mis s ion of Infe ctio us

■ S m (. . , m , **Dis e as es**

)

■ I z (. . , m , A - q (LAI) -

)

q -

4

PART 1 ■ Hematology Basics

m m m -

m m (. .,

m .

). T k

m

x m

, -

Am x

■

human immunode ciency virus (HIV),

L m

. S

■

Am ’

m k

■

m x

P k

.



x k k

T HIV-m CDC m OSHA. C

Ex HIV mm ,

m k

CDC , “R mm

- k m . HIV -

P HIV m H -C S ”

m . S m m

(MMWR, S S,) q .

m HIV.

T

E m

x k -

,

m . OSHA m m : m . HIV m m m m

■ E k -

m k q m m

m .

■ P q m , x m , T m HIV x

m k ,

■ M m

. T q mm -

m (AIDS) HIV U S

Occu pa t ion al Expo s ure Rela te d to HIV, HBV, a n d

CDC. S

HCV Tran s m is s ion

HIV/AIDS m , k

B m q m

m HIV. T m

HIV HBV x .

x HIV—

j (. . , m , m ,

Occu pa tion a l Exp os ure

k k , .). O m

A . , x m , HIV k x m .

k j US

N m HIV m -

. T N k P S A

k .

(C) m

U CDC , , m j - , . H k

x m , .

x HIV-k . % k

A x

m . I , ,

j , x m , k j , j , , .

m m m k (

T k m HBV C (HCV)

k , , m -

m x k

), x

HIV m . T k x -

, , - ,

HBV -

, ·

HBV mm

Am m -

k HBV. B ,

q HIV ,

B %. A

x m mm . C

m B

j k . R k

m , k-

m HBV

x .

■ A j

HBV x -

■ L - HIV

. HBV m

■ V j

°C . T k HBV

■ I j

m % %

,

. I HB ,

T m m HIV

k . % %; I HB , q k m k . % %).



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5

. A k j m -

.

Blo od-b orn e Virus Poste xp osure Issu es

A m m k HIV m

x , x m m

. T CDC

m m x

HIV mm x (PEP).

(U U.S. P H S G

M m O Ex HBV, HCV,

HIV R mm P x P x , MMWR, [RR-],).

FIGURE 1.1 P - . (C

A x

B D k , F k L k , N J .)

m m x m -

m . I x , T C

m m m

HIV HBV. Ex k

m . B k m

x . T k HCV

, k

k . % . ,

. I “ ”

, k

Sharps Prevention

.L

Transmission OSHA

,

CLSI -

m.

(F...).Tm

Interim, management ■ C, -, k

m

HIV. Tm -

m. I

■ A, m,

m,

■ L CFR. () () ()

m /

mm. T

■ Czm

x. Aj

■ L -,

m CFR. ()

m x .

() () (C). R m

CFR . () () (E)

Imm une S tatus : S cre ening and Vaccination

T m m -

S m -

q HBV. B

k m. S -

B , -

-

k B.

.

P m m

P m , - -

mm -

. N

B. I HBV

j m . U

m , . OSHA -

m q k

m m

m

B m m

m . T mm -

x

, , k ,

m . T x

m .

m , m , -

M k

m m .

m . I j

A - m m mm z

k m

m m m -

, , m

z ' m. T k

. M - x

m m HBV m

m .

m m z -

, z m .

6

PART 1 ■ Hematology Basics

SAFE WORK PRACTICES AND PROTECTIVE TECHNIQUES FOR

BOX 1.2

INFECTION CONTROL

Your 5 Moments of Hand Hygiene

Safety Manual, Policies , and Practices

E m - - m . T

BEFORE PATIENT CONTACT

WHEN? C

m m -

m

, ,

WHY? m m -

. S m

q m OSHA

m m . O m -

BEFORE AN AS EPTIC TAS K

J C mm

WHEN? C mm

(JC), C Am P (CAP),

k

CDC.

WHY? m m , -

E q

' m ,

.

T m m . A -

AFTER BODY FLUID EXPOSURE RIS K

WHEN? C mm x

' m

k (m)

, , m , WHY? -

.

m m m m

B m z

q , m biohazard . T

AFTER PATIENT CONTACT

m -

WHEN? C

k k

mm

m m .

WHY? -

T k

m m m m

m . I

m m , -

AFTER CONTACT WITH PATIENT S URROUNDINGS

WHEN? C j

, m .

' mm ,

L -

—

m . S

WHY? -

m q -

m m m m .

m .

A m -

Source: W H O z (WHO). . .

m .

S m , .

S tandard Pre cautio ns

Standard precautions

A WHO (B x .),

x . T m -

m - m ,

m

k . T

. I , m ,

m -

m . A CDC

.

, m -

W -

HIV, HBV,

m m

. I x - m

m . S m

, -

mm -

k Clostridium dif cile,

.

m .

T D m H H m S (CDC)

Handw as hing

H H H

S B x .). T f

F q m . I m m m m .

m m -

H m m

m . G j ,

. T A P I C

, .

E m x m q

CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

7

. I q m -

BOX 1.3

, k

.

■ W m m

Guide line s for Handw as hing and Hand

, m , mL

Antise ps is in Healthcare S ettings

,

,

. W m

. R m

m

. U .

m m .

. U - -

Pe rs o nal Pro te ctive Equipme nt

m , .

. W , OSHA q

m m -

q m (PPE) m. T m -

m .

:

. D m ’

■ A k z m z k .

. D m

■ P q m

.

■ Em m , m -

. D m m m m

.

■ R m k z

. D m m

j mm .

L

. D m m .

PPE m z . T

PPE m -

M m C D C P , U.S.

. A q

D m H H m S . G H

. F , PPE

H H S , MMWR Morb Mortal Wkly Rep,

(RR-): , .

. I m -

, .

Sele ct ion a nd Use o f Glove s

, k % % %

G m k

% x .

m x. T

H mm

x

k , , . I -

. E m k ,

m -

m mm . I

. L xm x

m . I m -

k k , m

, x- .

q , , :

C m k m

% . T

k j k . G

k m

m (F . .).

q .

A m q

I CDC ,

, q m ,

:

k .

T m

■ A m k

:

.

■ B , k , m k ,

■ M m k k

.

M m

m

■

S

q :

■

■ M m

■ W m

(. . , -),

Fa cial Ba rrie r Pro te ct ion a nd Occlus ive Ba nda ge s

m ,

F ()

, . F

m ' mm m

. M k m



8

PART 1 ■ Hematology Basics

FIGURE 1.2 G m . A. T

. **B.** T , ,

. **C.** W

, -

m

x . **D.** T

, x -

x . **E.** C m

. (R m M C

RE, k CM. Phlebotomy Essentials,

, B m , MD: L W m

& W k , : , m .)

m m -

. L

. A x k

m .

- m m . T

m , , k.

Sho e s

A CLSI m GP -A ,

La boratory Co at s o r Gow ns as Ba rrier Prote ction

m

A - , - q m

- . U

k-

, mm . F -

m . T

m m m , x m , , m -

mm .

m mm k .

C mm m -

Ele ctronic Devices

,

E , x m , m , I , MP

k . C m

, m , x

z

m .

.

D . C

De contamination o f Wo rk S urface s ,

z .

Equipm ent, and Spills

Na il Ca re

A k z

A CDC, m ,

:

q

(.) EPA- .

CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

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TABLE

1.1

Pre paration of Dilute d Hous ehold Bleach

Volume of Bleach

Volume of H₂O

Ratio

% Sodium Hypochlorite

% Solution

1 mL

9 mL

1:10

0.5

10

Note: A 10% solution of bleach is stable for 1 week at room temperature when diluted with tap water.

Disinfection m m

EPA HBV, HIV, Mycobacterium tubercu—

m m , x ,

losis m m . S

m j . I , j

m m -

q m z . T

m -

m -

(x m

- . S

m). M m -

m

m m

k . U m -

.

k

H H >

.

m m (°C) , q -

% % -

Dis in fe ct ing S o lut io ns

m . S m

S m m

m

. T m

.

U S q . % . % m

, . B ,

Dis in fe ct ing Proce du re

m m , x -

A , m

. I ,

z k

x , m x m -

: . I m

m m ,

z

x . A z m -

. I q m -

-

k q

, , . T

k k . S

E m P A (EPA) m

m HIV

x mm m m -

m .

m -

H

. D m m m

. I

m k “B z ”

. A ::

.

.

N HBV, HCV HIV m

C :: -

m m k (. ., -

, m m -

). H , m

. F m

z ,

(. .,),

m m . S

m

EPA- .

; (. ., B m -

) m . T -

,

mm m -

q EPA-

:

. I j ,

. W .

m

. A . R m m (:).

q m m .

A m

. U (:),

.

m

. W k

. T m

.



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PART 1 ■ Hematology Basics

. P m m

z .

. D m q m k

(:)

m . D

m

.

General Infe ctio n Co ntro l S afety Practices

A m m m z k

m , , .

S m , k -

FIGURE 1.3 B z m . (R m M C RE, m , q m k CM. Phlebotomy Essentials, ,
B m , MD:

m .

L W m & W k , : , m .) A , k m I m -

. T :

m , , -

. A -

m . B z m

m m

(F . . .). T

.

m m

. F k m k

m . A , m m . C , -

,

, z m m k

m m .

z .

I m , ,

. S m

m m m , m .

()

. R - -

x . I -

z q m m z

m m m m

(

k , m

).

.

. A .

W m m m

P m m -

, -

().

z . G z

. N .

m m m -

. N m -

. T m

.

. D k m k .

. A m

.

Pipe tting Safeg uards : Auto matic Device s

P m m m . S

q . I -m m m , m .

I q m m m .

S pecim en-Proce ssing Pro tectio n

P

m . S m -

k . P

FIGURE 1.4 A . (R m M C RE,

-

k CM. Phlebotomy Essentials, , B m , MD:

m .

L W m & W k , , m .)

CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

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m . S

m z . W

BOX 1.4

, k z

k .

W m ,

OS HA Regulation of Me dical Was te

k . C m

. T

■ C m m -

m -

;

k .

; k m. R

A k z

m

m x

m m

q m m m , -

m q m

m . T m -

.

.

■ S m m -

S

q

m m .

, -

T m ,

, . C m m

-

, , km . T m

m , .

, , m . L x m-

■ R (q m q

m m m m ; m m m m -

. S x

m q m q m ;

m .

m k

m

OS HA Medical Was te S tandards

m ; m ;

m

OSHA m m

m) m

m (B x .). O ,

,

m m infectious waste m -

k ,

OSHA. L

, m (

O H S A C

z z).

A A .

■ A , , , m

, k m m -

m -

NOTE: Now is a good time to complete the Review Questions

, q m

related to the preceding content.

. W m

m -

. C

QUALI Y ASSESSMEN IN HE

HEMA OLOGY LABORA ORY

.

■ L f x ; -

T m q

z

m m

m ; ,

-q . Q m m m -

,

:

m m z m ; -

- m ,

■ q

m ;

■ P

f x -

■ S m m

m .

■ S m

■ S m

■ L m

■ L m , ,

C m m

(x m)

(x m)

■ m

m m m m m

■ A

m .

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PART 1 ■ Hematology Basics

Re g u l a t i o n s and Organizations Impacting

Nonanalytical Factors in Quality

Quality

A s s e s s m e n t

Clinical La b o r a t o r y I m p r o v e m e n t A m e n d m e n t s

q -

I , U.S. C C L

, (x m)

Im m A m m (CLIA')

P (x m)

. T CLIA

(x m) m . F -

, L R q m R Q S m

m CLIA' m

C P Q ,

, m m m m m . I m ,

F R J , . E m CLIA m .

m m m

A -

. CLIA' **prof ciency**

% -

testing .

. O m m

m m m

Volu nt a ry Ac cre dit ing Orga niza t ion s

m

V , x m , J

m -

C mm A H O z

m .

CAP, q -

N q

m m .

:

. Q

IS O 15189

. L

T I O z S z (ISO),

. L m

k ,

. q

,

. P m m

. ISO m z

m . ISO

. S m , , ,

. P m q m

ISO m .

. A m

T , ISO , ISO/IEC ,

. A m

m , ISO . T

Qua li ed Pe rs onne l

CAP m

A , C O ,

T - x m m

m E . I U S ,

m m . V k

m .

m x m

ISO : m

k m .

q m m m

C m q m . P

m

m -

m z m m

m q m

.

m . P m m .

Co mpo ne nts o f Quality As s essm ent

Q m m m

k (x m)

A Q m m m j m-

: q -

m .

(q [QC]).

Q m m

La bora tory Policies

x m . A m

L -

q

m .

m .

E m - - m .

T (P) m

T m m

q .

, , ,

P

. S -

k :

m q m ,

. P (x m)

OSHA , m m .

. A (x m)

O m

. P (x m)

JCAHO, CAP, CDC.

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La boratory Proce du re Ma nu al

L

BOX 1.5

m m ,

. T m

Exam ple s of Po tential Pre analytical

m , m , (pre e xam inatio n)/ Analytical (e xaminatio n)/

, •

Postanalytical (po s texam inatio n) Errors

T m -

m m . T m

PREANALYTICAL (PREEXAMINATION)

m m CLSI m

m . CLSI z -

■ S m m

Q A m .

■ S m m

QC m, m m -

■ S m

m m .

■ B m

P m m

■ I m

m . T m

■ Im m

C k CLSI .

ANALYTICAL (EXAMINATION)

T CLSI mm m

z . E

■ O m

m m m . T

■ O - - QC

m m m :

■ W m

POSTANALYTICAL (POSTEXAMINATION)

■

■ P m

■ V

■ I m : L I m S m (LIS)

■ P m

m

■ QC m

■ C

■ R , , q m

■ F mm

■ P

■ R “ m ”

■

■ L m

(B x .). F x m , ,

■ P m

m , m j

■ A

. T m z

m m . U -

Te st Re quis it ion ing

m q m m , -

A q m

m m

, m , . T q ,

m . C m -

, m ,

(F . .) m k -

m m , -

m -

m , m . T m

. A m m m

m m m m x

m .

q . T m -

m

Spe cim e n Co lle ct io n, Tra nsp ort in g, Pro cessing,

k.

a nd S to ra ge

S m -

Pa tie nt Id en ti ca t ion , Spe cim e n Procu re m e nt , a nd

.

La be lin g

S m m f -

M k -

. S m q ,

m q m m

m mm .

q m m

S m

. C m

. T m m

m , q m

m

, , -

q m q m m

. I m m



.




k m .

P m . P (x-C m -

m) m mm

. S m m

LST/LIN (00000)000-19-97 999/999		2EM 0254-01	
DOE, JOHN		55 M	
			
ACCN# 96-270-0138		26SEP07 1252	
		TIME:-----	
		INIT:-----	
10.0ML CORVAC	RT/RT	5.0ML LAV	RT/RT
K		ABC	

(00000)000-19-97 DOE, JOHN		(00000)000-19-97 DOE, JOHN	
26SEP07 1252		26SEP07 1252	
2EM 0254-01 55 M		2EM 0254-01 55 M	
			
96-270-0138		96-270-0138	
K		ABC	
10.0ML CORVAC	CHEMISTRY	5.0ML LAV	HEMATOLOGY
RT 962700138	RT 962700138	RT 962700138	RT 962700138
DOE, JOHN	DOE, JOHN	DOE, JOHN	DOE, JOHN
			
K	K	ABC	ABC

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PART 1 ■ Hematology Basics

k . A

m

m q m .

Eq m m , , -

m k

. A m -

(“I m

P ,” C , . .) m q -

m (. . , - m). F m

q m

x .

M mm q

m m m m mm m -

m m . T m

:

■ I m m

■ R

■ M j m m

■ N

C m CLIA m -

’ q m m q , **FIGURE 1.5 B** . (R m M C RE, k m . CLIA q

CM. Phlebotomy Essentials, , B m , MD: L

(x m) m

W m & W k , : , m .)

m .

Appropria te Met ho dology

m .

W m , m k S m m .

. R -

I m , m . T

m mm k

(. ., m

m - -

m [P] m m [P]).

, ,

A CLSI (C , , P

.

B S m P m -B C

A M H m A ; A

Accura cy in Re po rt in g Re s ult s a nd Do cum e nt a tion

G —F E), m P

M

P m

delta check m m . T

m

,

- . T m m

x m

. C m

D k. A , ,

°C °C (. °F . °F)

m - m

°C °C (. °F . °F). T m

. D

m k - ,

k

k. E m -

, x m , m m .

m m m m

H m

z m . S

m D k m

m m . I m. A m ,

, m m

m m m

m m

m -

(x m) :

.

. O m m IV

Pre ve nt ive Ma in te na nce o f Eq uip m e nt

. S m

. A ’

M m q m -

m q m .

O q m () M , , q m q .

CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

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Re po rt ing Re s ults

I m m -

T m k

QC.

m

K m m

m k q -

m :

m m (CQI). T

. A Q

A m

m m

. I m , -

m q -

k ,

. Q m

CQI . E , A q m m -

. A q -

QC . CLIA , “T m

m

q

q .

m q (x m-D m m q -

) m

m . CLIA m m -

.” F

m m

m m x , CLIA m

. A m m

m q m :

, m -

P m m

m , - m .

■

m .

A q m q k F m ’ QC.

-

■

m. T m

QC m m

m m

m , , ,

m m x m .

q m . A QC -

m

Docum en ta t ion

, m ,

T m m m (x m)

x k . Q m

k m .

m q m , m -

D m QC m -

k . W

, m , QC -

m

. A

, k

(x m) m k

,

m . U QC m ,

, , . T

,

m m m -

q m .

.

T -

m m

Term s Us ed in Clinical Quality Co ntro l

.

I m , m

Q A m :

. Accuracy (F . .)

NOTE: Now is a good time to complete the Review Questions

. T m m m m .

related to the preceding content.

R m k

k .

. Calibration m m m -

QUALI Y CON ROL IN HE

m k .

HEMA OLOGY LABORA ORY

. Control specimen m m

QC m m m ' m . T

m .

m k . A m

T QC m:

m

x m k -

■ m

m ; m

■ A m

k m . C m

■ V , m

j k () m .

m m ,

C m m m

m

m m .

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PART 1 ■ Hematology Basics

Impre cis ion

Inaccura cy

■ D

()

■ D m m -

()

I QC m m

m , m

m k -

m , .

De te rmine d by:

De te rmine d by:

Re pe ate d a na lys is s tudy

1) Re cove ry s tudy

A

2) Interfe re nce s tudy

, -

3) Compa ris on of me thods s tudy

m

A

B

.

FIGURE 1.6 P . G A Ana l y s i s o f Q u a n t i t a t i v e D a t a m B

' - . (R m B M L, F E P, I m m -

S L E. Clinical Chemistry, , B m , MD: L

Q C.

W m & W k , , m .)

K m m

m :

. Precision (F . .)

. A Q

m

A m

m m . P -

. I m , -

. I m m k -

k ,

. T m

m m m ; m m

T e r m s a n d D e f i n i t i o n s

m m .

A q m

. Prof ciency testing CLIA q -

m . T m m .

m x

M m x m

P m m -

m . T m .

. P , m

: , , , , .

P m; -

M m m

z m ,

m . I m x

,

m m m , m

m m . I , m m m . T m QC m . L -

q CLIA .

Z : , , , , .

. Standards k m-M m m . A m m -

q . T m

m .

: , ,

S m . S

, , , , , , , , .

(. . , m m)

Mea s urem en ts of Varia t ion

.

I , m

. Quality control (QC) m

, , , f

, z .

m .

R m x

m m . T

Func tio ns o f a Quality Co ntro l Pro g ram

x m . T , x (%) , A m

x . I -

m m j :

μ

■ $P \rightarrow Q$ is true, P is false, Q is true

. % . % (X -

, q

$$), \cdot, , \cdot, \cdot, \cdot, \cdot, \cdot,$$

■ C m m

V_X -

m .

■ D q

Т m x m m m m () m . I m

CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

17

, m

S

S)

$$x_m.T : , , , , n \times - (x$$
$$S =$$
$$, , , , . T m m ($$

n n

(-)

m m) - , - , - , - , , T coef cient of variation (CV), -

, , , . m , q

, m -

. T m m

. T CV m

:

m m , , m .

, m x

$\sum(X - m)$

s =

m . T mN

x SD

m . T m :

$\sigma =$

X =

SD

$$C(\%) = \times$$

$$\mu = m$$

$$X$$

$$N = z$$

Standard deviation SD x

$$SD =$$

$$(m).$$

$$X = m$$

$$m \times m$$

$$T \ z \ m \ m$$

$$, q \text{ variance } SD.$$

$$m \ m \ m$$

$$SD, m, m$$

$$(F \ . \ . \ A). A \ z \ m \ m$$

$$m \ m \ m -$$

$$m,$$

$$m \ m \ . \ H, SD$$

$$z \ m$$

$$- \ m \ m.$$

$$m \cdot Tz$$

$$TmSDq$$

$$m \cdot$$

$$mmmq$$

$$mkm$$

$$qmm$$

$$,k$$

$$()m \cdot Tm:$$

$$zz \cdot Tmm-z:$$

$$\Sigma(X-X)$$

$$SD =$$

$$x-\mu$$

$$N -$$

$$z=s$$

$$\Sigma = m$$

$$X =$$

$$)$$

$$X = m$$

s

t

l

u

s

$N = m$

e

r

t

s

e

t

SD

(

s

n

$m, :$

o

i

t

a

v

r

e

68%

. A m m m . T -

s

b

o

f

m

o

r

e

95%

m m .

b

m

99.7%

. C (m).

Nu

A

−3 z

−2 z

−1 z

X

+1 z

+2 z

+3 z

. D m m m m .

. Sq .

)

s

t

l

u

. A q .

s

e

r

t

. D m m .

s

e

t

(

. F q m .

s

n

o

i

t

a

T SD. I m ,

v

r

e

68%

s

m m

b

o

f

m m

o

r

e

95%

b

m z . l

m

99.7%

, m , m -

Nu

B

−3 S D

−2 SD

−1 S D

X

+1 SD

+2 SD

+3 SD

mm , . T

FIGURE 1.7 F q . **A. Z . B. G**

m :

: m q .

18

PART 1 ■ Hematology Basics

,

x = m

. I

$$\mu = m$$

$$m, m$$

$$\sigma =$$

$$m, m,$$

.

Using Statistical Analysis of Results in

Quality Assessment

Types of Changes

S -

TQC mm Levey-k .

Jennings chart. W m

T mm L -

m z m m , m -

JQC (F . .):

m m

. S m

m .

. I

I, m :

. S

. T L -J

S m drift trend

. T m m (C m) m

m m

.

m . S m

. P

m -

. T m m

Th e Levey-J en nin gs Ch art

. D m -

QC

k -

m (. . ,

.

m). T L -J

Dispersion m k m QC (. . , m . T -

- -).

q m (. . , -

C m m m

m x

SD. T m m -

m).

m m m j

S m -

() . W m ,

. T

m . I mm \pm SD

m m q .

m .

Com pute d-Ba sed Con trol Syste m s

I L -J (F . .),

- x m x-x .

Cum ula tive S um (Cusum) Me thod

T x m

T m m . D

m

m m m SD

. I

m . T m

m ,

m m . I m j

FIGURE 1.8 L -J . T

m m

. T m -

+ 2 SD

(SD) m .

14.6

g/dL

Me a n

14.3

g/dL

– 2 SD

14.0

g/dL

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
29

Month of Fe brua ry

Hemoglobin a s s ay

Normal control lot No. 12C

Sta t la b – S pe c. 20

CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

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Other Statistical Applications in the

Hematology Laboratory

Frequency Distribution

Immature

, -

. Gram

m

q (.).

A

Histogram

Immature

. A

mm m

m; m.

Hmm m. T

m q -

, GD, m, mm. Tm, m

B

; , m

m , x m

. T m

(F . . B).

I - m , \pm SD %

, \pm SD % ,

\pm SD . % . F -

, m

\pm SD. W m ,

. I m -

,

C

m

FIGURE 1.9 QC . T k m m m

QC . **A.** D . **B.** D .

SD .

C. S . E -

I C , m m m m

.

- m . T

m m m

m , k ,

m ,

.

m (). C m

m L -J m.

Wes t ga rd Ru le s

W m z -

Tre nd Line Ana lys is

m . T

O

m m

SD

m m .

k . T k

m k m

m () SD (). I

An Exam ple o f a Fre que ncy

TABLE

1.2

x_m, m

Distribution of Grouped Data

.

Class Boundaries

Frequency (f)

Power Functions

0.5–35

3

T_m

QC_j

3.5–6.5

8

$z(x_m) \cdot T_m$

6.5–9.5

10

m_m 9.5–12.5

7

$m -$

12.5–15.5

4

m .

20

PART 1 ■ Hematology Basics

T W M P m

W , k

QC m \pm SD m

m . M

. A m j

k m

, W ' x m

W . A m m QC m k W

m m m. P

M P m .

W M P

j m % m

\pm SD m .

NOTE: Now is a good time to complete the end of chapter

S x W ;

Review Questions.

, m (B x .). T

,

m , q m . I -

CHAP ER HIGHLIGH S

W , k

An Ove rvie w of the He mato logy Laborato ry

. A

■ H m m

. R j m

. T m mm m

m m .

. I

■ M m ,

m

m m q z

m m

I m m m CLIA q -

.

m -

■ T m m

,

— m m .

- - . S m

■ M m -

m

m j .

■ R

■ S , k , m m , -

Patient S afety

m (. . , m , m)

■ P

■ P m

.

■ F m ’

■ T J C mm -

■ C m m , m ,

: , -

mm , .

Safety in the Hematology Laboratory

BOX 1.6

■ Mix q , μ mm.

Six Westgard Rules for Analysis of Quality

■ OSHA ASK

Control Charts

k.

■ OSHA-m O Ex B

(12s)

P q , m , m , m -

(13s)

.

(22s)

■ B m q m

same side of the center line

x k.

(R4s)

(41s)

Safe Work Practices and Protective

side of the center line

Techniques for Infection Control

10 x

■ E m - - m .

mean

■ S

SD, .

x -

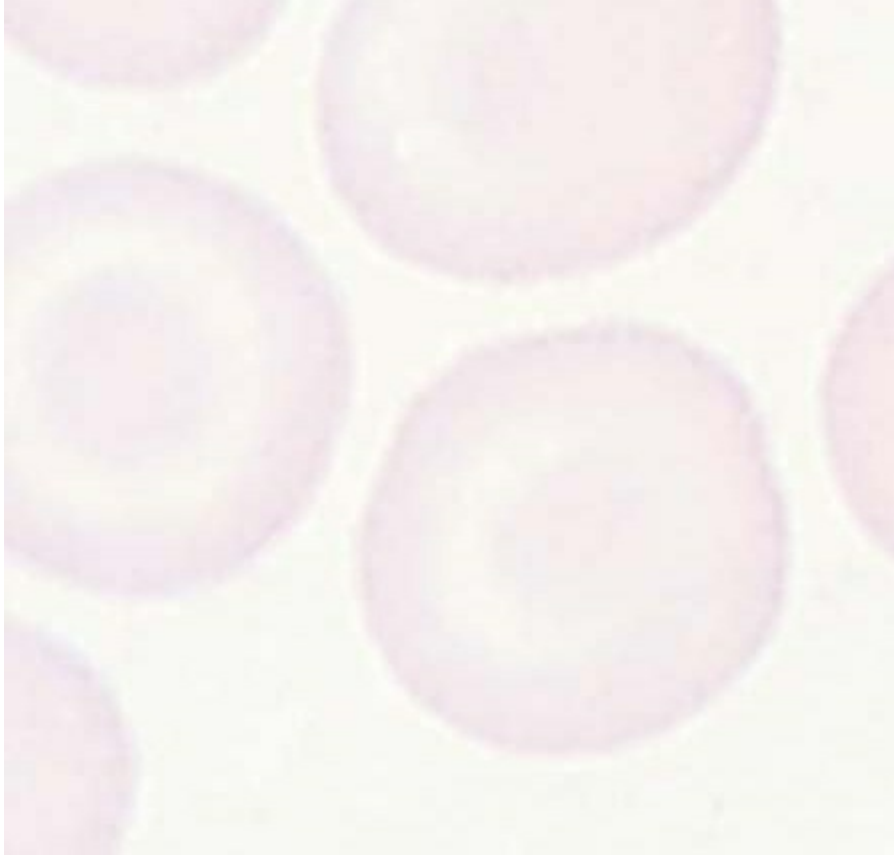
F m W JO, B PL, H MR, . A m - S

.

q m , Clin Chem, : , .

■ G j ,

, .



CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

21

■ A k z -

Quality Co ntro l in the Hem atolo gy

:

Laboratory

EPA- .

■ QC m -

Quality Assessment in the Hematology

m m . T QC

Laboratory

m m , m

, .

■ T m q

■ I m m -

m m

-q .

QC.

■ A m q -

■ S

m

L -

.

J . W m

■ A q m m m j m z m m , m m :

m m

q (QC).

m .

■ D k m m -

■ D k W R m

, , .

q m .

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

5. S -

_____ X

An Overview o the Hematology Laboratory

k HIV HBV.

Patient Sa ety

A.

B. m m m

1. T N P S G

C. k

A.

D.

B.

C.

6. Ex _____ m j

D. x

HIV HBV .

A. m

2. T m

m

B.

C.

A.

D. m

B.

C. m

q m

7 8. D k , q m , m k-D. m OSHA _____ () . T -

q _____ () mL mL

Safety in the Hematology Laboratory

H O.

3. I m

,

7.

A.

A. :

B.

B. :

C. %

C. :

D.

D. :

4. T k x HBV-8.

HIV—

A.

B.

A. (m)

C.

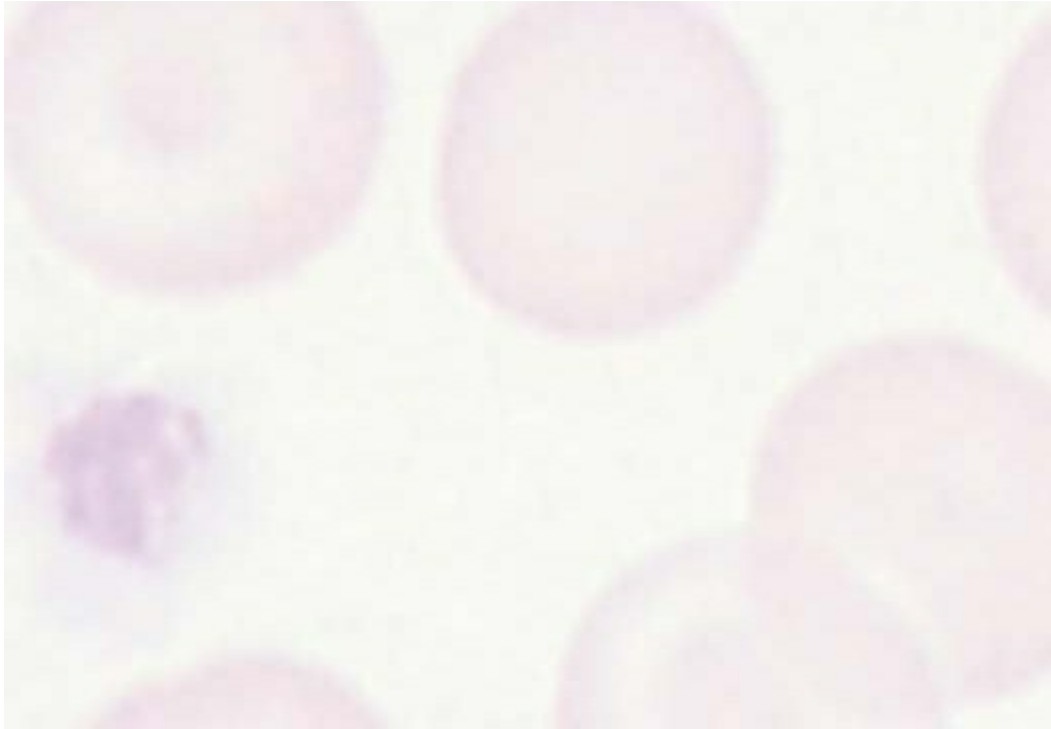
B.

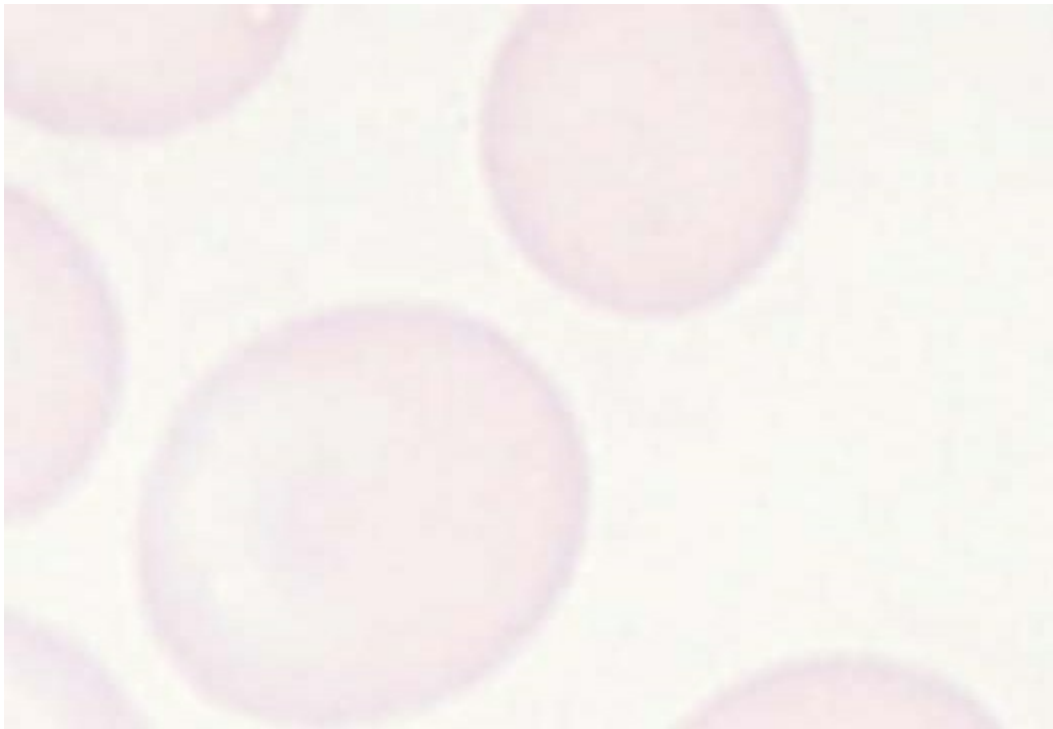
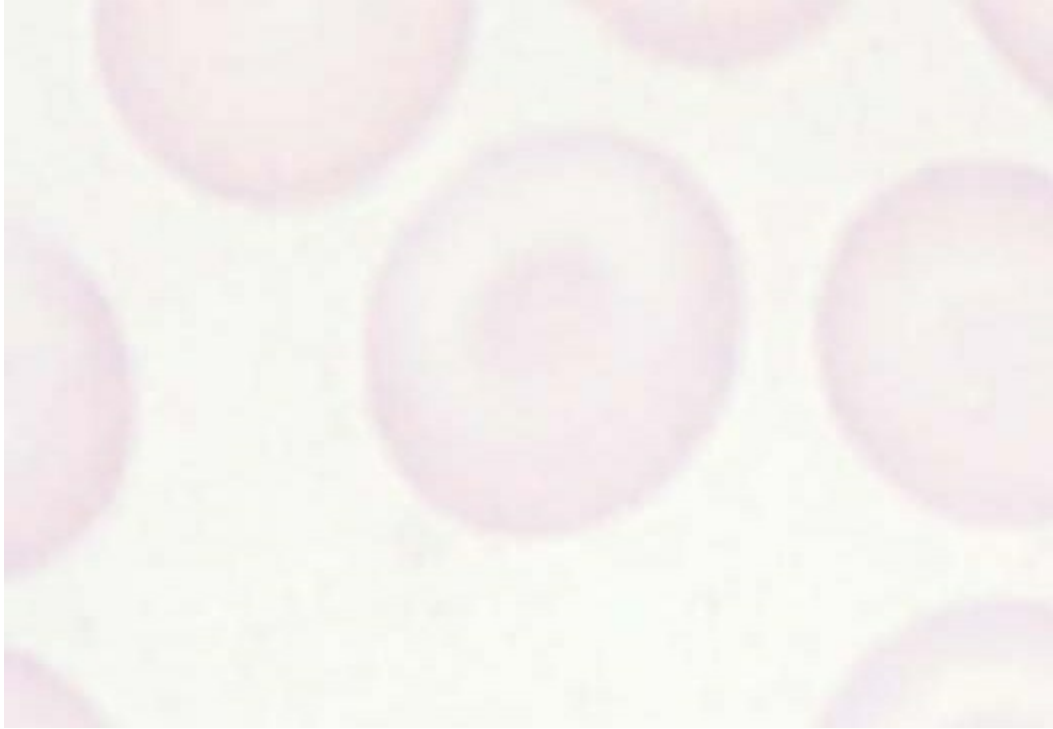
D.

C.

D. k

(continued)





REVIEW QUESTIONS (continued)

9. I -

16. T m m

permissible?

A.

A. W ’

B. k m

f k

C. m -

B. W ’

k

D. k

C. W m

17. T m m

D. N

A. k m m

m

Quality Assessment in the Hematology Laboratory

B. true

10. $T \rightarrow q \rightarrow m$

C. m

A.

D. $m \rightarrow k$

B.

C.

*18. $W \rightarrow \neg q \rightarrow$

D. $A \rightarrow B$

$Q \rightarrow C \rightarrow m$?

A. $M \rightarrow q \rightarrow m$,

11. $T \rightarrow k$

, q

A.

B. $C \rightarrow m \rightarrow m$

B. $m \rightarrow m$

C. $C \rightarrow m$

C.

D. $m \rightarrow m$

D. D

12. W

19. T

(x m) ?

A. m -

A. m

m

B. F m m

B. T x

C. C ER

C. T m

D. m

D. T

*13. I m m , ,

*20. T m m

_____, CLIA.

A. m q m m

A. O

B. q m m

B.

C. x

C. T m

m

D. D

D.

Quality Control in the Hematology Laboratory

*21. W ,

m m m m m -

14. A m

m m ?

A. k m m

m

A.

B.

B. true

C.

C. m

D.

D. m k

22. A m m

15. T m m

A. \pm m

A. k m m

m

B. m

C. \pm m

B. true

D. \pm m

C. m

D. m k



CHAPTER 1 ■ Safety Practices and Quality in the Hematology Laboratory

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REVIEW QUESTIONS (continued)

*23. A QC

25. W m G

A. m m

A. I .

B.

B. I f .

C. m m

C. I .

D.

D. I m - .

m m k

26. (SD) m m

24. A -

m

m m m

A. %

A.

B. %

B. m

C. %

C. m m

D. %

D.

C L S I (CLSI). Using Proficiency **COMPANION RESOURCES**

Testing to Improve the Clinical Laboratory, , W , PA,

:// . . m/

:GP -A .

C L S I (CLSI). Protection of Laboratory Workers from

Occupationally Acquired Infectious Diseases:

m .

Approved Guideline, , W , PA, :M -A .

H ,

C L S I (CLSI). Clinical

-

Laboratory Safety: Approved Guideline, , W , PA, :

.

GP -A .

C L S I (CLSI). Clinical Laboratory Waste Management: Approved
Guideline, , W , PA, :GP -A .

REFERENCES

C L S I . Training and Competence Assessment: Approved Guideline, , W ,

PA, :QMS -A .

. T J C mm . National Patient Sa ety Goals E ective Janu—

C L S I . Laboratory Documents: ary 1, 2015. Goals Related to a Laboratory Accreditation Program .j mm . Standards M , .

Development And Control: Approved Guideline, , W , PA,

. C D C P , U.S. D m

:GP -A .

H H m S . E m HIV U

C L S I . Statistical Quality Control S , – . HIV Surveillance Supplemental Report, (), or Quantitative Measurement: Principles and De nitions: Approved

, P D m .

Guideline, , W , PA, :C -A .

. O S H A m , US D m C L S I . How to De ine and L . H W H z -N k/S j .

Determine Re erence Intervals in the Clinical Laboratory: Approved

:// . . *SL C z* .

Guideline, , W , PA, :C -A .

m M , .

D k k LK. k S : A m - -

. O S H A m , US D m m, Med Lab Observer, (): – , .

L . S C , M , : CFR

G J. L : , Med Lab Observer,

. () () (A) ().

(): , .

. Z m BS. O x B C HIV

H -G B. P x -

x x : m-

. Med Lab Observer, (): , .

m , Medscape, , M , .

L EL. APIC

. L G, G GC, M zz C, . P : T

- , Am J In ect Control, : - , .

k m , Clin Chem Lab Med, L S, J ER. T -

: - , .

, Am J Clin Pathol, : , .

M P RA. L , I : Henry's Clinical Diagnosis and Management by Laboratory
Methods, , S , **BIBLIOGRAPHY**

:C .

O S H A m , US D m C L S I (CLSI). Use o Delta Checks L . S C , M , : CFR

in the Clinical Laboratory, , W , PA, :EP -E .

. () () (A) ().

PART 1 ■ Hematology Basics

O S H A m , US D m C H -C F , MMWR Recomm Rep, (RR): L . O Ex B P : F

– , .

R , Federal Register 56(235), :(CFR . , –

U.S. D m H H m S , C D

; P C F R).

C P (CDC). Exposure to Blood: What Health—

O S H A m , D m L .

Care Workers Need to Know, W , DC, .

O Ex H z C m L : F

U.S. D m H H m S C D

R , Federal Register 55(21), :(CFR . , –).

C P , H I C P

R WA, W DJ, H I C P

A C mm (HICPAC). G I

A C mm (HICPAC). G D

P H , .

S z H F , , . . (

U.S. D m H H m S C D

A ,).

C P , US D m H H m U.S. D m H H m S . C D

S . CDC -

C P , US D m H H m m x

S . U : -

m m , MMWR Morb Mortal Wkly Rep, (), .

m m mm , B , W JO, B PL, H MR, . A m - S

- , MMWR Morb

q m , Clin Chem, :

Mortal Wkly Rep, () : , .

, .

U.S. D m H H m S . C

W FH J . C : , ,

D C P . P O HIV

m ? Lab Med, () : - , .

m H P , F .

W m , D. A x U.S. D m H H m S , C D

m m , MLO Med Lab Obs, () : , , .

C P (CDC). H -

. . . S mm R M W ,

, MMWR Morb Mortal Wkly Rep, (RR) : - , .

O , .

U.S. D m H H m S , C D

Y -P J, P CA. T m QC q

C P . G E m I

, MLO Med Lab Obs, (): , - , .

CHAPTER

Principles o Blood Collection

2

KEY TERMS

a nticoa gula nts

h e m a to m a

p h le b o to m y

b a s ilic ve in

h e p a rin

p la s m a

b o n e m a rro w

in vitro

q u a lity a s s e s s m e n t

ce p h a lic ve in

in vivo

s e r u m

e d e m a

m e d i a n c u b i t a l v e i n

s o d i u m c i t r a t e

EDTA

P a t i e n t C a r e P a r t n e r s h i p

ve nipuncture

LEARNING OUTCOMES

Quality in blood collection

- Explain some techniques for obtaining blood from small or difficult
- Name four critical areas contributing to the quality of blood specimens—
men collection.
- Describe special considerations for pediatric and geriatric patients
- Describe various components of quality assessment that apply to
in the collection of a blood specimen.
blood collection.
- Name the six categories of phlebotomy complications and describe
- Explain the Patient Care Partnership and its importance.
the symptoms and treatment for each type of complication.

- Describe the role of a Phlebotomist as a laboratory ambassador.
- Describe the proper technique for the collection of a capillary blood specimen.
- Compare the strategies that can be used for a successful interaction with three categories of patients with special considerations.

Preparation of a blood smear

Safe blood collection equipment

- Describe the procedure for preparing a push-wedge blood smear.
- Explain the four systematic steps that make the collection of blood more organized.
- List the characteristics of a good push-wedge blood smear.

- Explain the factors that influence the preparation of a high-quality push-wedge blood smear.
- Name the three anticoagulants most commonly used in hematology and briefly explain their modes of action.

- Describe the coverslip method of blood film preparation.

- Name adverse situations related to evacuated blood tube antico-

Special collection procedures

agulants and describe the effects.

- Name the appropriate sites for bone marrow aspiration in adults

- Describe the various types of safe equipment used for venous and capillary blood collection.
- and children.
- Explain the proper technique for preparing bone marrow specimens.
- Compare the color codes of evacuated tubes with the additives contained in the tubes.

Routine staining of peripheral blood films

Describe four important environmental factors that can impact the

- Explain the principle of Wright's stain.
- quality of evacuated tubes.
- Cite the reasons Romanowsky-type stains produce too red or too blue an appearance on microscopic examination of blood cells.
- Discuss factors that are important in the determination of the expiration date of evacuated tubes.
- Describe the manual procedure of Wright's stain, including sources of error in the technique.
- Explain the order of draw for collection of capillary blood.
- Explain various considerations to meet specimen storage and handling requirements.

Case studies

Blood collection techniques

Analyze patient blood collection issues; discuss any errors and remediation of these errors by answering the critical thinking questions.

- Describe the proper technique for the collection of a venous blood specimen.

NOTE:

- Name and explain venipuncture site selection situations.

- indicates MLT and MLS core content

- Name and describe the solutions to eight typical phlebotomy

indicates MLT (optional) and MLS advanced content

problems.

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PART 1 ■ Hematology Basics

The Phlebotomist as Laboratory

NOTE: This is a good time to review Key Term definitions in

Appendix A

the Glossary and flash cards on

.

Appendix A

. Terms

QUALITY IN BLOOD COLLECTION

m .

I m m j

V , phlebotomy, -

m , m m -

. H k

. T m

j . P m . T

m m . I U S ,

k m mm .

m m

T m x x -

m , -

m . I m k

m m ,

' x , m x

.

, m m

C

m . I , z

m. B

■ Ex mm k

m m, m-

, k m ,

■ P m

m.

■ A q q

■ V m

Patients w ith S pe cial Co ns ide rations

■ A m

■ C j m

Pe dia t ric Pa tien ts

W k , m

Quality As s es s m ent

m m , m , k .

A m , z

T quality assess-

() m x

ment m . T q -

m .

m , ,

A k . B , -

. T m q m m

, ·

q m m

A m . A k

m . A m , m . O -

q m

m m m “ ” (. . ,

m , m ,

z).

m .

I , ,

. A k

Patient Care Partners hip

, m -

T

. I ,

.

m . W x ,

W m , m

m m .

m . D m .

T Am H A

Adoles ce nt Pa t ie nt s

Patient Care Partnership m , m -

P ' B R . P m ,

W m m ,

, x

m x x -

. A x m k '

m . G q

k -m k , mm ,

, m .

, m

q

.

— m — k

. A , -

Adult Pat ie nt s

m

A m x m

, ,

. C m m -

m

. T

.

m . T .



CHAPTER 2 ■ Principles of Blood Collection

27

I m q , m m m m ' , , m-

.

E , m m . I m m , m , x -

k .

I , ' m, k

m

. T -

q m k

. A

.

Ge ria tric Pa t ie nt s

I x m m -

. D m . I

m m M .,

M ., M . m .

FIGURE 2.1 G VACUE E m S m j . K

(G B -O).

x m .

S k q

m . A m q . T

m . m m

A m

, m m

q m . S

.

m .

P (x m)

Proble m Pa t ie nt s

, m , m j O , m .

.

T m , -

F m ,

. I m , m

m m q . W

m , ,

m x ,

. B j -

anticoagulants, plasma, m m m m , -

- , m : -

. T

, k , (m) (F . .).

,

W m

.

- , serum.

Anticoagulants

NOTE: This is a good time to complete Review Questions

E

related to the preceding content.

m . T -

m

. T

SAFE BLOOD COLLECTION EQUIPMENT

m . I q m x

m k m ,

m

m m :

m .

I m , q -

■ P m .

■ R m m m

.

■ m m (K E D A),

■ D m m m m

m m (K

(F . .).

ED A)

■ D m -

■ m

m m .

■

28

PART 1 ■ Hematology Basics

FIGURE 2.2 B m . **A. S**

Anticoagulant tube

Non-anticoagulant tube

- m . **B. C** , -

, m m . (M C RE, k

CM. Phlebotomy Essentials, , B m ,

MD: L W m & W k , .)

55%

Plasma

(Liquid)

S e rum

(ha s fibrinoge n)

(la cks fibrinoge n)

Buffy coa t

(WBCs &

pla te le ts)

S e pa ra tor ge l

Re d blood

ce lls (RBCs)

45%

Clotte d blood

(Forme d

e leme nts)

A

B

EDTA

m mK

.

EDTA K EDTA -

. ED A . m mL

S m

. T m

m m (P) m m

m z m (C +)

(P) W m -

. T m m

(ESR). T

.

.

ED A mm I C

A x x

S z H m (ICSH) CLSI

. B -

z . I

, m

m q m

m m .

m (CBC) m

(m , k m m m ,

Hep a rin

k , k ,

Heparin in vitro in vivo .

, modi ed W ' m -

H m , -

[ESR]).

- m X . T

T ED A m

m m x

m -

m (A) m z

. Ex ED A k , m . T m m m k

z m

m (m m).

q m

m .

Sod ium Cit rate

L m mm m

Sodium citrate . % ,

k

m . I -

I C mm

S z H m , I S

U/mL.

T m H m . I C

H “m ” () -

Am P (CAP) k

m . H

m .

m m ,

S m m m m -

W - m , m

m m. S m

.

CHAPTER 2 ■ Principles of Blood Collection

29

Handling of Bloo d Collection Tube sa

Num be r of

Additive

Investigations

Notes

Serum clot activator

5–10×

For complete clotting, allow tube to stand 30 min minimum.

Note: If clotting time is <30 min or delayed in mixing, it may result in delayed clotting.

Serum clot activator

5–10×

For complete clotting, allow tube to stand 30 min minimum.

with gel

Note: If clotting time is <30 min or delayed in mixing, it may result in delayed clotting.

Lithium heparin or

5–10×

Inadequate or delayed mixing may result in incorrect test results.

Sodium heparin

Note: Do not use lithium heparin additive tube for lithium assay. Do not use sodium heparin additive tube for sodium assay

Lithium heparin with gel

5–10×

Inadequate or delayed mixing may result in incorrect test results.

Note: Do not use lithium heparin additive tube for lithium assay.

K2 EDTA

8–10×

Spray-dried K2 EDTA or K3 EDTA are substantially equivalent.

K3 EDTA

K2 EDTA with gel

8–10×

Centrifugation using K2 EDTA with gel should be performed within 6 h after blood collection.

Note: For storage of 2 wk at –20°C or storage for longer than

2 wk at –70°C, transfer plasma to a secondary container and freeze immediately.

Sodium uride

5–10×

Inadequate or delayed mixing may result in incorrect test results.

Potassium oxalate

aGreiner Bio-One Preanalytic Pulse Sample Handling and Transportation, July 15, 2015.

Store tubes at 4°C–25°C (40°F–77°F) before blood collection.

Do not store tubes in direct sunlight.

Preferable transport tubes in an upright position.

Factor

Potential Adverse Effect

Correct Procedure

Incomplete mixing of If a tube with no anticoagulant, incomplete clotting of
Promptly mix the number of times specified by specimen serum tubes

the manufacturer.

If tube with anticoagulant, fibrin formation or micro—

clots in tubes

Abnormal formation

Incomplete clot formation results from fibrin strands

Tubes should be allowed to sit in an upright

of a clot

being trapped in the serum.

position for at least 30 min to allow for

complete clot formation and retraction.

Lack of upright

Clot or red cells adhere to the stopper or cap of the

Tubes should be kept in an upright

orientation of tube

tube.

position during handling, transport, and storage.

Transport by

Hemolysis can occur in the specimen.

Use quality assurance methods to monitor

pneumatic tube

specimen quality.

Transport by courier

Rough handling or exposure to extreme temperatures Careful temperature management during while in transit

transport. Quality control of storage guidelines

of specimens

(continued)

30

PART 1 ■ Hematology Basics

Factor

Potential Adverse Effect

Correct Procedure

Shipment of

Rough handling or exposure to extreme temperatures Pack specimens

according to DOT regulations specimen

while in transit

for biohazard material. Insulated containers

should be used for temperature maintenance.

Tubes should be in an upright position, if possible.

Temperature

Inappropriate hot or cold temperatures can produce

Specimens should be maintained at appropri—

inaccurate results leading to incorrect patient diagnosis and treatment.

Storage guidelines must be established at testing laboratory.

Time

Time

Extended delays in testing can produce erroneous

Samples should be centrifuged within 2 h after

results.

collection. Plasma or serum should be transferred to a secondary container and maintained at an appropriate temperature.

Centrifugation

Fibrin may form and adversely affect the analysis of Samples should be centrifuged within 2 h after plasma or serum constituents.

collection. Plasma or serum should be transferred to a secondary container and maintained at an appropriate temperature.

Sample separation

Continued cellular metabolism can adversely impact

Tubes with an inert gel barrier should be centrifuged

analysis of serum or plasma constituents.

centrifuged within 2 h of collection to minimize the

effect of cellular metabolism.

Recentrifuging a

Recentrifuging a specimen will produce a sample that Never recentrifuge specimens because the specimen

is a mixture of the original plasma or serum sample

sample will no longer be representative of

with additional serum or plasma that has been in

the original specimen. If a larger sample is

direct contact with blood cells.

required, collect a new specimen.

Adapted from Greiner Bio-One Preanalytic Plus, Sample Handling and Transportation, July 15, 2015.

Adverse Effects of Anticoagulants

SAFE BLOOD COLLECTION EQUIPMENT

Thrombosis.

ONM, , NKSP

A m . I :

■ Alteration o constituents: T -

m m m

■ R q m - -

. A m

m m -

. A x m

k x HIV,

, x , m -

C, -

. Ox m ; RBC m

■ Ex

(k), ,

j

z m m m

■ R q x - m -

x .

m m m

A x m -

m m m z x ;

m

m

W' . U

■ R q m

m ,

j , m -

k W' .

j

■ R q m m k-

■ Incorrect amount o anticoagulant: I

m

, . T -

. B m , m

q , m

q m m .

Safe Needle s

T

-G . B

NOTE: This is a good time to complete Review Questions

m q

related to the preceding content.

.







CHAPTER 2 ■ Principles of Blood Collection

31

T - . A

- . T

, -

. S

. V z . I -

, z . T

m , m m .

M - m

, -

. T m

m m

. W

,

. T .

Preattached Blo o d Co lle ction Ne edle and

Ho lde r

A , -

. T (F .

..) m -

k j , m -

m ' x m .

T k z . T

N P m A (NPA) %

m m , k x HIV, C

, B , -

k .

FIGURE 2.3 VACUE E® VISIO PLUS -

T U.S. O S H A m

(G B -O).

(OSHA) OSHA -

, OSHA B

m

P S [CFR . () () () (A)].

. N q q .

T m m

m m .

Oth er Co ns id e ra tions

I

B

. O venipuncture m ,

m . I

FIGURE 2.4

. **A.**

. **B.** V N -

P

.

(M C RE, k CM.

Phlebotomy Essentials, ,

B m , MD: L

W m & W k , .)

A

B

32

PART 1 ■ Hematology Basics

A -

BOX 2.1

m ,

m (

OS HA S afe ty and Health Info rm atio n

A x). T m m **Bulletin: Evaluation To olbox z**

m . T -

. Em m , , -

(. ,

j [SESIP]), -

m .

- SESIP .

A m x

. T k -

q (“ ”)

m -

j m .

. T CLSI ISO S -

D m

m

-

.

z .

T m m . A

. I , m .

. I m m -

m . T -

m (. . ,

mm m -

-

x °C °F. I

).

m ,

. I m m

k m -

.

, m m m .

. I , m m

E - m . U

m SESIP

-

. I , m m

m k m m -

. T q m -

mm

m .

m . T m m

CLSI

m m ' Ex C

m . W -

P .

m , “ ”

. I ,

m

j m ,

m - m

m

. E

k , m

-

m

. I , m

.

m (. ; F . .).

R m .OSHA. (M).

Environ m e nt al Factors As s ociate d w it h Evacu ate d

Blood Colle ction Tub es

, j

A m m q

k

. T

. m

x -

m

. E m

.

Evacuated Blood Collection Tubes

■ m

A

E m m -

■

H m

m . T m (F . .)

■

S

, ,

■

m m

.

Te m p era ture

E m (mL) z ,

I m ,

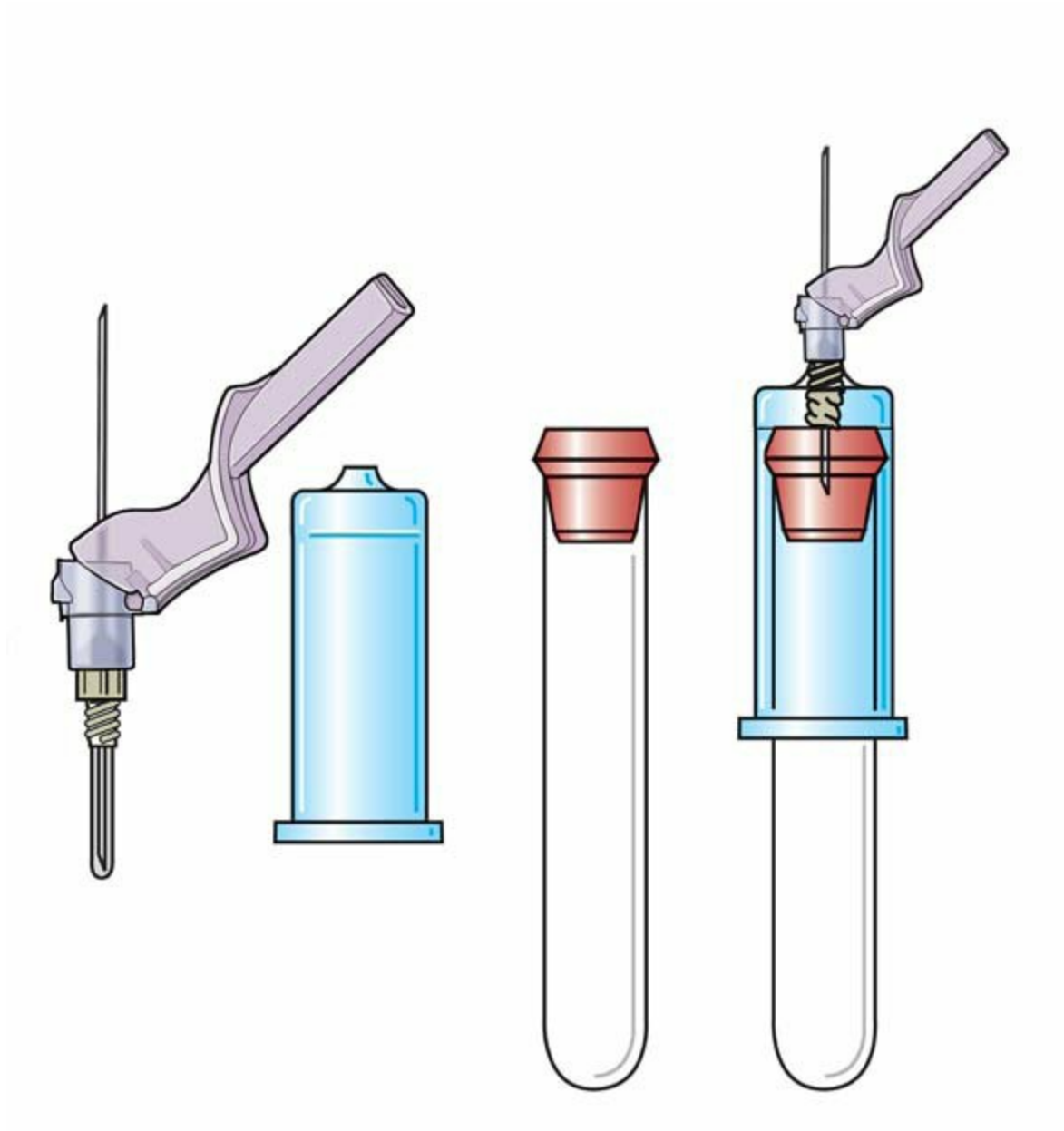
z , - . T

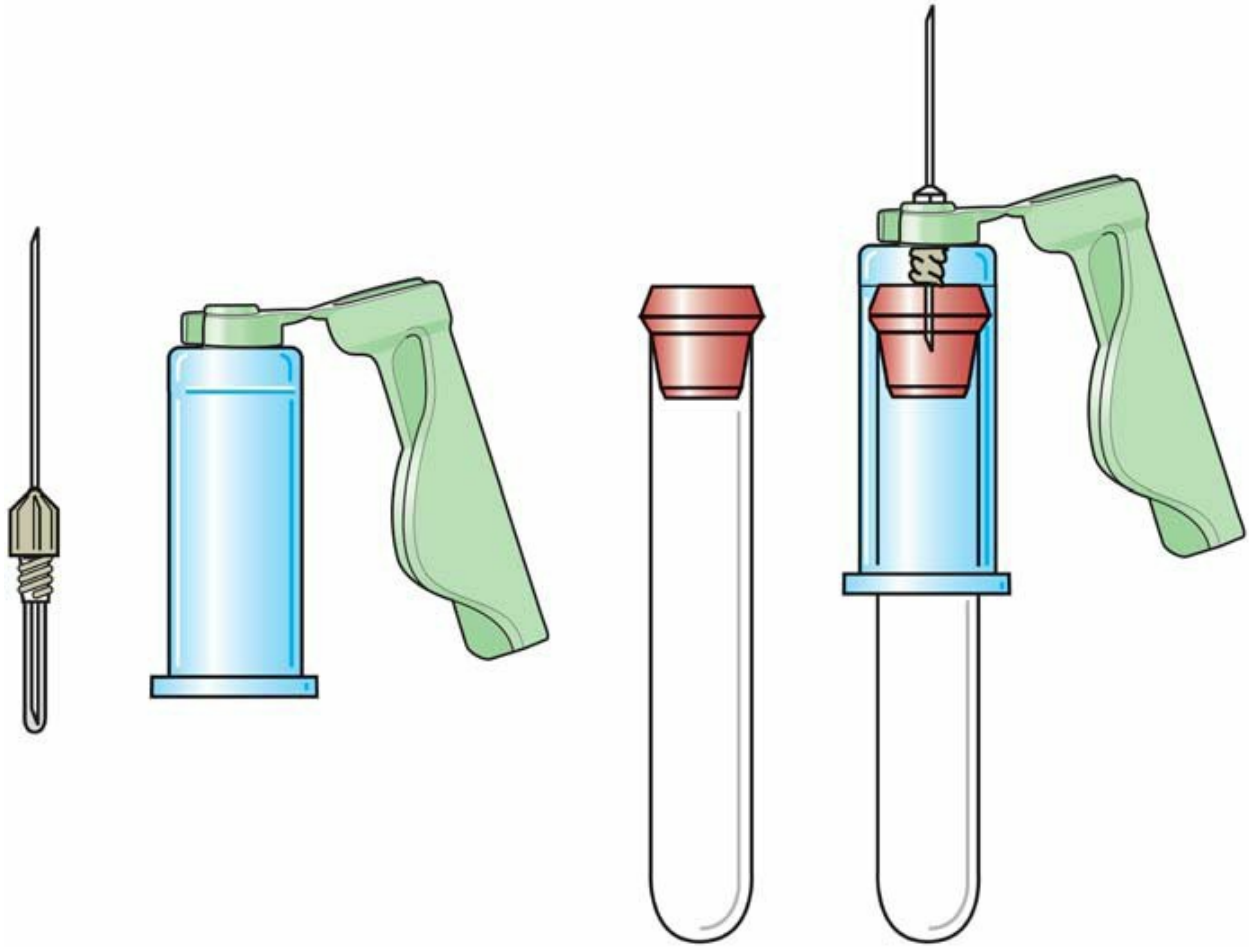
. T

m . C ,

(.).

m m .





CHAPTER 2 ■ Principles of Blood Collection

33

Needle

within

stopper

Bevel

shaft

Thre a de d

hub

Fla nge

Rubbe r

s le eve

ove r ne e dle

S afe ty

de vice

Multis a mple

Tube holde r

holde r

ne e dle

A

Eva cua te d

Asse mble d

tube

s ys te m

S a fe ty

de vice

Be ve l

S ha ft

Ne e dle

within

s toppe r

S a fe ty

de vice

Thre a de d

hub

Fla nge

Rubbe r

s le e ve

ove r nee dle

Multis a mple

Tube holde r

ne e dle

B

Eva cua te d

Asse mble d

tube

s ys te m

FIGURE 2.5 E m (E S) m . A. . B. S

. (M C RE, k CM. Phlebotomy Essentials, , B m , MD: L W m & W k , .) A ,
, x m , Altitu de

m , m

I (

m . G m-

,), m m . B

x

,

m (.).

34

PART 1 ■ Hematology Basics

Exam ple s of Sto pper Colo rs fo r

. T m .

TABLE

2.1

Venous Blood Colle ctio na

Hum idity

Colo r

Anticoagulant

T m m

m ,

Lavender

K2 EDTA (spray-coated plastic tube)

m m .

K3 EDTA (liquid in glass tube)

C m m

Pink

K

m -

2 EDTA (spray-coated plastic tube)

m , z . C

Green

Heparin

m m

Light blue or clear

Buffered sodium citrate (0.105M in

. I

glass, 0.109M in plastic)

m m .

Citrate, theophylline, adenosine,

dipyridamole (CTAD)

Light

White

K2 EDTA with gel

A m x -

Red/light gray clear None (plastic)

C AD (, , , mRed

Silicone coated (glass)

). T C AD m x m m z

Clot activator, silicone coated

. N m ,

(plastic)

m . T

aSee Appendices D and E for the comprehensive BD Vacutainer Venous

k m q m m z

Blood Collection Tube Guide and Greiner Bio-One VACUETTE Blood

x .

Collection Tubes.

bNew tube for use in molecular diagnostic test methods.

Expiration Date s of Evaluated Tubes

cNew red/light gray for use as a discard tube or secondary specimen tube.

Exam -

Adapted with permission from BD Vacutainer Venous Blood Collection

mm . S

Tube Guide, 2010 and Greiner Bio-One.

,

TABLE

Order of Draw of Multiple Evacuated Tubes Collection

2.2

Closure Color Mix

Required Number of Tube

Order

by Inverting

Type of Tube

Inversions

1

Yellow

Blood cultures-SPS—aerobic and anaerobic

8–10×

2

Light blue

Citrate tube

3–4×

3

Gold or red/gray

BD Vacutainer SST gel separator tube

5×

Red

Serum tube (plastic)

5×

Red

Serum tube (glass)

Orange

BD Vacutainer rapid serum tube (RST)

5–6×

4

Light green or

BC Vacutainer PST

Green/gray

Gel separator tube with heparin

8–10×

Green

Heparin

8–10×

5

Lavender

EDTA

8–10×

6

White

BD vacutainer PPT separator tube

8–10×

K2 EDTA with gel

7

Gray

Fluoride (glucose) tube

8–10×

aThe order of draw has been revised to reflect the increased use of plastic evacuated collection tubes. Plastic serum tubes containing a clot activator may cause interference in coagulation testing. Some facilities may continue using glass serum tubes without a clot activator as a waste tube before collecting special coagulation assays. Reflects change in CLSI recommended Order of Draw (H3-A5, Vol. 23, No. 32, 8.10.2).

bIf a winged blood collection set for venipuncture and a coagulation (citrate) tube is the first specimen tube to be drawn, a discard tube should be drawn first.

To ensure a proper blood to citrate ratio, use the discard tube to fill the air space with blood. The discard tube does not need to be completely filled.

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CHAPTER 2 ■ Principles of Blood Collection

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T x m m

m m m . Ex

z m -

m -

m . T x

m m

. E m

m m .

I m -

m . T m

m () , .

T

m , m

z . T m

m -

.

NOTE: This is a good time to go to Review Questions related to the preceding content.

= One inve rs ion

FIGURE 2.6 I m

Capillary Blo od

(G B -O). (M C RE, k CM. Phlebotomy Essentials,

, B m , MD: L W m & W k , .)

S (F . . .) m

. T

m . M

m (.).

m k -m . I m

S m

mm .

(F . .).

A

B

C

D

FIGURE 2.7 A–D. S () . (M C RE, k CM. Phlebotomy Essentials, , B m , MD: L W m & W k , .)



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PART 1 ■ Hematology Basics

A

B

FIGURE 2.8 A, B. S . (M C RE, k CM. Phlebotomy Essentials, , B m , MD: L W m & W k , .) **M m Specim en Sto rage and Handling**

m . m m -

Re quire m e nts

$z \cdot F, \lambda m -$

$m \cdot P - m m$

T

- k

. I ,

$m j m m m m$

$m m m$

$m \cdot E - m m$

.

$m M$

B E D A m

,

$q m \cdot I -$

$m \cdot H, M$

, E D A m m

$m k x$

$m x m m \cdot$

$m k$

S q m E D A :

k k. M

. B m m ED A

m k .

m m

T m m m

m (.).

.

. ED A m -

z ,

m m .

. ED A m

BD Microtainer Tubes w ith BD

TABLE

2.3

m (ESR) m m

Microgard Clo s ure Orde r o f Draw

m m

, .

Clos ure

Mix by

. ED A m

Order

Color

Additive

Inverting

mm, .

1

Lavender

K2 EDTA

10×

Tmm(P)mm(P)2

Green

Lithium heparin

10×

.% .I

3

Mint green

Lithium heparin

10×

m

and gel

, m ,

4

Gray

NaFl/Na₂ EDTA

10×

z m m . T C L

5

Gold

Clot activator

5×

S I (CLSI) m H -A () -

and gel

mm P m

6

Red

None

0×

m m m m

(°C °C). O

Reprinted with permission from Becton, Dickinson and Company. Lab

P , m

Notes, 20(1):7, 2009.

m m (°C) m-m







CHAPTER 2 ■ Principles of Blood Collection

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A

B

D

C

E

FIGURE 2.9 A–E. Examples of venipuncture techniques. (McCrackin, C. M. Phlebotomy Essentials, 8th ed., Baltimore, MD: Lippincott Williams & Wilkins, 2010.)

mm x mm

Order of Draw for Capillary

.

TABLE

2.4

Im, -

Specimens

mm OSHA qm

m (OSHA F.R., CAR.). S

Order

mm k q m m j

1

Blood gases

D m z

m . A -

2

EDTA tubes

z m m m

3

Other additive minicontainers

- . T m m

4

Serum

CLSI m , P L

Note: If multiple specimens are collected by heel or fingerstick puncture

W k m O A q I D .

(capillary blood collection), anticoagulant tubes must be collected first to

E m ,

avoid the formation of tiny clots due to prolonged collection time. Blood

(x m) ,

gases should be collected first, if the phlebotomy team is responsible for

. M m -

collection of these specimens.

(x m) m x m m . M

aOrder of draw for capillary blood collection is different from that for blood

.

specimens drawn by venipuncture.

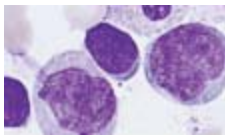
B m -

Adapted with permission from Becton, Dickinson and Company. Lab Notes,

m k q m

20(1):2, 2009.

CAP (:// . .).



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PART 1 ■ Hematology Basics

BLOOD COLLECTION TECHNIQUES

T x m m

mm m .

.

P . N P S G

q m , -

q m m -

m .

. A CLSI

m m m

GENERAL PROTOCOL

. L

. P m m

m :

x

, m , m m m q—

m . I k

. A

' , E

. T m

. E

m .

m S k .

. T ' m , q m , m

. P -

m , m

. C L S N P

. I m , m

S G m J C mm m -

m m

.

m . A m q m

q . T q m

m m .

mm

N : C m

m . T m ’

, . T -

-

-

.

.

I k m . I m -

VENOUS BLOOD COLLECTION (PHLEBOTOMY)

, m m m

Supplies and Equipment

m m m . C k

. q

. q

. W q - -

. A (%) z q

m -

. S (-)

. U , . A

. E () CAP-x m j -

()

:

. A q m m

— q m q

■ A

. S mm k (m

■ W

)

■ M m

. A

■ P m m

■ E m

Initiation of the Procedure

■ I m

. I .

W m ,

. A m q m ' .

m ,

. P .

k

. I , m

. A

.

q . A CAP mm -

I ,

m m

. T

.

m mm

T k k

. T

m , - - m , x m , . I m . N : D

k , x m ,

, m

(m C m). W

.

m , m k

Selection of an Appropriate Site

m m .

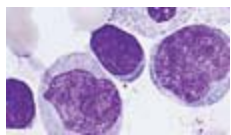
N : V -

. q k -

(IV) . I m m

m . A m

m, , m



CHAPTER 2 ■ Principles of Blood Collection

BLOOD COLLECTION TECHNIQUES (continued)

. I , IV

A. I q , q m m . W m m x -

m IV

. E j q

m q -

(F . . A).

. O m m IV

B. G q

k m x

' m. P

m .

q . C q

. W x , -

. V m . C m

m q

q (F . . B).

, , . I

C. S m q .

m, mm :

T m

cephalic, basilic, median cubital (F . .).

q ,

. A q . q (F . . C). N : P

. O ,

q m—

V m j m

. T m , m -

m.

(AS), m, , , ,

, .

. A k m k (m m z

'). T m k

m m . W x , ()

(F . . D). P m

, m-

. L

m . S m

. T

Ce pha lic ve in

m. I -

m, x m m

q m. D

q m m . V

Ba s ilic ve in

, ,

Me dia l cubita l ve in

; , x -

m m.

Ba s ilic ve in

Special Site Selection Situations

F f (x-m) . T

Ce pha lic ve in

Me dia n ve in

of the a rm

. E m x m

. IV

. S

. D

. P m m

Edema

Edema m m -

. V

m m x

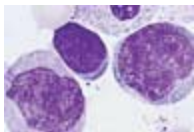
m k f , m m **FIGURE 2.10** A m m. I m, : , ,

m

m .

.

(continued)



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PART 1 ■ Hematology Basics

BLOOD COLLECTION TECHNIQUES (continued)

FIGURE 2 .1 1 S

.

A. A j q .

A j -

q . **B, C.** A

q . P

q ,

, m

q .

A q

m

m

. **D.** P . T

x

A

B

. T

,

m.

C

D

IV Lines

A m m m .

P IV m

B m m

mm m m . A m IV

m . T

m

m m . T ' m

m. I , q m

. I

, IV

.

m

I , k

m . T IV -

m -

m q . A m -

m m

,

.

.

Postmastectomy Patients

Scarring or Burn Patients

I m m m j

V f

m , m (k m

x . B m -

). S m m

(-

m . I

m). V m

, m m .

. A

T , m

.

m m m .

Dialysis Patients

Preparation of the Venipuncture Site

D m m

. A ,

, q , m

q .

. B m m

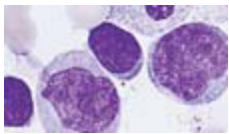
. U % -

(m) (

% , k

m).

. U m ,









CHAPTER 2 ■ Principles of Blood Collection

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BLOOD COLLECTION TECHNIQUES (continued)

m m . D

. U m k .

. U m

. A .

k m -

. T k .

Performing the Venipuncture (Fig. 2.12)

P ' m .

N : I -

. H

. I , m

-

. P

.

. T -

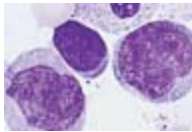
.

FIGURE 2.12 P m . A z q , A k m k m . B. T

' . C. A . D. R q . E. R m x . F.

A , m q z . A . (R

m m E DJ. Applied Phlebotomy, B m , MD: L W m & W k , .) (continued)



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PART 1 ■ Hematology Basics

BLOOD COLLECTION TECHNIQUES (continued)

. G k

. R . T

. T m m . I -

m x m

,

' m, q ,

m .

. I m

. D f m

. S k

.

m m.

. M m . m, M m

m m

.

. T m

T CLSI m -

, x m .

m -

C m x m

, . I

m m m

,

.

k .

. A q m . A Termination of the Procedure “ , ” k m -

. T q m

, . A x -

mm

m EDTA k

m .

, f m

. A k .

m m,

. A m , m .

z .

. Im . I m ,

. W

. F x m , m

mm z

m z m

(F . . E).

W ’ ,

. I , m

.

z . I

. S m m m

,

m m m .

.

A . Q k !

. P

Imm m q , z

. N : F f -

,

m m

m hematoma. I

(k

z

).

m .

. M x -

. B m . I

m . I , m

m , - ()

. B

. P m m

k ,

, -

. D

m m x

.

m .

. L q .

. F q . T

. C m k , m ,

. N : I ,

. I m j

m m , k

zz .

zz

Ten Tips for Locating and Drawing from Dif cult or Small

. D m

Veins

z .

I mm f

Phlebotomy Problems

m . m f

O , . D m m : m m m . I

. A j m.

m , m -

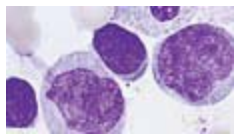
. U m .

. P m m

. U m .

:

. U m m .



CHAPTER 2 ■ Principles of Blood Collection

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BLOOD COLLECTION TECHNIQUES (continued)

. q .

S m m m -

. L q .

m m

. A k m.

:

. U q .

. V m

. U

m

m.

q m .

. U m

. V m

.

Special Considerations for Pediatric and Geriatric Patients

q / q

Pediatric Patients

.

. P , ,

P m m k q m mL . L m - mm -

m m -

m f m .

“ .”

P B x . m m-

. L -

m . P m

m ,

m q -

f . T m

/ m .

. I

. I x m m m x m , , x m m

m . U m-

.

m

m -

Geriatric Patients

k.

A

P m

k

(. ., m m).

m . A , x

B , -

' . W m k

m m m

m m

. I , -

x q z q j .

. B m m

P m

j -

mm .

m .

Specimens from Children Younger than Age 1

T m m -

m . T

G

:

mm

. C m k k

. C . E m

.

.

. k m “ ” -

. L k m , , , mm .

PROCEDURE Box 2.1

. N m

General Tips for Pediatric Phlebotomy

.

. W m k ’ m

■ W k q k m .

mm .

■ W m

. B q

.

k . q

■ C k

, m m

.

.

■ D m

. R m q j

k

m .

m m .

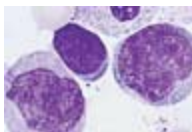
■ R mm -

. C m (. . ,

.

) m .

(continued)



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PART 1 ■ Hematology Basics

BLOOD COLLECTION TECHNIQUES (continued)

. U m m -

m k, m .

PROCEDURE Table 2.1

. U q k m ;

Vascular Complications of Phlebotomy

m .

CONDITION

DESCRIPTION

. N .

. V m k -

P m

F -

k

m m . T -

.

T m

T -

. A k m

. T m

m m ,

m -

. I m -

.

,

. P k . B

x mk m

. E z

m .

k .

R x m

O k

Phlebotomy Complications

P m m

P x m m m . m A

A m

x m j : (m m-

m), , , m , ,

.

m .

Vascular Complications

Cardiovascular Complications

B m m m C m -

m m mm m . T

, , k, .

m m x

O m m -

m (. .,

m -

). B

. T k m

. U mm m

.

q m,

S m m -

m , x m,

, , - , zz , -

m (P .).

, . T

, m , , m

Infections

, k, . A

T m mm m

m ,

. T m mm m

, , m m , , -

(mm) (m-

, . T m

m). O

m ,

m (), -

, , . m

(j), m

, -

(). I j -

, ,

m j

,

. O m

m z .

m k m m

S k m , m m , m j m m m. D

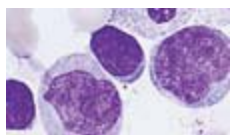
m k ; k ; . T

k m m

m m m , m

.

k , .



CHAPTER 2 ■ Principles of Blood Collection

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BLOOD COLLECTION TECHNIQUES (continued)

C m ,

, ,

, m , , .

m j .

m mm -

. T m m

m (CPR) .

; , m

Anemia

m. I ,

m m m

I m k m m , m .

- m , m m

. O m () m -

. P

. T m -

m k

q . I m

m . T m -

. S

q m , , m m .

m m k. I , m - m IV .

m .

Neurological Complications

O m m

m , -

P m x m

, k , k

m . T , z , ,

. mm, x ,

m . A mm .

,

Dermatological Complications

m .

T m mm m q -

Preparation of the Site

m

. H m x

. O m m -

.

, m (), .

. W % - .

. W z q . I

, m

NOTE: This is a good time to complete Review Questions

f .

related to the preceding content.

Puncturing the Skin

. U

CAPILLARY BLOOD COLLECTION

- .

Supplies and Equipment

. S m

. A (%) z q

m . T

. D m z q

, . I

. S

,

. Eq m ,

—

m , m CBC ,

(F . . B .).

m m

. W

m . T m x m

Selection of an Appropriate Site

.

. T (-

. A q

)

. A q -

m q . T m

q z . I q z

. D

, m m x

k , m .

.

T ()

(F . . A)

Collecting the Sample

m . N : T m -

. I m ,

,

x -

. mm - k

m .

m

. A m m m

. CLSI mm

- . T m

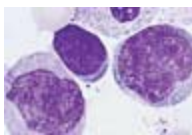
. T k

z

j . T

k m .

(continued)



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PART 1 ■ Hematology Basics

BLOOD COLLECTION TECHNIQUES (continued)

m . CLSI

, ,

m . B

, m

.

I m neonatal screening programs, m -

. S

;

k . T

m , q -

m m

q m. T -

m ,

m .

A

C ,

. W

,

m . A f

m

. T

-

. T -

-

.

PREPARATION OF A BLOOD SMEAR

B

T m m

' , ED A-

FIGURE 2.13 A, B. A . Shaded arrows

. m

mm . (M C RE,

m

k CM. Phlebotomy Essentials, , B m , MD:

L W m & W k , . [A]) **B.** F .

m . I , -

T shaded

m m .

m .

THE PUS H-WEDGE METHOD

. W q z q -

Specimen

m ,

E ED A- -

.

. I ED A , m m

Termination of the Procedure

. B

m , °C °C. A q m x

. W .

m .

. P z q . I

,

Supplies and Equipment

z q .

C (), N .

. L m .

, ()

. P - , m m m .

m , .

Procedure

Blood Spot Collection for Neonatal Screening Programs

. P m (. mL) -m x

M q

m

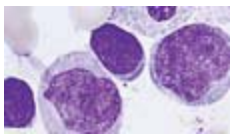
k x m . . m

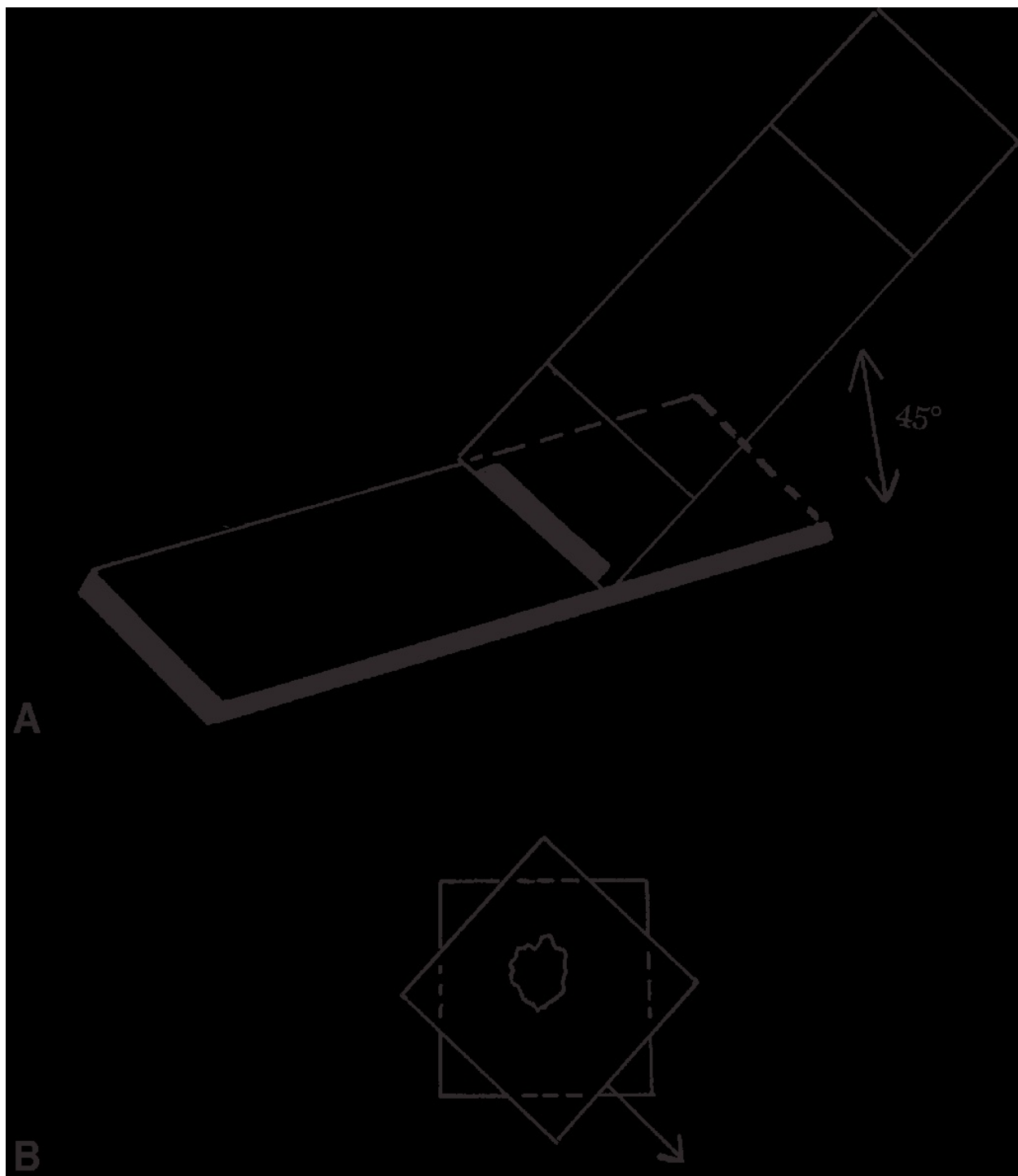
m , m ,

. I ,

. T

.





BLOOD COLLECTION TECHNIQUES (continued)

FIGURE 2.14 A - m '

. (M C RE, k

0 mm

CM. Phlebotomy Essentials, , B m , MD:

L W m & W k , .)

Va scula r be d

Rich in ca pillary

loops from 0.35 mm

to 0.82 mm in de pth

1 mm

Pa in fibe rs

Pa in fibe rs

incre a s e in

abunda nce

be low 2.4 mm

2 mm

. P -

mm

m . R -

m

.

m m -

. U ,

.

. T

m x m (F . . A).

. D k .

A

. D

. Q k

(m). T m -

m m m

.

. A m - . T

m .

A

. L N . . T m

k ()

.

Procedure Notes

. A mm -

B

m m - .

FIGURE 2.15 P - m . A. A . T

S m

x m . **B.** C

m

. O m

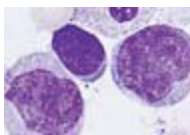
. A (mm × mm) m m-

, m

m .

z .

(continued)



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PART 1 ■ Hematology Basics

BLOOD COLLECTION TECHNIQUES (continued)

. - m mm CLSI

. I z .

m k

k, m . m

.

, m .

. N m , m . I -

. Im . T m

, m m m

, m . T

.

, k m .

. Im m m . S

Visual Evaluation of a Good Blood Smear

m .

A m (F . .) .

. Im . T ,

. I m k

m .

m m .

. H m m . H m

. I

. T

.

m

. I m , .

x m .

. I k , , , m k

COVERS LIP METHOD OF BLOOD FILM

.

PREPARATION

. I m

Procedure

x m

. H m

.

. -

m .

Causes of a Poor Blood Smear

. Imm

m .

. P -

. A . J

m . T .

m ,

. A m . I m m m z m mm

(F . . A).

. I ,

. P m -

m -

.

x m

m .

Procedure Notes

. D -q . S

. T m -

.

k .

FIGURE 2.16 I m . A

Examination

m

area

Blunt feathered edge

Point

m

Side margins

of

. T m

origin

m m

m

. (M m m

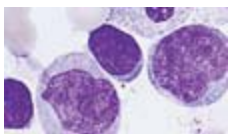
K k JA. S z m -

k , Lab Med,

: , . © Am S

C P © L

M .)



CHAPTER 2 ■ Principles of Blood Collection

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BLOOD COLLECTION TECHNIQUES (continued)

. B m m m , k

m m x m . T m m

.

m m

. O m z ,

. A m -

m ()

m .

.

Sites of Aspiration

Special Collection Procedures

A m m m k -

I

(F . .). A

m m , m

(), ,

m -

m. T m

' . T

m .

.

Supplies and Equipment

BONE MARROW PROCEDURE

B m / q m k -

q m -

Principle

m , m k

Bone marrow x m m

m. I m

m m -

q m , m :

. A m x m

m m k , -

. A , , z k m m m m .

FIGURE 2.17 S m

. T red-shaded -

k m

m .

(R m Dz z k E. O

m m

m , Curr Opin Hematol, (): ,

, m .)

S te rnum

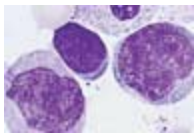
Ante rior

Pos te rior

ilia c cre s t

ilia c cre s t

(continued)



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PART 1 ■ Hematology Basics

BLOOD COLLECTION TECHNIQUES (continued)

. C z , m , % % , m : (M:E) , m , , .

m P ,

. A Z k ' m x .

mm .

. A m

q .

ROUTINE STAINING OF PERIPHERAL BLOOD

. T m q , **FILMS**

, , m

x m m ,

m

m . T m

.

m m

Procedure

m -

m .

A m , -

T m mm m

m k m

R m k - .

m ' . U m

STAINING PRINCIPLES

.

I , R m k M k

. P m x m

m m (x z) m -

. A m m m

. ,

L m , m

m

m m , -

m () m m

, m . ,

.

R m k - m

. D ,

q .

m m m : - ,

A R m k

, q q .

m / x -

. T -

, B Y. R m k -

q . Sq -

, W ' , G m ' , M -G ü

m m

, mm .

. S m

T -

m m

(F . . B).

R m k . T m -

. T m m , -

m m

m (. . , m),

H . . . T -

x .

, k

. A m m '

m, .

m. P m .

Stain Preparation

Bone Marrow Examination

T x m -

M m m m m m ; z A, B, C; m m f m

; m m . W

m m -

, m

m x m m

. Az B

m . I , f - m , m x -

m x R m k

(H&E)- m ,

. S q z B

m m m , -

R m k

, m , m k ,

m .

m mm

T z B Y m

. , m -

. S k m

m m m

z (. . , G m '). A k

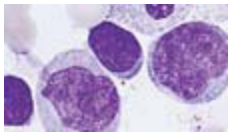
m k .

m x

M x m

m . T k

m , m , m H . m z .



CHAPTER 2 ■ Principles of Blood Collection

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BLOOD COLLECTION TECHNIQUES (continued)

Staining Reactions

S m . C mm , m m

T R m k -

m .

:

. A m , (H .) m . T

M ,

m (x) . D

m m .

x . S m -

T (. . , DNA

.

RNA).

. M x . A E , m m -

-m x m

- . T -

. T m m

(. . , m).

m . I C j ,

W m m x-

.

m , k

. W

. T .

. P k k

STAINING PROCEDURE

m .

. A - .

B m

R m k - . T

Sources of Error in Staining

- - m. E

P -q m : m m q .

. F , ,

I m , m x

m m . I m

m . T

, m

. T

m . Sm m m m -

x m . S

k m x m .

- m m . W '

. I H k R m k - m . T m -

; , x m k

m .

m . A

Reagents and Equipment

m m -

. S m - - m m

x m . I (k),

m

m m x m .

m ' .

. Im m

. A k C j .

m .

m m

Pro cedure

m x m . I m

, m k m

. P -

x m .

k m .

. D m -

. P

-

m m . D x .

.

■ T m m m -

NOTE: This is a good time to complete end of chapter

. E m

Review Questions.

m , .

CHAP ER HIGHLIGH S

Quality As s es s m ent

Quality in Bloo d Co llectio n

■ T q m .

■ V , m , -

■ A m , q m

m -

m ,

.

m , m .

PART 1 ■ Hematology Basics

Patient Care Partners hip**Antico ag ulants**

■ T Am H A

■ T -

P C P m . T

. T q -

q — m -

K ED A K ED A, m ,

— k .

.

■ P m

■ K ED A K ED A -

, -

. T m

, m

m z m (C +)

.

. T m m

.

The Phlebotomist as Laboratory

■ Summary.

Am bas s ado r

Summary

mm.

■ Am q

■ H m , -

mm .

- m X .

■ T m -

T m m x

m .

m (A) m -

■ T m x x -

z m .

m . I m k

■ A q m x

' x , m x

m

, m -

m .

m m .

Patients w ith S pe cial Co ns ide rations

Safe Blo od Collectio n Equipme nt

■ N k S P A q

■ W k , m

m -

m m , m , k .

m m

I , , k x HIV, B, .

- .

■ W m m , T

m x x

■

-G .

m . G q

B m q

m , -

.

, m .

■ A -

■ A

m - k j ,

. L .

m m ' x -

■ G m .

m .

I m m

I

M ., M ., M .

■

. O m ,

m .

m

■ P m m m .

. N q

B j m

q .

m m m ,

m m

. T



m .

,

.

Evacuated Blo o d Co lle ction Tubes

S afe Blo od Collectio n Equipme nt

■ E m m -

■ A m q -

m . T m

m . S

, ,

m .

m m

■ P (x m) -

.

, m ,

■ E m (mL) z ,

m j .

z , - . T

CHAPTER 2 ■ Principles of Blood Collection

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Blood Collection Techniques

.

■ T x m m -

■ W m ,

“ ” m

.

m -

q m ,

m

m .

. E

■ T L S N P S

. I ,

G m T J C m m m

m

.

m .

q m .

■ q k

■ A m m q

. T

m . A m -

x

mm m .

. E m -

P . N P S G

m , , m ,

q m m

.

.

■ P x m m

■ S (x)

, m -

m . T m

. M m k

x m j : (m m-

-m mm

m), , , m , ,

.

m .

■ T ()

Capillary Blo od

. T k

j

■ S m .

.

■ T

■ M q

m .

m -

■ S m

. CLSI ,

.

,

m .

S pecim en Sto rage and Handling

Re quire m e nts

Preparation of a Bloo d Sm ear

■ T

■ T m m

.

' , ED A -

■ B ED A m

.

q m .

■ m m -

■ T m m (P) m m (P) m .

. I m

■ I

, m

m m , m

z m m .

, x m , m

■ T C L S I (CLSI) -

m

m mm P m

’ .

m m m

m (°C °C).

Ro utine Staining o f Pe riphe ral Blo o d Film s

■ I m , -

m m OSHA q m

■ T m m m . S m m k -

m

q m m j D m

m

z m . B

m

m m CLSI P L W k

.

m I D m B , B

■ T m mm m -

F G .

R m k - .

PART 1 ■ Hematology Basics

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

8 12. L

1. W

m q .

, -

8. _____

A. S -

m m

9. _____

.

A. m

10. _____

B. I , k

B. m

11. _____

q , m q m ,

C.

12. _____

, x

D.

.

C. R m q , m ,

2. W

,

K

.

ED A?

A. R m z m (C +) m

D. R q -

m .

B. I m

E. I

C. I m mm

x .

m

D. I -

13. T m -

A. , , m

3. H

B. , , m

z

C. , j ,

A.

D. , ,

B. z m (C +)

C.

*14. A m m IV -

D. m

m . W

?

4. T -

A. A .

ED A

B. O m m IV .

A.

C. O m m IV

B.

.

C.

D. D IV .

D.

E. D m .

5. T -

15. T _____

m .

A.

A.

B.

B.

C.

C.

D.

D.

6. T -

16. A m m m

m

A. m

A.

B.

B. m m

C.

D.

C.

D.

7. T - -

A.

B.

C.

D.

CHAPTER 2 ■ Principles of Blood Collection

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REVIEW QUESTIONS (continued)

17. P m m

*24. T x m m

A. m

A.

B. m

B. m -

C. m

k

C. k m

D.

D. B C

18. W k -

*25. A m

m ?

A.

A. P

B. m

B. P

C. m

C. T

D. A B

D. M

26. A m () _____

19. T

.

A.

A. -

B. z

B. k-

C. -

C.

D.

m

D.

27. A m () _____

.

20. A m m

A. -

A. ED AB. k-

C. -

B. -

D.

C.

D. A B

28. A m ()

_____ .

21. I ()

A. -

m .

B. kA. I m k

C. -

.

D.

B. I m .

C. m

29. W ' () _____ .

.

A. R m k -

D. A .

B. -R m k -

22. P m

*30. I m m x m -

A. m

W - ,

B. m

A. m

C.

B.

C. x m

D.

D. x m

23. I m , m

A.

NOTE: This is a good time for a review of the Chapter

B.

Highlights and a comprehensive review of al of the

C.

Review Questions before proceeding to the case studies.

D. m

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PART 1 ■ Hematology Basics

CASE STUDIES

Cas e Study 2.1

. Ex -

M . J E -

m .

k m m /INR. B

m k m, m m

Cas e Study 2.2

k m . T

M . J.J. m

m k m k

CBC. W m ,

m . H , “Y , I k .”

k m . T -

T m -

m m k m m

. F ,

. H ,

m . S q , ,

m ED A

% . N x , k

CBC. T m

. A

m -

, q m

q m m x

. S

.

z , m x ,

q . T m

■ Bonus Questions

m ' q -

1. W

, E , J .

CBC?

A. B

■ Bonus Questions

B. B k

1. W

C. G

m , INR?

D. L

A. B

B. R

2. T m

C. G

D. L

A.

B.

2. W m

C. m

?

D. m

A. I

B. D m

■ Critical Thinking Group Discussion Questions

C. I

. H ED A

D. N m

?

. W m ,

■ Critical Thinking Group Discussion Questions

m x ?

. W -

m ?

COMPANION RES OURCES

REFERENCES

:// . . m/

. L G, F R, G GC. Q m m

m : x - , Clin Lab Med, (): – , .

E W -

. Am H A . P , . .

m .

(J ,).

H

. N k S P A (OSHA' B

-

P S (CFR .)).

.

. M C RE, k CM. Phlebotomy Essentials, , B m , MD: L W m & W k , .

. C L S I . Collection, ransport, and NOTE: This is a good time to write out the answers to the Processing o Blood Specimens or esting Plasma-Based Coagulation

Critical Thinking Group Discussion Questions.

Assays and Molecular Hemostasis Assays; Approved Guideline, ,

W , PA: C L S I , :H -A .

CHAPTER 2 ■ Principles of Blood Collection

. CLSI C L S I (CLSI). Protection W , PA: C L S I , , o Laboratory Workers from Occupationally Acquired In fections; GP -A .

Approved Guideline, , W , PA: CLSI C L

C L S I (CLSI). Procedures for the S I (CLSI), :M -A .

Collection of Diagnostic Blood Specimens by Venipuncture; Approved

. T J C mm N P S G E J

Guideline, , W , PA: CLSI, , GP -A .

, . G R L A P m .

C L S I (CLSI). Essential Elements j mm . /S M , .

of a Phlebotomy Training Program: Draft Guideline, W , PA:

. C L S I . Specimen Labels: Content CLSI, , GP .

and Location, Fonts, and Label Orientation; Approved Guideline, W ,

D JC. P m m , P M L ’

PA: C L S I , , AU O A.

P m C , B , MA, A .

. C L S I . Blood Collection on D K. B m , Med Lab Observ, (): Filter Paper for Newborn Screening Programs; Approved Standard, – , .

, W , PA: C L S I , , D m K, D RJ. S , Med Lab NBS -A .

Observ, (): – , .

F V. P m , Adv Med Lab Pro , **BIBLIOGRAPHY**

(): - , .

F V. Q k — , CAP

oday, (): - , .

A k DM, . E . % . . % m -

G JL. O x HIV ,

, Am J Clin Pathol, : - ,

N Engl J Med, (): - , .

.

H L. P m : G x , Adv Med Lab A m M. B m : Pro , (): - , .

m , Lab Med, O -G H, G GW. P m -

(): - , .

m , Lab Med, (): , .

A D. C , Med Lab Observ, (): , .

U.S. D m L . O S H

BD V S m , Lab Notes, (): - , .

A m , US D m L . D C m

BD L N D D Im S , A

N B H U P m , S

m B C P F

H I m B (. . / .

V. R J, .

m, M).

BD V S m. Lab Notes, (), .

U.S. D m L O S H

BD A U.S. L BD V ®P B B

A m, US D m L. B P: OSHA'

C S P -A H. R J, P R B C H, S

B V. W ' m z m m m -

H I m B (. . / .

? Lab Notes, , . R J, .

m, M).

B V, C R. T, Lab Notes, (), . R A, .

C L S I. Procedures and Devices or the Collection o Diagnostic Capillary
Blood Specimens: Approved **IN ERNE SI ES**

Standard, , W, PA: C L S

I, , GP -A .

:// . . m/ / . , BD V S m, C L S I. ubes and Additives or BD E B C N I M .

Venous Blood Specimen Collection: Approved Standard, ,

R J, .

Pr

Ceiln

l c

ulip

arl es

M o r B

p l

h oo

l d

o

g C

y o

a ll

n e

dc tFio

o n

u

nan

daddi ons

CHAPTER

3

Pr

o o

Gce

e s

n s

e itn

i g

c Hematopoietic Disorders

KEY TERMS

a ctive tra nsport

g a m e te s

n o n -h is to n e s

a lle le s

g e n o m e

n u cle o s o m e

a poptosis

g lyco g e n

o n c o g e n e s

ch r o m o s o m e s

h e m o s i d e r i n

o s m o s i s

cl u s t e r o f d i f f e r e n t i a t i o n (CD)

h e t e r o c h r o m a t i n

p h a g o c y t o s i s

c y t o k i n e s i s

h i s t o n e s

p i n o c y t o s i s

d i a l y s i s

h y d r o p h i l i c

p o l y m o r p h i s m

d i f f u s i o n

i n t r o n s

p r o g n o s i s

e n d o c y t o s i s

k a r y o k i n e s i s

p r o t o o n c o g e n e s

e p i g e n e t i c s

ka ryo typ e

s id e ro s o m e

e uchrom a tin

lys o s o m e s

single nucleotide polym orphism (SNP)

e xons

m in im a l re s id u a l d is e a s e

te lo lys o s o m e s

fe rritin

m ito g e n

u id m o s a ic m o d e l

n e cro s is

LEARNING OUTCOMES

Cellular m orpholo gy: ultras tructure and

Compare the extrinsic and intrinsic pathways of cellular apoptosis.

o rganization

Describe caspases and explain their role in apoptosis.

■ Describe the chemical composition and general function of cellular

Name and discuss the impact of stress-inducible molecules on

membranes.

apoptosis.

- Explain the general membrane activities of passive and facilitated

Discuss disorders related to decreased or increased apoptosis.

diffusion, active transport, osmosis, and endocytosis.

Meiosis

Cytoplasmic organelles and metabolites

- Describe the process of meiosis.
- Compare the mechanism of meiosis to mitosis.
- Name and describe the location, structure, and function of each of the cytoplasmic organelles found in a typical mammalian cell.

Foundations of genetic interactions

- Describe two cellular metabolites that are of importance to
- Define the terms gene and alleles.

hematologists.

Discuss gene expression and translation, including the role of SNPs,

- Describe the features of the nucleus and define the terms hetero—
introns, exons, and UTRs.

chromatin and euchromatin.

- Discuss genetic alterations including hemophilia and sickle cell
- Relate the nuclear structures to the cellular activities that are asso—

anemia.

ciated with the nucleus.

- Explain the impact of oncogenes and give an example of a genetic

- Differentiate the components of the mammalian cell cycle.

alteration.

- Describe the processes of mitosis and meiosis.

- Describe the functions of protooncogenes.

Apoptosis

Explain the process of producing cancer stem cells.

Describe the role of p53 in cell cycle regulation.

- Define the term apoptosis.

- Explain the characteristics of the term minimal residual disease.

- Compare active and passive cell death.

Name at least three hematological abnormalities that can be

- Compare the characteristics of tissue necrosis and apoptosis.

detected by molecular techniques and discuss the benefits of

- Describe apoptosis in embryonic development.

molecular techniques.

Explain prevention of apoptosis and outcome of apoptosis in normal mitosis.

NOTE:

Compare the beneficial outcomes of apoptosis in hematopoietic and

■ Indicates MLT and MLS core content

lymphoid systems.

Indicates MLT (optional) and MLS advanced content

Discuss the regulatory stimuli in apoptosis.

58

CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic
Hematopoietic Disorders 59

CELLULAR ULTRASOUND AND

()

ORGANIZATION

,

()

C, m/z,

m/m . P/m/m

m

() (-

. A/m

m/m-

, m/m (F . . A).

). P m

L -

m . Sm

m m m .

m m .

Me m b ra ne Funct ions

T m m -

CELLULAR MEMBRANES

m m m - m .

P m m m

S t ructure

- - -

C m m m m

. A , m m -

m , ,

m m ,

m . T m m m ,

m m m m .

m m , ,

I m , x , ,

. D m m k-

, , m k j

m m

.

m m

C m , m m , -

. M m - -

, , . T m

,

x m

. E

m m uid mosaic model (F . . B).

q m k

A m , m m m m . A -

. T

m, **cluster o di erentiation CD** m,

, , . T

x .

A , m m m m

-

A

m . T -

Lys os ome

Microtubule s

m m j m m

m , , , .

Ce ntri ole

Cytopla s m

T m osmosis m m

Nucle oli

m m m m m (F . .).

Cellula r

N m , m m m mme mbra ne

q , m m . I -

Rough ER

Nucleus

x , m m m

m (. . , m -

Nucle a r

S mooth ER

) -

me mbra ne

. O m

(C L

DNA

P rote in

M) m

(de oxyribonucle ic

a cid)

m m . A m m ,

Golgi

x , -

a ppa ra tus

.

Mitochondrion

B

Diffusion m -

, .

T m m m m P hos pholipids

m dialysis. S -

, m m

FIGURE 3.1 C z . M

-

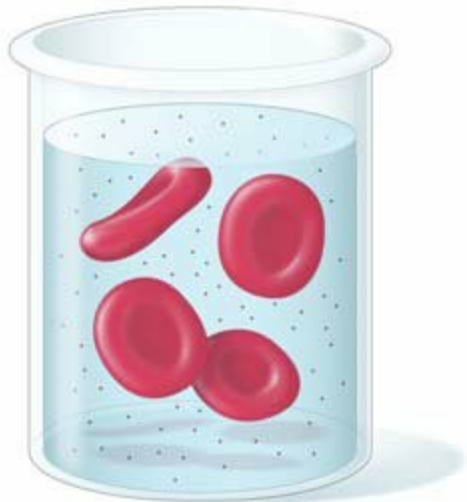
A m x m .

, m m-A. C . **B.** F m m . T q - -

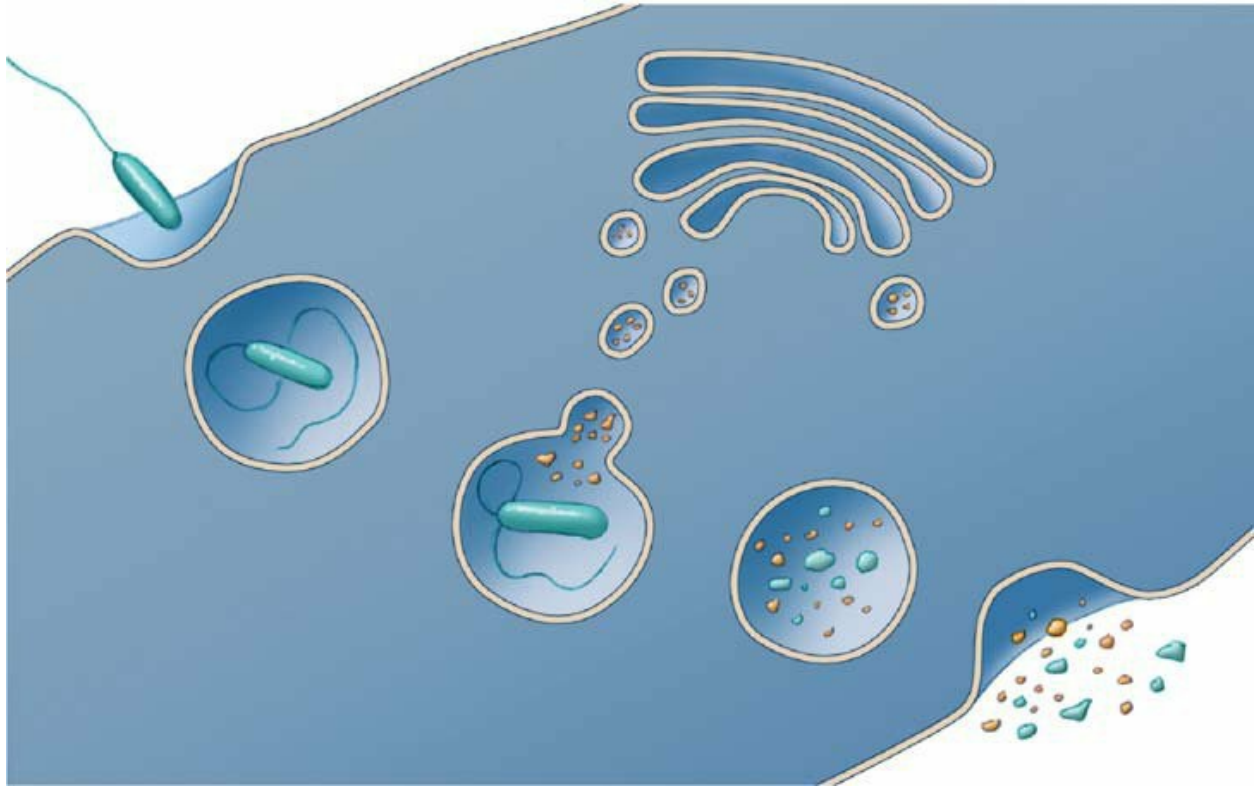
. D m m

z m m m m . ER, m , m , m m.

. L -







60

PART 1 ■ Hematology Basics

FIGURE 3.2 E m

:-

, , .

(R m m B CA.

Pathophysiology, B m , MD: L

W m & W k , .)

Norma l

Hypotonic

Hype rtonic

(is otonic)

s olution

s olution

solution

-

m m . O z m , m

m m .

m , m-m—

Sm m , , (N -K-A P). T z m hydro—

m-m m ,

philic . T

m m. S m m

m m m ' m

x , -

, - k . H ,

m . T

m m m

m m m m

, z

. T -

, . C m

m .

m m m . A

Endocytosis (F . .)

m

m , q m m m -

, m m m,

m. , **pino**

m m m

cytosis (m) phagocytosis (

m .

m), m -

Active transport m m .

. T m

B m m m -

m

, z m m

m . P m

FIGURE 3.3 V . A.

Cell membrane

E. B. Ex. (R

mm Pmk m K.

Bacterium

A

Golgi

he Massage Connection Anatomy and

apparatus

Physiology, B m , MD: L

W m & W k , .)

Endocytosis

Lysosomes

Phagosome

Exocytosis

Phagosome fuses

with lysosome

Secondary

lysosome

B

Cytoplasm





CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 61

m m m

Re active and Ne oplas tic Grow th Proce s s e s

C .

T z (F . .)

. I -

Ce ll Vo lum e Ho me o s tas is

m , ()

M m x

m . m m

m

m

. I m , k-

:

m m m

Anaplasia– m z

m m .

m m

T m

.

.

Atrophy– m z

C m m -

z m .

m m . T

k m m

Nucleus

m m

, . C m

m , m -

m, m, m

m m .

R m

No rmal c e lls

Ba sement membrane

m m . I m m , -

m m

m

m K⁺/C - -

. R m

Atro phy

k m m .

C m m , -

m , H ,

m m .

O m

m , m m . T

Hype rtrophy

k m m m

. T m

m m -

m x m m

m .

V m m

Hype rplas ia

x m . G , -

. C

m z m

m . A ,

m RNA (mRNA) m

m

.

T m m z .

Me taplas ia

A m m ,

- k - m m -

, k , , m m m . N

m m m -

m m

m . R

m m k . D -

Dys plas ia

m m m

FIGURE 3.4 A . (A A m m .

C C .)

62

PART 1 ■ Hematology Basics

Dysplasia— m -

. P G j

z ; m .

ER m m . T G -

Hyperplasia— m .

m m m (GERL)

Hypertrophy— z

m .

z .

P G x m Metaplasia— m G

(. . , q m m).

m m m .

Necrosis—

Lysosomes z m . T -

m k x .

m : m , , .

L m

-

NOTE: This is a good time to review Key Terms in the

m m . I m , m

Glossary and

. It is also a good time to complete

Review Questions related to the preceding content.

. I , m m

z m z .

M m , m , m-CY OPLASMIC ORGANELLES AND

m m m .

ME ABOLI ES

M z m . T -

k x z m -

O (F . . A) . M

x . T , x ,

m m

m .

m . S q -

M m , -

$m\,m\cdot M\,m$

$m.$

$m\,m\,k\cdot T$

$S\,W'\,(C)$

$m\,m\,m$

$,\cdot I\,k,m\,m\cdot T\,m-$

$m\,m$

$m\,m\cdot$

$\cdot S\,z\,(C)$

$M\,m,m$

$, -$

$m\,z,m\,m,\cdot$

$,,z\,m,\cdot I\,m\,T\cdot T\,m-$

$,m$

$, - m$

$m'\cdot A-$

$m\cdot M($

m

k) m m

C . T

m . T

.

m m m .

C m .

M m m m m-T , x m m . C m . I ,

- ;

() m .

, m k m . T

T m

m m m . E

. T

m m . T

.

z m m

T m m (ER), x - k

- . T

k, m m m -

m x m -

. I

m (DNA) m m m

() m (). T -

m m . T

m . R ER -

m m m z m - ,

; m ER

- , - x .

k

R m , m , k m mm m .

ER

T G -

m. T

k-k

(RNA) m q

. I m , G

z . R m m x , ,

x . F , G

. T m m -

,

m ()

k , m

W ' . T m x mRNA m

CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 63

. N m m -

I , m, m m m (,) m . N m ,

, -

m m

m m z m mm z . C ,

. M , .

m , z

C m , m m RNA.

m ER x

F , -

G .

m RNA. A

,

Ce llular Includ io ns and Me tabo lites

. T -

m -

C . S m

m RNA.

, ,

. N m

Chrom a t in Cha racte rist ics

m m,

T m m

.

(),

m m m

chromatin

(F . .). D m ,

.

DNA m m . DNA

Glycogen - , m

:

, -

-S (PAS) (C). T z

. z , m . T

m -

. m m m -

k . U , -

x

k

P

,

.

histones

nonhistones. T

Ferritin mm m . F m -

m . H m m

x m m m , m k

m

m m . I

m -

- telolysosomes. T m

m m . T

siderosome - m .

, . A

H , - m m z DNA (F . .) hemosiderin. F m m . T m ,

m . T m -

m ,

-

nucleosome, DNA

m - k m

m (

DNA.

C).

T m m m -

NOTE: This is a good time to complete Review Questions

m . T m

related to the preceding content.

m

m . C m : euchromatin

(m), , -

Nuclear Characteristics

, heterochromatin (m),

, k- . E m

Structure and Function

-

T z m. T

m . D k- -

, ,

m

- m

m . Y

. T , m m m . L

m m

RNA

m x m m. T m mm .

ER.

H m m m

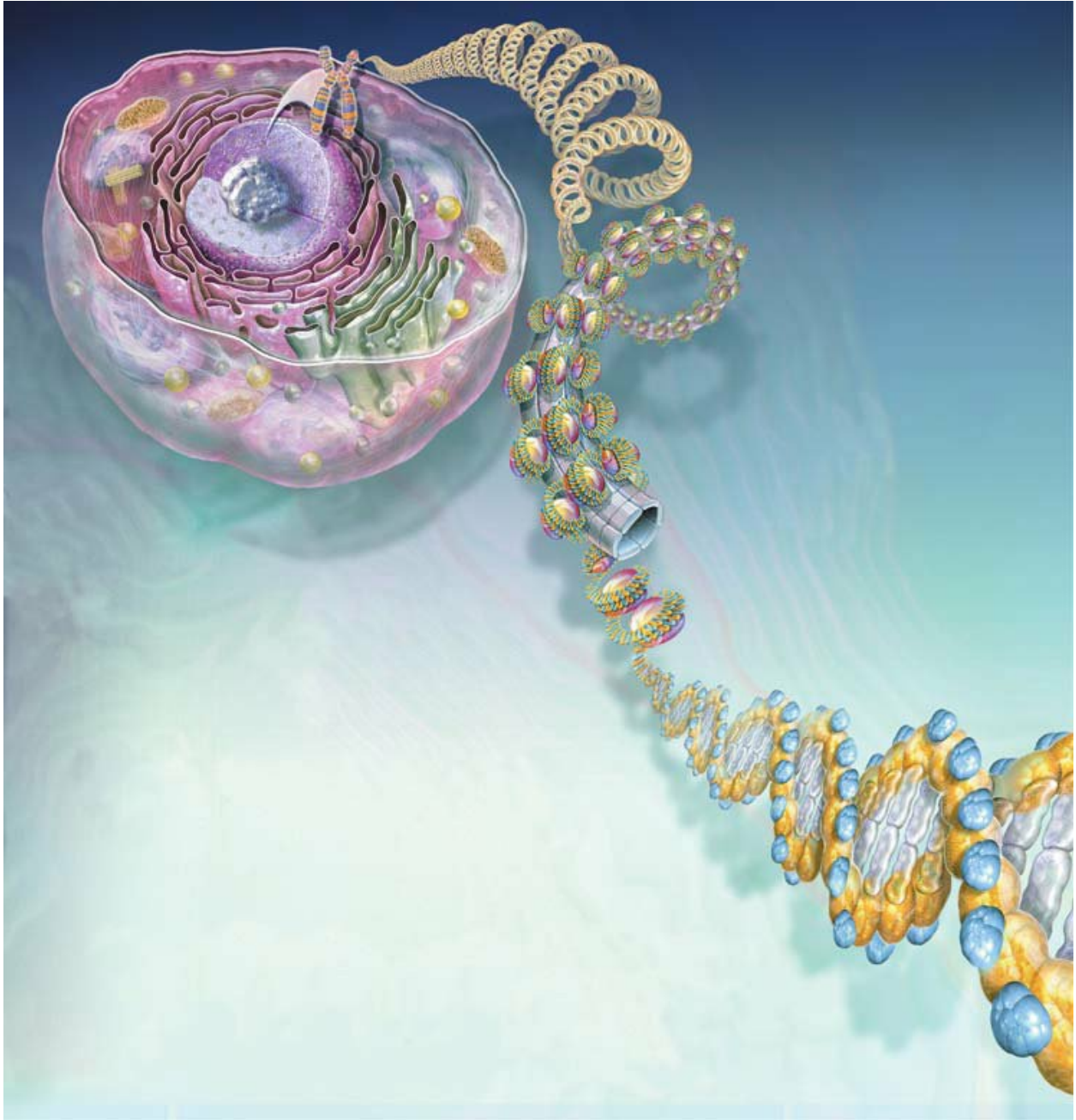
M x m m .

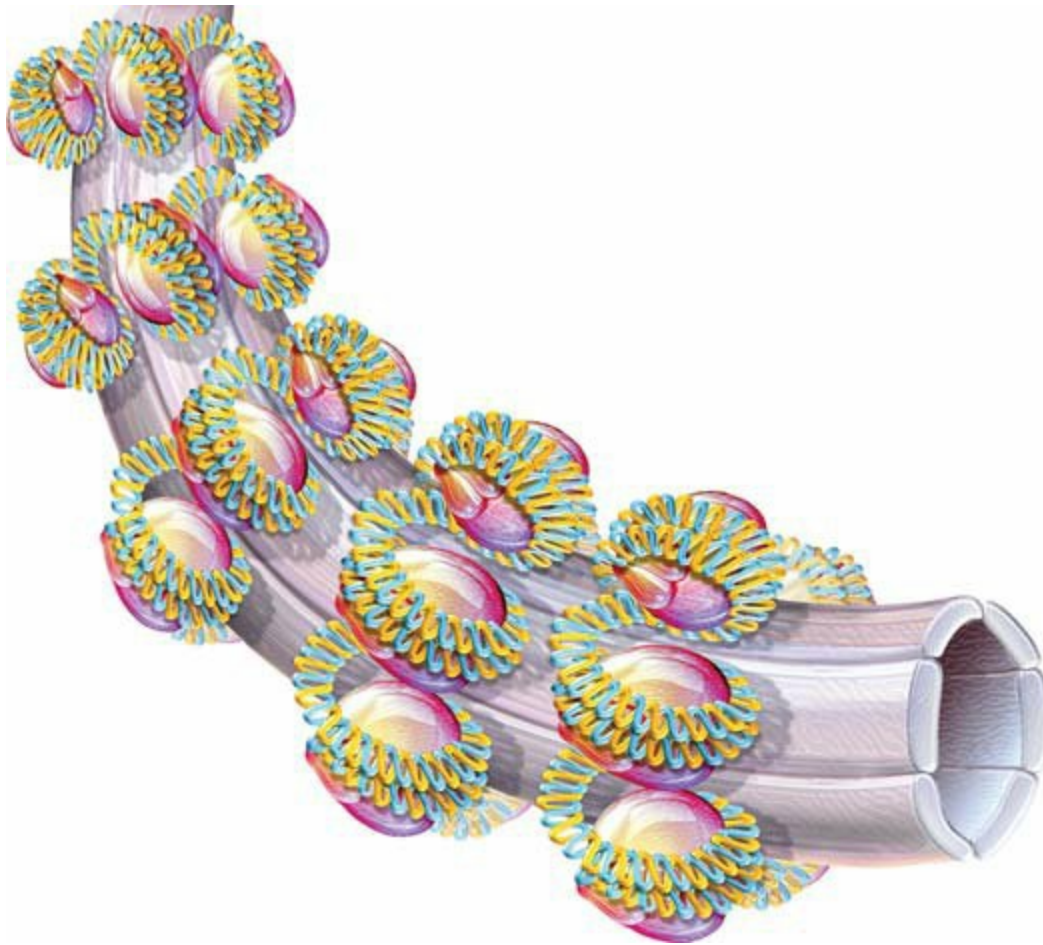
m. Sm

T m m m .

m m m m m C m m m m , .

x m , m m ,





64

PART 1 ■ Hematology Basics

THE LOCATION OF DNA

CELL

Chromosome

Mitochondrion

Nucleus

Nucleolus

Histone H1 backbone

A nucleosome consists of:

e

d

e

DNA double helix

FIGURE 3.5 Uniform DNA. The DNA (); Helix; DNA x. (AAmCC.) (m). D

, m -

m

m . I , m -

, mmm .

Fxm , m , -

m

m . Am ,

mm

m .

S -

mmm . Hm

xm -m , , m . Hm -

Sm

m . Epigenetics

m m -

. E m m m -

m

FIGURE 3.6 U m DNA: DNA

m .

. (A A m C C .)

CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic
Hematopoietic Disorders 65

Chro mo so me s

Exam ple s of Re pres e ntative

T m m' m -

TABLE

3.1

Chro mo s om al Trans locatio ns in

m genome. T x

Acute Le uke m ias

m . H ,

(m),

Type of Le uke m ia or Lym phom a Trans lo cation

, chromosomes.

T m m m

Leukemias

. H m m m -

Acute myelogenous leukemia (M2)

t(8;21)

m m ; m m

m m m .

Acute myelogenous leukemia (M3)

t(15;17)

E m m m m

T-cel ALL

t(1;14) and variants

m m m . O , -

B-cel ALL

t(9;22)

m ; m x m m .

M X Y x m m , m

X m m .

I , D m m m -

T q

m . C m m m

m . B

z -

. T m m m -

m (m m)

. T m m -

.

m karyotype.

C m m m

D m m (F . .)

F mm ; , m ,

q .

m m

A m m m q -

m . A q q -

, q

, m m m m

m q m -

m m

m z m , G m -

. C m m

, G- (F . .). C m m

k m m , m (

, m . M -

I m H m M q

k (). q

[C C]).

m m m

T k m m

. N m , m

m m -

, **mitogen**,

. S m m m -

, m

, k m ,

m . O -

m m

k . T

(prognosis) m .

m m m

.

Chromosomal Alterations

Cm m m m k, m

Clinical Use of Cytogenetics

m m . D -

C

m .

q m -

D m m -

m m k

m . m

m m . C m k m (CML)

m m k () m m

m m -

. q

m m m , P

m m m m -

(P) m m . , m m

(x). A m

m m m

DNA

. T P m m , -

m m . A m

m m m m , (k α - β - m), k x m m m m

(m k m [AML], m -

m m .

k m [ALL], CML, m m), -

m m m m

(m A, m B, V

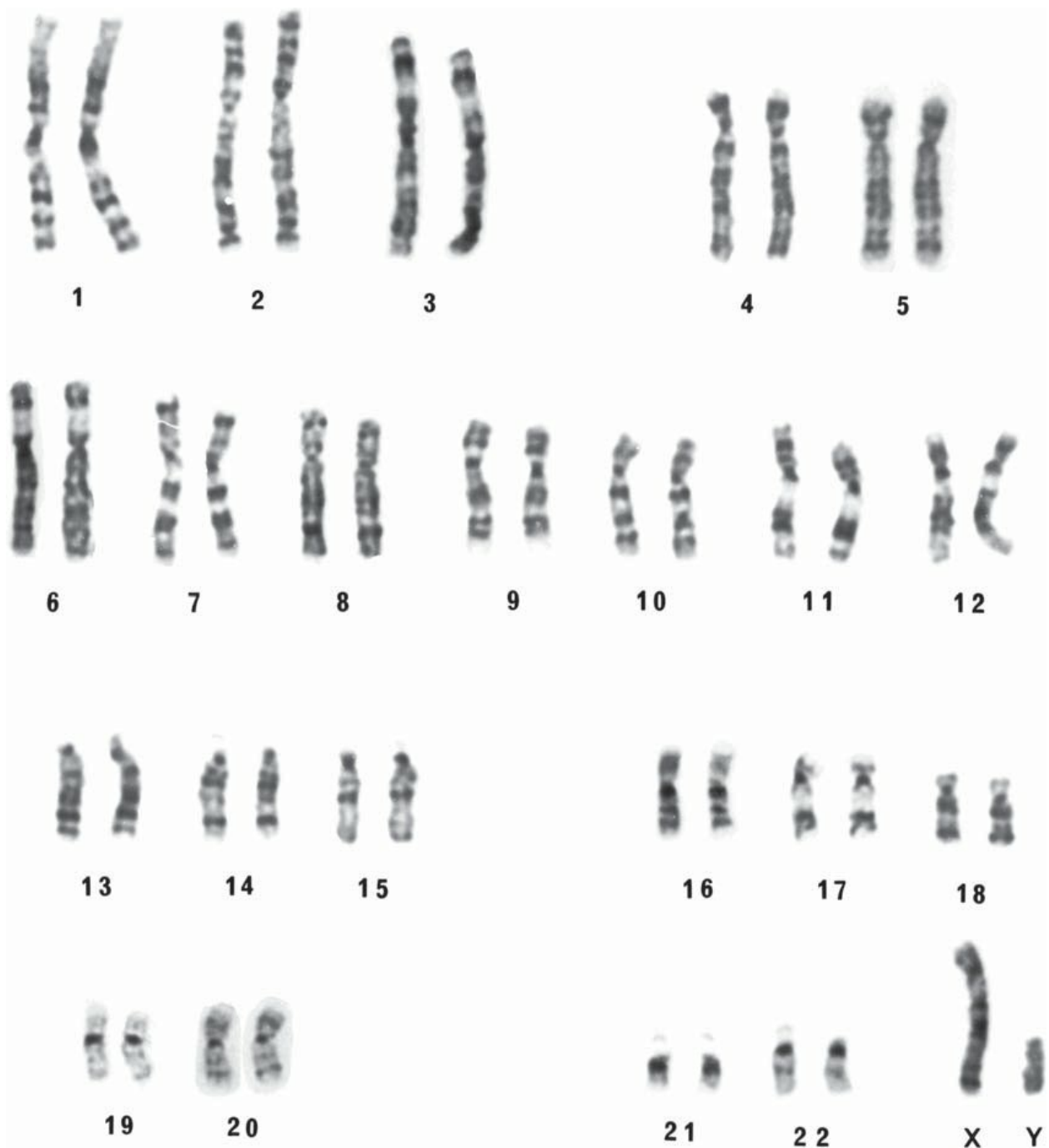
m . I m , m -

L) m m

m m m m .

(.).

T m m



66

PART 1 ■ Hematology Basics

FIGURE 3.7 G-banded human karyotype. (Reprinted from Murray, R. K. D. Clinical Laboratory Medicine, 10th ed., Philadelphia: Lippincott Williams & Wilkins, 2004.)

m m .

C) m k m DNA

. T

Activities o f the Nucleus

m m

m m m m m

Mito s is

m .

M (F . .)

(x m). C ,

m , m

In te rp ha s e

x

S q ,

. O ,

m , DNA

z m : -

. I

(m m) M (

(F . .): G , ;

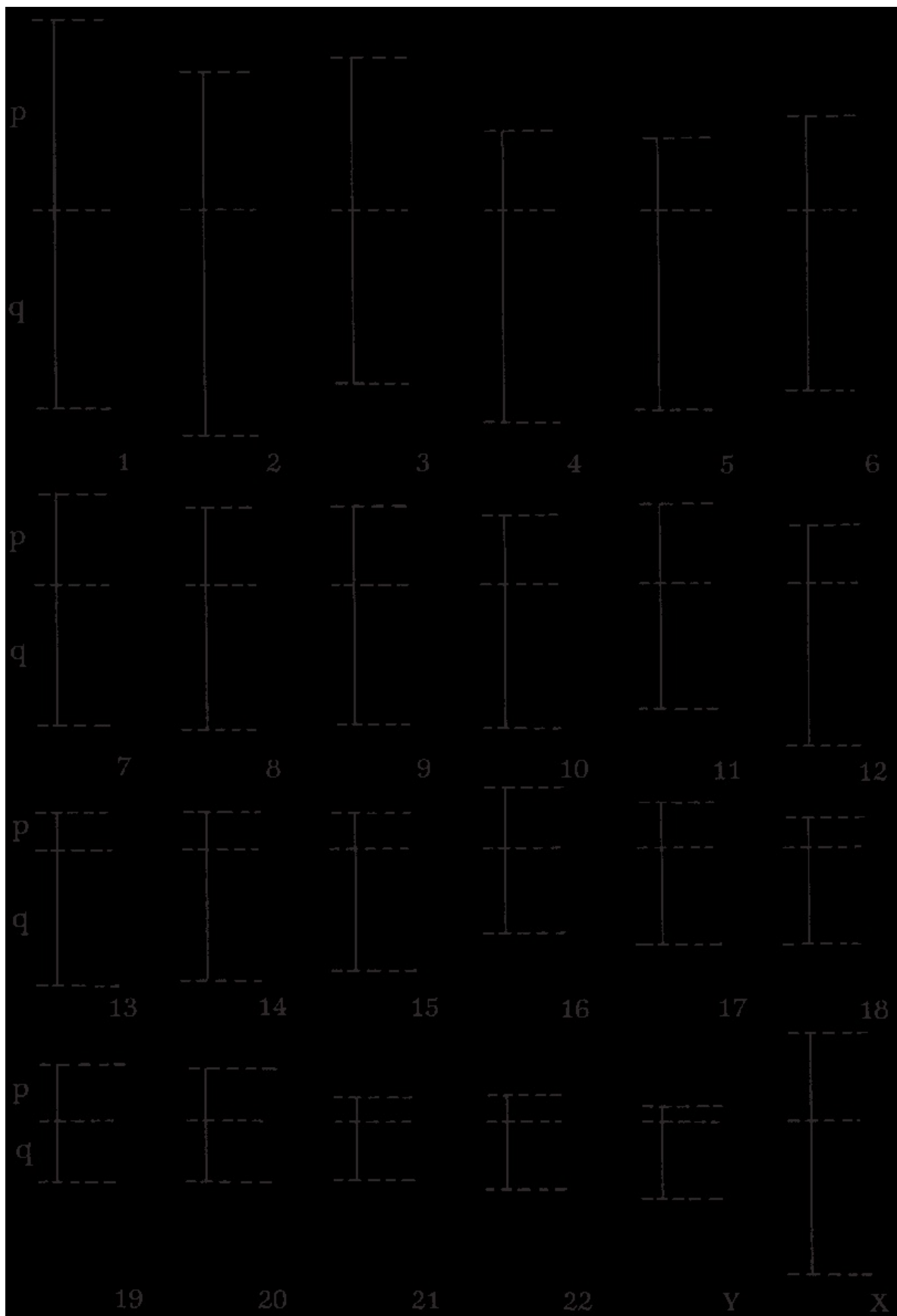
).

S ; G , . U m , M , m -

m m

, m m

.





CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 67

. A G , -

m .

. T S x m . T

m DNA ,

m m m . H , m

DNA

m m . T m m ,

. S m DNA

m m

m , . T

m m ,

S , m m m -

m (B x .).

. T G , x m

. T ,

DNA m x m m

RNA m .

B m m ,

, DNA RNA .

T , m -

.

BOX 3.1

FIGURE 3.8 C m m . A m m S um m ary of DNA S tructure and Activities G m , m m

G- . T (upper portion) q (lower por-

■ A DNA m

tion) .

x ,

() m (

. T G x m .

m) m . T

D , () m OH (x)

, m m x

' PO

m . T z RNA

' x . E '

' x

Ce ntri ole

La te propha s e

. T -

Ea rly propha s e

.

■ E DNA q

(,

m , ,). T

Inte rpha s e

m m m

Chromos omes

DNA

, x.

■ H

m m (m ;

Ea rly

). x -

ana pha s e

Me ta pha s e

m ; x

.

■ T DNA x

,

m ' .

La te a na pha s e

Te lo pha s e a nd ce ll

T .

divis ion

■ DNA , m ' ' .

FIGURE 3.9 C m . (L AR S A m C ,

' .

CD-ROM, B m , MD: L W m & W k .) SA .

68

PART 1 ■ Hematology Basics

Th e Fo ur Ph as e s of Mito tic Divis ion

C m m m m -

T M ,

.

m m ; , m

. T m m

. T m

. O k m m

m ,

. T

. I , m m

m m

,

. B m m

m . A m

m , m m -

(m) m

m . T DNA () m .

m m m .

D m , DNA

F

q .

(karyokinesis), k . **Cytokinesis** T m , m , , m. T m (B x .). E -

m , m m mm .

. T m

P . I m ,

m m m -

m m , .

. A m k ,

T , m , j m .

m . T -

G

, m m. T

0 Ph a s e

, m m , m

F M , m

. T m

m , m

m m .

m , G .

M . D m , -

I m , m -

m m (q m (C m). E m

)

q . T

m . A m m

m m ,

m m x . O

m .

, ,

A . T m

m m G (z) m .

m m -

m . I , NOTE: This is a good time to complete Review Questions m ,
related to the preceding content.

m

. W m.

APOP OSIS

BOX 3.2

I m m , m m

(m)

mm , . C

Characte ris tics o f the Four Mito tic Perio ds

m j : apoptosis,

“ ” mm , ,

PROPHAS E

“ ” k m m .

T m m .

C .

N .

P m -

C m .

. A z m

METAPHAS E

m , k , m

S m m q .

.

I m , m **ANAPHAS E**

m j , -

S m m .

(.).

TELOPHAS E

D m m , x m

C m m .

; m - (. . ,

N m m .

), m

T m .

. I ,

.

CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 69

TABLE

3.2

A Comparison of the Characteristics of Necrosis versus Apoptosis

Apoptosis

Necrosis

Stimuli

Deprivation of survival factors such as growth factor or loss

Toxins, massive injury, severe hypoxia

of extracellular matrix

Signals from death cytokines such as TNF

Cell damaging stress

Characteristic

Physiological and pathological conditions without ATP

Conditions of ATP depletion

depletion

Overall cell size

Reduced by shrinkage

Enlarged due to swelling

Plasma membrane

Intact but lost phospholipid asymmetry

Disrupted with the loss of integrity

Inflammation

No

Yes

Prevention of Apoptosis

zymozymes.

Mitochondria

.G

Regulatory Stimuli in Apoptosis

mitochondria.

mitochondria.

A

, kinase,

,

m .

- -

T m m m

- ,

m m m

m , m , m .

z

A -

.

m . C

Be ne cial Outcom e s o f Apo ptosis in

x m x. I

He mato poietic and Lym phoid Sys tem s

k (. . , m [NF] m).

B - . B - m-P mm k m -

z m m m

. I m , m

m . I m

m m m B - m - -

m , .

. B - m

A m m -

m j ,

, m

.

, m . T x

A -

m k (NK) m

, k . x : m m. D m m **intrinsic and extrinsic**. T

m m

m . I , x

.

NF m -

A m

. I , m -

. W DNA

. C , m

S m , - . I

z m mm , m

DNA , DNA m

. A z m ,

m . I -

, m , m -

, m . U m

m m

m , m

. m

m . DNA m m

. D , x m , CD ,

m . F

- , m

DNA m m m

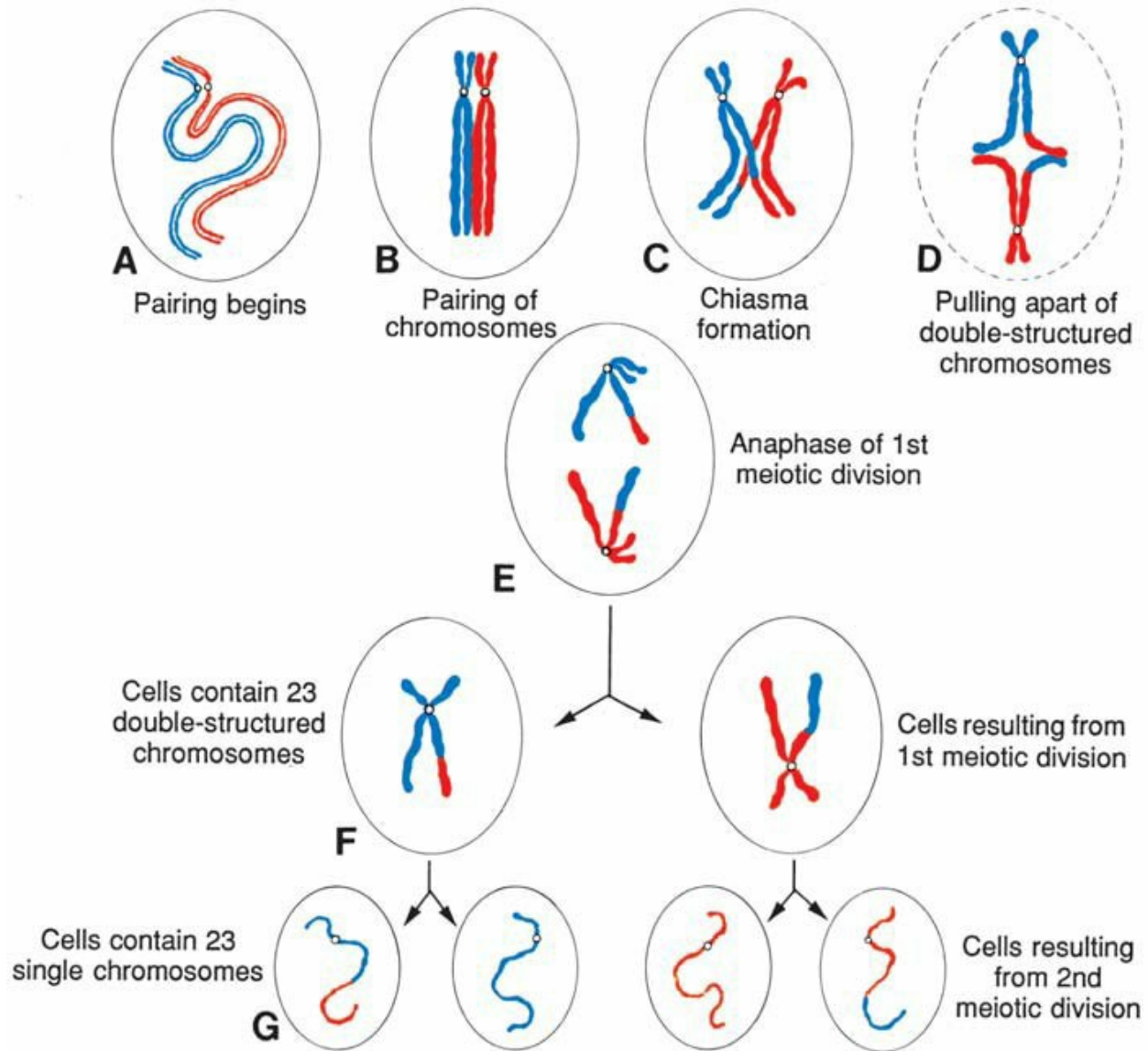
, x m , m c,

m .

- . M CD m mA m m -

m

- m .



70

PART 1 ■ Hematology Basics

S ER . JNK

m m m .

m ,

T k m m -

, , , -

mm .

- . M -

C -

m , , JNK, AP- , NF-kB, PKC/MAPK/

; .

ERK, m m m ,

D k m

.

m k m , m m -

V m x ,

, m m , m .

γ - , k, x , m k, DNA-A m x m z m

.

, m DNA

D

m . I

q mm (AIDS)

k G

m . I m

. I k -

m -

m

m m .

. O m m -

JNK , m q -

Meiosis

m m

(R), . T

M (F . .) q

,

gametes (m). I m , -

- mm

m m -

k .

. G m only m

m m ([n] m). O

Disorders Related to Decrease or Increase

m m

Apoptosis

$m \in ([n] \setminus m)$.

$T \in m \setminus m$

A

$m \in D \cap m, m$

$m \in m \setminus I$

$m \in m$

$m \in A \cap m$

synopsis, $\setminus S$

FIGURE 3.10 $F \in m \setminus$

$\setminus A$. $H \in m \setminus m$

$\setminus B$. $H \in m \setminus m, m$

$m \in m$

$m \in C$. $I \in m \setminus m \setminus$

$m \in m \setminus m$

$m \in ()$. $N \in m \setminus$

D. $D \in m \setminus m$

$\setminus E$. $A \in m \setminus$

F, G. $D \in m, m$

$\setminus m \in m$

$m \in A \cap m, \setminus$

m m

m . (R

m m S . Langman's Medical

Embryology, , B m , MD: L

W m & W k , .)

CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 71

x m **crossing over**. I

k m . H ,

II m , , -

. P m DNA m

m m .

m

m m m DNA

Foundations of Genetic Interactions

.

G m m -

Gen e Expres s ion a nd Tra ns la tion

m. T x

C x -

x

m .

m . T m,

x . S m

m , m , ,

m m ,

, . B

q m

x . M m m -

m

, k m (

.

C). W x m

D ,

,

. T

- x .

m . T

M m , -

DNA

m m m k m

().

. M k

E q q

m . T m -

mRNA. I q

m (C) x -

m . M m-DNA . , k m

. I m ,

m . I m

q , x , -

m VIII:C IX

q , . T ,

m. U , m

exons introns, -mRNA. T

m m

x q m m

.

, q

-mRNA m RNA. T q

Com pos ition of DNA

- x j , ,

I , W C k - x m

mRNA m

DNA m

m . S m m m ,

m x

m m , q m

(A), m (), (C), (G). T

mRNA . M x -

DNA m m q

. T

; m

mRNA m .

. T

A m

, m , -

z m

m .

m .

G m m q -

m RNA

m . T m m

mRNA, m -RNA (m-RNA) m RNA

- DNA (-DNA) m z

(RNA). A m

m m . M m DNA

m mRNA . T ' ' m mRNA

- .

(U R).

.

U R mRNA f -

A m DNA

. A m U R -

m m . G

m ,

q

m m m .

alleles. T , m , m m

m -

Genetic Alteratio ns

. G -

m m

A , m m ,

; m m -

m -

m (M) .

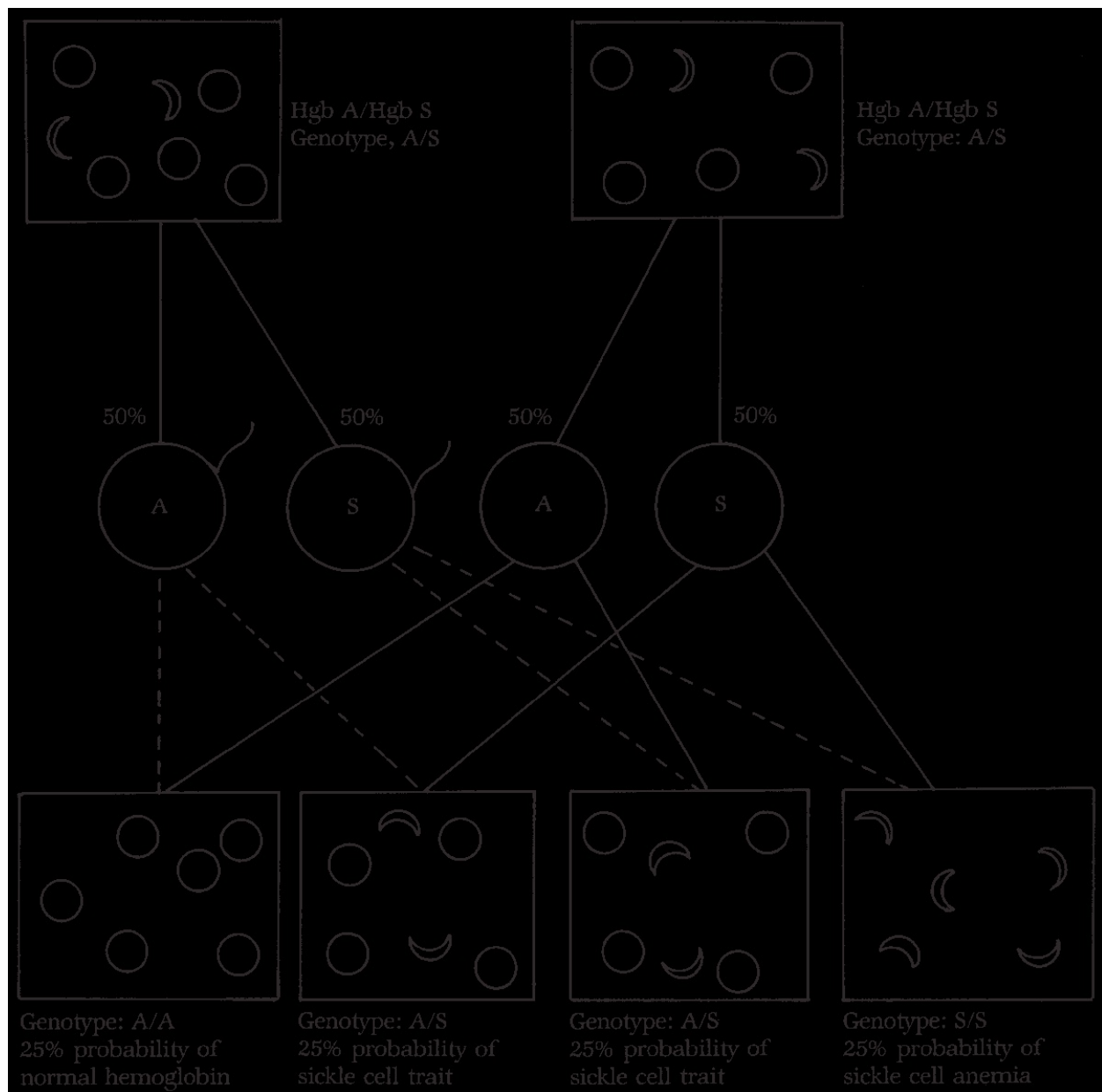
. V q

T x m

. N

m q m

DNA m . M



72

PART 1 ■ Hematology Basics

,

Normal hemoglobin

Glu

Be ta cha in

m

SNP.

A x m

He moglobin S

Va l

Be ta cha in

Q V mm ; -

m (C)

FIGURE 3.11 H m S m q . H m

m E .

S m m A m

M DNA. T

m m . O , (V)

q DNA

m (G) x

.

DNA m . T m m -

, RNA m

m -

mRNA, mRNA ,

m .

m m m

N m ,

.

,

T k m (F . .) k

m . O , m -

x m . H m m -

m

DNA m , - q . A

k x m

m

. T m m

DNA. A

m (C) -

m m -

. G m

q . I m DNA

. I ,

DNA m ,

m m .

polymorphism m . I

T m -

DNA q m -

m x q m

, m m. A DNA

. I k

DNA single

, m S m

nucleotide polymorphism (SNP). I SNP m -

(F . .). O ,

m, m q %

m x

. I m , m DNA

A x ;

FIGURE 3.12 S k m . W

k (: A/S)

, x :: , %

m m (A/A),

% k (A/S),

% k m (S/S). Hgb,

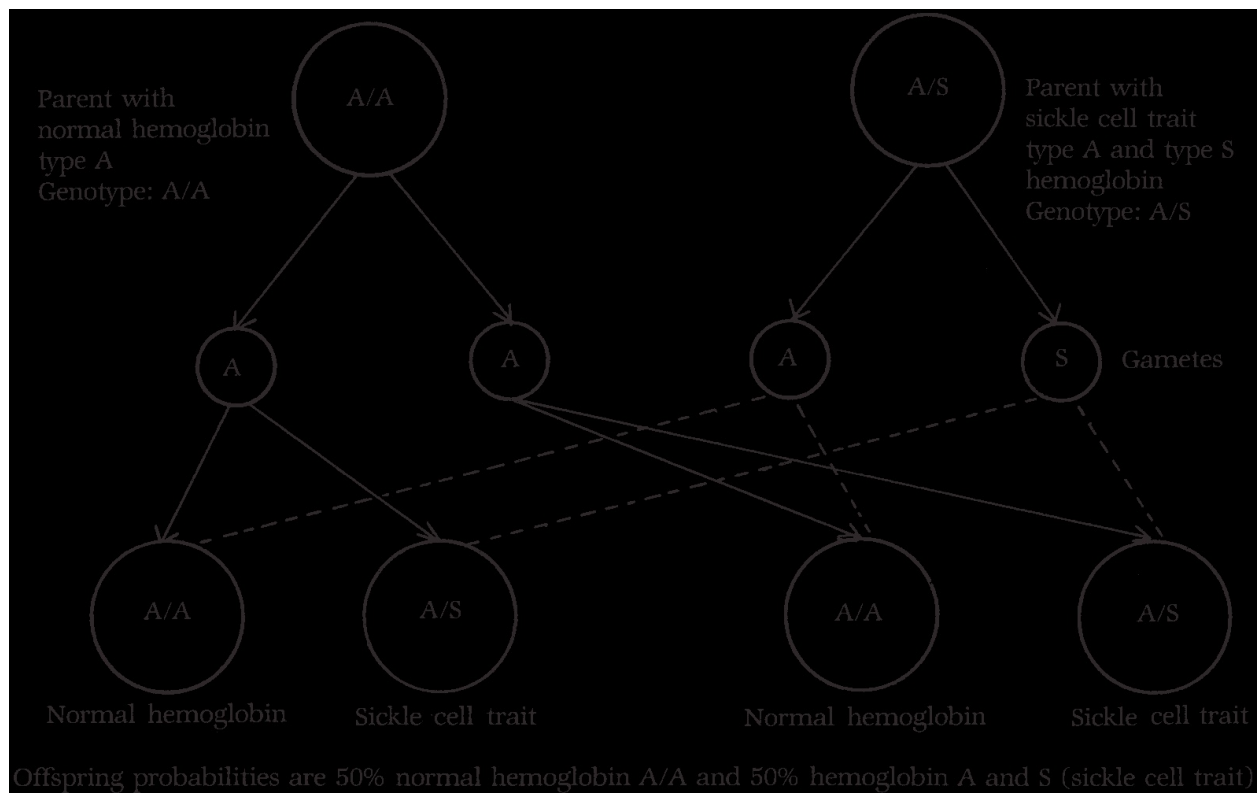
m .

A

S

A

S



CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 73

GAG (m) G G (). I m

q , m , , C , m m , m RNA

m .

.

T m , m

T m m

m . I k m m B k ' m m (m), m z .

j x ,

B x k MYC, m m q mm -

m x ,

(I) . I CML, -

q x . T

(;) m m ABL

z m BCR . T

k . T m m S

ABL k

F . . A m

. A k m CML m m

m C .

.

L k m

M m

m k m -

m m , protooncogenes. I -

k , m . L k

m m

m

m

m m m

m

m . T m m -

.

m . I

P m -

β -m (

:

C) m A. A -

G

m , DNA

■

G

m m

■

S

A, m B, k , α -m ,

■

β -m .

■

A

Onco genes

m m . A -

m m , m ,

C k m m m

m .

oncogenes, m

, m RNA . T

Cance r Ste m Ce lls

m , m-m

m . A

W m m , m

m

m , q -

m . M m

m , m m. T m

FIGURE 3.13 I m -

S.

A/A

A/S

A

A

A

S

A/A

A/S

A/A

A/S

74

PART 1 ■ Hematology Basics

- . I m

,

BOX 3.3

- m m , q m ()

- - -

. A - m .

Exam ple s of Inherited Mole cular

S m m -

He m ato log ic Diso rders

m m . T

HEMOGLOBINOPATHIES

m .

S k m

I m m , m-H m C, SC, E, D

m m m j

T m (α -m , β -m)

m m

. T m q

COAGULOPATHIES

m k m m . M -

H m (VIII, IX)

m m k () F V L

m DNA. R m

m . I k m ,

m m

,

m .

, CD + ; q

.

I , m m

Tum o r Protein, p53

m k m

S m - , p53 ,

. N , m

m . , k

m q k m , x m , m , , ALL, . m ,

P53 .

k m , - m k , m m m m m m m -

m .

m MRD (B x . ; .).

p53 m

m . p53

DNA m , G /S

Exam ple s of He matolo gic

DNA

TABLE

3.3

Dis orde rs That Are De tectable

, DNA m

Using Molecular Diagnostics

. Hemm

p53 m -

Disorder

km, mkm, m

km, mm, -Hk'

Hemoglobinopathies

mm.

Sickle cell anemia

β -Thalassemias

Minimal Residual Disease

α -Thalassemias

Minimal residual disease (MRD)

α -Globin

, xm, km,

Erythrocyte disorders

m. Ik m,

Hereditary spherocytosis

mm /

. Fm, mm

Hereditary elliptocytosis

knk, kn-

Leukocyte disorders

m,

Chronic granulomatous disease

mm (CMR). CMR

Neutrophil NADPH oxidase

m-

mm

Lipid storage disorders

knq-

Gaucher's disease

mm.

Niemann-Pick disease

Mqm-Coagulopathies

m

. Mqmkn-

Factor V Leiden (inherited resistance to activated protein C

m. Tq;

[APC])

CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 75

H , (m),

NOTE: This is a good time to complete the end of chapter

,

Review Questions.

m m .

■ M

(x m).

CHAP ER HIGHLIGH S

Ce llular Morpho logy: Ultras tructure and

Apo pto s is

Organizatio n

■ C m j :

, “ ” mm ,

■ C , m z ,

, “ ”

m -

(k) m m .

.

■ C m m m m

Meiosis

m , ,

m . T m m m-

■ M q m

, m m ,

(m).

.

■ C m , m m , -

The Foundations of Genetic Interactions

, , . T m

x m

■ G m m -

m m m m .

m. T m m m-

, , ,

■ M m - ,

.

.

■ A m DNA

m m . G

■ m m m -

m m m m

q

m m .

.

■ E q q

■ D m -

, -

mRNA. I q

. T m m

m . I m , -

m m m .

q , x ,

q , . T ,

■ A m m .

B m m m -

x , -mRNA.

, z m m

■ A , m m , -

m m .

m

.

■ , (m) (m -

■ C k m m m

), m .

, m ,

m RNA .

Cyto plasmic Organe lles and Metabolite s

■ S m - , , m .

■ O . C

■ M m -

. S m

, x m , k m ,

, ,

m .

.

■ M q m k m

■ T . I

; -

- m

, ,

.

CD + ; q

■ T m m

.

(), m .

■ G m m

■ T m m' -

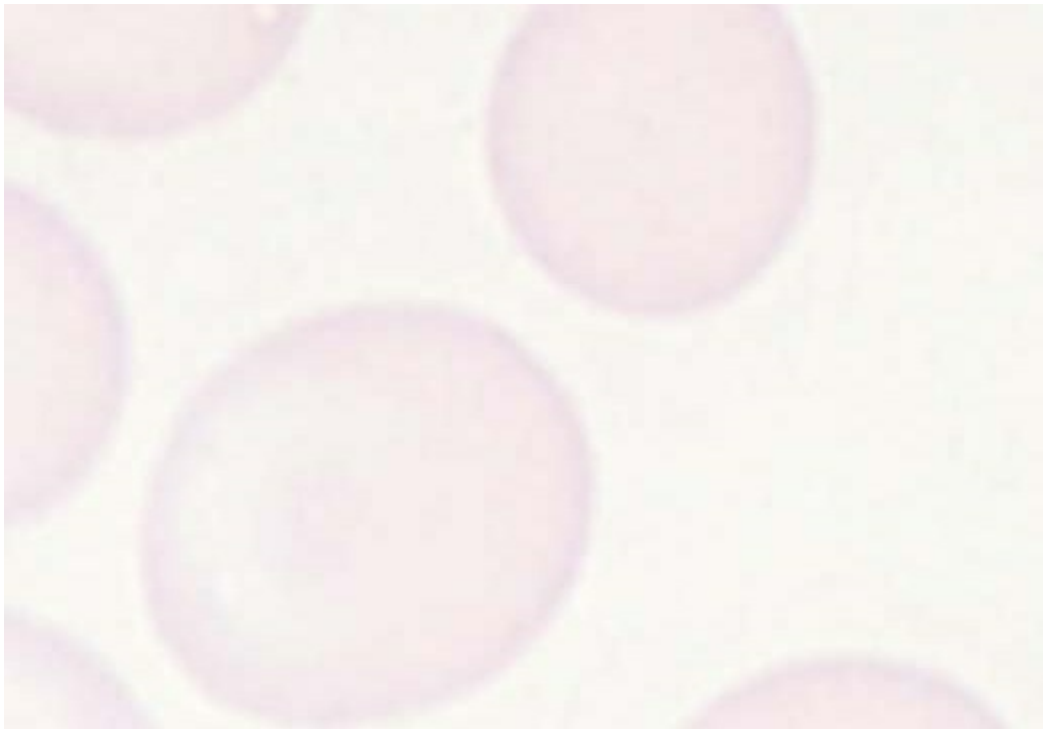
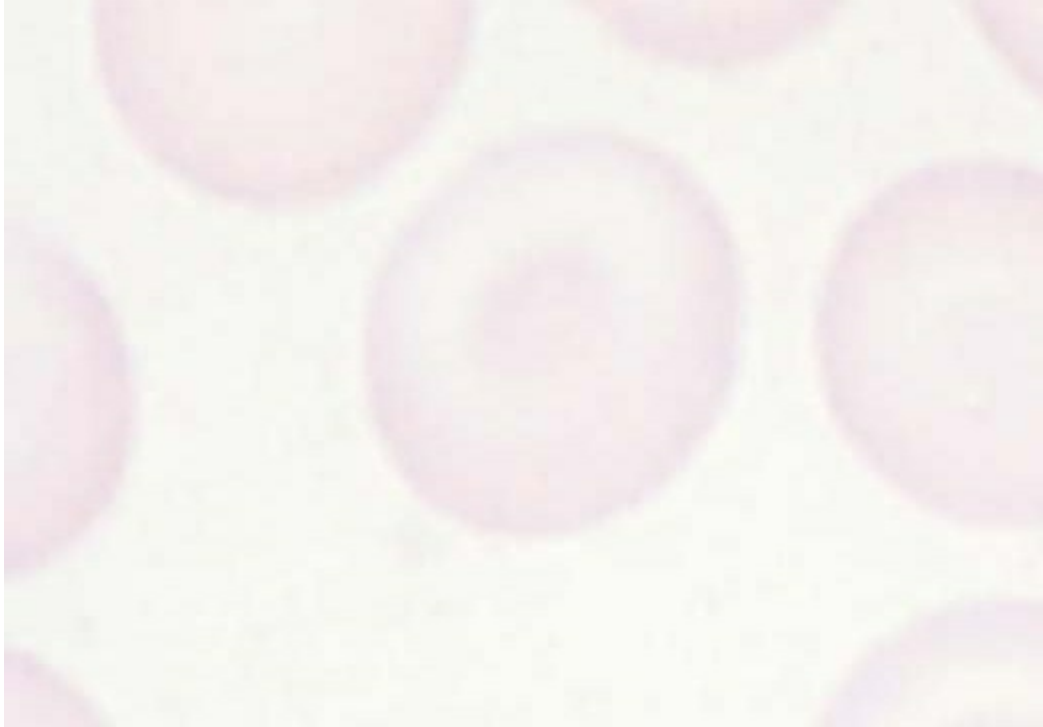
m

m m m . T x

m m

m .

.



REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

9. T G

A.

Cell Morphology: Ultrastructure and Organization

B.

1. T m z

C.

A.

D. DNA

B.

C.

10. T m

D. m

A. m

B.

2. T m m ' major m

C. k

A.

D.

B.

C.

11. T m

D.

A. m

B.

3. W m ?

C. k

A. R q (A P)

D.

B. M m m

C. A

12. T m

D. R q m

A. m

B.

4. W

C. k

?

D.

A. R q (A P)

B. M m m

13. T m

C. R q m

A. m

D. A

B.

C. k

5. P

D.

A.

B. m m

14. G

C. m m

A.

D. B A C

B.

C.

Cytoplasmic Organelles and Metabolites

D. m

6. T

15. A mm

A.

m

B.

A.

C.

B.

D. m

C. A

D.

7. T ER

A.

16. T

B.

A. m , , m

C.

B. m , , m

D. DNA

C. DNA, RNA, m

D. DNA, RNA, m

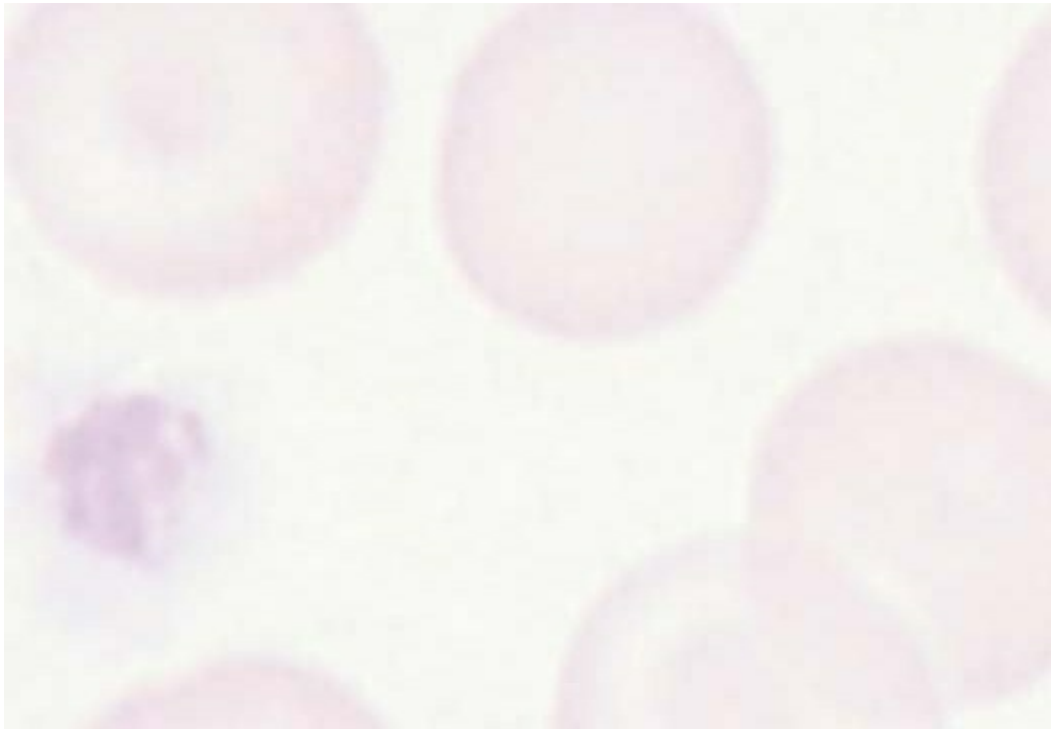
8. T m ER

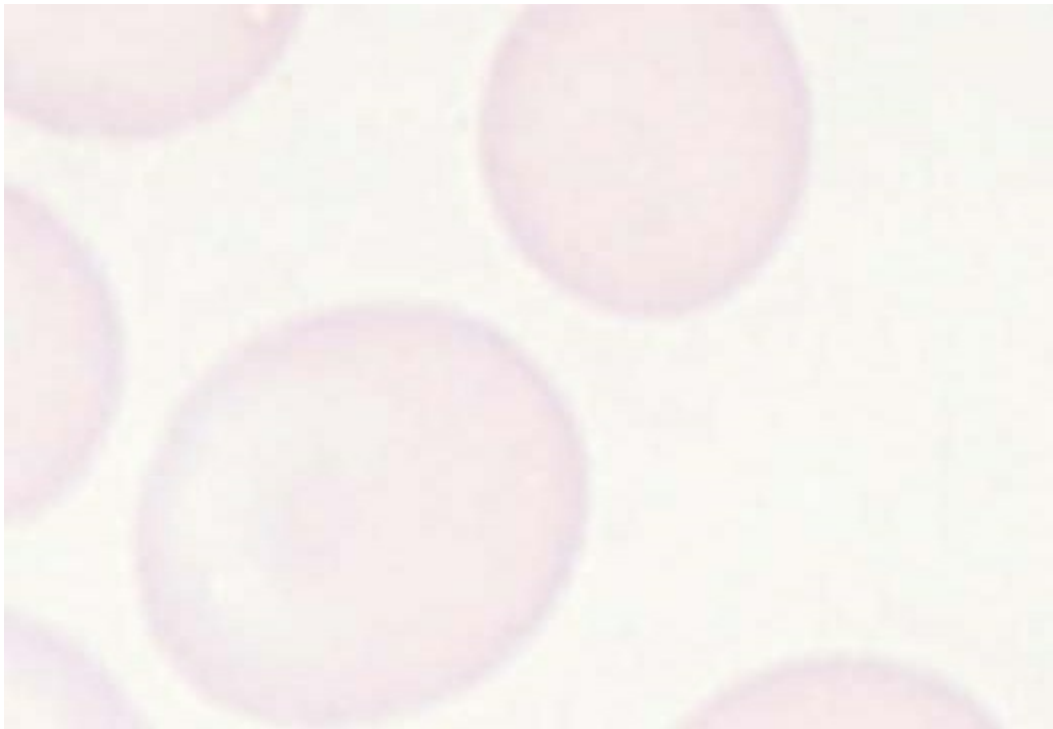
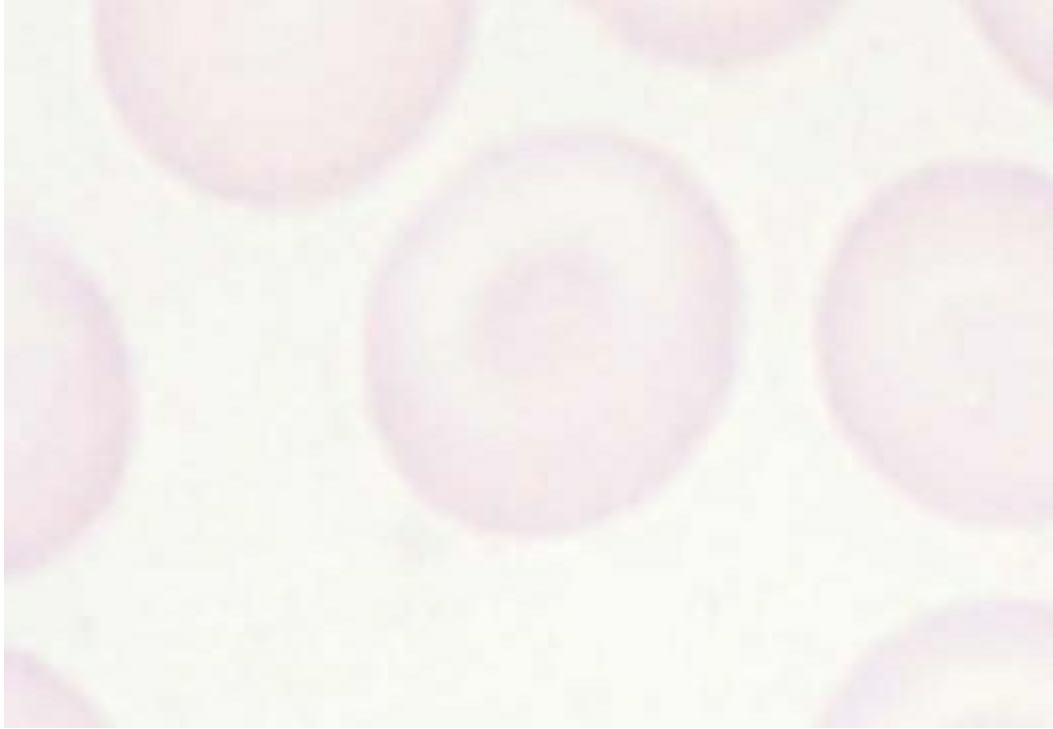
A.

B.

C.

D. DNA





REVIEW QUESTIONS (continued)

17. T DNA

25. I

A. z m

A. C m m ' q

B. m

B. m

m

C. D m

C. m m

D. C m m

D. m

26. I m

m

A. C m m ' q

B. m

*18. H m

C. D m

A.

D. C m m

B. m

m

C.

D. B A B

27. I

A. C m m ' q

19. C m m

B. m

A. q m m m

C. D m

m

D. C m m

B. m m

m

C. m m m

k m m

28. I

D. A

A. C m m ' q

B. m

20. A m m

C. D m

A. m m

D. C m m

B. m m m

m

C. m m m

D. x m

29. I k

A. C m m ' q

21. I G m , _____.

B. m

A. DNA

C. D m

B. m

D. C m m

C. mm m

m

D. m m

Apoptosis

30. A

22. I S m , _____.

A.

A. DNA

B.

B. m

C.

C. mm m

D.

D. m m

*31. A z

23. I G m , _____.

A. m m

A. DNA

B. k

B. m

C. m

C. mm m

D.

D. m m

*32. A

24. I G , _____.

A.

A. DNA

B. m

B. m

C.

C. m

D. A B

D. m m

(continued)

PART 1 ■ Hematology Basics

REVIEW QUESTIONS (continued)

33. B m

The Foundations of Genetic Interactions

x :

41. T m -

A. z m

m

B. m m

A. m A

C. m m

B. V L

D. m k (NK)

C. k m

D. CML

*34. T

A. m

42. G m :

B. m (NF) m

A. m m

B. m , , -

C. B -

D. m m (ER)

C. m

D. q

35. A m

A.

43. A x x : B. m m

A. (A)

C.

B. m ()

D. m m

C. (C)

D. (U)

*36. T x

A. m

*44. T -mRNA m

B. m (NF) m

A.

C. m m

B. x

D. CD

C.

D.

*37. A m j

A. m

*45. Ex m m

B. m m

A.

C. , -

B.

, m

C.

D. NF-kB m

D. m m

RNA

*38. D

A. m

*46. A U R

B. m

x :

C. m m

A. mRNA

D. q mm m

B. f

C. m

Meiosis

D.

39. I m ,

A. n m m m

*47. A DNA single DNA B. m m

C. m m

A. m

D. m m

B. m m

C.

40. H m .

D. SNP

W ?

A. S k

*48. M

B. S k m

A. DNA

C. H m

B. m DNA

D. A

C. mRNA

D. B A C



CHAPTER 3 ■ Cellular Morphology and Foundations of Genetic Hematopoietic Disorders 79

REVIEW QUESTIONS (continued)

*49. I m k m (CML),

*50. A (CD)

x :

A. (;) m m A. m m

B. ABL BCR

B. m -

C. k x

D. m

C. k m

CML

D. A B

G JP, . Wintrobe's Clinical Hematology, , , **COMPANION RES OURCES**

P , PA: W K H /L W m & W k , : - .

:// . . m/

H I, D K-M. C

, Blood, (), .

E W -

K m HW, S m CM. F m m : m .

m , Med Lab Observer, H

(): - , .

-

M E. R m PCR m -

.

, Adv Med Lab Pro , (): - , .

R JC. B - - m m m :

, Blood, : - , .

BIBLIOGRAPHY

S m SL. R m m , Current Concepts in Clinical Pathology Conference, J : .

B YP, . SN m m m DNA S z M, V J. P m DNA, N

, Nucleic Acids Res, (): – , .

Engl J Med, (): – , .

Bé é MC, K JS. H m m

ML. Immunology and Serology in Laboratory Medicine, ,

k m , Haematologica, (): – , .

S . L , MO: M , .

B DE, A ER, B CA. Fundamentals o Molecular

U S. B m , Adv Diagnostics. S . L , MO: E , .

Med Lab Pro , (): – , .

C CM. O , N Engl J Med, (): – , W k J, F D, R K. M m , Curr Opin .

Hematol, (): – , .

G m A, H VL, S z DJ. Im m -

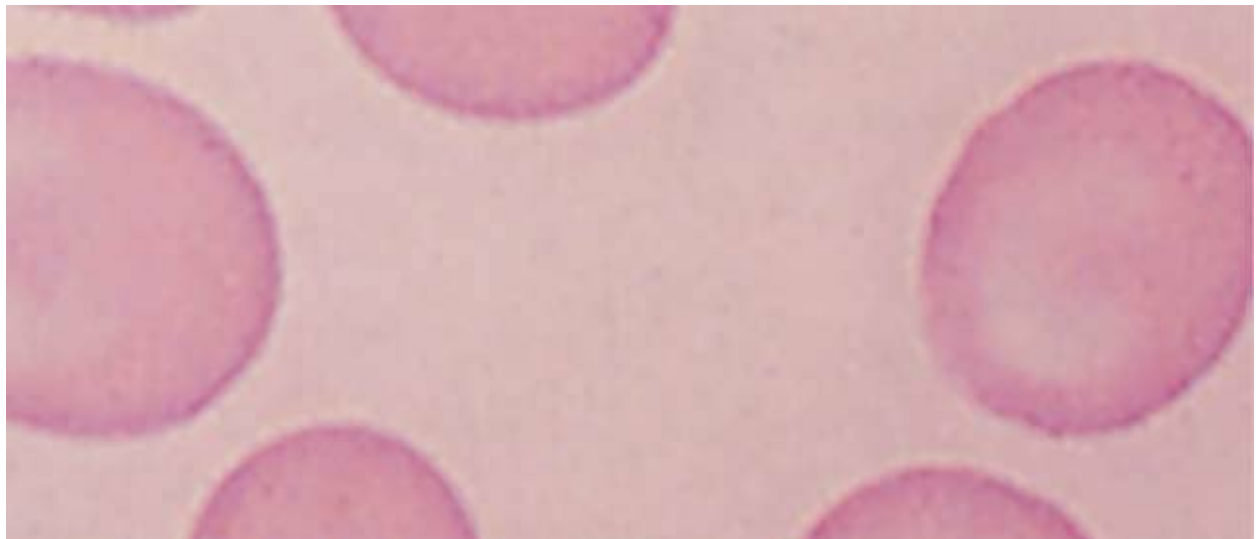
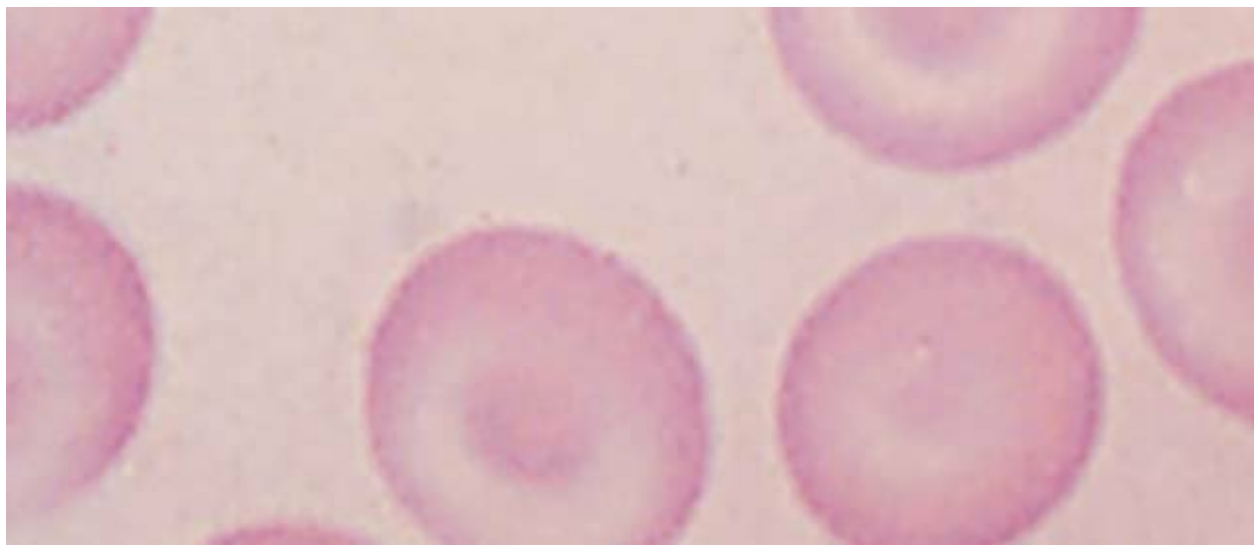
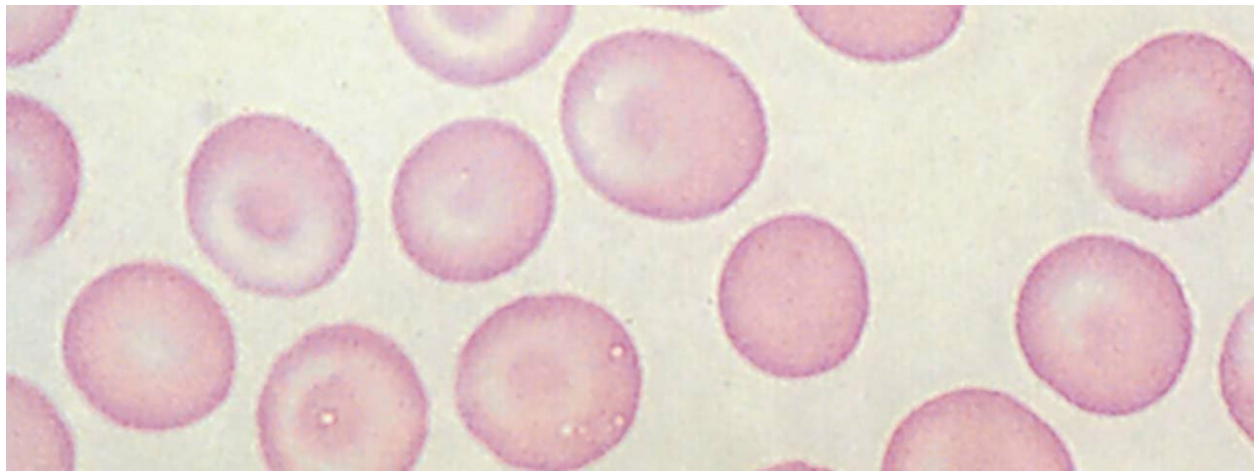
W RL. ARUP's Guide to Molecular Diagnostics Clinical Laboratory

m . ADV or Admin Lab, (): – , .

esting, , S L k C , U : ARUP L , .

G k CD. M m m , Clin W m JL. A m Lab Sci, (): – , .

m , Clin Lab Sci, (): – , .



PART TWO

Hematopoiesis and Cellular Maturation

CHAPTER

Principles of Blood Collection

4

Hematopoiesis

KEY TERMS

nucleus

extramedullary hematopoiesis

nucleocytoplasmic ratio

basophilic

hematopoiesis

pancytopenia

basophilic

hematopoietic progenitor cells

primitive hematopoiesis

cytokines

hepatosplenomegaly

progenitor blood cells

definitive hematopoiesis

human stem cells

e osinophilic

m o n o n u c l e a r p h a g o c y t e s y s t e m

LEARNING OUTCOMES

Ontogeny of hematopoiesis

Compare and contrast the phenotypic characteristics differentiating

- Define the terms hematopoiesis and extramedullary hematopoiesis.

the hematopoietic stem cells and progenitor cells.

- Name the major anatomical sites of the hematopoietic system pro-

- Contrast the features of erythropoiesis, granulopoiesis, lymphopoi—
gressing from embryonic to adulthood.

esis, and megakaryopoiesis.

Differentiate between primitive and definitive erythropoiesis.

- Name at least three growth factors and associate each factor with

- Compare the developmental events of embryonic, hepatic, and early
target cells.

medullary phases of hematopoiesis.

Assess the overall functions of interleukins and hematopoietic

growth factors in the hematopoietic microenvironment.

Hematopoietic organs and tissues

Summarize the impact of positive and negative cellular regulators.

■ Name the mature organs and tissues of the hematopoietic system.

Exam ination of maturing blood cells

Assess the comparative features of hematopoietic organs and

■ Name the cells in developmental order that will mature into eryth—
tissues.

rocytes, thrombocytes, plasma cells, and the various leukocyte types.

■ Compare the anatomical locations and functional differences

■ Name and describe in detail the two overall features of a cell that
between yellow and red marrow.

are important in the identification of a cell and that may vary as a

■ Name the sites and cells found in primary and secondary lymphoid
cell matures.

tissue.

■ Compare the nuclear characteristics of shape, chromatin pattern,

■ Define the term extramedullary hematopoiesis.

and nucleoli in specific cell types and according to the age of the

Identify sites of extramedullary hematopoiesis.

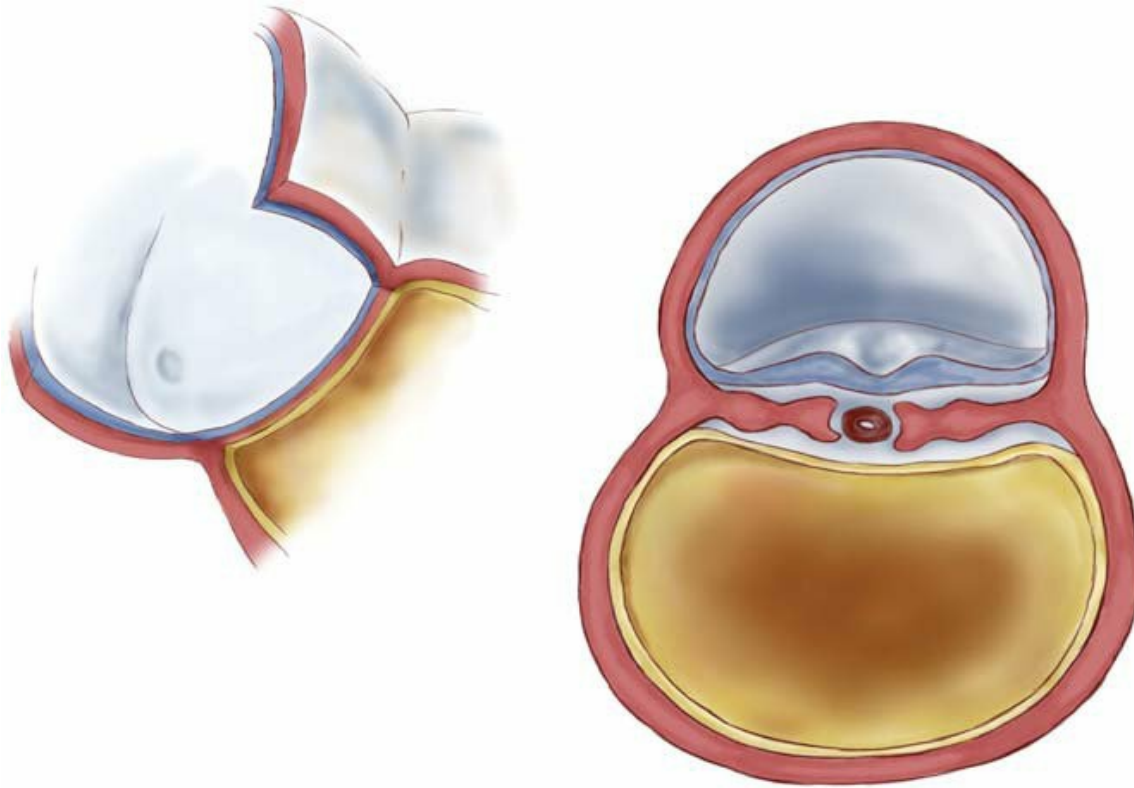
cell.

■ Describe two conditions that can produce hepatosplenomegaly.

- Compare the cytoplasmic features of color, granulation, shape,
- Describe the characteristics and functions of the thymus.
- quantity, vacuolization, and inclusions to cell maturity.
- Describe the characteristics and functions of the spleen.
- Name and describe the average percentage and cellular characteristics of the six mature leukocytes found in normal peripheral blood.
- Compare the functions of lymph nodes.
- Name the two functions of blood as a lymphoid tissue and effector organ.

NOTE:

- Cellular elements of bone marrow
- Indicates MLT and MLS core content
- Describe the functional characteristics of human stem cells.
- Indicates MLT (optional) and MLS advanced content
- Compare the extrinsic and intrinsic regulation theories.



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PART 2 ■ Hematopoiesis and Cellular Maturation

ON OGENY OF HEMA OPOIESIS

Def nitive erythropoiesis

m m . T

Hematopoiesis m -

m - HSC m m

m m

m k AGM. T

m (HSC) q

HSC mm . HSC

m .

z -

H m -

. T - . T m mm

m m . T m j

m

k , - -m (AGM) ,

m .

, m , m . F

m m

Fetal Hepatic Phase

m .

H m m -

The Embryonic Phase

m . I

k z z Em , x m , , m

, m m m

z , , m , , -

m m m , m m

m m m .

(F . .). T m j m m -

H m k m k () m . H m

m (F . .).

x m m

H m

. T m m

k k . T m

. V -

m . I m , m m

k m

k . T

m , -

k -

m . T m

. Primitive hematopoiesis , m -

m m F (

, m

m).

. P m ,

Head

. Thorax, G

axial. Abdomen

Pelvis

muscles.

Amniotic cavity

Notochord

Lateral plate

mesoderm

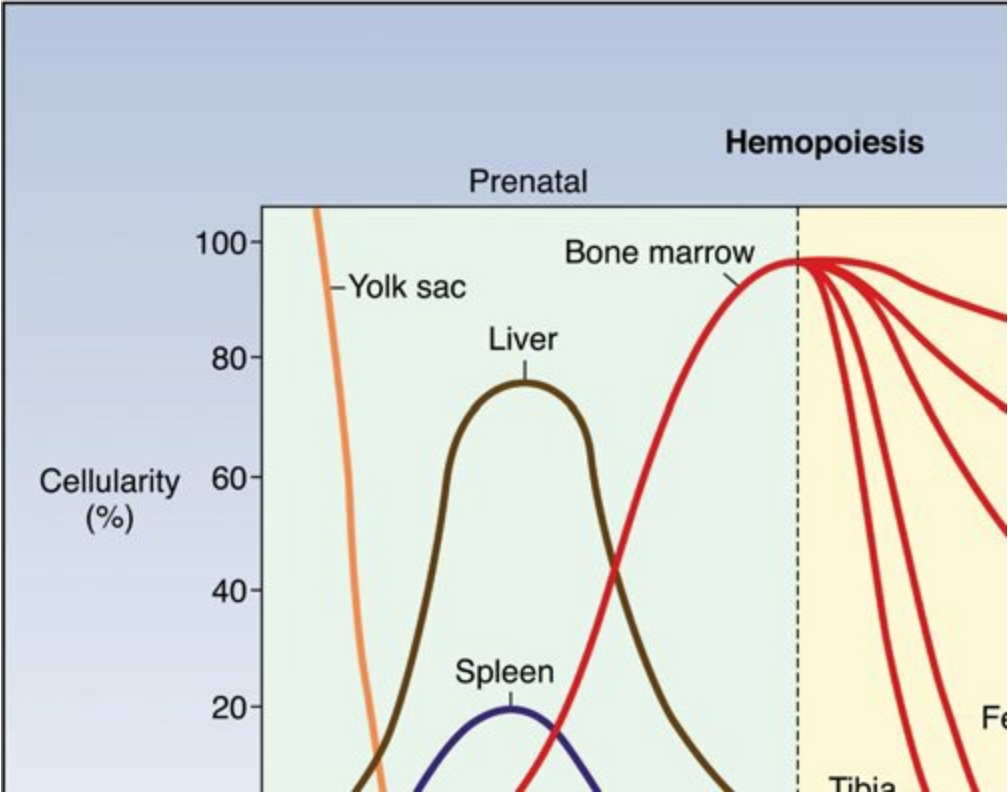
Intermesoderm

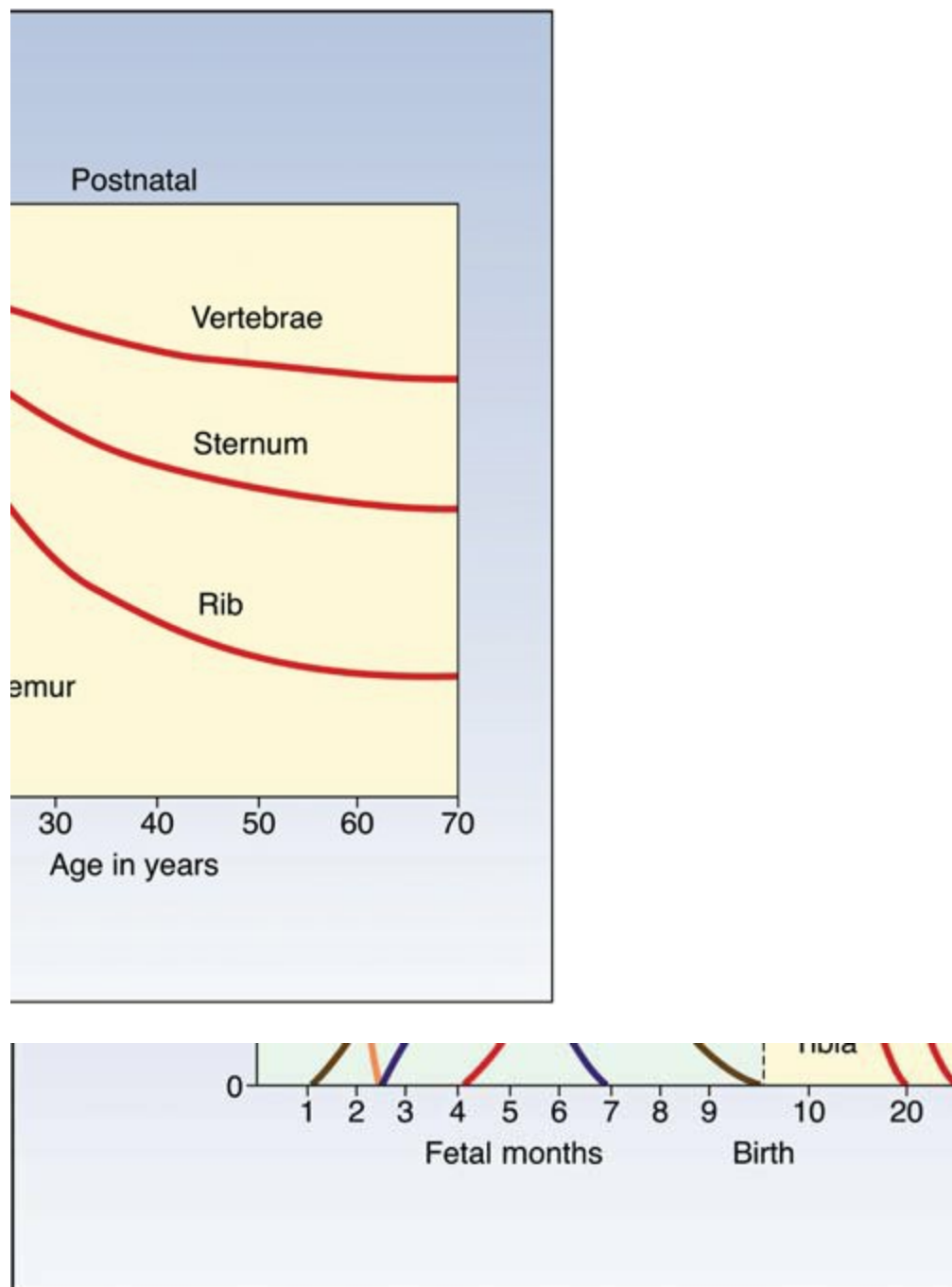
mesoderm

Paraxial

mesoderm

FIGURE 4.1 C - mesoderm. Thorax, . Thorax. Thorax. Thorax. Thorax (), mesoderm (), axial. (Rosen H W. Fundamental Anatomy, Boston, MD: L W & W k, .)





CHAPTER 4 ■ Hematopoiesis

83

FIGURE 4.2 Hematopoiesis. (Rommersley, F. J. L. Pathology, 1998, PA: Lippincott Williams & Wilkins, Inc.)

T , m , m -

, k

m . T m

m .

. T -

T m m

m

:

m . P m k

. T m

.

m .

Early Medullary Hematopoiesis is

. T m x

m m m

B m ,

m . S m z

m m . A

m m , m

m , m m m

HSC , . T m

m m (m m -

m m m

). H m F m A

(HPC) . V

m . U -

m x m x m

, m m

m .

(extramedullary hematopoiesis) .

. T HPC HSC m

C m m .

. E , - m -

, -m – m -

Prim ary Hem ato po ietic Organs and Tis s ues

. M

m :

Bon e Ma rrow S ite s a nd Fu nct ion

: .

B m

m m : m , -

m m m () , NOTE: This is a good time to review the definitions of Key m , m

Terms in the Glossary and ash cards on

.

m k , , m

(F . . - .).

HEMA OPOIE IC ORGANS AND ISSUES

T m ' .

I x m . % %

T m m -

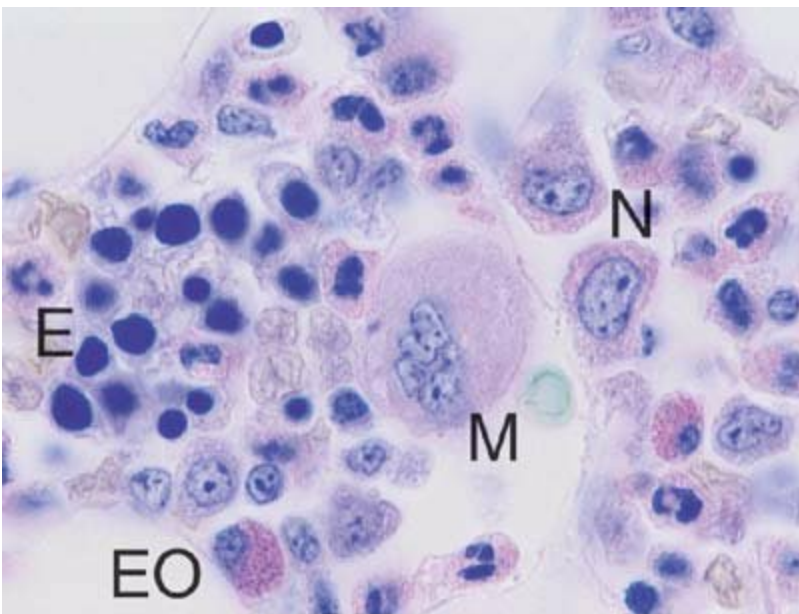
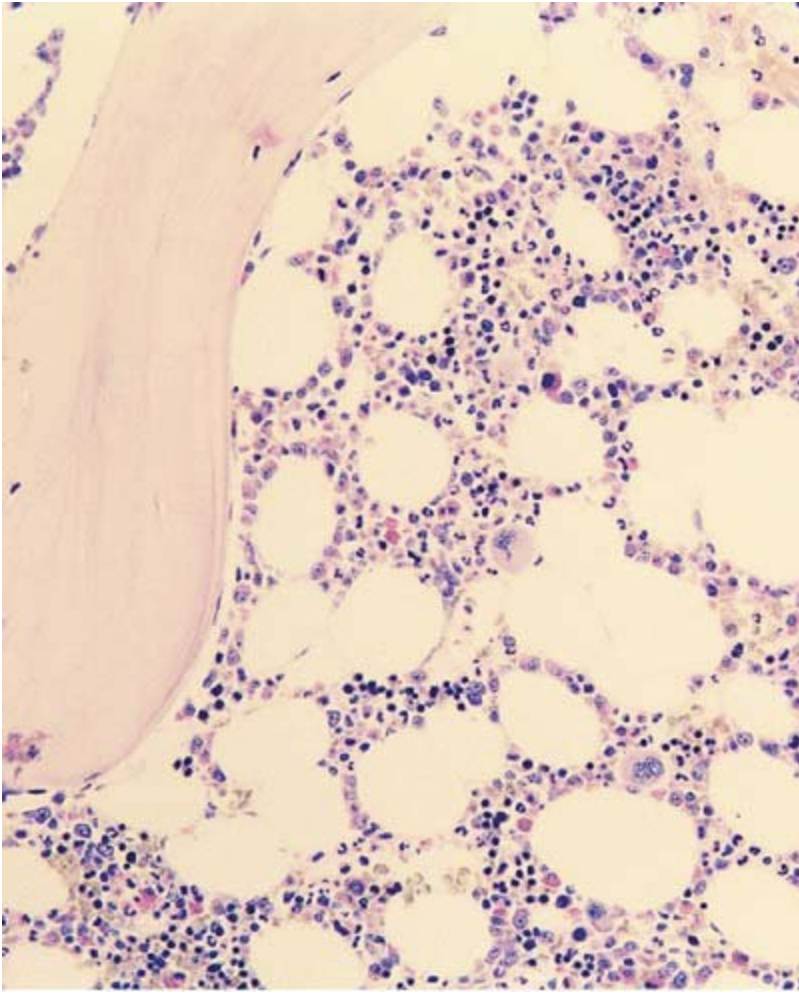
, ,

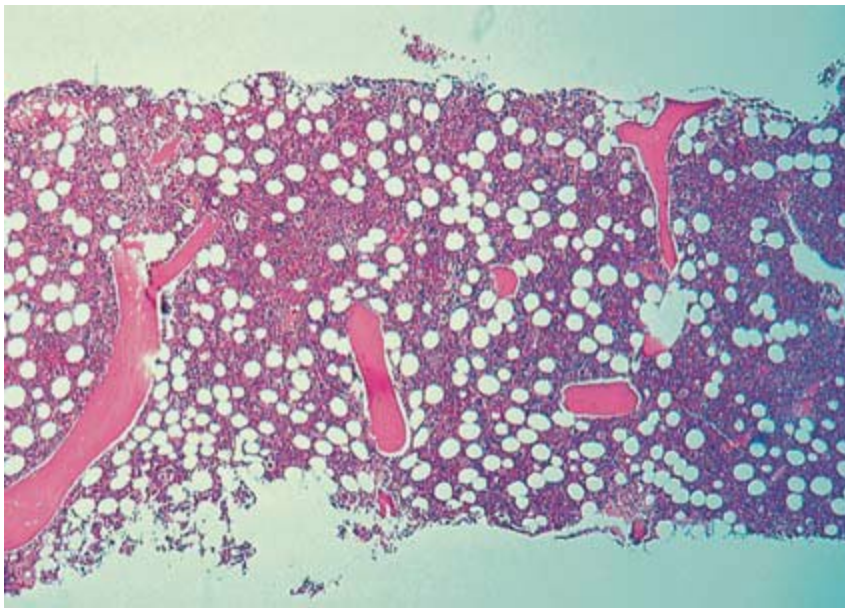
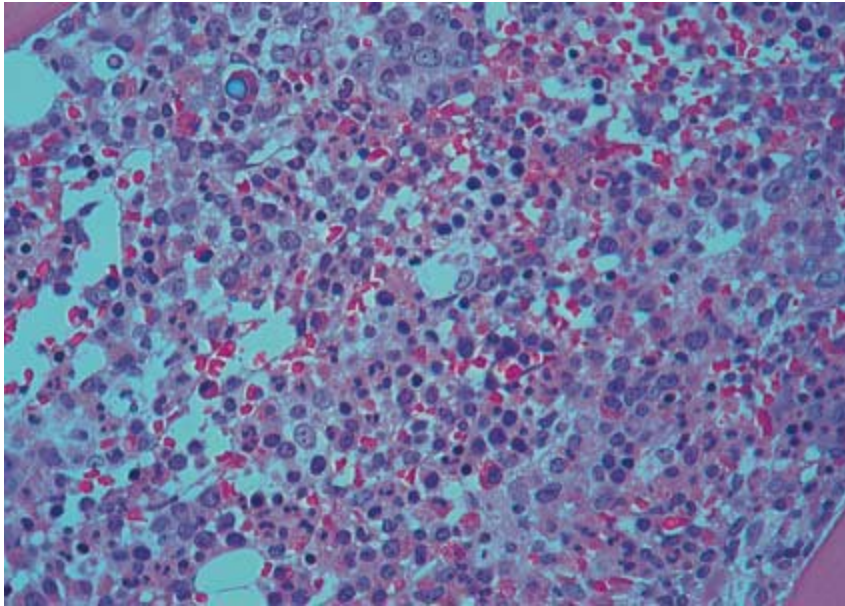
m m , m , , ,

m m z

m . B m

(F . .). T m





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PART 2 ■ Hematopoiesis and Cellular Maturation

FIGURE 4.4 B m m m

. A x m % % -

- - m . (R m m

M C KD. Clinical Laboratory Medicine, , P , PA: L W m & W k , .) I m m ,

A

k mm

x m . I , m

, hepatosplenomegaly, q

x m . T -

m

m .

T :

. W m m

m , m ,

(. . , k m).

. W m m m

, m m (

B

C

.)

FIGURE 4.3 N m m . S

m , , : -

Bo ne Ma rrow S t ructure an d Fun ction

(E), (N), (E), m k (M). G m : $\times \mathbf{A} \times$, \mathbf{B} .

T m x (R m m H RI, L x SE, S P.

. I m

Blood: Principles and Practice o Hematology, , P ,

PA: L W m W k , .)

m (, m , m ,

m k), () ,

, m . H m

m m z . F m

m , m

, z ,

(F . .).

D , m

. T m

x k (F . . A)

m

x k x m

FIGURE 4.5 B m m -

(F . . B). B , m

m . V % m m

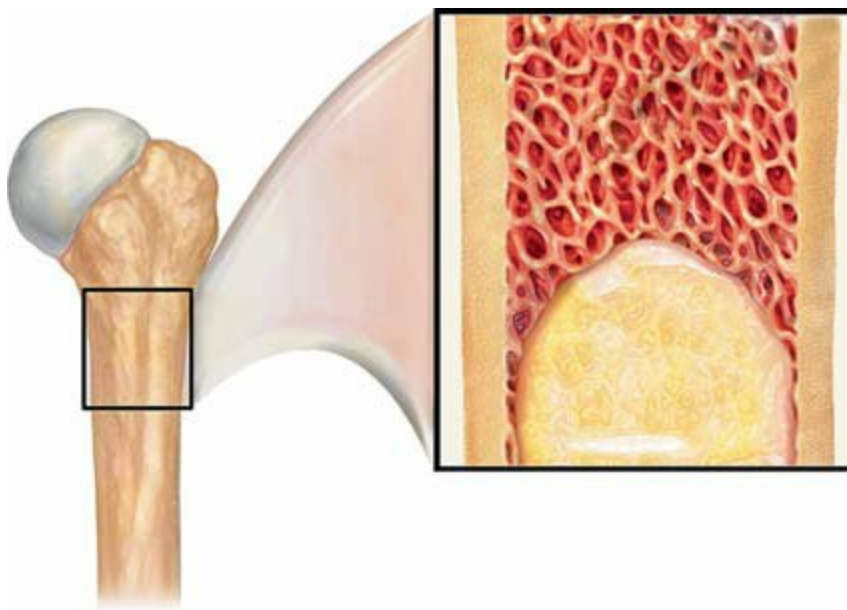
, , m, k , , ,

. (R m m M C KD. Clinical

m x , x m m

Laboratory Medicine, , P , PA: L W m m .

& W k , .)



CHAPTER 4 ■ Hematopoiesis

85

FIGURE 4.6 T m : m , -

, m , m . (A -

A m C C .)

m k , x m %

$m \cdot B m m j m ,$

$m m , -$

$, , m , m ,$

$.$

$H m k q m m$

$m m x$

$m x. F m , m$

$m m z m -$

$m (. . , k)$

$q , , m -$

$m m m . T m -$

$m m$

$, , m , , , -$

$, (. . ,) m$

$x m x.$

A

B

$H m m m z$

FIGURE 4.8 $S m . A . C : R$

$m k -$

m (red-shaded areas) k

m (); -

m . **B.** A : Y m m

, m k

(dark-shaded areas) k m. R m -

j m. U m , k . (R

m m Dz z k E. O m

m m , Curr Opin Hematol, (): , .)

F

m , ,

m;

m m -

E

m k

m .

A

D

, , m

m .

m . A x m

m m (E).

B

C

E m k

m m m m -

mm

mm ;

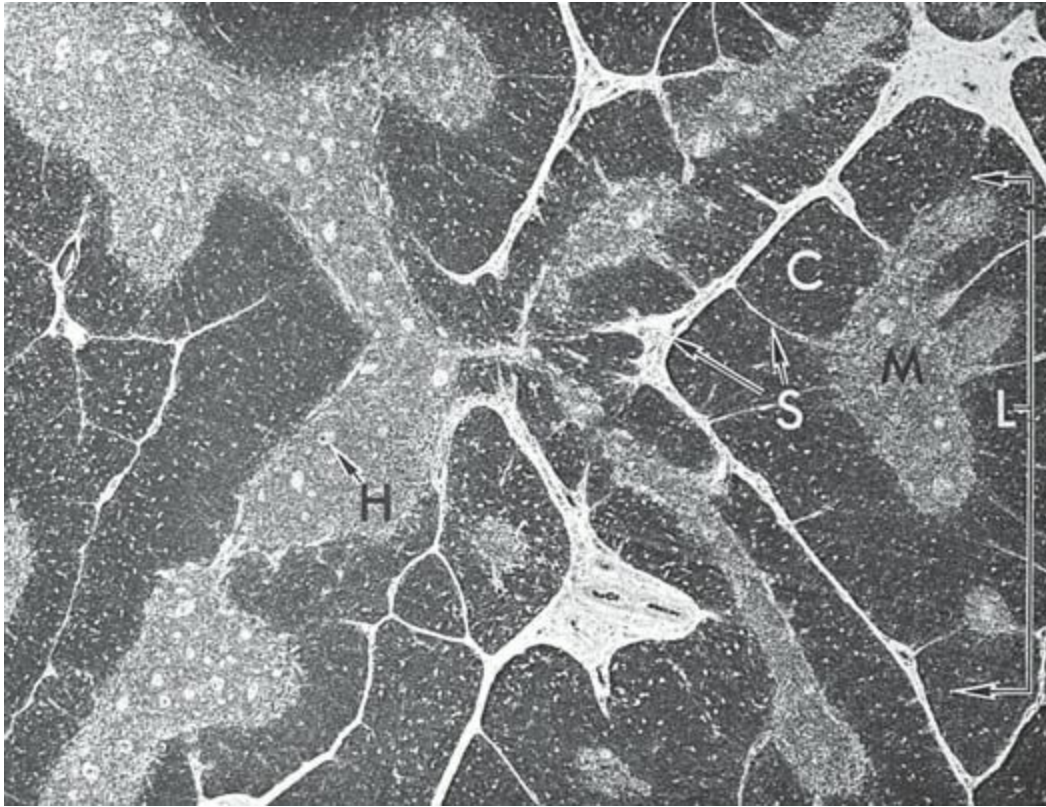
FIGURE 4.7 N m . **A.** L m .

. H m

B. B . **C.** E . **D.** S m .

, , ,

E. M . **F.** B m .



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PART 2 ■ Hematopoiesis and Cellular Maturation

m , m . T

. F x m , , x ,

m q m m

m ;

m . T -

.

m m m -

H m x -

m **immunocompetent**

m

(mm), m -

, m , mm , x ,

. T m mm -

.

m m .

T x m z

Thym us

m; q .

I “ -

E m m , m m-z ” . W

m m m

m m m m ,

. T ,

CD CD m k (-

m m, x mm -

) x-m j -

m. I m

. C k m

m m m ,

m x x CD CD

x m m m m k (). A m .

m , m

I , m m m

x CD CD (m k).

m k . A

m k m j m

m

. I m ,

m . I , - m

m - -

m m m .

, x

m m m

, m cytokines. M

m m m

k . T

m . T CD +

m , , m x , m .

m x.

M m -

L m m , m , m -

z , m m m m -

m (F . .). m -

. V m .

m m ,

T m m m

m m, mm

- m . F x m ,

(),

D G m m -

, j . P

m m m q

m m

m m .

E , m m

m - -

, m , m -

. A m ,

m x . T

m mm m m -

m ,

. L m x

- x

m z m -

. T m % m

z m . A

m , m

m , -

. T

m m mm m m

(C D L m).

T m B , -

, , m .

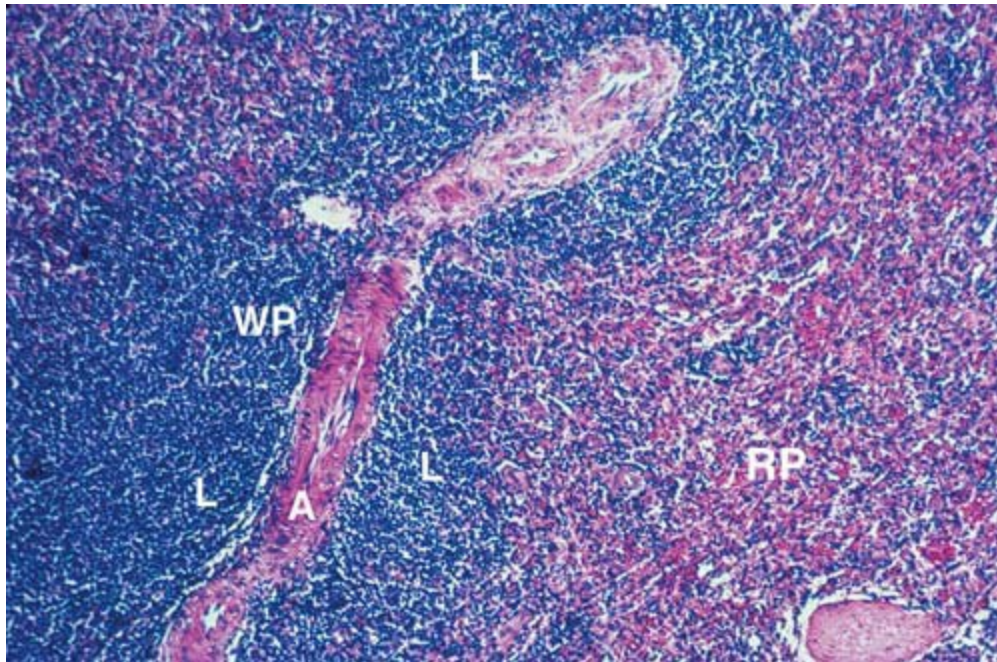
FIGURE 4.9 H m (L) -

x (C) m (M). H. H , S.

I m -

(). (G JP (). Wintrob's Clinical Hematology, , mm m m . T m V . , P , PA: L
, W m & W k , : .)

% m



CHAPTER 4 ■ Hematopoiesis

87

We ight

(g)

30

Fa t

20

Cortex

10

Me dulla

Pre natal

10

20

30

40

50

80

(months)

FIGURE 4.11 S . W (WP); m -

(L) k (A); (RP)

Age (ye a rs)

m , ,

FIGURE 4.10 T m m .

. (G JP (). Wintrob's Clinical Hematology, ,

V . , P , PA: L , W m & W k , : , FIG . .)

(F . .). H , m

m m -

m .

() mm - . I T m -

m m -

m , -

mm m

mm . T

m mm m m

m m

. M

. T m k z mm ,

z : , , m z

B m , mm (-

(F . .).

-) m x , , T

m mm ,

m,

m . Imm m

. T m ,

, m-

,

m , m .

. T ,

(),

. I m , L m

SECONDARY LYMPHOID ISSUES

.

T m m , P ' k , - m (GAL),

m m. B

, - m (BAL), k -

m , . L m -

m . R m m , m

z

m (. . , m ,

m m k . P

). M m (. . , m m -) , m (C).

B -

O m ,

(.).

m j B m

P B m -

. T m

m m

m m

m .

mm m (-

) mm . T m m

S pleen

,

m . A , m T m j -

m B .

m m

T . A /

, - m x -

q . W

z m m

(m), m

88

PART 2 ■ Hematopoiesis and Cellular Maturation

m q , m . R

m x m m m

, q x -

.

m m . R m M m m -

m ,

m m m m . T

m

.

mm . T m

H m m m

m

z . T m

. A m m , -

m x m , k -

x -

, , pancytopenia, .

m m , , CD .

S m % % -

W m k m , m -

m .

I m

m .

- m. O

B m m m m

m m q

G ' . N m

, , m . B-m

m m m

m x

m . I m

- - , m , -

m m , - , m -

m .

plasma cells. S m B -

I m ,

m m B , -

m. A q

.

m m k m .

B m m

A , x , m

m B -

k . T k m -

(. . , m). B m

m k m m

k x RBC . C m

m m

m m mm . B % %

m .

m . M m B m

, m B

Lym ph Nodes

.

I m m m

L m m , m , m -

m

m , , .

m m. L m -

L m m m

m :

m . I m m m

k , m . M

. S m m

m z

. F m , ,

.

m

. P mm

Blo od

A m , m -

x

B m m mm -

m m .

. C m

M m m m

-

k , , m-

. I ,

. F , m j

q mm k

m , , m , B , B

.

m .

B m q m m .

L m k (NK) I m m -

, m , B m . B lymphocytes

m . A

m

m ,

. lymphocytes

.

q , m

m m (. . ,). I -

NOTE: This is a good time to complete Review Questions

m

related to preceding content.

x , m .

CHAPTER 4 ■ Hematopoiesis

TABLE

4.1

Hematopoietic Micro environment**Cellular Stroma****Extracellular****Components****Functions****Components****Function**

Adipose cells

Expression of homing receptors

Soluble factors

Regulation of hematopoietic

Endothelial cells

Production of soluble growth and

Cytokines and growth factors

stem/progenitor cell differentiation and expansion

Fibroblasts

differentiation factors

Extracellular matrix

Macrophages

Production of integral membrane

Collagen

Structural support

proteins

Glycosaminoglycans

Cell-to-cell interactions; local—

Osteoblasts

Production of extracellular matrix

Cytoadhesion molecules

ization of growth factors

T lymphocytes

components

Adhesion of hematopoietic precursors to extracellular matrix

proteins

CELLULAR ELEMENTS OF BONE

q (GO ;

MARROW

C).

I HSC m m

M , m m

-

m m

. T m m m

m . T m m m

HSC

m m m

HSC . I z m -

m x m x m

m (.). T m m

m k m mm m /

. B m

m

m m m

, m .

m .

S - m mm HSC

x HSC -

HSC . O x HSC . HSC

HUMAN STEM CELLS

m z

m m

A m m

m m . HSC

human stem cells (HSC). HSC -

mm . I -

m m

, m

.

m . S m

N m . S - m

m -

(S R) - m (L R)

, m. HSC m

. S R m

m

m m m k

. HSC m -

- .

z m . HSC -

S R m m -

-

' m m m m

- m . S

HSC .

m HSC .

HSC m , -

S m m m -

m , -

, m , m ,

(FACS), m m -

m .

m m . HSC

S m m m -

m k CD +, T - +, . T - m -

m ' m . D

m k j CD .

m , m m

D m - , HSC

q q (F . .). D

m m , -

HSC m -

, HSC m z

m, m m j m

x m

(%); m m

m .

90

PART 2 ■ Hematopoiesis and Cellular Maturation

Young

Old

Fa s te r

Shorte r

DNA re pa ir

L

o

n

Tra ns cription

g

(a)

(a)

e

vi

Intra ce llula r prote in

t

y

Te lome re le ngth

s e lf-re newa l

High

Low

(b)

diffe re ntia tion

(b)

Ma ligna ncy

Ag

Re plica tive s e ne s ce nc e

i

n

g

DNA damage accumulation

(c)

(c)

Chromosomal instability

Slower

Longer

Qualitative changes

Quantitative changes

Hematopoietic stem cell (HSC)

Hematopoietic progenitor cell (HPC)

FIGURE 4.12 Old HSC (a), middle (b), (c). Examine, middle. In q, q HSC
P, middle DNA, - k, middle. T, middle DNA, middle, middle - -

HSC, middle. B, q, q HSC, z, middle. F, HSC, middle -

q, , . (R

middle L, W, V, Z, G. G, middle-middle, Curr Opin Hematol, (): , .) T, , middle

z, middle, middle k-

.

middle. S, middle, middle, middle z

P, middle, j

, “ middle, ”

: m m . T

m m

m m m

. R

B / m . T m (m) m

“m ” m

- m , -

m m .

- -m -m k (CFU-GEMM).

T “m ” m

T m CFU x m

(MAPC). MAPC x z m

- m

m k m . I , MAPC

. T

x m m , , , ,

CFU-GEMM m

.

mm . T CFU-GEMM

m CFU- -m /m -

Progenitor Blood Cells

(CFU-GM), CFU- (CFU-E), CFU-

(CFU-B), CFU-m k (CFU-M). I -

A x , HSC

, CFU-GEMM - m

m q

, (BFU-E). E CFU -

m ,

m

m ' m m -

.

. I m , -

Hematopoietic progenitor cells (HPC) m

x z , - m m -

m m k . N

m m m

m -

x m x , ,

. H m

CHAPTER 4 ■ Hematopoiesis

91

m (UCB). UCB

Ma s t Ce lls

m -

m , m m m .

, m m . M

C x m UCB .

m m m

(KI), CD

Erythro po ies is

m m k , KI .

E m

CD , m , , -

, z

m . P q m

, , . E

m , q m

. W , - -

m m .

x m x , -

M m j

k, m .

x

E % %

- k - I E. - -

m .

-

, m , ,

Granulopo ie s is

z m . A ,

m m C C , k m ,

M % %

m . A m

m m . G z

IL- .

m . E

I m m

. N m

m (. ., m -

m

).

(C). M

A

. I , m -

m mm m

x

m m m

.

.

Lym pho poiesis

Ma cro pha ge s

M , m , U k , m m m . m

m . L m m

(F . .)

. L m m

. -

, . P m

m, **mononuclear phagocyte system** (

. L m

F . .), -

% % m

m, m

m .

m , m ,

m .

Megakaryopo i e s is

“ m” mm -

M k k j -

, m m , ,

m. M k

m I G

m m

m j m C .

. M k

M m m -

x m .

m m -

. C m m

Other Ce lls Found in Bo ne Marrow

m m m m . T

mm , mm

Ma rrow S t ro m a l Ce lls

m -m -

T m k m m m

, m m m

, , , . T

. M m

m

. C m m -

m . S m x -

m

m x m (. . , -

m .

). T x m x

M , k

m m -

, m , m , -

m .

z m m.

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PART 2 ■ Hematopoiesis and Cellular Maturation

F , m m m

CY OKINES

m k -

m m m .

Cytokines m

M

. C k k , - m -

m - k -

, . C k m -

mm (IFN- γ) - m

x . C

(G-CSF), m m z

m m m x

m m m . T -

m (k) .

-m mm . I

B k -

, m x x

, , -

m , m (NF- α ,),

. T

m

-

.

,

T m , -

(. ,

k .

m - m -

). G ' m -

He mato poietic Grow th Facto rs

z . T m

z m mm

E m

. B m m

. T m m m

. C m m G-CSF . T

, m -

.

..

T m j m

Bon e Ce lls

HPC

O m x- z m m

m . A

. T m

m

m

m , m -

.

m m

H m

m . O m m -

m m

k . T - m .

. E , GM-CSF, G-CSF, M-CSF, IL—

TABLE

4.2

Exam ple s of Hum an He matopoietic Grow th Facto rs

Grow th Facto r Ce llular S ource

Proge nito r Ce ll Targe t

Mature Ce ll Targe t

Erythropoietin

Peritubular cells of the kidney,

CFU-E, late BFU-E, CFU-Meg

None

Kupffer's cel s

IL-3

Activated T lymphocytes

CFU-blast, CFU-GEMM, CFU-GM, CFU-G, Eosinophils, monocytes

CFU-M, CFU-Eo, CFU-Meg, CFU-Baso,

BFU-E

G-CSF

Monocytes, broblasts, endothelial CFU-G

Granulocytes

cells

M-CSF

Monocytes, broblasts, endothelial CFU-M

Monocytes

cells

GM-CSF

T lymphocytes, monocytes, eosino—

CFU-blast, CFU-GEMM, CFU-GM, CFU-G, Granulocytes

phils, monocytes, broblasts,

CFU-M, CFU-Eo, CFU-Meg, BFU-E

endothelial cells

G-CSF, granulocyte colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; CFU-blast, colony-forming unit, blast; CFU-GEMM, colony-forming unit, granulocyte, erythrocyte, monocyte, and megakaryocyte; CFU-GM, colony-forming unit, granulocyte and macrophage; CFU-Eo, colony-forming unit, eosinophil; CFU-Meg, colony-forming unit, megakaryocyte; BFU-E, burst-forming unit, erythroid; CFU-G, colony-forming unit, granulocyte; CFU-M, colony-forming unit, macrophage; CFU-E, colony-forming unit, erythroid; CFU-Baso, colony-forming unit, basophil.

CHAPTER 4 ■ Hematopoiesis

93

, ,

m DNA .

TABLE

4.3

Regulators of Hematopoiesis

S j

 $(\cdot, \cdot)_m$

Regulator

Effect

m).

H m m z

Interferons

Suppress hematopoietic

HPC . A k

progenitor cells by inhibiting

. Ex m

proliferation

$$\vdots$$

Induce apoptosis

. G-CSF GM-CSF m m .

Transforming growth

Suppress hematopoietic

. IL-m B m .

factor β

progenitor cel s by inhibiting

. IL-NK .

proliferation

T m z HPC m m

Induce apoptosis

m -

Tumor necrosis factor α

Suppress colony growth fac—

m HPC

tors: CFU-GEMM, GMP, and

m x m x. M z

BFU-E

HPC m , m -

E-prostaglandins

Suppress granulopoiesis and

, m , k . HPC

monopoiesis by inhibiting GMP,

m z M . S m

CFU-G, and CFU-M

m HPC m m z -

Stem cel inhibitor

Maintains stem cel in the G

VCAM . H m -

O

phase of the cell cycle

Macrophage in amma—

m m

tory protein-1 α

m . O , -

HPC G G .

m

T m j m -

m m -

m m m . T

m m m -

m j :

. Im k m k

. IL- , IL- , GM-CSF

m -

. IL-IL-

.

. IL- , IL- , IL- , IL- , IL- , IL-

Interleukins

S m k . A x m T m, k , -

m , k , m x -

JAK-S A . A m JAK-S A

, m , m ,

m j m (C).

. T m k j m -

I k , m -

-

.

x . D m

D -

m -

m , m

m . Ex m -

k (F . .). I m k

m

m -

m (.).

, m , x m , m ,

m m k q

Trans cription facto rs

. M z -

m mm ,

A ' m

, .

x . G m

I k mm -

m m

(inter—) (leuk—

. C m -

m k). I k m

x

m m

-

. I k m m

. A ’

mm m. T

x -

k (A x F). T

k . M

k mm m k

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PART 2 ■ Hematopoiesis and Cellular Maturation

P luripote nt

He ma topoie tic

S te m Ce ll (P P S C)

SCF

S CF

SCF, IL-3

Re d Blood

GM-CSF, EP O

Myeloid

SCF, IL-3

Lymphoid

Cells

Basophils

Stem Cells

Stem Cells

SCF

SCF

SCF, IL-3

IL-3

IL-3

GM-CSF

GM-CSF

SCF

IL-2

IL-6

Platelets

Colony - Forming Unit -

Eosinophils

IL-3

IL-7

Granulocyte - Macrophage

IL-7

(CFU-GM)

IL-3

IL-3

B-Lymphocytes

T-Lymphocytes

GM-CSF

GM-CSF

M-CSF

G-CSF

Monocytes

Neutrophils

GM-CSF

M-CSF

Macrophages

SCF = stem cell factor, IL-3 = interleukin-3,

GM-CSF = granulocyte-macrophage colony-stimulating factor, EPO = erythropoietin, G-CSF = granulocyte colony-stimulating factor, M-CSF

= macrophage colony-stimulating factor.

FIGURE 4.13 Schematic diagram of the DA, RVF, and Foye's Principles of Medicinal Chemistry, 5th ed., PA: Lippincott Williams & Wilkins, 2006.

1. I (C

mmj

"L k D C " C),

. T -

x m , , . R

, , mm ,

x m

, m

.

.

m (F . .

m z mm m

m (

NOTE: This is a good time to complete Review Questions

"B M Ex m " C),

related to the preceding content.

m k q m m (.) m . S m -

EXAMINATION OF MA URING BLOOD

m m

CELLS

, , .

A m x m m

x m m m

General Cellular Characte ris tics

. f - mm -

T m

m q

m

m m m

(.). m

(C).

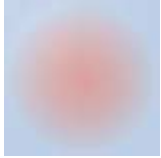
T x m m

m m m

. O z

(CBC) , m

. N - m (N:C)



CHAPTER 4 ■ Hematopoiesis

95

**Pluripotent hematopoietic
stem cell**

Myeloid progenitor cells

Bone marrow

Lymphoid progenitor cells

Released from

Cells in bone marrow

bone marrow Cells in blood

Function

Allergic

reactions

Myelocyte

Basophilic

Basophilic

Basophil

metamyelocyte

band cell

Allergic

reactions

Antiparasitic

reactions

Myeloblast

Myelocyte

Eosinophilic

Eosinophilic

Eosinophil

metamyelocyte

band cell

Phagocytosis

Acute

s

l

l

inflammation

e

c

Mye locyte

Ne utrophilic

Ne utrophilic

Ne utrophil

d

i

o

me ta mye locyte

ba nd ce ll

l

e

Coa gula tion

My

Mye loid

Me ga ka ryobla s t

Mega ka ryocyte

P la te le ts

proge nitor ce ll

Oxyge n

tra ns port

Erythrobla s t

Normobla s t

Re ticulocyte

RBCs

P hagocytos is

Antige n

ca pture

Monobla s t

P romonocyte

Monocyte

Blood, lymph node s ,

bo ne marrow, tis s ues

Ce llula r

immunity

T ce l

s

l

l

P re -T ce l

Thymus

e

Humora l

c

d

immunity

i

o

h

P las ma ce l

p

m

Lymphoid

Ly

proge nitor ce ll

Humora l

immunity

Bone

B cell

Pre-B cell

marrow

FIGURE 4.14 Hematopoiesis: maturation of B cells. (McH. Nature of Disease, 3rd ed., PA: Lippincott Williams & Wilkins, 1996)

PART 2 ■ Hematopoiesis and Cellular Maturation

TABLE

4.4

Blood Cell Development: Normal Differentiated Cell Lines

Erythrocyte *

Megakaryocyte

Granulocyte

Monoocyte

Lymphocyte

Immature

Rubriblast

Pronormoblast

Megakaryoblast

Myeloblast

Monoblast

Lymphoblast

Prorubricyte

Basophilic

Promegakaryocyte

Promyelocyte

Promonocyte

Prolymphocyte

normoblast

Bone

Rubricyte

Polychromatophilic Megakaryocyte

Myelocyte†

marrow

normoblast

Metarubricyte Orthochromatic

Metamyelocyte†

normoblast or

nucleated red

blood cel

Circulating

Reticulocyte or polychromatophilic

Band or stab†

blood

erythrocyte (diffusely basophilic)

Mature

Erythrocyte

Thrombocyte or

Segmented

Monocyte

Lymphocyte

platelet

neutrophil†

Macrophage

B cel T cel

(tissues)

Plasma cell

(bone marrow)

*Comparable terms.

†These cell types may be neutrophilic, eosinophilic, or basophilic.

Overall Cell Size

nuclear cytoplasmic N:C ratio. T z

T z m

decreases m . C q , N:C

z m . Ex m k

decreases m m . B m

m , k decrease

, k , m k

z m .

(:) N:C . A m ,

: : m , x m , m -

Nu cle a r-Cytoplas m ic Ra t io

, m k . T m

T m -

k (anuclear), m mm

q : : N:C .

CHAPTER 4 ■ Hematopoiesis

97

. I m , **S um mary of General**

TABLE

4.5

m

Maturation Characteristics*

.

Morphological Feature

Usual Development

■ L m

. S m m m .

General cell size

Decreases with maturity

■ M k – ,

Nuclear-cytoplasmic ratio

Decreases with maturity

mm .

Nucleus

■ M , , -

Chromatin pattern

Becomes more condensed

m m .

T m m

Presence of nucleoli

Not visible in mature cells

.

Cytoplasmic characteristics

Color

Progresses from darker blue

Presence of Nucleoli

to lighter blue, blue-gray, or

Tan -

pink

. Tan, pink -

Granulation

Progresses from no granules

, pink

to nonspecific to specific

. Amorphous, .

granules

T

mRNA.

Vacuoles

Increase with age

T m ,

*The characteristics of specific cells vary.

x m :

■ L m .

Nuclear Characteristics

■ M .

N m -

■ M -

. Im

m .

■ E m m

. C m

m k

. N

.

. P

■ M k .

Chromatin Patterns

Cytoplasmic Characteristics

T m m m -

A m m

. T m

m . T

m m . I ,

m - k -

m m m m . T m

. S

: m

. G

m , , , , m ,

. S

m , k (m). Ex m m-

. Q m

m m .

. V z

. I

■ L m x m m

m m m

, m m m .

Staining Color and Intensity

■ Gm

TW -

m .

mm . I ,

■ M , m

mm k (

m .

)

■ Emm

km . Mmmmm , x m (k-m. Imm

)(x)mm .

k-mm k

zm . Am , m -

Nuclear Shape

k - m

T

. Vmm

; , mm

m .

PART 2 ■ Hematopoiesis and Cellular Maturation

Granulation

. A

T, z, m

--

. I,

. S, (. ., m no nonspeci c speci c m), m m -

.

m k m k -

T, m k m k -

.

, x

In clus ion Bod ie s

. T

k . T

C m A A m m k (C) m m -

C .

m m

G :

. V (C

. I z , m

) k (C)

. I , (azurophilic), (basophilic),

. S m

(eosinophilic)

m W - m ,

. I m

() q

q .

Cyto pla sm ic S h ap e

T m -

. T m m

MA URE BLOOD CELLS IN PERIPHERAL

m m , m , m k .

BLOOD

P m m m

m k m . T m k

I m x m

m m .

m m m m

m m

Qu a nt it y of Cytoplas m

. T m l m , q m m m

. T m k , ,

k , -

x q m. A m

.

m q m m

m k

m.

m W - m

Va cuoliza tion

..

M q

m -

NOTE: This is a good time to complete the end of chapter

. Ex m , z -

Review Questions.

m mm m

Normal Adult Values and Selected Characteristics of Mature Leukocytes in Peripheral TABLE

4.6

Blood

Nuclear

Cytoplasmic

Average

Type

Shape Average Chromatin

Color

Granules

Color of Granules Percentage (%)

Segmented Lobulated

Very clumped Pink

Many

Pink, a few blue

56

neutrophil

Band form

Curved

Moderately

Blue/pink

Many

Pink

3

neutrophil

clumped

Lymphocyte Round

Smooth

Light blue

Few or absent Red

34

Monocyte

Indented or

Lacy

Gray-blue

Many

Dusty blue

4

twisted

Eosinophil

Lobulated

Very clumped Granulated

Many

Orange

2.7

Basophil

Lobulated

Very clumped Granulated

Many

Dark blue

0.3

CHAPTER 4 ■ Hematopoiesis

99

CHAPTER HIGHLIGHTS

■ Bone marrow is the site of hematopoiesis.

■ Hematopoiesis is a continuous process.

■ Hematopoiesis is regulated by cytokines.

■ Hematopoiesis is a highly regulated process.

, m .

. T m m

■ T m

m ,

m m , , m

m , (. . , -

, m .

k m), m m

■ P m m , m -

m , m m .

, m

■ T m m

. P m , -

m m m ,

m m , G

x

P .

m .

■ D m -

HSC m m m -

Secondary Lymphoid Tissues

key AGM.

T cells,

■ HSC (HSC)



macrophages. T cells

, GALT, BALT, KMT

HSC.

, .

T cells. S

■ S, ,



m, m,

m x m.

m.

■ L:

m;

■ H

m. I

m, ,

k z -

m ; mm

z , , m -

.

z , , m , ,

■ B m m mm

m m .

.

■ Em , x m

, m m m

Ce llular Ele me nts o f Bone Marrow

m m m , m m.

M , m

■ A m , m

■

m m m m

m m m

m .

He mato poietic Organs and Tis s ue s

■ S m - -

.

■ T m m -

■ M MAPC . MAPC x

m m , m , , ,

z m , m , k m .

m .

■ T m m m j

■ T m

: m m .

m .

■ E m -

■ T m x m -

mm . E CFU

m m

m

m .

.

■ T HPC HSC m

■ T m m m m m .

m m m

■ T m

m -

m m : m

m . B m m m m

m m .

m .

■ T m ' . I

■ HPC m z m m -

x m . % %

.

m m z

■ E m

.

, z -

■ D , m

, , .

k

E % %

x k .

m .



100

PART 2 ■ Hematopoiesis and Cellular Maturation

■ G z m .

■ H m m z M -

HPC . A k

. I , m

.

x

. M %

Examination of Maturing Blood Cells

% m m .

■ T x m m

■ U k , m m

m m CBC. I ,

m . L m

x m , , .

% % m

R x m m .

.

■ M k q m k

■ T m

m m

m .

. M k

m -

x m .

z N:C .

■ T m k m m m

■ T z m

, , , .

z m . Ex m -

■ m m m .

k m , k -

■ M , m ,

decrease z m .

m . T -

■ T m -

m ,

m N:C

(. . , m -

. T z decreases

m). G ' m -

m . C q , N:C decreases m

z .

m . B m ,

■ O m x- z m-k , m k (:) N:C

m .

. A m , :

: m , x m , m -

Cyto kines

, m k . M m-

■ C k m

q : : N:C .

.

■ T m .

■ C k k , - m -

■ N m

, .

. Im

■ D

m , ,

m , m

. P m

k .

m m .

■ T m k

■ A m m

m , k , m x

m . T

, m , m , -

, , ,

. T m k j m -

q m, z , .

m

.

Mature Blood Cells in Peripheral Blood

■ T lymphocytes

HPC

■ Immature

m

m m m m

.

m m .

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

2. T lymphocytes

A. k — m —

Ontogeny of Hematopoiesis

B. k — m — —

1. m

m

m

C. k — — m

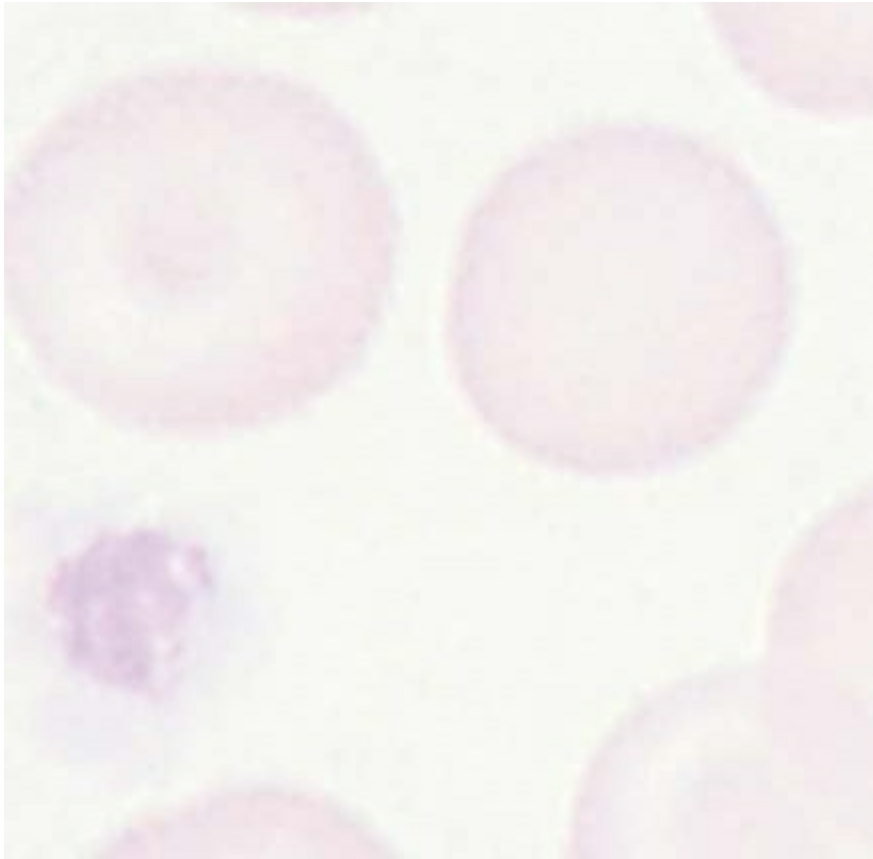
A. m

D. — k — m

B.

C. m m

D.



CHAPTER 4 ■ Hematopoiesis

101

REVIEW QUESTIONS (continued)

3. T m q m ()

*9. W k x m m -

A. m k — m k —m k -

, m m

—m m k — m

_____ .

B. B m k —m k —m m -

A. m m

k — m

B. m

C. m k — m k —m k -

C.

— m

D.

D. m k — m k —m m -

k — m

Cellular Elements o Bone Marrow

10. S m

4. T m q () () A. — — —m -

A. -

— —m

B. m

B. — —m — -

C.

—m

C. m — m — -

D. A B

m m — m m -

— —m

11. T MAPC

D. A C

A. m

B. m

5. T m q m -

C. m m

D. m

A. m — m — m — m -

m — — m

12. E z (-

(PMN)

)

B. m — m — m — m -

A.

m — — m

B.

(PMN)

C.

C. m — m — m — m m -

D.

— — m (PMN)

D. m — m — m m —

*13. E _____

— m (PMN)

m m .

A. % %

Hematopoietic Organs and issues

B. % %

6. T m m () C. % %

A. m

D. > %

B. m

C. m m

*14. D , m

D.

_____ .

A. <

7. B m x m _____

B.

m . .

C.

A. . % . %

D. > k

B. . % %

C. % %

*15. O ,

D. m %

A.

8. B , m

B.

A. k

C.

B.

D. >

C. m

D.

(continued)



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PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

*16. M _____

23. T

m m .

A. mm

A. % %

B. , x m

B. % %

C.

C. % %

D.

D. > %

24. I m k ,

*17. M m

m

G ' _____ m.

A. k k

A. - m

B. k

B. -

C.

C. z

D. m kD. m

Mature Blood Cells in Peripheral Blood

*18. T m k

25. A m

A. m

A.

B. k

B.

C. m , C. , k - m m , m ,

D. m WBC

D.

*19. T m j m

26. A m m

A.

A. HPC

B.

B. HPC

C. , k - m

C. m

D. k –

D.

27. A m m

A.

Examination o Maturing Blood Cells

B.

20. A m , m

C. , k - m

m

D. k –

A.

B.

28. A m

C. m m

A.

B. , k - m

21. A m , -

C. k –

m (N:C) m

D. x m % m k -

A.

B.

C. m m

29. A m

A.

22. m , m ,

B.

m

C. , k - m

A. m m m

D. k -

B. m m

C. m m

CHAPTER 4 ■ Hematopoiesis

103

K MJ, M m N, R MM. O m **COMPANION RES OURCES**

. I : Wintrobe's Clinical Hematology, , . ,

P, PA: W K L W m & W k ,

:// . . m/

: - .

L YG, V Z G. G m-m E W -

, Curr Opin Hematol, (): - , .

m .

M LR, . H m m , , m H ,

. C

-

. Protein Sci, (): - , .

.

M D. H m k , Blood, (): - , .

O z A, O'M DP. B m mm m , Advance, (): - , .

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P J, S GB. H m . I : P J , K k K, L m MA, . (.). Williams Hematology, A AK, L m AH, P S. Cellular and Molecular Immunology,

, N Y k: M G -H .

, P , PA: E /S , .

P k F. m NK . I : G JP, F J, B k U, K G, K S. S m-R GM, . (.). Wintrobe's Clinical Hematology, , , Blood, (): - , .

P , PA: W K H /L W m & B HF. N m , Blood, (): - W k , .

, .

P m k MR, J m D, C m MB. D . I : C JA, M N. E : -

G JP, F J, R GM, . (.). Wintrobe's Clinical

, Blood, (): - , .

Hematology, , P , PA: W K H /

D C, Dz z k E. Em m L W m & W k , .

m , Haematologica, (): - , .

T m J, L F, L k DC. M m m z m -

Dz z k E. O m m m ,

- m , Curr

Curr Opin Hematol, (): - , .

Opin Hematol, (): - , .

F m MD, K k JL, Sk A . Ex m m . I : GS. N m , , m -

H RI, L x SE, S P (.). Blood, , P ,

. oxicol Pathol, (): - , .

PA: L W m & W k , : - .

ML. Immunology and Serology in Laboratory Medicine, ,

H k RJ. H m , Lab Med, (): - , S . L , MO: M , .

.

W ZL, . H m : m K k K. H m m , , k .

, Blood, (): – , .

I : L m MA, B E, KI J, . (.). Williams Z J, Em SG. H m k , Hematology, , N
Y k: M G -H , : – .

mm m . Oncogene, : – , .

Erythrocytes: Erythropoiesis,

Prin

i ci

c p

i l

p e

l s

e

s o

o Bl

B o

l o

o d

o Co

C l

o lle

l c

e t

c io

i n

o an

a d

CHAPTER

Maturation, Membrane Characteristics,

5

Proc

o e

c s

e s

s i

s n

i g

and Metabolic Activities

KEY TERMS

a cidosis

He in z b o d ie s

p o l y c y t h e m i a

a n u c l e a r

h e m e

p r o n o r m o b l a s t

a p o p t o s i s

Howell-Jolly bodies

pernicious

basophilic

hypoxia

relative polycythemia

erythroblastoid islands

intravascular coagulation

reticulocytes

erythroblasts

maturation erythrocyte

rubricast

erythrocytosis

megakaryoblastic maturation

pernicious

erythropoiesis

methemoglobinemia

secondary polycythemia

erythropoietin

polychromatichromoblast

shift reticulocytes

e xtra va scula r ca ta bolism

p o lych ro m a to p h ilia

s tre s s re ticu lo cyte s

LEARNING OUTCOMES

Erythropo iesis

Mem brane characteris tics and m etabo lic

■ Name the sites of erythropoiesis from the early embryonic stage of

activitie s of erythrocytes

development until fully established in adults.

■ Describe the general characteristics, including the physical proper-

■ Name the basic substances necessary for proper erythropoiesis.

ties, of the erythrocyte membrane.

■ Explain the normal condition that stimulates the production of

Summarize the cellular membrane changes that take place at the

erythropoietin.

end of the erythrocytic life span.

Describe how erythropoietin in uences the production of erythrocytes.

Explain the importance of enzymes in energy-yielding cellular

Describe the various types of conditions that can produce disorders

reactions.

of erythropoietin production.

Describe the importance and physiology of the Embden-Meyerhof

General characteristics of maturation and

glycolytic pathway.

Explain the physiology of the oxidative pathway and the effects of

development

a defect in this pathway.

■ List the maturational times for the various erythrocyte developmen—

Explain the importance of the methemoglobin reductase

tal phases.

pathway.

■ Describe the major morphological features of each of the erythro—

Describe the function of the Luebering-Rapoport pathway.

cyte maturational stages.

■ Explain the events that occur during reticulocyte maturation.

Case study

Analyze the patient history, clinical signs and symptoms, and labo-

Disorders related to the erythrocyte maturation

ratory data for the stated case study, answer the related critical

and production

thinking questions, and decide the most likely diagnosis.

- Compare the terms secondary polycythemia and relative polycythemia.

NOTE:

- Compare the morphological characteristics of defective erythrocyte
- indicates MLT and MLS core content

maturation and megaloblastic maturation with normal development—
indicates MLT (optional) and MLS advanced content
tal features.

ERYTHROPOIESIS

x m -

, m m -

H m m m -

m , ,

m k ,

.

104

CHAPTER 5 ■ Erythrocytes: Erythropoiesis, Maturation, Membrane Characteristics, and Metabolic Activities 105

T m -

, f

m .

I m , , m ,

.

m x - x -

. T m , -

Erythro po ietin

, m

. A

T erythropoietin (EPO) m

, m z m z ,

k (% %). EPO -

m m () , ,

, m

.

EPO . P

T m , , -

k . Ex

m m

. T m ,

m m -

m , , m

, , m

. T m

. EPO m m

erythropoiesis. E

.

m m m m B EPO x -

(HSC) m . E

. T , mU/mL

m z z

m x m . N m m -

x .

EPO % m

E -

m m mU/mL. T

, erythroblastic islands. T -

m j -

erythroblasts **reticulocytes**

m z x , m

m .

m m m x

I m

k . hypoxia, x

. A -

, m -

, m

EPO. A m

m . T

x - m m. T m m

mm . m

m m m EPO, m

, m

, m k k

m m ,

k . T

m x m %

EPO, m m - -

. E

, x -

m k -

, m x

.

.

M z I , EPO x . T

mm

m m (m)

m EPO,

. M ,

m -

, m m .

EPO. R ,

C m -

- m

, , ,

m

apoptosis.

m .

x

EPO m mm

x m m

, - m , (CFU-E), m

heme m m , z

.

m . T -

I m m m m -

m m m

, - m ,

() , , m B , m B , (

(BFU-E), - - m

m m m B m x), m

. EPO . C

k . I m ,

m m

m q m

. D -

m m . T m j m m . I -

m

m ,

m m m; x , q -

m m

B -x.

106

PART 2 ■ Hematopoiesis and Cellular Maturation

EPO . T

C EPO m

:

m m , , -

x k . A m

■ A mRNA (m

m m ,

)

m m -

■ D m m m

m , EPO. A EPO

(m)

m x . I ,

■ S m m mm

q EPO .

, , m m

A m (AC H), - m -

■ I x

m (SH), x , -

()

, , , , '- () I m EPO, m m k . D

m

q -

(RNA) k . T

mm . A m (-

x

P IV E D , C)

(DNA) . T m

, m B ,

m

.

-

. A m

, z NOTE: This is a good time to review the de nition of the m . I m -

Key Terms in the Glossary and ash cards on

. It is

m . Q m

also a good time to complete Review Questions related to

m EPO . T

preceding content.

m m m -

.

EPO k - , -m -

General Characteris tics o f Maturatio n and

- m (GM-CSF), k - ,

De ve lopme nt

m - m m

E m . O m -

m k . R m m EPO

(F . .), m

m m mm

. B m -

m

m .

. I

. O , m m , m m

m EPO k

x m . % . %

.

.

Nutritio nal and Re gulato r Facto rs

De ve lopme ntal S tage s

As s o ciated w ith Erythro po ies is

Ea rly Ce lls

A m m

A m m , q -

mm m . B

. T :

m m , , ,

m m . m z -

■ E

, -

■ I

m . A m m z

■ V m

m mm -

■ H m

, ,

. A m ,

Eryt hro poiet in

m , m ,

I EPO secondary m . W z polycythemia. S m m -

z q

x

, , m

x f m ,

m m x m.

m , , EPO

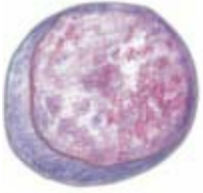
W m , q

. Sm k mm

m ,

.

m m m , m





Characteristics, and Metabolic Activities 107

Pronormoblast (Rubriblast)

Rubriblast (pronormoblast)

Basophilic Normoblast (Porrubricyte)

Porrubricyte

(basophilic normoblast)

Polychromatophilic Normoblast (Rubricyte)

Rubricyte

(polychromatic normoblast)

Orthochromic Normoblast (Metarubricyte)

Metarubricyte

(orthochromic normoblast)

Polychromatophilic Erythrocyte (Reticulocyte)

Reticulocyte

Reticulocyte appearance

with asupravital stain

Mature Red Blood Cell (Mature Erythrocyte)

FIGURE 5.1 E m . (R m A , SC. Anderson's Atlas of Hematology, P , PA: W

Mature erythrocyte

K H /L W m & W k , C , **FIGURE 5.2** E m . T m -

m.)

m m . T

q . I

- m , - -m -

, m

m k (CFU-GEMM)

, , , .

(F . . , C).

I , CFU-GEMM

W x , m BFU-E. T BFU-anuclear m (F . .).

E. L k HSC , BFU-E . M

G

Ru b rib la s t (Prono rm ob la st)

/G .

T x m CFU-E .

T rubriblast (F . .) pronormoblast (.)

CFU-E . M S

m x m μm. T -

. CFU-E

- m (N:C) : . T ,

. U EPO, CFU-E

, k ,

mm m , -

m .

m m . A CFU-E

T m (basophilic)

- , m -

W k . T

G . R

RNA

q m . S

m . I

m m

DNA (. . , m x)

m k .

m q DNA

(. . , m B

Prorubricyte (Basophilic Normoblast)

), m m .

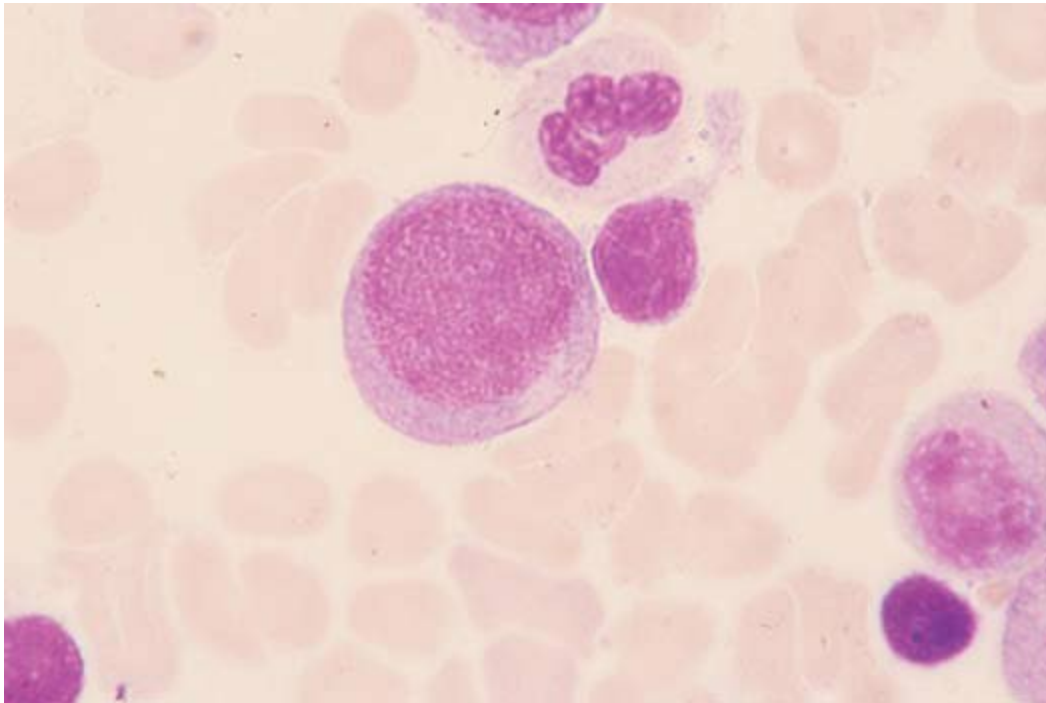
W , m -

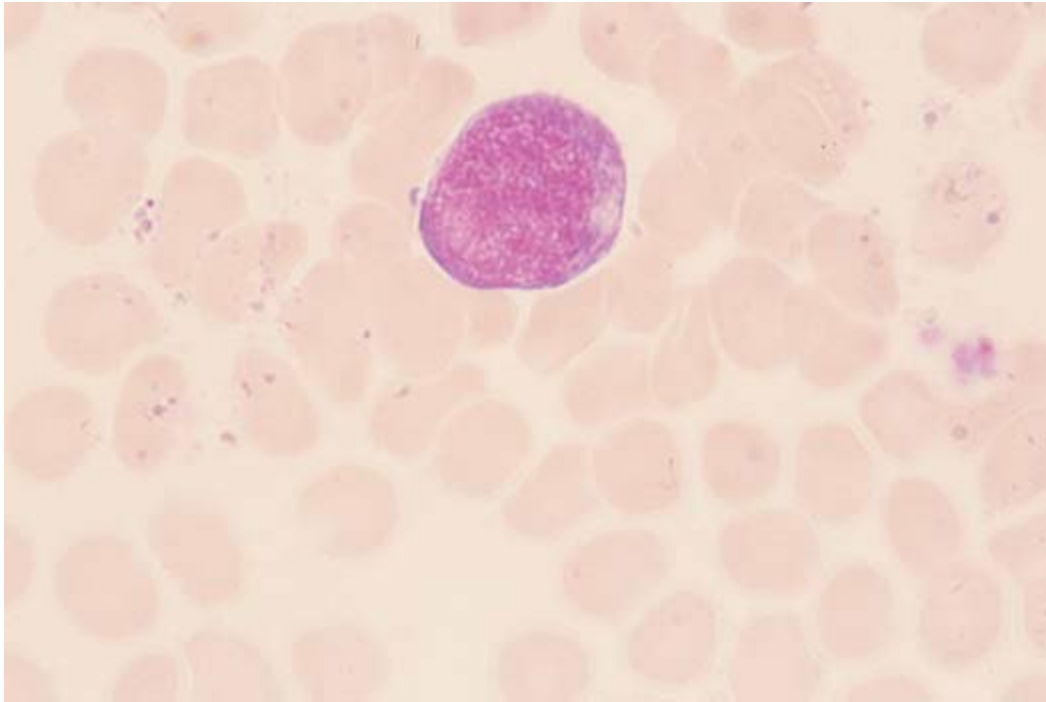
T , prorubricyte (F . .)

m , m μ m

m (C).

m . T N:C





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PART 2 ■ Hematopoiesis and Cellular Maturation

FIGURE 5.3 P m (). (R m **FIGURE 5.4** B m (). (R

A , SC. Anderson's Atlas o Hematology, P , PA:

m A , SC. Anderson's Atlas o Hematology, P ,

W K H /L W m & W k , C

PA: W K H /L W m & W k ,

, m .)

C , m .)

m (:); , m m -

m .

Rubricyte (Polychromatic Normoblast)

Thrombocyte

Hydrophilic

Thrombocyte

, rubricyte (F...) polychromatic normoblast.

W.T

A, z μm

km

m.Fm -

m.

m N:C : .

TABLE

5.1 Dual Nomenclature and Developmental Characteristics of Red Blood Cells Name

Cellular Features

Name

Cellular Features

1. Rubriblast or

Size: 12–19 μm in diameter

3. Rubricyte or polychromatic Size: 11–15 μm in diameter

pronormoblast

N:C ratio 4:1

normoblast (polychromic

N:C ratio 1:1

erythroblast)

Nucleus

Nucleus

Large, round nucleus

Increased clumping of the

Chromatin has a ne pattern

chromatin

0–2 nucleoli

Cytoplasm

Cytoplasm

Color: variable, with pink

Distinctive basophilic color

staining mixed with basophilia

Without granules

2. Prorubricyte or Size: 12–17 μm in diameter

4. Metarubricyte or

Size: 8–12 μm

basophilic

N:C ratio 4:1

orthochromic normoblast

Nucleus

normoblast

(orthochromic erythroblast)

Nucleus

Chromatin pattern is tightly

(basophilic

or nucleated RBC (NRBC)

Nuclear chromatin more

condensed

erythroblast)

clumped

Cytoplasm

Nucleoli usually not apparent

Color: reddish pink

Cytoplasm

(acidophilic)

Distinctive basophilic color

5. Reticulocyte (supravital

Size: 7–10 μm

stain) or polychromatic

Cell is anuclear

erythrocyte (Wright stain)

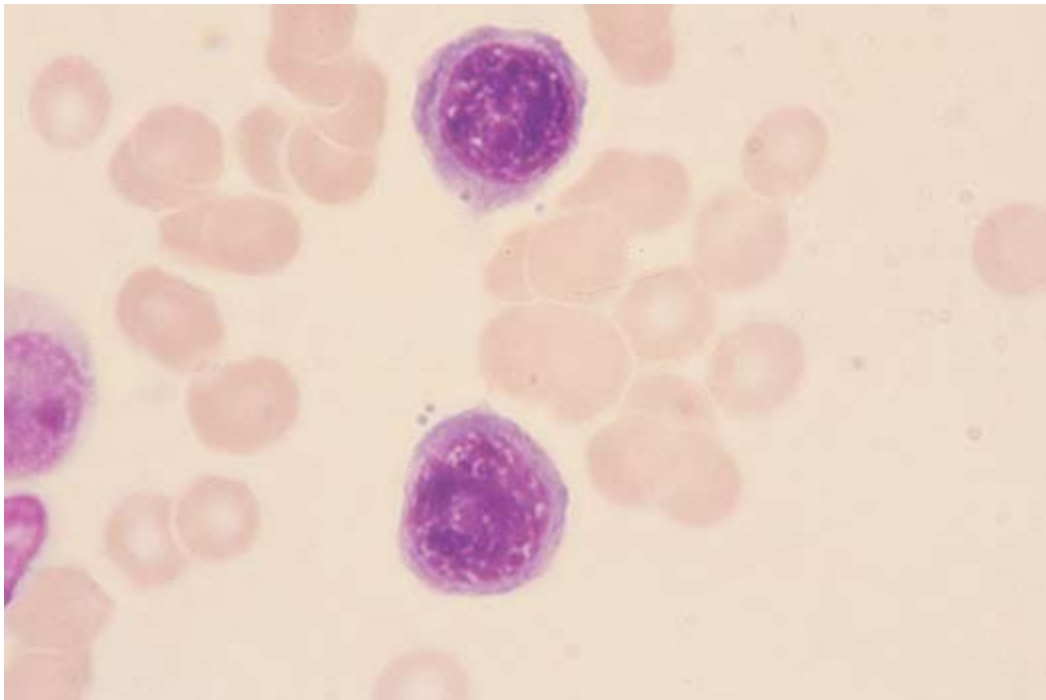
Diffuse reticulum

Cytoplasm

Overall blue appearance

6. Erythrocyte

Average diameter: 6–8 μm



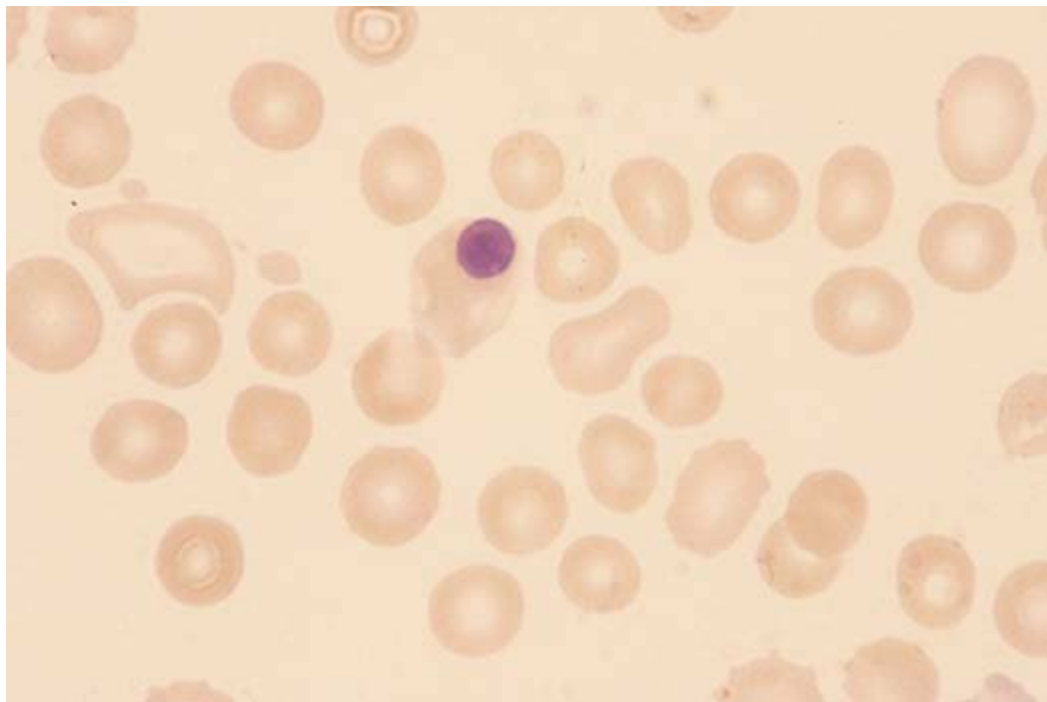
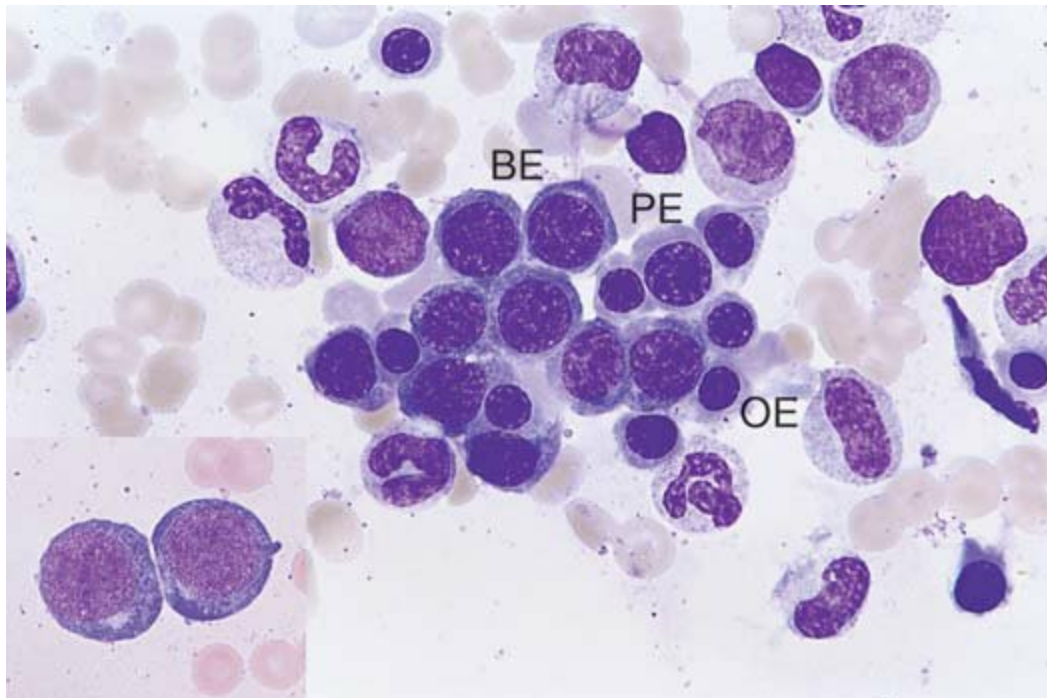


FIGURE 5.5 P m m ().

FIGURE 5.7 E m **BE** , (R m A , SC. Anderson's Atlas o Hematology, **PE** m , **OE** m .

P , PA: W K H /L W m & (R m H RI, L x SE, S P. Blood: Principles W k , C , m .) and Practice o Hematology, , P , PA: L

W m & W k , , m .)

T m m m .

T m m

- -

k m x ;

m . m

m , .

. A ,

m .

Met a rubricyte (Ort ho ch rom ic Norm oblas t)

Re t iculocyte

T m m (F . .) m m . T m (

T x m . P

μm). T m

m ,

m k (

k (F . .).

m). I ,

x m . T m z **Re ticulo cytes**

(- k) m. T

A , m m q m

m . A

(F . . .). T m

, mm () m

m -

. D m -

, mm

.

A k ,

, m , x

m m . T m m

m ;

, RNA , m -

. D m , RNA

z , m . T -

m m , m z

, m k m

m .

U m , q

m q

. m

FIGURE 5.6 O m m (m).

, m m-

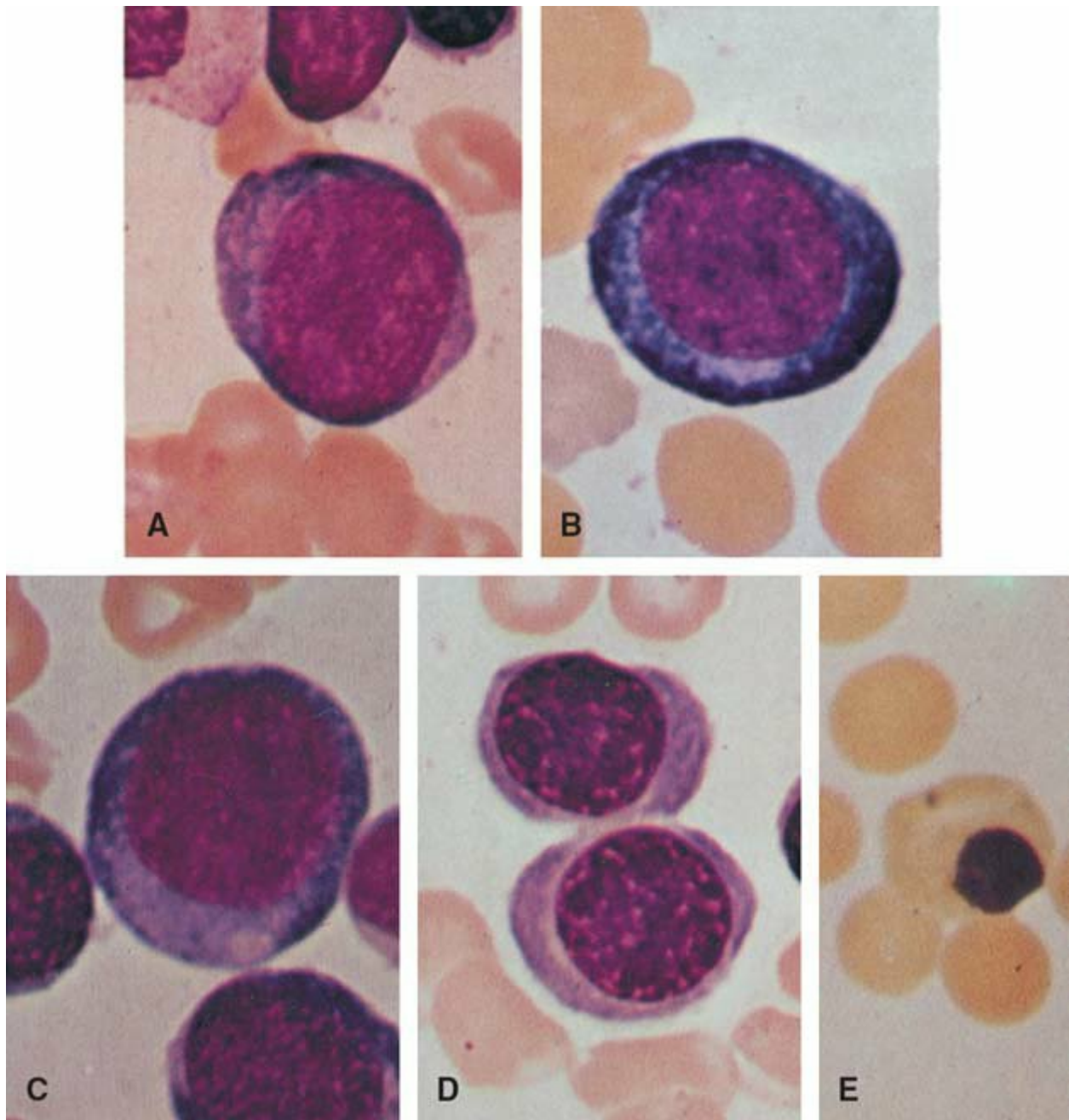
(R m A , SC. Anderson's Atlas o Hematology,

P , PA: W K H /L W m &

.

W k , C , m .)

B m m ,



110

PART 2 ■ Hematopoiesis and Cellular Maturation

FIGURE 5.8 E m . RBC m /

m . P m A ; m -

B ; C D m

m ; m m

E . M , , × ; W . (R

m G JP (). Wintrobe's Clinical Hematology,

, P , PA: L W m & W k ,

, m .)

/ m , stress reticulocytes shift q m

reticulocytes. T

.

mm k

I , m EPO, m

.

m m

T m -

m , m RNA

FIGURE 5.9 C m , m 18

Blood he moglobin

14

, , -

conce ntra tion

m . T ’

To

16

Tota l body

12

t

. D k , m -

a

L

n

i

l

d

/

he moglobin

b

b

m

g

o

o

l

(g

d

n

, ·

g

14

10

y

r

o

o

i

a

t

h

T m m m m

m

a

e

er

ms

t

h

k z

)

n

o

de

12

8

g

m m . A k , m

c

o

l

n

o

o

b

,

Bl

co

10

6

i

n

m . T

m m ,

, z x m

)

m m m . (R m M G, 10

Re

s

Re ticulocyte count

3000

m

t

. Avery's Neonatology Pathophysiology and Management o the

i

a

c

r

u

g

Newborn, , P , PA: L W m & W k , (

lo

6

t

c

, m .)

h

2000

y

g

t

i

e

e

s

w

2

%

y

1000

d

Bo

0

2

4

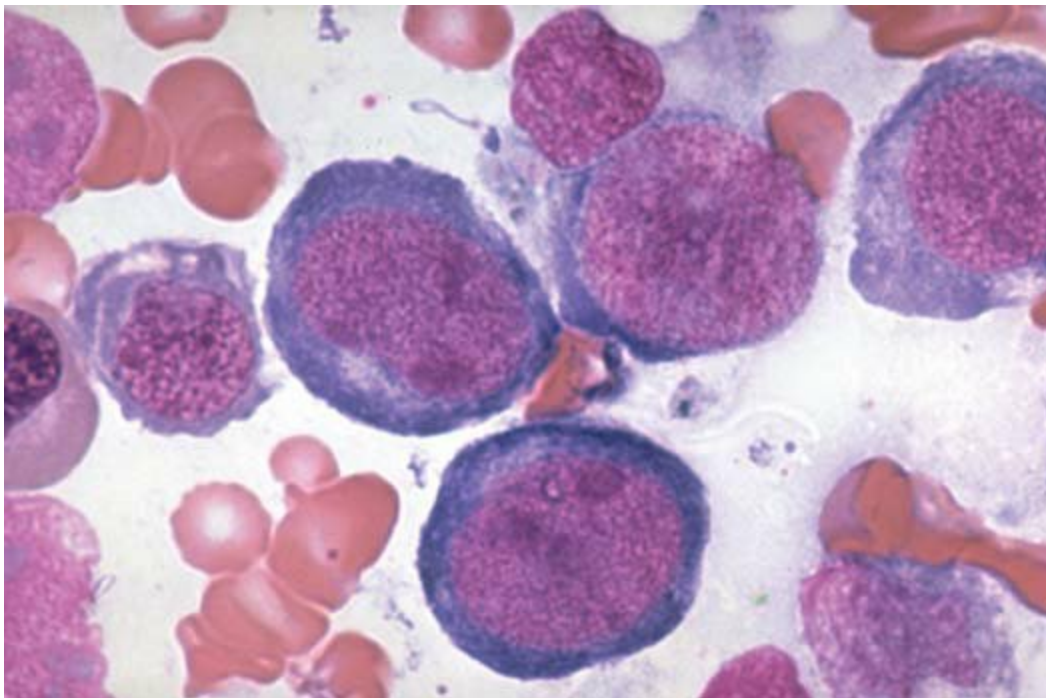
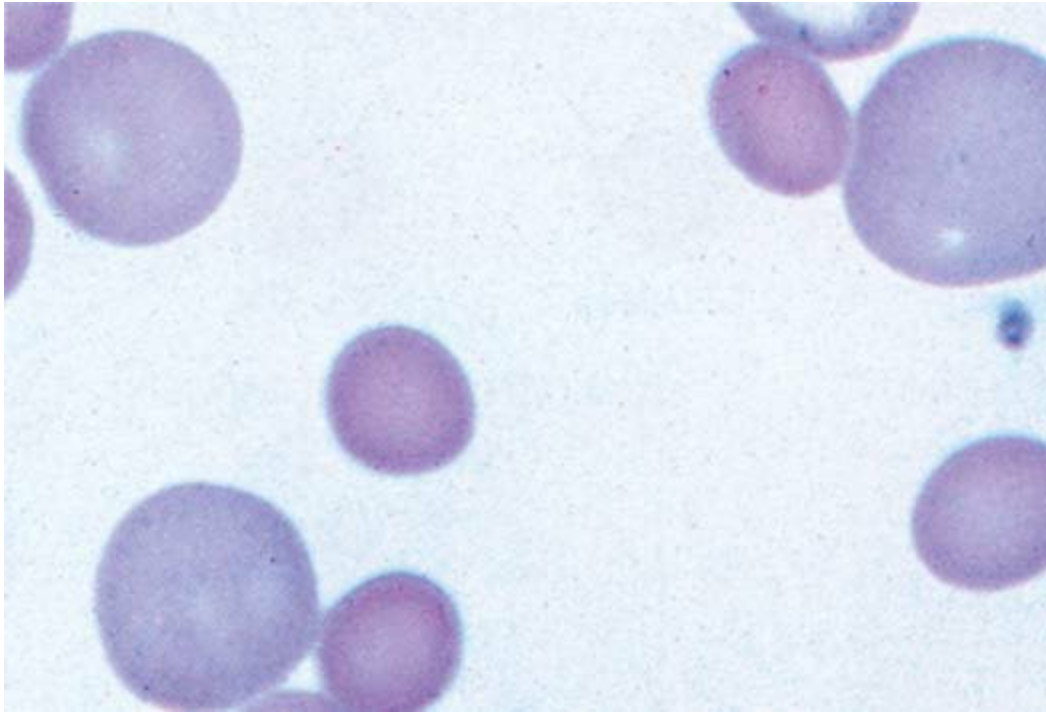
6

8

10

12

Time (we e ks)



Sm k mm .

C

m m m , ,

x k .

A m

m m , m m

. A

m x . I

, q -

.

Re d Ce ll Incre as e s

I m

EPO . T

FIGURE 5.10 P m . (R m A , SC.

relative polycythemias.

Anderson's Atlas o Hematology, P , PA: W K

A m x

H /L W m & W k , C , k m (m) m .)

m m . T -

m . I k RBC

, m . I W -

m -

m , m RNA m m .

, poly—

L m m

chromatophilia (F . .). I m

, , -

W -

.

m ,

. T

De fe ctive Nuclear Maturatio n

m m μ m. T

A m k

.

megaloblastic maturation

(F . .) m , m Ma ture Erythrocyte

B (C). T m -

m -

A , mature erythrocyte m .

m m . B

T m μ m. T -

m z DNA,

m -

m .

m m (C). A

T m

m m (C).

m m

NOTE: This is a good time to complete Review Questions related to preceding content.

DISORDERS RELATED TO ERYTHROCYTE

MAURADION AND PRODUCTION

Disorders of Erythropoietin

Polycythemia m -

(erythrocytosis)

m . S -

m EPO

not m (C) m .

S m -

FIGURE 5.11 M m . A m m m x m B (m)

x -

m m . (R m m , m , R E, F JL. Pathology, , P , PA: L

, .

W m & W k , , m .)

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PART 2 ■ Hematopoiesis and Cellular Maturation

k m ,

x m m . O m

Metabolic Pathw ays in the

TABLE

5.2

m m

Erythrocyte

m m

z . T m

Me tabo lic Pathw ay

Function

x m k . G -

k m q

Embden-Meyerhof pathway Maintains cellular energy by
m .

generating ATP

Oxidative pathway or

Prevents denaturation of globin

hexose monophosphate

of the hemoglobin molecule by

NOTE: This is a good time to complete Review Questions
shunt

oxidation

related to the preceding content.

Methemoglobin reductase Prevents oxidation of heme
pathway

iron

MEMBRANE CHARACTERISTICS

Luebering-Rapoport

Regulates oxygen affinity of

AND METABOLIC ACTIVITIES OF

pathway

hemoglobin

ERY HROCY ES

ATP, adenosine triphosphate.

T m

x -

m

. A m m m

m j Em -M -

. T m -

m , -

m z m k m -

m m . T

x m m.

Em -M -

E (F . .) m

(A P). T

m x k . T

m -

m m , -DPG

, L -R . I ,

H₂O₂

H₂O

x m -

m

Gluta thione

m m x . A

pathway

Glucos e

(.)

x m

NADP

NADP H

q .

G-6-P

Pe ntos e

CO

F-6-P

2

Hb

phos pha te

NAD

Ga -3-P

pa thway

Ribos e

Mem brane Characteris tics

phos pha te

MHb

s

NADH

i

Re t iculocyte Mem bra n e

ys

l

o

R m m -

yc

l

. E

G

2 ADP

Ra poport—

m -

Lue be ring

2,3 DP G

. A x , mm

s hunt

2 ATP

m

. V m , x

mm ,

m - - x m x m La cta te

. D - -

FIGURE 5.12 E m m . M -

, m m m

x ; m j

z -

x . ADP, ; A P,

.

; , -DPG, , - ; F- -P,

D m m ,

-- ; G- -P, -- ; G - -P, -

m m z . I k

- ; H , m ; MH , m m -

m m m m

; NAD, NADH, m , NADP,

. T m m m -

NADPH, m . (A m G JP (). Wintrobe's Clinical Hematology, , m m . M j -

P , PA: L W m & W k , : , z m m m F . , m .)

RBC m m

CHAPTER 5 ■ Erythrocytes: Erythropoiesis, Maturation, Membrane Characteristics, and Metabolic Activities 113

m . R

m m m . B . R -

m m m . T -

z actin. P

m m

m j k . . -

m m . T

k k . D

m -

k

m .

m (C).

T m -

M m m -

m x

, m

m m m . T

. A , A B

:

R , m m .

A m m

. I

. S m m m m , x m-

. L (%) m m-

, CD , L , -

m (ICAM-),

. A q

.

O m mm -

C m m

m -

m (.). T :

. I , mm

. W , q

x ,

. G (GLO I GLU), m/

m .

x

. N -K-A P

NOTE: This is a good time to complete Review Questions
related to the preceding content.

Examples of Transporter

TABLE

5.3

Membrane Proteins and Functions

Ma ture Red Blo od Ce ll Me m bra ne

T m m x m %

Prote in

Function

, m ,

; x m %

Aquaporin 1

Water transporter

k . G

Band 3

Anion transporter, support

m -

Anion exchanger 1

system for surface antigens of

x m % , m .

ABH blood group antigens

T m

Duffy blood group antigens Ca²⁺ transporter

m m x m m-x m . T . I m -

GLUT1

Glucose transporter, supports

(m C).

ABH blood group antigens

R m

Glycophorin A

Transports negatively charged

m . T m x -

sialic acid, supports MN blood

m m x .

group antigens

T m m m

Glycophorin B

Transports negatively charged

m m k

sialic acid, supports Ss blood

m m k -

group antigens

.

Glycophorin C

Transports negatively charged

T m m m k .

sialic acid, supports Gerbich

D m m - k k blood group system antigens , m

, z z

ICAM 4

Integrin adhesion

m m m . T m j m m m

Kell blood group antigens

Zn²⁺ binding endopeptidase,

k α - β - , . T

supports Kel blood group

m , , m

antigens

x. S m z m k

Kidd blood group antigens

Urea transporter

x m m ankyrin. A k

Rh blood group antigens

Supports D and CcEe blood

m m “band 3”

group antigens

anion exchanger protein. T band 4.2 m

z k k x .

Rh antigen expression

Supports DCcEe antigen

S k m m gly-protein

expression, gas transporter

cophorin C k band 4.1. T m k

(probably CO₂)

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PART 2 ■ Hematopoiesis and Cellular Maturation

T m m m

Metabolic Activities

m H

. C

Em bde n-Meyerhof Pat hw ay

A P m -

T m j

. x m m :

. I k m

k

, A P m x

.

, A P .

T A P - -

Aging Red Blood Cell Membrane

m'

A - RBC membrane. PMCA, membrane, membrane C + (PMCA) membrane, H₂A,

membrane-membrane x. T

membrane k membrane RBC. A -

A P membrane -

x PMCA H₂A

: membrane

RBC. PMCA RBC. T

A P

RBC membrane. A

membrane

RBC, membrane

A P. T

C + k RBC.

Em -M membrane -

membrane x -

Cytoplasmic Characteristics

.

I membrane, membrane

Oxidative Pathway or Hexose Monophosphate

max -

Shunt

m, , m

Tmax

, zm . TEm -M

NADP (m

xm % ' . Ef

) NADPH (m NADP),

mm - zm . T

qq . T '

zm zm

x -

f :

. W , m

mfzx . T

■ Mm (F +)

, -

Heinz bodies (C).

■ Dmm

C H z m z m (N +) m (K+) m

. A m m , m

■ M , z m , m .

m m

■ P m m

Me t he m og lob in Re duct a s e Pa th w ay

I m k q ,

T m m m

m .

m Em -

D m m :

M

k m . A x -

. F f ,

, x m , m

m m x

m

. I f - z m

m x , m m

m (NADH), -

x m .

m

I m m

(NADPH), A P

q m m , x

T m mm z m ,

x m m m .

Em -M , -

M m m x

k . I ,

(, F +) m (, F +) k , - z m -

m. I m, m m

, m . T

x ; , x

m ,

m . M m

. A -

(F +) q

- (G PD) m

NADH (m m -

NADPH, x -

), Em -M ,

m .

z m m m . I

CHAPTER 5 ■ Erythrocytes: Erythropoiesis, Maturation, Membrane Characteristics, and Metabolic Activities 115

, % m x z

m ,

% % m m

.

. N f k

E m , , m -

m m . A

extravascular catabolism and intravascular

m m m

catabolism. Ex m m j -

m m ; , -

() m m

m m

m . T

x .

m

Methemoglobinemia—

m (Howell-Jolly bodies)

m , m

(C)

x z — m

m .

m NAD

. T m q .

NOTE: This is a good time to complete the end of chapter

Lue be ring-Ra po port Pat hw ay

Review Questions.

T m x -

. B ,

- m m x

CHAP ER HIGHLIGHT S

x

Erythro po ies is

x - . T L -

R m m , -DPG.

■ T m , E DPG m m x -

m . H m ' ,

x ,

m j , x x .

x .

■ E RBC ,

R . W

k m m -

x , -

m m .

x x m m

■ E , z m

. A x m

k , x .

(x m)

DPG m . T

General Characteris tics o f Maturatio n and

m m H -

De ve lopme nt

q DPG A P. A DPG A P

■ A m , m

x .

m m : -

I acidosis, ,

, , , m , -

x , , -DPG j f -

m . R

m z x . I k -

m

, k .

.

Aging Erythrocyte s

Dis o rders Related to Erythro cyte Maturation

Ex m -

and Production

m , m

■ D m

, ,

.

m . B – -

A , :

-

. T m m m x .

m m .

. T m .

■ D m , x m , m -

. E z m , , m .

m .

W , Mem brane Characteris tics and Metabo lic m m -

Activities o f Erythro cytes

z . T m

m . B

■ T m -

m ,

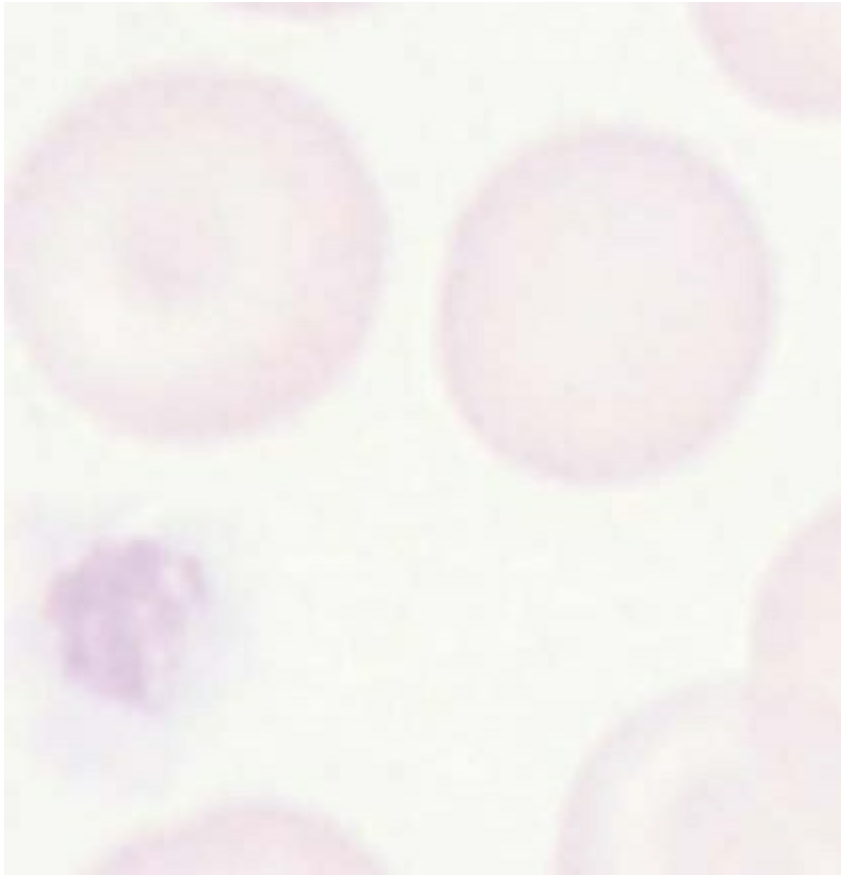
.

m m . T x

■ E m m x -

. I

Em -M





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PART 2 ■ Hematopoiesis and Cellular Maturation

m , x m , x

■ A , m m m x-

, x m .

(m m m), m m-

■ I x x , -

; m ;

, , H z

z m , , m .

.

W ,

■ T m m

m m

x m .

z .

■ T L -R m x -

- .

CASE STUDY

Cas e Study 5.1

A - - m

■ Critical Thinking Group Discussion Questions:

ABO-m m

. W m ?

. S ,

m .

. W q m

?

■ Laboratory Data

RBC . \times /L

H m %

NOTE: This is a good time to write out the answers to the

H m . / L

Critical Thinking Group Discussion Questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

4. S m

A. x

Erythropoiesis

B. m

1. T m

C. mm

D.

A. k , m ,

B. k , , m

5. T m q

C. m , , k

A. , , m ,

D. , k , m

-

B. , , , m -

2. W () ()

?

C. m , m , -

A. G

m m , m m ,

B. S

C. S k

D. B C

D. A

6. W mm

3. W

m : μm

?

m , N:C : , ,

A. P m .

m?

B. G m m .

A. R (m)

C. M .

B. R

D. C .

C. M (m m)

D. P (m)

CHAPTER 5 ■ Erythrocytes: Erythropoiesis, Maturation, Membrane Characteristics, and Metabolic Activities 117

REVIEW QUESTIONS (continued)

7. T - k -

*14. R m x

m m

A.

A. (m m)

B. m x

B. m ()

C. m m

C. m (m m)

D. m m

D. B C

15. W () () m -

8. W m , m

m ?

A. C m k m

A.

m .

B. .

B. N m m

C.

m .

D.

C. C m m

m .

General Characteristics o Maturation and Development

D. E m m .

9. I W - m, -

. T

Membrane Characteristics and Metabolic Activities o

A. m m

Erythrocytes

B. m

*16. I m , -DPG _____ x f -

C. m

m m .

D.

A. I

B. D

10. I m , C. D

A. m m m

B. RNA z m

17. A m m x -

C. m z

m , m

D. B C

A. x , -DPG

B. x f

Disorders Related to Erythrocyte Maturation and

C. m x

Production

D. m x

*11. I

m

*18. T m m

A.

B. m k

A. RNA

C. m

B. m

D.

C. m x m m

D. DNA

Questions 12 and 13 Re er to Case Study 5.1

*19. W m ()

*12. W m x

m ?

?

A. H m

A. S

B. H m

B. S

C. C z m

C. W

D. D

D. B A C

*13. W ' m ?

*20. T Em -M _____

A. C

% ' .

B. A

A.

C. L k q EPO m

B.

D. H

C.

D.

(continued)



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PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

*21. T Em -M A P -

*27. m m m

-

, m m

A. m m m

m (+) ?

B. m m-A. m B

m m m x

B. , -DPG

C. x

C.

m m

D. NADH

D.

*28. W H z

*22. T Em -M

?

m m

A. m

A.

B. D m - x f

B.

C. I

C. - -

D. D A P

D.

*29. T L -R

*23. T A P Em -M -

A. m m , -DPG

B. m

A.

C.

B.

D.

C.

D.

*30. I ,

A.

*24. T m mm z m

B. x

Em -M

C. H

D.

A. A P

B. k

Aging Erythrocytes

C. - -

31. A ,

D.

A. m m m m x

m m

*25. I x (x m -

B. m

), ?

C. z m , ,

A. I f m

D. m m -

B. D

.

C. P H z

D. A

32. Ex

A. m

*26. T m m

B. m

C. m

A. x m

D. m

B. m m m

C.

D.

BIBLIOGRAPHY

COMPANION RES OURCES

L VL, . E - m m

:// . . m/

m m , Blood, E W -

(): - , .

m .

L J, . M m m m , Blood, (): - , .

H ,

U EF, . E - m - m -

-

, N Engl J Med, (): - , .

.

W MJ, S CO. C , Blood, (): - , .

CHAPTER

Erythrocytes: Hemoglobin

6

KEY TERMS

b iliru b in

h e m o g l o b i n e m i a

s i d e r o b l a s t s

c a r b o x y h e m o g l o b i n

h e m o g l o b i n o p a t h i e s

s u l f h e m o g l o b i n

c o n j u g a t e d b i l i r u b i n

h e m o s i d e r i n u r i a

u n c o n j u g a t e d b i l i r u b i n

g l y c o s y l a t e d h e m o g l o b i n

H o w e l l - J o l l y b o d i e s

u r o b i l i n o g e n

h a p t o g l o b i n

K l e i n a u e r - B e t k e

H e i n z b o d i e s

m e t h e m o g l o b i n

LEARNING OUTCOMES

Characteristics of hemoglobin

Describe the physiological event that produces dark-staining gran-

■ Explain the genetic inheritance of hemoglobin.

ules when examining a peripheral blood smear.

- Describe the chemical composition and conformation of a normal

Associate the appearance of dense, dark-staining particles in adult hemoglobin molecule.

a Wright-stained peripheral blood smear with a specific medical diagnosis.

Hemoglobin function

- Define the term porphyria and name three categories of classification—

Explain the physiological role of the regulator of hemoglobin function of porphyrias.

2,3-diphosphoglycerate (2,3-DPG) or 2,3-bisphosphoglycerate (2,3-BPG) in the oxygenation of the hemoglobin molecule.

Ontogeny of hemoglobin

- Name the embryonic hemoglobins and describe their chemical composition and site of formation.

Oxygen Dissociation and Alterations

position and site of formation.

Interpret the oxygen dissociation curve to the oxygen-binding activities

- Explain the types of chains, developmental formation, and quantities of the hemoglobin molecule, when given specific information

ties of fetal hemoglobin.

such as pH, temperature, CO₂, and fetal hemoglobin concentration.

- Identify the types of chains, site of formation, and quantities of adult

Cite at least two examples of clinical conditions that can alter oxyhemoglobin A and A2.

gen dissociation, and explain what effect these conditions have on

Describe the formation and concentration of glycosylated hemoglo—
the oxygen dissociation curve.

bin in normal and hyperglycemic environments.

Describe the Bohr effect and other physical or chemical factors that

Contrast the differences in oxygen affinity of embryonic, fetal, and
affect the oxygen dissociation curve.

adult hemoglobin and associate the differences to the structure of

Carbon Dioxide Transport

the molecule.

- Explain the elimination and transport of carbon dioxide.

Variant forms of normal hemoglobin

Biosynthesis of hemoglobin

- Name three variant forms of hemoglobin.
- Describe the difference between normal hemoglobin and variant

Prepare a flow chart to explain the overall molecular synthesis of
types of hemoglobin.

heme in the cytoplasm and mitochondria.

Describe the physiology of carbon monoxide poisoning.

Describe the sites and mechanism of transport and insertion of iron

Describe the formation of Heinz bodies.

in the production of hemoglobin.

Explain the etiology and physiology of methemoglobin.

■ Explain the factors that regulate the synthesis of globin in hemoglobin production.

Abnormal hemoglobin molecules

■ Specifically describe the outcomes of a deficiency in the production

■ Name an example of an abnormal hemoglobin molecule.

of globin.

Compare the characteristics of hemoglobin S and hemoglobin C.

Disorders related to hemoglobin biosynthesis

Assess the oxygen affinity of abnormal, acquired hemoglobins and explain how this affects oxygen transport.

Cite an example of an acquired disorder of heme synthesis and describe two points of impairment of hemoglobin biosynthesis.

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Analysis of hemoglobin

■ Explain the overall impact of intravascular destruction in normal

■ Name at least four laboratory techniques for studying hemoglobin.

erythrocyte physiology.

Describe at least four hemoglobin analysis methods and explain the

■ Name the three products of renal processing of filtered

purpose of each procedure.

hemoglobin.

Explain the mechanism of intravascular erythrocyte destruction and

Catabolism of erythrocytes

the subsequent products of catabolism of hemoglobin.

■ Name the three major components of a disassembled hemoglobin

molecule.

NOTE:

Explain the mechanism of extravascular erythrocyte destruction and

■ Indicates MLT and MLS core content

the subsequent products of catabolism and recycling of hemoglobin

Indicates MLT (optional) and MLS advanced content

components.

CHARACTERISTICS OF HEMOGLOBIN

β_2

β_1

I, F x S m -

. H m m m -

. F ,

m x . ,

m x k m-x .

Genetic Inheritance of Hemoglobin

N m m A

M ' L I . T

A/A. A m m

m m ; $\alpha 2$

$\alpha 1$

x m . M

FIGURE 6.1 S m . (R m m m -

P CM. Pathophysiology Concepts o Altered Health States,

m

, P , PA: L W m & W k , ,

. D **hemoglobinopathies** (

m .)

C) m -

m .

m m .

T m -

x m m , -

Chem ical Com po s ition and Co n g uration

x . M

o f Hem og lobin

m

N m m (m A) (F . .) -

m m m

m

m .

m . T -

I m , x .

z . E

m (F . .). N m

HEMOGLOBIN FUNC ION

m m

m . T

T R , -D , -B

q m k m

T m j m m

m m -

x . T x f

m .

m m -

I m m , m m -

m m

, , -

. E

, - (, -DPG , -BPG)

m , m -

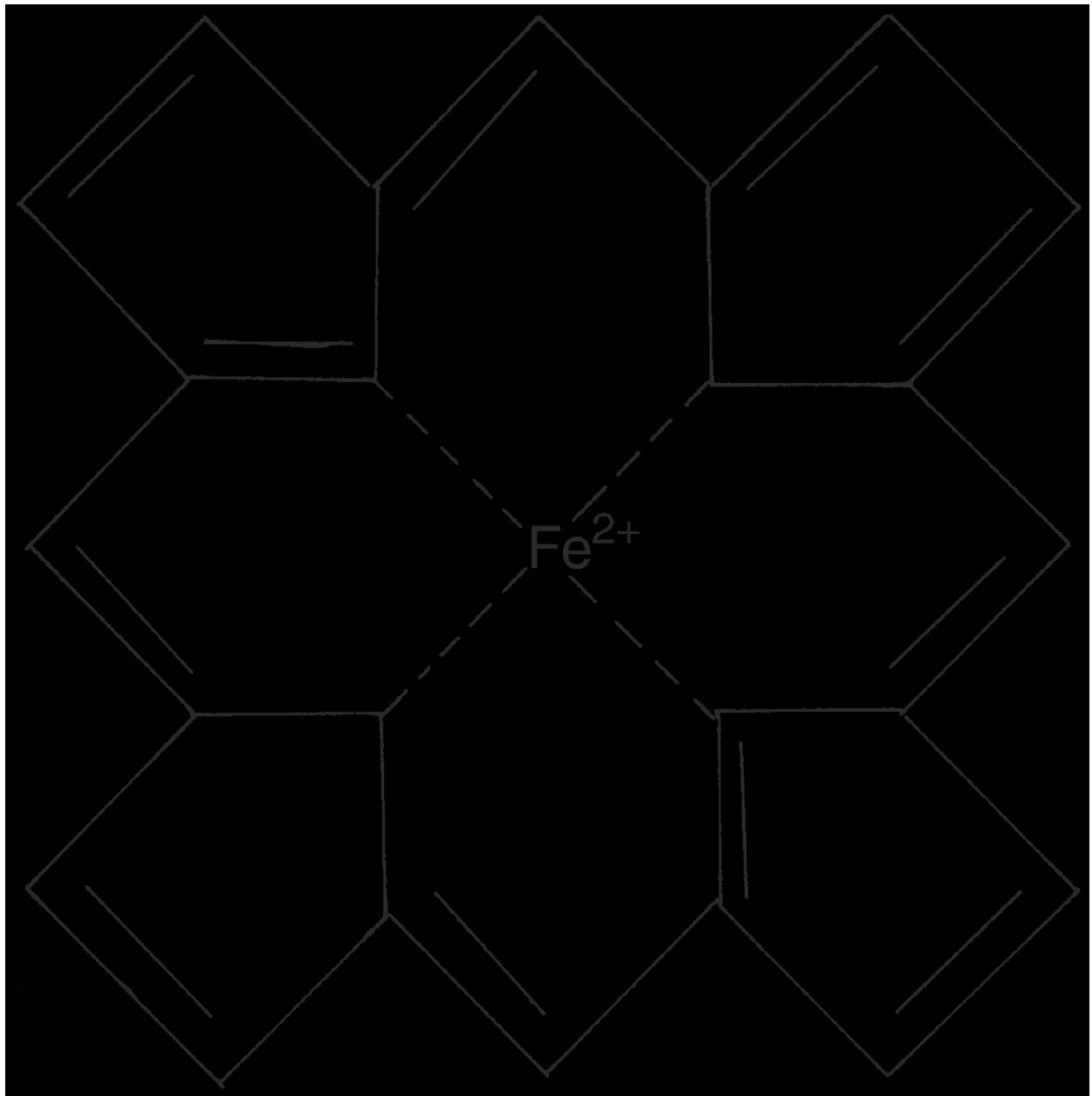
. T m , -DPG

m . I

m (x m) x

m m x ,

f m x. B , , -DPG m



CHAPTER 6 ■ Erythrocytes: Hemoglobin

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x . C x f m

m

x .

Oxygen Dis s ociatio n

T x m

(F . .)

x () -

x (PO). F m , -

x P . T

P x q m , x -

m (m) q

FIGURE 6.2 T m m m

x m (x m)

(F +) m j

. A m m m m m -

H m . I m , P

, m .

. mm H -

H . m . °C. A

x m m m -

x f m

' f x (F . .).

, x f

W m x -

.

, . T m

I , -DPG, x -

, -DPG m

x .

. T

f m x . W x k

, q k ;

O2 Affinity

, x , -DPG; f

O2 Re le a s e

80

m m x .

n

I x , x m m m -

o

i

t

a

O₂ Affinity

, m x m

60

r

u

O

t

2 Re le a s e

. T

a

S

m , -DPG, x f **b**

40

H

m m . I x ,

2

O

, -DPG m , -

%

DPG f m

20

m x .

“P 50” va lue s

20

40

60

80

OXYGEN DISSOCIA ION AND

PO₂ torr

AL ERA IONS

FIGURE 6.4 T x m .

T x % x (P) x -

T m m m k

m . A , x f -

m

m , m x

x . W ,

. A H m

f m x . (R m M G,

. Avery's Neonatology Pathophysiology and Management o the

O2

O2

Newborn, , P , PA: L W m & W k , α

α

α

α

, m .) N : T green line m

x . T blue line

H⁺ (H), CO , m -

β

D

β

, , -DPG. A

P

m F m x O2

O

G

β

β

2

f m . M m -

m x -

f , x

x . T orange line

Oxyhe moglobin

De oxyhe moglobin

H⁺ (H), CO , m ,

FIGURE 6.3 H m m .

, -DPG (, -BPG).

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PART 2 ■ Hematopoiesis and Cellular Maturation

Ox m x m

m x . O m

- m . I x , m

m q m m m

z

x x m tense () structure . I x m , m m .

k , m m

A m m m -

relaxed (R) structure . T

m x . D

m m m

, m m m

R q

m - m . T

m x . T

m ζ-k-

- x - f m R -

m - m m -

- x m .

m α- .

Ox m m

T m -m m m -

f x m

; k

. T q m

ζ -x β -m x m . T

β (H H). C m

x m ,

k m m x

- m . T

m m .

x -

T m H P -

m , m

$(\zeta \beta) < H P - (\zeta \gamma) H G - (\zeta \varepsilon) < H$

. T , -DPG

G - $(\alpha \varepsilon) < H F < H F (\alpha \gamma) < H A (\alpha \delta)$, , x m m , x m m . T m f , x m .

m m -m m -

T m m

m x (m \rightarrow

m m m x

\rightarrow).

.

Oxygen Dissociation Curve

Oxygen Alterations

Ox m

F m (m F) f

(x f)

x (B x .). T f x

H (B), m ,

-

x m .

x x . T x -

A H

,

x

, -DPG x

m m x . T

f m F.

x m .

T x f m -

Genetic Hemoglobin Abnormalities

m m .

H m m

Ox m x m .

x . T

B x m m k

m m m m

x m , k H m

, , -DPG . T ,

, m f x . I m-m , m x m BOX 6.1

m , -DPG f x .

CARBON DIOXIDE TRANSPORT

Adjustments in Hemoglobin-O₂ Affinity

T m -

Hemoglobin F

Increase in O₂

x m

. C x

Altitude

Decreased PO₂

m m . T m m

Plasma O₂

Increased O₂ to tissues

m .

Increased 2,3-DPG

I m m m, x m -

Exercise

CO₂ and acid H⁺ and heat

m x , x

Produces O₂ from tissues

, z z m -

, m .

CHAPTER 6 ■ Erythrocytes: Hemoglobin

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H O + CO

→ H CO

Form atio n o f Hem e fro m Po rphyrin

H m (F . .) m x T k -

m . O ,

x m , k

m m m m (-

m .

) . H m -

H CO → H + + H CO-

m m

m .

F

T m m x

m x m (C -) -

m z m . T m -

. T .

(F . .) -C A

B k m . I m - m . A m , k m III, x m

m . I z m ,

.

IX

A x m x-m .

x

T m , m m. I m m, x m -

m m , -

x . T x—

z m A (C A) . A

m

m , m m -

m m m .

x - m

T m m

(ALA). T

x m m . T z

m q m B

x m x f . A x m

. T m m -

m

% x m

-ALA, z z m ALA -

.

. T z m

x

BIOSYN HESIS OF HEMOGLOBIN

(m B).

F m -ALA m -

S m m

, m.

m m : A, A , F, m

m ALA m m

m . N m m A m

(PBG). T z

m . T A/A. I

z m ALA . F m PBG

- m -

m I III.

: .

T III m , -

G m

III , .

m

T , m ,

m m m . T

m

m . O -

m m . F x

k m k m .

(F +)

T m j m m m -

z m m . T m

. H m z m -

m m , m

m . A x m % m

m

m z x ,

.

m % z -

. T m m -

, m , m (.). V

The Role of Iron in Hemoglobin Synthesis

m .

I , m m T

x



k .



I m m .



I k .



I k m .

■ m k

M m m m



; m m

■ m (x m , m -

m m x

m , m)

m . T , m ,

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PART 2 ■ Hematopoiesis and Cellular Maturation

TABLE

6.1

Value s in the Meas urem ent o f Erythrocyte s

No rm al Adult Value s

Me n

Wom e n

Conve ntional Units

SI

Conve ntional Units

SI

Hematocrit

Packed cell volume

41.5%–50.4%

0.415–0.504 L/L

36%–45%

0.36–0.45 L/L

Erythrocyte count

$4.5\text{--}5.9 \times 10^6/\mu\text{L}$

$4.5\text{--}5.9 \times 10^{12}/\text{L}$

$4.5\text{--}5.1 \times 10^6/\mu\text{L}$

4.5–5.1 × 10¹²/L

Hemoglobin

Concentration

14.0–18.0 g/dL

145–180 g/L

12.0–16.0 g/dL

120–160 g/L

Normal value of MCV

80–96

80–96 fL

80–96

80–96 fL

Normal value of MCH

27.5–33.2

27.5–33.2 pg

27.5–33.2

27.5–33.2 pg

Normal value of MCHC

33.4%–35.6%

32–36 g/dL

33.4%–35.6%

32–36 g/dL

Representative Average Pediatric Values

At birth (cord blood)

Packed cel volume

51%

Erythrocyte count

$4.7 \times 10^{12}/L$

Hemoglobin concentration

16.5 g/dL

MCV

108 fL

MCH

34 pg

At 6–12 y of age

Packed cel volume

40%

Erythrocyte count

$4.6 \times 10^{12}/L$

Hemoglobin concentration

13.5 g/dL

MCV

86 fL

MCH

29 pg

SI, Systeme International d'Unites.

Source: Perkins SL. Normal blood and bone marrow values in humans. In: Greer J P, *et al.* (eds). Wintrobe's Clinical Hematology, 11th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2004:2607; Handin RI, Lux SE, Stossel TP. Blood, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2003:2216, Appendix 25

Red Blood Cell Values at Various Ages.

One femtoliter (fL) = 10^{-15} L = 1 cubic micrometer; one picogram (pg) = 10^{-12} g = 1 micromicrogram.

. %

F m

m m m

. F x m m

m . M

, m m

m m , m

, m

mL RBC . I -

. H (m C)

, m m ,

m m

, m x

m , , m -

.

m m k

I m,

. F x

(+) k m m

m m , m m

m -

, m

(DM). DM m k

(F . .).

. O , m

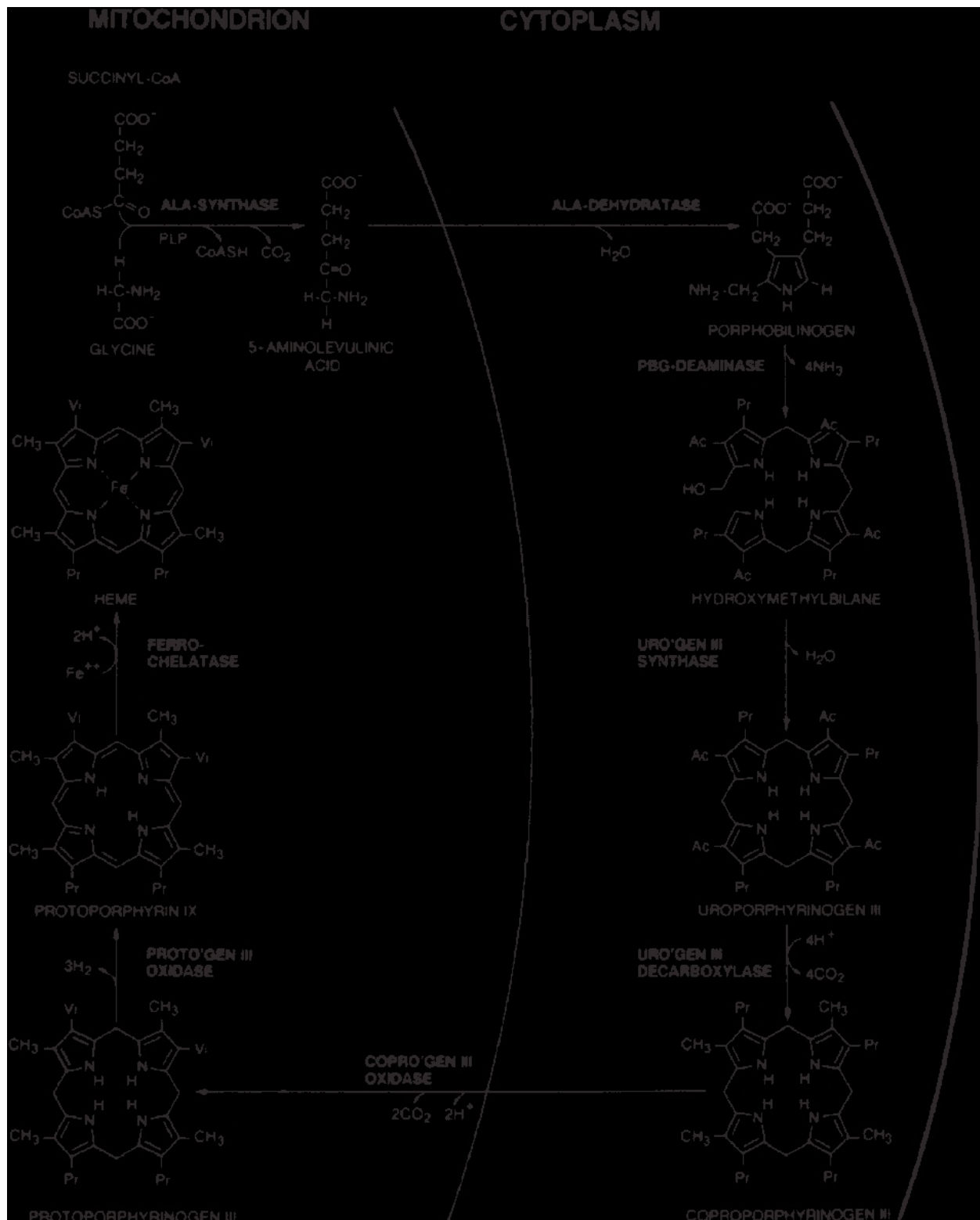
I m , (+) ,

x -

m m -

, ().

, (R). I



CHAPTER 6 ■ Erythrocytes: Hemoglobin

FIGURE 6.5 H m . A , ; ALA, σ -m ; C A, z m A; C AS, -C A; C ASH, m z m A; COPROGEN, ; URO'GEN, ; V , . (R m G JP (). Wintrobe's Clinical Hematology, , P , PA: L W m & W k , : , m .) HFE R

DNA m

k .

(mRNA).

Globin S tructure and Synthe s is

Alph a Globin Locu s

B m -

E m m

m (F . .). T

m m . F

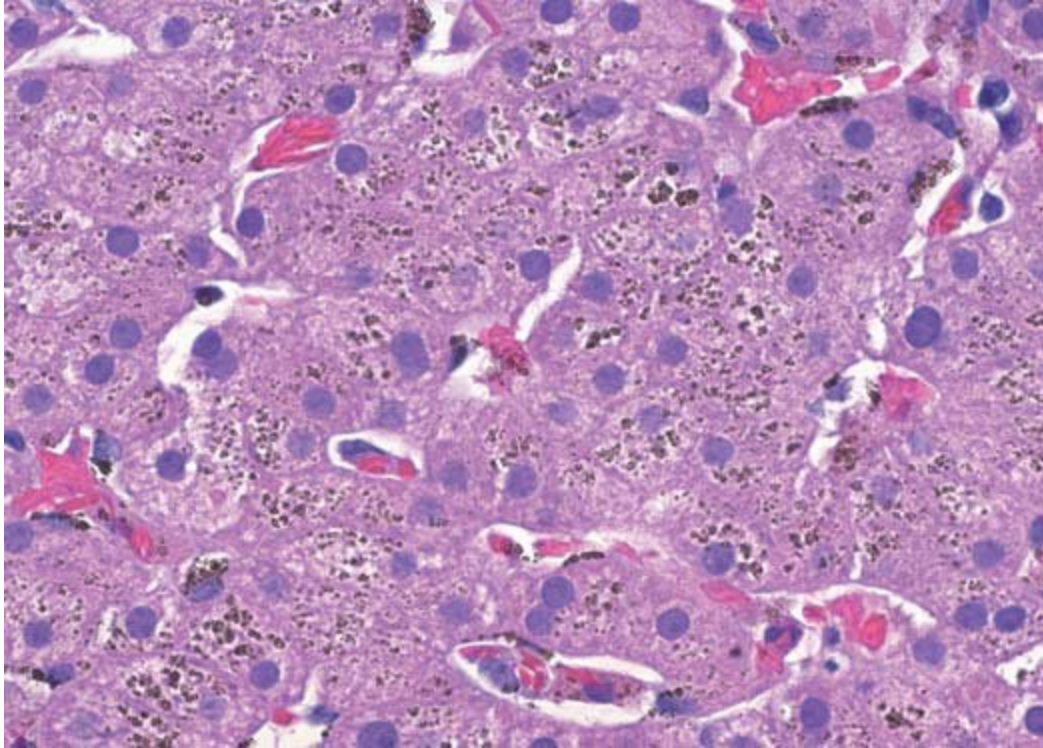
q m -

, . E

DNA , . T

m m ;

x . E



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PART 2 ■ Hematopoiesis and Cellular Maturation

Succinyl CoA + glycine

q m

ALA synthetase

De la t-amin-

. T m m x

le vulinicaid

k .

Vitamin B6

Body cells

(Δ ALA)

T,

Δ ALA dehydratase

, m. T -

Δ ALA

m

m m m. I m m -

Prophobilinogen

(m A),

.

Uroporphyrinogen

Mitochondrion

Summary of the Assembly of a Hemoglobin

Molecule

Coproporphyrinogen

Protoporphyrin + iron

Am m m q.

T:

Protoporphyrinogen

. T α - β -m mRNA.

. O m

He me s ynthe ta s e

, -D

He me

.

FIGURE 6.6 S m .

. T α β

.

. A m m x -

q m m $\alpha\beta$ m .

. T m m k .

. m m m m

T x m

m , α

m , z ,

β m .

.

NOTE: This is a good time to completely review the Key

Be t a Globin Locu s

Terms in the Glossary and on

. It is also a good

T q . T

time to complete Review Questions related to the preceding

q , mm , , . T

content.

mm m m .

T . E

, m m

Be ta Glo bin Ge ne Clus te r

. T x

Chromos o me 11

e ps ilon

ga mma

de lta

be ta

G

A

5'

3'

Hb F

Hb A2

Hb A

Alpha Glo bin Ge ne Clus te r

Chromos o me 16

Ze ta 2

Ze ta 1

Alpha 2

Alpha 1

5'

3'

FIGURE 6.8 T . T

FIGURE 6.7 B m (β) m m . T mm H&E . I -

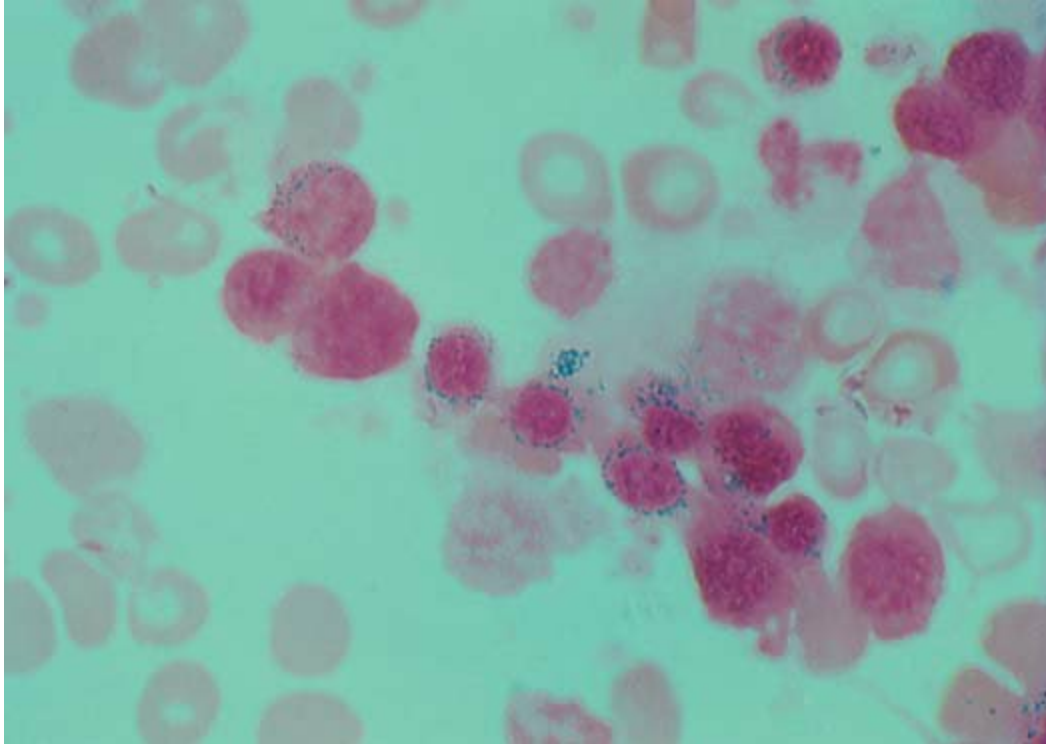
m F. T

m. T m

“ ” , β , m . T m -

m . (M. Biopsy Interpretation (α) m m . E

o the Liver, P , PA: L W m & W k , .) α - α - .



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DISORDERS RELATED TO HEMOGLOBIN

BIOSYNTHESIS

Disorders of Heme (Porphyrin) Synthesis

Defects in the synthesis of heme lead to a group of disorders called porphyrias.

.

Acute intermittent porphyria (AIP) is a rare, inherited disorder of heme synthesis.

It is caused by a deficiency of the enzyme uroporphyrinogen decarboxylase (UROD).

Patients with AIP often experience severe abdominal pain, vomiting, and constipation.

, ALA PBG

m . N m

m (C), q ALA m x m **FIGURE 6.10** I m . B m

m .

m (N m green granules -

P m m m m). (R m M C KD. Clinical m m

Laboratory Medicine, , P , PA: L W m x m x & W k , , m .)

/ .

P :

- -

■ C (.)

. PBG m x m m

■ S z m

; , m

■ S z m m

m , m

E ' . I

C , -

PBG, m m

m k m . S m

m .

m m . D m

W m , m

:

m , m x

m

. T -

m .

P m G k , porphyrin,

, P (F . .). T

m . T - m ()

sideroblasts.

Glycine

δ -ALA

Porphobilinogen (PBG)

Synthesis

Succinyl CoA

δ -ALA

(4-P BG)

Hydroxymethylbilane

Heme

Heme proteins

Fe²⁺

Protoporphyrin IX

Uroporphyrinogen III

Uroporphyrinogen I

Uroporphyrin

(excretion)

Protoporphyrinogen IX

Coproporphyrinogen III

Coproporphyrinogen I

Coproporphyrin

(excretion)

FIGURE 6.9 Tetrapyrrole biosynthesis. (R

and M M W, . Green field's Surgery Scientific Principles and Practice, , P, PA: L

W m & W k, , m.)

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PART 2 ■ Hematopoiesis and Cellular Maturation

Disorders of Globulin Synthesis

Fetal Hemoglobin

G -

Fm (mF) mm -

. Wm ,

. Tm -

. Sm , -

mm . Tmm

m , x .

m , . H ,

H , k

mm m . mm m m .

x , m . E -

W , m

mm .

m .

Fmk

Dm -

m . Tm

m (C).

. A

m m -

ON OGENY OF HEMOGLOBIN

, m m

m k .

I m m m , m -

A m F x k

. T m m

, β -m -

A A

x mm (γ) m

, m A , m ,

m m . E m

F . G , m F

m

m

(.). M m -

m F (%) . T k

; , m m m m , m .

m , m -

. I m , m F

Embryo nic Hem o glo bins

(% % m)

m (HPFH).

Em m m m m mm k . T m -

He mo g lo bin A

G I, G II, P . T

m m x m

T m m m -

k . I m , z

β - . A

m -

m m A (% %).

m m mm m

H m A m -

m m . T

(α β).

mm , , .

H m A m

. T m

m . S

m , A

Com parative Chain Co mpos ition

TABLE

6.2

. T

of Hem oglobin Type s

m A z /

. T , m A

Po lype ptide

m . % m .

He m oglobin Type

Sym bol

(Globin) Chains

Embryonic

Glyco s ylated He mo glo bin (Hem og lobin A1)

Gower-1

$\zeta_2 \epsilon_2$

2 zeta

A m m A m A .

2 epsilon

T m **glycosylated hemoglobin**

Gower-2

$\alpha^2 \epsilon^2$

2 alpha

m m -

2 epsilon

A , A , A . T m m

m . B

Portland-1

$\zeta^2 \gamma^2$

2 zeta

m z

2 gamma

m , m k m

Hemoglobin F

$\alpha_2 \gamma_2$

2 alpha

m m . T m

2 gamma

m ,

Hemoglobin A

$\alpha_2 \beta_2$

.

2 alpha

C q , m

2 beta

,

Hemoglobin A2

$\alpha_2 \delta_2$

2 alpha

k m

2 delta

.

CHAPTER 6 ■ Erythrocytes: Hemoglobin

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G m m

m x m x ,

m m A x addi—

m x m m

tion m

x .

. T m A % %

m % % -

Sulfhe mo glo bin

— .

T m . I

x , m

NOTE: This is a good time to complete Review Questions

m m .

related to the preceding content.

T m

m m

x , -

VARIAN FORMS OF NORMAL

m **Heinz bodies**.

HEMOGLOBIN

S m x , m-m x m x m .

C x m , m , m m

S m m x -

k m m m -

z m m -

m . U k m m m -

m Clostridium welchii

m m m ,

. C m -

m m m -

m % m x

m x . T

% ' m . E

m x .

m m .

T m m x

x , x m , m

Methe mo glo bin

m , , x m , m x .

Methemoglobin m , -

M m m q ,

, m x . I

x m .

m m (“M m R

P ”) m m -

Carboxyhe m og lobin

m m -

C m x m mm

m m . T m

U S . C m x ,

m m

- m m -

m m

, x m m . C

x

m x x . A

m m m k m . V

m , m x

m m M -

m m

m m .

m m (. . , m -

N m , % m m m . A

m x). H m

, m m m

m m x m

x -

x , f m m

. H m m -

m x m . T f -

m x m -

m . C m m x

m x m

%; x x %.

m x x m . T m m

I , m m

x m m , m

m m

m x . **Carboxyhemoglobin** x x m . O

x x .

m , m m .

A m x m % %

I m m m -

m x m -

m F m

m - m m x x .

m m . I ,

S (% k m k

q z m . H

) m k . C

q , , m -

.

m .

L % % x m -

m m zz , , , m -

ABNORMAL HEMOGLOBIN MOLECULES

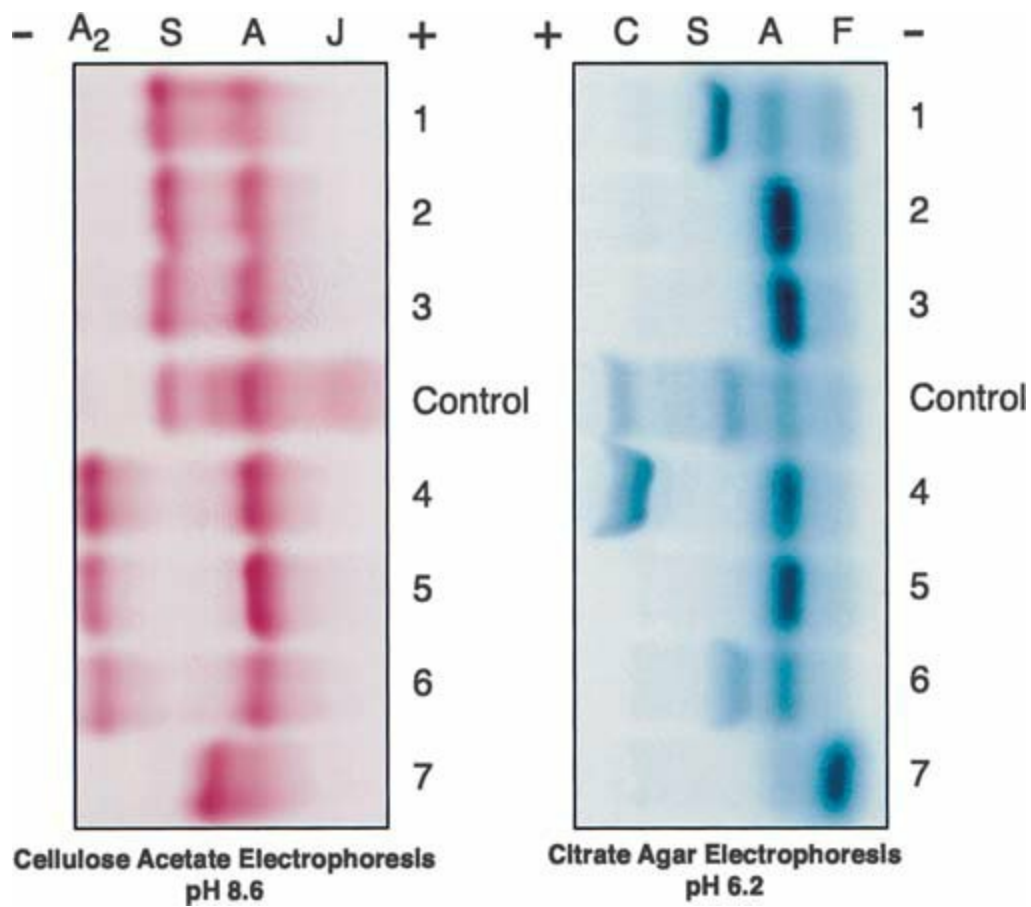
k . A (%

%) m %

A m m m

. m

k m (k) m m ,



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PART 2 ■ Hematopoiesis and Cellular Maturation

m m m . M m

Normal hemoglobin

Glu

Beta chain

mmmm, mm. Tm -

mm -

. Tm

Hemoglobin S

Val

Beta chain

mm. Ak -

FIGURE 6.11 Cmmkm

mm

m. G, m; V, .

' m .

Ammm -

m (C). Pm

mmmmmm z (S/S) z (S/A) m (SLS) m SLS-

. Tkm C, E, D,

mm. I, SLS

SC, SE, SD. Im

x .

S, m m m m x

Alkaline Electro pho res is

m . T m -

T m m

k x -

m m k -

m S m z m

m

m m m

m. T -

m x . A -

m A, F, S, C m -

m S m

(F . .). T m

m

m A H . m -

. I m m C, m

. Ex m m B m

m m x

, m H m

m . A m l. H m C m m m .

m q

V m , , ,

m m A F . .

k , m .

T m f -

ANALYSIS OF HEMOGLOBIN

. F x m , k H

. T q m

S m , S, F, A, C, A .

q m -

. T m q m

m m m . T m

m m -

m . O m m

m m ,

, , ; ; -

m k

. A m m

mm (F . .).

He mo g lo bincyanide (Cyanmethemoglobin)

mm ,

mm . Am

mm

(HCN), x mm ,

m . F , m -

, mm , m

mm m .

FIGURE 6.12 Ex mm mm

T mm m

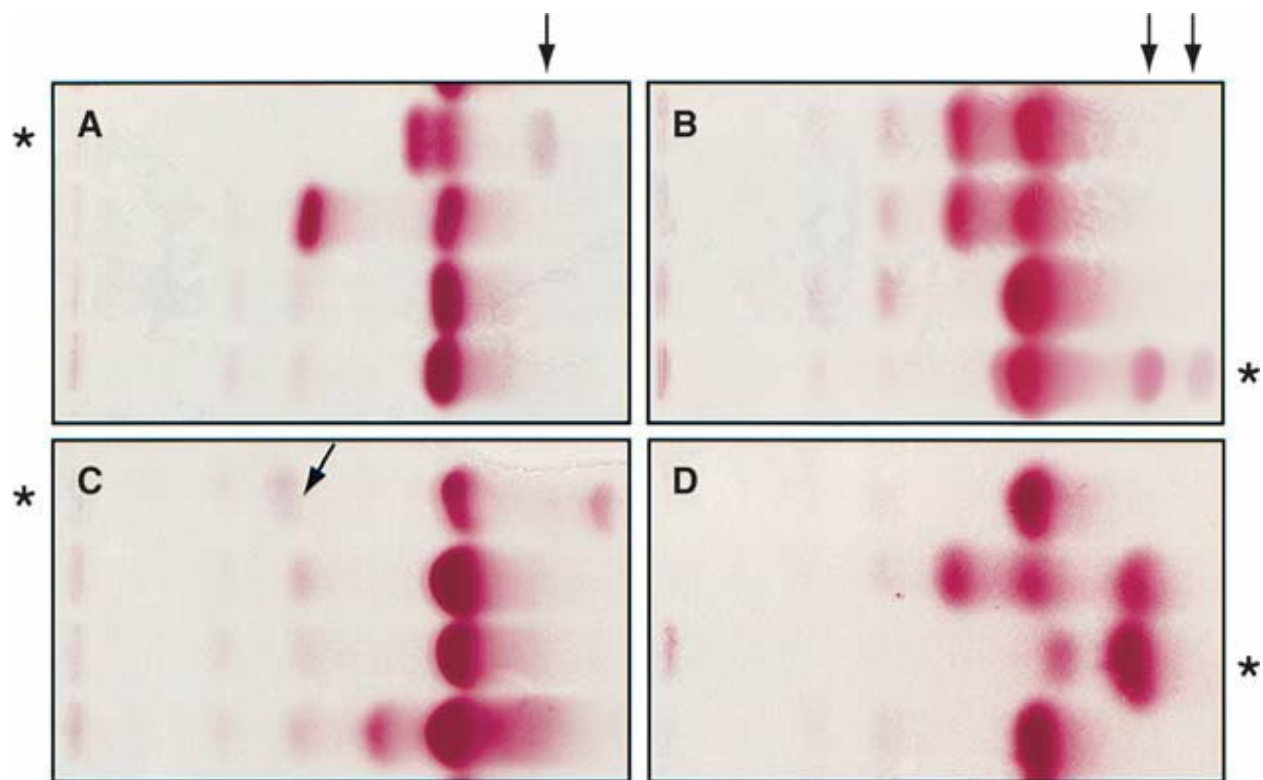
. , HS ; , H

mm m -

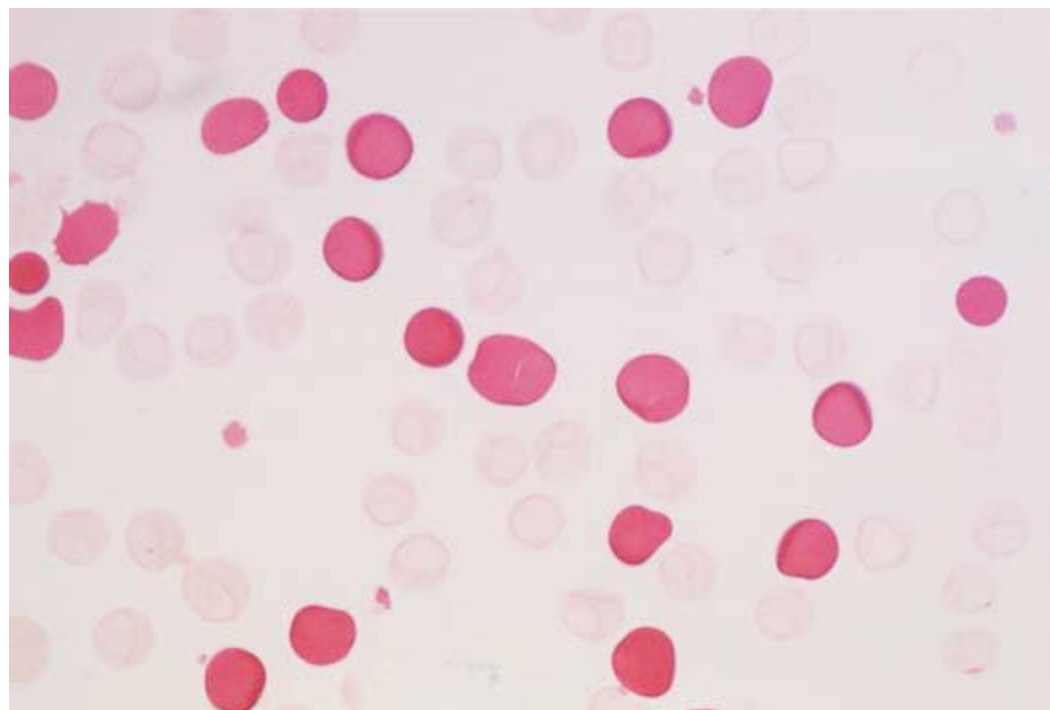
G-P ; , HD-P j ; , HC ; , HE ; m . I , m -

, HO-A ; , HF . (R mm m .

MC KD. Clinical Laboratory Medicine, , P , T mm m PA: L W m & W k , ,
m .)



**Cellulose Acetate Electrophoresis
pH 8.6**



CHAPTER 6 ■ Erythrocytes: Hemoglobin

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FIGURE 6.13 C .

A. S m m . H -B

(arrow) m . **B.** S m m

-m - . I H -B ,

m m H H (double arrow). **C.** H H-H -

C S . T H A

H -C S .

D. H -B . T H A -

. T m j m H -B ,

m m H P . (R m

M C KD. Clinical Laboratory Medicine,

, P , PA: L W m & W k ,

, m .)

Citrate Agar Ele ctro pho res is

, m .

O , m () m T k H. I m , m -

m m –

m x

. T -

m , , . C

m F - m , m

m m -

m , x m hemoglobinuria (PNH).

. T m S, D, G, C, E,

O. A m m m -

Chro mato g raphy

k m -

. T m m

Q m A m -

m m m .

x m m m . H ,

q

De naturatio n Pro cedures

m m A . C

j -

A mm m m

x m m

m x m

m . O

Kleihauer Betke (F . .).T

m m -

m . F m

q m (HPLC).

Molecular Tes ting

T m x m m m

(C). G m -

m m .

F x m , β -m

m . HPFH

m β -

m m

m (H F) .

CA ABOLISM OF ERY HROCY ES

W ,

m m -

FIGURE 6.14 K -B k m z . A , : (H). R H F ; H A k . (R m . T m m m x .

G JP (). Wintrobe's Clinical Hematology, , P ,

. T m .

PA: L W m & W k , , m .)

. E z m , , m .



132

PART 2 ■ Hematopoiesis and Cellular Maturation

T m

m

m . B m

m m m .

, m m

G z m

. T x

m . T -

. I

k m m

m ,

x z m . T m -

. T m

x . T (bilirubin) m

m (

m m ,

Howell Jolly bodies) (

j x .

C) m .

B unconjugated bilirubin conjugated bilirubin -

Ex m

m . B x

m , m

, , x

, , -

. A m m urobilinogen

.

x .

A m

, m m m -

Intravas cular Catabo lis m

m m m m m mm m . T m m

I -

k (F . .). T m

■ x m

% . A

■ m

, m

m

Extravas cular Catabo lis m

m , q k m

haptoglobin. T m m

W z

- m m x x -

m m m, m m . T m x m m m m (F . .). T -

m ,

m , , . I

m m m

FIGURE 6.15 Ex m -

. U j

. C j m -

Bo ne marrow

.

Tra ns fe rrin + iron

Globulin

He moglobin

P hagocytos is

of e rythrocyte

rotoporphyrin

P

O

He me

2

oxida s e

Exha le d through the

CO₂

re s pira tory s ys te m

Indire ct bilirubin

Amino a cid pool: blood

– unconjugate d

+

Liver

Plasma albumin

Conjugated bilirubin

Glucuronide

– H₂O soluble

albumin-bound

Bile

bilirubin

Gallbladder

Pancreas

Intestines

(bilirubin

glucuronide)

Stercobilinogen

excreted in

Kidney

the feces

Urobilinogen

CHAPTER 6 ■ Erythrocytes: Hemoglobin

RBC

FIGURE 6.16 I -

destruction

m .

Hemoglobin

Haptoglobin

+

alpha and beta dimers

Haptoglobin-hemoglobin complex

Kidney

Catabolized

Hemosiderin is

formed and

excreted in

the urine or

Liver

Hemoglobin

remains in

tubular cells

Hemoglobin

He moglobin

He me

Me the moglobin

or

He mos ide rin

He mopexin

+

Methe ma lbumin

Albumin

k . B m m -

H m x m x - -

- m m x,

m m m m m m

m m .

m m x . O , m x O m -

m , m x q -

, m m -

z .

m k ,

T m m x

, m . T

m m m m hemosiderinuria

k m /

m m -

m . O k

m . T

x , m m m

m m m m m x with-

. T m -

out m

(. ., - m).

. H m , m

. Ex m

NOTE: This is a good time to complete end of chapter

. Ex m m ; -

Review Questions related to preceding content.

q m m ,

hemoglobinemia

H m

CHAPTER HIGHLIGHTS

xxzmm -

Characteristics of Hemoglobin

. Tmmm

k, mx.

■ Tmxk

Hmxmm

mx.

f. Hmx - mm

■ NmmA

-- (LRP/

M'LI..T

CD, mx, A/A.

, m/m,). T

■ Ammm

mm

m; xm

x - m.

.

PART 2 ■ Hematopoiesis and Cellular Maturation

■ H m -

x , m % z

m m .

.

■ N m m (m A)

■ T m j m m m .

m

■ H m m x

m . T z

m . O ,

.

m m m m

■ M -

() .

m m -

■ T m m x m -

m m m

z m . T m -

. I m

-C A

, x .

m - m . A m

III, m -

He mo g lo bin Function

. I z m ,

IX m .

■ T m j m m

■ T m , x . T x f m m , -

m m -

z m A (C A) . T

m m -

m

, , -DPG .

q m B .

■ A m , ,

Oxygen Dis s ociatio n and Alte rations

m , m

m m . T

■ C x f m

m m m .

m

■ I m m .

x .

I k -

■ A x

m .

x (-

■ M m m m

) x (PO).

; m m

■ T P x m m x q m ,

m .

x m (m) -

■ M m m

q x m (x m)

. I , m , -

H m .

, m x

■ F m (m F) f

.

x . T f x -

■ O m m, m

x

x x .

, .

■ A m m m -

■ F m

m x .

. F x m m -

O m m q

, m m

m m m x

, m .

x m m m .

■ B

m m . E

Carbon Dioxide Trans po rt

m m . E

m m ;

■ T m -

x .

x m

■ E ,

. C x -

m m . T

, m m m,

x q

,

m . I m m . A x m % x m (m A),

m .

.

Bio s ynthe s is of He mo glo bin

Dis o rders Related to Hem og lobin

Bio synthe s is

■ H m z m -

m . A x m % -

■ D m m -

m m z

m q .

CHAPTER 6 ■ Erythrocytes: Hemoglobin

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■ I m -

Variant Fo rms o f No rm al He mo glo bin

, .

■ C x m , m , m m -

■ A q ,

m .

k m m m .

U k m m m

■ P m m m m m

m m m , -

x m x -

m m m

/ .

m x .

■ C m x m mm

■ W m , m

m , m x

U S . H m

. T -

m m x

,

m x , f

P . T .

m m m x m

. T f

■ G

. W m , -

m x m m x m -

.

m x

x m . C x m x

■ W , m m . D

x .

m m .

■ S m m

. I m m

x -

Onto geny o f Hem og lobin

m H z

■ I m m m , m -

.

.

■ M m m ,

■ E m m m m

, m

m m m k . T

x . V m

m G I, G II, P

m M m -

. T m m

m . H q , ,

x m k .

m m

■ F m (m F) m

.

m . T

m m m .

Abnormal Hemoglobin Molecules

Forsk

Ammon

m. Tm

■

k (m) mm, m -

. A

.

mm -

Pmmz (S/S)

, m -

■

z (S/A) .

mmk .

■ Tkm C, E, D,

■ G, mAmF

SC, SE, SD .

m

I mm C, mm

mF (%) . Tk

■

m x

m m m , -

m .

m ,

m .

Analys is o f Hem og lobin

■ I m , m F

(% % m)

■ S q m -

m .

. T m

■ A m m A m A .

m , , , ;

T m m

; m

m A , A ,

k .

A . T m m

■ A m m m m . A m m m .

m A (% %), A

■ A k m

m q (% %). H m A

m m

m .

k m

H m A m

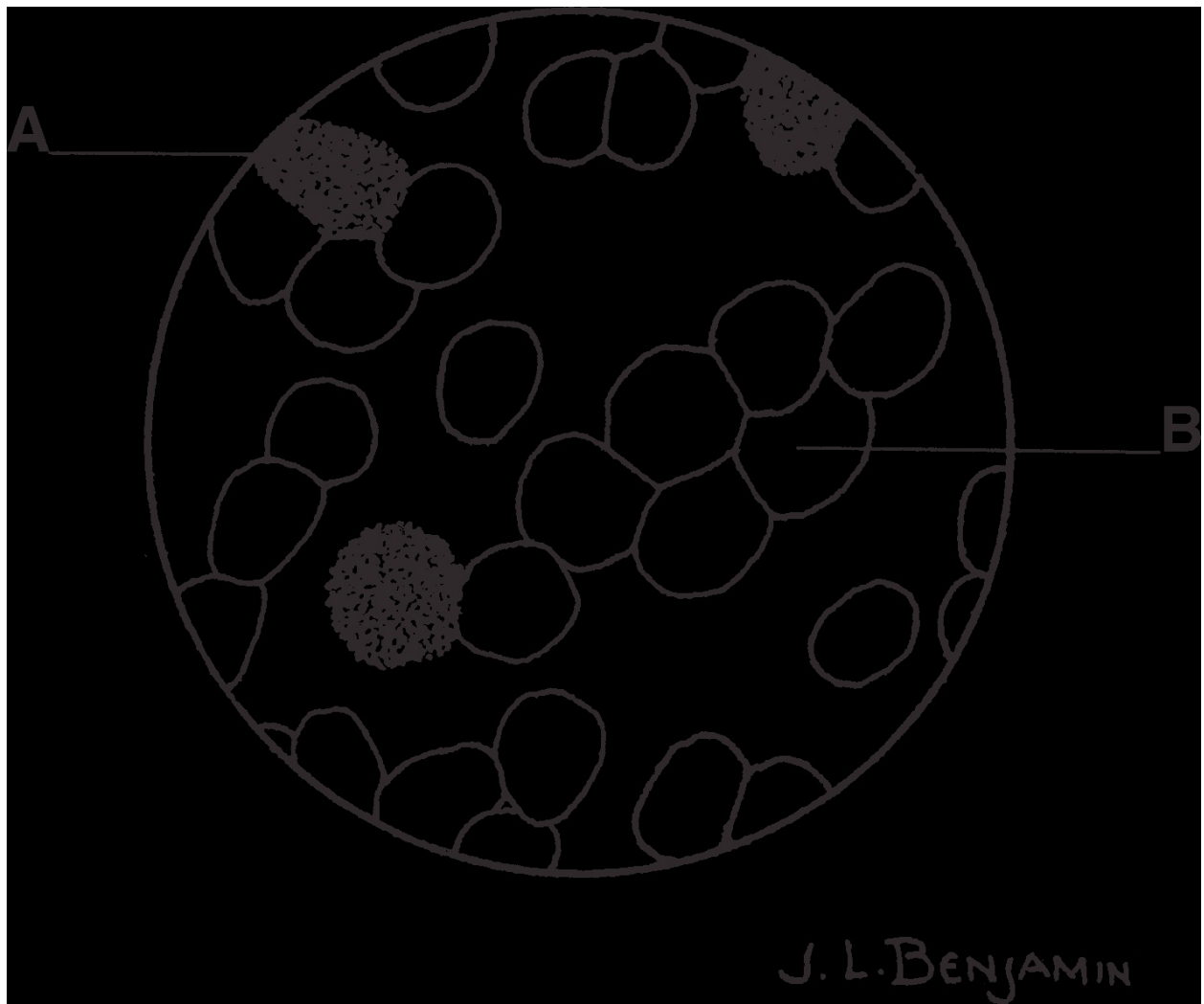
m. T -

. T m A m

m A, F, S, C

. % m .

m . T



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PART 2 ■ Hematopoiesis and Cellular Maturation

mm AH .

■ Gzm m . Exmm Bm -

m .

, m H m -

■ Tk m

I. H m C mm m .

m x z m . T

■ C k H.

m x . T ()

T m S, D, G, C, E, O.

m m -

■ A mm m m

m , j

m x m -

x . B j j

K -B k . T

m .

m . F m -

■ B x ,

, m . E

, x -

m F - m , m

. A m m

m , x m m (PNH).

x .

■ C m m x

m m m . O m

In trava s cula r Ca t ab olis m

m - q

■ I

m (HPLC) m m .

k .

■ T m m m

■ T m % -

m m m -

.

m

■ H m m m ,

Catabo lis m o f Erythro cyte s

q k m .

■ m m -

Ext rava s cular cat a bolis m

m m x m m

■ W z

,

m m m, m m m k .

m m m . T m-

■ O m -

, , .

m , m

■ I m -

m m

m m

k , , -

m .

m .

CASE STUDIES

Cas e Study 6.1

■ Critical Thinking Group Discussion Questions

. A x m K -B k ,

A R - m , , m

' ?

' m . A f

, - m . A

. W ' -

m m k R

?

; m R

.

. W

m ?

■ Laboratory Data

T ' m

R (D+) .

■ Follow-Up Testing

B m -

- - m ,

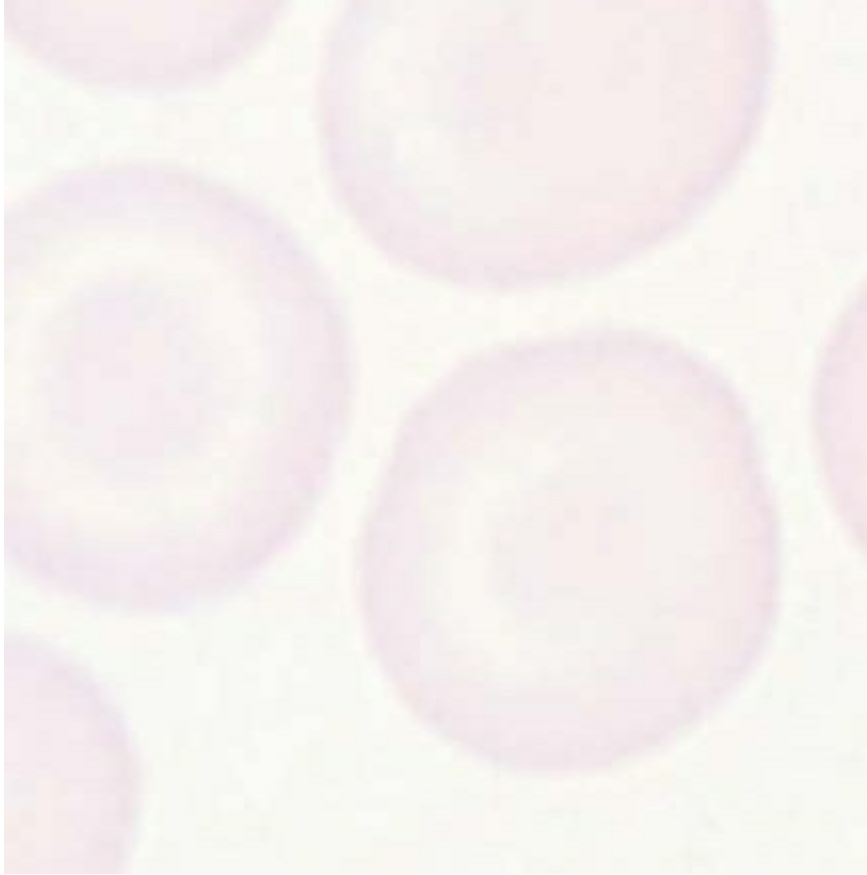
K -B k m

mm D f -

m x ' R - RBC . A

m m m . T

m K -B k .



CHAPTER 6 ■ Erythrocytes: Hemoglobin

137

CASE STUDIES (continued)

Cas e Study 6.2

RBC . \times /L

WBC . \times /L

A -m - P R

N Y k m

T m , kN Y k C . H m

m RBC .

k . S .

H

■ Critical Thinking Group Discussion Questions

, m m . H

. W k- ?

m -

. A CBC .

. W ?

■ Laboratory Data

T m :

H m . /L

NOTE: This is a good time to write out the answers to the

H m %

Critical Thinking Group Discussion Questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

*6. A m m x -

m , m

Characteristics o Hemoglobin

A. x , -DPG

1. N m m

B. x f

A.

C. m x

B.

D. m x

C.

Oxygen Dissociation and Alterations

D.

*7. I m (A) m F m². T m m m m

, m _____ f x .

A.

A.

B.

B. m

C.

C.

D.

*8. Ox m _____ x m .

3. M m m

A. k

A. m

B.

B. m

C. m z A/A

*9. I m m m

D. A B

m m x -

, m

4. T m m m

A.

A. m α β

B.

B. m , α

C. m m

C. m , β

D.

D. m , $\alpha \beta$

Carbon Dioxide transport

Hemoglobin Function

*10. I x m x ,

' x x

*5. I m , -DPG _____ x f -

A.

m m .

B.

A.

C. f x

B.

D. m

C.

(continued)

PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

Biosynthesis of Hemoglobin

*18. T m , m -

11. H m z m

, k

A.

A. '

B. m

B. ' m

C. m

C.

D. A B

D. m

12. T m m

Disorders Related to Hemoglobin Biosynthesis

m

Q . R .

A. . . / L

*19. T kB. . . / L

m m P B

C. . . / L

x m

D. . / L

A. m m m

B. m

*13. W , -

C.

mRNA

D.

A. x f

B. x f

*20. T ' m

C. x f

, k-m

D. , -DPG m -

m

m

A. m

B. m

*14. T

C.

m

D. k

A.

B.

Ontogeny o Hemoglobin

C. x m

21. T m j m z -

D.

m

A. A

*15. I f , -

m _____ .

B. F

C. A

A.

D. A

B.

C. m

22. W m m j

D. m

m ?

*Q : T

m m

A. A

k () _____ q () _____.

B. S

C. A

D. A

16.

A.

23. H m A

B.

A.

C. m

B. z mm

D. m

C.

D. mm

17.

A.

*24. H m A

B. m B

A.

C. m B

B. z mm

D. m D

C.

D. mm

CHAPTER 6 ■ Erythrocytes: Hemoglobin

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REVIEW QUESTIONS (continued)

*25. H m F

*32. k m mm

A.

m ____.

B. z mm

A. C

C.

B. F

D. mm

C. B

D. H

*26. Em m

A.

Analysis o Hemoglobin

B. z mm

33. T k

C.

m

D. mm

A. A C

B. F

27. I m , m (m F)

C. C

D. S

A.

B. k

Q . R . .

C. m

*34. T m

D.

K -B k

A. A

Variant Forms o Normal Hemoglobin

B. A

*28. A m

C. F

m , m -

D. S

m . W m-

, m .

*35. T m -

W k m ?

K -B k m

A. H m F

m

B. H m A

A.

C. M m

B.

D. C x m

C. k mm

D. f x

29. H z

A. m F

36. C H . m

B. m A

C. m

A. S

D. x m

B. H

C. A

*30. A m m

D. A C

A. m x

B. m x

Catabolism o Erythrocytes

C. H z

37. A ,

D. m

A. m m m x

%

m m

Abnormal Hemoglobin Molecules

B. m

C. z m , ,

*31. A m S m

D.

A. m

*38. E m m

B. m -

m

A. m

C. m m m

B. z m

x β -

m

m

C. m m

D. m m x

m m , j ,

β -m

x

m

D.

(continued)





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PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

*39. W m -

*40. I m m

?

- m

A. I % m

k .

m m , m

B. H m .

?

C. A m .

A. Ex m

D. A .

B. I m

C. D m

D. T m

F R, H m z PR. N - m -

COMPANION RES OURCES

, Hematology Am Soc Hematol Educ Program, – , .

:// . /

J GF. U : , Adv Med Lab Pro , (): – , .

E W -

K k K. L - m , N Engl m .

J Med, : : – , .

H

K m H, . S m

-

, Clin Chem, (): – , .

.

K FL. M , Lab Med, (): – ,

.

L VL, . E - m m

m m , Blood,

BIBLIOGRAPHY

(): – , .

M JM, . I m x A NC. F : , Blood, , FEBS J, (): – (): – , .

, .

A NC. U m , N Engl J Med, : –

P ML, F k EL. P , Clin Lab News, (): – , .

, .

P CA. C m x , N Engl J Med, D D m I, L E, W DM, . H m m D G

(): – , .

- JAK

W MJ. H m , Blood, (): – , .

- , Blood, (): – , .

Z A, E CA. M m m m m -

D k m H, . F m m : m ,

, Hematology Am Soc Hematol Educ Program, – ,

, The Hematologist, (): – , .

.

Erythrocytes: Morphology and

CHAPTER

7 Inclusions

KEY TERMS

a be ta lipoprote ine m ia

h e lm e t ce lls

p o ikilo cyto s is

a ca nthocyte s

Ho w e ll-J o lly b o d ie s

p o lych ro m a to p h ilia

a gglutina tion

h yp o ch ro m ia

p u n cta te s tip p lin g

a niso chrom ia

h yp o ch ro m ic

p ykn o cyte s

a nisocytosis

ke ra to cyte s

re ticu lo cyte

b a b e s io s is

kn izo cyte s

ro u le a u x fo rm a tio n

b a s o p h ilic s tip p lin g

le p to cyte s

s ch is to cyte s

b ite ce lls

m a cro cytic

s ickle ce lls

b lis te r ce lls

m a cro cyto s is

s id e ro tic g ra n u le s

b u rr ce lls

m a la ria

s p h e ro cyte s

Ca bot rings

m icro cytic

s p icu la te d e ryth ro cyte s

d e g m a cyte s

m icro cyto s is

s to m a to cyte s

drepanocytes

normochromic

target cells

echinocytes

normocytic

teardrop cells

elliptocytes

oval macrocytes

Heinz bodies

Pappenheimer bodies

LEARNING OUTCOMES

Erythrocytes: normal and abnormal

Select methods for differentiating various erythrocyte inclusions.

- Name and describe the variations in the size of a mature erythrocyte.
- Correlate at least one clinical condition with the following erythrocyte
- Describe the artifacts that can cause a variation in cell size.

inclusions: basophilic stippling, Cabot rings, Heinz bodies, hemoglobin C crystals, Howel-Jolly bodies, Pappenheimer bodies, and siderotic granules.

Types of variations in erythrocyte size

Alterations in erythrocyte distribution

- Correlate at least one clinical condition with each of the erythrocytic size variations: normocytosis, macrocytosis, and microcytosis.

- Define the alterations in erythrocyte distribution that may be

- Define the term anisocytosis.

encountered when examining a blood smear.

Briefly describe the chemical reasons for alterations in erythrocyte

Kinds of variations in erythrocyte shape

distribution on a peripheral blood smear.

- Explain the terms used when a mature erythrocyte assumes an

- Name the clinical conditions associated with alterations in erythrocyte shape.

erythrocyte distribution on a blood smear.

- Explain the chemical or physical reasons for differences in cell shape.

Parasitic inclusions in erythrocytes

Correlate at least one clinical condition with each of these erythrocytic

- Name and describe the morphology of various types of malaria parasites—shape variations: acanthocytes, blister cells, burr cells, crenated red cells, and sickle cells on a peripheral blood smear.

elliptocytes, keratocytes, knizocytes, leptocytes, poikilocytosis, pykno-

- Name and describe the morphology of Babesia on a peripheral blood

cytes, schistocytes, sickle cells, spherocytes, stomatocytes, and teardrops.
smear.

Alterations in erythrocyte color

- Name and describe the morphology of Leishmania parasites on a
- Compare the chemical basis for differences in erythrocyte color on peripheral blood smear.
a stained blood smear.

Case study

- Describe the alterations in color that can be seen in an erythrocyte.
Analyze the patient history, clinical signs and symptoms, and laboratory data for the stated case studies; answer the related critical thinking questions; and conclude the most likely diagnosis.
- Correlate at least one clinical condition with the conditions of hypochromia and polychromatophilia.

Variations of erythrocyte inclusions

NOTE:

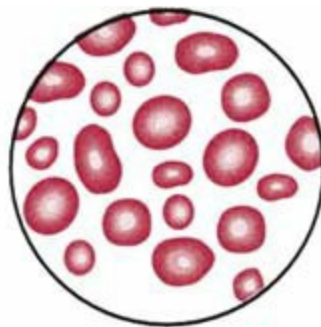
- Name and describe the appearance of inclusions that may be seen
Indicates MLT and MLS core content
in a variety of abnormal conditions.



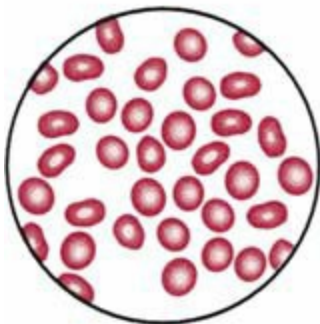
Indicates MLT (optional) and MLS advanced content

- Explain the cellular or chemical basis of inclusions.

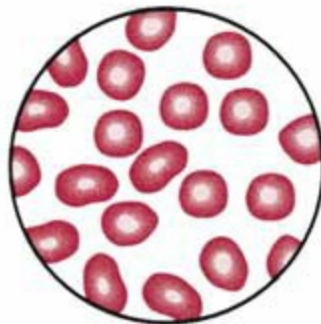
141



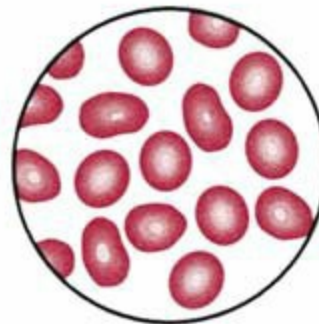
Anisocytosis



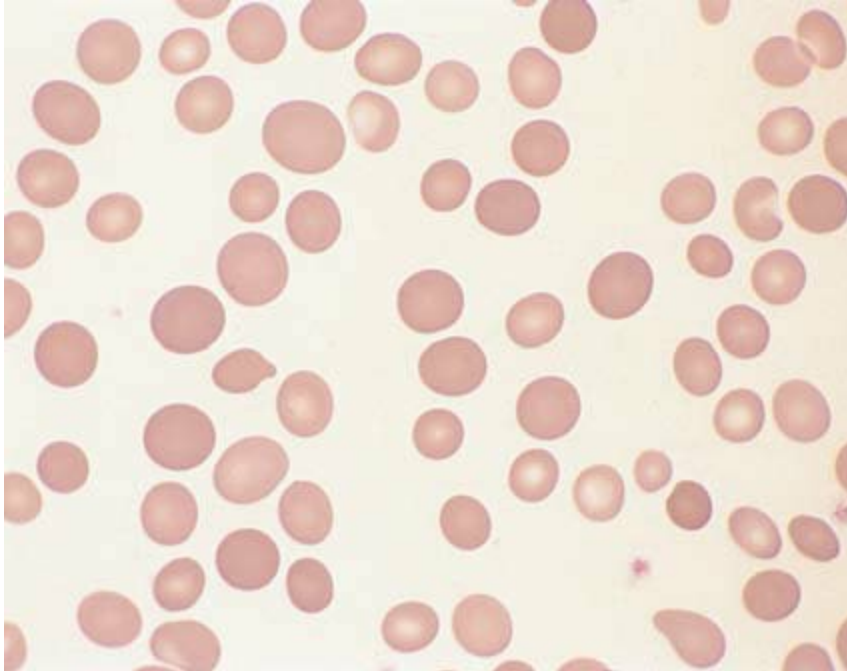
Microcytic



Normocytic



Macrocytic



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PART 2 ■ Hematopoiesis and Cellular Maturation

ERYTHROCYTES: NORMAL AND ABNORMAL

Normal ()

1. Size ,

, color

normal WBC -

. I m , m m .

T m m

. V z

. V

. A

. I

. A

FIGURE 7.2 V z .

m

S m m m .

A m

-

T m m

. H , m

z anisocytosis (F . .). T

m , , . T

m

-

. A m

m , m -

m .

, .

T m m , m , m

m . C -

m m z

YPES OF VARIA IONS IN

m . I m

ERY HROCY E SIZE

,

m m m

A m (F . .) m

, m z m ,

. μm . . μm . T x m

z .

z m . . μm .

Macrocytosis

T m z **normocytic**. E

m m . m

m m (macrocytic) m m -

m (**microcytic**) (F . .). M -

m B . T m

x . - μm m m , m

m m -

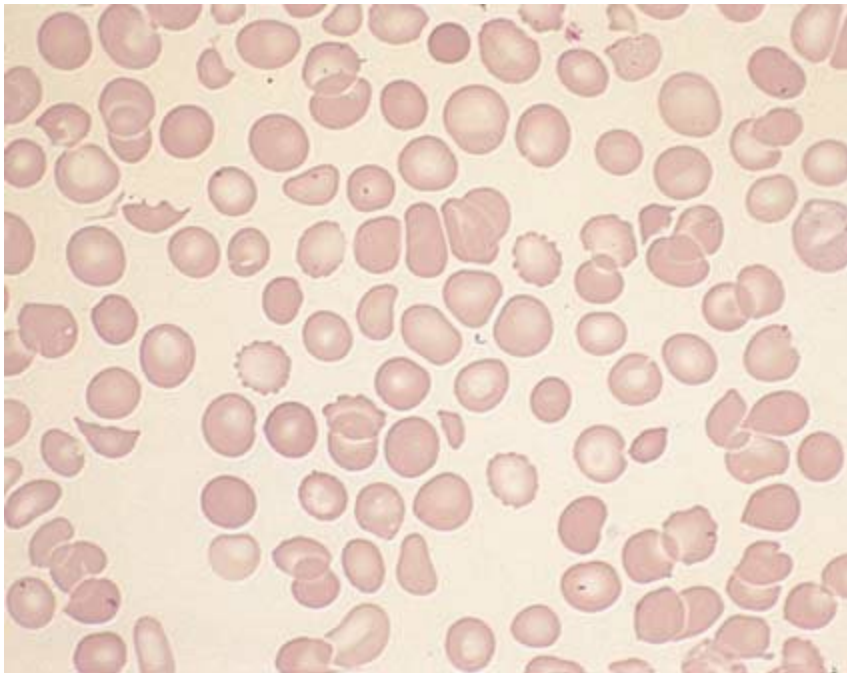
m . - μm m .

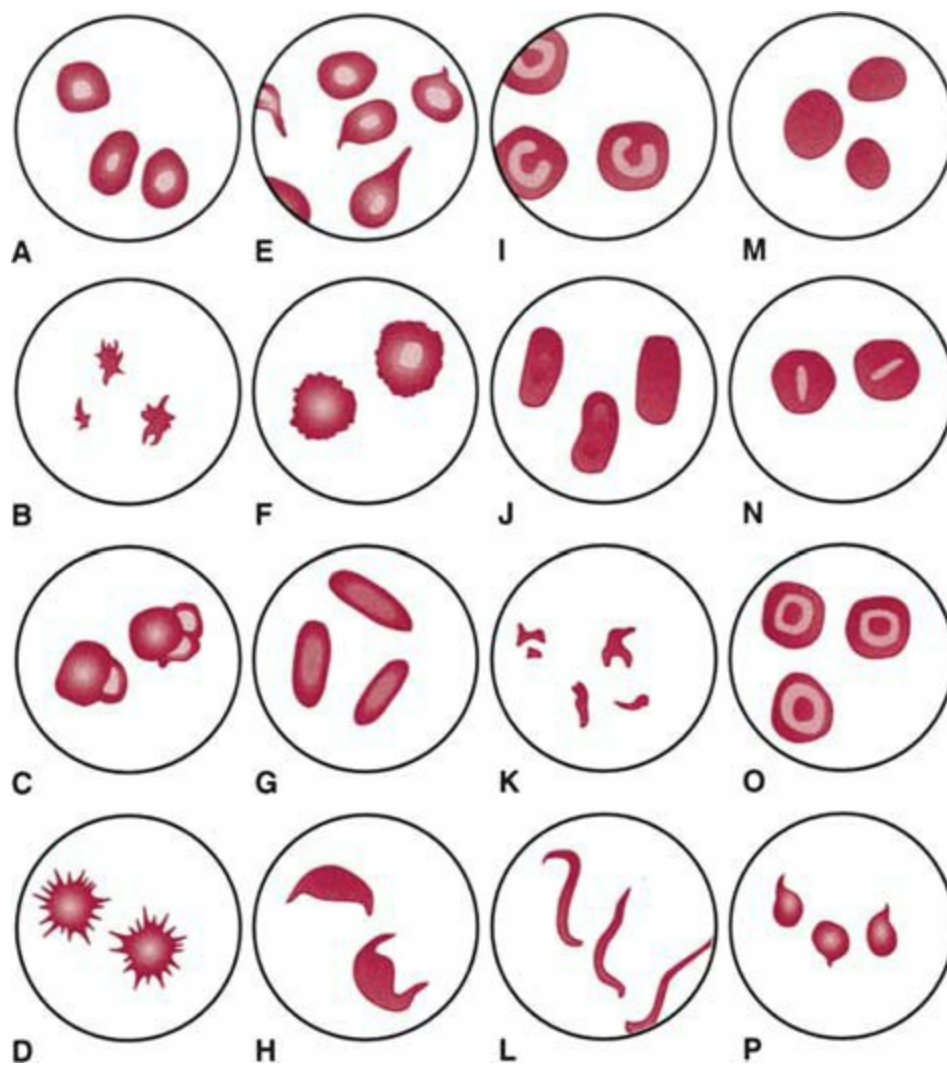
. B , m , **FIGURE 7.1** S m m RBC

FIGURE 7.3 A : PB, ×, W -G m . (R

m . (A S , S P L , m M K z SB. extbook o Hematology, , B m , MD: S S /P R
)

L W m & W k , , m .)





CHAPTER 7 ■ Erythrocytes: Morphology and Inclusions

143

. T

m

m , m

. T m

. T

m m

m m . **Microcytosis**

m . T

m m -

, m , m -

m m

m m . D m -

m m m ,

m , m

, m .

NOTE: This is a good time to review the definitions of Key

Terms in the Glossary and ash cards on

.

KINDS OF VARIATIONS IN

FIGURE 7.5 V . A. Normal RBC

ERYTHROCYTE SHAPE

(RBC) (). **B. A . C. B . D. B .**

E. Polychromatophilic . F. C RBC (). G. E .

T m m

H. H m (z). **I.** L . **J.** O m m , -

(m). **K.** S (z). **L.** S k (-

m , , poikilo-

). **M.** S . **N.** S m . **O.** (-

cytosis (F . .). P k m m

). **P.** ().

(F . .) q m mm j

, , . S m

m . T m k

TABLE

7.1

Erythro cyte Nom enclature

k , ,

, , , , k -

Com mo n Te rms

Synonyms (Greek Terminology)

, , k , , k ,

, , m ,

Acanthocyte

Acanthocyte

, . A m (.)

Blister cel

m m mm

Burr cel

Echinocyte

m .

Crenated erythrocyte

Echinocyte

Elliptocyte

Elliptocyte

Helmet cell

Schizocyte

Normal erythrocyte

Discocyte

Oval macrocyte

Megalocyte

Ovalocyte

Elliptocyte

Pyknocyte

Schistocyte

Schizocyte

Sickle cell

Drepanocyte, meniscocyte

Spiculated erythrocyte

Spherocyte

Spherocyte

Stomatocyte

Stomatocyte

Target cel

Codocyte

Teardrop

Dacryocyte

FIGURE 7.4 H m m : k

Keratocyte

, m . (R m

Knizocyte

M K z SB. extbook o Hematology, , B m , MD:

L W m & W k , , m .)

Leptocyte

PART 2 ■ Hematopoiesis and Cellular Maturation

D m -

Acanthocytes (F . .) m , k - k

m

j -

m m . I m , m m m z . U k , x m m k . H ,

. A

k

: abetalipoproteinemia,

m m m . E k

, m . I -

m m

m , m

m m

m . T m

(.). T

m -

.

. T m ,

TABLE

7.2

Red Blood Cell Morphology and Related Conditions

Associated Clinical

Associated Clinical

RBC Morphology

Conditions

RBC Morphology

Conditions

Variation in size

Pyknocytes

Acute, severe hemolytic

anemias

Anisocytosis

Significant in severe anemias

G6PD deficiency

Macrocytes

Megaloblastic anemias and

Hereditary lipoprotein

macrocytic anemias (pernicious—

deficiency

cious anemia and folic acid

de ciency)

May be seen in smal numbers

during the rst 2–3 months of life

as infantile pyknocytes

Microcytes

Iron de ciency anemia

Schistocytes (schizocytes)

Hemolytic anemias related to

burns or prosthetic implants

Hemoglobinopathies

Variation in shape

Renal transplant rejection

Acanthocytes

Abetalipoproteinemia

Sickle cells (drepanocytes)

Sickle cell anemia

Cirrhosis of the liver with asso-Spherocytes

ABO hemolytic disease of the

ciated hemolytic anemia

newborn

Following heparin administration

Acquired hemolytic anemias

Hepatic hemangioma

Blood transfusion reactions

Neonatal hepatitis

Congenital spherocytosis

Postsplenectomy

DIC

Blister cells

An indication of pulmonary

Storage phenomenon produced—

emboli in sickle cell anemia

ing microspherocytes in the

recipient

Microangiopathic hemolytic

anemia

Burr cells (echinocytes)

A variety of anemias

Stomatocytes

Acute alcoholism

Bleeding gastric ulcers

Alcoholic cirrhosis

Gastric carcinoma

Glutathione deficiency

Peptic ulcers

Hereditary spherocytosis

Renal insufficiency

Infectious mononucleosis

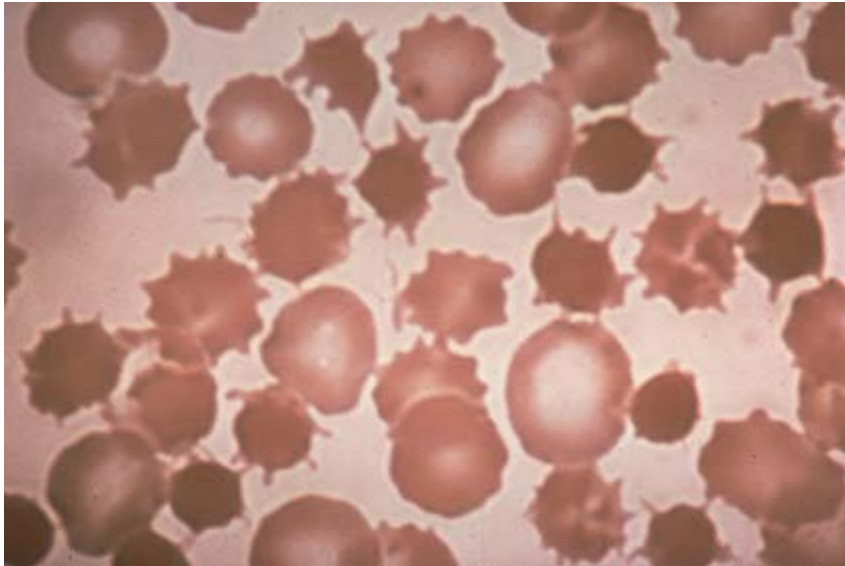
Pyruvate kinase deficiency

Lead poisoning

Uremia

Malignancies

Thalassemia minor



TABLE

7.2

Red Blood Cell Morphology and Related Conditions (continued)

Associated Clinical

Associated Clinical

RBC Morphology

Conditions

RBC Morphology

Conditions

Crenated RBCs (echinocytes) Diseases—none

Transiently accompanying a

hemolytic anemia

Result from osmotic imbalance

Elliptocytes

Anemias associated with

Target cells (codocytes)

Hemoglobinopathies: Hb C

malignancy

disease, S-C, S-S, sickle cell

thalassemia, thalassemia

Hb C disease

Hemolytic anemias

Hemolytic anemias

(occasionally)

Hereditary elliptocytosis

Hepatic disease with or without

jaundice

Iron deficiency anemia

Pernicious anemia

Iron deficiency anemia

Sickle cell trait

Postsplenectomy

Thalassemia

Artifact

Keratocytes

DIC

Teardrops (dacryocytes)

Homozygous beta-thalassemia

Myeloproliferative syndromes

Pernicious anemia

Severe anemias

Knizocytes

Hemolytic anemia, including

hereditary spherocytosis

Leptocytes

Hepatic disorders

Alterations in color

Iron deficiency anemia

Hypochromia

Iron deficiency anemia

Thalassemia

Polychromatophilia

Rapid blood regeneration

Poikilocytosis

Hemolytic anemias

Myelobrosis

Pernicious anemia

Thalassemia

RBC, red blood cell; G6PD, glucose-6-phosphate dehydrogenase; DIC, diffuse intravascular coagulation; Hb, hemoglobin.

m m . T m m

m x

. A

m m , m -

; m m ; ;

m .

Bite cells m m m .

I .

Blister cells (F . .)

m m k . T

m m . T m .

FIGURE 7.6 A . I m

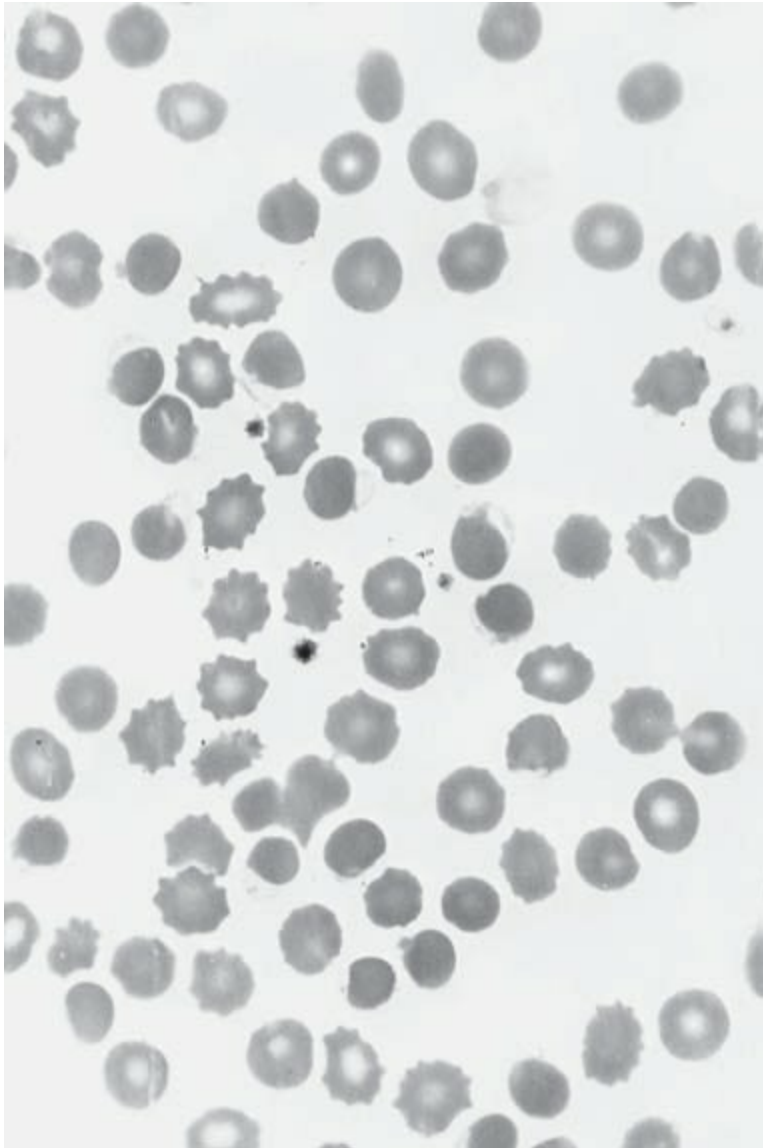
I , ([F . .])

m m ,

m ([F . .]) . T

m j m . (R m m m m (. . , R E, F JL. Pathology, , P , PA: L
) . B m m

W m & W k , .)



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m m . T j

m . C

(

C m). N

, m

m m .

Elliptocytes (F . .) m m . T , , . T m m

m m -

. A -

, m m , m (H) C , m m (), m , m , k , m .

Helmet cells (z) -

(F . .) m -

m

m . T m m

. (S -

.)

Keratocytes (F . .)

m . T , m ,

m . U , k

FIGURE 7.7 B H z –m

-m . T

m . B

m () (DIC).

m m . (R m M C KD. Clinical Laboratory

Medicine, , P , PA: L W m & W k , Knizocytes m . T m , m .)

m m ,

.

Leptocytes m () ,

.

m m

C , m

m m . T -

m m k m m -

, m ,

m m .

m .

Burr cells m -

Oval macrocytes (m) - k j m m . T q (F . .). A m m q m .

, m m

B . I ,

. I , -

. P j

m - z m . I

m . T

m m B

m . T m

m -

.

.

m , m ,

Pyknocytes (F . .) , -

. T m -

m . T

m .

, m m ; - - -

C , m , (G PD) ; , m , ,

m m m

f , k , m .

m k .

T m .

Schistocytes (z) (F . . F . .) Degmacytes ()

m m

G PD x - m -

. B

. T (RBC)

k ,

m , z m m . I -

. I m

, RBC m m x z . T

m m

RBC m H z .

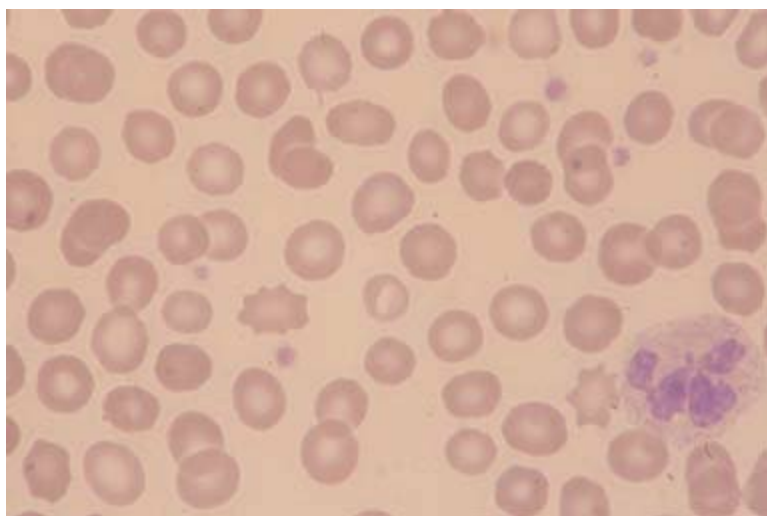
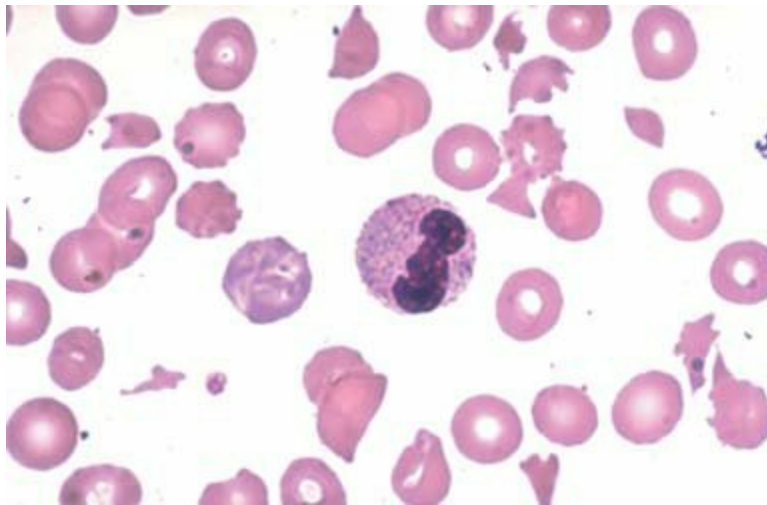
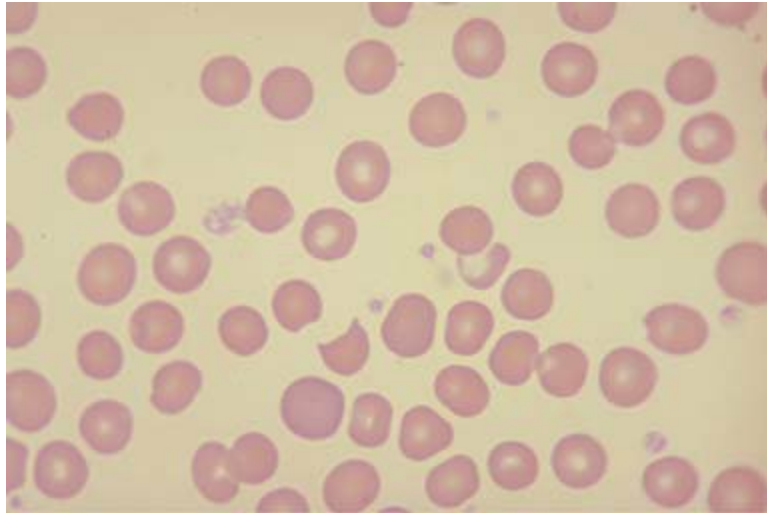
m j .

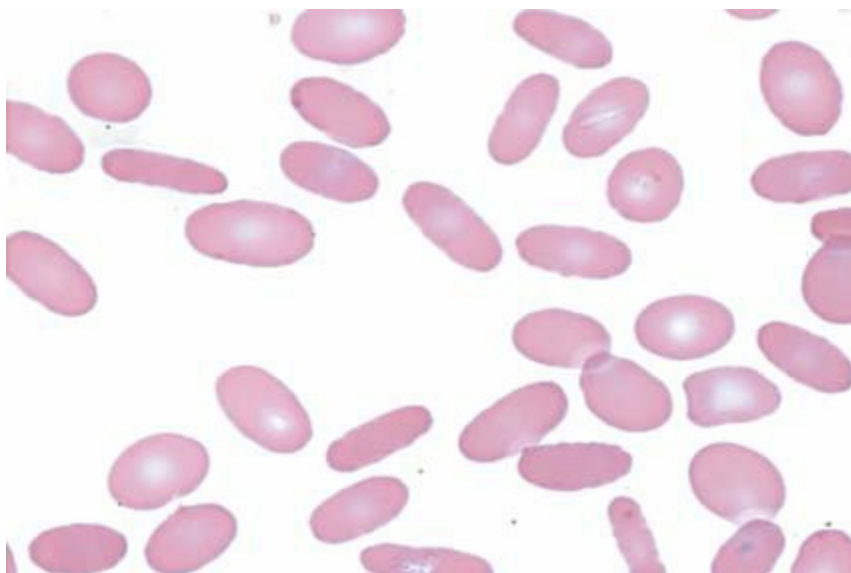
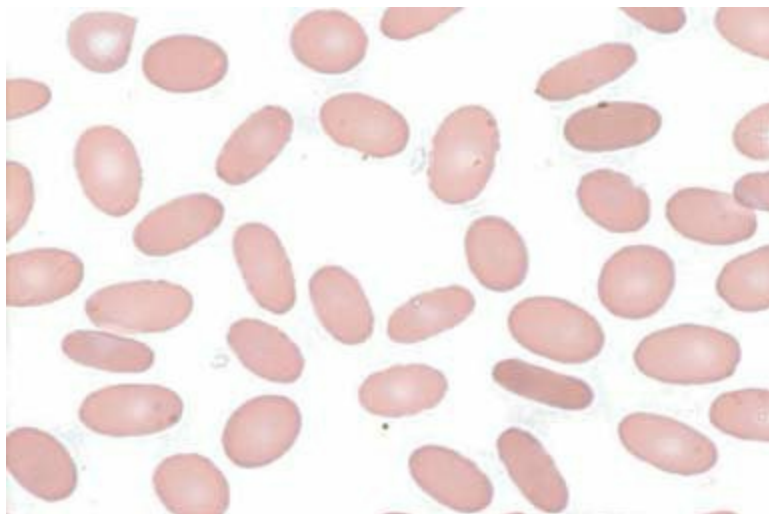
Echinocytes ([F . .])

Sickle cells () m (F . .).

, , k - k j

A m . G ,





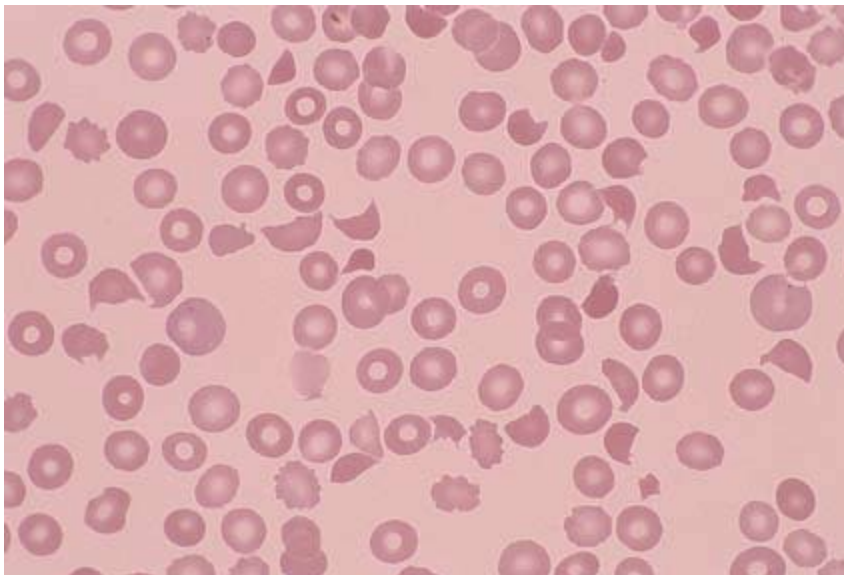
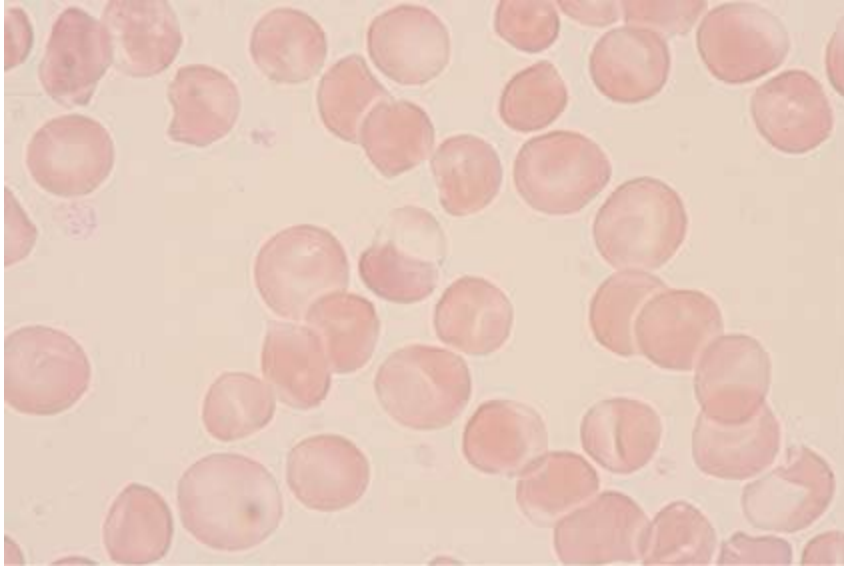


FIGURE 7.12 P k , , k -

. RBC : () (le), () (middle), k (m) (right).

(L AR m L W m & W k . A

.)

FIGURE 7.8 K (). (R m A SC.

Anderson's Atlas o Hematology, P , PA: W K

H /L W m & W k , , m .) **FIGURE 7.13** O (). (R m A SC. Anderson's Atlas
o Hematology, P , PA: W K

H /L W m & W k , , m .) **FIGURE 7.9** M m m . I , -

m () m DIC. (R m R E, F JL. Pathology, , P , PA: L W m & W k , ,
m .)

FIGURE 7.14 P k (arrow) (). (R m A

SC. Anderson's Atlas o Hematology, P , PA: W K

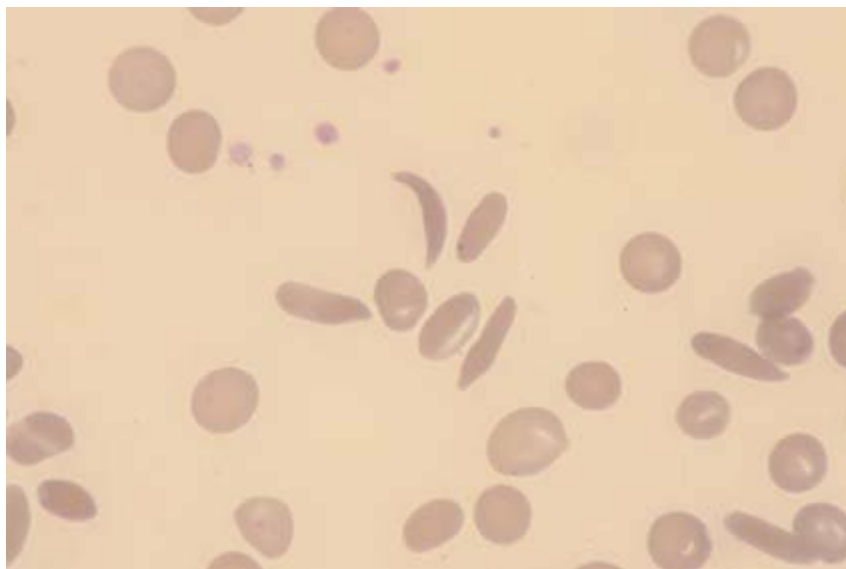
H /L W m & W k , , m .) **FIGURE 7.10** E (). (R m A SC. Anderson's Atlas
o Hematology, P , PA: W K

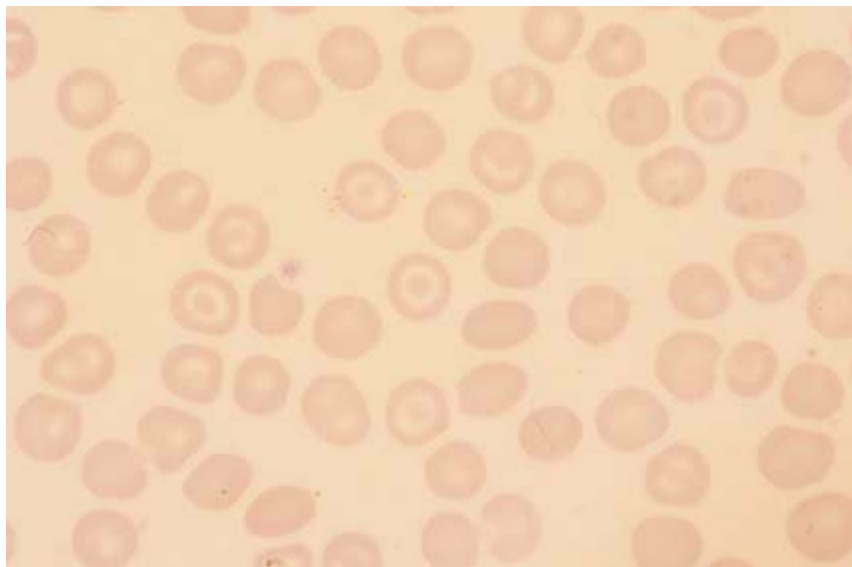
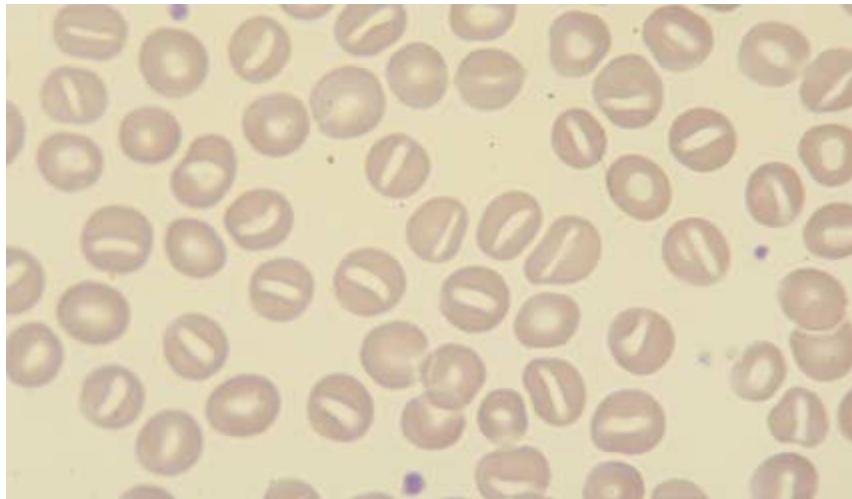
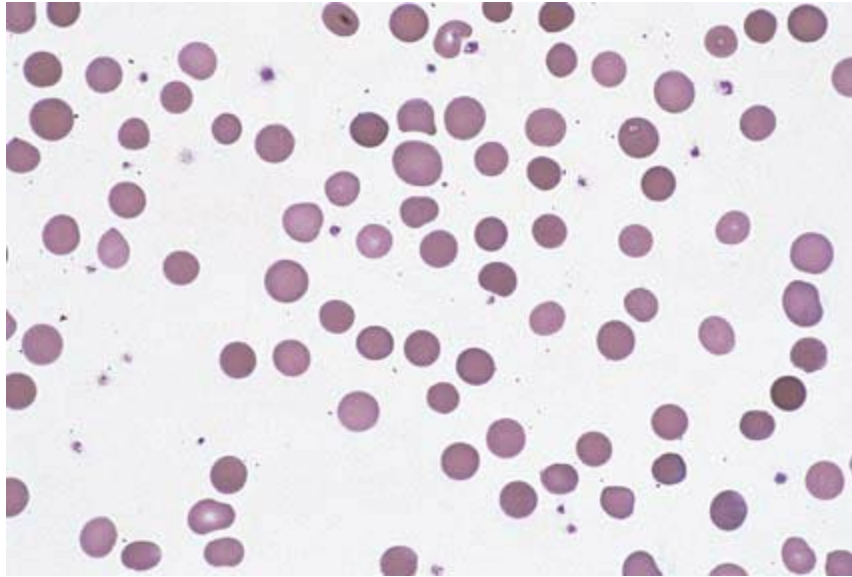
H /L W m & W k , , m .) **FIGURE 7.15** P m **FIGURE 7.11** H (HE). (R m m
m -

A SC. Anderson's Atlas o Hematology, P , PA:

. (R m F D. Pathology o Bone Marrow

W K H /L W m & W k , , and Blood Cells, , P , PA: L W m & m .) W k , , m
.)





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FIGURE 7.18 S m . (R m A SC.

Anderson's Atlas o Hematology, P , PA: W K

FIGURE 7.16 D (k). (R m A SC.

H /L W m & W k , , m .) Anderson's Atlas o Hematology, P , PA: W K

H /L W m & W k , , m .) m m j . C

m m m , m

q m -

. S k m mm , , , z x H S. P m z H S -

DIC. M ABO m -

x

mH. A m m m

m

. T x m

. H -

m x m

m m m .

m . A

Spiculated erythrocytes -

m m . T k

. S m

k m .

, , k , , , Spherocytes m . T m **echinocyte acanthocyte** -

(F . .). T x m

m .

m , . I m μ m

Stomatocytes (F . .) - k m-

- . S - k

m . T - k .

m x m

S m m m (N +)

m m . S

m (K+) -

m

m . C

m mm m,

. T m m m m -

, , ,

. M m m

m , , m , -

m .

m m , m m -

S m m

m . T m

m m m

R , k R m x.

Target cells () m (F . .). A ' -

. T

FIGURE 7.17 H , . N

m m .

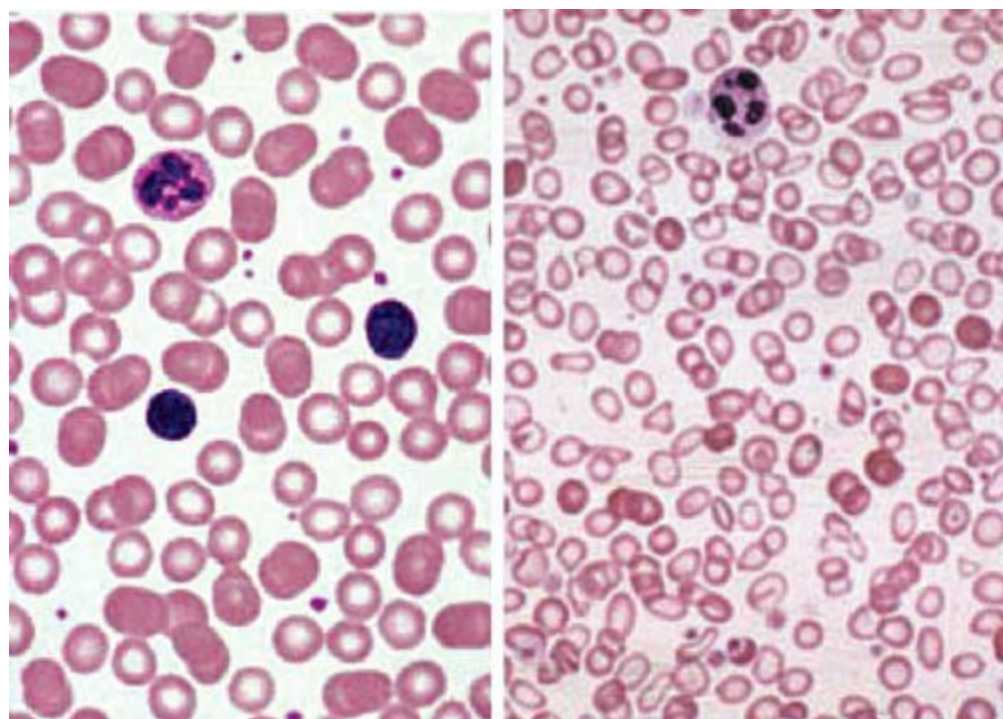
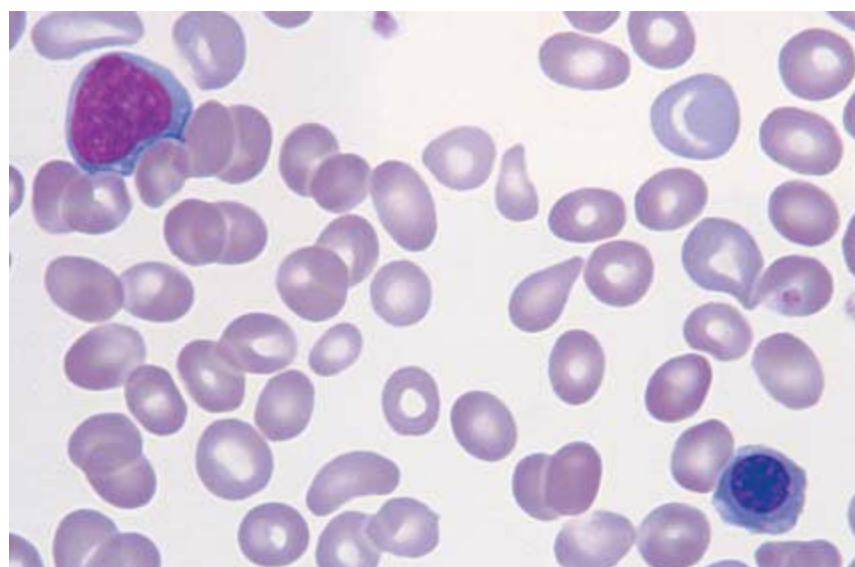
(R m F D. Pathology o Bone Marrow and Blood

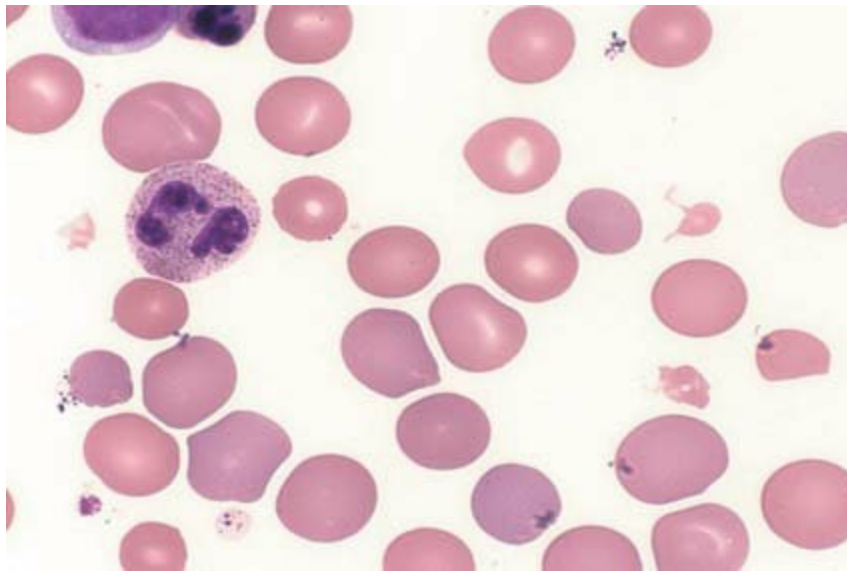
FIGURE 7.19 C (). (R m A SC.

Cells, , P , PA: L W m & W k , Anderson's Atlas o Hematology, P , PA: W K

, m .)

H /L W m & W k , , m .)





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FIGURE 7.20 m -

m k RBC .

(R m M C KD. Clinical Laboratory Medicine,

, P , PA: L W m & W k , ,

A

B

S ma l , pa le RBCs

m .)

FIGURE 7.21 T m . **A.** N m m . **B.** B m m . T

m , m x -

m (m) (m). (R

m m m . D -

m T m HM. T e Nature o Disease: Pathology or the Health

m m m ,

Pro essions, P , PA: L W m & W k , , m , m . I m m .)

, m m ,

m m . I z m

. I m , q

, m

m . D

m

m x q

m m . C ,

k -

m (H C , S-C S-S ,

m . H m -

k m , m), m m , m .

j ,

A m

m m . L -

mm . T m polychromatophilia

.

-

Teardrop cells () m -

(F . .) W . T k

m (F . .). A m m ,

m m ,

m . T m

RNA m.

m z - m , m -

U , m

m , m , m .

m . I , m -

k reticulocyte (F . .).

NOTE: This is a good time to complete Review Questions

A m, ,

related to preceding content.

AL ERA IONS IN ERY HROCY E COLOR

A m m k - -

-

. T m

m . T m -

, m x

' m

. U ,

normochromic. N m m

m m .

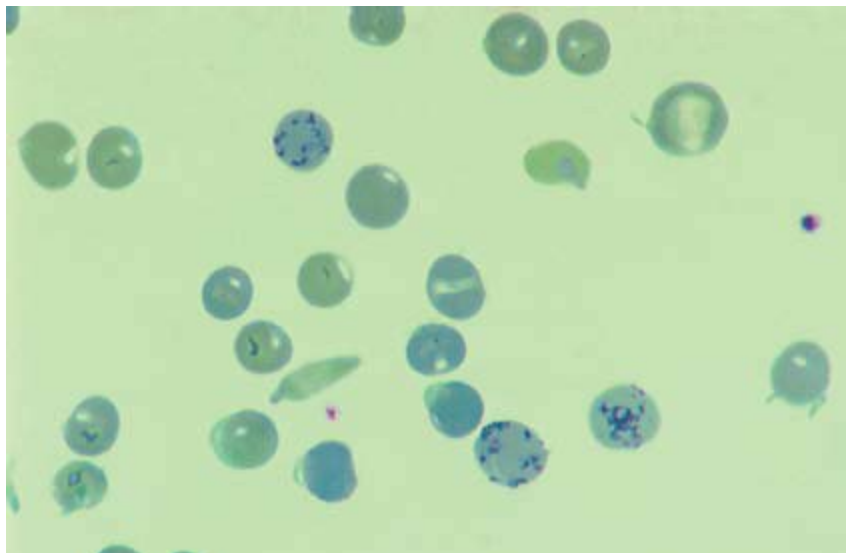
FIGURE 7.22 E . P m , T m m RBC , .

anisochromia. A m m, hypochromia, m

(R m A SC. Anderson's Atlas o Hematology, mm x P , PA: W K H /L W m
&

' m (F . .)

W k , , m .)



PART 2 ■ Hematopoiesis and Cellular Maturation

q m m

m .

Basophilic stippling () , , - -

, k- . T -

q x m

m. C (F . .) m -

m punctate stippling. T

m m

m . S

m m RNA

m . S

(-

FIGURE 7.23 P m ; m), , m .

m . T -

Cabot rings (F . .) - , - , -

, RNA. (R m M C KD. Clinical

. O , m m

Laboratory Medicine, , P , PA: L W m

& W k , , m .)

m . T

. T m

m m m m .

- k .

H , -

I m m

m m . C

m .

m .

C H C - k -

q m . T -

VARIE IES OF ERY HROCY E

H C (F . .). H H m

INCLUSIONS

, m . T

S

m HB A.

W . I

Heinz bodies (F . .) , . . μm z , m (x),

m

. T , m -

(DNA RNA), m ,

m

m , . T

m , G PD , m m

(

, m m .

m), C , H z , H -J ,

Howell-J olly bodies (F . .) , - , P m , .

k- , μm z . I , M W . H , H -J . A m (.). T

m q m

TABLE

7.3

S taining Characte ris tics of Erythrocytes and Inclus ions

S tain

Inclus io n

Fe ulge na

S upravitalb

Wright

Basophilic stippling

Negative

Positive

Positive

Cabot rings

Negative

Negative

Positive

Howell -J olly bodies

Positive

Positive

Positive

Polychromatophilia

Negative

Negative

Positive

Reticulocytes

Negative

Positive

Negative

Pappenheimer bodies

Negative

Positive

Positive

Heinz bodies

Negative

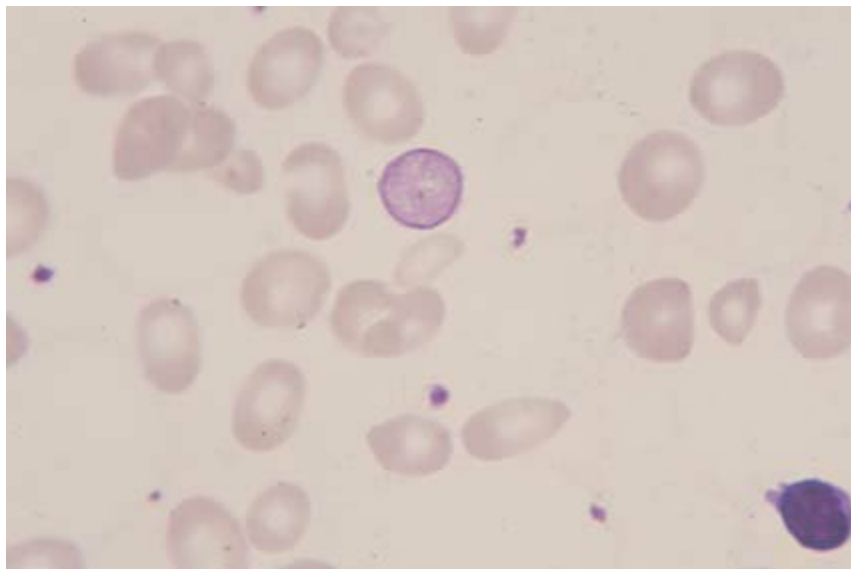
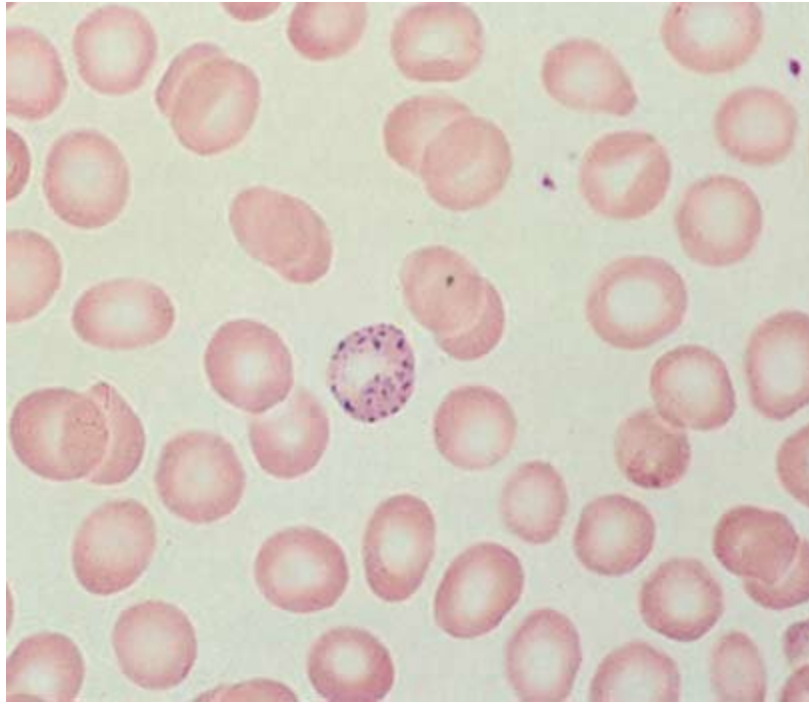
Positive

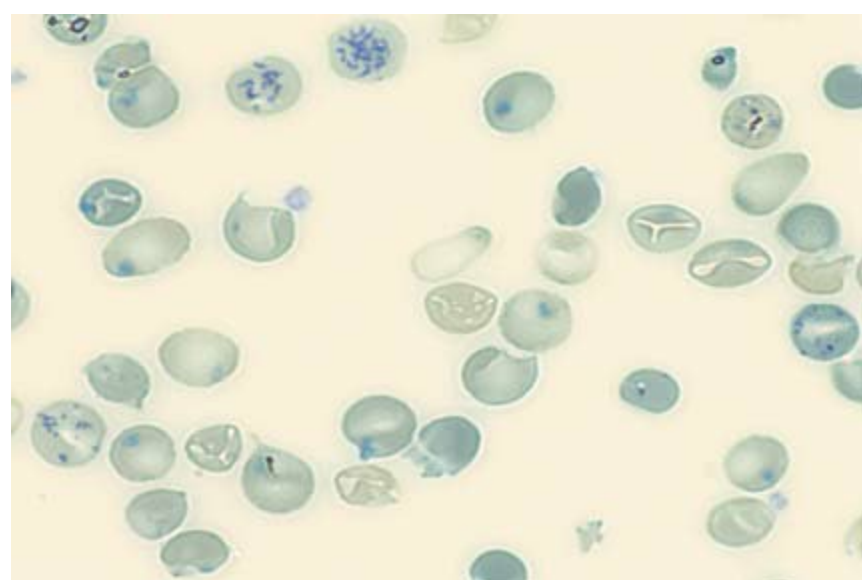
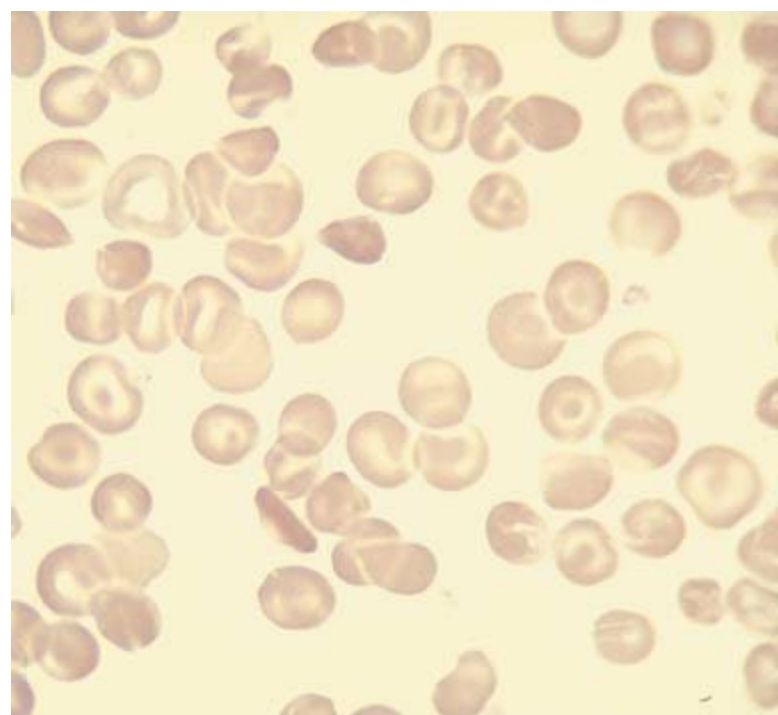
Negative or positive

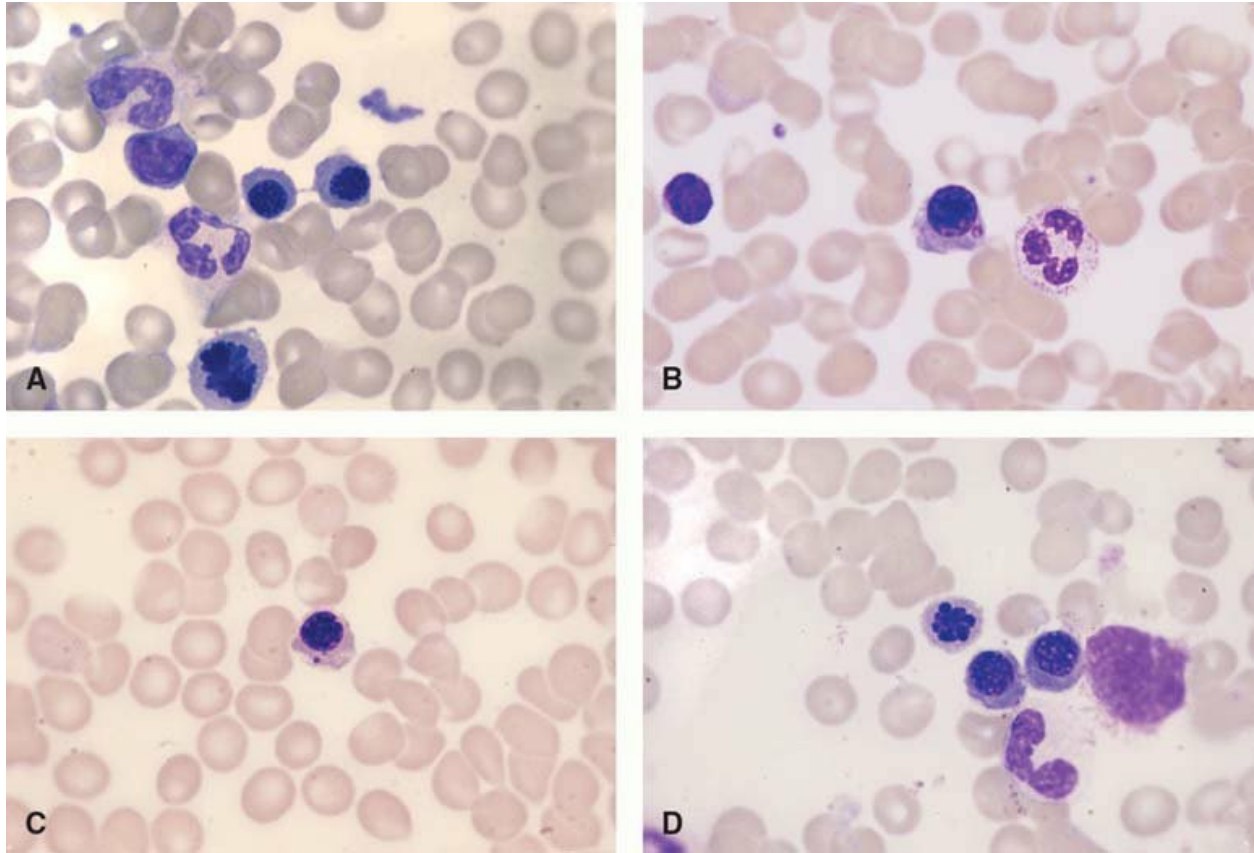
aFeulgen stain demonstrates the presence of DNA.

bSupravital stains (e.g., new methylene blue or brilliant cresyl blue) demonstrate the presence of RNA.

cCan be demonstrated with crystal violet stain.







CHAPTER 7 ■ Erythrocytes: Morphology and Inclusions

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FIGURE 7.24 H m -

FIGURE 7.26 H m C (arrow). (R m

. (R m G JP (.). Wintrobe's Clinical Hematology,

A SC. Anderson's Atlas o Hematology, P , PA: W

, P , PA: L W m & W k , ,

K H /L W m & W k , , m .) m .)

FIGURE 7.27 H z . (R m A SC.

FIGURE 7.25 C . (R m A SC.

Anderson's Atlas o Hematology, P , PA: W K

Anderson's Atlas o Hematology, P , PA: W K

H L W m & W k , , m .) H L W m & W k , , m .) **FIGURE 7.28** M

m -

m m .

A. I m

. **B.** L H -J

m .

C. m H -J

m .

D. K x -

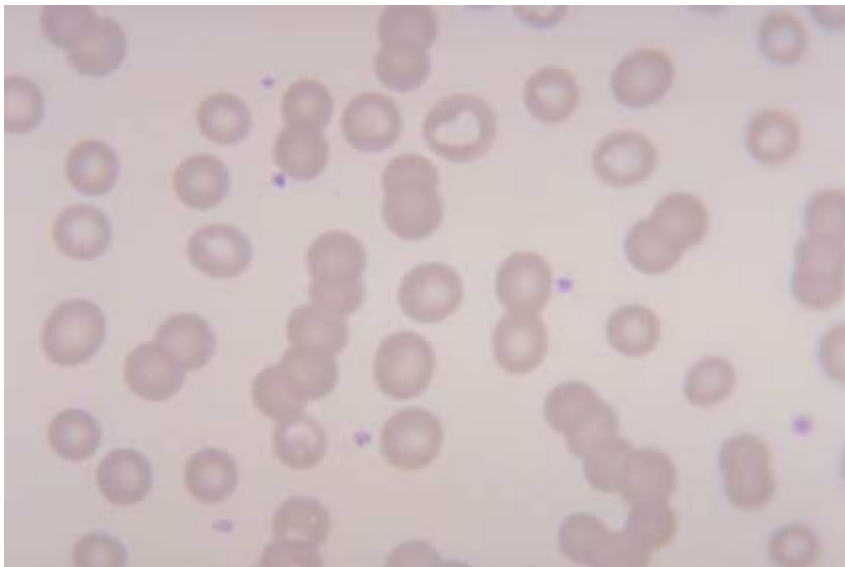
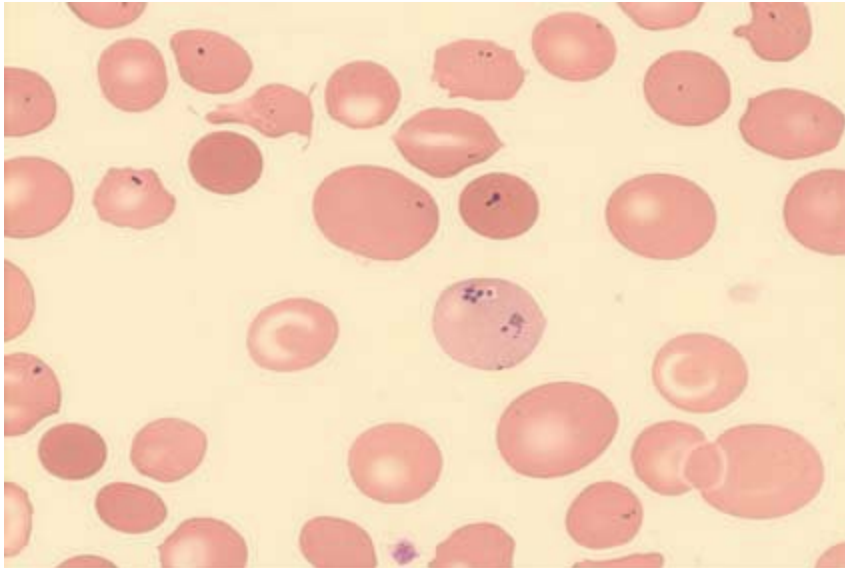
m . (R m

S EM. Histology or Pathologists,

, P , PA: L

W m & W k , ,

m .)



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FIGURE 7.29 P m (). (R m **FIGURE 7.30** R x (k). (R m A SC.
Anderson's Atlas o Hematology, P , PA: W

A SC. Anderson's Atlas o Hematology, P , PA:

K H L W m & W k , , m .) W K H L W m & W k , , m .) k , m mm , -

PARASITIC INCLUSIONS IN

. H -J -

ERYTHROCYTES

m . T m m

m DNA. H -J

Malaria

m -

, k

I m m, m m m j m m . T H -J -

- m : m ,

m m , m ,

, m m . S m

m ,

m , m j

.

m - m . O

Pappenheimer bodies (siderotic granules) m

, z mm

W - m . T

m m . M

q m . S

m , A , m -

k- -

m x . m .

—P .

T (F +) .

Et io logy

P m (F . .)

T m

. P m

m m m m : m , m , .

Plasmodium vivax, P. alciparum, P. malariae, P. ovale.

C , - m , T Plasmodium m m, m m .

m q .

Th e Dis e as e Pha s e in t he Mos quito

AL ERA IONS IN ERY HROCY E

T (F . .) m **DIS RIBU ION**

m q m q

A x m

m . T ,

m

m m m m ,

m .

m m q . I m q , m

Agglutination, m -

, m .

m m m

Rouleaux formation (F . .),

m m k

m m . C , m

, k m

m m m m m ,

m . I x x

m z m m m z .

m j

T z k -

, x . -

. T -

m m

. T , x m

- k z ,

.

m q . T z

m q

x m q . T

Plasmodium

NOTE: This is a good time to complete Review Questions

m m . I m m

related to preceding content.

P. vivax P. malariae.



CHAPTER 7 ■ Erythrocytes: Morphology and Inclusions

153

I , x k

. T , z ,

9

8

m

. A z -

, ,

m z , . D

, m

z . H m -

m , -

. E k

7

. A m m

z m (, , m

m)

k . C -

6

1

z m k -

. T z m k

k m .

5a

U , m , m

. G m ,

2

5

m m z , m m m m . G m m m

m q x , m ,

3

4

m q .

FIGURE 7.31 M m m. P m m—

: (1) z RBC; (2) “ ” -

Symptom s

m ; (3) m m ; (4) x ; T m m m -

(5) ; (5a) RBC

m . T m

m ; (6) m x m ; (7) x -

m m m q k m

; (8) z ; (9)

z . (C N O. H , W , C .)

. A ' m m °F °F.

T m m m

Th e Dis e as e Pha s e in Hum a ns

, m .

S z j m m

m q m m -

La boratory Da t a

m . I

T m m

, x m

(C m -

. T m m -

). M m

z . S q

m ,

m z .

(.).

TABLE

7.4

Red Blo od Cell Mo rpho log ical Fe atures o f Malarial S pecies

Pla s m odium **sp.**

Size

Inclus ions

Cytoplas m

Me rozoites

P. vivax

Enlarged

Schüffner dots

Blue discs with red nucleus

12–24

Accolé forms

Signet-ring forms

P. falciparum

Normal

Maurer dots

Minute rings

6–32

Two chromatin dots

Accolé forms

Gametes crescent shaped

P. malariae

Normal

Ziemann stippling

One ring with one dot

6–12

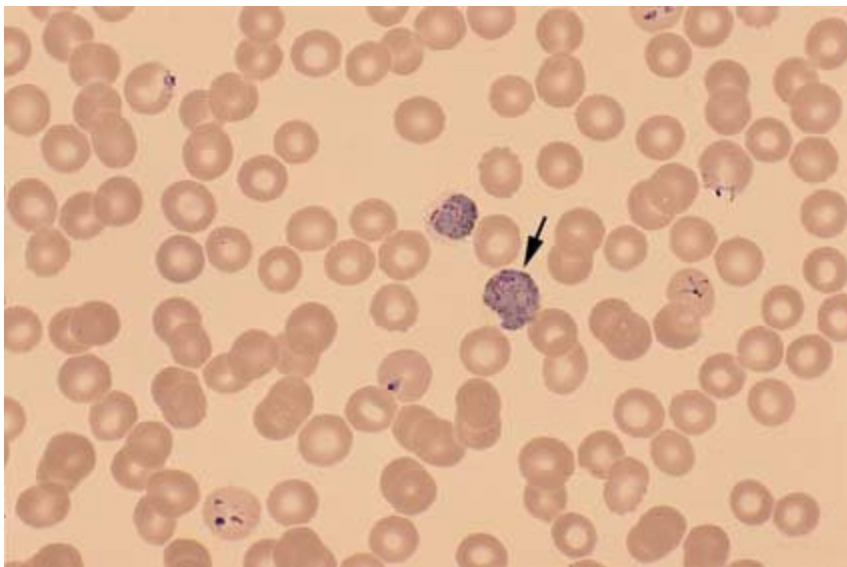
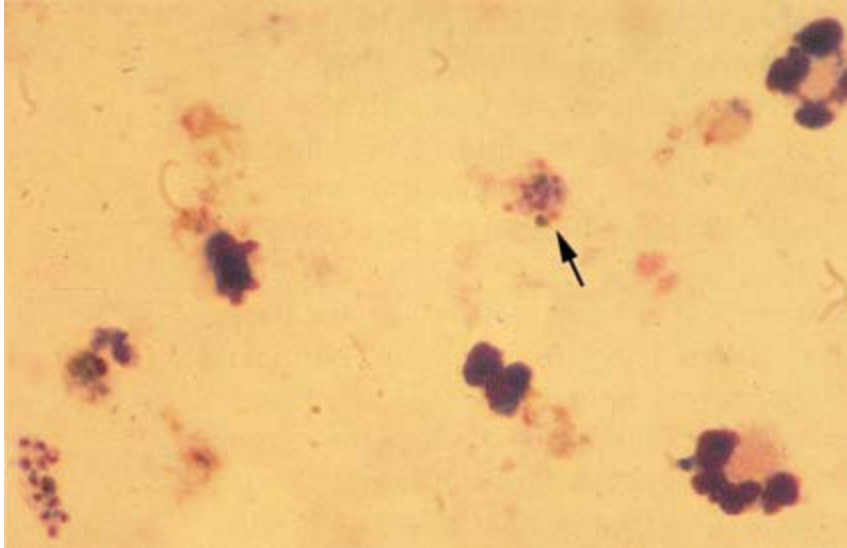
P. ovale

Enlarged

Schüffner dots

One ring form

6–14



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PART 2 ■ Hematopoiesis and Cellular Maturation

B , z x m z , m .

I -

. T m m

m k k S ü

. T S ü m .

A S ü m ,

m

. I ,

P. vivax P. ovale. D

x , -

FIGURE 7.32 A k m m P. vivax -

z ,

z (arrow), z , k .

. T . D

, k z . T

M

m m m m m

RNA PCR k (. .), M , I ,

. T

m

k m z ,

- II (HRPII) P. alci-

(), -

parum -m , mm m

. T m m z .

m (.I M).

F m m m m

m m . M m m -

Plas m odium viva x

m m . T

Plasmodium vivax m m

m m ,

. I x x

m m m , k.

m m (F . .

A m m

.). T x

k-m , m

m k

. Y z m cannot -

. I m m , m j

m m P. ovale P. alciparum.

m

G m P. vivax, P. ovale, P. malariae

, z . G m - m m -

m . P. malariae m k

k m

not S ü .

. S m m , z -

- m (é Plas m odium fa lcipa rum m). A , m

Plasmodium alciparum m m

m , m -

. I m

. A , m

. F , m m m

. m m m

m m , m m

, . V z -

. T

m m m .

O P.

alciparum O m - m .

S z k

. Y z m

m .

Y z m . M m -

q , m m m . M m mm .

T m P. alciparum (F . . .)

. I -

- . T m m

, m -

m m

. I ,

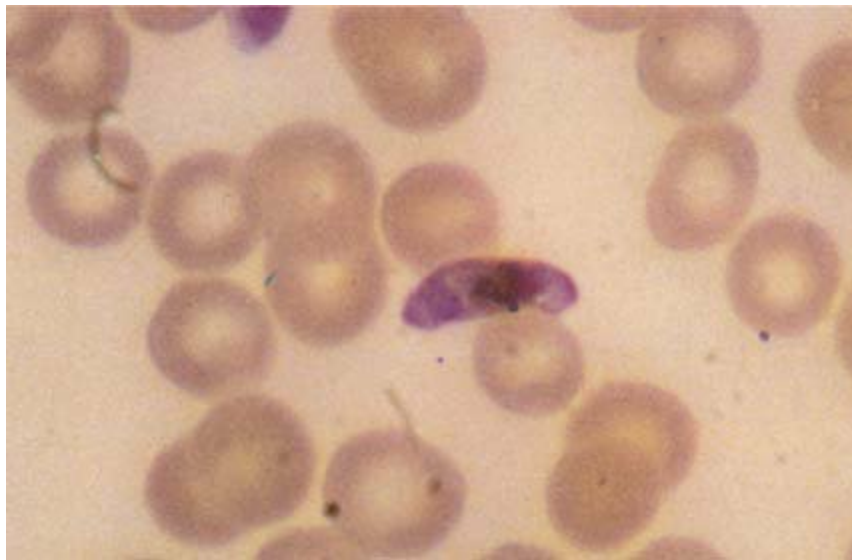
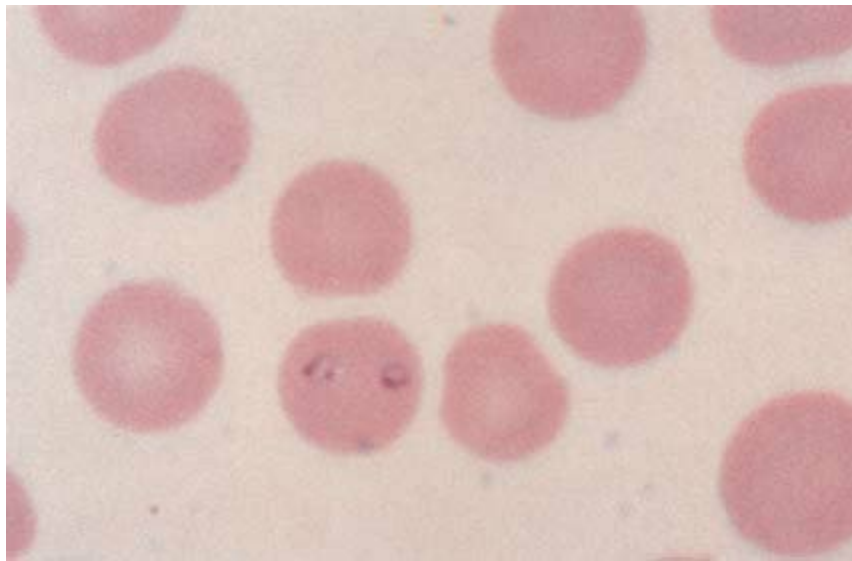
FIGURE 7.33 A m m P. vivax

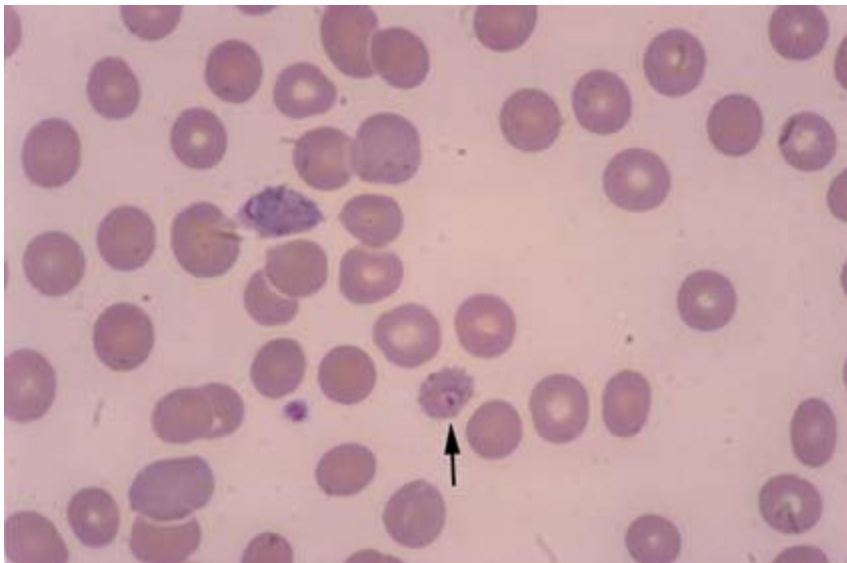
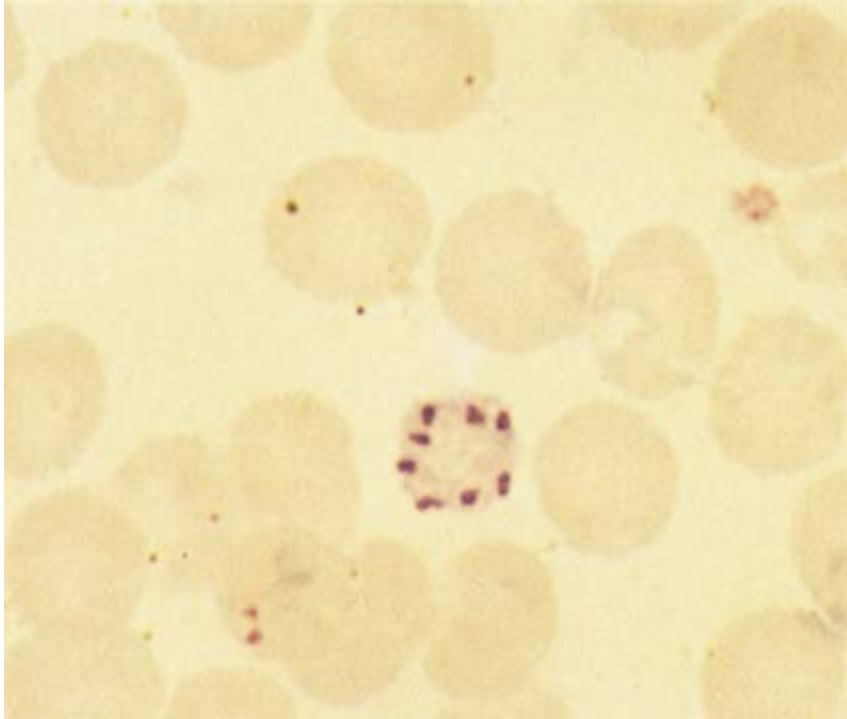
m m m . T m -

z , m k -

m m (arrow). G m , k- , - - k × , .

M .





CHAPTER 7 ■ Erythrocytes: Morphology and Inclusions

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FIGURE 7.34 A (center)

m *P. alci*parum, m .

I k m , m z

FIGURE 7.36 *Plasmodium malariae*: z

. B , q

m .

m mm .

G m z , m q k. T m m z -

m m.

, m mm

P x m *P. alci*parum RBC

m m m . ,

m m k

m z .

m . T RBC m mG m f m

, m , -

z . W m , m

, q

m z , ,

RBC .

m m z

. T k m m m

Plas m odium m a la riae

m

Plasmodium malariae m

m m

m m

m. O z m , -

q P. vivax P. alciparum. A

m m m .

m m

(F . .). T x P. malariae

Plas m odium ova le

m x m .

Plasmodium ovale

R m P. malariae m A P. vivax q W A P. vivax. O m

. I S Am A .

, m -

A m m

m . I . A

m .

, m m z .

m Plasmodium m

T z m z . G , P. vivax. I m m m m , (F . .). O m

m m - ,

- k , Z m . T

. P m

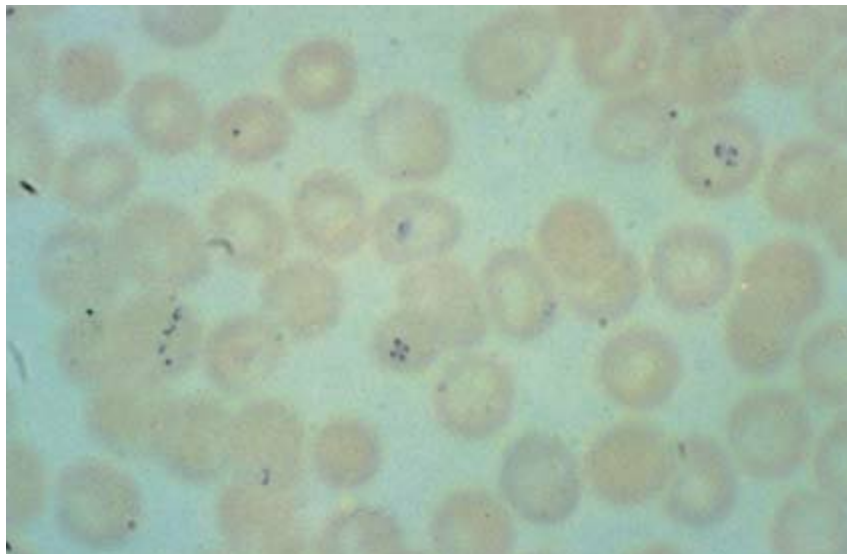
FIGURE 7.37 A m m P. ovale

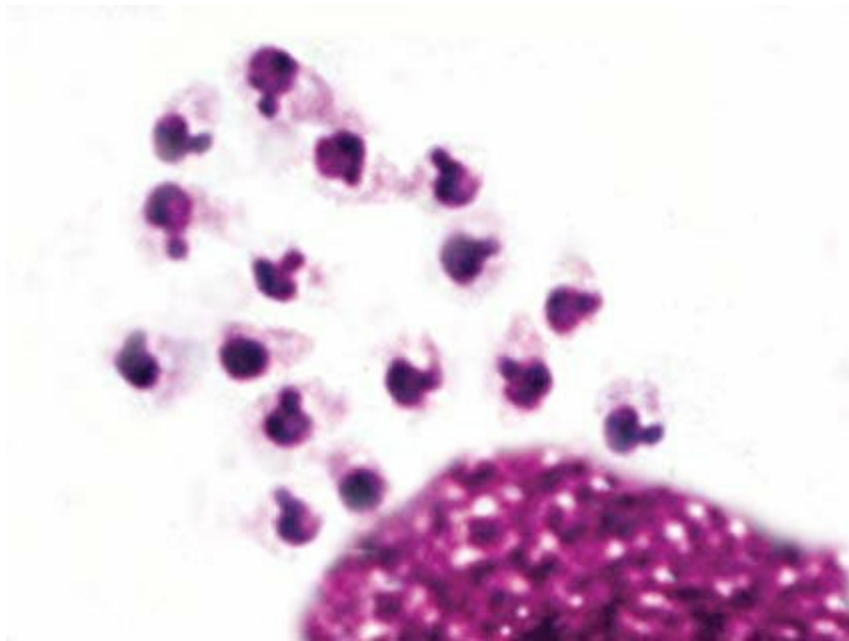
mm z m. O

m

FIGURE 7.35 A m P. alciparum. G m , × , .

(arrow). G m , × , .





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PART 2 ■ Hematopoiesis and Cellular Maturation

, m -

m k ; , m .

mm m -

T m z z . T

. I U S , m

,

N k , MA, . B m -

, x S ü . T m

U S

, -

, , . E m

, , .

N Am -

T k m m P. vivax. S z

U S , L I , N Y k,

P. malariae m

N k M ' V , MA.

m z . S ü .

Signs an d Sym pto m s

Other Paras itic Includ ions

T m m

S .

m RBC Babesia (F . .). F , m -

T m rypanosomatidae, m -

m , m m m Babesia

, z m . T

. A m , k /m

m

m RBC m , x

, m , -

, , , m m .

. Leishmania (F . .) m

W m , m m m m m m m m RBC m m

m .

. RBC m m .

B (F . .) B-m

Ba be sio sis

- m . A m , - -m

mm m mm ,

Etiolo gy

mm m

Babesiosis k- , z -

x B-m .

m - k m m m

m . I m P. alciparum,

La bora tory Findings

m m m

Hematology

x .

T m m m -

H m U S

m - k

Babesia microti, m . T

m (F . .). M -

m N Am

m m

m -

Babesia m . M

m Babesia microti. B m

m m -

m .

% m

Ep ide m iolo gy

. P

m .

H m Babesia

W G m m

m k . A , , mm m -

m . P Babesia ,

m , m

m m z

FIGURE 7.38 Leishmania . m m -

, k . (R m **FIGURE 7.39** A m -

C P . Color Atlas and ext o Pulmonary Pathology, P ,

. A m . G m , PA: L W m & W k , , m .) × , .

CHAPTER 7 ■ Erythrocytes: Morphology and Inclusions

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m M . m -

■ m m

m . B m m z .

m m m z ,

■ M m -

.

m .

O m

■ M m (CBC) m (ESR). A CBC

.

k H -J , k , m-

, m . T ESR .

Kinds o f Variatio ns in Erythro cyte Shape

Chemistry

■ T m m

S m -

m ,

mm B-m -

m , ,

- m Babesia. L

k .

k -

■ P k m m q

m (SGP /AL), k

m mm j , , -

, m , .

. S m m

T m m .

.

A

■ D m -

m . A m

m

k m . T q

m m .

m

Alteratio ns in Erythro cyte Co lo r

Babesia .

■ A m m k -

Immunology/Serology

-

Imm M (I M) I G mm

.

(IFA) B. microti m

■ T m m

.

m (. ., m). A I M IFA

■ N m

: . I I G

m m .

IFA Babesia x

■ T m m

. P m m

m . A m m, m ,

Babesia

m mm x

m

' m .

q .

■ A m

mm . T m -

m

NOTE: This is a good time to complete end of chapter

- W

Review Questions

.

■ I m m

CHAP ER HIGHLIGHT S

m -

.

Erythro cytes : No rmal and Abnorm al

Varieties of Erythrocyte Inclusions

■ Normochromic

pink.

■ Schistocytes

W, m

■ Iron, membrane, lipid

.

mm

m.

■ Anisocytosis

■ Iron,

.

m -

(DNA RNA), m -

Types of Variations in Erythrocyte Size

, m, .

■ N

■ Normochromic

(m), C, Hz,

■ E m m (m -

H -J , P m ,

) m m (m).

.

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PART 2 ■ Hematopoiesis and Cellular Maturation

Alteratio ns in Erythro cyte Dis tributio n

Paras itic Includ ions in Erythro cytes

■ A x m

■ O , z m

mm m m .

m .

■ T m

■ A , m , m

m m Plasmodium :

.

Plasmodium vivax, P. alciparum, P. malariae, and P. ovale.

■ R x m k -

■ B k- , z m m .

m - k m m .

CASE STUDIES

Cas e Study 7.1

Z m , . A m , -

m x . A

A - - mm N k

k Z m , k

I , MA, m k

m . H m

m . H

x k .

- k m m . H

m . H

H x m m m—

CBC .

. H

. CBC, , m

■ Laboratory Data

. A z m

. RBC . × /L

.

. H m %

. H m . / L

■ Laboratory Data

. WBC . × /L

. H m

. P × /L

. H m . / L, m %

. WBC . × /L

■ Follow-Up

. P . × /L

B ' m m mm -

■ Blood Smear Examination

, PCR mm -

S m %, m %, m

.

%, % = WBC .

U m .

■ Critical Thinking Group Discussion Questions

. W ?

■ Chemistry

A m , x

. W ’

(LDH) x

B ?

.

■ Follow-Up

. C B ?

T m Em

D m m , x -

Cas e Study 7.2

/ .

A m , - - m -

T x

m m

f . H m Em

k - - k,

D m , m .

m . F , x -

, ,

■ Critical Thinking Group Discussion Questions

. W m

. T m

x ?

. °F . H

k f . H k

. I m

- - m ,

m , -

.

m ' ?

T m -

. H - k

C C , M , m

Note: This is a good time to write out the answers to the

m m . H

Critical Review Questions Group Discussion Questions

CHAPTER 7 ■ Erythrocytes: Morphology and Inclusions

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REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

*8. M m

Erythrocytes: Normal and Abnormal

A. m

1. T m m _____

B. m

µm.

C. m

A. .

D.

B. .

C. .

Kinds o Variations in Erythrocyte Shape

D. .

9. T k

A. z

types o Variations in Erythrocyte Size

B. m

2. T m

C. m m

A. z

D.

B. m

C. m m

10. T q m m

D.

A. m

B.

3. T m

C.

A. z

D.

B. m

C. m m

11. T q m m

D.

A. m

B.

4. T

C.

A. z

D.

B. m

C. m m

12. T q m

D.

A. m

B.

*5. W

C.

m ?

D.

A. M

B. S k

13. T q m k

C. M

A. m

D. A

B.

C.

*6. W

D.

m ?

A. M

14. A m

B. S k

A. , , k - k j

C. M

D. A

B. m

C. - m

*7. M m -

D. m

A. m

B. m

15. A m m

C. m

A. , , k - k j

D.

B. m

C. - m

D. m

(continued)

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PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

16. A m

*23. M m

A. , , k - k j

A.

B. m

B. m

C. m

C. -

D. m

m

D. m

*24. C m

A.

17. A m

B. m

A. , , k - k j

C. m

D. m

B. m

C. -

*25. D m

m

A.

D. m

B. m

C. m

18. W m

D. m

m ?

A. M

Alterations in Erythrocyte Color

B. S k

26. A m m -

C. M

D. A

A. m

B. m

*19. R m m z x -

C. m

H S

D.

A. M

B. S k

27. P m

C. M

A. -

D. A

W

B. RNA

*20. A m -

m

C. q

A. m

B. m

D.

C. m

D.

Varieties o Erythrocyte Inclusions

28. B

*21. E m -

A. DNA

B. m

A. m

C. m m RNA

B. m

D. , m , m

C. m

D.

29. H -J

A. DNA

B. m

*22. L m

C. m m RNA

A.

D. , m , m

B. m

C. m

30. P m

D. m

A. DNA

B. m

C. m m RNA

D. , m , m

CHAPTER 7 ■ Erythrocytes: Morphology and Inclusions

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REVIEW QUESTIONS (continued)

31. H z

38. Plasmodium alciparum z

A. DNA

A. z m z ; -

B. m

m ; m

C. m m RNA

Z m

D. , m , m

B. m m ;

m z ; m S ü

*32. B

C. m

A. m

; m S ü ; m -

B. G PD

z z

C. m

D. z m

D.

; m -

- - -

*33. H -J

; M m

A. m

B. G PD

39. Plasmodium malariae z

C. m

A. z m z ; -

D.

m ; m

Z m

*34. H z

B. m m ;

A. m

m z ; m S ü

B. G PD

C. m

C. m

; m S ü ; m -

D.

z z

D. z m

*35. P m

; m -

A. m

- - -

B. G PD

; M m

C. m

D.

40. Plasmodium ovale z

A. z m z ; -

Alterations in Erythrocyte Distribution

m ; m

36. W m

Z m

m k

B. m m ;

m ?

m z ; m S ü

A. A

C. m

B. P k

; m S ü ; m -

C. A

z z

D. R x m

D. z m

; m -

Parasitic Inclusions in Erythrocytes

- - -

; M m

37. Plasmodium vivax z

A. z m z ; -

m ; m

41. N k I m

Z m

A. P. vi x

B. P. m

B. m m ;

m z ; m S ü

C.

D.

C. m

; m S ü ; m -

z z

42. Babesiosis m m m -

m

D. z m

; m -

A. Plasmodium alciparum m

- - -

B. L m

; M m

C. E

D.

(continued)

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PART 2 ■ Hematopoiesis and Cellular Maturation

G FK, . F m -

COMPANION RES OURCES

Plasmodium alciparum, Blood, (): – ,

:// . . m/

.

H DE, L A. Im RBC m m , Adv Med E W -

Lab Pro , (): – , .

m .

K m DM, R BS. E m -

H ,

m m , Am J Med echnol, (): – ,

-

.

.

L m D. M , Adv Med Lab Pro , (): – , .

M M, . C m

BIBLIOGRAPHY

m m , Haematologica, (): – ,

.

C CM, Dz k WH. T ABO m Plasmodium

R PJ. H m alciparum m , Blood, (): – , .

, Curr Opin Hematol, (): – , .

C BA, B B. B , M (. m . m), W G, W M. S m m : ?

m O , .

Blood, (): – , .

Leukocytes: T e Granulocytic and

CHAPTER

8 Monocytic Series

KEY TERMS

agranulocytosis

g ra n u le s

m ye lo cyte

azu ro p h ilic

g ra n u lo cytic kin e tics

n e u tro p h il

Au e r Ro d s

in a m m a tio n

n e u tro p h il e xtra ce llu la r tra p s (NETs)

ba so p h ils

le u ko e ryth ro b la s to s is

o p s o n in s

Ch a rco t-Le yd e n crys ta ls

m a cro p h a g e s

o p s o n iza tio n

che m o ta xis

m a r g i n a t i n g p o o l

p h a g o c y t o s i s

c i r c u l a t i n g p o o l

M a s t c e l l s

p r o l i f e r a t i v e c o m p a r t m e n t

c o l o n y - s t i m u l a t i n g f a c t o r

m a t u r a t i o n - s t o r a g e c o m p a r t m e n t

p r o m y e l o c y t e

d i a p e d e s i s

m o n o c y t e s

s e g m e n t e d n e u t r o p h i l s

e o s i n o p h i l s

m o n o n u c l e a r p h a g o c y t e s y s t e m

e t r a v a s a t i o n

m y e l o b l a s t

LEARNING OUTCOMES

Intro ductio n

No r m a l m a t u r a t i o n a l c h a r a c t e r i s t i c s o f

■ Name the source of the cellular elements of the blood.

g r a n u l o c y t e s

- Name the three major categories of the cellular elements of the
- Describe the nuclear and cytoplasmic characteristics of the neu—
circulating blood.

trophils, eosinophils, and basophils throughout the maturation

Granulocytic series : production of neutrophils ,

process.

- Explain the appearance and etiology of the various morphological

eosinophils , and basophils

abnormalities encountered in mature granulocytes.

Briefly explain the general factors related to the development of

- Define terms associated with an increase and decrease in
multipotential progenitor cells into specific leukocyte cell lines.
granulocytes.

Compare growth factors and inhibitory factors in granulocyte

- Explain the term, shift to the left
development.

Describe the abnormalities associated with mature granulocytes in

Granulocyte sites of development and

body fluids.

maturation

Leukocyte surface markers

■ List each type of immature neutrophil found in the proliferative com-

■ Define the term leukocyte surface marker.

partment of the bone marrow along with the percentage of each

■ Name one technique for identifying cell surface markers.

Cite the approximate time spent in each developmental stage of

■ Name the system for identification of cell surface markers.

neutrophils in the proliferative compartment.

■ List each type of neutrophil found in the maturation-storage com-

The monocytic-macrophage series

partment of the bone marrow along with the percentage of each.

■ Associate the various terms for macrophages depending on their

Cite the approximate time spent in maturation-storage compartment

anatomical site.

phase.

Discuss the differentiation of monocytes and macrophages from the

multipotential stem cell.

Distribution of neutrophils, eosinophils, and

■ Compare the bone marrow maturation of the monocyte with that of

basophils

the neutrophils.

Describe the chemical factors and cellular characteristics that per-

- Describe the general functions of macrophages.

mit neutrophils to exit the bone marrow and enter the peripheral

Compare the life span of monocytes and macrophages in the circu—
circulation.

lating blood and tissues.

- Define the terms marginating and circulating pools.

- Describe the nuclear and cytoplasmic characteristics of the mono-

- Discuss the length of time the neutrophils, eosinophils, and baso—
cyte as it develops.

phils spend in each marginating and circulating pool.

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Normal reference ranges and variations

Explain the physiological events that alter the number of circulating

- List the normal relative reference values for neutrophils, eosino—
granulocytes and monocytes in the peripheral blood.

phils, and basophils and monocytes in normal peripheral blood.

Describe how neutrophils exit the peripheral blood circulation and

- State the (neutrophilic) granulocyte reference range.

migrate to the site of an infection or injury.

- State the normal relative reference range for monocytes.

Discuss the specialized functions of eosinophils and basophils.

- Describe ethnic differences and daily fluctuation of granulocytes.

Compare differences in phagocytosis by monocytes versus neutrophils.

Functional properties of monocytes /

Define the abbreviation, NETS, and briefly describe.

macrophages

Case study

Explain the function of monocytes/macrophages.

Analyze the patient history, clinical signs and symptoms, and laboratory data for the stated case studies, answer the related critical

- Describe the general function of monocytes

laboratory data for the stated case studies, answer the related critical

Compare the phenotypic differences of the three subsets of monocytes—thinking questions, and conclude the most likely diagnosis. cytes in the circulating blood.

Phagocytosis

NOTE:

Explain the general characteristics and specific details of

■ Indicates MLT and MLS core content

phagocytosis.

Indicates MLT (optional) and MLS advanced content

IN RODUC ION

, m -

. S m k m ,

T m m m-m . C

m , m m . T

m . S q m -

m j -

m : , k , BOX 8.1

m .

L k

The Imm une Sys tem

, m -m , m-

- m . T

NATURAL IMMUNE SYSTEM

k , , **Cellular**

, , m -m .

■ M

E k q

■ N

, m ,

■ M

. T m m -

(PMN) k m -

Hum oral

m /m . T

■ C m m

“ - k” m m

■ L z m

mm m (B x .), m ,

■ I

, CD m . L m (C)

ADAPTIVE IMMUNE SYSTEM

m

. P m

Cellular

m j .

■ m

■ B m

■ P m

GRANULOCY IC SERIES: PRODUC ION OF NEU ROPHILS, EOSINOPHILS, AND

Hum oral

BASOPHILS

■ A

■ C k

T m m m m

Cell-Mediate d Im munity

m m x

■ m

m m m , k ,

■ D - -

x m x. C k m mm

x m

■ D , -

x -

m , m , j

. T m ,

CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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mm -

HS C P roge nitor ce lls

Ste m ce l pool

m x m .

0.1%–0.2%

Mye lobla s ts 1%

15 hours

Grow th Facto rs

P romye locyte s

24 hours

Mitotic pool

F

3%

colony-stimu—

Myelocyte s

5–7 days

lating factor (CSF) k . T

12%

Pos t Mitotic

CSF k , m m

m

(**myeloid**) m -m

. I

m , CSF m , **A**

m .

Grow th Inhibitors

Me ta mye locyte s

C m (CDD) k

45%

-m - m

(GM-CFC). V

Ba nd form

Se gme nte d

m , m m

ne utrophils

ne utrophils

m - β , m

35%

20%

$-\alpha$ m $-\beta$, m mm -

- , .

B

FIGURE 8.1 B m m m . A. P .

GRANULOCYTES ES OF DEVELOPMEN

B. M - ().

AND MA URA ION

m m m . T

T m , , -

x m %, %, %, -

, ,

. T m m -

granulocytic kinetics . T , m m q m .

, m

T - - . I m . T m -

m m m

, m , m , m

m - . E

m . M .

m . , m

W - m , - -

, x m .

m k (CFU-GEMM) -

N k m m

- m , -m

m m — m , -

(CFU-GM) , m mm

, . M m -

m ; m -

m m m

m m m m

m . I ,

m k

m

m ' m m (F . . A). T

. I ,

mitotic pool DNA

. I GM-CSF G-CSF

.

mm ,

T myeloblast -

. M x m %

k .

m . T x -

m . T x , **promyelocyte**,

DIS TRIBU ION OF NEU ROPHILS,

x m % m . T

EOSINOPHILS, AND BASOPHILS

. T **myelocyte** x m -

, x m %

T neutrophils m m

x . T m m **metamy**

m m x . C -

elocyte . . O m m (F . .) m m -

,

m

proliferative phase m .

m , m m . S m

F , mat

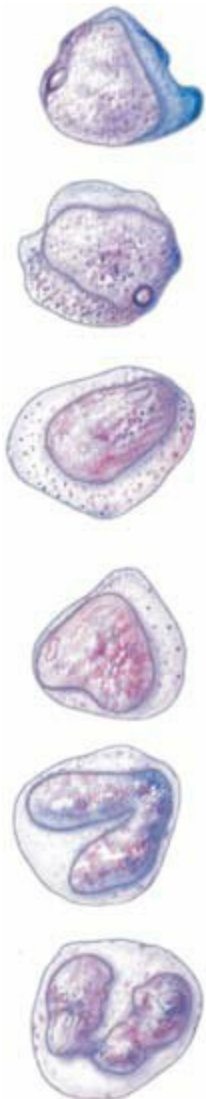
-

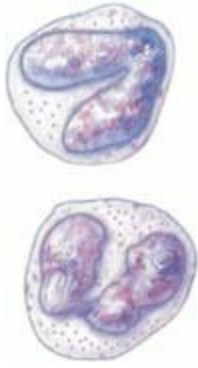
uration storage compartment (F . . B). T m m -

k . C -

m m m

z m - m . T





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PART 2 ■ Hematopoiesis and Cellular Maturation

Immature cell

Mature cell

NOTE: This is a good time to review definitions of the Key Terms in the Glossary and flash cards on

. It is

a good idea to complete Review Questions related to the preceding content.

Decreased overall

cell size

NORMAL MATURATIONAL

CHARACTERISTICS OF GRANULOCYTES

Myeloblast

Decreased nuclear:cytoplasmic (F...;...),

cytoplasmic ratio

m

myeloblast (F . .). T m-

µm. T m -

, - . T m m m m k . **Auer rods** (F . .),

Increase flexibility

m , m , - k -

and mobility

m . T m

. A , m , .

FIGURE 8.2 C m m . A m , m **Pro myelocyte**

m z , -

T **promyelocyte** (F . .) m -

m , x m .

. T

m m

x m m

m .

m m

T m azurophilic

.

z m m x (MPO)

T q

. T m ,

q z : **circulating pool**

marginating pool. T m

m . S m

. M

Mye lobla s t

. T m m m

P romye locyte

diapedesis. O , -

, ,

phagocytosis.

T m -

Ne utrophilic myelocyte

x m .

O m m ,

, -

, x , m m .

Ne utrophilic me ta mye locyte

E

. B

m . .

I x m

Ne utrophilic ba nd

, m

Charcot-Leyden crystals ,

m .

I m

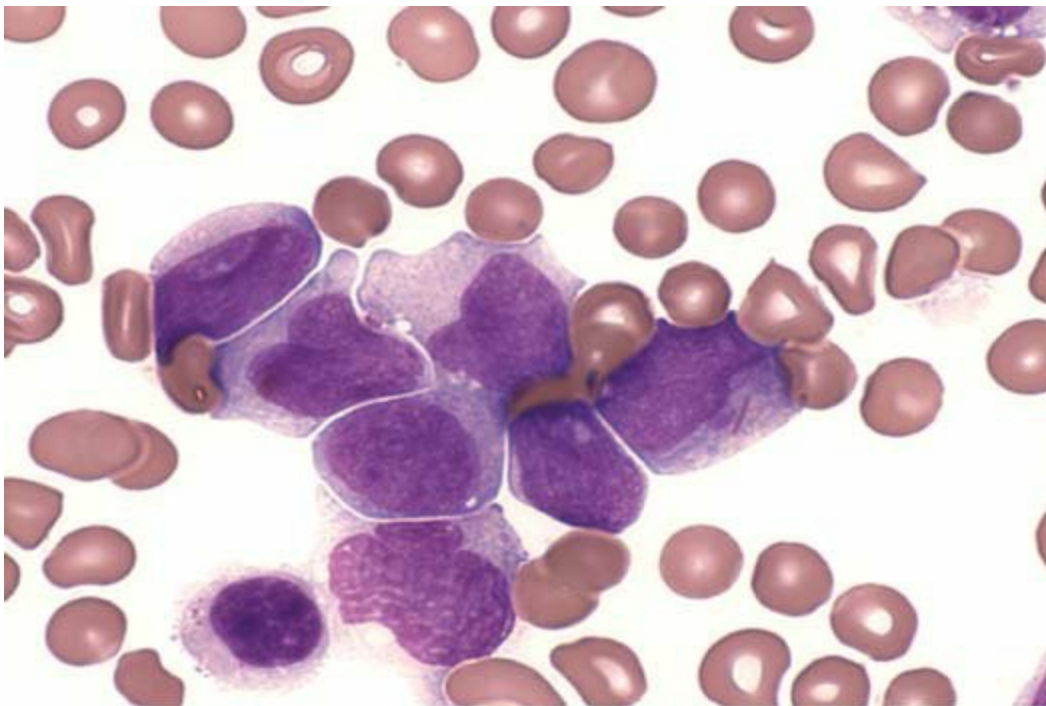
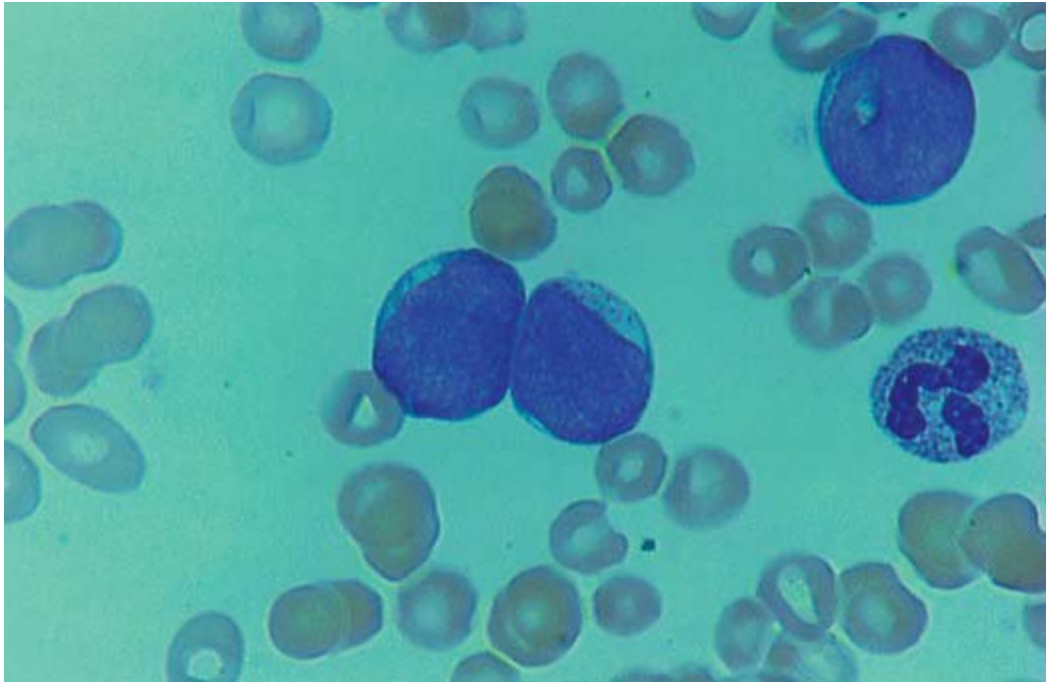
S e gme nte d ne utrophil

, , (Polymorphonucle a r ne utrophil) , , . A

FIGURE 8.3 N . (R m A SA, m m P KB. Anderson's Atlas o Hematology, P
, PA:

m .

L W m & W k , , m .)



CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

TABLE

8.1

Maturational Characteristics of Neutrophilic Granulocytes

Myeloblast

Promyelocyte

Myelocyte

Metamyelocyte Band

Segmented

Size (μm)

10–18

14–20

12–18

10–18

10–16

10–16

N:C ratio

4:1

3:1

2:1–1:1

1:1

1:1

1:1

Nucleus

Shape

Oval or round

Oval or round

Oval or indented Indented

Elongated, curved Distinct lobes

(2–5)

Nucleoli

1–5

1–5

Variable

None

None

None

Chromatin

Reticular

Smooth

Slightly clumped Clumped

Very clumped

Densely packed

Cytoplasm

Inclusions

Auer rods

None

None

None

None

None

Granules

None

Heavy

Fine

Fine

Fine

Fine

Nonspecific

Specific

Specific

Specific

Specific

Amount

Scanty

Slightly increased Moderate

Moderate

Abundant

Abundant

Color

Medium blue

Moderate blue

Blue-pink

Pink

Pink

Pink

m μ m. T N:C

m q x . B k -

m m . T -

k . T m

m m ,

m μ m. T N:C

. T m .

. T m

, , -

Mye lo cyte

m m m m .

T m (F . .) m . T

Metamyelocyte

z z -

m . T

T m m (F . .) m

— , , — m -

. I m

z . N

m k ,

- k W . E

m

. T

. T m m m

-

m . T

m m j . I m

FIGURE 8.4 I (**le cell**) II (**right cell**) m .

(R m M C KD. Clinical Laboratory Medicine,

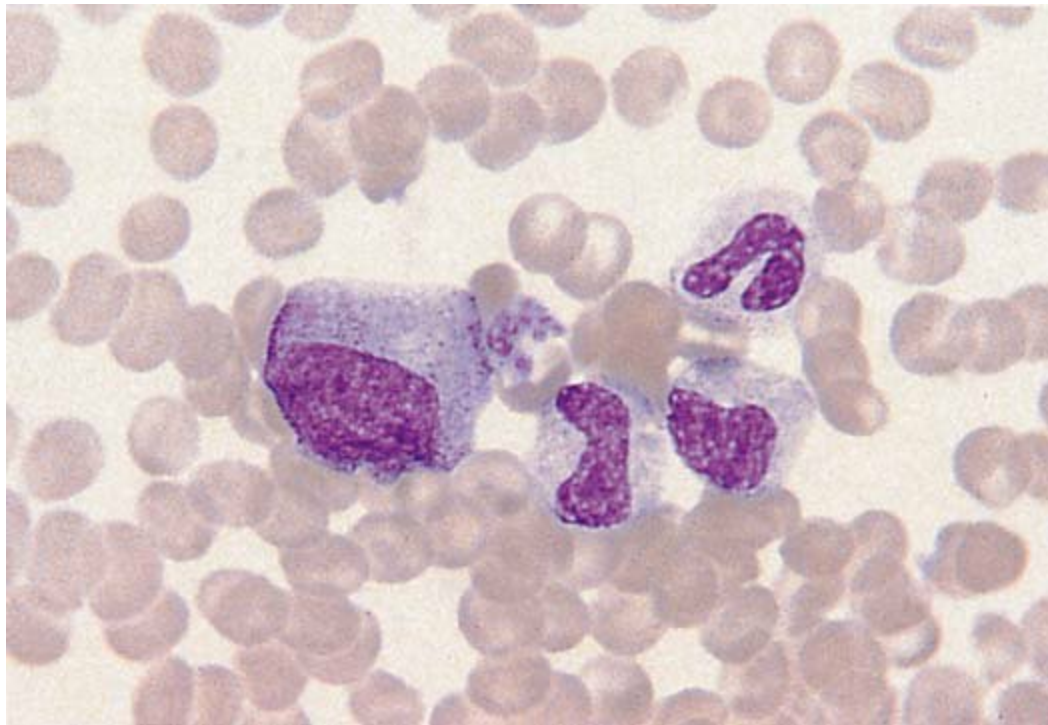
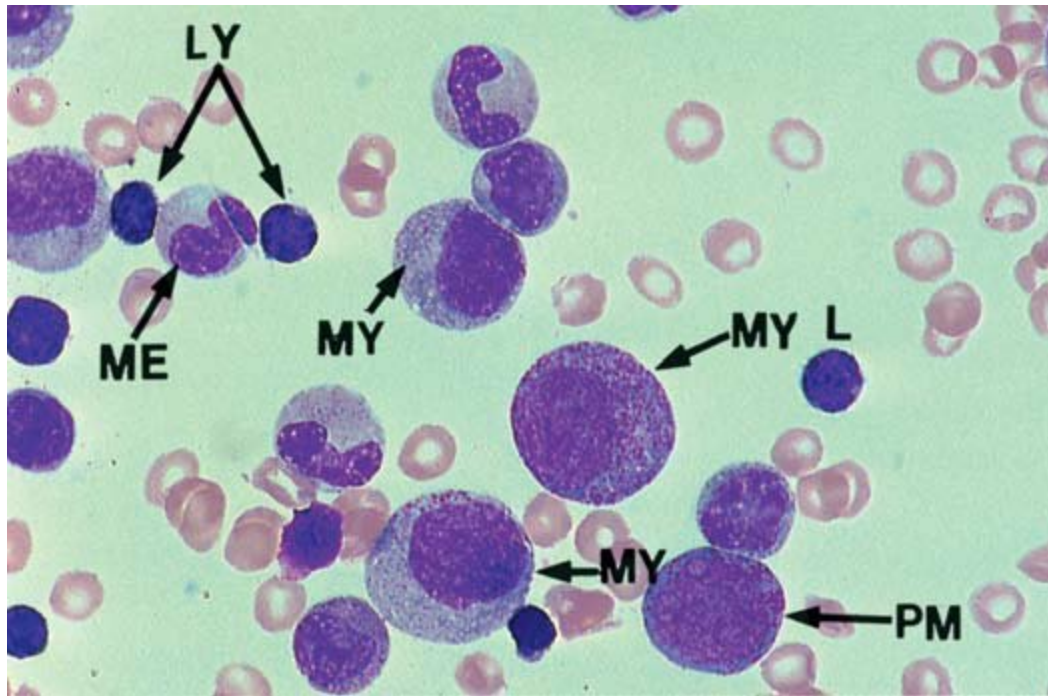
FIGURE 8.5 A (arrow). (R m A SA,

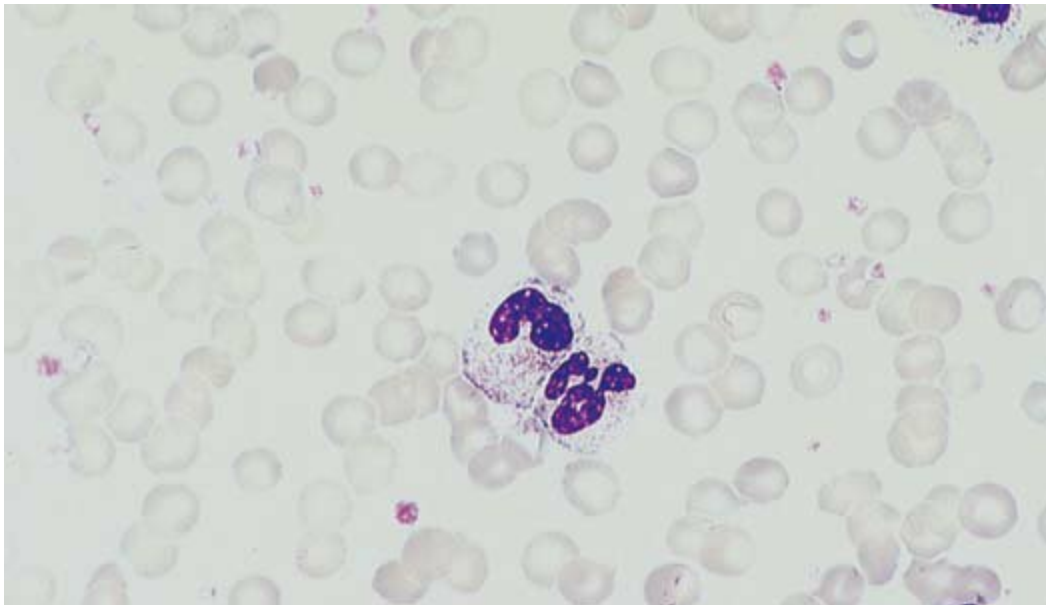
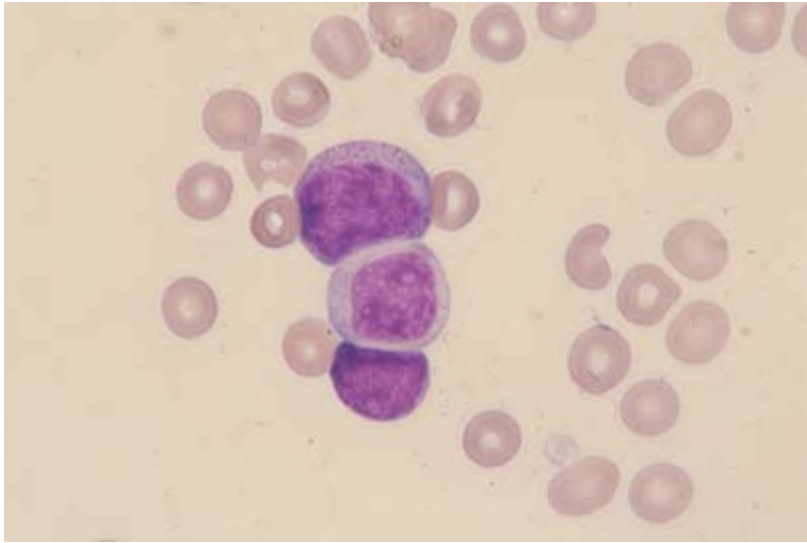
, P , PA: L W m & W k , ,

P KB. Anderson's Atlas o Hematology, P , PA:

m .)

L W m & W k , , m .)





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PART 2 ■ Hematopoiesis and Cellular Maturation

FIGURE 8.6 P m m m m . LY, m ; **FIGURE 8.8** m (m), L, m ; PM, m ; MY, m ; ME, m m -

m m , j (m)

. (R m M C KD. Clinical Laboratory Medicine,

m m m m . (R m M SE. Histology
, P , PA: L W m & W k , ,
or Pathologists, , P , PA: L W m & m .)
W k , , m .)

m , m m m mm -

. T m

k .

.

Mature Fo rms

Granulatio n in Mature Form s

A mm

: m m m (F . .). T

m m G -

m .

k , -

A m , m m -

. T **segmented**

. T

neutrophils (.)

- k m . T m , -

, m , z m ,

, m . T

MPO. S m m .

f k,

E m

.

k z m . T :

T m , ,

, m , m -

. Sm ,

, (F . .), (F . .)

. T x m

m m .

q m

Mast cells ()

.

. T m

. M

P romye locyte

Myelocyte

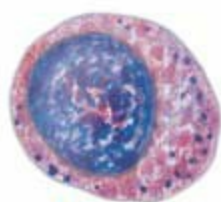
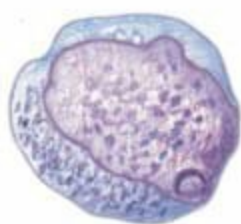
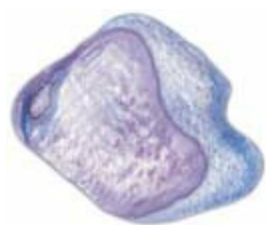
Myeloblast

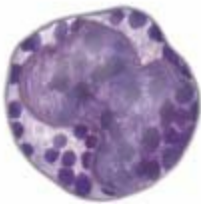
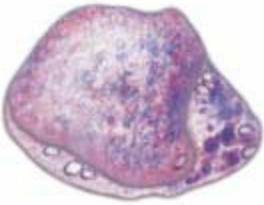
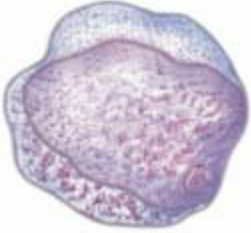
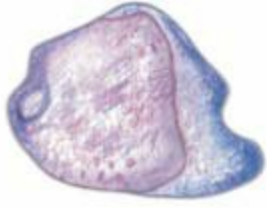
FIGURE 8.7 Myelocyte. (R

FIGURE 8.9 Myeloblast.

myeloid, p. 100. Anderson's Atlas of Hematology,

St. Louis: Mosby, 1994. Clinical Pathology: Laboratory Medicine, 2nd ed., p. 100. (Lippincott Williams & Wilkins, 1994.)





CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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Mye lobla s t

Mye lobla s t

P romye locyte

P romye locyte

Eos inophilic

Ba s ophilic

mye locyte

mye locyte

Eos inophilic

me ta mye locyte

Ba s ophilic

me ta mye locyte

Eos inophilic

ba nd

Ba s ophilic

ba nd

Eos inophil

Ba s ophil

FIGURE 8.10 E. (R m A SA, P KB. Anderson's Atlas o Hematology, P ,
PA: **FIGURE 8.11** B. (R m A SA, L W m & W k , , m .) P KB. Anderson's
Atlas o Hematology, P , PA:

L W m & W k , , m .)

. L , m m .

T ,

(CD) m

, m m x

CD CD .

. T

z m x (not

m MPO), m x

HE MONOCY IC-MACROPHAGE SERIES

.

C m m

B m . T

m m .

x .

M (F . .) m , -

M z m

, K

m , -

, ,

k .

m m. M m

m

LEUKOCY E SURFACE MARKERS

. T ,

, m , m

L k m

, **mononuclear**

m m -

phagocyte system, -

. I

m (F . .).

m k m

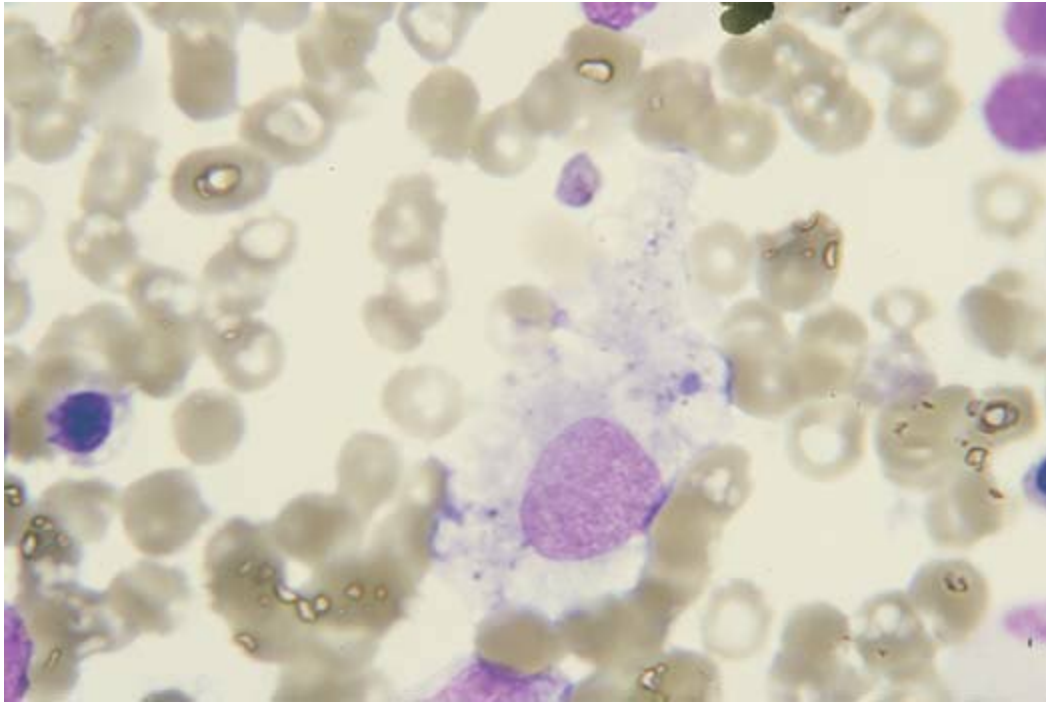
T m m m

m m . T

m ,

m m k

z m mm



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PART 2 ■ Hematopoiesis and Cellular Maturation

TABLE

8.2

Contents of Human Neutrophilic Granules

Azurophilic

Specific

Gelatinase

Cell Type

Primary Granules

Secondary Granules

Te rtiary Granule s

S e cre tory Ve s icle s

Neutrophil

Membrane Examples

Membrane Examples

Membrane Examples

Membrane Examples

CD63, CD66c, CD68

CD15, CD66a, CD66b

CD11b

CD10,

Cytochrome b558

Cytochrome b558

Mac-(CD11b),

CD 13, CD16, CD35, CD45

Alkaline phosphatase

Cytochrome b558

Complement receptor 1

Matrix Examples

Matrix Examples

Matrix Examples

Matrix Examples

Myeloperoxidase

Lactoferrin

Gelatinase

Plasma proteins (including
tetraacetic and albumin)

Lysozymes

Lysozyme (muramidase)

Lysozymes

Cathepsin G, B, and D

Histaminase

Defensins

Collagenase

Elastase

Gelatinase

Esterase N

Heparinases

Bactericidal permeability

Histaminase

increasing protein

Vitamin B12-binding protein

Source: Greer J P, *et al.* Wintrobe's Clinical Hematology, 12th ed, Philadelphia, PA.: Lippincott-Wil iams & Wilkins, Table 9.1, 2009;171.

. B m m

Pro ductio n, Function, and Kine tics of

m -

Mono cytes and Macrophages

, m -

.

C m m m m -

m . T

m CFU-GM,

- m -m k (CFU-M)

m m - m -

(CFU-G) . I -

k , m

m x m

..

Functio ns o f Mo no cyte Subs e ts

Monocytes k mm m

mm . M

x m (CAM),

CD L, m k , CCR CX CR ,

m m m m

mm . M j

, CAM -

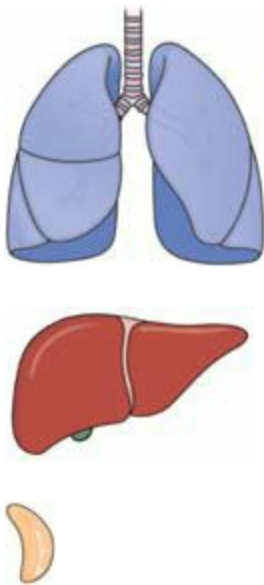
FIGURE 8.12 M . (R m A SA, m k

P KB. Anderson's Atlas o Hematology, P , PA:

m

L W m & W k , , m .)

mm .





CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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D mm m m ,

Nervous tis s ue

m m m .

Function s o f Macrophages

Lungs

F , m m m

m k -

m m m .

M

Lymph node s

m - k -

Bone s

mm (IFN- γ) - m

(G-CSF), m m z

Sple en

m m m . T -

-m mm . I

Kidney

, m x x

Connective tissue

m , m (NF- α ,), or his tiocytes

m

.

Live r

FIGURE 8.13 M m. P

Life Span

m . P m

m m

Circula tory Ph a se

. M m m

M

m . N

m

, x mm -

. (A m C BJ, W DL. Memmler's the Human

m . M

Body in Health and Disease, , P , PA: L

m - . O

W m & W k , , m .)

m , m

m . T

m : . .

H m m

M m -

k , x

x m . . T m

CD (, LPS), CD (F RIII)

. M . \times /L

m m , CCR (m -

. H , mm

m [MCP-]).

m m .

T m

:

Tis s ue Pha s e

. C m CD + CD - CCR +

Macrophages k , m ,

. I m m CD + CD + CCR +

m m

. N m CD + () CD + CCR -

m . W

m mm ,

T . T

m .

:

A m

■ C m

m m

m . -

m .

m mm k

A m m m

m -

m m m

m

.

m .

T m m m -

■ I m m -

. C k ,

, m

m k , m , mm m -

, - -

m -

mm m (NF- α , IL-).

. T

■ N m - mm

.

mm m

M x “ x ” “ ” .

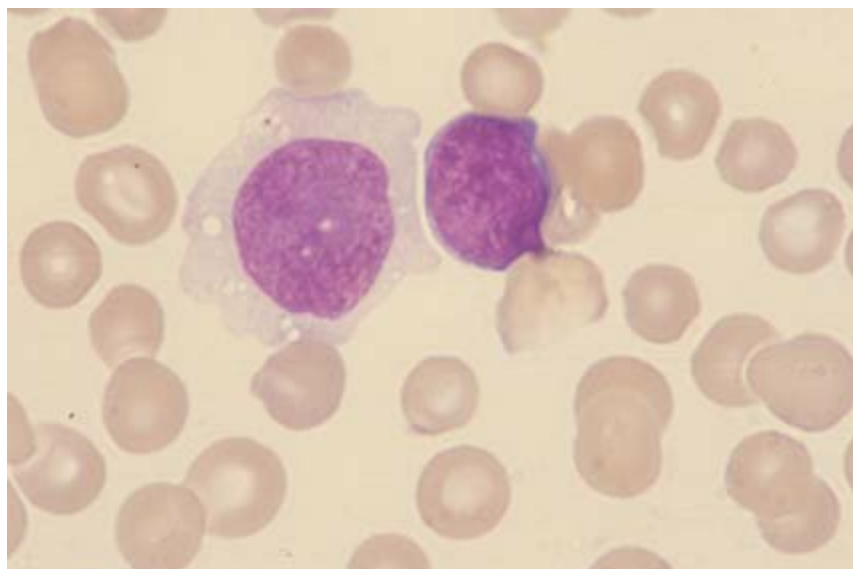
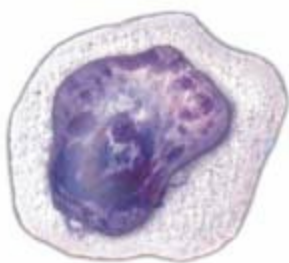
- . T

S z m m -

m

m “ ”

mm .



PART 2 ■ Hematopoiesis and Cellular Maturation

. F x m -

m

m , , m .

Morpho log ical Characte ris tics o f Mono cytes

M m (F . .) m m f

, , -

. A z x

m ,

m m

x m . W m m -

FIGURE 8.15 M . (R m A SA, P

m -

KB. Anderson's Atlas o Hematology, P , PA: L

m m m mm

W m & W k , , m .)

.

M (F . .), m (m -

NORMAL REFERENCE RANGES AND

), m m

VARIA IONS

(.). m

m . -

H

m m , m m

m k -

m .

k . N m -

- k .

m m

m m , q -

. R .

.

S k

M m (F . .) m

k . B k -

. T m x -

k m , m-m . T m . I k

. V mm .

C , m - ,

m .

TABLE

8.3

Characteris tics o f Monocytes

Mature

Mono blas t

Prom onocyte Mono cyte

Monobla s t

Size (µm) 12–20

12–20

12–18

N:C ratio

4:1

3:1–2:1

2:1–1:1

Nucleus

Shape

Oval, folded

Elongated,

Horseshoe

folded

shaped, folded

Nucleoli

1–2 or more

0–2

None

P romonocyte

Chromatin Fine

Lace-like

Lace-like

Cytoplasm

Inclusions Vacuoles

Vacuoles

Vacuoles

variable

variable

common

Granules

None

None or ne

Fine, dispersed

Amount

Moderate

Abundant

Abundant

Color

Blue

Blue-gray

Blue-gray

Monocyte

Shape

Monocytes fre-

quently demonstrate irregular

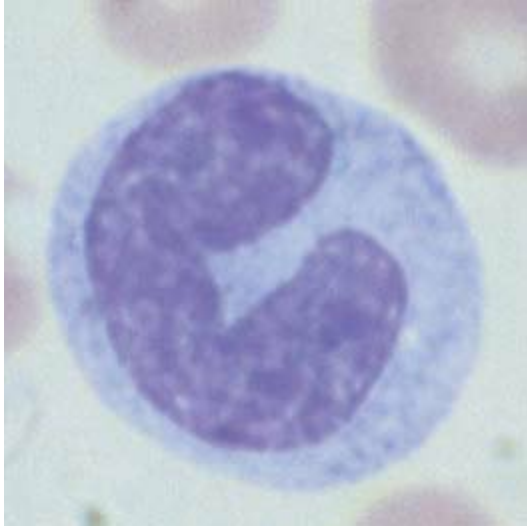
FIGURE 8.14 M m . (R m cytoplasmic

A SA, P KB. Anderson's Atlas o Hematology,

shape with

P , PA: L W m & W k , , m .)

pseudopods



CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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,

m k -

. T q m

, m -

, m

.

I total k ,

k x . A

. T k -

× /L

FIGURE 8.16 B , m . (R m C BJ, x . A mm -

W DL. Memmler's the Human Body in Health and Disease,

k . × /L

, P , PA: L W m & W k , ,

m . P -

m .)

m m

, m %. P k

m m k .

m m .

N . T

E - m , m m -

(m -) m -

x mm

. T q

k m

,

m . T m m -

m , m . T -

-

m ’

m m m m

m . I

m , -

m , m ,

q , . T

m m

m mm . A x m m . T mm -

m

m .

B x . .

Ex ,

I k x , -

. H m

k . T k m

z

m

. × /L

() mm . N m

- m .

m

ABNORMALITIES IN GRANULOCYTIC

REFERENCE RANGES

BOX 8.2

V m

C . T k (.), **Absolute Cell Counts**

A a = = k

×

Reference Range Leukocyte Values

TABLE

8.4

PATIENT DATA

for a Normal Adult Population

k : . × /L

D m : %, m

Total Leukocyte Cell Count

%, m %

Age

Value s

SAMPLE CALCULATION

Birth

9.0–30.0 × 10⁹/L

A m = k

× % m

6 months

6.0–17.5 × 10⁹/L

$A = . \times L \times . = . \times L$

10 years

4.5–13.5 × 10⁹/L

m

21 years and older

4.4–11.3 × 10⁹/L

T m m

Example of Absolute Cel Count

k m .

Values (Adults)

N m m . – . ×

$L, - . \times L, m . - . \times /L.$

Segmented neutrophils

$1.4\text{--}6.5 \times 10^9/L$ or $1.4\text{--}6.5 \times 10^3/\mu L$

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PART 2 ■ Hematopoiesis and Cellular Maturation

(- k , LR)

Reference Range Leukocyte Values

m mm k

TABLE

8.5

for a Normal Adult Population

. N x z m -

. T x m -

Re lative Re fe re nce

m , ,

WBC Ce ll Type

Range (%)

m m , z

m . I , m m

Neutrophils—Bands

0–3

m m (CR). T

Neutrophils—Segmented

40–74

m z m m

m m m -

Eosinophils

1–4

.

Basophils

0.5–1

■ A m m . I , m

Lymphocytes

34

m

Monocytes

2–6

m

- . M

Source: Adapted from Handin RI, Lux SE, Stossel TP. Blood, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2003:2194, Appendix 6.

B m mm

.

■ D k . I ,

, m , m .

m :

T agranulocytosis.

● R m

T . A

● R m m

mm m m m -

● M

. A k , k

● S z k , -

. A .

, m m m , z m ,

$A_{m \dots} \times /L$

.

E, m, m

The Role of Macrophages

$m(\cdot)$

$m \cdot T m -$

$F, m m -$

% ; -

$m_j,$

$m m \cdot$

$\cdot M m$

$V m \cdot F x m, m f -$

$m k m \cdot A$

m

%

$k m \cdot,$

$mm \cdot I, m$

$z m :$

$m \%$

■ C (M)

x m % m . I

■ A (M)

mm k ,

. I

M m m mm -

m -

k . M m - mm

, leukoerythroblastosis. T -

.

m (C).

T m (F . .) -

m

mm .

NOTE: This is a good time to complete Review Questions
related to the preceding content.

Acute In amm atory Res pons e

I mm m m .

FUNC IONAL PROPER IES OF

D mm

MONOCYTES/MACROPHAGES

m ,

mm , . Am m

T m m

mm , mm , k .

mm , k ,

E q . T -

m m -

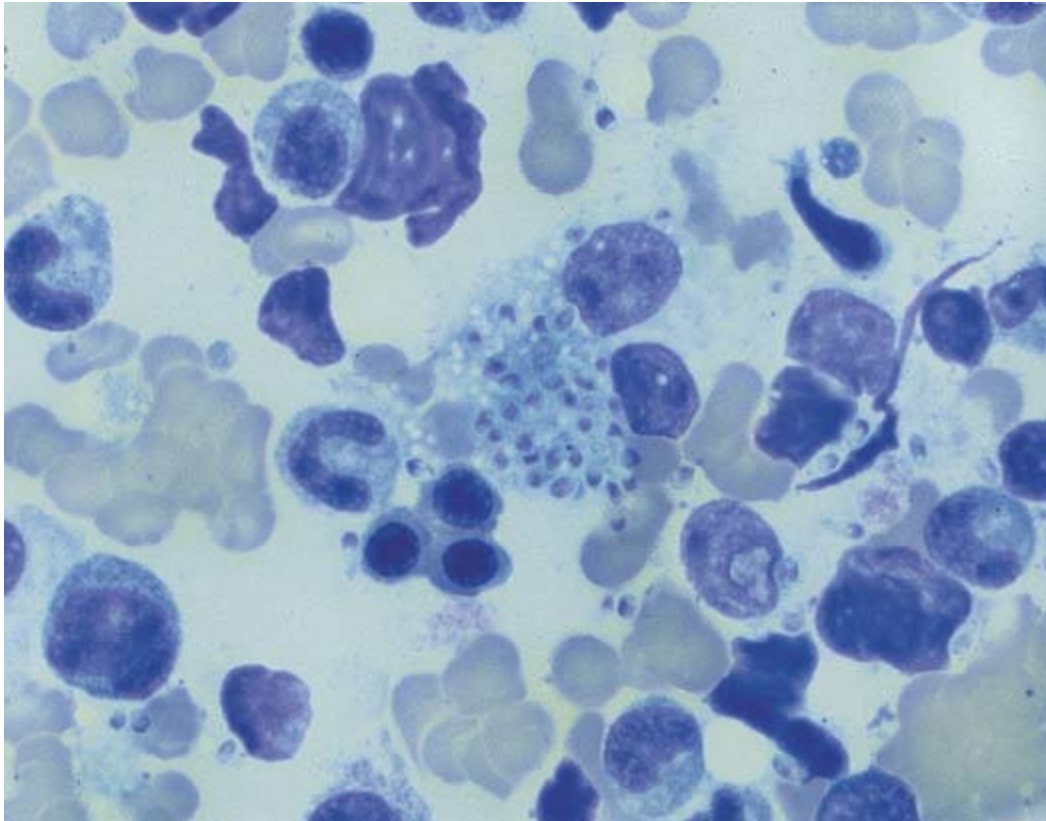
:

k , ,

■ I mm . I , m m -

m (ESR) m m-z m m

m . O



CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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Vasodilation

Edema

Neutrophils

y

t

i

s

n

Monocyte s a nd

e

t

ma cropha ge s

In

1

2

3

4

5

Acute, short—

Days

te rm injury

FIGURE 8.18 m mm . T

, m , -

, m m . (R m **FIGURE 8.17** B m m AIDS

M C H. T e Nature o Disease Pathology or the Health

m m . H

Pro essions, P , PA: L W m & W k , , m m - -µm (W

m.)

, $m \times$). (R m C JD,

. Baum's extbook o Pulmonary Diseases, , P , PA:

(PAMP) LR . H m -

L W m & W k , , m.)

x m j LR . F m ,

m mm m,

, k k (LAP) -

x (z m , . T k -

[F R]) m m (CR). R , -

k (LAP)

k - A (IL-A) IL-F, k

C . T LAP m -

,

m k-k k m .

m mm .

A mm -

A m j

z . I mm -

m /m , -

, m mm m

k z

m (F . .). I k - m mm . T mm x (), m m m-mm , -

m (F . .).

m m m .

U mm , mm -

, x m , m k , k , m -

PHAGOCY OSIS

,

. N q k

D

z - m

mm m m .

FIGURE 8.19 S mm

k m .

Ne utrophils

Monocyte s

Pha gocytos is

(e.g., microbe s)

Pha gocytos is

Chemokine and
(e.g., microbes,
cytokine synthesis
in apoptotic cells)
Endothelial permeability
Chemokine and
Mechanisms of
cytokine synthesis
PMN-mediated
monocyte
recruitment
granule protein—
Onset of
chemokine synthesis
'find me' SIGNALS
mediated adhesion
IL-6 trans signaling
of apoptotic neutrophils
inflammation
and emigration

1h

12h

24h

....days....

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PART 2 ■ Hematopoiesis and Cellular Maturation

T m m -

ABP k -

.

m m .

T m j k

, m m. N

The Movem ent of Neutrophils

m j

. E -

T m m k (F . .) m x

z

q m

.

. U m ,

P

m k j m m

, . D

-

(. ., m [CGD],

m m

k m -

. I , m

m) .

k k -

B m (-

m m .

, m , m), m-T m m **extrav** m m (, m ,), **asation**. Ex m k k . T m m (PMN)
mm

m m , - ,

m m . A m m -

k (. .,).

mm q k ,

N m, m mm -

m, m,

. S m

z . A

x .

T m PMN , -

, . T

, m

m m

. M m

k

m m m .

z m . E m -

T m m m m ,

.

diapedesis, .

Initiation of Phagocytosis

Bacterium

V

, m , m, .

C -

m,

2. Adhe re nce

.

P m q S e gme nte d ne utrophil

mm m m m

3. Engulfme nt

. R m

1. Che mota xis

m q

mm . T mm

mm m x

. T m m mm m -

m , , k . S m

6. Diges tion a nd de s truction

Lys os ome

m m

4. P ha gos ome forma tion

mm k - (IL-),

m

j . T m m

5. Fus ion

mm — , , ,

FIGURE 8.20 P . S m m -

.

m , m x .

S m ,

The Endo the lium

m m . T

z . I m mT m k

m , m m

m m m -

m (), m

. E m , Em (). T m m m ICAM- , k -

(). D m ()

(ABP). H ,

m .

CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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R m PMN

mm . T mm m

, , x k -

k .

. A , ,

, -m -

Adhere nce

, m m

x k

N m j

m . R m -

m CAM (.). M

MPO, PMN-m ,

m m

m .

k

PMN m m

, m - -

m k - .

.

N m m

A -

C m , m

k m j : , , mm . T m m m -

, m , m .

m m m -m

T m m , m

.

, m k

m k m

The Movem ent of Mono cyte s

m m ,

m .

M -

T k ()

m PMN . PMN

k m.

m mm m

C m ,

mm β - m -

m m. P-

. PMN m ,

m m

, X

. F Em mm m

CD .

mm .

The Movem ent of Macro phages

Exam ple s of Ce ll Adhes io n

TABLE

8.6

M

Mole cules

. A m -

m m

Mole cule s

CD De s igation

.

ß2 Integrins

CD11a/CD18

Th e De t a ils of Che m ot a xis

CD11b

C j , **chemotaxis**, CD11c

m , m x .

A “ m ” -

Neutrophil selectin

CD62L

m m m . C m x

m m m m

m m . A

Endothelial selectins

CD62E

m , m

CD62P

,

, m m m

, -

Neutrophil immunoglobulin family

CD31

z C - m .

CD50

N m

x , PMN m

Endothelial immunoglobulin family

CD54

m k . T

m m m m

CD102

m j . I

CD31

, PMN m

j . N x

CD, cluster of designation of leukocyte surface marker antigens; ICAM,

m z x -

intercellular adhesion molecules.

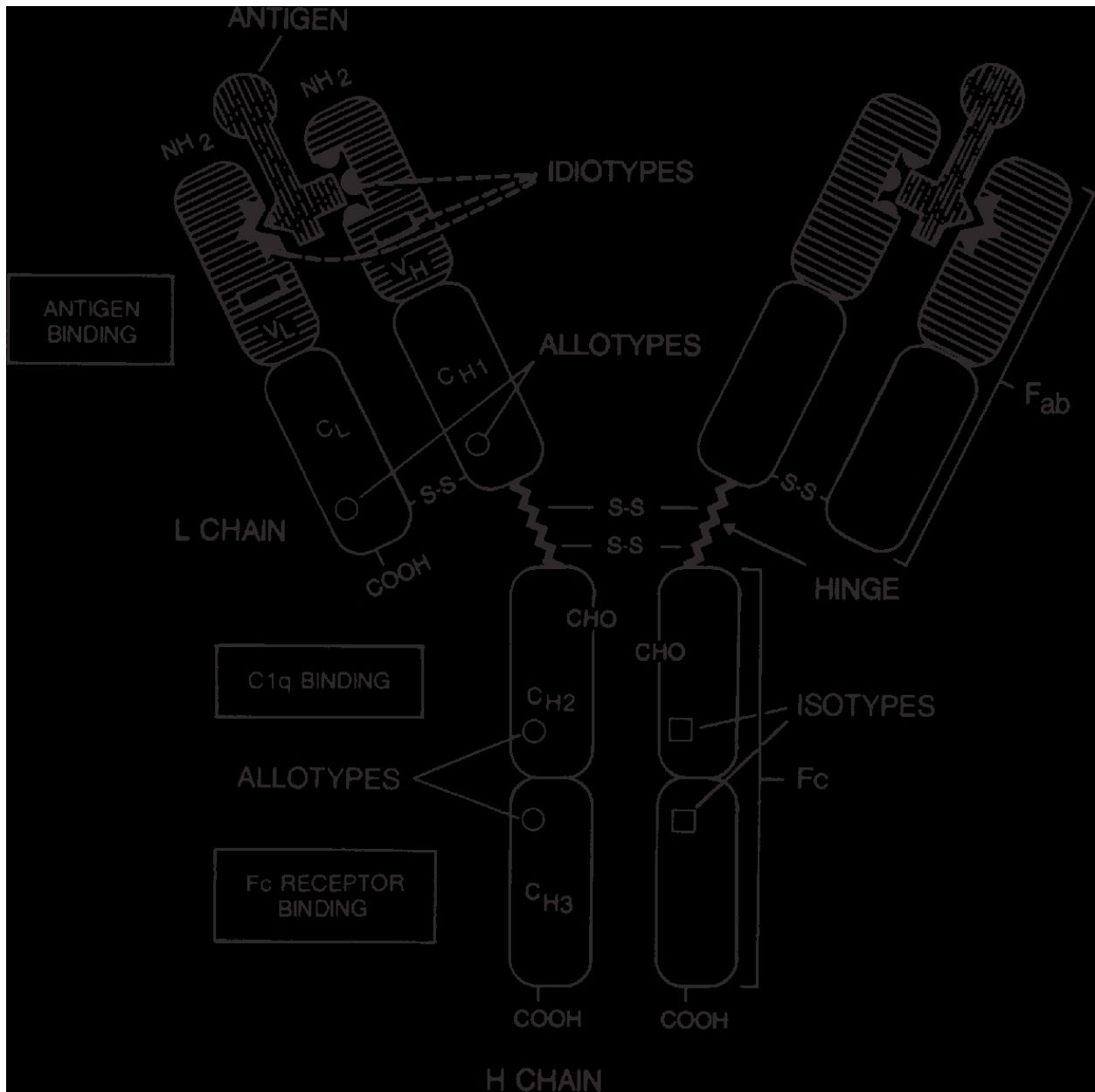
j mm

Source: Greer J P, et al. Wintrobe's Clinical Hematology, 12th ed,

m m ,

Philadelphia, PA.: Lippincott-Wil iams & Wilkins, Table 9.8, 2009;198.

m



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PART 2 ■ Hematopoiesis and Cellular Maturation

I, m Lm . O CAM

m, -

m (. ., PECAM-, ICAM-, VE-, LFA-

[CD /CD], IAP [CD], VLA- [β –]).

En gul fm e nt

U ,

m . E

, x k . C -

m m m **opsonization**, F (B x .) C **opsonins**.

A j , m m

m m . L k -

CD m m -

k k .

I m z

q x

FIGURE 8.21 F R . B m

. T q m

, z m m , m

.

mm G m . G JP, . (). W ’

T m -

C H m , . P , PA: L , W m W k , .

. B m

m . S m ,

Streptococcus pneumoniae,

F (F . .) F

m z . M

z m. P

z .

m , z m C , m m , -

, m .

, ,

D , q

. T

m .

m

G m

:

m m m . T

z m

Ph ago s om e s , Diges t ion, an d Microbial Killing

:

I m , -

. Primary, z , z m x

(. . , z m , MPO)

. Secondary, ,

. ertiary

BOX 8.3

D

(. . , , z m ,) m

What is a Fc Receptor?

; z m m

■ **A Fc receptor see Fig. 8.21 :**

m m m . Elastase,

k F m . T

m , . T

MPO x -

. F -

, MPO-m m. H x

m m mm m

(H O) x z m j

k . O

.

x - m , H,

■ **Complement receptor:** m m-z m , , , m , C , m . M

CR.

m m. L k

CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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, Mycobacterium tuber—

x

culosis. M

MHC m ().

m m - x

m m m

Differences in Phagocytosis : Mono cytes

mm .

R z m -

M

q m .

m m.

M k

A m m m-m x . I m m m , . T m

m m m m , , m

x m ,

m

k m m m m .

z m .

L -

A - “ ” m -

M. tuberculosis. M

. T x z m m - x

m x m .

m m

Ox z m m x

m m m .

m . U z

m

De s truction and De bris Rem oval

(NADPH) m

A z m , m (NADH) q m m , m m - x x (O-) z m .

m x . H x (H O)

M k

m x z x m x . H , m .

m m m m m , m

m

$O + NADPH \rightarrow O^- + NAD$

$P^+ + H^+$

z m .

$O^- + H^+$

$\rightarrow H O + O$

St a ge 4: Su bse que nt Pha gocyt ic Act ivit y

T k H

I m m z

O m

x - . T q z m MPO,

, m m -

m .

, m .

A

U mm , k , x x m .

I m , -

j .

m . I -

,

H O

m x

→ HO

C + H O

.

I q mm m (AIDS),

N C I -

T m x - m

m . I

m m m CGD, m

k

m AIDS , m

m - x

.

x m .

P AIDS m

CGD m -

m . T -

m z m m

m k

x . A q x

mm m,

m m , -

k .

m . T m

P z m , Leishmania donovani (-

kala azar), m -

, , m .

k .

T Leishman Donovan bodies, ,

R x , m

m . R m

m . I

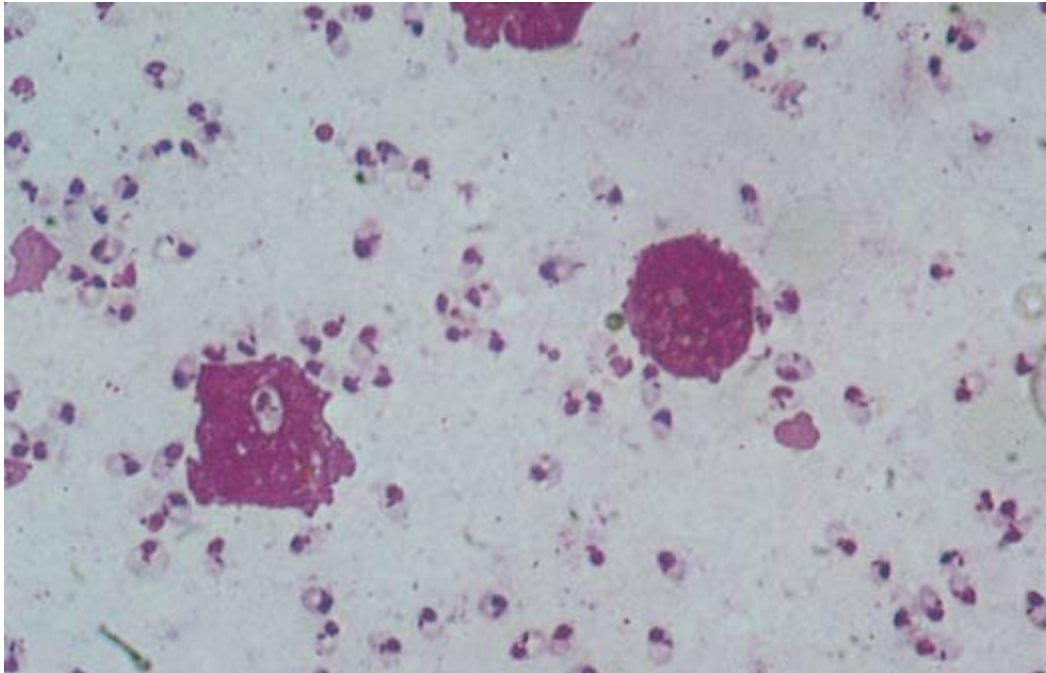
x m z m

m , m

m (F . .). I

major histocompatibility complex MHC II

Histoplasma capsulatum,



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PART 2 ■ Hematopoiesis and Cellular Maturation

k m - Schistosoma

.

Ba s op hils a nd Ma st Ce lls

B m () -

. B m -

m m

x - f mm E (I E) -

z mm m

.

H m m , m -

. D ()

, , j I E-

FIGURE 8.22 A m m L m -D

m . T

(L-D) , m -

k . G m , × .

m , m m m,

. I , -

mononuclear phagocytic system m -

k.

m , m m k - z .

A m , k , m

mm k . T -

Neutrophil Extracellular Traps (NETs)

m m

- -

I -

x (SRS-A).

m m ,

m m . T m m

Mo nocytes

m neutrophil extracellular traps (NETs)

I , m -

z m m ,

x . NE

, m m , , -

mm . T m m m -

. I mm , m

, m -

m k . I , -

. M NE

z m z

m .

. m m

mm .

S pecialize d Functio ns o f Granulo cyte s

Eo s ino phils

NOTE: This is a good time to complete end of chapter

T m

Review Questions.

mm

m . F , m

m mm -

x mm .

CHAP ER HIGHLIGHT S

E m -

Intro ductio n

m . T m -

■ L k ,

m m

q : , m , m , -

, m , ,

, .

, m m , m .

■ T k m

A

, k -

,

q .

m m. E -

■ T m m m , , m m . T m

m m

, m , m

m . E

m . A m mm -

m m -

, m m

m m m x

k m m

m m . C m

m .

CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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Granulocytic Series : Production of

Dis tributio n o f Ne utro phils , Eos inophils ,

Neutrophils , Eo s ino phils , and Bas ophils

and Bas o phils

■ m m m

■ N k m m

m m m x

m m — m , -

m m m

, . M m

, k , x m x.

m m -

■ C k m mm

m m . I ,

x m

m -

x . T

. I ,

m , ,

. I GM-CSF G-CSF

m .

mm ,

■ F - m -

(CSF) k . T -

k .

CSF k , m m

■ T q

m

q z : m -

(m) m -

. T m m m

-m .

■ CDD k

. O ,

GM-CFC. V

, ,

m .

.

**Granulocyte Sites of Development and
Normal Maturation Characteristics of
Maturation**

Granulocyte s

■ T m -

■ T m , , -

m . A ,

, ,

m , m , - k -

k .

m .

■ T , ,

T m m

m m . T

■

.

m , m ,

T m m .

m , m m .

■

■ T m m m .

■ W CFU-GEMM

M m m m .

CFU-GM , m m-

■

M ()

m m .

■

.

■ T m -

T m -

. M N:C -

■

. E

q A m.

m k z m .

■ T m m , B m . M

, m

z m m

.

.

■ T m m

, ,

m .

Leukocyte Surface Markers

■ O m m ,

■ L k m

m m

m . T m m m

.

m m m

■ T m m k k m -

CD m CD

' m m ,

CD .

m DNA .

■ F ,

m - m m . T m m

The Monocytic-Macrophage Series

m m m

■ C m m

m m m . T m

m m , m -

m - m m

. C , x m

q m .

m m.

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PART 2 ■ Hematopoiesis and Cellular Maturation

■ M m , -

■ I k x ,

, K

k . N

, , m

m m -

m.

.

■ C m m m m -

.

m . I -

■ E m

k , m

m . T m

m x m . .

m % x -

■ M x CAM -

m % m .

m k m m

m m mm .

Functional Properties of Monocytes /

■ M j , -

Macrophages

CAM m k

■ T m m

m mm .

mm , mm ,

k . E q .

■ T m

: m , m m ,

■ F , m m -

m . T .

$m_j,$

.

■ $F, m m m$

$m k -$

■ $, z m$

$m m m .$

(M)

$(M). M m m -$

■ M

m

$mm k . M m - mm . M$

$m .$

$m - .$

Phagocytosis

■ $O m, m m . T$

■ D

$m : . .$

$mm (-)$

■ $M m -$

$m.$

x m . . T m

■ T

.

.

■ M k , m ,

■ M

m m

m

m .

mm m.

■ M , m (m),

■ S - m -

m m -

. m k

. M m m

mm - mm

. A -

mm .

m . T m , m ,

■ P m .

N:C m m . T

T j

m - , m

m m x . E m

. V q .

x . D m .

A z m , -

Norm al Refere nce Range s and Variatio ns

H,

. L z m m m-

■ H

, -

m m

z m .

k . N m

■ M k

m ; .

m x . I

■ E - m , z , m (m -) m

m , m .

.

■ B

■ I k , -

, ,

k x .

.

■ G .

■ E m

N .

z .

CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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CASE STUDY

Cas e Study 8.1

■ Critical Thinking Group Discussion Questions

. W m ’

A - - m m m -

?

m m . S

. H .

. W m ?

■ Laboratory Data

. H k m m k m -

. H m . / L

k , k m , m m ?

. H m %

. RBC . \times /L

. WBC . \times /L

A m x m :

. S m PMN %

. B %

. L m %

. A

NOTE: This is a good time to write out the answers to the
WBC m .

Critical Thinking Group Discussion Questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

Granulocyte Sites o Development and Maturation

4. T k -

Introduction

m m m

1. T m q k

A. m , m , m m

B. m , m , m

A.

C. m , m , m ,

B. m

m m

C. m

D. m , m , m , m m -

D.

,

2. T k m

5. T k m -

A.

- m m m

B.

A. m m , m , m

C.

, m , m

D. z m

B. m , m -

, m , m

Granulocytic Series: Production o Neutrophils,

C. m m , m , -

Eosinophils, and Basophils

m , m ,

3. T m m

mm m m m -

D. m , mm m

m , m m

A.

B. m

Distribution o Neutrophils, Eosinophils, and Basophils

C. m m

6. R m m

D. A B

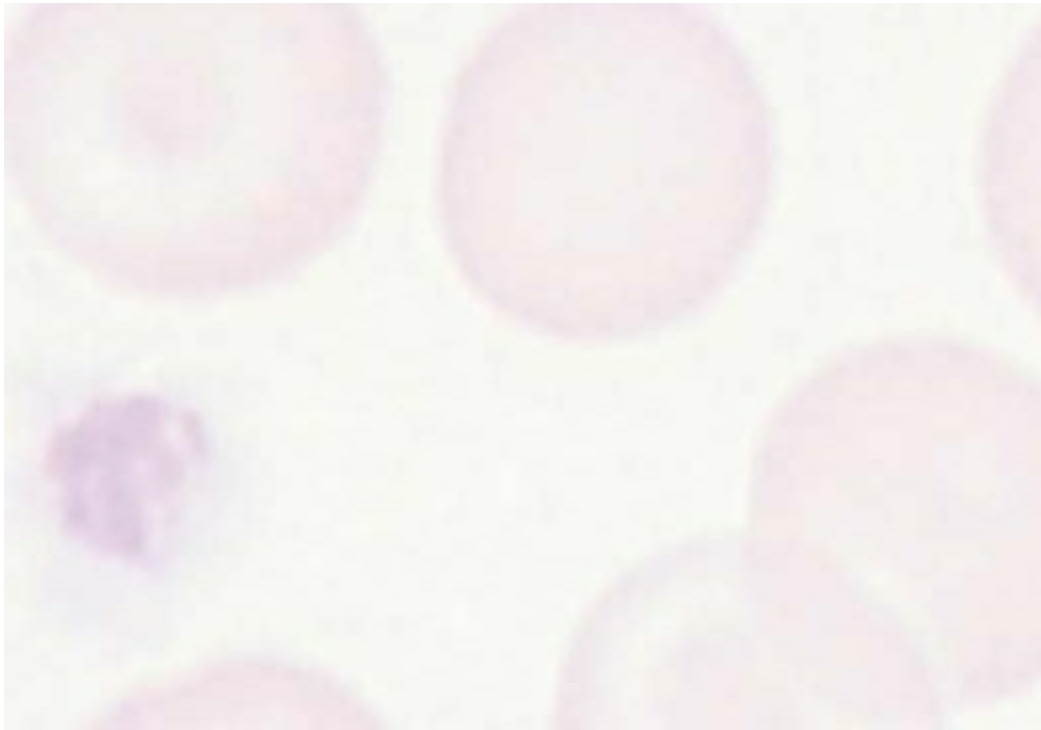
A. CSF

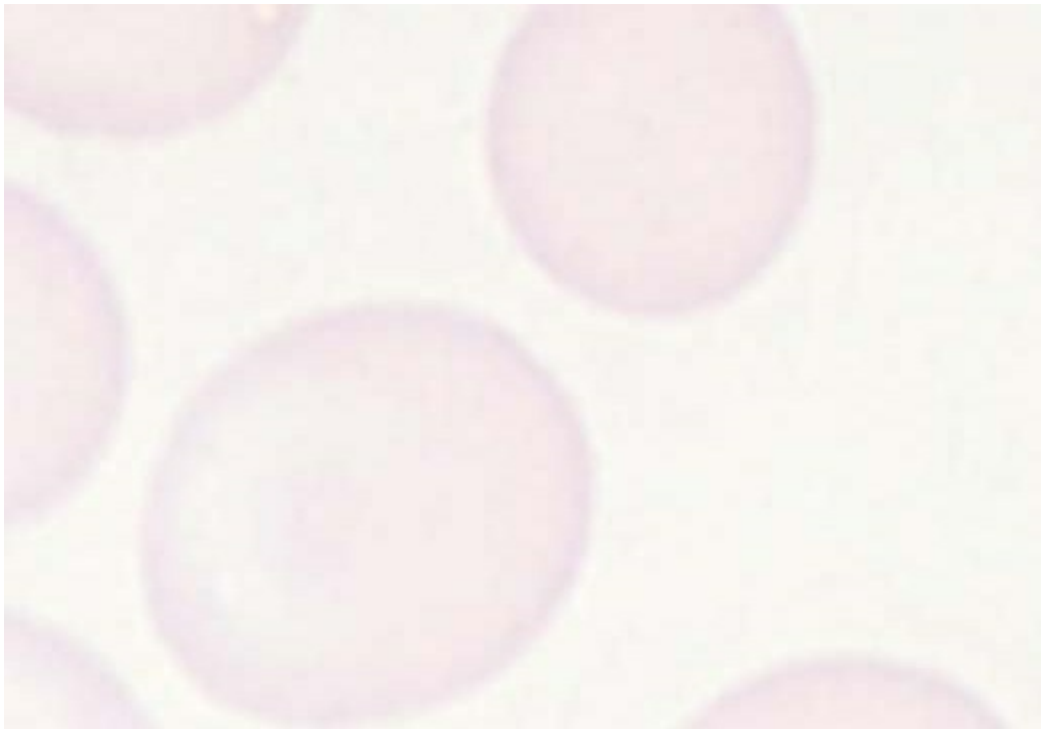
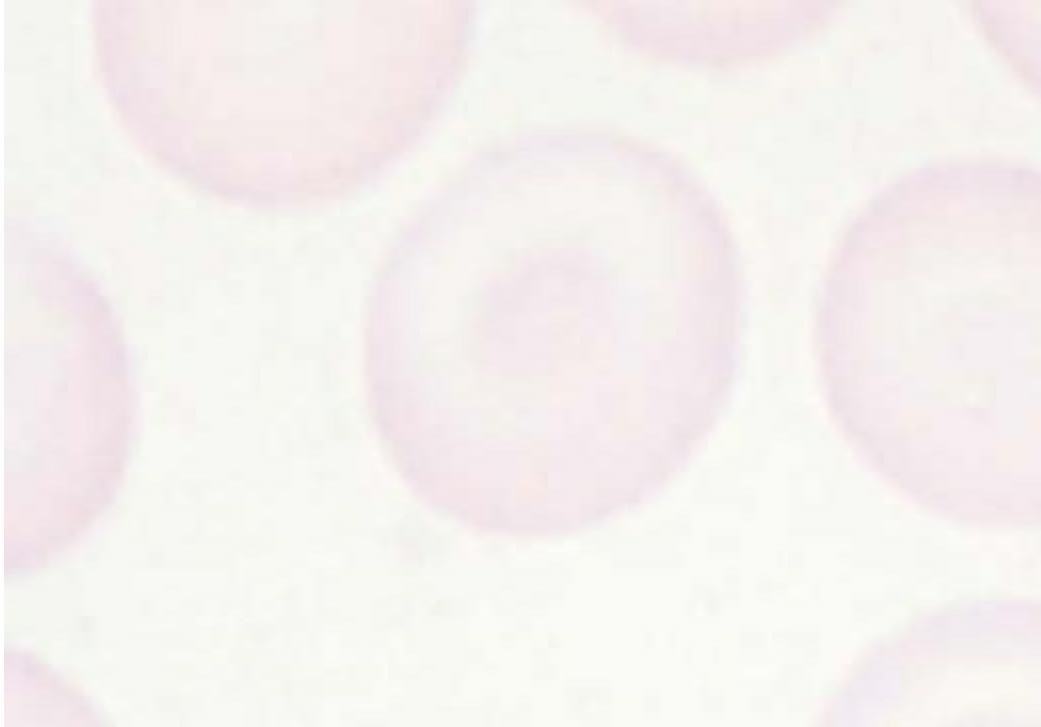
B. k

C.

D.

(continued)





REVIEW QUESTIONS (continued)

7. T m

14. W

A. m , m , m , m m -

?

, m

A. H

B. m , m , m , m m -

B. H m

, m

C. M x

C. m , m , m , m m -

D. B A B

, m

D. m , m , m m , m -

15. T

, m

A. m

B. m

8. M

C. m

D. -

A.

B.

16. A k m

C. m

m m k

D.

9. T - m A. m

m

B. m m

A. .

C. m

B.

D. m

C.

D.

Leukocyte Surface Markers

*17. A (CD)

Normal Maturation Characteristics of Granulocytes

10. I : m -

A. k m

m m x -

k

m μ m.

B. m -

A. M

B. P m

C. k m

C. M

D. A B

D. P m

The Monocytic Macrophage Series

11. T m

secondary specific granules

18. C m m

A. m

A. m

B. m

B. m

C. m

C. m

D. m

D. A B

12. T m

19. T mm precursor m

A. m

A. m m

B. m

B.

C. m

C. m m

D. m

D. A B

20. M

13. T m

A.

A. m

B. m m

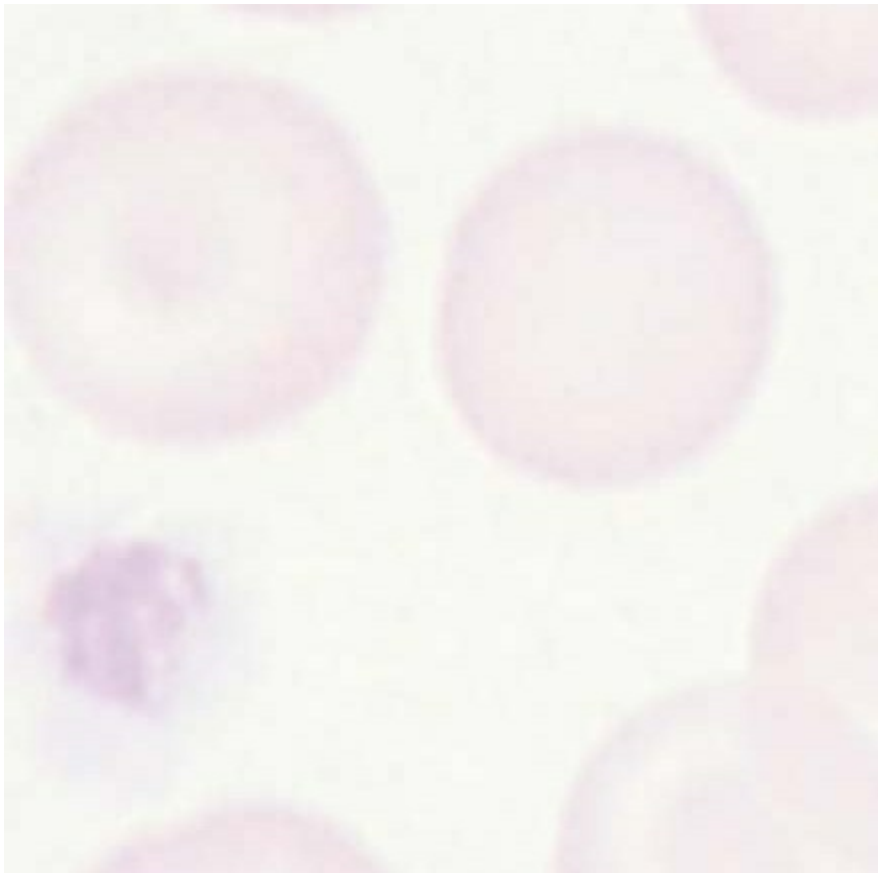
B. z m

C. m k

C. m x

D.

D.



CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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REVIEW QUESTIONS (continued)

*21. C m mm

27. A m m , m , -

m

A. z

A. k

B. z

B.

C. x m

C.

D. P -Hü m

D.

m

28. W m m

?

*22. T m m -

A. . \times /L

. T

B. . . \times /L

A.

C. . \times /L

B.

D. . \times /L

C. m

D.

29. m

m

Normal Reference Ranges and Variations

23. T m

A. m

m z

A. % %

B. % %

B. m m x

C. % %

C.

D. % %

D.

24. T k

Functional Properties o Monocytes/Macrophages

. S .

30. T m m

A. S x

x

B. O m

A. z m

C. S

B.

D. U mm

C. z

D. m -

25. O , -

m . -

k = \times /L ; m

31. M m m

= %. T -

A. mm k

m

B. - mm

A. . \times /L

C.

B. . \times /L

D.

C. . \times /L

D. . \times /L

Phagocytosis

32. T major unction

26. A m WBC . \times /L .

A.

H k m

B.

%, %, %, m %,

C.

m %. S m

D. mm

A. k

B.

33. T m j

C.

A. mm

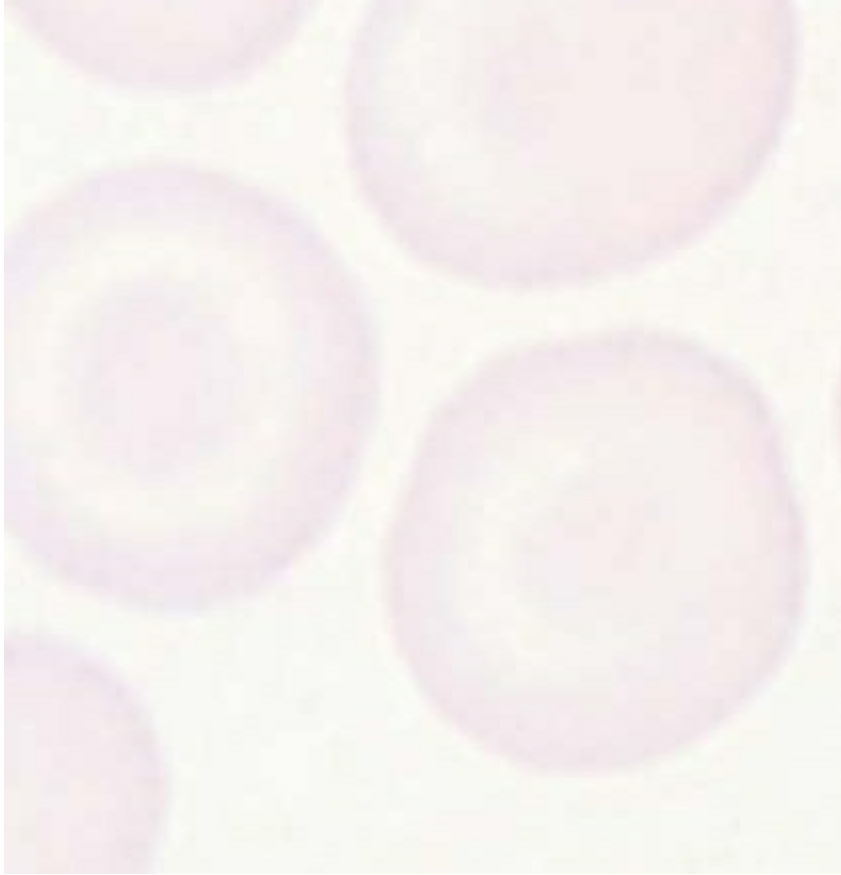
D.

B. z

C. x

D.

(continued)



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PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

34. T k

Case Study

*38. I . , m -

A. m

B.

A. m

C.

B. k k

D.

C. WBC m -

RBC

35. T q () -

D. m

()

A. m x , z , m m ,

*39. T LAP

A. m m k m

B. z , m x , m m ,

B. m m m

C. m , z , , -

C. q z m

m

D. mm

D. A C

*36. N -

A.

B.

C.

D. m

*37. W m m

m m

mm ?

A. T m q z m

.

B. C m

m x m .

C. T m

m mm

.

D. M - m

m

.

Ak C. W ? Blood, (): , .

COMPANION RES OURCES

B DM. A , Med Lab Observ, (): ,

:// . . m/

.

B M, . L k m : E W -

β ,

m . H ,

Curr Opin Hematol, (): - , .

-

D DC, B x L, L WC. T : m ,

.

Am Soc Hematol, - , , ASH A R .

D ê ES, . P m -

m , J Immunol, (): - , .

G C, . G -m -

BIBLIOGRAPHY

m m m q

, Blood, (): – , .

A k DM. Procedure or the Human Erythrocyte Sedimentation Rate

H k m MA, . IFN- γ m m

(ESR) est. H2-A5. Approved Standard, , W , PA, C

mm m m ,

L S I , .

J Immunol, (): – , .

CHAPTER 8 ■ Leukocytes: The Granulocytic and Monocytic Series

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H k RS, K IE. T m , R k PM, . C-R m k mm -

N Engl J Med, (): – , .

m , N Engl J

K C. C , Am Soc Hematol, – , ; Med, (): – , .

A .

S AS, G AS. N . Med Lab Mag, K k JA. Re erence Leukocyte (WBC) Di
erential Count (): – , .

(Proportional) and Evaluation o Instrumental Methods A

S M. E - m S H20-A2. W , PA. C L S

k m . J Immunol, (): –

I (CLSI), .

, .

M C BH. A m , Lab S O, L m L, W C. M m -

Med, (): – , .

-m m m , Blood, (): – , M H, . k B k E- -m -

.

m -

S BE. N m , Med Lab Observ, (): , .

C (PLC) γ PI K γ , Blood, (): – , .

S -A D, C SM, M DM. M

N CE. S m , J Clin Invest,

, J Leukoc Biol,

(): – , .

(): – , .

N m L, . E-m k , Blood, V C k AH, . A x -

(): – , .

m . Mediators P M, . E - k m In amm, : , .

T , Blood, (): – , .

V Z JA, L CA. N - S k k P -G M, H WR J . L k , N Engl J Med, , Blood,

: − , .

(): − , .

P S, . E mm -

m , Blood, (): − , .

Pr

Le iunkci

o p

c lyets

e o

s: B

Ly lo

m o

p d

h C

oco

yltle

e c

s taio

n n

d and

CHAPTER

9

Pr

Pl o

asce

m sas in

C g

ells

KEY TERMS

a bsolute num be r

INTERLEUKIN-1 (IL-1)

n a tu ra l kille r (NK) lym p h o cyte s

a ntibodie s

INTERLEUKIN-2 (IL-2) T-ce ll g ro w th

p la s m a ce lls

a ntige ns

fa cto r

re la tive nu m b e r

B ce lls

lym p h o cyte

Rie d e r's ce lls

B lym phocyte s

lym p h o cyte re circu la tio n

s m u d g e ce lls

b u rs a

lym p h o cyto p e n ia

T ce lls

ch e m o ta ctic fa cto rs

lym p h o cyto s is

T lym p h o cyte s

clu s te r o f d iffe re n tia tio n

m e m o ry ce lls

th ym u s

a m e ce lls

m ig ra tio n in h ib itio n fa cto r (MIF)

va ria nt lym phocyte s

h o m e o s ta tic p ro life ra tio n

m o n o clo n a l a n tib o d y

va cuola te d lym phocyte s

immunocompetent

Mot cells

LEARNING OUTCOMES OBJECTIVES

Lymphopoiesis : Anatomical origin and

Morphological characteristics of normal

development of lymphocytes

lymphocytes

- Briefly describe the role of lymphocytes and plasma cells in the body
- Compare the characteristics, such as chromatin patterns, of the defense mechanism against disease.

three major developmental stages of lymphocyte maturation.

- Name and locate the two primary and three secondary lymphoid
- Discuss the morphological abnormalities of specific variant (activated or reactive) lymphocytes.

activated or reactive) lymphocytes.

- Identify the anatomical sites populated by T cells and B cells.

State at least three conditions associated with specific lymphocytic abnormalities that may be seen in peripheral blood.

Lymphocyte kinetics : Lifespan and

proliferation

Characteristics of lymphocytes

- Compare the length of the life span of the T and B lymphocytes.

- Differentiate the functions of the three major categories of

Summarize what influences proliferation of T and B lymphocytes in lymphocytes.

primary and secondary lymphoid tissues.

Compare the functional differences between various subtypes of

Explain homeostatic proliferation of lymphocytes.

B cells.

Explain the antibody-independent roles for B cells in immune

Lymphocyte kinetics : Recirculation of

responses.

lymphocytes

Briefly describe the production and laboratory application of mono-

- Explain the process and importance of lymphocyte recirculation.

clonal antibodies.

Describe the migration of naive T cells from blood to tissue.

Major lymphocyte membrane characteristics

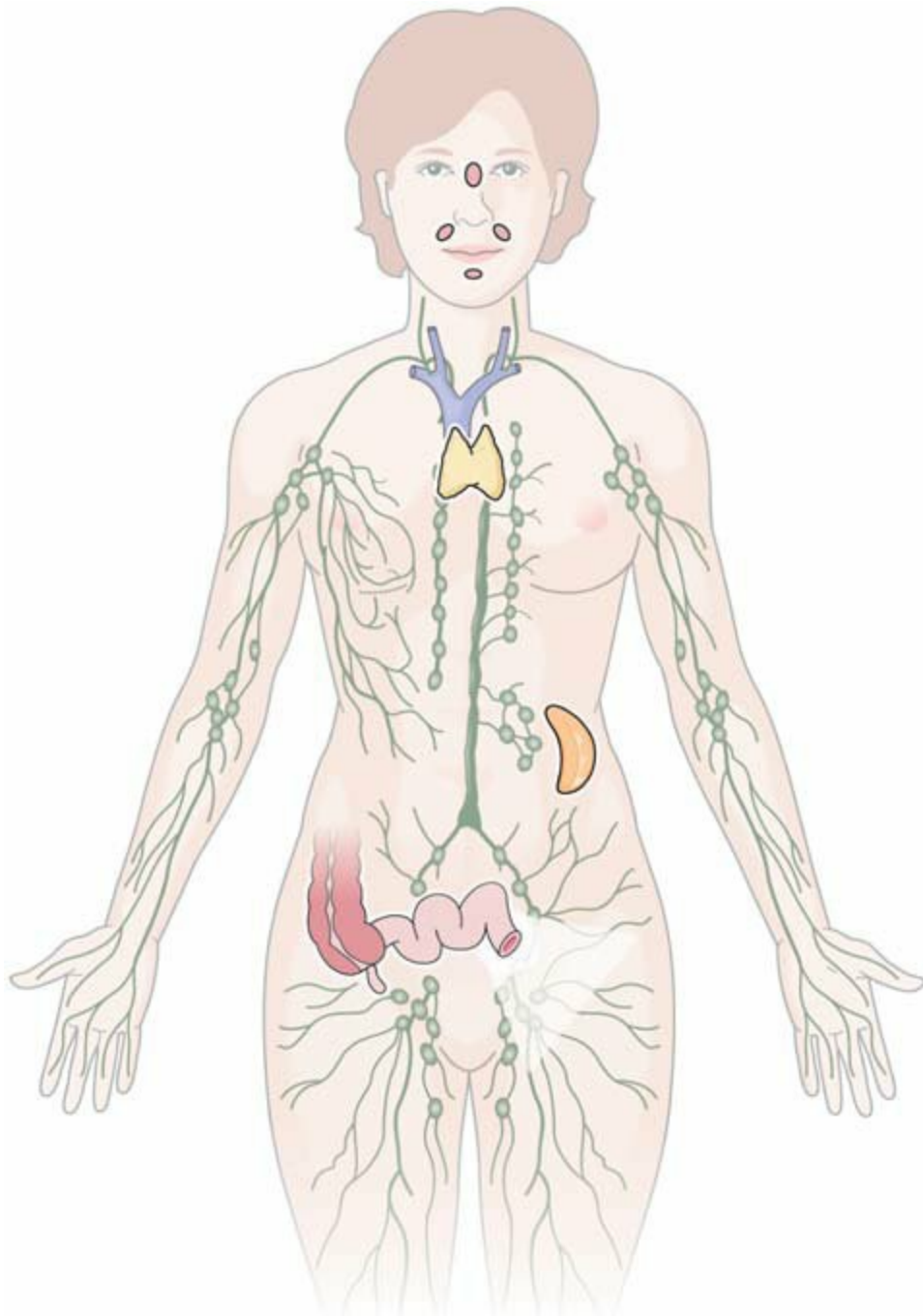
Normal reference values

and development

- Compare absolute and relative numbers of lymphocytes.
 - Describe the production of monoclonal antibodies.
 - Calculate the absolute value of a lymphocyte example.
 - Briefly describe membrane marker development in T cells.
 - Cite the percentage of T and B cells found in the peripheral circulation—
- Summarize major lymphocytic phenotypes and the molecular characteristics used to differentiate lymphocyte subsets.
- Compare the major types, normal reference value percentages, and
 - Name four cytokines or chemokines produced by T cells and describe quantities of lymphocytes at different ages ranging from birth to their function.
- adulthood.
- Name several applications of lymphocyte subset testing.
 - Define the terms lymphocytopenia and lymphocytosis.
- Compare and contrast the immunologic features and major function
- Classify conditions associated with an increase or decrease in
- of T, B, and natural killer (NK) cells.
- the absolute number and relative values in the peripheral blood circulation.

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CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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T lymphocytes, B lymphocytes, and plasma cell

Differentiate the characteristics between short-lived and long-lived

kinetics

plasma cells.

Describe the activation and function of T cells, B cells, and

■ Describe the appearance and cytoplasmic contents of Russell's bodies—
plasma.

ies, Mott cells, and amorphous cells.

Plasma cell development and maturation

NOTE:

- Describe the pathway of plasma cell development.
- Indicates MLT and MLS core content
- Identify the maturational characteristics of plasma cells.

Indicates MLT (optional) and MLS advanced content

ANATOMICAL ORIGIN AND

lymphoid tissue

DEVELOPMENT OF LYMPHOCYTES

are immunocompetent (-

mm) m . I

I , m , m q m (C), lymphocytes

m . I m m bursa B m

plasma cells

m . M m

antigens

; m m

antibodies.

.

Se con da ry Lym ph oid Tissue

S ite s of Lym pho cytic De ve lo pme nt

T m m

D m m , m m (F . .), , P ' (

k .

L m , m m m -

m . C m

interleukin-1 (IL-1) ILm m . C m

Pa la tine tons il

m

Ade noids

m m . H m

Lingua l tons il

m

Thymus

-B m . T m j

gla nd

T lymphocytes B lymphocytes

Node s

(.). A m B .

Prim a ry Lym p hoid Tis s u e

I m , m thymus -

S ple e n

m m (F . .)

m . S m m

m

Approximate Percentage of T and B

TABLE

9.1

Lymphocytes in Lymphoid Organs

Lymphoid

T Lym phocyte s B Lymphocyte s

Organ

(%)

(%)

Appe ndix

Peye r pa tche s

Thymus

100

0

(in inte s tine)

Blood

80–85

10-15

Lymph nodes

60

40

Spleen

45

55

FIGURE 9.1 L m . (R -

Bone marrow

10

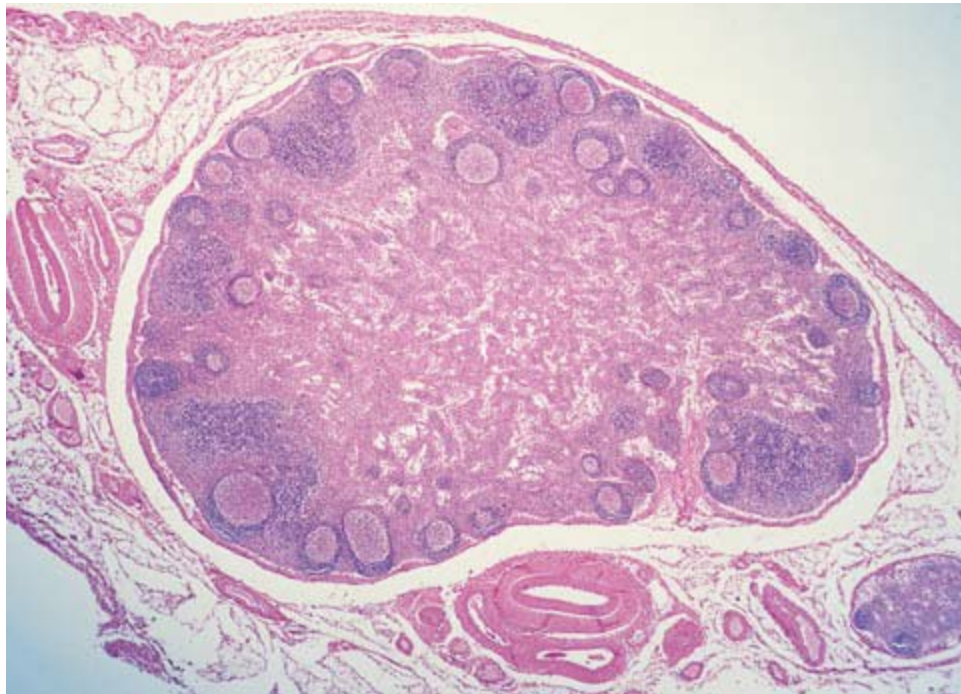
90

mm C BJ, JJ. Memmler's Text Human Body

in Health and Disease, , B m , MD: W K

Modified from Claman HN. J Am Med Assoc. 1992;268(20):2792.

H , .)



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PART 2 ■ Hematopoiesis and Cellular Maturation

FIGURE 9.2 S m . A.

Flow of lymph

Arrows m

Valve

. B. S m

Germinal center

m (). (A. R

m m C BJ, JJ. Memmler's

The Human Body in Health and Disease, ,

Capsule

B m , MD: W K H , ;

B. R m m C m k Afferent DH. Essential Histology, , P , lymphatic Trabecula

PA: L W m & W k , .)

ve s sel

Medullary

sinus

Medullary

cord

Efferent

lymphatic

Cortical

ve s sel

nodule

Subcapsular

sinus

Hilum

Flow of lymph

A

Cortical nodules

with germinal

centers

Capsule

Subcapsular sinus

Medullary Medulla

Hilum

B

sinus

cord

F . .). B m q

. F - m (GAL) x m ,

. M m

q m .

T m **cells** (F . .)

Lymphocyte Kinetics : Life Span and

Proliferation

• P m

• M m

T m m m

• P

, B m

• T m

• L m -

chronic

T B m **B cells** (F . .) m

lymphocytic leukemia CLL m m m -

:

m (ALPS).

• F m (m) P B m m

m m -

• P m

m • T m



CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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FIGURE 9.3 B m . T m x m m (F), m j B-m z -

. T z (DC), x, m m m

. T m z mm m .

m -

m

. T z m m

- z m m m

mm m. T m

m. L m m j

(),

m (F . .). L m , m , m . W - x m m

x m , x m ,

m x

. D m mm , -

m . T m m

- m , - ,

m m .

% m m . S

L m m , m-k mm , -

, m , -

(APC) - m

m . D m

mm . T m m

f k . - m

m

m m

k . T homeostatic prom m , m -

li eration, m .

x m .

A m , m ,

C m k q k “ m ”

m , m

- z m .

m B m . H , T m m m m x m ,

m x m

,

$m \times L$. H m

m . T B , m m - m x m m

(MAL) z

m .

- z .

M k m

Lym pho cyte Kine tics: Recirculatio n o f

q . T

Lym pho cytes

m m :

N m m

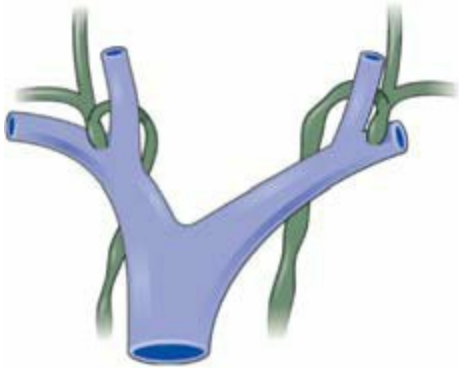
■ L - f k

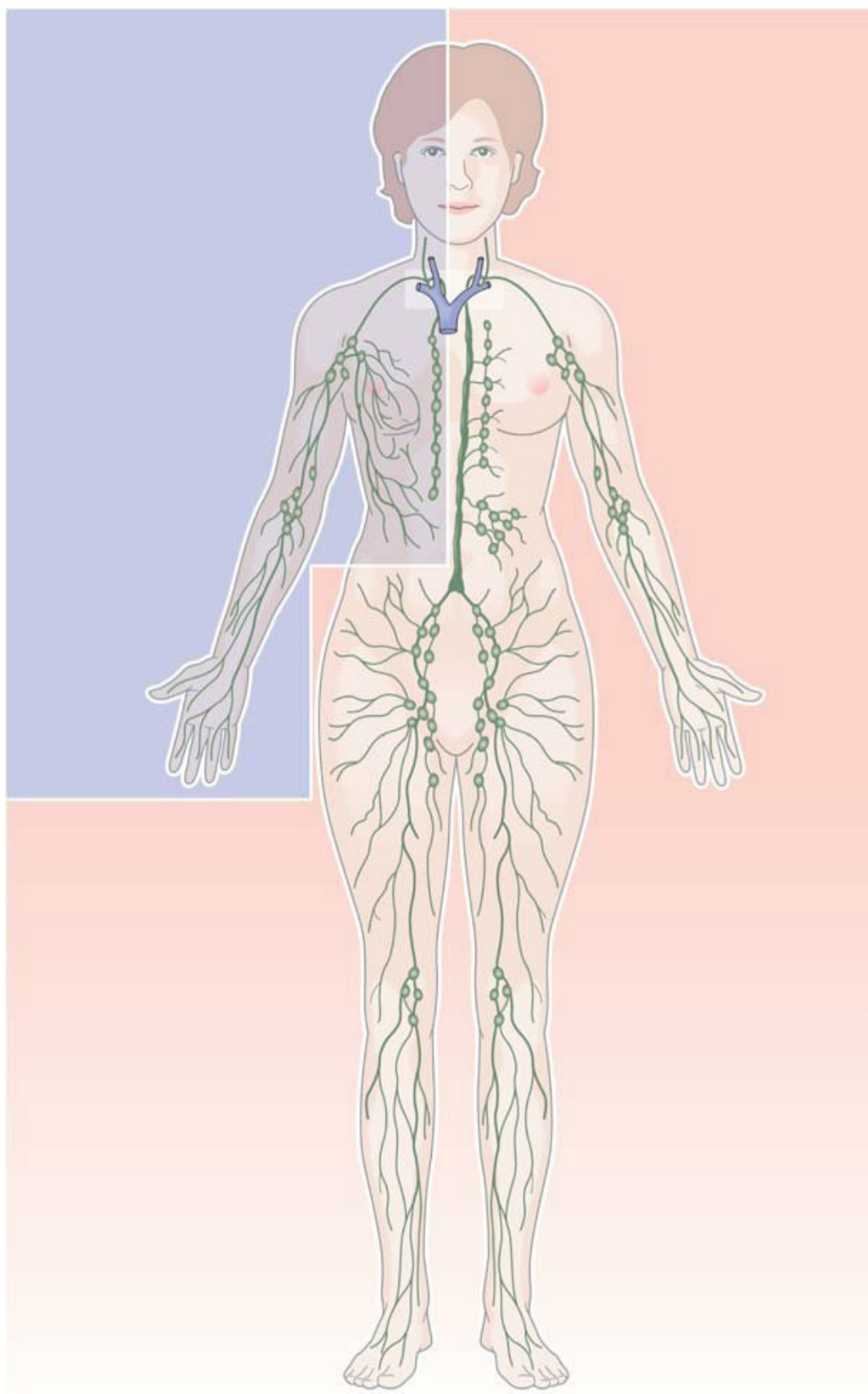
m . T ,

m

lymphocyte recirculation, m m

.





PART 2 ■ Hematopoiesis and Cellular Maturation

Vessels in purple are a

Vessels in red are a

drain into right lymphatic

duct

duct

duct

Occipital

Parotid

nodes

nodes

Right lymphatic

duct

Cervical

Mandibular

nodes

nodes

Axillary nodes

A

Mamma ry

Thora cic

ves s e ls

duct

Mes e nte ric

Right inte rnal

Le ft inte rna l

node s

jugula r vein

jugula r vein

Cubita l

Right

Thoracic

node s

Lumbar

lymphatic

duct

node s

Cis terna

duct

chyli

Right

Le ft s ubcla vian

s ubclavia n

vein

ve in

Ilia c node s

Right

S upe rior

Le ft

and ve s s e ls

bra chioce pha lic ve na cava

brachiocephalic

ve in

vein

Fe mora l ve s s els

Inguina l node s

B

P oplitea l nodes

Tibia l ve s s els

FIGURE 9.4 V m m. **A.** L m . **B.** D

m . (R m m C BJ, JJ.

Memmler's T e Human Body in Health and Disease, , B m , MD: W K H , .)
■ C m k -

E -

■ L k -m m

x m

m

m -

■ m

. C m

m

N m m

, m -

z -

m k x .

. T x

m m m -

Norm al Refere nce Values

. N - m - m -

(GAL) m m m . T

A m , x m % mm m -

m . S x

.

m

CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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m

Average Lymphocyte Values in

TABLE

9.2

m W mm z

Peripheral Blood

..

Lymphocytes

U m , m m

Total White

Age

Relative Percentage

. M

Cell Count

m . A B

Birth

$18.1 \times 10^9/L$

31

R m k -

6 months

$11.9 \times 10^9/L$

61

m , m m m , m

m B .

10 years

$8.1 \times 10^9/L$

38

21 years

$7.4 \times 10^9/L$

34

and older

NOTE: This is a good time to review the definitions of the

Key Terms in the Glossary and ash cards on

. Also,

Source: Dallman PR. Blood-forming tissues. In: Rudolph AM (ed.).
Pediatrics, 9th ed, New York: Appleton-Century-Crofts, 1977:1178.

a good time to complete Review Questions related to the
preceding content.

m m , x m %

m B m . m

Maturationa l S tage s

(.). L m -

% k ;

Lym p hob la s t

, m m k -

T m (F . .) m

. M m .

m m

I , m x m %

m . T z m μm,

k .

- m (N:C) : .

T . O

m . T m

Absolute and Relative Lymphocyte Values

$k \cdot T$ m m m m m

T absolute number m m

$m k - \cdot N \cdot$

m m m

$k \cdot T$ **relative number** m -

Prolymphocyte

m m m

T m m

$m k \cdot T$ m

$m m (F \cdot \cdot) \cdot T$ m

$m k$

$m, m, m-$

$\cdot T$

$\cdot T z m ($

$, m, \cdot$

$\mu m) m \cdot T N:C m :$

Ex m m \cdot

$:\cdot$

$T \cdot T$

$$A_m = k \times \%$$

$$m_m . T_m -$$

$$m$$

$$. T_m m -$$

$$k = . \times /L$$

$$m_m m , k_m . A$$

$$R_m m = \%$$

$$z_m .$$

$$A_m = . \times /L$$

$$T_m m$$

$$\text{Ma ture Lym pho cyte}$$

$$\% \%$$

$$M_m (F . .) z_m$$

$$T_m$$

$$(\mu m) m (\mu m)$$

$$. . \times /L.$$

$$. T N : C_m : :$$

$$A_m m$$

$$: .$$

$$\text{lymphocytopenia. } W_m$$

T m -

m ,

(). N . T m

lymphocytosis.

m . T m k

. A z m .

E m

MORPHOLOGICAL CHARAC ERIS ICS

m m (F . .).

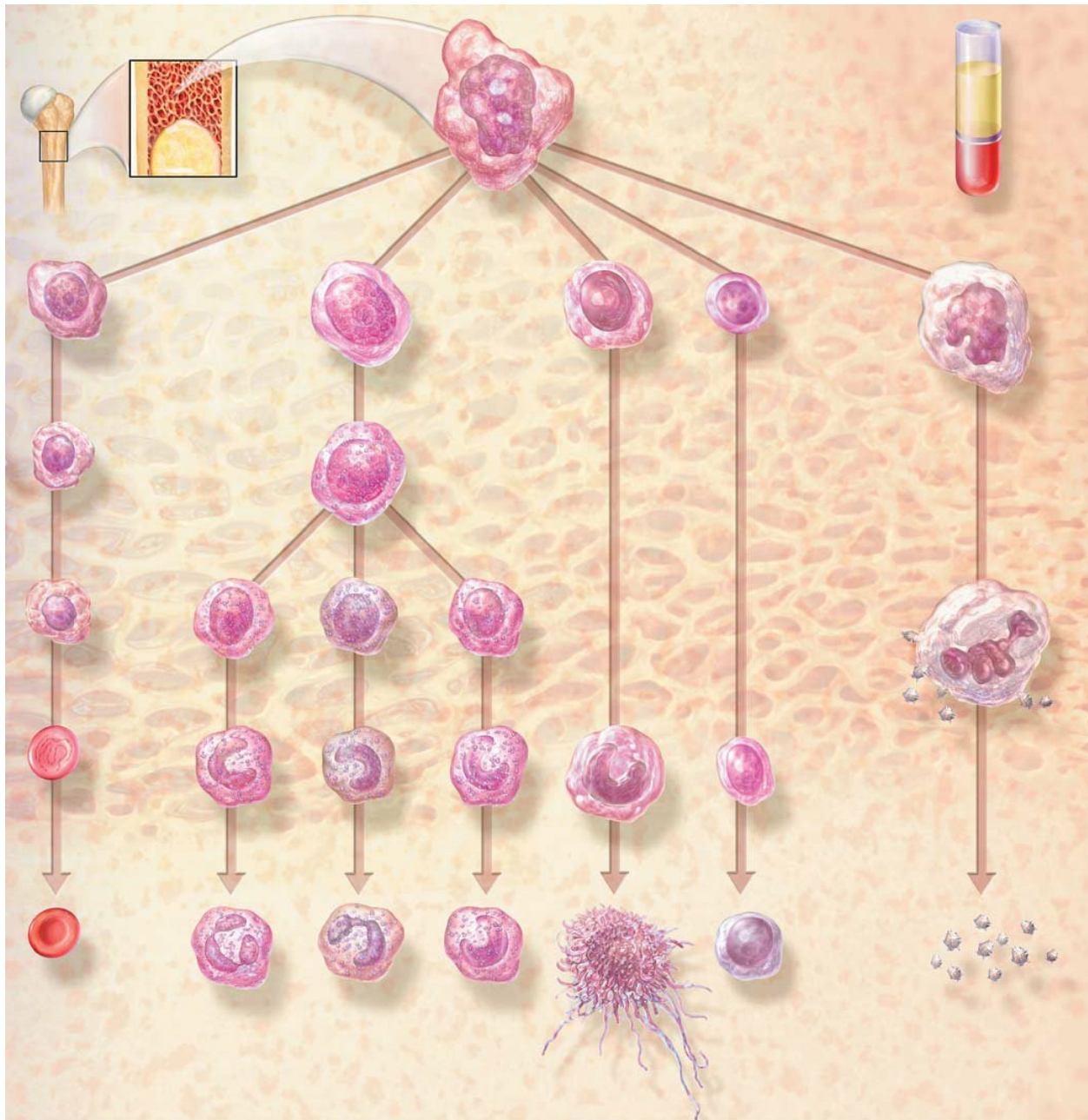
OF NORMAL LYMPHOCY ES

Ge n era l Va ria t ion s in Lym p hocyte Morpho logy

T m m (F . .)

V m m m , m , m , m m . T

m , D ,



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PART 2 ■ Hematopoiesis and Cellular Maturation

Cortical bone

Plasma

Red bone marrow

White blood ce lls

Platelets

Ye llow bone marrow

Re d blood ce lls

He mocytobla s t

Hume rus bone

Blood s a mple

(Ste m cell)

P roe rythrobla s t

Monobla s t

Lymphobla s t

Mye loblas t

Me ga ka ryobla s t

Erythrobla s t

Progra nulocyte

Normobla st

Ne utrophilic

Eos inophilic

Ba s ophilic

mye locyte

mye locyte

mye locyte

Me ga ka ryocyte

Re ticulocyte

Ne utrophilic

Eos inophilic

Ba s ophilic

ba nd ce l

ba nd ce l

ba nd ce ll

Monocyte

Lymphocyte

Erythrocyte

P la s ma ce ll

(Re d blood ce ll)

Ne utrophil

Eos inophil

Ba s ophil

Thrombocyte s

(Pla te le ts)

Macrophages

Granulocytes

(White blood cells)

Nongranulocytes

(White blood cells)

FIGURE 9.5 Diagram of the immune system, showing the flow of information and the interaction of the various components.

Diagram of the immune system, showing the flow of information and the interaction of the various components.

Diagram of the immune system, showing the flow of information and the interaction of the various components.

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Diagram of the immune system, showing the flow of information and the interaction of the various components.

, m , .

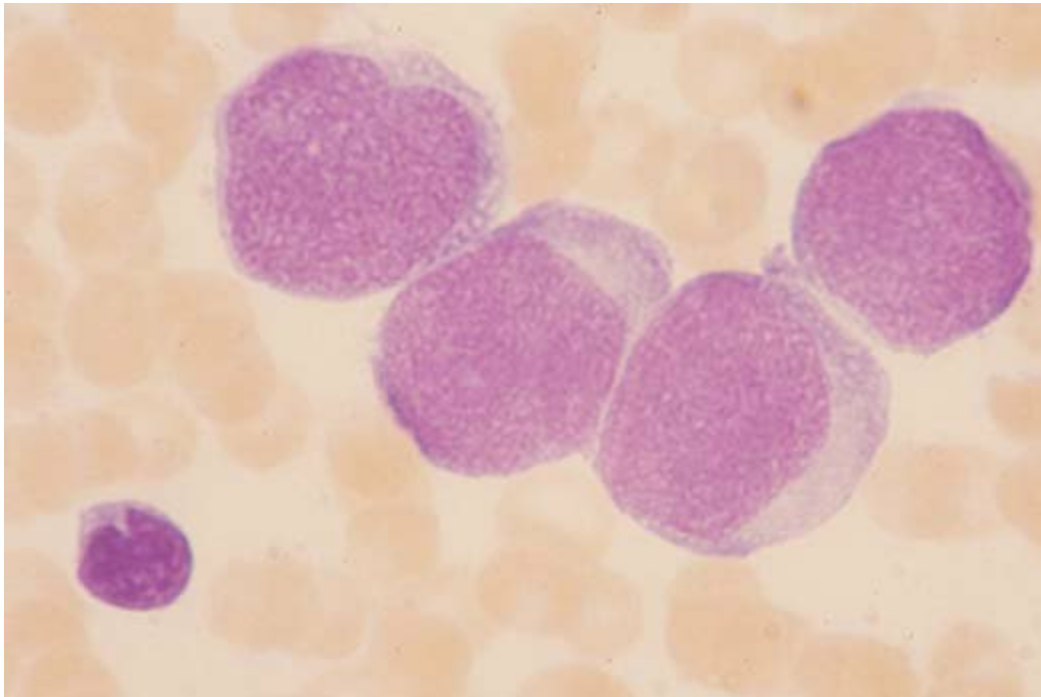
D z mm

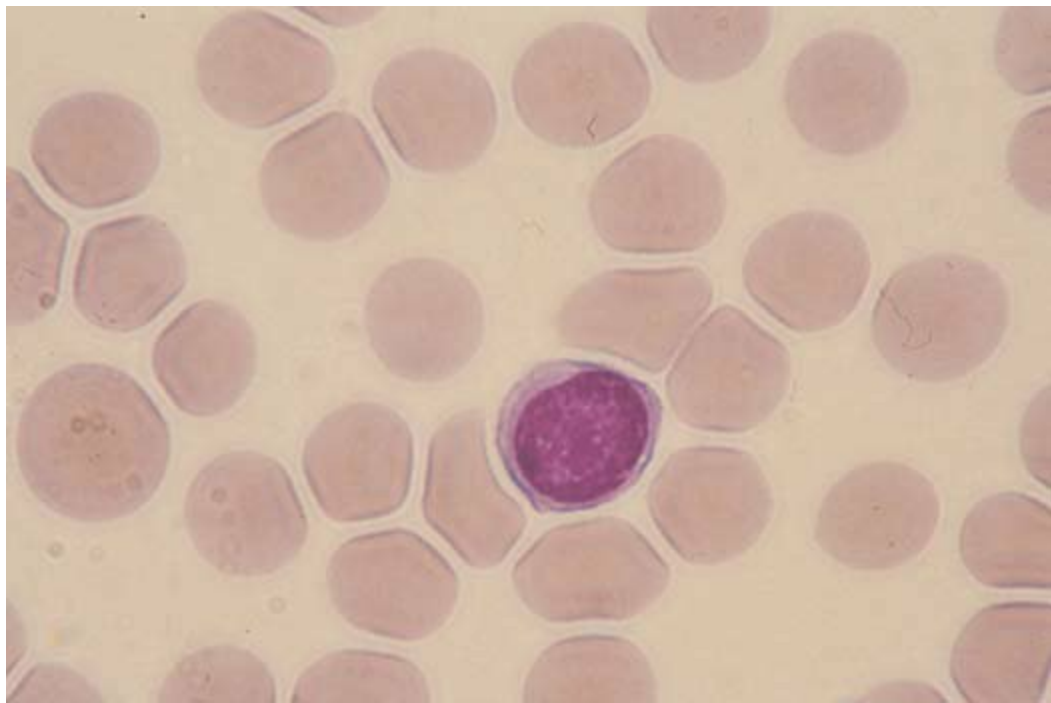
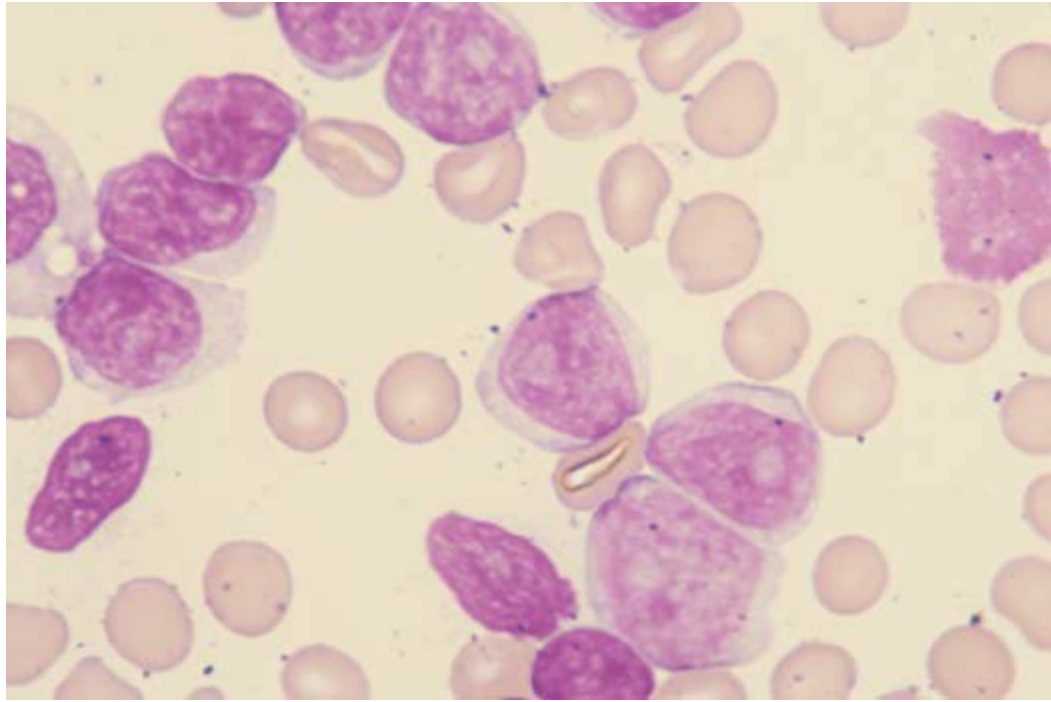
m m (

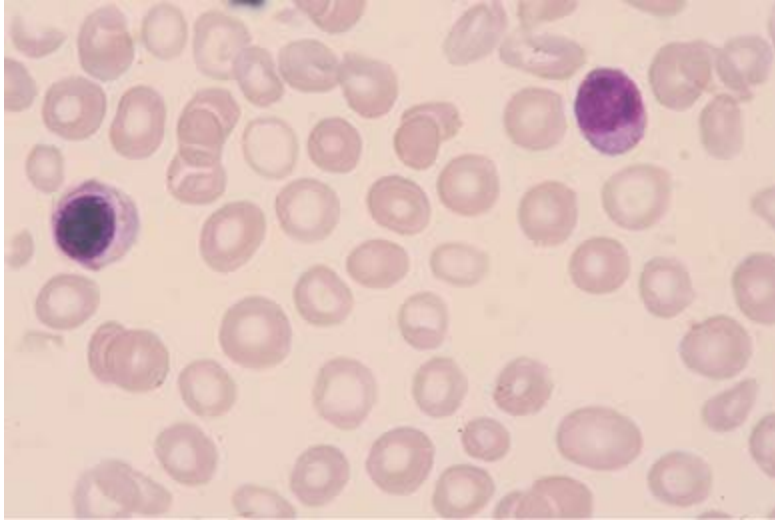
m m m

F . .), , m z mm m memory m

cells.







CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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TABLE

9.3

Comparative Characteristics of Lymphocytes

Lymphoblast

Lymphocyte

Prolymphocyte

Mature

Size

15–20 mm

15–18 μm

Small, 6–9 μm Large, 17–20 μm

N:C ratio

4:1

4:1 to 3:1

Small, 4:1 to 3:1 Large, 2:1

Nucleus

Nuclear shape

Round or oval

Oval, slightly indented

Round or oval; may have clefts

Nucleoli

1 or 2

0 or 1

Absent

Chromatin

Delicate

Slightly condensed

Dense and clumped

Cytoplasm

Granules

None

May have a few azurophilic granules

Few azurophilic granules may

be present

Amount

Small

Small

Very scanty

Color

Medium blue, may have a

Medium blue with a thin rim of darker blue

Light blue

darker blue border

FIGURE 9.6 L m . (R m m **FIGURE 9.8** M m . (R m A SC. Anderson's Atlas o Hematology, P , PA: m A SC. Anderson's Atlas o Hematology, P ,

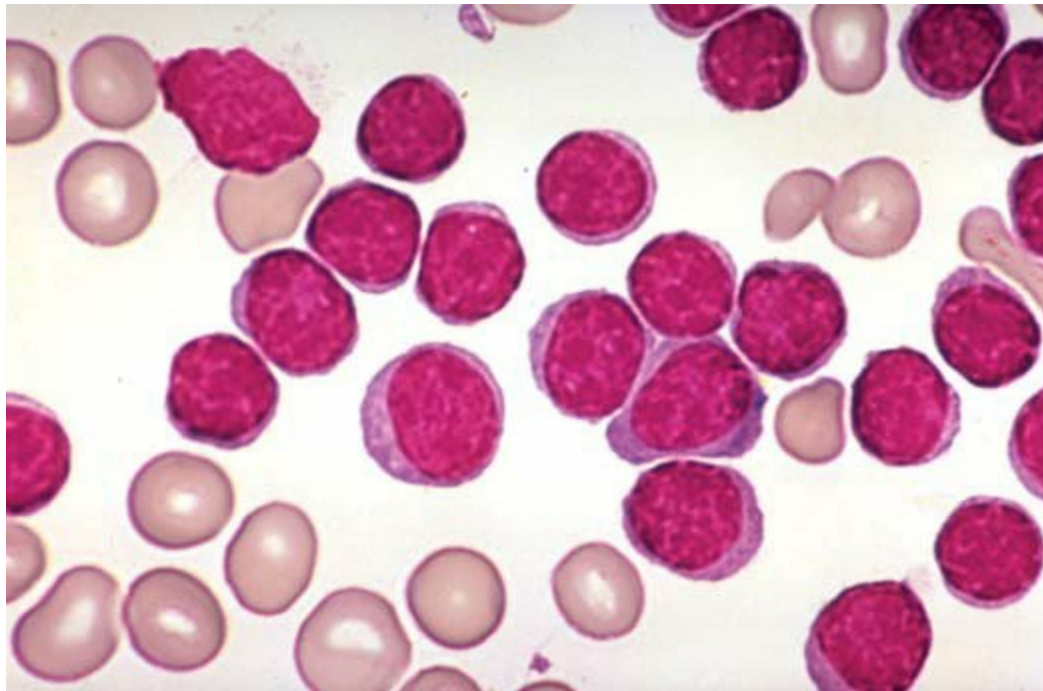
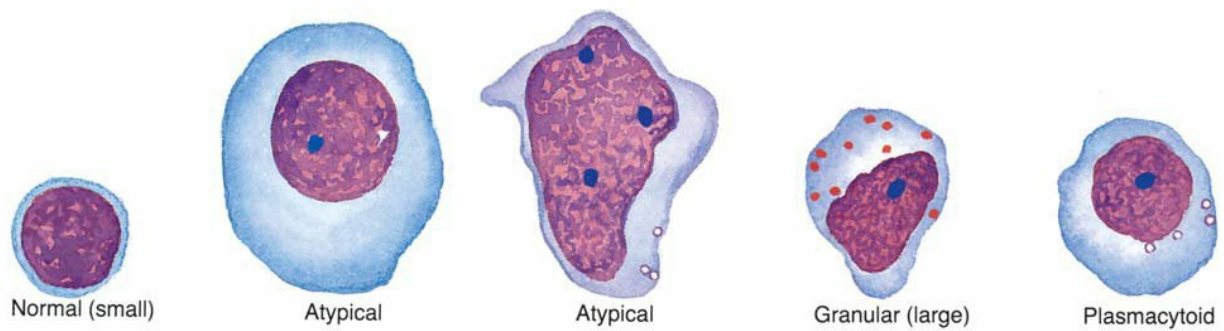
W K H /L W m & W k , .) PA: W K H /L W m & W k , .) Lymphocyte Polychroma tophilic

normoblasts

FIGURE 9.9 L m m , m .

FIGURE 9.7 P m . (R m m (R m m A SC. Anderson's Atlas o A SC. Anderson's Atlas o Hematology, P , PA: Hematology, P , PA: W K H /L

W K H /L W m & W k , .) W m & W k , .)



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PART 2 ■ Hematopoiesis and Cellular Maturation

Variant lymphocytes

(NK cells)

FIGURE 9.10 L m m . T m “ m ” m m . A m x () m ; . L m m m - - m m k m . T m , m NK , m m . P m m m m . (R m m R R , S D S . Rubin’s Pathology: Clinicopathologic Foundations o Medicine, , P , PA: L W m & W k , .) T **variant lymphocytes** CLL (C)

:

m .

. U z (μm).

Vacuolated Lymphocytes

. T m .

. T m m

Vacuolated lymphocytes

N m -P k , -S , H ' -

m (m) -

m , B k ' m m . V

m .

m ,

. C m m

, m .

.

. O m .

Smudge Cells

. T m q m

.

Smudge cells (F . .)

m . T

. C m m m

m . I

.

m k m -

. G m .

m . S m m m

S peci c Lympho cyte Morpholo gical

Variatio ns

A m m m - k

. T :

Binuclea ted Lym p hocytes

T . I m %

m ,

m k m k m (m

m m).

Crysta llin e Inclus io ns

C m mm m . T z mm .

FIGURE 9.11 C m k m . A m -

Ried er's Ce lls

m m - m m-z m .

A m . (R m -

Rieder's cells m m m x

m R E, F JL. Pathology, , P , PA:

, , - k . T

L W m & W k , .)

CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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TABLE

9.4

Lymphocyte Characteristics

Peripheral Blood

Type

Function(s)

Phenotypic Marker

(% of Total)

Helper T (Th) Stimulate B-cell growth and differentiation

CD3+, CD4+, CD8–

50–60

cells

(humoral immunity)

Macrophage activation by secreted cytokines

(cell-mediated immunity)

Cytotoxic T

Lysis of virus-infected cells, tumor cells, and

CD3+, CD4-, CD8+

20–25

(Tc) cells

allografts (cell-mediated immunity)

Macrophage activation by secreted cytokines

(cell-mediated immunity)

Natural killer Lysis of virus-infected cells (antibody-dependent Fc receptor for IgG or cells CD16

~10

(NK) cells

cellular cytotoxicity)

B cells

Antibody production (humoral immunity)

Fc receptors, MHC class II, CD19, CD21

10–15

k

B, m m

k . Sm -

B . O m

m , CLL.

m m

m B m .

I monoclonal antibody MAb

NOTE: This is a good time to complete Review Questions

m m m k-related to the preceding content.

m (F . .) . I

m , m k m

m , m m ,

CHARAC ERIS ICS OF LYMPHOCY ES

k m , m mm -

.

Major Lymphocyte Categories and Functions

C m z MA

(m) S m j m z m k (m , . T **cells**, **B cells**,

“m k,”). O ,

natural killer NK lymphocytes (.).

m k m

Bo ne

Thymus

Pe riphe ral

marro w

lympho id

Corte x

Me dulla

o rg ans

Lymphobla s t

Ea rly

Common

Ma ture

Ma ture

thymocyte

thymocyte

thymocyte

T ce l

TDT+

CD4

CD4 (T4)

CD10

CD9

CD9

He lpe r ce l

CD4

CD10

CD10

CD10

CD8

TDT+

TDT+

CD8

CD8 (T8)

CD10

Pre -T ce l

Suppres s or ce ll

FIGURE 9.12 Ex m m k - m . - m (-

) m m , mm m , m m . S m -

m m m k . CD () CD () m k m k m .

PART 2 ■ Hematopoiesis and Cellular Maturation

m, m m m

. I m, m k -

BOX 9.1

,

MA , m m cluster of differentiation (CD).

Interleukin 21 (IL-21) Regulation of B-Cell

M

Function

m m m-

■ E CD L, BCR

. T q

m

(C).

■ I x m k A/CDA, XBP- ,

T Lym ph ocyte s

.

■ I CSR I G , I G , I A

mm (-

■ P m B m m

m mm)

I M, I G, I , I E

B m -

■ S z IL-CSR I G

. S z m m

I E

m ,

■ S m m -

, , z . T

m

j .

■ G m m , B Lym pho cyte s

. . , m m

■ R q S A m

T m B m-

. T m B

m x mm -

(I) ,

B k ,

mm (mm).

m B- . D m ,

S m B m x B , -

, q m m

z m k I (z , ,), x .

, B . B m

T B- :

. T

B- , CD , B , m z B , B .

j m

N I M (I M) B mm

B .

q m . Imm mm -

(I) - -

Na tura l Kille r Lym p hocyte s

.

NK m NK m

B :

. , z q

m mm m

. CD m m m B , m .

m , x m m B

NK , m CD + m -

m ,

, mm m. U k

mm m .

B m - mm

. B z -

m, NK - mm -

. B MZ z -

m m .

.

NK m B :

. R B , m ,

m x

m IL-m .

m

. A - B (ABC)

x . NK - , B- . T k m , k m m CD CD x . T CD /

.

m k x B . F , ABC

O W - m , NK

.

m m m

C , m k

m z

m B-IL- (B x .).

m.

B m

T m NK -

m () . P B

. mm -

m mm m

NK , CD

m m , q

CD m, . CD m

CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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TABLE

9.5

Stages of T-Cell Maturation

Double Positive

Single Positive

Stages of

Maturation

Stem Cell

Pro-T

Pre-T

CD4+ CD8+

(Immature T Cell) Mature T Cell

Anatomic

Bone

Thymus

Thymus

Thymus

Thymus

Peripheral blood

location

marrow

Response to

None

None

None

Positive and Negative

Activation (proliferation

antigen

Selection

and differentiation)

Surface mem—

c-kit⁺

c-kit⁺

c-kitⁿ

CD4⁺CD8⁺

CD4⁺CD8[−]

CD4[−]CD8⁺

brane markers

CD44⁺

CD44⁺

CD44[−]

TCRCD3^{lo}

TCRCD3^{hi}

TCRCD3^{hi}

CD25⁻

CD25⁺

CD25⁺

Modified from Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology, 8th ed, Philadelphia, PA: Elsevier (Saunders), 2015:191 [Figure 8.12].

Note: Only the stem cell and early Pro-T and mid to late Pre-T lymphocytes can proliferate. Expression of RAG occurs in the Pro-T, very early Pre-T, and in the early to mid stage of CD4⁺CD8⁺ development. TdT expression occurs in most of the Pro-T stage and extremely early in the Pre-T phase.

m x m

Monoclonal Antibodies

- x (ADCC)

U m , m k

- x CD

z :

. B m , CD m m -

- k m mm

. S m m k

(IFN- γ), GM-CSF, IL- , IL- ,

m (. .).

mm . F , -

. S m m k x ,

mm (IFN- γ) NK—

m .

k , T mm , -

F x m , CD

APC MHC I x ,

m , m m

m k , CD +, CD +, CD -; m x m -

m - -

CD +, CD -, CD +. I

m .

CD

TABLE

9.6

Stages of B-Cell Maturation

Stages of B-Cell

Maturation

S t e m C e l l P r o - B

P r e - B

I m m a t u r e B

M a t u r e B

Anatomic location

Bone

Bone

Bone marrow

Bone marrow/

Peripheral blood

marrow

marrow

peripheral blood

Response to

None

None

None

Negative selection (deletion), Activation (proliferation and

antigen

receptor editing

differentiation)

Surface membrane CD43+

CD43+

B220lo

IgMlo

IgMhi

markers

CD19+

CD43+

CD43 (neg)

CD43 (neg)

CD10+

Immunoglobulin

None

None

Cytoplasmic μ and Membrane IgM (μ^+ kappa or Membrane IgM and IgD

expression

pre-B receptor—

lambda light chain)

associated μ

Modified from Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology, 8th ed, Philadelphia, PA: Elsevier (Saunders), 2015:185 and 191 [Figure 8.12].

Note: Only the stem cell and early Pro-B lymphocytes can proliferate. Expression of RAG occurs in the late Pro-B/Pre-B stages of development and in the early immature B phase of development.

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PART 2 ■ Hematopoiesis and Cellular Maturation

mm, CD mm z mm

, m :

mm . B ,

mm ,

■ P m - -

k

■ m

(), B , .

T m B m -

M m x

q -

: m m m -

- m . L m

m m .

m m q -

T x

. A - m

m . T m -

x B ,

, m -

x m -

, . T

z . L m

m k m m .

m , m ,

I m , m k

, m , m

m m

m -

m . V x

m .

m m k . T

m m m k

T Lymphocyte s

m m , -

m (. . , q mm -

m q m

m [AIDS]), k m (C),

m . B m m -

m mm .

m . S m

q m k , CCR CCR . T m

Major Lymphocyte Mem brane

N

- m – m .

Characteristics and De ve lopme nt

M (F . .) z

T m m m

m m m k . E B

m . T m-z m D , CD , CD ,

(x m m m k)

m . H , m k ,

. . C mm

CD CD , mm

. O m k q m -

. S m m k m .

Cell Differentiation Antigen

TABLE

9.7

T m k m , CD

Distribution

CD , m k m k m m

.

Ce ll Diffe re ntiation Antige n

I , CD m

Antige n

Dis tribution Clus te r De s ignation

: T T . T m

CD2

78%–88% T cells, NK cel s

k -

. T mm -

CD3

68%–82% mature T cells

m m ; T mm

CD4

35%–55% mature T cells, monocytes

m x , m -

CD5

65%–79% T cells, B-cell subset

. A m T m

CD7

75% T cel s and NK cells

- mm ; T

m mm .

CD8

20%–36% mature T cells

, CD -

CD10

90% of common ALLs, granulocytes,

m x : T , T , T , -

(CALLA)

immature B cells

().

CD19

5%–15% B cells

CD mm . T

CD20

5%–15% B cells

CD

CD21

5%–15% mature B cells, dendritic cells

■ H B m m k

CD22

5%–15% B cells

■ I m m

CD34

Bone marrow progenitor cells, TdT+ cells,

■

some acute leukemias

R , ,

mm

Adapted with permission from Turgeon M. Immunology and Serology in

■ P k m k mm Laboratory Medicine, 4th ed, St. Louis, MO: Mosby, 2006:63.

CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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C mm -

TABLE

9.8

Characteris tics o f CD4 T Ce lls

B (.). T -B m k

: m x (D) m k-

Re pre s e ntative

: CD , CD , mm (CALLA

S ubs e t Major Activitie s

Cytokine s Produce d

[CD]).

T q -B

Th1

Autoimmunity intracel-IFN- α , IL-2, IL-10

mm m.

lular pathogen defense

Imm m

Th2

Extracellular parasites IL-4, IL-5, IL-10, IL-13,

. T (μ) I M z

defense active in

IL-25

z -B . O m k D ,

asthma and al ergic

CD , CD , CALLA, HLA-DR. P -BCR

reactions

k m -B

k m .

Th17

Autoimmunity extra—

IL-10, IL-17a, IL-17f,

I x m , mm B

cellular bacteria

IL-21, IL-22

m (I) mm (I)

defense fungi defense

m m m

iTreg

Regulation of immune

IL-10, IL-35, TGF β

I M. T - I M m responses immune

I M m m m .

tolerance lymphocyte

R m m F (m , homeostasis

c z) mm (I G)

IFN- α , interferon gamma; TGF β , transforming growth factor beta.

. A

m , m .

T m B mm -

, I M I D. W , B

T z

x ,

m , x -

m (-). S m

. S . mm

m m m m

m j CD . T m j mm

m B-m ;

CD +

m - - ,

.

m .

B Cells

Function al Tes ting o f Lym phocyte s

Af m

- m m m -

f -

. S m (cytokines) m -

m , m

, m , ,

m I B

- - mm . Im k

f . I

:

m B k z , I V

. Migration inhibition factor (MIF): m m -

m x m .

M I V m m .

. **Interleukin 2 IL 2 cell growth actor** : m j

B m f -

m -

.

. **Chemotactic actor:**

A - B mm

. **Interleukin 1 IL 1** : m -

■ P - B

m CD + CD + . I MHC II k-

, m x

LYMPHOCY ES, B LYMPHOCY ES, AND

mm z .

PLASMA CELL KINE ICS

■ P k m k

mm

H – B- -

m mm .

q -

■ A mm m B- -

z B m m .

m

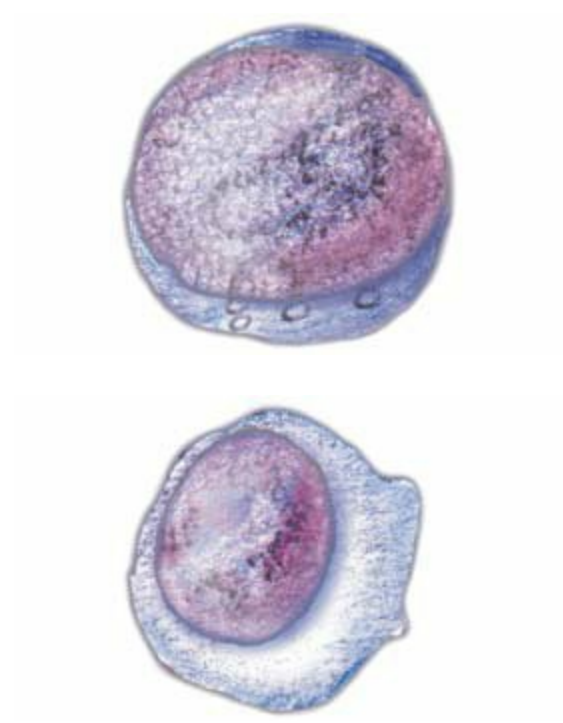
T m m

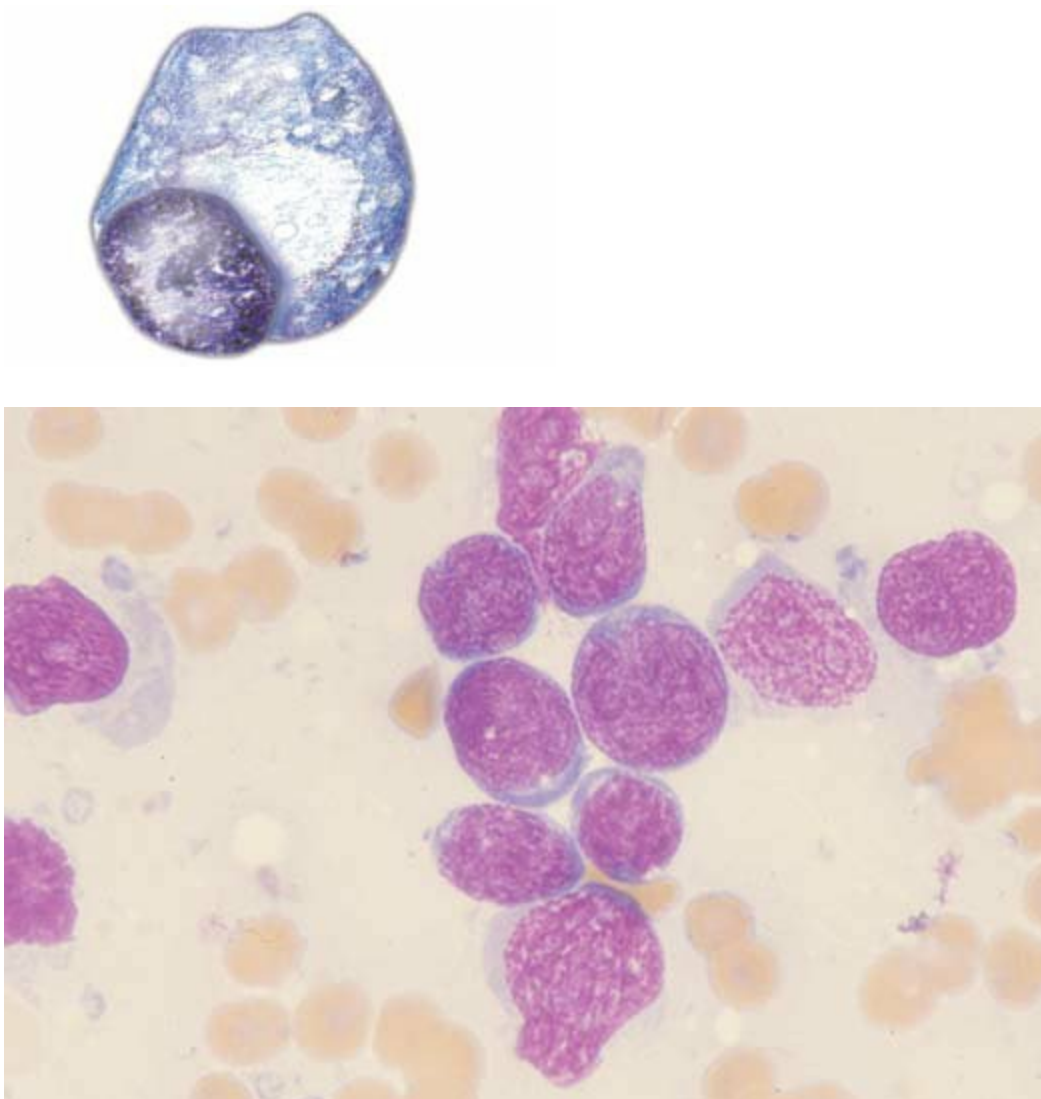
CD + CD + , ,

, B

x - m .

.





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PART 2 ■ Hematopoiesis and Cellular Maturation

A x CD L,

CD B , k k B . T m CD k m B-

.

B m

m . P -

m . A B—

q CD +

.

NOTE: This is a good time to complete Review Questions
related to the preceding content.

FIGURE 9.14 P m . (R m m PLASMA CELL DEVELOPMEN AND

A SC. Anderson's Atlas o Hematology, P , PA:

MA URA ION

W K H /L W m & W k , .) T m x mm (). P m -

mm . A

m

(

m m x

F . .) , - B

%. P m B- -

m -

m .

m m

T (F . .) m B m

.

- z m B

P m m , m -

m m -

B mm -

. T mm -

. T B

B m

m BCR, CD , - k (LR),

k .

T k m :

. S - m

- - -

x B- . T

Pla s ma bla s t

m

m .

. L - m - -

m . S m B-IL- -

m , m .

P m m ,

Proplasma cyte

- CD -

m B .

L - m - -

. P m mm

m m m -

. T m m

q m m m

Plasma cell

B-m , , m

m m m B- . P ,

k , LR m m B -

- m m

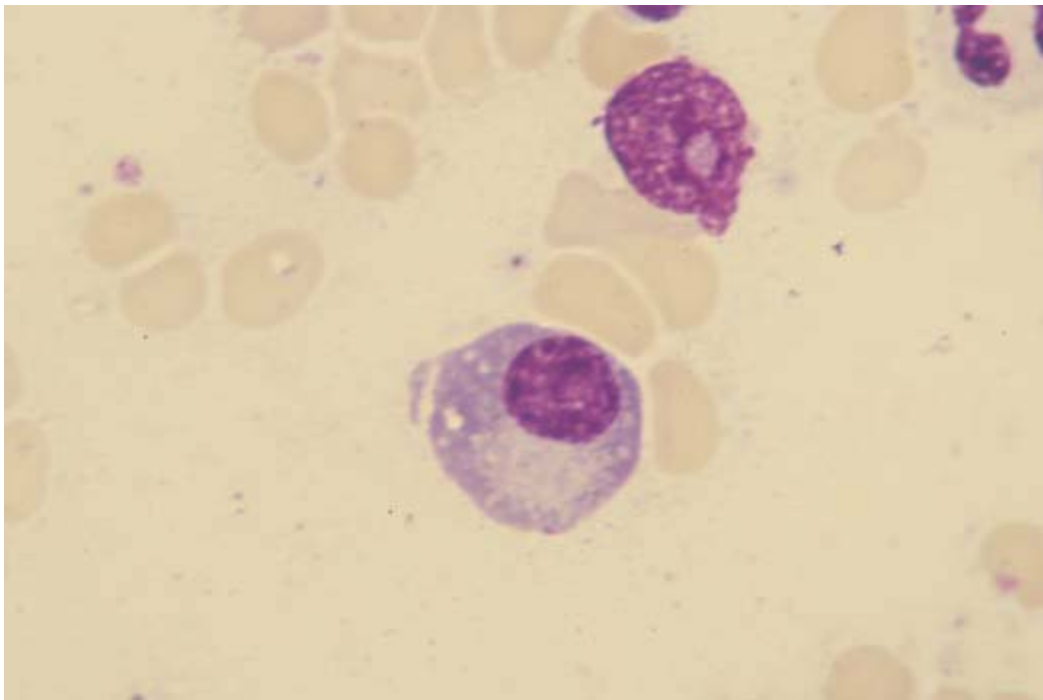
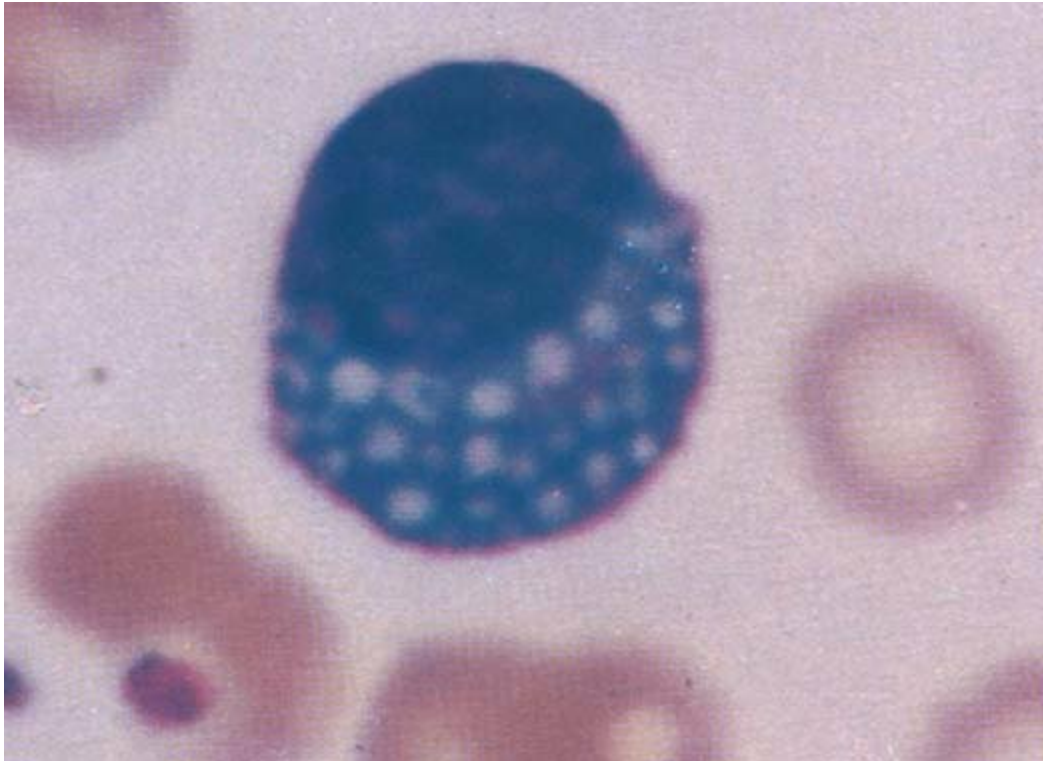
FIGURE 9.13 P m . (R m

.

m A SC. Anderson's Atlas of Hematology, P ,

P m m -

PA: W K H /L W m & W k , .) m m



CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

203

- m . T m m -

k m m m

. T ,

. A k

mm z - ,

m m m j .

P m m m

m

. A

- m

. S

m , m m

.

D B - m

m j m

FIGURE 9.15 P m . (R m m m m , A SC. Anderson's Atlas o Hematology, P
, PA:

I I

W K H /L W m & W k , .) m m m m. W

, m ,

mm () . O

m . T

m m , m -

ER m m , m

, :

m B

m .

. G Mott cells, m m

R ' (F . .)

Maturational Morphology

. Flame cells, m -

q

Ma ture B Ce ll (After Bla s t Tran s fo rm a t ion)

m m

T z μ m. T m

m eccentrically placed (m

). T m m . T

Plasma Cell Disorders

m , m m ,

A m m -

m .

,

Plas macyto id Lymphocyte s

m :

. V (. . , , k x, m m ,

T z μm . T

m)

. T m -

. A

. N m . U , m

k , ,

x . T

G .

Pla s m a Ce ll

T m m (F . .) m

; , % % m – k

m m .

T z μm . T m

. M m

. T m

.

A m k , -

. T - , m

m,

x . T k-m

().

FIGURE 9.16 M (R '). (R m -

T m . G

m O'C BH. A Color Atlas and Instruction Manual o

, mm . C m

Peripheral Blood Morphology, B m , MD: L W m

R ' ,

& W k , .)

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PART 2 ■ Hematopoiesis and Cellular Maturation

. C

- z m m m

. C

m.

C m k q k “ m ”

I m , m m



- z m . T m m -

m m m ,

m m

, x m , W m' m m m -

x m m

m m (C).

× /L .

NOTE: This is a good time to complete end of chapter

Norm al Refere nce Values

Review Questions.

■ A m , x m % m-m .

CHAP ER HIGHLIGHT S

■ S x m

m m x m

Anato mical Orig in and De velopm ent of

% m B m .

Lym pho cyte s

■ m .

L m % k -

■ L m m

; , m

m -

m k . M

.

m . I , m

■ F m m ,

x m %

m m

k .

m . F m

m m mm — m

■

m m m

m — — m

k . m m-

, , GAL .

m -

■ I m , m

m m m

m j m : B .

k .

Lym pho cyte Kine tics: Life S pan and

■ A m

m . W m

Pro liferatio n

m ,

■ T m m

m .

m , B

m .

Morpho log ical Characte ris tics o f No rm al

■ L m

Lym pho cyte s

m -

k m (CLL) mm m -

■ T m m m -

m (ALPS).

, m , m m .

■ P B m

■ W m W ,

m m

m

m .

k .

■ T m m m-

■ L m m m .

k

S m -

m ,

m m , -

m .

, m .

■ I , m , m , -

% k ,

Characte ris tics of Lym pho cyte s

% m

, % % %

■ T m z -

B .

, B , NK .

■ m mm **Lym pho cyte Kine tics: Recirculatio n o f -**

Lym pho cyte s

B m .

■ B m m -

■ N m m

m mm () .

m . T ,

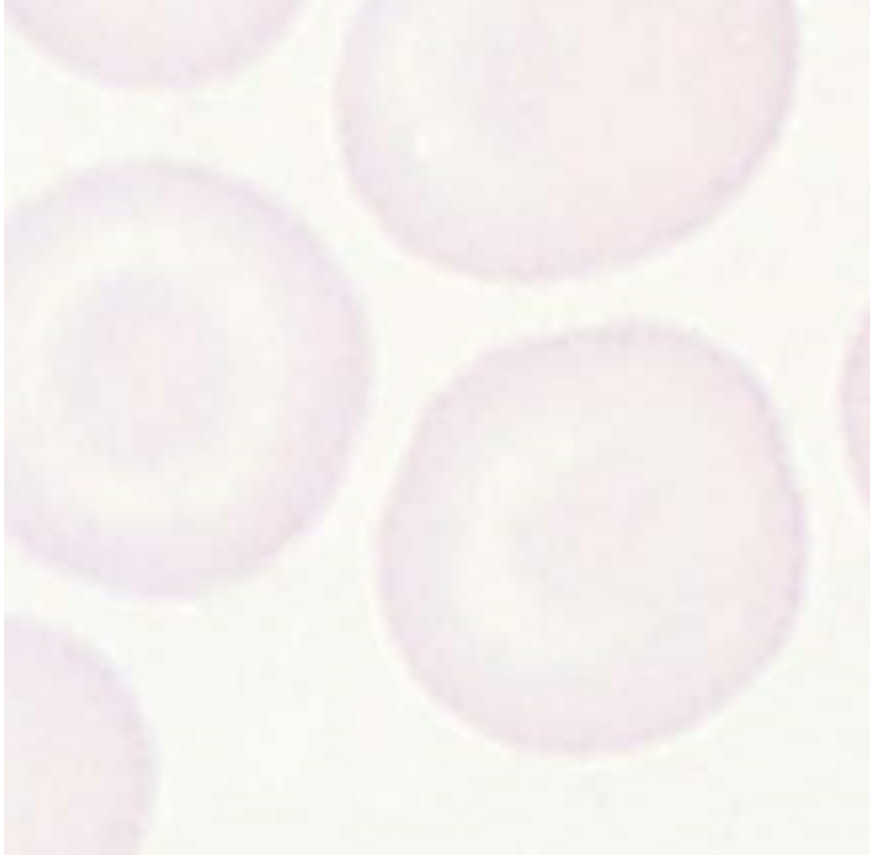
■ NK m k m m k

m , m m

B . T -

m

x .



CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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Functions and Membrane Characteristics of

T Lymphocytes, B Lymphocytes, and

Lymphocytes

Plasma Cell Kinetics

■ Imm ,

■ H – B- -

m k

q -

.

z B m m .

■ T m B m

■ B m

q -

m .

- m .

■ P m B-

■ E B z m D , CD , m .

CD , m .

■ M k , CD CD ,

Plasma Cell Development and Maturation

mm .

■ C mm

■ P m m

B. -B m x -

m m .

(D) m k : CD ,

■ P m m m B m .

CD , mm ALL (CALLA

■ I m m

[CD]).

m , m m m .

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

6. P ’

A. m m

Lymphopoiesis: Anatomical Origin and Development o

B. m

Lymphocytes

C. m

1. L m

D. m

A. m m

B. m

Lymphocyte Kinetics: Life Span and Proliferation

C. m

7.

D. m

A. m

B. x m

2. L

C.

A. m m

D.

B. m

C. m

8. A m j B-m z -

D. m

A. m

3. S

B. z

A. m m

C. x

B. m

D.

C. m

D. m

Lymphocyte Kinetics: Recirculation o Lymphocytes

9. T m m

4. R m

A. m m

A.

B. m

B. m

C. m

C. m - z m m

D. m

D. mm m m B

5. T m

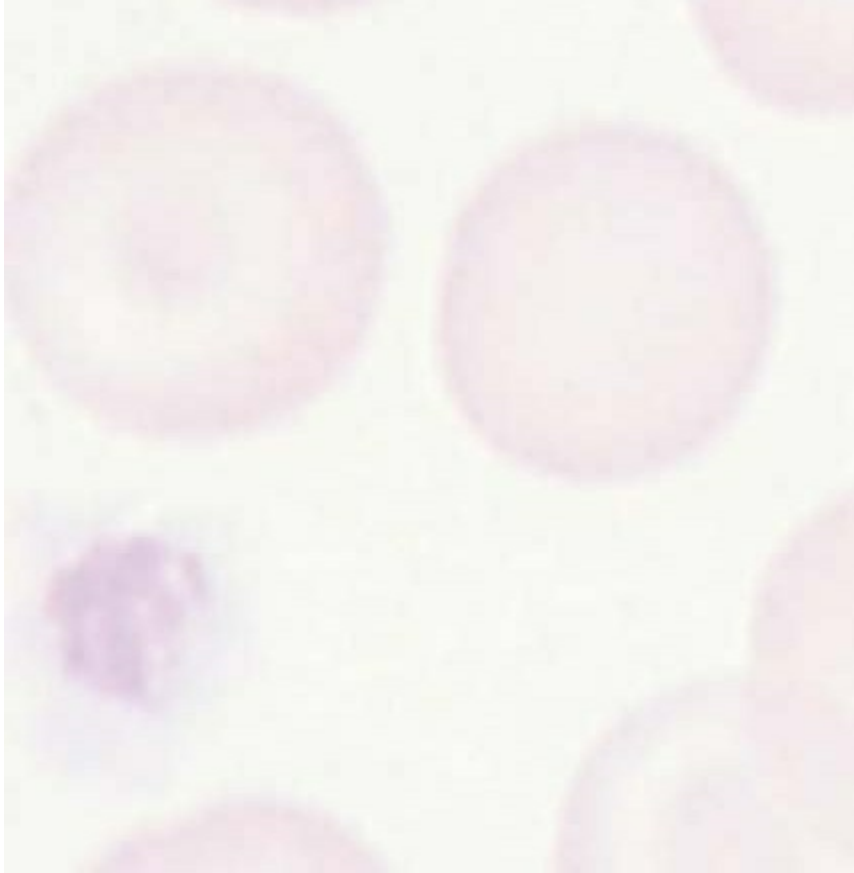
A. m m

B. m

C. m

D. m

(continued)



PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

Normal Reference Values

17. A m m , z

10. m _____% m -

_____ .

.

A.

A. -

B.

B. -

C. m m

C. -

D.

D. -

18. A m m , m

11. L m x m _____%

A.

k .

B.

A.

C. m m

B.

D.

C.

D.

19. A m m , m m

A.

12. T m m

B.

k _____

C. m m

m .

D.

A.

B.

20. A m m , q m C. m m

A.

D.

B.

C. m m

13. I k

D.

$\times /L \% m$

, m _____ \times /L .

21. T m m -

A.

m

B.

A. z , , C.

m

D.

B. z , ,

m

14. I m x

C. m , - m,

m ,

A. m

D. , x , k-B. m

m

C. k

D. k

*22. R ’

A. N m -P k B k ’ m m

15. I m

B. CLL

. × /L k m

C. k m

%, %, m %,

D. N

m %, m

A. m

*23. V m

B. m

A. N m -P k B k ’ m m

C. k

B. CLL

D. k

C. k m

D.

Morphological Characteristics o Normal Lymphocytes

16. A m m , - m

*24. C

A.

A. x

B.

B. - m

C. m m

C. B-m

D.

D.

CHAPTER 9 ■ Leukocytes: Lymphocytes and Plasma Cells

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REVIEW QUESTIONS (continued)

*25. Sm

*33. A - B mm

A. N m -P k B k ' m m

B. CLL

A.

C. k m

B. k

D.

mm

C. k m

Characteristics o Lymphocytes

mm

26. A

D. k - m x

A.

B. m

*34. T IL-B m

C. m

A. m B m

D. m

m

B. m B m

27.

C. m

A. m mm

D. m m

B. -m mm

C. G

Major Lymphocyte Membrane Characteristics and

D.

Development

*35. O CD m k -B

*28. F z m

m m B m

A.

A. CD

B. j

B. CD

C. mm

C. CD

D. A B

D. CD

*29. A m j CD + (T) m

36. L m m m

A. x

q

B.

A.

C. x

B.

C.

30. A AIDS m k

D.

A. CD +

B. CD +

*37. A mm B

C. m

A. m (I) mm

D. m

B. mm (I) I M

C. mm (I) I G

*31. T CD m k CD CD -

D. A B

A. NK

*38. T m k B m

B. m

A. CD +

C. B m

B. CD +

D. m

C. CD +

D. I M

*32. T B m -

IL-

*39. M B mm (I) A. CD

A. I M

B. B

B. I D

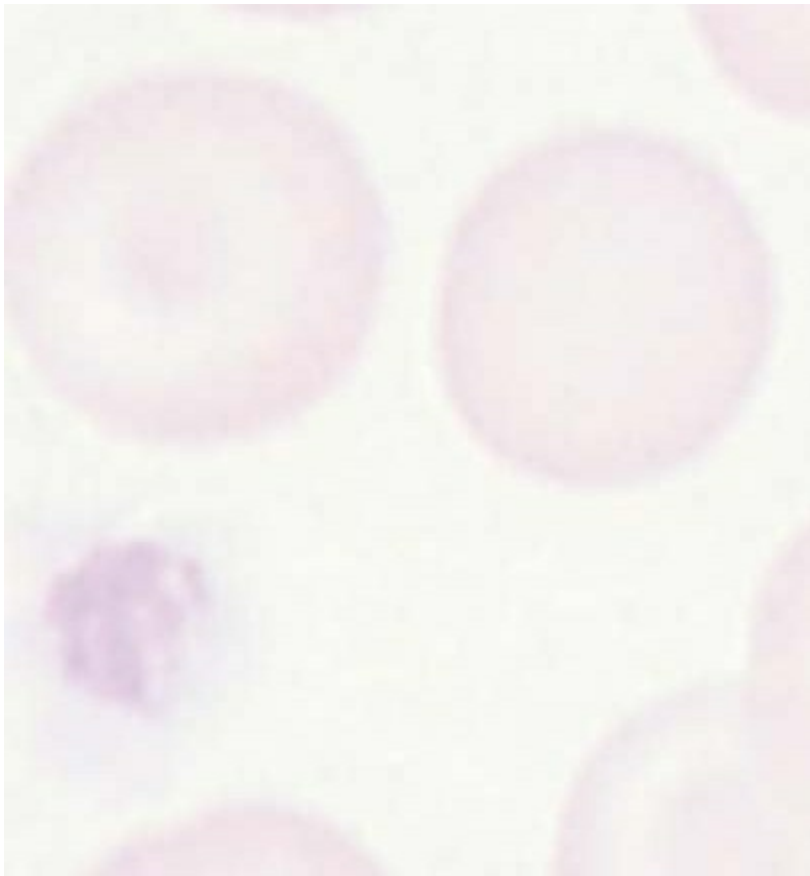
C. B

C. I A

D. B

D. A B

(continued)





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PART 2 ■ Hematopoiesis and Cellular Maturation

REVIEW QUESTIONS (continued)

*40. A m k NK

*43. L - m

A. CD

A. - m

B. CD

B. - m

C. CD

C. B-m

D. CD

D. B-m

Lymphocytes, B Lymphocytes, and Plasma Cell

*44. A m m - m **Kinetics**

41. B m

A. R ’

A.

B. M

B.

C.

C. mm

D. m

D. A B

Plasma Cell Development and Maturation

42. P m

A. CD +

B. CD + B

C. m () B

D.

G A, S AG, P m M. E m -

COMPANION RES OURCES

m , Med Lab Mag, ; : - .

G F, P N, M I, C PM , M M: C

:// . . m/

I I B M L m , H m

; (): ECR , .

E W -

H JB. Clinical Diagnosis and Management, , P , m .

PA: S , : - , , , - .

H ,

L B W, F. B m : ,

-

Blood, ; (): - .

.

M KL. B m . AAI Advanced Course in Immunology,

B , MA: Am A Imm , A ,

.

BIBLIOGRAPHY

R RA. M m : q , Blood,

; (): - .

A AK, L m AH, P S. Cellular and Molecular Immunology,

R K, . A - B : - -

, P , PA: E (S), .

mm , J Immunol,

B k JW, D JE. Medical Parasitology, , S . L , MO: M ,

; (): - .

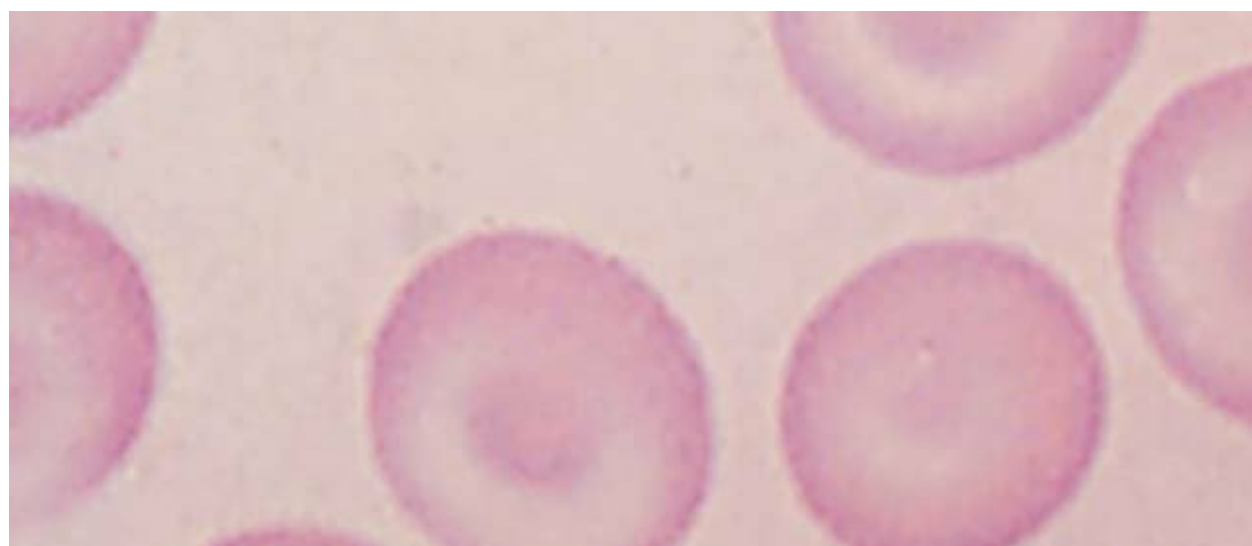
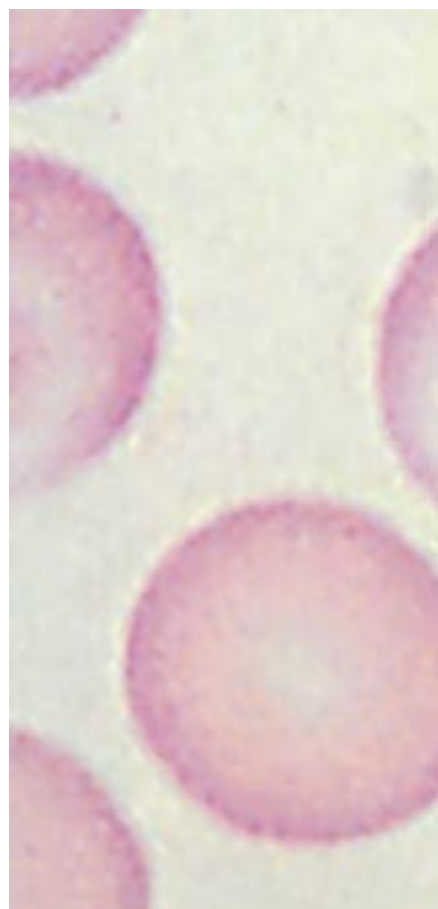
: - .

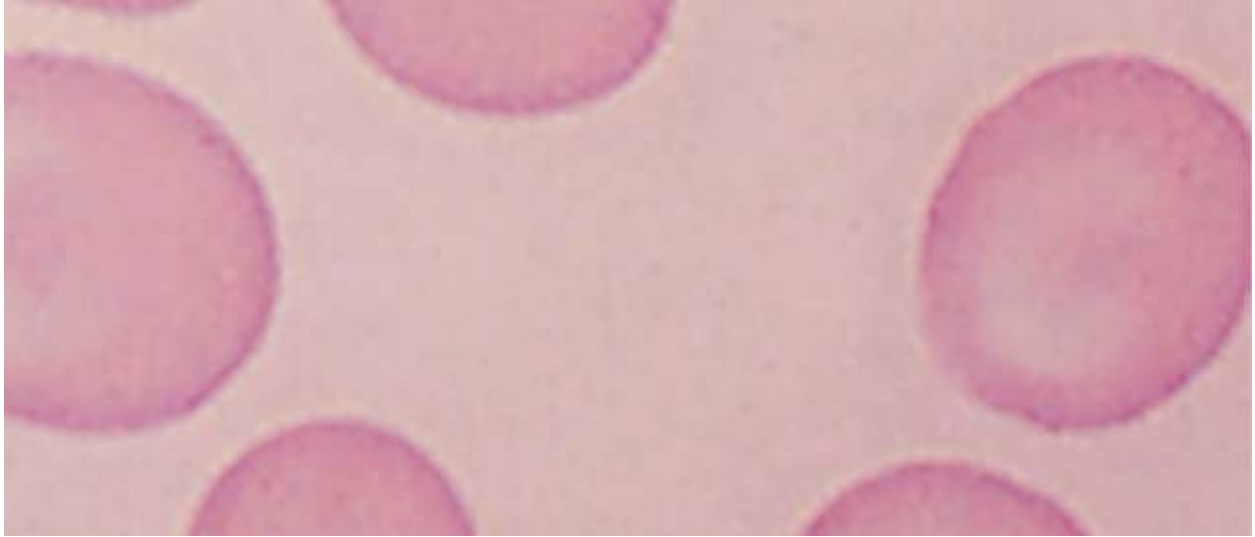
Z X, J j Y. - NK- , C MA. H m k , Blood, ; (): - .

Blood, ; (): - .

C MA, B BW. N k : m , , Z J, P WE. CD : , , Blood, mm , Hematologists, ; (): .

; (): - .





PA RT THREE

Hematology Laboratory Assessments

Basic Laboratory Assessment o

CHAPTER

Principles of Blood Collection

10 Erythrocytes, Leukocytes, and Platelets

KEY TERMS

anisocytosis

mean corpuscular volume (MCV)

polychromasia

erythrocyte sedimentation rate (ESR)

mean corpuscular hemoglobin (MCH)

polychromatophilia

hemoglobin

mean corpuscular hemoglobin

reticulocyte

leukocytosis

concentration (MCHC)

rouleaux

leukocytopenia

poikilocytosis

LEARNING OUTCOMES

The Complete blood count

Quantitative assessment of leukocytes

- List components of a complete blood count (CBC).
- Calculate a manual WBC cell count when given the dilution and numbers of cells counted.
- Define abbreviations: RBC, WBC, Hgb, Hct, and retic.
- Name two conditions that can produce leukocytosis.

Quantitative assessment of erythrocytes

- Name two conditions that can produce leukocytopenia.
- Calculate a manual RBC cell count when given the dilution and numbers of cells counted.
- Calculate an absolute cell count.
- Compare some reasons for eosinophilia and basophilia.
- Describe the principle of the cyanmethemoglobin assay for determination of hemoglobin.
- Explain the method and clinical applications of the neutrophilic hypersegmentation index.
- Describe the measurement of a microhematocrit.
- Correct a total white blood cell count when nucleated red blood
- Compare RBC, hemoglobin, and hematocrit values using the rule

cells are present.

of three.

- Define each of the erythrocyte indices: mean corpuscular volume

Other leukocyte-related assessments

(MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular

Critique the purpose and clinical applications of assessing neutrophilic function and the killing ability of granulocytes.
hemoglobin concentration (MCHC).

phobic function and the killing ability of granulocytes.

- Apply the appropriate formulas and calculate the MCV, MCH, and

- Name a classic application of the leukocyte alkaline phosphatase

MCHC when given the erythrocyte values.

(LAP) test.

- Classify RBC morphology based on erythrocyte indices.

- Explain the purpose of the erythrocyte sedimentation rate (sed rate).

- Compare the morphological appearances of reticulocytes stained

- Discuss the classic history of the sed rate.

with Wright stain and a supravital stain, such as new methylene blue.

Quantitative assessment of platelets

- Give the normal value of the uncorrected reticulocyte count.

- Define the terms shift or stress reticulocytes.

- Describe the appearance of a mature platelet in a platelet count

When given the necessary laboratory results, calculate the corrected preparation.

reticulocyte count, absolute reticulocyte, and reticulocyte produc-

- Describe the manual calculation of a platelet count.

tion index and compare the values to normal reference ranges.

S em iquantitative g rading of erythrocytes

Explain the terms reticulocyte hemoglobin concentration, immature

Describe the process of evaluating a peripheral blood lm, including selec—

reticulocyte fraction, and reticulated hemoglobin content



tion of the correct area and observations to be made at each magni cation.

Correlate polychromatophilia on a blood smear with reticulocyte

De ne the terms anisocytosis and poikilocytosis

results.



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PART 3 ■ Hematology Laboratory Assessments

- De ne the terms rouleaux and agglutination.

Compare the clinical conditions associated with rouleaux or agglu—

Compare the clinical conditions associated with rouleaux or agglutination observed on a peripheral blood smear.

tion observed on a peripheral blood smear.

S em iquantitative as s es s m ent o f leukocyte s

S em iquantitative as s es s m ent o f leukocyte s

- Estimate WBC when given the number of cells observed per eld

- Estimate WBC when given the number of cells observed per eld

and the magni cation of the objectives.

and the magni cation of the objectives.

- Describe the value of comparing semiquantitative and quantitative

- Describe the value of comparing semiquantitative and quantitative leukocyte counts.

leukocyte counts.

- Explain the corrected leukocyte count.

- Explain the corrected leukocyte count.

S em iquantitative as s es s m ent o f platelets

Quantitative as s es s m ent of platele ts

- Explain the purpose of a semiquantitative estimate of a total plate-

- Describe the appearance of a mature platelet in a platelet count

let count.

preparation.

- Explain platelet satellitism and clumping.
- Describe the manual calculation of a platelet count.
- Estimate a platelet count when given the number of cells observed
- Explain various sources of error in counting platelets.

power and the magnification of the objectives.

Peripheral blood film evaluation

Leukocyte differential count

- Describe the appearance of a properly stained blood film.
- Explain the principle, specimen, quality control, and procedure for a
- Explain the proper care and use of the microscope.

differential leukocyte count.

- Describe the starting point for the evaluation of a blood film.
- Name conditions associated with increases in the various types of
- Compare the types of blood film evaluations that can be made with leukocytes.

10×, 40×, and 100× objectives.

- Define the term shift to the left.

Assess the opinions regarding the separation of band forms from

Semi-quantitative grading of erythrocytes

segmented neutrophils in a leukocyte differential count.

- Describe the process of evaluating a peripheral blood film, including selection of the correct area and observations to be made at each

NOTE:

magnification.

- indicates MCV and MCHC content

- Define the terms anisocytosis and poikilocytosis

indicates MCV (optional) and MCHC advanced content

- Define the terms rouleaux and agglutination.

THE COMPLETE BLOOD COUNT

MCV (MCHC)

MCV. TMC

T complete blood count CBC -

MCV, MCH

MCV. A CBC

, MCH,

MCV, MCH,

(RDW), MCH.

, MCH -

MCV -

, m

C .

m . T

- .

Manual Erythro cyte, Leuko cyte, and Plate let

Co unts

Ove rall Quantitative Meas ure me nts

I m , m

Q m m , k

x (.), m m ,

m m . A

m q m m (

m , m (C , F . . .).

M P) m . I m , RBC

I k , m WBC m m m m RBC WBC m

x m k m

m . (WBC m RBC

,

.) T m

(C , B F). M

m . I -

m m - m -

, m z RBC

m m m q

m m m , k m .

. T -

T RBC m m (MCV),

m

m m (MCH), m

m RBC .

CHAPTER 10 ■ Basic Laboratory Assessment of Erythrocytes, Leukocytes, and Platelets 211

He mato crit (Pac ke d Ce ll Vo lum e)

TABLE

10.1 Manual Total Ce ll Counts

T hematocrit Hct , packed cell volume, m -

m k RBC m-

S tandard

, m m q . T

Bloo d

Micro s cope

m m . A m -

Type of S pe cime n

Obje ctive

Ce ll

Dilutio n

Dilue nt

Pow e r

m .

W ,

RBC

1:100

0.85% NaCl

40×

m ,

WBC

1:20

3% acetic acid,

10×

. T m

1% ammonium

RBC m . I x

oxalate, 1% hydro—

SI m.

chloric acid

A m m m -

Platelet 1:100

1% ammonium

40×

m m . T m

oxalate

phase microscopy

m m (MCV)

m

RBC m m m . H m

m m

QUAN I A IVE ASSESSMEN OF

m .

ERY HROCY ES

S m m m m -

m m .

Hemoglobin Measurement in the Laboratory

S m

T m **hemoglobin Hb** m

q ED A

CBC. A m m -

RBC k .

m m m , m .

m m m . A m -

P m m m m q k m m .

m k m .

T m m , H C, m -

Rule of Thre e

m m m D k ’

A q k k m m m

m ; m ; -

“ .” T m m -

m (KH PO),

m m

m m ;

$m(H \times = HC \pm \%).$

$m m z RBC . W$

Example: $I m . / L$

$m m m x -$

$m m \%, m$

$m , m H CN m m -$

$m m . T$

$q m .$

$(\% \%).$

$H m m m -$

$T m m , m$

$m m m$

$. A m -$

$m . T m - m$

$m m m .$

$D k ' , m m .$

$S m m m$

Blo od Vo lum e Me as urem e nt

$m m . H m$

$m (m), x m$

I m , m

WBC , m -

H

C m S.

m m

S m-mm m ,

. I m m -

, .

, m m q m

m , m m

. % , m x m , m . I m m m m .

x m WBC ,

P m m m m . A -

m m

m m -

,

m , E , I-m ,

, %

m- , j m -

m . I m C S

m j

, m :

m . R

m .

m m mm , m

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PART 3 ■ Hematology Laboratory Assessments

m m m -

Re d Bloo d Cell Indice s

x , %

m m m m .

T m m

P m m m m m -

z m .

m , . T -

T

C m m mm . R

. **Mean corpuscular volume MCV**

m m m m m

. **Mean corpuscular hemoglobin MCH**

m m .

. **Mean corpuscular hemoglobin concentration MCHC**

MCV, MCH, MCHC

NOTE: This is a good time to review the definition of the Key

m -

Terms in the Glossary and ash cards on

. It is also

m m x . F

a good time to complete Review Questions related to the

x m , m , m

preceding content.

m m m

m m m m

m . I m x m m

Manual Erythro cyte Co unt

m m

R C C M m

.

, m -

T MCV x m

m m q .

m , MCH MCHC m ,

I ,

m . T MCH MCHC

. B m

m q

x m (

m m

m),

m .

. H , m k k -

, m m

Me an Co rpus cu la r Vo lum e

m

T MCV x z m -

. T RBC m

. T m

m z % (CV) m m m m

m % m m .

P ' k m m (L/ L)

MCV =

= L

Calculation:

E (

× / L)

N . RBC = RBC q × -

× m

Example: I ' m %, . L/L,

. q -mm q .

. × /L, MCV -

. T m .

m :

. T m . T m -

. L/ L

m q m

MCV

= . × - = . L

=

. μL. T m

. ×

(. µL) m (. /µL).

aO m (L) = - L = m m (µm)

Example: I m RBC , R () MCV L

RBC

Clinical Conditions:

× × = . × /L

T MCV m -

A m m , , m z . E MCV

× . T RBC ×

m m m m

, = , , × /L.

m . L

Sources o Error:

MCV m , -

I m m

z m , m mm -

, , m m ,

/ m .

m m .

Sources of Error:

Reference Ranges:

A

Normal

mm

..

MCV. Mean ± MCV -

V

L, x mm ,

. A RBC

mm Hg, mm , mm , mm -

(mm / L) mm -

, km . A

, MCV.

RBC mm .

L km MCV .

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TABLE

10.2 Values in the Measurement of Erythrocytes

Normal Adult Reference Values

Me n

Wom e n

Conve ntional Units

S I

Conve ntional Units

S I

Hematocrit

Packed cel volume

41.5–50.4%

0.415–0.504 L/L

36%–45%

0.36–0.45 L/L

Erythrocyte count

4.5–5.9 × 10⁶/μL

4.5–5.9 × 10¹²/L

4.5–5.1 × 10⁶/μL

4.5–5.1 × 10¹²/L

Hemoglobin

Concentration

14.0–18.0 g/dL

145–180 g/L

12.0–16.0 g/dL

120–160 g/L

Normal MCV

80–96

80–96 fL

80–96

80–96 fL

Normal MCH

27.5–33.2

27.5–33.2 pg

27.5–33.2

27.5–33.2 pg

Normal MCHC

33.4%–35.6%

32–36 g/dL

33.4%–35.6%

32–36 g/dL

Representative Average Pediatric Values

At birth (cord blood)

Packed cell volume

51%

Erythrocyte count

$4.7 \times 10^{12}/L$

Hemoglobin concentration

16.5 g/dL

MCV

108 fL

MCH

34 pg

At 6–12 years of age

Packed cell volume

40%

Erythrocyte count

$4.6 \times 10^{12}/L$

Hemoglobin concentration

13.5 g/dL

MCV

86 fL

MCH

29 pg

SI, Systeme International d'Unites.

Source: Perkins SL. Normal blood and bone marrow values in humans. In: Greer J P, *et al.* (eds.). Wintrob's Clinical Hematology, 11th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2004:2607; Handin RI, Lux SE, Stossel TP. Blood, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2003; Appendix 25, Red Blood Cell Values at Various Ages, p. 2216.

One femtoliter (fL) = 10^{-15} L = 1 cubic micrometer; One picogram (pg) = 10^{-12} g = 1 micromicrogram.

Mean Corpuscular Hemoglobin

Clinical Conditions:

TMCH x () m -

Immm ,

. Im

m , mm

mz . Tm

, MCH .

Sources of Error:

Hm (

× / L)

MCHmmm -

MCH =

=

m m

E (

× / L)

m m m . C mm m m -

Example: I ' m / L

m MCH .

× /L, MCH q

/ L

Me a n Corpu s cula r He m og lob in Conce nt rat io n

MCH

= × - =

=

T MCHC x m -

× / L

m m . I

bO m () = - = m m m (μμ)

m

R () MCH . .

m . T m

PART 3 ■ Hematology Laboratory Assessments

H m (/ L)

Re ticulo cytes

MCHC =

= /

k m m (L/ L)

L

A , m m m . A **Example:** I ' m / L

, mm () m

m % . L/L, MCHC q :

m -

. D m -

/ L

MCHC =

= / L

, mm

. L/ L

reticulocyte.

A k ,

R () MCHC . % . %

, m , x

Clinical Conditions:

m m . T m m

F

m ;

m MCHC. T m k

, RNA , m -

m mm

. D m , RNA

MCHC. O m MCHC

z , m . T -

/ L m (m

m m , m z

m) . -

, m k m

.

m .

Sources o Error:

U m , q

T MCHC m

m q

m m H m

. m

m m

, m mm k .

.

I m m

B m m ,

, MCV

/ m , RBC . -

q m

m m MCHC.

.

, m m

I , m RNA m °C m m , m

O MCHC

m m m -

m -

, -

m m m m . H m

. T

MCHC.

mm k -

W x m

.

m z k m C ,

MCHC x / L. T

m . F H

Re t iculocyte Count

m z .

P m m W

m m m . T

Re d Cell Dis tribu tion Widt h

m ,

T (RDW) m -

m C , polychromato—

m q m -

philia **polychromasia**.

z m .

S m **reticulocyte**

RDW -

m

m m m

RNA m . I ,

m m , -

m , , m m

RNA m - , m k k.

RDW z m

S z W -

m , m

m z

m (RDW, m MCV)

m m , m z m (m RDW, m . W , MCV), q m x m m k k.

. RDW

T q m

m ,

-

. U , x -

NOTE: This is a good time to complete Review Questions

Time related to the preceding content.

, . % . %, % %

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k .

k .

TABLE

10.3 Maturation Time Correction Factor

T

- - m ,

Hematocrit (%)

Maturation Time (Days)

(RBC) m

q m RBC ,

45

1.0

m - . W

35

1.5

RBC m , RBC -

25

2.0

. N m

,

15

2.5

m . A

m RBC . R

m m .

m

Ca lcu la ting a nd Expre s s ing Tra ditiona l

.

Re t iculocyte Va lue s

A m mm

, x

x (RPI). T m

m -

m m -

(. ., %). H , m -

m m . T RPI m -

m

. T

m

m -

m .

.

m m m -

q , x

q RNA m

m m m

,

. T m

m m m

, m . T

(.). T CLSI mm RPI

C L S I (CLSI)

m .

m , ,

Ca lcula tion of th e Re ticulocyte Pro duction Ind ex

m m m -

m k RBC m (m).

%

RPI =

Correcte d Reticulo cyte Coun t

m m

C = (%)

I . % ’

’ k m (m)

m . L/L, RPI

× m m

.

RPI = = .

.

Example: I m m % (. L/L)

%,

Abs olute Re ticulo cyte Cou nt

T m m m -

C = %

, ,

. L/ L

m . T m

× . L/ L(m m)

(L) m (μL) .

T m () × /L.

. L/ L

= . ×

.

. %

I × /L,

. L/ L(m m) = =

m .

T m m

A m z

m m

, m m

. % . %.

Re t iculocyte Prod uct ion In de x

A =

A m

% ×

m

q m . .

*

m k m. T

*T m /L

, ,

/L.

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Example: A - - m m (RBC) . ×

NOTE: This is a good time to complete Review Questions

/L, % m -

, . %. H -

related to the preceding content.

$$. \% \times . \times / L$$

$$= \times / L$$

QUAN I A IVE ASSESSMEN OF

I , ' m q

LEUKOCY ES

m m .

Total Le uko cyte Co unt

Ne w Re t iculocyte As s ays

T m k

M , m m m WBC = WBC \times -

m z . T m m

\times m

m x -

. T m k

. T m m

m m .

x m -

. T : m ,

m .

. T m . . T -

A m m

m (. μ L) m (. μ L).

m m

m q m m RNA

Ex m : I k

m B -

, k :

m RNA m m RBC , m mm -

$\times \times . =$

(IRF) . T

. \times /L.

IRF m x m

m mm ,

A m m \times

m RNA, m . T IRF

\times m

m -

.. Ex m : $\times = . \times /L$.

m m m .

S m , E m m m ,

m

k m .

B

C , m m -

m m . I

, IRF

.

m , m ,

m WBC m .

.

. \times /L . A WBC (k)

A m m m

m m m **leukocytosis**. A

m . T m m

m m m **leukopenia**.

m m m m -

L k m ,

m , ,

m . A ()

m m

WBC m m k

. T m

m m .

k .

T k (C , A m m m -

L M) , k , (CH). CH m

\times /L x -

MCH. T m

. A mm

m q MCHC.

k . \times /L m -

. T m ,

k , m

As s e s s m e n t of Bone Marrow Re s p o n s e

() mm .

N m m RPI x .

L k m m , -

I m m , -

, m , m -

-

, , , m m

m m , x m

m , m m , k m .

m m . I m m , I mm m m .

, m B ,

D mm -

, (), x

m ,

.

mm , .

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Am m -

O HER LEUKOCY E-RELA ED

mm , k

ASSESSMEN S

, m

m k (

Neutrophilic Hypersensitivity Index

m),

, m (ESR)

M m

m mm .

(m). C m m m m x (NHI). A m

Absolute Cell Counts

m , x m , -

T m m

, . T NHI m x mm

B (m) .

k

T m x NHI:

m m . T

. L . T m m

m m m

m , x m , ,

. A m

m -

mm . A x m

m . T . . .

m

. P m . C

B x . . I , m

m m m ,

.

x m , . A m

m , m-

. T %.

As s e s s me nt of Eo s ino phils and Bas ophils

. H m x. x,

Ex m m m m-m m m m .

x m % . A

N m m ×

, ,

m . B m

N m

m m q ,

, m m

V . -

m q m , . T q

m . T m m -

x m m

m .

m m m

T m .

.

N m , m m %

, . A , ,

m -

k m m .

BOX 10.1

Neutrophilic Functio n

A m k -

Abs o lute Cell Co unts

m , m x , ,

A * = = k

m . I

×

m m

m m

PATIENT DATA

m x m . A

k : . × /L

, x m , k

D m : %, m

(LAD), m .

%, m %

A k

SAMPLE CALCULATION

z m (NB) . I

A m = k

, q m .

× % m

F m x -

A = . × L × . = . × L -

m ,

m

k, .

*T m m

Leukocyte Alkaline Phosphatase Test

k m .

N m m . . ×

P m

$L, . \times L, m . . \times /L.$

C . T m -

m m k m .

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Erythrocyte Sedimentation Rate (ESR)

P ; m m m k Ex m m , **erythrocyte sedimentation**

m.

rate ESR m-M m , . $\mu\text{m mm m . T}$

m . P ,

W m CLSI -

m j . P -

m . T ESR, ,

k ,

. A ,

.

m mm m .

T -

V ESR. A m m -

S , F ,

(C).

. H , m

M m m

G k m

m . T q

. T G k m ()

m q m m

m bc

m . I m m

A . T

m , m m . T -

m . O -

,

m m .

m :

, m, , k .

Plate let Co unt

I , N z m ,

, k

T m

. T k N

m . T

m -

m -m -

m, m m m -

m x : %

G k

x . T RBC -

V . W m F -

. W m ,

m G k m , W

k

k q .

x.

T . I T m m m ,

z . I

mm/ m mm/

m . F , -

. I q .

mm/ m mm/

I : m

m .

m , m

E m ,

m m ' . T

k , rouleaux m

m x

ESR. T m

m × /L. T m

ESR. A ESR

× /L.

m : x,

Example: I

, m

m m , -

, ?

m . C -

+ = = \times L.

ESR m , ,

C :

mm , (m -

W q

), , m m m .

(mm) (\times), m

.

N m m :

NOTE: This is a good time to complete Review Questions

P / μ L = m q

related to the preceding content.

$\times \times$ m

Example: I

q ,

QUANTITATIVE ASSESSMENT OF

$\times \times = \times /L$

PLATELETS

T

mm -

Am m , m .

thrombocyte. P m

T : ; megakaryocytes, m .

. T m

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TABLE

10.4 Observations at Various Microscopic Magnifications

Low Power (10×)

High Power (40×)

Oil Immersion (100×)

Erythrocytes

Rouleaux, agglutination

Locate appropriate examination Evaluate size, shape, and color and inclusion area for 100×

bodies

Leukocytes

Scan for abnormal cells

Determine estimate of total WBC Evaluate and differentiate 100 WBCs;
observe

such as smudge cells

count

any inclusions or other abnormalities

Platelets

Scan for clumps and

Determine estimate of total platelet count

satellitism

Evaluate platelet size and granularity

Microscopic Examination

. /L.

V

T m A x

. A ,

G. O

mm m

(×), j m

. A m

(×) mm (×). E m

m m m . O

m (.).

k m m -

Low Pow er (10×)

m . I -

m m . O

T x m

m m

m. A m ,

m , m , m .

m q , ,

Sources o Error:

. I m

A .

m m ,

T m , m ,

. T

, , -

m RBC

m . A

x

. C m m -

(.). L

q m x q . I m , m

mm m m , m . I m , m , m . C m m : : .

m m .

I m m ED A—

High Pow er 40×

m ,

m . Sm

A m m × j

m . I

(×), m,

m x m ,

.

Red Blo od Cell Dis tribution

TABLE

10.5

PERIPHERAL BLOOD FILM EVALUATION

Disorders

P m -

Terminology Description

, m ,

rouleaux

Overlapping red blood cells, often referred

to as a “stack of coins” caused by abnormal—

to as a “stack of coins” caused by abnormal—

mal or increased plasma proteins. May be

- m . D

an artifact, if anticoagulated blood specimen—

k . U m -

men is allowed to stand for hours or if the

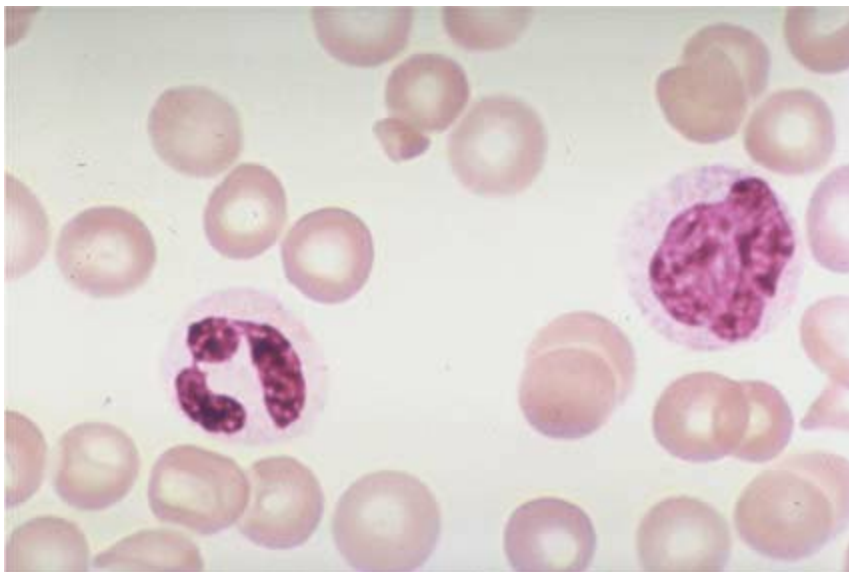
x m , m RBC

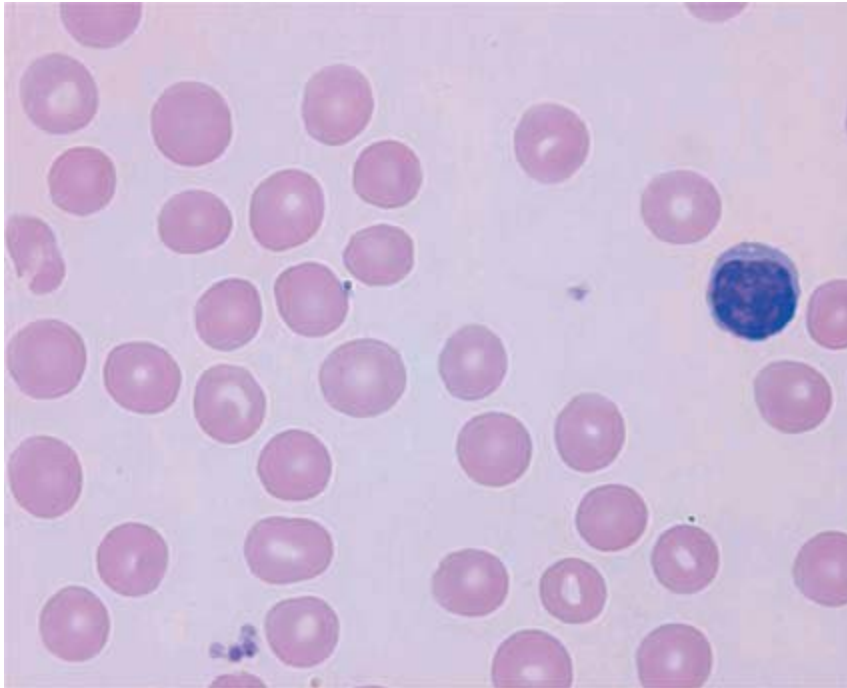
thick part of blood smear is examined.

k k ; WBC x

agglutination

Clumps of red blood cells looking like
k . G
bunches of grapes. This condition can
m. N
falsely decreased the automated, calcu-
. T
lated hematocrit and falsely increase the
, k -
MCH and MCHC.
, . B k .





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Grading of Erythrocyte

TABLE

10.6 Morphology

Num e rical

S cale

De s cription

0

Normal appearance or slight variation in erythrocytes.

1+

Only a small population of erythrocytes displays a particular abnormality; the terms slightly increased or few would be comparable.

FIGURE 10.1 Normal T -

m z . A

2+

More than occasional numbers of abnormal

m . (R m R E, F JL.

erythrocytes can be seen in a microscopic

Pathology, , P , PA: L W m & W k , eld; an equivalent descriptive term is mod-

, m .)

erately increased.

3+

Severe increase in abnormal erythrocytes

RBC ,

in each microscopic eld; an equivalent

k (WBC) m .

descriptive term is many.

Oil Im m e rs io n Le ns (1,000×)

4+

The most severe state of erythrocytic
abnormality, with the abnormality prevalent

$A \times ()$

throughout each microscopic field; compa—

RBC j , k

table terms are marked or marked increase.

m z k m. T

k

m

m m m q

m (F . .

m . V -

.).

m RBC z anisocytosis; m RBC

poikilocytosis.

SEMIQUANTITATIVE GRADING OF

E mm

ERYTHROCYTE MORPHOLOGY

m , m m k ,

m , +, +, +, +. T -

D m m -

m m

m mm

m

m . I -

..

m (C),

SEMIQUANTITATIVE ASSESSMENT OF

LEUKOCYTES

A m q m WBC q m -

m WBC q . T m

m . I m , m

m m

.

A mm m

m B x . .

SEMIQUANTITATIVE ASSESSMENT OF

PLATELETS

A m m -

. Exam

FIGURE 10.2 P m m -

m m m -

m RBC , , m m . (R

m EJ, . textbook o Cardiovascular Medicine,

m m

, P , PA: L W m & W k , ,

m m q

m .)

m .

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Specimen

BOX 10.2

P , m , m ,

, m . W

Total White Blood Cell Estimate

m m m m ED Am - .

. Sm

Sm m -

× m (RBC j).

m ED A m m m

. C m WBC

m . U m

(m m m).

, m

. C m WBC

.

μL m m WBC/

\times , .

Re a ge nt s , S up plie s, a nd Equ ipm en t

. C m m q -

. A m

m m m WBC

. M , mm ,

Example: I WBC

Qu a lit y Co nt rol

\times j ,

m \times , . T WBC m , / μL

x x m mm -

mm . \times /L.

m m . A

m

S : M m JC. L k . I : m m S -M EA, L -S CA, K k JA (.). Clinical Hematology: Principles, Procedures, Correlations, , P , m k .

PA: L -R P , : .

P q m

m x m m .

Q m m

Ex m m

.

m m . A m

m m not q -

Proce du re

m m , - k

. B x m

q m m .

m (C m).

N m , mm

. F m × j ().

m (RBC

S m k , m ,

). A x m ,

m . A mm

m m

\times (mm) j . B

, x m

m (F . .). Ex

.

x m m x m

Example: I

\times j ,

. It is important

m \times , . T

to examine cellular morphology and to count leukocytes

m , μ L mm . \times L.

in areas that are neither too thick nor too thin. I

Note: I m , k, -

, m x , m :

m f x m . I

A m / \times RBC

, m k k

RBC /

.

. A k .

Ex k

. C k k

LEUKOCY E DIFFEREN IAL COUN

(F . .). E mm

Principle

(), (m),

m (PMN); m ;

A m x m m

m ; ; . A k

k

m (.). R

m . I m k

, m k

mm k

m .

m -

. A m k , ,

mm k m . E -

. N not

m m m . P z m .

(m).

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TABLE

10.7 A Com pariso n o f No rmal Le ukocytes in Pe ripheral Blood

S e gm e nte d

Ne utrophil

Band Ne utro phil

Lympho cyte Monocyte

Eosinophil

Basophil

Nuclear shape

Lobulated

Curved

Round

Indented or twisted Lobulated

Lobulated

Chromatin

Very clumped

Moderately clumped Smooth

Lacy

Very clumped Very clumped

Cytoplasmic

Pink

Blue, pink

Light blue

Gray-blue

Granulated

Granulated

color

Granules

Many

Many

Few or absent Many

Many

Many

Color of granules Pink, a few blue Pink

Red

Dusty blue

Orange

Dark blue

Average

56%

3%

34%

4%

2.7%

0.3%

percentage

Re po rt ing Re s ults

m

R , , m ED A m . V m .

S k .

, x

. C

, , -

Proce du re No te s

m , S m m

■ T m q

m m ,

C mm

m (B x .).

m . I m

WBC x m ,

■ A m m m k m -

(C)

k , k

k .

× /L.

■ T k m -

■ T , m , m

-q . T m ,

m.

k ,

L % k

m m . C

m x m -

m . I m ,

. I

BOX 10.3

Technical Issues: Total Cell Counts and Corrective Action

Cell Type

False Impact on Total Cell Count

Resolution

>5 Nucleated RBCs/100

Elevated WBC

Calculate WBC correction, if not automatically cor—

WBCs

rected by blood cell counting instrument.

Agglutination of RBCs

Decreased RBC count

Warm blood specimen to 37°C for 15 minutes and then

retest.

Rouleaux

None

No correction available.

Platelet clumps or plate—

Decreased platelet count

If collected in EDTA anticoagulant, redraw blood speci—

let satellitism

men in citrate anticoagulant and multiply the platelet

count by a factor of 1.1 to correct for dilutional effect of

liquid citrate anticoagulant.

Alternate strategy: Obtain a capillary blood specimen

and prepare blood smear at the patient's point of care.

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and Platelets 223

BOX 10.4

Formula for Leukocyte Count Correction*

$L k C \times$

$= C L k C$

$+ N m N E / L k$

$* I m / , \times (\times) m .$

Example:

$. \times / L \times$

$$= . \times / L$$

+

$$, . C$$

$$V -$$

$$(m \, k)$$

$$m \, k (B \, x \, .).$$

$$“ , ” m \, m$$

$$P \, m$$

$$.$$

$$m \, m$$

$$\blacksquare T$$

$$m \, k . T \, m$$

$$m \, m$$

$$\% ;$$

$$m \, m . I \, m$$

$$m \, m . A -$$

$$\% -$$

$$, m$$

$$mm . T \, m$$

$$(B \, x \, .). S \, m \, m$$

m % x m %

z -

m .

.

Shift to the Left

BOX 10.5

W m mm -

m m m ,

m m shif to the left .

Disorders Associated with Increases in

T m , , k

Distribution of normal types of leukocytes

m mm

NEUTROPHILS

.

. B

S m -

. I mm

m k

. S

. C k m

m . T C L S

I mm

LYMPHOCYTES

-

. V

-

. W

m .

. C k m

MONOCYTES

NOTE: This is a good time to complete the end of chapter

.

Review Questions related to the preceding content.

. R m

. F k

EOS INOPHILS

CHAP ER HIGHLIGHT S

. A

. I

The Complete Blood Count (CBC)

BASOPHILS

■ A CBC measures ,

. U

m , , ,

. H m

k

m m .

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■ Q m m , k ,

, q m

.

m m .

■ I , m , m-

■ I m , RBC WBC m

m m

m x m k

m

m m . M

,

m m

.

, .

■ P m m W

■ T RBC m m (MCV), m m m m m (MCH), m -

m

m (MCHC) -

m .

m CBC. T

■ A , m , -

m m m , m -

m RNA mm

m , -

m - , m k k.

m , (RDW),

■ T x

m .

. T m . % . % .

■ I m , m

I , . % . % ,

x

k

m m , m

.

q m m .

■ q -

, x

Quantitative Assessment of Erythrocytes

m m m

.

■ T m m (H) m -

■ B m RNA

(m m) m .

m m RBC , m mm

■ T m (H), k m , m -

(IRF) . I

m k RBC

m

m .

m .

■ A m RBC

■ A m m m

m q m m m -

m . T m -

m

m m

.

■ T m m

m -

z m

m .

.

■ A m m m -

■ RBC MCV x m

(CH). CH m

, MCH x (-

MCH. T m -

) m , MCHC

m q

x m

MCHC.

m

■ N m m q

m m .

m . I m m ,

■ A , m m m . A m m ,

, mm m

m x m . I

m

m m ; ;

. D m

m B

, mm

, , ,

RBC m .

.

■ T m m ,

Quantitative Assessment of Leukocytes

m z , m k m

m .

■ T k m m

■ U m , q

WBC $\times \times$ -

m q

m .

. B m

■ I m m m m-m , / m . A m m

CHAPTER 10 ■ Basic Laboratory Assessment of Erythrocytes, Leukocytes, and Platelets 225

m , m m -

■ M m m

, .

m .

■ A m m -

■ T q m -

m m , k

q m m

, m

m .

m k ,

, m

Peripheral Blood Film Evaluation

(ESR) m mm .

■ T m

■ L k ,

m ,

m .

x .

■ U m x m , m

L k m m

RBC k k ; () mm .

WBC x k

.

■ A -

.

■ D m -

m m mm -

■ T m m

x mm

m .

k

■ V m RBC z ;

m m .

m RBC k .

■ A m m q m WBC

■ E m

.

q m m WBC q

.

■ B m -

k m m .

■ T m m ,

, q m m

■ M m

. C m m

. I m , m

m m x

m m . C k

(NHI). A m

m .

m , x m ,

■ N m , × () mm -

, . T NHI

m m -

m B

m .

(m)

.

■ A x m

m q m .

■ A m k -

m , m x , ,

■ G

m .

m. N -

. T

■ A , x m , k -

(LAD), m .

, k , . B

k .

■ F m x -

m ,

■ W m mm -

k , .

m m m

, m m

■ L k k (LAP) m -

m m

.

k m .

■ T C L S I mm

■ T m (ESR), ,

m -

-

mm

-

m .

m .

■ P m

■ T ESR m-mm m .

m m

T W m CLSI

m k . T m

m .

% ; -

m m .

Quantitative Assessment of Platelets

■ A % -

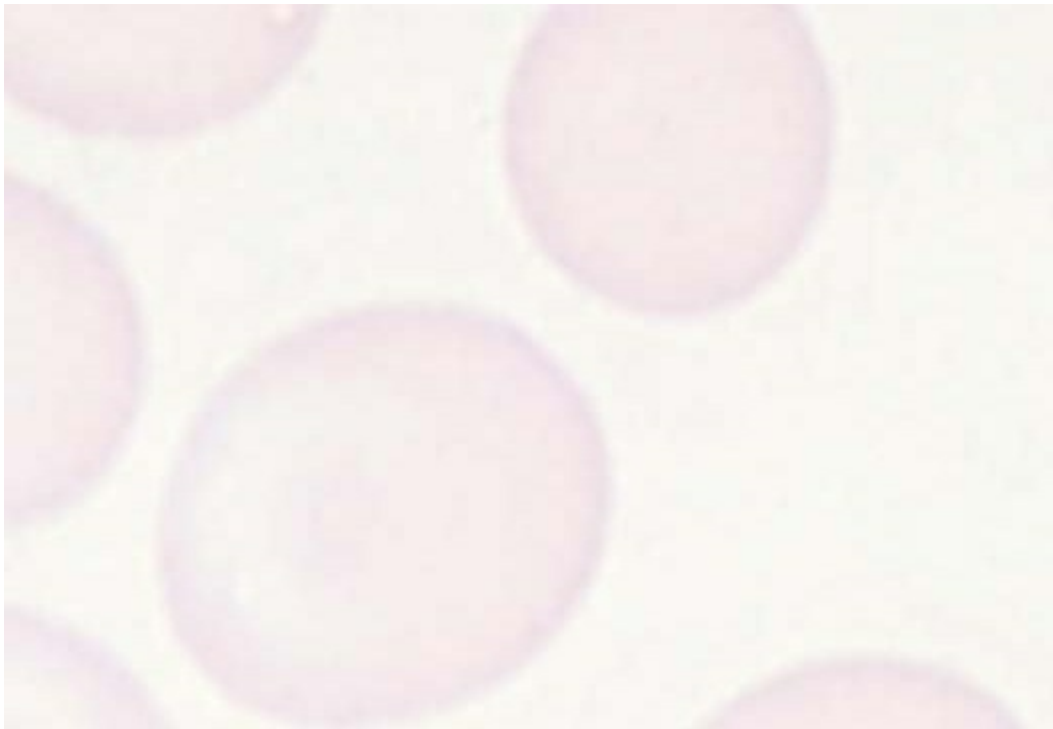
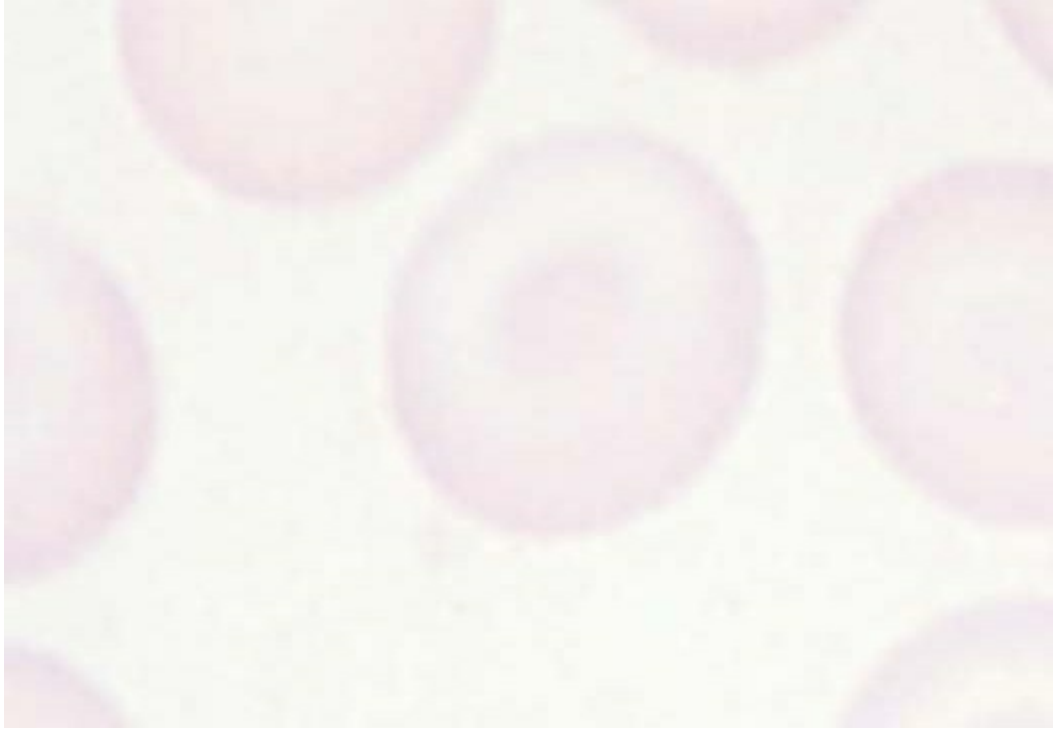
mm . I

■ A m m ,

, m m

m .

% x m % m .



REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

*7. I m m z ,

RBC m ?

Quantitative Assessment o Erythrocytes

A. MCV

1. W m

B. MCH

C. MCHC

m m m m

D. R

m ?

A. L m

Laboratory Assessment

B. Ex m WBC

8. W m m -

C. H m S

?

D. H m F

A.

B. F

2. I m m % m -

. / L, “ ”

C. R

_____ m ().

D. H m

A.

9. T m

B. .

A. % . %

C. .

B. . % . %

D. .

C. . % . %

D. . % . %

3. T m MCV

A. k m m (L/L)/ -

(×

10. I m . %

$$/L) = L$$

$$k\ m \cdot L/L, \text{ corrected}$$

$$B. m\ (L) k\ m\ m -$$

?

$$(L/L) = /L$$

$$A. \cdot \%$$

$$C. m\ (\times /L)/$$

$$(\times$$

$$B. \cdot \%$$

$$/L) =$$

$$C. \cdot \%$$

$$D. m\ m\ \times =$$

$$D. \%$$

$$4. T\ m\ MCH$$

$$11. I\ m \cdot \%$$

$$A. k\ m\ m\ (L/L)/ -$$

$$k\ m\ \%, RPI?$$

$$(\times /L) = L$$

$$A. \cdot$$

$$B. m\ (L) k\ m\ m -$$

$$(L/L) = /L$$

B. .

C. .

$$C. m (\times / L)/$$

(\times

D. .

$$/L) =$$

$$D. m m \times =$$

12. W

?

5. T m MCHC

A. N m .

$$A. k m m (L/L)/ -$$

(\times

B. P x B.

$$/L) = L$$

C. S k

$$B. m (L) k m m -$$

.

$$(L/L) = /L$$

D. A .

C. $m (\times / L) /$

$(\times / L) =$

13. O W - m , stress

D. $m m \times =$

shi

A. $m m$

*6. I MCHC / L ' ,

$m , ?$

B. $m z m$

C. m

A. I RDW

D.

B. H m RBC

C. A RBC

D. I m RBC m

CHAPTER 10 ■ Basic Laboratory Assessment of Erythrocytes, Leukocytes, and Platelets 227

REVIEW QUESTIONS (continued)

*14. I - - m -

21. W

%, x -

?

m W

A. I

?

B. B

A. P m

C. V

B. P k

D.

C. > / WBC

D. A m

*22. I WBC m

- - m , -

15. T -

?

A. mm/

WBC . \times /L : m

B. . % . %

%, m %, m %, %.

C. \times /L

A. L k

D. % %

B. A

C. R

Quantitative Assessment o Leukocytes

D. A m

16. A m :

% . I

23. W ' WBC

. \times /L % m ?

q m m , '

A. . x /L

WBC (\times /L)?

B. $.x /L$

A. $.$

C. $.x /L$

B. $.$

D. $.x /L$

C. $.$

D. $.$

24. W m m

?

17. T k

A. A k $\times -$

A. mm/

$\times m$

B. $. \% . \%$

B. A k $\times -$

C. \times /L

$\times / m q$

D. $. . . \times /L$

C. A k \times

D. A k \times m

18. W m

q

k ?

A. A

Other Leukocyte Related Assessments

B. Imm

*25. A k (LAD),

C. I mm

D. A m m

A. m

B. m

19. W

C. k

?

D. k

A. I

B. B

*26. T LAP

C. V

A. m m k m

D.

.

B. k m

20. W

C. k m

m ?

D.

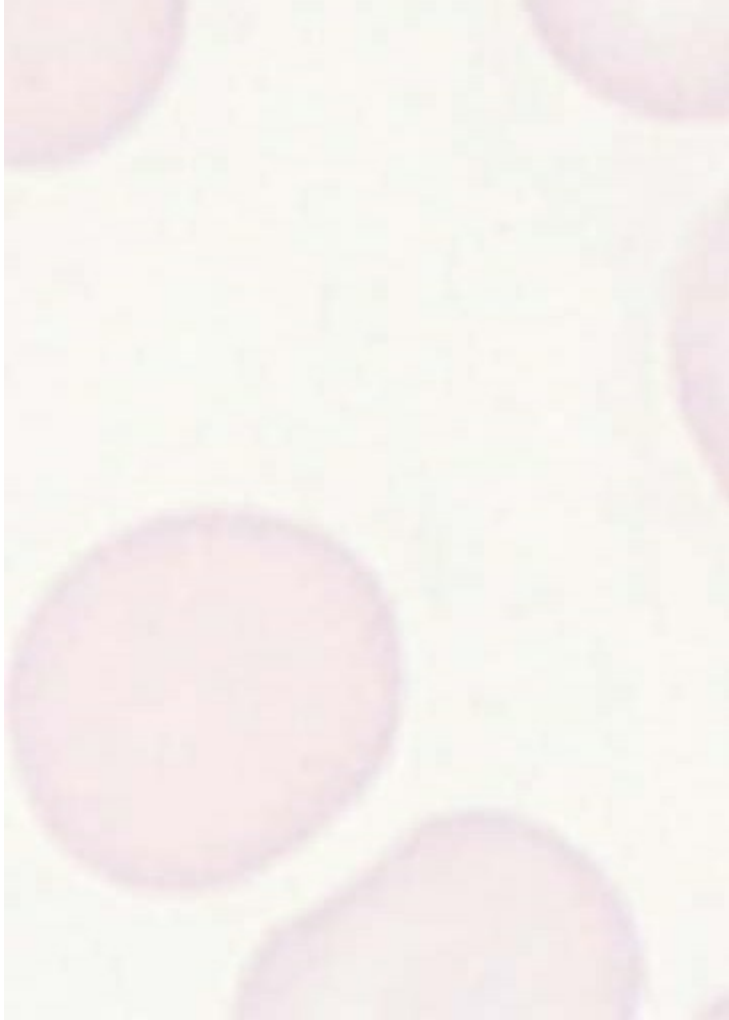
A. I

B. B

C. V

D.

(continued)



PART 3 ■ Hematology Laboratory Assessments

REVIEW QUESTIONS (continued)

27. T ESR

*33. I m

m

m /

A.

m m , -

B. mm

k ?

C.

A. R m

D.

m .

B. R m m

28. T W ESR m

°C m z .

m)

C. R ED A-m

A. mm/

m x m .

B. . % . %

D. R m

C. × /L

x m .

D. % %

34. I z **Quantitative Assessment o Platelets** m m ,

29. T m

k m ?

A. B

A. :

.

B. :

B. L .

C. :

C. C j m k

D. :

m .

D. C m m .

30. W

m : ,

Semiquantitative Grading o Erythrocytes

35. I z

N m .

+ many

A. m

m m m ,

B. m

A. +

B. +

C. m

C. +

D. +

D. m

m

36. R x _____ x m

m m .

31. T m

A.

A. mm/

B. m

B. . % . %

C. k

C. \times /L

D. m RBC

D. . . \times /L

Semiquantitative Assessment o Leukocytes

32. I m x

37. S m -q m k -

m , m

m m

m ?

A. C m

A. L k

B. C m

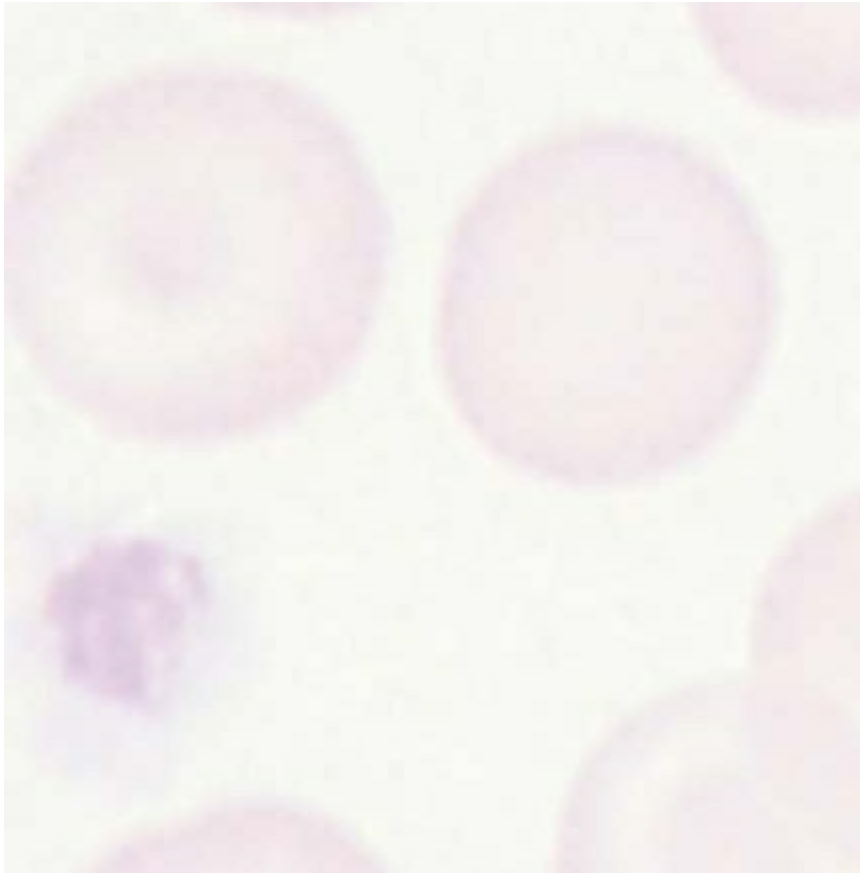
B. E

C. S

C. P

D. I

D. M m



CHAPTER 10 ■ Basic Laboratory Assessment of Erythrocytes, Leukocytes, and Platelets 229

REVIEW QUESTIONS (continued)

Semiquantitative Assessment of Platelets

Leukocyte Differential Count

38. A m m m

40. I ,

x m _____ (m x m m) m

WBC m ,

mm -

?

j .

A. L m m k

A.

.

B.

B. T m m

C.

(HDFN).

D.

C. $N \times$

.

*39. -

D. $D,$

$m \times m \ m \ q -$

$m .$

$m . I,$

$m : , , , ,$

41. $I . \times /L$

$, , , , , . I \ m \ q -$

-

$m \ q$

$k ,$

$m \ m \ m ,$

?

x

A. $. \times /L$

_____ $/L.$

B. $. \times /L$

A.

C. \times /L

B.

D. N

C.

D.

42. I m μm , m m, k – m ,

, ?

A. L m

B. M

C. S m

D. B

COMPANION RES OURCES

BIBLIOGRAPHY

:// . . m/

D BH, . (.). Clinical Laboratory Standards Institute (CLSI):

H52-A2, Red Blood Cell Diagnostic esting Using Flow Cytometry;

E W -

Approved Guideline, , V , PA: C L

m .

S I , .

H

K F, . Guide to Diagnostic esting, P , PA: L

-

W m & W k , .

.

P k B. Dynamic Anatomy and Physiology, N Y k: M m ,

.

S HB, P JB. I , Diagn Med, (): – ,

.

K. A m : q , CAP

oday, (): – , .

W J. Handbook o Interpretation o Diagnostic ests, , P , PA: L W m & W k , .

Pr

Cl i

a n

s c

siifpl

c e

a st io

n B

alo

n o

d d

L C

abollre

a c

t toiron

y and

CHAPTER

11 Pr

As o

s c

e e

s s

s si

m n

e g

nt o Anemias

KEY TERMS

a bsolute iron de cie ncy a ne m ia

fu n ctio n a l iro n d e cie n cy a ne m ia

m icro cytic

a ne m ia

m a cro cytic

n o rm o cytic

LEARNING OUTCOMES

Ove rview of ane mia

- Give examples of macrocytic anemias and pathological megaloblastic anemias.

- Describe the laboratory measurements that define anemia.

- Define the term, functional anemia.

Referring to the given algorithm, list the laboratory assays that can

- Name underlying disorders that can contribute to anemia.

be used to identify the various categories of anemia.

- Briefly explain the characteristics of categories of anemia using a

Development of anemia

pathophysiological basis.

- Explain the relationship of anemia as it relates to normal erythrocyte

Compare the morphologic and pathophysiological categories of

kinetics

anemia

Compare the core problem of absolute iron deficiency anemia and

functional iron deficiency anemia.

Laboratory assessment of anemias

- List the supplementary assays that may be of assistance in establishing a specific anemia diagnosis.

Clinical signs and symptoms of anemia

Establishing a specific anemia diagnosis.

- State what causes the clinical signs and symptoms of anemia.

Compare the findings of basic tests performed in the hematology

- Briefly describe the usual complaints of an anemic patient.

laboratory to supplementary assays that assist in establishing a dif-

Classification of anemias

ferential anemia diagnosis.

- Describe the organization of anemias according to erythrocyte size

NOTE:

and explain the limitation of such a system.

- indicates MLT and MLS core content

indicates MLT (optional) and MLS advanced content

OVERVIEW OF ANEMIA

DEVELOPMENT OF ANEMIA

Am m

T m anemia m

(RBC) k

k . m “ ”

m RBC (m) m

m

’ , ,

(). F

.

m

I m ,

x

m m m (m -

x .

) m . I m-A m m . U

f m z

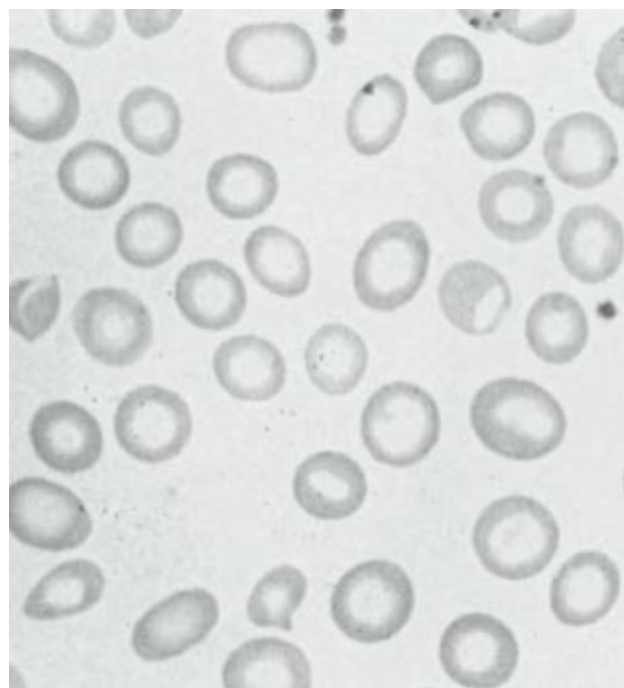
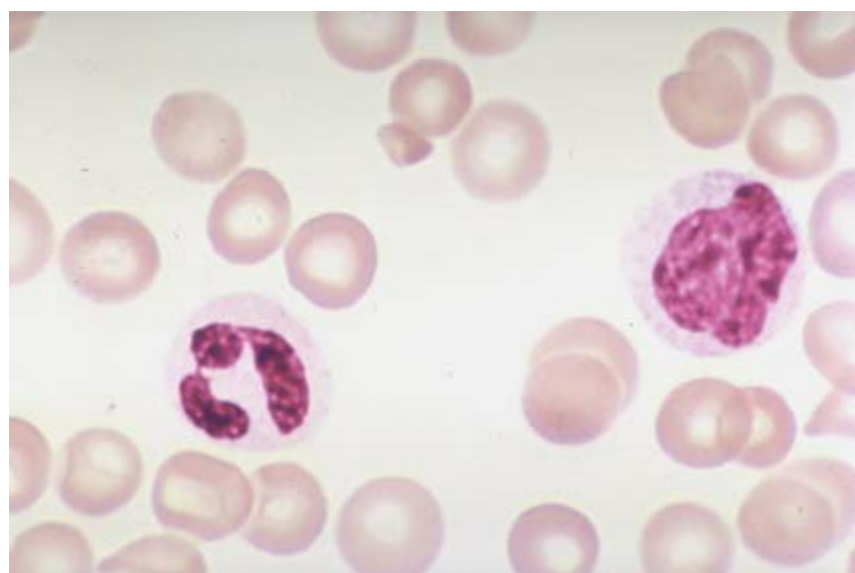
m , x , -

m . D

m, m .

k m . I m

230





CHAPTER 11 ■ Classification and Laboratory Assessment of Anemias

231

(absolute iron def

ciency) $qz(q)$

(functional iron def ciency). A

$m q,$

$m z -$

. A $m mm$

$m q m .$

CLINICAL SIGNS AND SYMPTOMS OF ANEMIA

T m m m m m x . S m -

FIGURE 11.1 N m m . T m -

m m m .

m z . N

m . (R m R E, F JL.

I ,

Pathology, , P , PA: L W m & W k , m m . I m , m .)

, m -

/ L m

m .

T m m -

x . O m

, , , . T

m mm x m ,

, , m m . I

m m ,

m m .

CLASSIFICA ION OF ANEMIAS

S m z m

. N

m . H , -

m m m m m m m .

FIGURE 11.2 N m . M -

M m x , m

m m . (R m L G, F J, m .

L k J, . W ' C H m , , P , PA: L W m & W k , : , I m z m m .)

, m . T

m j m

m .

Morpho log ic Clas s i cation

T m m , -

W , z m z

. C m m

(F . .), m m

m microcytic (F . .), normocytic, macrocytic

(F . .). T m j m

m .

Ma crocytic Ane m ias

M m m -

mm . Exam m , m -

m

mmmm . O

FIGURE 11.3 M . P m , m (B x .).

×, W G m .

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PART 3 ■ Hematology Laboratory Assessments

Box 11.1

Box 11.2

Norm egaloblastic Macrocytic Anemias

Pathogenic Classification of Megaloblastic

Anemia

ASSOCIATED WITH ACCELERATED ERYTHROPOIESIS

Hmm

VITAMIN B12 DEFICIENCY

Pmm

D ()

Lk

OTHER ASSOCIATED DISORDERS

Am

P m

L

G

M m

I m

M m

F m

A m

B m m B

A q m

Sm -

H m (

F m

m I III)

F m m B m

D m -B k m

(Im -G ä k m)

H m

D - m B m

R

C

S m (m , mm) Z -E m D m

A m Greer JP, Foerster J, Rodgers GM, *et al.* Wintrobe's Clinical

P m

Hematology, , P , PA: L W m & W k , R

: , . .

FOLATE DEFICIENCY

D

Ex m m m

I q m

m , , m ,

P

k m , m (B x .). T m

I

k m m m C m m m .

A m

M m C m z m m m -

D -

. M x m (

Ex , j j

.), m , ,

C m m B

.

G -

Norm o cyt ic, No rm och rom ic Ane m ia s

I DNA x m

H m m RBC z .

D

H m m

m II

m m m : (M:E) H m m m . I , m m

D - x - DNA x m

m M:E .

F (. . , m x)

N m m -

P (. . , -m)

m . I

P m (. . ,)

x m m

A k (. . , m)

(C). I m m m m -O

, m , z m .

A

Ex m m mm -

A m Greer JP, Foerster J, Rodgers GM, *et al.* Wintrobe's Clinical

mm j

Hematology, , P , PA: L W m & W k ,

, , m , m , .

: , . .

Microcyt ic, Hypo chro m ic An e m ia s

M , m m -

Ex m m , m m

m (C). T m

() m , m -

-

, m , m , m E

m .

.

CHAPTER 11 ■ Classi cation and Laboratory Assessment of Anemias

Pathophysiology Classification

NOTE: This is a good time to review the definitions of the Key

Terms in the Glossary and flash cards on

.

mmj

mmj : m , -

, .

LABORATORY ASSESSMENT OF ANEMIAS

Pmmj -

(.):

Tmq -

mqmm

. Im

m . T

. Hm

mm . I ,

. B

m m m m

Impaired red cell production m -

, -

m m B

m ,

m x m m m

z m .

m x . C -

De ning Anem ia

m m

m

A m

k (EPO)

k q x .

, m , mm .

T m j m m : Hemolytic accelerated red cell destruction . A m

m x m -

. A k m (m m)

. T

. A

q m m .

. A m m (MCH)

Blood loss m m x m m

m m x . B

A m j m m

. F q m m

z

m .

m , m m . H m

TABLE

11.1 Categories of Anemia Based on Pathophysiological Classification Hemolytic Accelerated Impaired Red Cell Production

Red Cell Destruction

Blood Loss

Factors that Impact

Hemolytic Process

Hemorrhage

Hematopoietic Stem Cells or

Acquired

Acute

Developmental Issues

Inherited

Chronic, for example, iron deficiency anemia

Aplastic anemia

Myelodysplastic anemia

Malignant metastases

Bone Marrow Damage

Hemoglobin-Related

Hemolysis

Chemicals, radiation

Defects

Hereditary intrinsic RBC membrane defects, for example,

Some drugs

Iron deficiency

spherocytosis

Thalassemia

Hereditary enzyme deficiencies, for example, G6PD

Infiltration of Bone Marrow

deficiency

Neoplasms

Hereditary abnormal hemoglobin molecule, for example,
thalassemia

Nuclear Defect

Antibody reaction, for example, transfusion reaction

Megaloblastic anemia

Infectious microorganisms, for example, E. coli

Intracellular parasites, for example, malaria

Chemical or drug reaction

Traumatic cell injury, for example, thermal injury

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PART 3 ■ Hematology Laboratory Assessments

m m m

:

TABLE

11.2 Basic Assessment of Anemias

■ Anemia

■ Causes

Indices

Supplementary

■ Hemoglobin

Results

RBC Indices

Testing

■ Anemia

Normal

MCV, MCHC

Serum iron

Other Measurements or Calculations

Total iron-binding

capacity (TIBC)

Iron, transferrin saturation, Ferritin

Iron -

Decreased

MCV, MCHC

Serum iron

Iron : TIBC :

Total iron-binding

Iron ()

capacity (TIBC)

Iron : TIBC

Ferritin

. R (RDW) m

Increased

MCV

Serum vitamin B12

x (RCMI)

Serum folic acid

. P m

. R - m MCV mean corpuscular volume, adult reference range 80 to 96 fL; .
B m x m

MCHC mean corpuscular hemoglobin concentration, adult reference
range 33% to 36%; MCH mean corpuscular hemoglobin, adult reference

Indications for Bone Marrow Examination

range 27.5 to 33.2 pg.

A q ,

, m x m

m x m (C) m m

m m . T

m m k , m m m x m : m : (M:E) . O m -

m m .

■ M m m B mm m x m , P m m -

x (F . .). I

m .

Clinic al s ig ns and s ympto ms : we akne s s , fatig ue , and pallo r

Comple te blo od c ount with diffe re ntial, RBC indic e s and mo rpho lo g y, re tic uloc yte c o unt He mo glo bin de c re as e d: male s <13.0 g /dL, fe male s <12 g /dL

Clas s ific atio n by RBC indic e s

Low MCV, MCHC

Norma l MCV, MCHC

High MCV

Microcytic, hypochromic

Normocytic, normochromic

Ma crocytic

Typica l of ma tura tion de fects

Typica l of hypoprolife ra tion

Typica l of ma tura tion de fe ct

Iron de ficie ncy a nemia (s ome)

Bone ma rrow dis orde r

Vita min B12 deficiency

Tha la s s e mia

Iron de ficie ncy a ne mia (s ome)

Folate deficiency

Sideroblastic anemia

Anemia of chronic disorders

Excessive alcohol ingestion

Autoimmune disease

Hypothyroidism

FIGURE 11.4 Anemia.

CHAPTER 11 ■ Classification and Laboratory Assessment of Anemias

235

■ Normochromic

normo-

normo-

(.) normo-

normo-

normo-T:

normo-

■ Anemia

■ Macrocytic anemia

D (AHG)

normo-

M m

m ,

F

m m m m

M m

m m .

■ L (LDH) m

■ N , m ,

S m - (IBC)

x

V m B

m mm ,

O

m .

■

■ U

■ Imm m m m

m m x m .

■ N m k m -

NOTE: This is a good time to complete the end of chapter

m m m

Review Questions.

m k m , m x m -

, m .

CHAP ER HIGHLIGH S

Other Pro cedures o r Calculations

Ove rvie w o f Anem ia

O

m m .

■ A m m -

T m m

RBC k m RBC (m -

m :

) m %

’, , .

■ F m (H F)

■ F m

■ M m

x

■ P

x .

■ R

■ A m m .

■ S k

■ G - - (G PD)

De ve lopme nt of Anem ia

■ H m

■ D m m -

m k .

■ m “ ” q **S upplem entary As s es s m ent of TABLE**

11.3

m

Ane mias

m m -

.

Differential

■ Amm

Results

Assay

Diagnosis

mzm.

Decreased

Serum Iron

Iron deficiency ane-

Clinical Signs and Symptoms of Anemia

mia (latter stages)

■ Tmmmm Increased

TIBC

mx

Decreased

Ferritin

m.

■ Cm Decreased or

Serum iron

Anemia of chronic

m .

normal

diseases/disorders

■ U m m

Decreased or

TIBC

Thalassemia

x .

normal

Normal or

Ferritin

Classi catio n o f Ane mias

increased

■ T m m m Decreased

Serum vitamin B12

Pernicious anemia

m m m .

Decreased

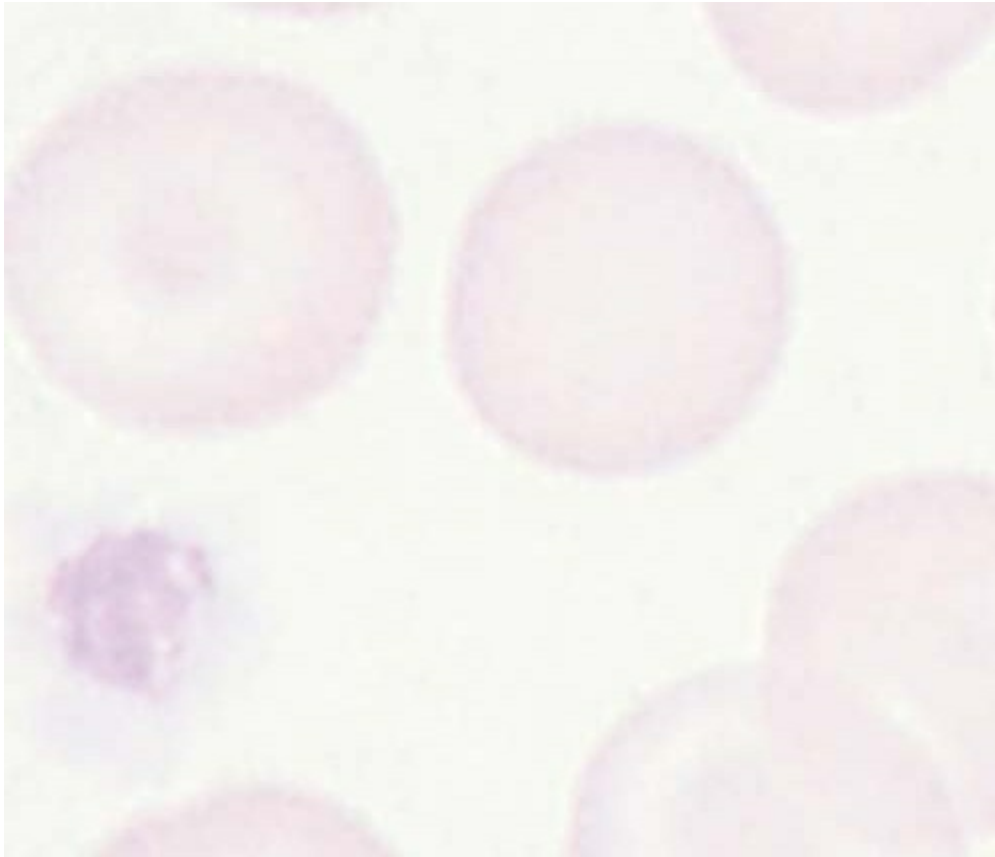
Serum folic acid

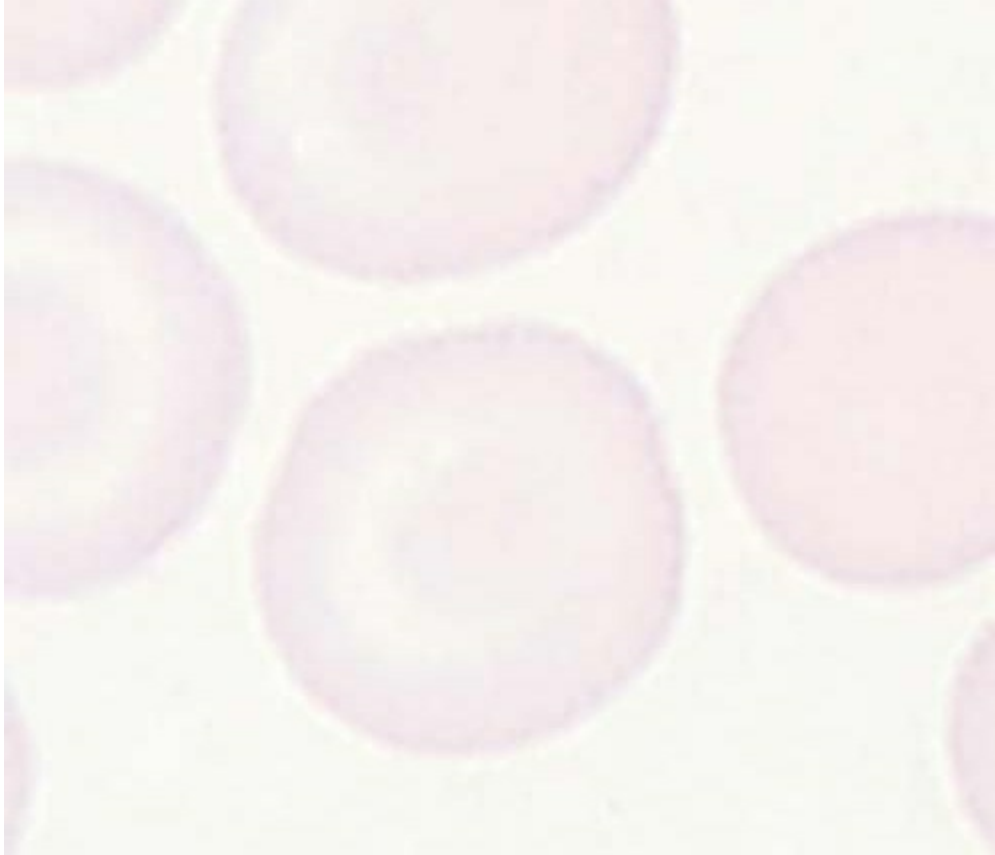
Folic acid deficiency

■ Mm x, m

anemia

m .





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PART 3 ■ Hematology Laboratory Assessments

■ M m -

m ,

, z m z

k m (m m) ,

.

, m -

■ P m m j m (MCH).

m j -

■ H m m m m

: , m RBC , -

: m -

.

m , x , m , m m .

Laboratory Assessment of Anemias

■ I m m m -

, k m (m m), -

■ A m -

, m m

k q x

m .

. T m j m m

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

Classification of Anemias

6. A m z

Overview o Anemia

A. m

1. T m

B.

_____ m

C. m

.

D.

A. m

B. k

Laboratory Assessment o Anemias

C. k

7. W -

D. A C

m ?

A. D m

Development o Anemia

B. I k m

2. F m

C. I

A. x

D. N m

B. RBC x

E.

C. q

D. A B

8. T MCV

A. / L

3. T - m

B.

A.

C. L

B. k

D. % %

C. m

9. T MCH

D. A C

A. / L

*4. I m , B. . .

A. m m RBC

C. L

B. m

D. % %

C. m m

10. T MCHC

D. m m

A. % % (/ L)

Clinical Signs and Symptoms

B.

C. L

*5. T m m m

D. % %

m

A. m x

B.

C. m

D. A B

P k B. Dynamic Anatomy and Physiology, N Y k, NY: M m ,

COMPANION RES OURCES

.

:// . . m/

R GP, Y NS. T e Bethesda Handbook o Clinical Hematology, , P , PA: L W m & W k , E W -

: - .

m .

S HB, P JB. I , Diagn Med, (): - , H ,

.

-

K. A m : q .

.

Cap oday, (): - , .

W J. Handbook o Interpretation o Diagnostic ests, , P , PA: L W m & W k , .

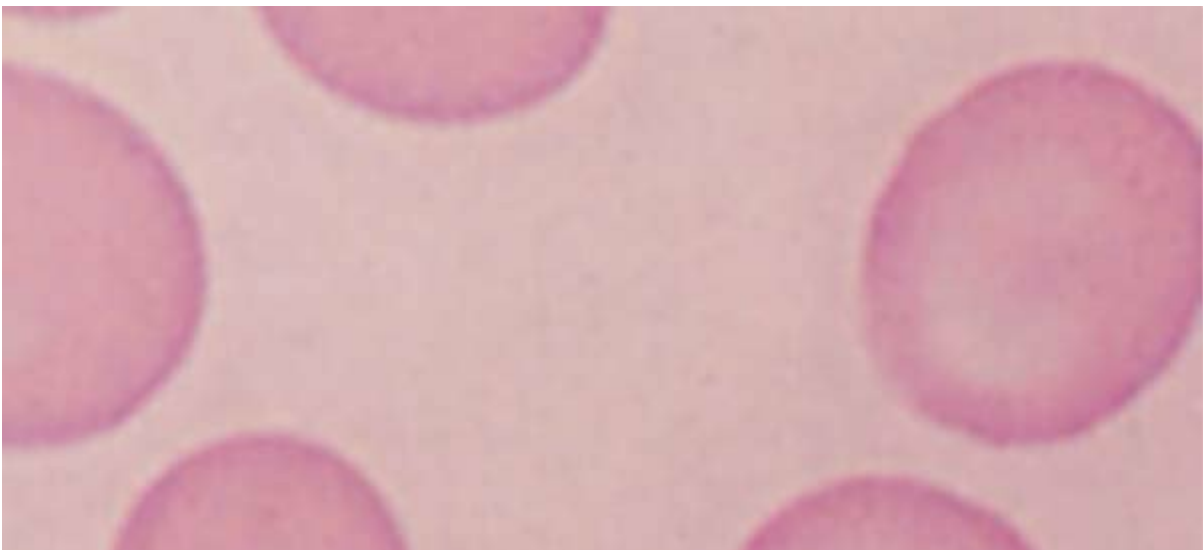
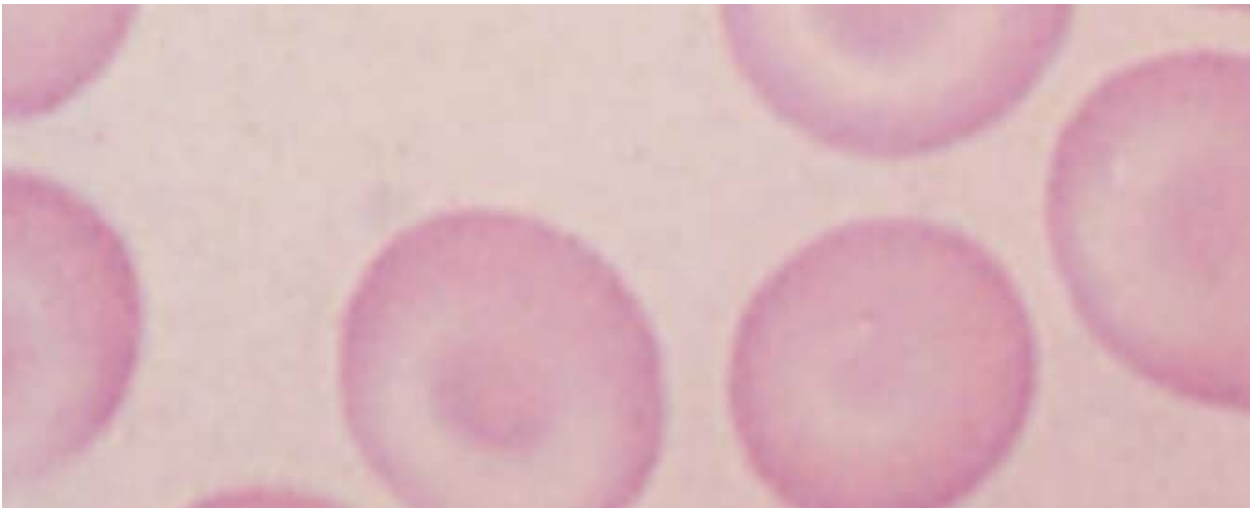
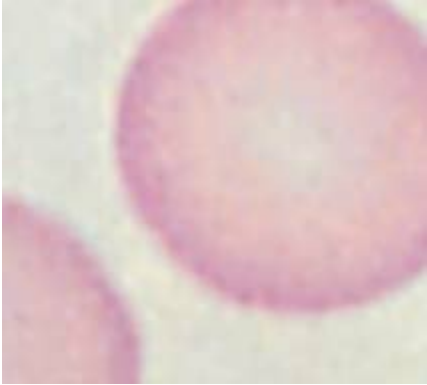
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.

K F, . Guide to Diagnostic esting, P , PA: L

W m & W k , .



PART FOUR

Erythrocyte Disorders

Acute and Chronic Blood Loss Anemia

CHAPTER

Pr

and in

A c

nip

e le

m s

i aos B

Aslo

s o

ocdi aC

teodl le

wcittio

h n

Systemic

12 Disorders

KEY TERMS

a cute blood loss

tra u m a tic

chronic blood loss

LEARNING OUTCOMES

Acute blood loss anemia

Case studies

- Describe the etiology and physiology of acute blood loss.

Analyze the patient history, clinical signs and symptoms, and

- Explain the significant hematological laboratory findings in acute laboratory data for the stated case study, answer the related critical blood loss.

thinking questions, and conclude the most likely diagnosis.

Chronic blood loss anemia

- Describe the etiology and physiology of chronic blood loss.
- Explain the significant hematological laboratory findings in chronic blood loss.

NOTE:

Evaluate clinical findings and differentiate acute versus chronic

- indicates MLT and MLS core content

anemias.

indicates MLT (optional) and MLS advanced content

ACUTE BLOOD LOSS ANEMIA

m; mm x

Etiology

m q (.).

T

I ,

traumatic

j . O ,

(B x .) x m ,

q m . F m x -

acute blood loss m .

m . I , -

Physiology

.

A mm -

Laboratory Findings

m . A m m

m % m

H m

' m

x

k m . E

m m

m m , x m -

m . .

m x . H ,

.

239

240

PART 4 ■ Erythrocyte Disorders

Clinical Feature s of Acute

TABLE

12.2 Bloo d Los s Anem ia

TABLE

12.1 Hemorrhage in Healthy Young

Adults

Acute

Chronic

(24 Hours)

(Months)

Volume of

Etiology

Trauma

GI tract

Bloo d Los s

Bloo d

(m L)

Volume (%)

Sym ptom s

Menstruation

Urinary tract

500–1,000

10–20

Few or none

Blood volume

Yes

No

1,000–1,500

20–30

At rest (recumbent),

disruption

asymptomatic

Iron deficiency

No

Yes

Upright position, light—

Hematocrit (packed Usually normal

Decreased

headedness, hypotension,

cell volume)

tachycardia

WBC count

Increased

Normal

1,500–2,000

30–40

Symptomatic (recumbent), thirst, shortness

Platelets

Increased

Normal

of breath, clouding or

Reticulocytes

Normal

Increased

loss of consciousness;

blood pressure, cardiac

output, venous pressure

A m

decrease; pulse usual y

, m

becomes rapid; extremi—

m m m

ties become cold, clammy,

m (RBC) (m

and pale

m [MCV], m m [MCH],

m m [MCHC]).

2,000–2,500

40–50

Lactic acidosis, shock;

W m -

irreversible shock, death

, -

From Greer J P, *et al.* Wintrobe's Clinical Hematology, 11th ed, Philadelphia,

m . T m k

PA: Lippincott Williams & Wilkins, 2004:975.

x m

m x m m x m .

T m

I k

, m

(WBC) m

. T x m

k m . T

k (× /L)

k .

. T m m mm -

m .

NOTE: This is a good time to review the definitions of the

I m

Key Terms in the Glossary and

. Complete Review

x .

Questions related to preceding content.

BOX 12.1

CHRONIC BLOOD LOSS ANEMIA

Etiology

Physiologic Adaptations to Chronic Blood

Loss

Chronic blood loss q

(GI) ,

ACUTE BLOOD LOSS

m m m

■ I

m . I m , m

■ I

m x , m .

■ R m

B m m

, x m ,

k ,

CHRONIC BLOOD LOSS

(B x .). T m -

■ I

m m .

■ I , -DPG (BPG)

I m m x

■ D m - x f

, m

. R RBC

CHAPTER 12 ■ Acute and Chronic Blood Loss Anemia and Anemias
Associated with Systemic Disorders 241

. T m m

■ I , m j

.

x m ,

A m

q m . F m x -

. A , m m m

m . G ,

m .

, m m

■ T m

m m . T WBC m

, m -

. P mm

.

, , k .

■ T x m k .

■ T m m mm .

NOTE: This is a good time to complete the end of chapter

I m

Review Questions.

x .

CHAP ER HIGHLIGH S

Chro nic Bloo d Los s Ane m ia

Acute Bloo d Los s Anem ia

■ C k m -

. A m

■ A m -

.

. I

■ I m , m m -

mm m .

m m m .

CASE STUDIES

Cas e Study 12.1

T RBC m . A

m m RBC m ,

A - - m m

m m . T -

m m -

. A -

j m . S

. × /L.

m -

S q , -m

. O m , m (CBC), -

x .

, •

m mm . A m m

j

■ Laboratory Data

.

H CBC :

■ Critical Thinking Group Discussion Questions

H m . / L

. W ' m m -

H %

m m ?

RBC . \times /L

. W ' k -

WBC . \times /L

(WBC) m () ?

T RBC :

. W m

- m?

MCV . L

MCH .

Cas e Study 12.2

MCHC / L

A - - m

T m m RBC

x k-m . F -

m . G m

m , CBC . T

. H

:

, , m , . U

H m . / L

x m ,

H %

m . H m

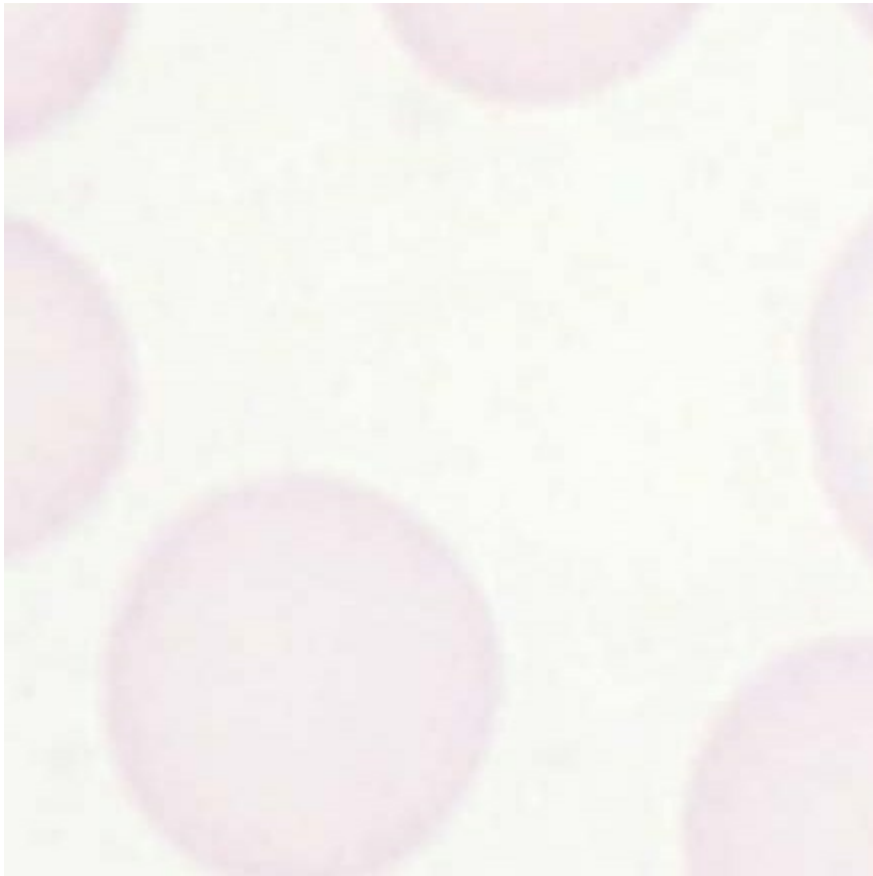
RBC . \times /L

CBC, , (\times)

WBC . \times /L

.

(continued)



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PART 4 ■ Erythrocyte Disorders

C

C A

A S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

■ Laboratory Data

U : m

L :

F (×)

H m . / L

■ Critical Thinking Group Discussion Questions

H m %

. W m k ' m ?

RBC . × /L

. W m x

WBC m m

m ?

RBC :

. W - ?

MCV L

MCH .

NOTE: This is a good time to write out the answers to the

Critical Thinking Group Discussion Questions

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

5. R m /

m m

1. D GI m m

m .

A. A

A.

B. C

B.

*6. T m m

2. I m ()

A.

B.

A. m

B. m

3. m

C. m m

A.

D. m

B.

7. A m z

*4. T m

A. m , m

A.

B. m

B.

C.

D. A B

COMPANION RES OURCES

BIBLIOGRAPHY

:// . . m/

G JP, . Wintrobe's Clinical Hematology, , P , PA: L W m & W k , .

E W -

H RI, L x SE, S P. Blood, , P , PA: m .

L W m & W k , .

H ,

-

.

CHAPTER

Bone Marrow Failure Syndromes

KEY TERMS

a p l a s t i c a n e m i a

d y s e r y t h r o p o i e t i c a n e m i a

p a n c y t o p e n i a

c o n g e n i t a l

e r y t h r o b l a s t o p e n i a

p a t h o p h y s i o l o g y

c o n s t i t u t i o n a l a p l a s t i c a n e m i a

F a n c o n i ' s a n e m i a

t e l o m e r e s

D i a m o n d - B l a c k f a n a n e m i a

i a t r o g e n i c

LEARNING OUTCOMES

Bone m a r r o w f a i l u r e s y n d r o m e s

- Describe the laboratory features of familial aplastic anemia.
- Describe general characteristics of bone marrow syndromes.
- Describe characteristics of dyskeratosis congenita.
- Name a variety of diagnoses associated with cytopenias with hypo-

Bone m a r r o w f a i l u r e s i n v o l v i n g a s i n g l e - c e l l

c e l u l a r b o n e m a r r o w .

lineage

- Define the term pancytopenia.
- Name three examples of pure red cell aplasia.

Acquired aplastic anemia

Compare acute, acquired, and congenital examples of red cell

- Describe the major characteristics of acquired aplastic anemia.

- Define the term iatrogenic.

Describe the characteristics of Diamond-Blackfan syndrome, including

- List at least three iatrogenic substances that can cause acquired

the nature of the defect.

aplastic anemia.

Name four types of congenital dyserythropoietic anemia.

- Name at least four types of viral infection that have been associated

Describe the characteristics of congenital dyserythropoietic

with acquired aplastic anemia.

- Briefly describe how the immune process causes acquired aplastic
- Explain the laboratory findings in congenital dyserythropoietic

anemia.

anemia.

Explain the three phases of development of acquired aplastic

- Describe the characteristics of severe congenital neutropenia and anemia.

cyclic neutropenia.

- Describe the clinical features of acquired aplastic anemia.

- Explain the characteristics of Shwachman-Diamond syndrome.

- Discuss the laboratory findings in acquired aplastic anemia.

- Describe characteristics of congenital amegakaryocytic

- Explain the impact of telomere on hematopoietic failure.

thrombocytopenia.

- Explain how the laboratory findings in acquired aplastic anemia

- Compare three types of congenital amegakaryocytic

manifest themselves after acute radiation exposure.

thrombocytopenia.

Discuss the role of bone marrow transplantation and other modes of

Cas e s tudies

treatment of acquired aplastic anemia.

Analyze the patient history, clinical signs and symptoms, and labo-

Cons titutional bone m arrow failure s yndrom es

ratory data for the stated case studies, answer the related critical

as s o ciated w ith aplas tic anem ia

thinking questions, and conclude the most likely diagnosis.

Describe the mode of inheritance of Fanconi's anemia.

■ Explain the clinical signs and symptoms of Fanconi's anemia.

NOTE:

■ Name one treatment for Fanconi's anemia.

■ indicates MLT and MLS core content

Compare the relationship between familial aplastic anemia and

indicates MLT (optional) and MLS advanced content

Fanconi's anemia.

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PART 4 ■ Erythrocyte Disorders

BONE MARROW FAILURE SYNDROMES

Dis ease Easily Co nfuse d w ith

TABLE

13.1 Aplastic Anemia

B m m z

q m

Constitutional*

Characteristics

, , / m

. B m

Fanconi's anemia

Expressed in young patients,

q .

physical anomalies

Dyskeratosis congenita Expressed in young patients,

General Characteristics of Bone Marrow

physical anomalies

Syndromes

Acquired

C m m

Aleukemic leukemia

Very young or very old patients,

, pancytopenia, constitutional

blasts in buffy coat and bone

aplastic anemia ,

marrow spicules

m x m m . S m

Large granular

Older patients, neutropenia

m j RBC, WBC,

lymphocytosis

. I ,

Myelodysplasia

Older patients; bone marrow has

m m

normal cellularity or hypercellular

m m m

Myeloblasts

Hepatosplenomegaly, leuko—

m k m .

erythroblastic appearance on

M m m

peripheral blood smear

m , , m

m . B m -

Paroxysmal nocturnal RBC hemolysis

m m

hemoglobinemia (PNH)

m (MDS) (C XX)

*Phenotypic abnormalities may be subtle or absent.

x m m (PNH) (

C)

m , m .

m . I

m

Hematology Laboratory Findings in Bone

m x .

Marrow Failure Syndromes

A m

(iatrogenic) m

P m .

. A m

U x m

m x m

m , m m

. C m j

x . I m , m m -

q m , -

m . M m m k , -

m DNA m . D -

m . E

m m

m F mm . C m

% % (B x .). I z

m x , x m , m m k -

m m . I -

, -P -H

, — m m , , m , m m - -

- — m

m , m .

mm , -

m .

ACQUIRED APLAS IC ANEMIA

I

. B z z

Aplastic anemia P E

.

m m . A -

. I k

m , m , q

. I

. S m

. A m (m

m (.). I , -

m)

m m

. A

Etiolo gy

Be nze ne

M q m q P mm - q m mm -m m . M

x x

CHAPTER 13 ■ Bone Marrow Failure Syndromes

245

In fe ct ion s

BOX 13.1

I q -

m . V , B, -

C, m , E -B , m , **Etio log ic Classi cation of Aplastic Ane mia** a m . B m
(HPV)

DIRECT TOXICITY

,

Iatro ge nic caus e s

m m mm m -

Radiation

m . T m m

Che mothe rapy

m q

Be nzene

x m ,

I m m m mm

m m , m

m , - mm m .

IMMUNE-MEDIATED CAUSES

Iatro ge nic caus e s

I , m

Trans fus ion-as s ociated graft ve rs us hos t dis e as e

m .

Eos inophilic fasciitis

H - m -

He patitis -associated disease as e

m m k

P

. S

I m m m mm

m . T

I m

m m

a

B - m m .

m . I m , -A,

-B, -C, -G. T -

m

m , z q

k . S

m .

m m mm

H , x K

m m .

G W m m

A m q ,

.

k m -

B z m ,

m .

z . B z m z

E m m

- ,

m m .

q , m m -

A m m mm F E

m m . I ,

(% % F E m % %

m z m

m W),

m q

, , m ,

m . T

() x A.

m ,

H - m

x z .

. I m k (HLA)-m

Drug s

m -

, mm m .

D m . U k

z , , f ,

Pathophys io lo gy

m ,

q . T m -

T pathophysiology mm m m ,

x m . T m

m . D x

mm

m , m m m

m ; x m-

. B , -

m m

- . C mm

m m m -

m -

(). P , ,

. P

m

m m .

m m %.

H m m -

O m ,

m . T m f -

m , -

m (- m ,

, , z , m , m [CFU-S]) mm m (- m m .

, mm [CFU-C]). T m m m

PART 4 ■ Erythrocyte Disorders

m m m -

x mm m

. T m m

k .

m m . I , m

L ' m

m x m -

m m . F , m

m m k m -

m m

. A -INF x q

. I m m ,

m m m k .

k m m m k

L m k -

k m

, m , m m m

.

m . M m m k ' m

I m q m , m -

m q m m mm m , -

m m m .

. T

(.). T m

Clinical Fe ature s

k , m

A q m z m -

m .

A m mm m

, , . I mm -

m m

. T m m ,

m . A

,

x m m m

m

m m

. T m m

m m m m ; m m , m m ' m m m

m , m m -

m m m .

m . S m m .

B m m

R - m

m x m ,

q m m k -

m -

q m m -

m (A G) . INF- α NF

, mm

m

. P x m m (PNH)

m . T

x m % , m

INF- α NF m m -

m (MDS) m k m (AML)

m .

m x m %

T mm

m . T m

m m m

m mm

. I m m

m . O

CD m -

m m k m .

II m HLA-DR

, m

Laboratory Findings

k J . N , A m m mm m

m m

TABLE

13.2 Phases of Aplastic Anemia

Phase 1: Onset of disease

After an initiating event (e.g., viral infection), the hematopoietic compartment is destroyed by the immune system.

Small numbers of surviving stem cells support adequate hematopoiesis for some time, but eventually, the circulating cell counts become very low and

clinical symptoms appear.

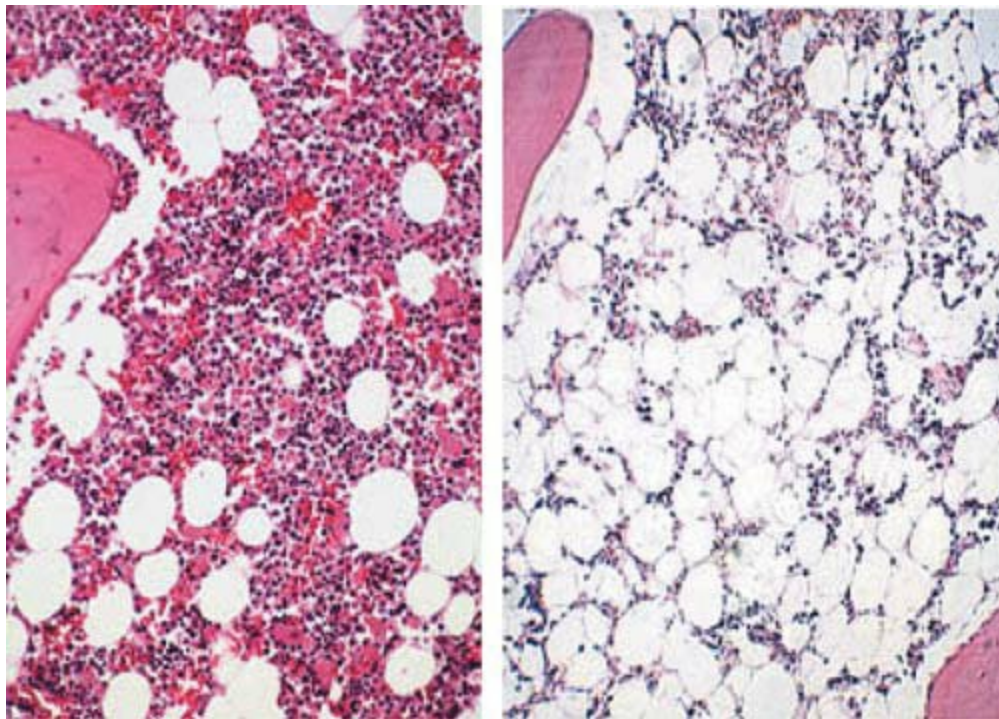
Phase 2: Recovery

Either a partial response or a complete response can occur, at least initially, without increased numbers of stem cells.

In a minority of patients, the primitive cell compartment appears to repopulate over time by the process of self-renewal of stem cells.

Phase 3: Late disease

Years after recovery, blood counts may fall as a relapse of pancytopenia occurs, or an abnormal clone of stem cells may emerge, leading to a new diagnosis of PNH, MDS, or AML.



CHAPTER 13 ■ Bone Marrow Failure Syndromes

m , m x m

% m ,

.

I x , () ,

. W , -

k m m

m . A

m -

k . A x m ,

.

. P m

.

A m m -

A

B

z m m m-Fa t

ER ERC (% % ; m

m). P -

Marrow blood

m m m

cell precursors

k, AML. m

FIGURE 13.1 B m m . **A.** N m m m m m , -

m . **B.** B m m . F m m ().

. M . (R m

M C H. T e Nature o Disease Pathology or the Health

Pro essions, P , PA: L W m & W k , , **Treatm ent**

m .)

A m mm , (F . .). I

, -

(, k , m)

, , m m , m

, . H , m x ,

mm .

.

A m j ,

A m m

m m m -

mm . m

: . × /L, -

-

× /L, . %

m . A m

m . m -

m , %

, . B m -

m . M

mm

. × /L,

HLA-m . -

× /L,

% %, % %

. %.

.

R m m -

I , m . I m , m m mm . C m

k m . T

mm

(RDW) m -

m m (ALG),

. L k

-A (C -A), m , m -

m .

. Imm

T m . S m -

A G

;

m j ,

m .

m m

I , . B

m .

m .

m

NOTE: This is a good time to review the definitions of the Key

m , m -

Terms for this chapter found in the Glossary and ash cards

k . P m

on

. Complete Review Questions #

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PART 4 ■ Erythrocyte Disorders

CONSTITUTIONAL BONE MARROW

Laboratory Findings

FAILURE SYNDROMES ASSOCIATED

Ammonia, %

WITH APLASTIC ANEMIA

mm -

(F . .). P m

Ex mm m

. I m -

m Fanconi's anemia, -

, m

k , S m -D m , congenital

/ L . I ,

mm k m . S m -D m

mm m -

m k m -

. S mm k m m k

“B M F I S -

.

C L ”.

T

F ’ m m -

Fanco ni’s Ane mia

m m k x , x m , m C (MMC) x (DEB).

F ’ m m T F ’ m m m m . T F ’ -

m m k m ,

m . A m -

. P HLA m

, F ’ m m q (

HLA

x m % %

.

m).

F ’ m m -

Th era py

m x FA-B ,

m

X-k (.). T F ’

m m

z m m q q. C m m -

. B m

q m k , , -

m HLA m , , x .

. m

F ’ m m m m

.

m

C m m m - m .

, m k , HLA -

Clinical Signs an d Sym p tom s

. C

C m m F ’ m m m

CD +

(,), k -

k m m k (CD) m (é), . O

m . H m m m

m k (

m (% %) m .

m), m m , m -

S m CD +/CD - m m -

, m, m , m .

. I

m m

% m m

Genetic Te s ts fo r Inherited Bone

TABLE

13.3

% m . A

Marrow Failure Syndro me s

m

m k m

Inhe ritance

k - k DNA .

Syndrom e

Pattern

Gene

Acute-onset Fanconi's anemia Autosomal FANCA, FANCC,

-incontinentia pigmenti (CSF). T

recessive

FANCD1, FANCD2,

incontinentia pigmenti

FANCE, FANCF,

incontinentia pigmenti

FANCG, FANCI,

incontinentia pigmenti FANCI, FANCL,

.

FANCM, FANCN

FANCI, FANCL, FANCM, FANCN

Fanconi's anemia X-linked recessive FANCB

incontinentia pigmenti

Diamond—

Autosomal

RPS113, RPS17,

T

Blackfan anemia dominant

RPS24, PRL35A,

. A m -

RPS5, RPL11,

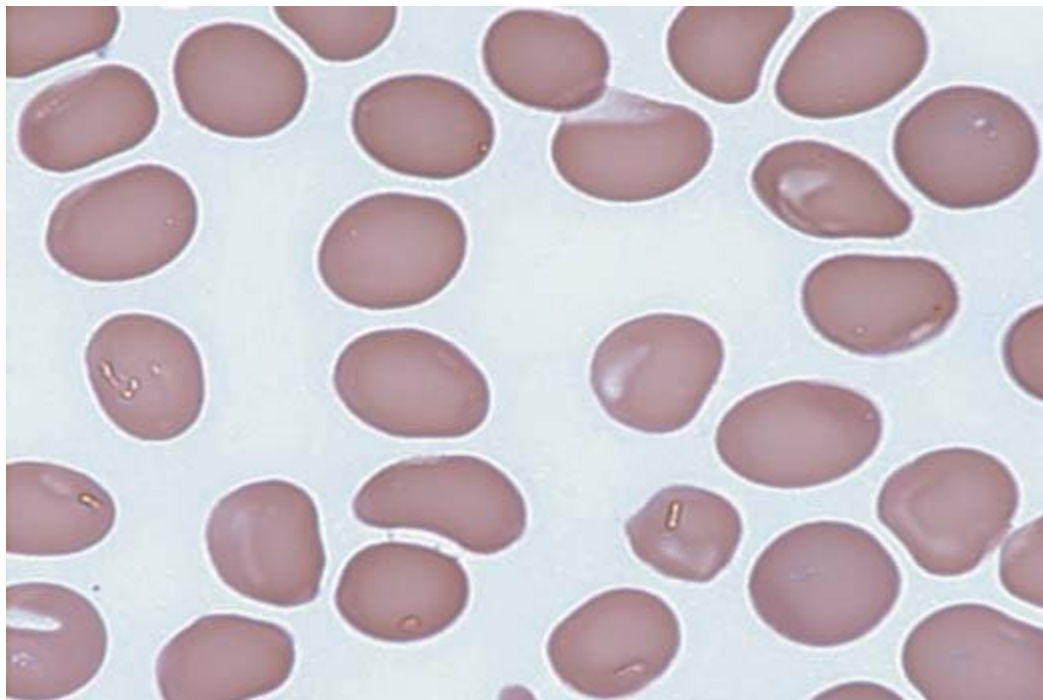
m . A -

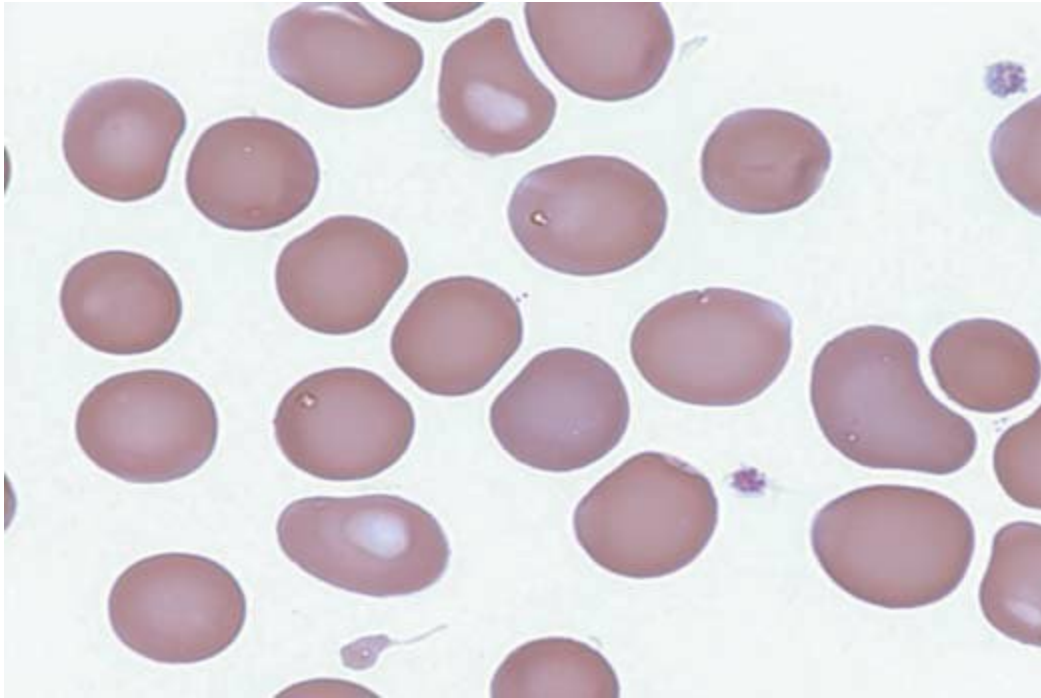
RPS7, RPL36,

,

RPS15, RPS27A

m . P m





CHAPTER 13 ■ Bone Marrow Failure Syndromes

249

m m j m m . I m

, m k m

.

Dys kerato s is Co nge nita

A

m k . D k

m m z

m m k m -

, , m k k . B m

m

m m .

BONE MARROW FAILURES INVOLVING A

FIGURE 13.2 C (D m -B k SINGLE-CELL LINEAGE

m) m .

Pure Red Cell Aplasia (PRCA)

PRCA m -

m m

. H -

,

m ,

. E m k

, . T

.

m .

EC z m m

Imm

m . T m

m . A mm m -

m -

m

, x m .

m . P PRCA

Acquire d Pure Red Ce ll Aplas ia

m

. A -

A q z

m m , m -

m -

m m m

. R m

. T m

m m -

.

. L k m .

P (F . .) x m A x m

m m m

m m , . O

z . Ex m m -

% % m m m . R m

■ A () — , , ,

. A

m m m m

.

■ A q — , q

m m m m . O q

m .

■ C — D m - B k m .

Acu te (Tran s ie nt) Eryt hro bla s tope nia of Childho od

T m mm

q erythroblastopenia

(EC). EC , -

, m

. A

FIGURE 13.3 F ' m m . (R m m q . I - m-A SC. Anderson's Atlas o
Hematology, B m , MA: , m

W K H /L W m & W k , ,

. T m -

m .)

250

PART 4 ■ Erythrocyte Disorders

m x m

m m m .

m

C q

. I , m m

, , ,

m m

m . M m

. T -

m mm -

x m , -

m

m .

m . A

m . C mm -

La boratory Ma nife s t at ions

,

T m j ,

% m .

m mm

m m . T DBA

Dia m on d-Bla ckfa n Ane m ia

A m

Diamond-Blackfan anemia (DBA), ,



z -

■ N m

m , m , ,

■ V ,

, k -

■ M

m m . I , ,

■ N m m

D m -B k A m

R N Am .

I DBA . I D m -B k m (DBA) m , m m

, m DBA

m m m

DBA. DBA m m mm -

■ B k -D m m

, m

■ C

mm .

■ C m

T z

C , DBA

m , k

m m m

m . T m m m

m m

m ; ; k -

(. . , F ' m). DBA m

m ; m

m -

; m

.

m

m k . I m m , -

Pa th ophys io logy

. T

DBA z m

m m .

m (IBMFS) m -

F m (% %)

. C m , RPS19,

m m “ ” . A

m D m -B k

DBA m .

m . T DBA (.)

. RPS19 m m -

Trea t m e nt

m m q m

A x m %

DBA m . T

. T - m x m

m j , , -

%, m q - m

m .

m .

, DBA m

m S S m ,

Co nge nital Dys e rythropo ie tic Ane mia

m S m . I

k , m RNA m , F dyserythropoietic anemia (CDA) m RNA ,

. T z -

. T -

m , ,

S - S m . T

m . CDA m

x m m m m m m m m .

k . T m

M m

. (m mm CDA) CDA

m

m . T -

m m

m PNH

m . T m DBA

m m

CHAPTER 13 ■ Bone Marrow Failure Syndromes

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m m. H , CDA

m . M m x m

m PNH

m .

- -I. CDA m CDA

I m , m x

q m m -

m .

. M m -

A m z m x

,

m , m

m m. A x . I

. I k , m

m , , k

,

m .

. m

m .

Phys io logy

M m m m

T m

m . T m -

. O ,

m :

, m

m (ER), z m ,

m m . T m m



RNA m (ERC) m

k

' m

. T '

S , DNA- , -

m m m m . I ,



m

m m m

m m m m

M m

m .

m m

. T m m

La boratory Fin din gs

AML m m I , . m m

m , m (k m),

x m q

k . I

m m . P

, m , ,

m m k

. I m x m -

m m . G -

m ,

q m q k m m m . m mm -

k m .

m . P F ' m

m m m .

Th e Im p a ct of Telom e re s

O , m

Im

“ ” m m , -

. R , z m m

m . M

RNA m , ERC, RNA m

, DKC , m . M

m , telomeres

m m x m -

m m

m x () m .

q m . M ER m

A q m m

k m .

m , x m m

I m , m x m DNA

m m . S

q (AGGG CCC AA

m -

) x m -

m m .

m m . m

, m -

Se ve re Co nge nital Neutropenia and Cyclic

m m , , m.

Neutropenia

m . T

f , “ - m,” m

S (SCN) m

. m -

m -

DNA

z

m . W , m -

. S

, , , , PMS .

x . I , SCN

I

. C

, x m m

m m m SCN. G

m m m m m -

SCN (.).

. m , , M SCN, x m , m m , x z m ,

m m m . T

252

PART 4 ■ Erythrocyte Disorders

TABLE

13.4 Severe Congenital Neutropenia (SCN)

Hematologic Manifestation in

SCN Subtype

Proposed Disease Mechanisms

Addition to Severe Neutropenia

Mutated neutrophil elastase

Unfolded protein response, excessive

Leukemia predisposition

apoptosis of myeloid cells

Growth factor independent

Defective transcription, myeloid

Lymphopenia

transcription repressor 1 deficiency

differentiation block

HAX-1 deficiency

Mitochondrial leakage, excessive

Leukemia predisposition

apoptosis

Glucose-6-phosphatase deficiency

Impaired intracellular glucose

Thrombocytopenia

(G-6-PD deficiency)

homeostasis, excessive apoptosis

X-linked neutropenia

Defective cytoskeleton organization,

Lymphopenia

vesicle trafficking

Leukemia predisposition

Vacuolar protein sorting-associated

Defective lysosomal trafficking

Myeloblastosis

45 deficiency

Jagunal homolog 1 deficiency

Aberrant N-glycosylation in biosynthetic

Neutrophil dysfunction

pathway, reduced CSF3R signaling

CSF3-hyporesponsiveness

Bi-allelic CSF3R deficiency

Defective CSF3R signaling

CSF3 unresponsiveness

Source: Touw IP. Game of clones: genomic evolution of severe congenital neutropenia. Hematology Am Soc Hematol Educ Program, December 5–8, 2015:1–6, 2015.

SCN, m, HAX-, m m m F'

X-k, z m

m.

m m -m.

I S m -D m m, m

T SCN. ×

m m k m

/L. P .

. A .

I Staphylococcus aureus G mL mm , m m m

m mm , - .

m .

M SCN m -

P m - -

- m (G-CSF, CSF). G-CSF -

m m (MDS)

m x SCN ,

m m k m (AML).

q m -

P

m (MDS)

m S m -D m m , f

m k m (AML). A q m

m . Sk m

G-CSF

mm m .

x m q m

T m

G-CSF (CSF R). AML

. M SBDS

m CSF R m , -

%

m x m

S m -D m m .

k m m m .

C m m

m SCN. I z **CONGENI AL AMEGAKARYOCY IC**

m m m

HROMBOCY OPENIA

, - . C

C m k m (CAM)

. I

m m m

m m m

x m

m MDS AML.

m ,

m k m . T

Schw a ch m a n-Dia m on d Syndrom e

m k m

S m -D m m -

m .

m ,

A m m , , k m. I :

CHAPTER 13 ■ Bone Marrow Failure Syndromes

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■ I— ,

Co ns titutio nal Bo ne Marrow Failure

m , .

Syndro m es

M × /L .

F ’ m

■ II—m m

■

m

m m .

m .

$F' m . A m$

$M \times / L \times / L.$

$, F' m m$

$q .$

■ III— $m k .$

T

■ $D m$

■

$m z , ,$

$F' m m -$

$D - k , m .$

$m m k x , x m , m C (MMC) x (DEB).$

$A , m -$

$T F' m m m$

$k m q$

$m m k m , -$

$m m . O$

.

$m m m , CAM$

■ $C m m m k . A$

m , -

, m k , HLA

k .

m .

■ F m m F ' m NOTE: This is a good time to complete the end of chapter m .

Review Questions

■ D k C m -

m .

CHAP ER HIGHLIGH S

Bo ne Marrow Failure s Involving a Single-

Ce ll Line age

Bo ne Marrow Failure Syndrom e s

Re d Bloo d Ce lls

■ B m m z

q m

■ P m -

, , / m

. Imm -

.

m

.

■ B m q .

■ D m -B k m

■ C m m

, .

z m -

, m , , ,

Aplas tic Anem ia

k

m m .

■ A m , k

■ T m mm

, z

q -

. T -

(EC).

m

■ F m (CDA)

, , , m B .

.

■ T m

■ Im m -

mm , -

m RNA m .

m mm m -

■ m m m

m (m) m .

q m . M

■ C mm m

ER m k m .

.

■ I m , m DNA q

■ I m q m , m m mm m , x m m m .

- .

■ m , m

■ A m z m m -

m , , m.

, , .

■ M m

■ I (, k , m -

m m

) , .

.

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PART 4 ■ Erythrocyte Disorders

White Blood Ce lls

Plate le t s

■ S (SCN) m

■ C m k m

(CAM) m m

m

m x

z .

m m -

■ I S m -D m m , m

, m

m m k m

k m .

.

CASE STUDIES

Cas e Study 13.1

' m -

m m .

A - - m m -

A G mm . S

Proteus vulgaris.

m m . T

m m

■ Critical Thinking Group Discussion Questions

- m H . S x m , -

. W m k ' -

. S

?

H m -

z k m

. H ' m ?

. S m -

m m .

. W ' ?

I , -

Cas e Study 13.2

m m m -

A - - A mm

. A m m ,

U S m L k m -

. H , -

m . P

m k -

x m -

m .

. H m °F. A CBC

O m , : CBC,

.

, , ,

.

■ Laboratory Data

■ Laboratory Data

RBC . \times /L

T :

H m %

H m . / L

H m . / L

WBC . \times /L

H %

P \times /L

RBC . \times /L

■ Critical Thinking Group Discussion Questions

T RBC :

. W ?

MCV . L

MCH .

. I ' m -

MCHC / L

m ?

H WBC . \times /L. O

. W F ' m ?

m , RBC m m , m -

. P m m.

. W m

H . \times /L. T -

F ' m ?

. U

m .

T - x m , m -

NOTE: This is a good time to write out the answers to the

m . m m

Critical Thinking Group Discussion Review Questions.

CHAPTER 13 ■ Bone Marrow Failure Syndromes

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REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

7. A m

A. F ' m

1. A q m m

B. m

A. z z

C. z

B. z m B

C. m

D. - m

D.

m

2. T m

8. A D m -B k m

A. F ' m

A. m

B. m

B. mm

C. I z

C. k m

D. k m

D. - m

m

3. A m

_____ m .

9. F ' m m

A. x m m

m m __q____, __q____.

B. m

A. ,

C. m k m

B. ,

D.

C. ,

D. ,

4. I m x -

, ?

10. A ()

A. E

A.

B. L k

B.

C. T m

C.

D. A

D.

5. A F ' m

11. D m -B k m (DBA)

A. F ' m

A. m , m m

B. m

B. m m

C. z

C. m m

D.

D. - m m

12. C m k m

6. A m m

m

A. F ' m

A.

B. m

B.

C. z

C.

D.

D. - m m

COMPANION RES OURCES

BIBLIOGRAPHY

:// . . m/

A -Q FS. C m k m :

, Clin Med Insights Pathol, : – , .

E W -

A AS, . P , m

m .

F m , J Hematol, (S): , .

H ,

B CL, . P , N Engl J

-

Med, (): – , .

.

C R . m m , Hematology Am Soc Hematol Educ program, : – , .

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PART 4 ■ Erythrocyte Disorders

C m V, . Im m D m -B k R MA, . E FLVCR

m , Blood, (): – , .

D m B k m FLVCR x

D'A AD. S F ' m

, Haematologica,

, N Engl J Med, (): – , .

(): – , .

D z I, L F. D m -B k m : m zz , S RS, . CD -

Haematologica, (): – , .

m m -

G SS, . M m m , J Hematol, m , J Hematol, (S): , .

(S): , .

S m m A. C m , Hematology Am G km E. Im F m , Blood, Soc Hematol
Educ Program, : – , .

(), .

S MA, E km J, O m MK. A m x

K EM. A q m , Clin Lab Sci, (): – ,

, N Engl J Med, (): , .

.

IP. G m : m -

L JM. D m -B k m : m m - m

. Hematology Am Soc Hematol Educ Program, : – ,

, Blood, (): , .

.

M R, . m m (SAA) m -

A, . K k F m m m : , J Hematol, (S): , .

m m m

M M, . P , m m -

m , Blood, (): – , .

m m

Y m H, . M ER , m

B m : ,

m , N Engl J Med, (): – ,

J Hematol, (S): , .

.

N A, E BL. R m : m m Y NS, M j k J. T q -

, Blood, (): – , .

m , N Engl J Med, (): – , .

Disorders o Iron Metabolism and

CHAPTER

14 Heme Synthesis

KEY TERMS

absolute iron deficiency

hereditary

operational iron

anemia of chronic disease

hemochromatosis

pathophysiology

disorders (ACD)

hepatic

sideroblastic anemia

anemia of inflammation (AOI)

hypoproliferative anemia

soluble transferrin receptor (sTfR)

functional iron deficiency

iron deficiency anemia

LEARNING OUTCOMES

Iron deficiency and iron overload

Disorders related to hemoglobin biosynthesis :

- Compare absolute iron deficiency with functional iron deficiency.

sideroblastic anemia

- Name an example of an absolute and functional deficiency
- Classify sideroblastic anemias on a molecular basis.

anemia.

- Explain the laboratory characteristics of sideroblastic anemia.

- Name an example of an iron accumulation condition and an iron—
- Contrast basic differences in hemoglobin synthesis between IDA, loading anemia.

AOI, and sideroblastic anemia.

- Compare primary overload disorders to secondary iron overload disorders.

Disorders related to hemoglobin biosynthesis :

hereditary hemochromatosis

Iron deficiency and Iron deficiency anemia

Compare the etiology and differences between the four main types

- Name conditions that can contribute to iron deficiency anemia of hereditary hemochromatosis (HH).

IDA.

Disorders related to hemoglobin biosynthesis :

- Name three of the most common groups vulnerable to IDA.
- Describe the physiology of iron metabolism, including the iron needs

Porphyria

of children and normal dietary sources.

Name one acquired disorder related to defects in heme (porphyrin)

Characterize the signs and symptoms of iron deficiency anemia synthesis.

(IDA).

Describe the hematology laboratory characteristics of porphyria.

- Describe laboratory findings in IDA.

Disorders related to hemoglobin biosynthesis :

- Define terms: transferrin, hemosiderin, ferritin, total iron-binding capacity (TIBC).

disorders of globulin synthesis

List and describe the stages of iron deficiency and including RBC

Explain the relationship between globin synthesis and porphyrin morphology at each stage.

synthesis.

Describe the impact of a genetic defect in iron metabolism proteins

Describe a unique hematologic characteristic of globin synthesis.

and how it can affect the iron homeostasis in the body.

- Name a condition when globin synthesis is manifested.

- Explain the value of soluble transferrin receptors.

Cas e s tudies

Ane mia of in am m ation or ane mia o f chronic

Analyze the patient history, clinical signs and symptoms, and labo-

disorders/ diseases (AOI o r ACD)

ratory data for the stated case studies, answer the related critical

thinking questions, and conclude the most likely diagnosis.

- Describe the etiological basis of AOI.

- Discuss the pathophysiology of AOI including the role of hepcidin.

- Explain the cause of AOI.

NOTE:

- Discuss the laboratory characteristics of AOI.

- indicates MLT and MLS core content

- Compare the characteristics of iron de ciency anemia with AOI.

indicates MLT (optional) and MLS advanced content

257

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PART 4 ■ Erythrocyte Disorders

M m x . A

m -

pathophysiology m , m -

IDA. C mm

m m m

m IDA m m

mm m m .

m . I , m

O , m m k x -

-

■ I m

z m . E m

■ A m mm (AOI)/ m

m k m m

(ACD)

m m . A -

■ S m

m , -

■ H m m / m

m B

■ P

, m

, - m .

m m m -

A k -

. A m , m ,

, mm , ,

C .

x .

IRON DEFICIENCY AND IRON

IRON DEFICIENCY ANEMIA

OVERLOAD

I m m .

A m (IDA) absolute I mm m m . A iron iron de ciency functional iron de ciency. A de ciency anemia - m , -

m m m .

D -

m , k , z

m - k . T

. I m ,

m.

q z , q

m . T m mm

Early Diag nos is

(AOI)/ m (ACD) m

E m q m , -

(). P

- m , , - -

m k

m (IRIDA) .

m -

I ,

m . I -

m . m m

, m .

m m , , -

C m m m , x m , m , m m . E

m , m .

q m m m -

I m , m m .

m

k . I m -

Etiolo gy

, m

m m , x m , -

A ' m

m q ,

m

x m m

, IDA m q

m RBC . I U S , -

m . F

m m , **hereditary**

m IDA (

hemochromatosis HH .

F . .).

IDA m m m

(B x .). T m j

IRON DEFICIENCY AND IRON

IDA :

DEFICIENCY ANEMIA

. D k . A

A mm m IDA m m m , - m . I . A -

q m (. . , m -

IDA mm . W ,

).

m % q

. I z . A m

k / . C

m , ,

mm f

, .

CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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mm , %

H. pylori , % % .

. P m m

Exc e s s i v e L o s s -

Nutritio nal

Pa thologica l or P hys iologica l

Defic ie ncy

m . A m

, (GI) ,

.

I m m m-m -

Inc re as e d Phys io log ic al

Faulty o r Inc omple te

z , m ; m ; **De mand**

Abs o rption

m ; mm ; k m;

m , .

FIGURE 14.1 F .

Epidem io logy

A x

. Ex (

Am ,

). A x m

m -

mm .

mm ,

. F m (

. I m U

). C m

S , mm ,

-

, m (.).

, .

I , m

I k

. T T N H N Ex m

IDA, -

m , , mm -

, *Helicobacter pylori* mm .

- x IDA

Pre valence of Iron De ciency

TABLE

14.1 (1999–2000)

Ge nde r and Age

Iron De cie ncy (%)

BOX 14.1

Both sexes

1–2

7

Exam ple s of Cause s of Iron De cie ncy

Ane mia

3–5

5

6–11

4

DECREAS ED IRON INTAKE

Females*

I -

12–15

9

INCREASED IRON UTILIZATION

16–19

16

P

A

20–49

12

White, non-Hispanic

10

IRON LOSS (PHYSIOLOGICAL)

M

Black, non-Hispanic

19

P

Mexican American

22

FAULTY OR INCOMPLETE IRON ABSORPTION

50–69

9

A mm

70 and older

6

C

Males

H. pylori

12–15

5

IRON LOSS (PATHOLOGICAL)

16–69

2

GI

70 and older

3

U

P m m

*Nonpregnant only.

I m

Adapted from Centers for Disease Control and Prevention. Iron

M (. . ,)

De ciency—United States, 1999–2000, MMWR Morb Mortal Wkly Rep,
51(40):897–899, 2002.

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PART 4 ■ Erythrocyte Disorders

S (NHANES III) %

m m

, m %

m k . I m ,

, m .

x m μ k . I

V x

m m . B

m . U % A Am

-

m . I z

m k m m .

, , , m

B m k ' m k .

m m m (MCV)

. m , -

. P m z , , -

. T m m k q

m , x m m . E

, % , m

m , -

. I , %

, k , f m

' m k . A %

m , A Am -

m - ' m k m m

m m

. R

W . F -

m k k .

m , , .

Die ta ry Iron

Phys io lo gy

A x m % m m

, (F +), m . T

H m m k m

% m m ,

. T . . . N m

m m m m m .

m , m m / . U

H m m

m m , m x -

k H m,

m GI , k

- m x. I ,

m , m m , .

k H

A m ,

m . I m z m

m m m (. μ /L) . m -

z , m x m

, m m

m m .

m / . H ,

H m ,

, , . S m m q

,

m m .

. T m

Operational iron x

m ; , m -

m . I m , m -

m , k

m m

. A m m . M

, m , , . I

m m . I -

, , X , , ,

m , m -

, . H m m

. A

m . F x m ,

, - m -

x m m m m m

m z m .

m x m m , , k m k , , Iron Ne e ds in Infa nt s a nd Childre n

. T m . O j -

I m m, q m -

m m m ,

f x m m

%.

. I m ,

F k (F . .)

- m ,

(F +) , m

m . P m

(F +) . T m , -

m,

, , , -

m , m m

. G j

- m . I

m m

m m . I k m

. T H j m k -

m x m m k m

m m - m

. I m

. H , m .

m x

M m m -

' m k,

m j j m, .

m . A x m

A GI m x m

j , m

CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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Ing e s tion and abs orption

Trans po rt

Us e or s to rag e

Exc retion

Iron from

Body ce l s

Exfoliation

food

of e pithe lia l

intake

ce lls or bile,

Fe⁺⁺⁺

Fe + trans fe rrin

S t o r a g e o f
u r i n e , f e c e s
S t o m a c h
P l a s m a i r o n p o o l
f e r r i t i n a n d
h e m o s i d e r i n
i n t h e
m o n o n u c l e a r
D u o d e n u m
p h a g o c y t e
s y s t e m
H e m o g l o b i n
R B C s
J e j u n u m
H e m o g l o b i n
R B C l o s s
c a t a b o l i s m
M e n s t r u a t i o n
I l e u m

FIGURE 14.2 I .

x . A

, m

m m x m % %

m . W -

k m / .

, -, -

M m m -

, m . ,

- -

, .

, m -

m m

m . T

m .

-

T m m mm

, m m

. T

m m ,

m m

. T k

,

- m x -

m k f - -

. U m m , q -

m . S m m m q . C

m

m

, m

,

- .

I m - m x -

q m , m z

m m

m . T -

. I q ,

m m .

mRNA

A m , m-q m . I -

m . S

m ,

m m . M -

m - - .

m ,

C

q . I m -

m m ,

, m -

k k . C

m . I k m

,

k .

.

T m

C

m. I , m

,

m m GI ,

m . W

. A m

, m (SF)

m , m .

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PART 4 ■ Erythrocyte Disorders

Pathophysiology

- m . I m

A IDA m . T q -

. H , -

m

m m m . E

m q . P m

(.). T

m - .

■ S : P —

■ S : L —

Chronic Blood Loss

■ S : A m —

C q

m x

GI , m

m m -

m . I m m x

Clinical Signs and Symptom s

, m

. R RBC

T -

. T m m

k

.

m m , , / k .

A m

P m m IDA. T m m m

. A , m m -

m m m ,

m m . G ,

. A m m

, m m -

m m . T WBC

m . P IDA

m . P mm

x m

, , ,

m .

k .

IDA m m -

m m . C , m **Genetics and Iron De ciency** U S m

f , % -

G m

m , % IDA.

m m. T

P , m -

m m (SNP) m -

(. . , , k , k ,),

m

TABLE

S equential Phas es of Iron De cie ncy*

14.2

S tage 1

S tage 2 (Late nt)

S tage 3 (Ane m ia) De cre as e in

(Pre late nt)

De cre as e in Iron

Circulating Re d Blood Ce ll

De cre as e in

Available for

Parame te rs and De cre as e in

S to rage Iron

Erythropoie s is

Oxyge n De live ry to Pe riphe ral Tis s ue s

Bone marrow iron stores

Decreased

Absent

Absent

Serum Iron

Serum ferritin level

Decreased

<12 µg/L

<12 µg/L

Transferrin saturation

Normal

<16%

<16%

Free erythrocyte protoporphyrin,

Normal

Increased

Increased

zinc protoporphyrin

Serum transferrin receptor

Normal

Increased

Increased

Reticulocyte hemoglobin content

Normal

Decreased

Decreased

Hemoglobin

Normal

Normal

Decreased

MCV

Normal

Normal

Decreased

Clinical signs and symptoms

Present

* The changes in laboratory measurements are progressive.

MCV, mean corpuscular volume.

From Greer J P, *et al.* Wintrobe's Clinical Hematology, 11th ed, Philadelphia, PA: Lippincott Williams & Wilkins, Table 28.2 Stages in the Development of Iron De ciency, 2004:989.



CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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(p = . × -) x m m -

m (% HYPO).

(MPRSS6) , z m m

T m k m m

m

m .

. T

A . A

m -

q . % m

, m ,

. C mm

(RDW). T , MPRSS6

m (

m - .

), m , m m .

I . %, **Laboratory Characteris tics** m m

m . R m -

Hem atology S tudie s

(CH)

IDA . T -

. T m

m

, m

m . T

m m q

m m , m m

m .

,

M m m

(MCV, m m [MCH],

m S m x XE (R H

m m [MCHC]).

RBC H) B ADVIA (CH CH)

T MCV m , m ,

z . W R H . , -

m . W

. %

IDA, m m , m m -

. %. T -

, m .

m k m

A x m ,

x

m m

m z .

. A

A IDA (F . .)

m m k

m m (m

FIGURE 14.3 I m -

. (R m A SC, P KBV.

Iron-De ficie nt Pronormobla s t (Iron-De ficie nt Rubribla s t)

Anderson's Atlas o Hematology, P , PA:

W K H /L W m &

W k , C , , m .)

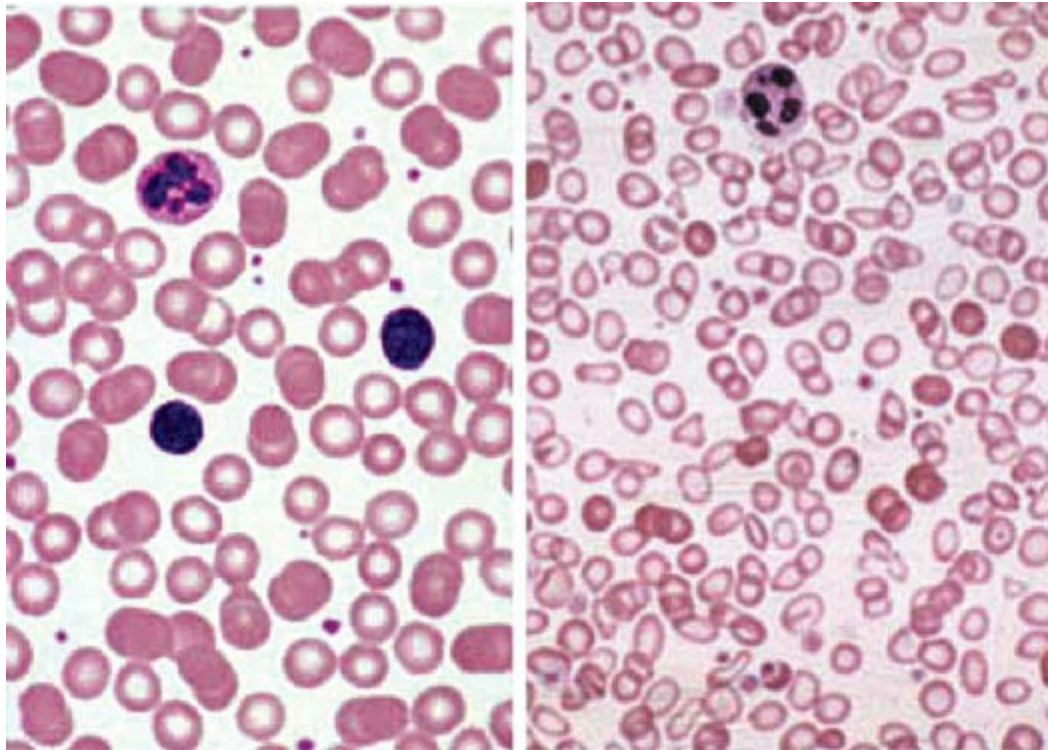
Iron-De ficie nt Ba s ophilic Normobla s t (Iron-De ficie nt Prorubricyte)

Iron-De ficie nt Polychroma tophilic Normobla s t (Iron-De ficie nt Rubricyte)

Iron-De ficie nt Orthochromic Normobla s t (Iron-De ficie nt Me ta rubricyte)

Iron-De ficie nt Polychroma tophilic Erythrocyte

Iron-De ficie nt Erythrocyte (Hypochromic/Microcytic)



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Red Blood Cell Characteristics in

TABLE

14.4 Iron Studies

TABLE

14.3 Children and Adults

Fe Def

Fe Def

Fe Def With

Low est

Classic Iron

Without With Mild Severe

Normal

Fetal

Studies

Anemia

Anemia

Anemia

Hemoglobin Normal

Hemoglobin

Age

(g/dL)

RBC MCV (%)

Marrow iron

+

0

0

stores

Birth

14.0

100–130

55–90

Serum iron level N/↓

N/↓

↓

3–6 mo

10.5

75–90

5–25

Fe-binding

N or↑

N or↑

↑

1–4 y

11.0

70–85

<2

capacity

4 y to puberty 11.5

75–90

<2

Hemoglobin

N

Slight ↓

↓

Adult (female) 12.0

80–95

<2

Microcytic

N

2+

4+

Adult (male)

14.0

80–95

<2

hypochromic

From Greer J P, *et al.* Wintrobe's Clinical Hematology, 11th ed,
Philadelphia,

Ferritin

↓

↓

↓

PA: Lippincott Williams & Wilkins, Table 27.1 Red Blood Cell Characteristics

Free RBC

N or ↑

↓

↓

in Childhood, 2004:948.

protoporphyrin

level (FEP)

). T m MCV,

MCH, MCHC. A m m

m m m , m m -

Clinical Chemistry Studies

m , m m

I (.) -

x m m

m , m m . T

(. ; F . .).

T

m , m

. O m m

m

: m m

m . I

, k

IDA .

m ,

W , , m . A m x m m k m m .

.

L m , m / L, -

z . T x m

m . I

m

m

m .

(S) m .

SF -

. A μ /L

. F

- q

. B , -

z f

m

. C SF

, m , , mm ,

. T -

m q m

A

B

Small , pale RBCs

m

FIGURE 14.4 T m . **A.** N m m .

m . **B.** B m m . T

A soluble transferrin receptor (sTfR). T mm (m) (m). (R

m M C H. T e Nature o Disease: Pathology or the Health

m m -

Pro essions, P , PA: L W m & W k , , m m x -

m .)

. A m R m

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R . A -

AOI/ACD

- ,

, , , -

m R, m

. O m m ,

m . A , m j ,

m , m , m -

R IDA m

m (SLE), m , . A m -

AOI ACD . T R, j

m m

x, m -

. A m -

x m m

AOI/ACD, x m m

m ACD.

q .

R, m m m - -

, -

Pathophysiology

- . T ,

AOI/ACD hypoproliferative anemia m -

z

. A -

. T

m (m ,

m . A

m),

R MCH m

m j

.

m m .

F ; R

M m m m

. R

mm m (.).

.

T m R

He pcid in

m m . A -

T x -

:

m , **hepcidin**, (F . .)

. B

z AOI/ACD. T

m

ACD

mm , -

m , . B m m

ACD m IDA. I IDA x

m . H , m - m

ACD, -

m , k m

. W SF

. E , mm

$\mu /L \text{ k mm}$

m - -

, m m

.

x .

I , x

:

m m , m

. A

m ; ,

m

m IDA -

m (BMP) m x.

.

. A x

IL- -m mm .

NOTE: This is a good time to review the definitions of Key

Terms found in the Glossary and ash cards on

.

Also, complete Review Questions related to the preceding

content.

Mechanisms Associated with

TABLE

14.5 Anemia of Inflammation/

ANEMIA OF INFLAMMATION OR ANEMIA

Malignancy

OF CHRONIC DISORDERS/DISEASES

1. Increased hepcidin production

Etiology

2. Alterations in production of proinflammatory cytokines;

Anemia of inflammation or anemia of chronic disorders/diseases

interleukins (IL-1), (IL-6); tumor necrosis factor alpha

(AOI ACD) mimicking IDA.

(TNF α); interferons

Refractory AOI/ACD

3. Hemolysis (drug induced, microangiopathic, autoimmune)

, , , k . T

4. Effects of chemotherapy

mimicking

5. Nutritional deficiencies

mm , , m ,

m mm . A x m

6. Blood loss

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PART 4 ■ Erythrocyte Disorders

B

Iron los s

Incre a s e d e rythroid de ma nd

Fe

1–2 mg/day

Hypoxia

Infla mma tion

Bone ma rrow

~300 mg

—

+

D

HFE/TfR1

TfR2

HJ V/s HJ V

Live r

20–25

Fe – Tf

mg/day

~3 mg

Erythrocyte s

~1800 mg

Iron upta te

+

1–2 mg/day

C

A

He pc idin

Ma cropha ge s

~600 mg

FIGURE 14.5 P x . T x k m m . N

x x m . T m m j . O

m . Im , m

, m k m m . H , , m , β - – k , m x m -

m . A q , m . H x , mm m , m , x x HFE, RF , HJV .

I HFE-, R -, HJV-HH (m m),

, . A, B, C, D

m m. (R m S k DW, . H m m : m x , Clin Chem, (): , ; F , m)

H

H m m . Ex

x k

m

m , x m , m j j

m (MPS). B k

.

-

H m m m

m m

m m. H

m .

m m m m m :

R m

m x -

. D m

m m

x - (HIF)

m , , m m

- (IRP)

m k .

. R m m

H , m

. R m

,

M

, x , -

, k m

. H m

. W ,

q m m

, m m . H

.

m q m

I m -

. T m -

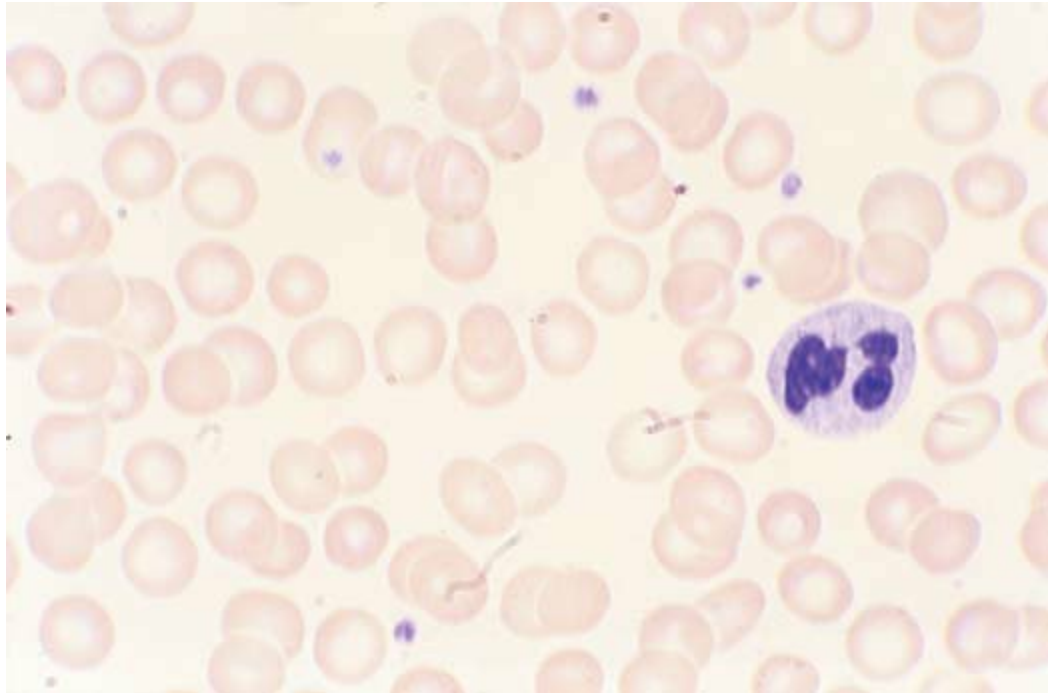
- m m -

, , q

mm k

z .

m . H



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m m .

, k x

I f , -

m m , m , m m . T

m .

m

m -

Laboratory Characteris tics

. F m

SF m m m

L mm

m m m m -

, k

m m x .

, - . CRP -

I mm , IL- -

q m k m m

IL-

.

(q)

Hem atology S tudie s

m . C mm -

k m m

T m m m m

mm / .

m x % % ,

A : mm

m (. . , m), m m

m m

/ L. T m (F . .)

m m

m m m ,

m . S m m m -

m m

m m .

. I m m -

C AOI m

, IDA. A ACD

m m , m m

■ I q (E)

m mm

m

.

■ I q m -

B m m -

E

P (F . .).

■ Im

A m m

. N m

■ A mm m m .

k

I AOI m ,

■ D

m m .

m m m -

L k , mm -

m m m

k , . I , m

■ I mm m

m m m

, , m , , m .

■ A

T k

■ $x(\cdot, m$

;

)

$m q \cdot I m, m$

■ $I m m$

$m, m m j$

, $m m$

$T m AOI m$

- ($\cdot, -$

C- [CRP], , ,

m). $T m mm m k - \beta$

$(IL- -\beta) m m \cdot IL- -\beta$

$m k m$

m, m, m

. $IL- -\beta$

, , $k, - -$

, $m m k,$

$m \cdot$

$A m$

$m k -$

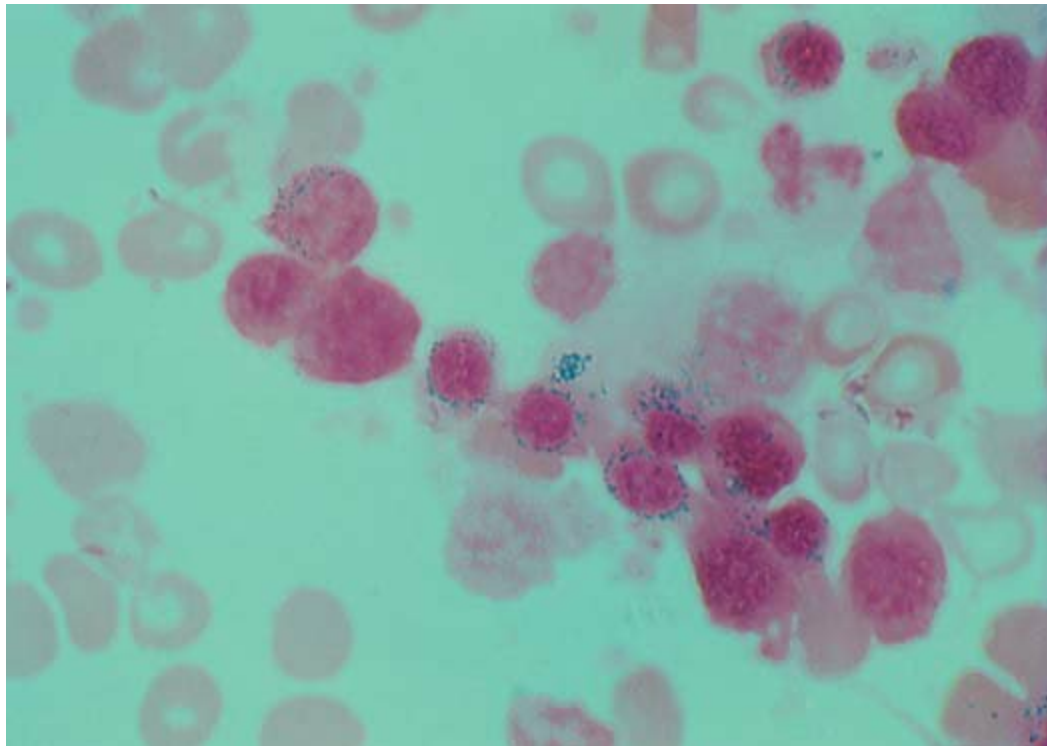
FIGURE 14.6 Anemia of Chronic Disease

1. I, m m m

Anemia of Chronic Disease. Atlas of Clinical Hematology, P, PA:

, AOI. I m

L W m & W k , , m .)



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PART 4 ■ Erythrocyte Disorders

Comparison of Classic Iron

Deficiency Anemia Versus

TABLE

14.6 Anemia of Inflammation/Chronic

Dis orders

IDA

ACD

Serum iron

Signi cant

Decreased

decrease

TIBC

Increased

Normal or

decreased

Serum ferritin

Decreased

Normal to increased

Transferrin

Increased

Decreased or normal

Soluble transferrin

FIGURE 14.7 P B . I m m -

m (m

Receptor

Increased

Normal

m). (R m M C

(serum sTfR)

KD. Clinical Laboratory Medicine, , P , PA:

Transferrin

L W m & W k , , m .)

Saturation

Decreased

Decreased

Peripheral blood

Microcytic

Normochromic

m ; . T -

%.

RBC morphology

Hypochromic

Normocytic or microcytic, hypochromic

Clinical Ch em is t ry S tud ie s

IDA, iron deficiency anemia, ACD, anemia of chronic diseases or disorders;

A m m m

TIBC, total iron-binding capacity.

. S m (-

) . S m

m m m . T -

DISORDERS RELATED TO HEMOGLOBIN

(IBC) m , S

BIOSYNTHESIS: SIDEROBLASTIC ANEMIA

. I -

-

T sideroblastic anemias -

mm . T

q z

m mm

m . Ex -

m . m -

m m mm -

. I

. T m m

IBC , IDA m . SF

m

. R m -

q . S m

mm k . T m m

m m -

- m

() .

m ACD (.).

C q

I mm AOI x IDA, -

m (.)

m . I , -

. N m m

IDA ACD x .

. I m

. M m

Treatm ent

. M q m

m m m .

D m

I m , m . I m , m m k ,

m ; m

. R m m ,

SF;

H -EPO (P ,), m -

m .

. A AOI

m x m . T

, m

q m , -

k (. . , IL-).

m x-k m

C m m

. O m

H -EPO m m .

x ,

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TABLE

14.7 Classification of Inherited and Acquired Sideroblastic Anemias

Laboratory and Clinical

Classification

Condition

Molecular Basis

Characteristics

Nonsyndromic congenital sideroblastic anemias

X-linked sideroblastic anemia

Germ-line mutation, ALAS2

Mild-moderate hypochromic
gene

anemia, liver parenchymal iron
overload caused by ineffective
erythropoiesis and increased
iron absorption and release of
stored iron

Autosomal recessive

Germ-line mutation,

Defective heme synthesis,
sideroblastic anemia

SLC25A38 gene

severe microcytic anemia

Autosomal recessive

(Rare) Germ-line mutation,

sideroblastic anemia

GLRX5 gene

Inherited syndromic conditions

X-linked sideroblastic anemia

Germ-line mutation, ABCB7

Moderate hypochromic

and spinocerebellar ataxia

gene

anemia

Myopathy, lactic acidosis,

Germ-line mutation, PUS 1

Anemia, lactic acidosis

and sideroblastic anemia

gene

(MLASA1)

Myopathy, lactic acidosis,

Germ-line mutation YARS 2

Anemia

and sideroblastic anemia

gene

(MLASA2)

Lactic acidosis

Thiamine-responsive

Germ-line mutation SLC19A2

Thiamine-responsiveness

megaloblastic anemia

gene

macrocytic anemia

Pearson marrow-pancreas

Mitochondrial DNA deletion

Pancytopenia with sideroblastic

syndrome

anemia

Sideroblastic anemia

Germ-line mutation TRNT1

Sideroblastic anemia associated

associated with B-cel

with B-cel de ciency

de ciency

Myeloid neoplasms with ring sideroblasts

RARS

Somatic mutation SF3B1 gene

Myelodysplastic syndrome with

Refractory anemia with ring

isolated erythroid dysplasia,

sideroblasts

ring sideroblasts

RCMD-RS

Somatic mutation SF3B1 gene

Myelodysplastic syndrome

Refractory cytopenia with

with isolated multilineage

multilineage dysplasia and

dysplasia, ring sideroblasts

ring sideroblasts

RARS-T refractory anemia

Somatic mutation SF3B1 gene

Myelodysplastic syndrome/

with ring sideroblasts with

myeloproliferative syndrome

marked thrombocytosis

with mild anemia, resembling

essential thrombocythemia

Miscellaneous acquired

Ethanol-induced and drug—

sideroblastic anemias

induced sideroblastic anemia

Modified from Cazzola M, Malcovati L. Diagnosis and treatment of sideroblastic anemias: from defective heme synthesis to abnormal RNA splicing. Hematology Am Soc Hematol Educ Program, 2015:18, Table 1, 2015.

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. C m

. T k -

m m

m . T m m , k m .

% %

. I % , m -

Etiolo gy

m . S

S m

(%) x m , SF m m .

. G

A k m x m % .

. D z (INH), m ; m x , , .

NOTE: This is a good time to complete the Review Questions

. D (. . , m , ,

related to the preceding content.

mm)

. M (. . , m , x ,

)

DISORDERS RELA ED O HEMOGLOBIN

. I

BIOSYN HESIS: HEREDI ARY

Phys iolo gy

HEMOCHROMA OSIS

I m , q

I

m . T

m , ,

m -

m , m m

m m

(HH) m m -

(m). A m z m m (

m -

m) GI . T m

m (-ALA) . R -

, , . I m m m x m -

m (m)

x

. A P B -

, m -

. I m

. T C D C P

m. O m m

(CDC) m m m Am

m .

m m .

I m ,

P , m ,

m z m .

, m m

HH.

La bora tory Cha racte ris tics

H mm -

I m

m , x

. S m m

m m . G

, m (-

m j m m . S

m), m . T

m β -m ,

m . T , m -

. H

, m -

HH.

. T m ,

m m / m -

Clas s i ca tion a nd Cha racte ristics

.

T , -

S m -

m

m . T -

m m :

m m , , , m RBC , k . Hemochromatosis ype 1: High Iron Fe (HFE-gene) related.

m . F m % % -

A HH . T

m .

q HFE-m m

M m m

U S x m ,

. I , S ,

m z q x m

% x .

. T m HH, m

I m

E “ z ,”

m m . T m -

E , -

m , m m

C . I m m-

. S m

m m m ,

CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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k HLA-A m m

T HH m

. E m

SF /mL.

m z

SF

k m .

,

T HLA-A m

. A SF

HFE. mm m HFE , C282Y

, x , H63D, HH. I m , x k . SF -

.

(-) -

T m m HH.

.

G

M m q .

. T m S SF

A HFE m -

.

m m

A m m

k

m m ,

m - , m m z k

m ,

IDA.

,

. Hemochromatosis ype 2: Juvenile hemochromatosis.

. F m , z

x .

, m

m HJV ,

. T , m

m m q . m -

m m

z m m -

S . I -

, m m q .

%

H m j x , k

,

m , . M m m j

HH %. A

m .

m .

HAMP . M

m j j m -

Tre a tm e nt

m z

m m

. N

. R m (mL)

m k j m m .

k

. Hemochromatosis ype 3: Mutation o rans errin receptor

m - m m HH -

2 (R2) gene. T m

m m m .

m . T m

E x m m . I m FR ,

- m ,

, m m m q . I

m .

.

. Hemochromatosis ype 4: Mutations o Ferroportin-1

DISORDERS RELATED TO HEMOGLOBIN

gene. I ,

BIOSYNTHESIS: HEME (PORPHYRIN)

SLC40A1 .

SYNTHESIS

Am m

m . T m

D (F . .) m m m m m q . I

m

m , -

m MPS. T m

.

kk HLA .

A q ,

m . I ,

Pathophysiology and Laboratory Characteristics

zm , m , m -

HH m x

, ALA PBG

. T m -

m . N m

m . A

m , q ALA

m m -

m x m m

x m

.

, , x

P m m m m m m.

m m

A -

x m x

m HH m / L.

/ .

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Glycine

δ -ALA

Porphobilnoge n (P BG)

S yntha s e

Succinyl CoA

δ -ALA

(4-PBG)

Hydroxymethylbilan

Heme

Hemoproteins

Fe²⁺

Protoporphyrin IX

Uroporphyrinogen III

Uroporphyrinogen I

Uroporphyrin

(excretion)

Protoporphyrinogen IX

Coproporphyrinogen III

Coproporphyrinogen I

Coproporphyrin

(excretion)

FIGURE 14.8 The heme synthesis pathway.

(Rosenfeld MW, et al. Greenfield's Surgery: Scientific Principles and Practice, 6th ed, P, PA: Lippincott & Wilkins, 2004.) P: Smith, J.

m, x -

■ C (.)

. H ,

■ S z m

k m m

■ S z m m

. W , -

m m .

C , -

D m -

m k m . S m

m (C).

m m . D m

:

m

NOTE: This is a good time to complete the nal end of

m .

chapter Review Questions.

P m G k , porphyra,

m . T - m ()

. PBG m x m m

CHAP ER HIGHLIGH S

; , m

m , m

Iro n De cie ncy Ane mia

E ' . I

■ A ' m

PBG, m m

m

m .

. I m (IDA)

W m , m

m mm m .

m , m x

■ IDA m m ,

. T -

m , m

m , x .

, P B (F . .). T

■ I m m mm -

sideroblasts.

Z ,

m ; m ;

DISORDERS RELATED TO HEMOGLOBIN

m / m-

BIOSYNTHESIS: DISORDERS OF

m ; k m;

GLOBULIN SYNTHESIS

m ,

.

G -

■ N m m , m

. W m ,

m / . I m x

CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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k , ,

Disorders Related to Hemoglobin

x .

Bio synthesis : Hemochromatosis

■ m , m -

mm / . H ,

■ I

, ,

m , ,

.

m , mm

mm -

■ Mm

m .

(m)

GI .

■ Imm, q

mfxmm

■ Ix -

.

m

x , m

■ T . A x m

% m m

.

m . T %

■ H mm -

m m , m

m , x

m m m m .

m m .

T , - -

■ A m m x

■

m m .

m

m m : m m

■ S m (SF)

. H , SF, -

: HFE- — , m m : j

, q

m m , m m : m

.

(R), m m

: m - .

■ A R. F ; R .

■ A m m

m m ,

m ,

Anem ia o f In amm atio n o r Anem ia of

,

Chro nic Dis o rde rs / Dis e as es

.

■ A m .

■ A m mm (AOI) (AOI/

ACD) - - m mm -

, , m , m

Dis o rders Related to Hem og lobin

.

Bio synthe s is : He m e (Porphyrin) Synthe s is

■ AOI/ACD .

■ D m m -

AOI/ACD

m q .

m m m

■ A q ,

mm .

m .

■ A RBC AOI/ACD,

■ P m m m m j m m . T

m m

m .

x m x -

■ I m , m

/ .

P B .

■ P , z m , z m **Dis o rders Related to Hem og lobin**

m .

Bio s ynthe sis : Sidero blastic Ane mia

■ W m , m

m , m x

■ S m m

. T -

m (-

,

). .

P B . T .

■ C m , q , m m , -

Dis o rders Related to Hem og lobin

, x .

Bio synthe s is : Dis o rde rs of Glo bulin

■ D m

Synthe s is

m , m mm ;

■ G

m SF;

.

P m

■ W , m

.

m .

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CASE STUDIES

Cas e S tudy 14.1

Cas e S tudy 14.3

T -m - - - m

A - - m m -

- . T -

. T

' f

x m m j m ,

. A m (CBC) .

. CBC

m .

■ Laboratory Data

T m :

■ Laboratory Data

H m . / L

H m . / L

H m %

H m %

$$S m \mu / L N = \mu / L$$

$$RBC . \times /L$$

$$S m m$$

$$(WBC) \times /L$$

$$S m . \mu /L (x m)$$

$$T k m -$$

$$S m S \%$$

$$k ; ,$$

$$F - : A x m$$

$$m m .$$

$$m . m m -$$

■ Critical Thinking Group Discussion Questions

$$. W q m$$

$$. L m -$$

$$' ?$$

$$m P B .$$

■ Critical Thinking Group Discussion Questions

$$. W MCV, MCH, MCHC ,$$

$$. W ' ?$$

?

. W m mm m ?

. W m x

m , -

. W -

?

?

Cas e S tudy 14.2

Cas e S tudy 14.4

A -m - P R

A -m - C Am

N Y k m

.

N Y k C . H m

T m

k . S . H

. T : m

, , , m -

, m m . H

, m IBC, x m

m -

, , . T :

. A CBC .

■ Laboratory Data

■ Laboratory Data

H m . / L

T m :

H %

H m . /L

RBC . \times /L

H m %

WBC . \times /L

RBC . \times /L

T RBC :

WBC . \times /L

MCV . L

T m , k—

MCH

m RBC .

MCHC / L

■ Critical Thinking Group Discussion Questions

T m -

. W ?

, m , m , k . A

m . A

. A m ?

:

P × /L

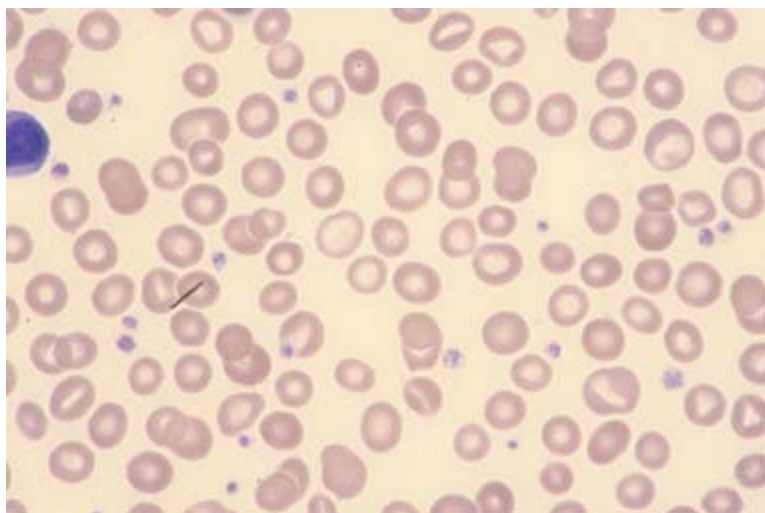
. W k- ?

R . %

m . m / L

. W ?

S m μ / L



CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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CA

CA S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

nttiin

n u

u e

e d

d))

IBC μ / L

% S % (, % %)

P . %

F μ /L (, μ /L)

S m /mL (, /mL)

A x m , ,

RBC /mL (, /mL)

.

V m B /mL (, /mL)

■ Follow-up

T m

m B , m / . T

-m - :

H m / L

MCV L

IBC μ / L

RDW

F μ /L

I μ / L

R m A SC. Anderson's Atlas o Hematology,

P, PA: W K H /L W m & W k ,

% S %

C , , m .

B m m

■ Critical Thinking Group Discussion Questions

■ Critical Thinking Group Discussion Questions

. W m m -

. I ?

RBC m ?

. W RDW ?

. W

?

. W m B ?

. W m ' m ?

. W m ?

Cas e Study 14.5

Cas e Study 14.6

A - - m k. T

A - - m I m

m m k . A m -

m , m , . S

m m m . T

m . S

m -

m .

. T x m

P x m m m . S m

m q m . S k -

, . H m .

- m ' -

A CBC, , ,

. T m ; k

.

- mm ,

- m x - . T

■ Laboratory Data

m q CBC

H m . / L

, , IBC, % S , . N m -

RBC . × /L

GI . B m GI

H m . %

. P

MCV L

mm , H. pylori m .

MCH .

MCHC / L

RDW %

■ Laboratory Data

H m L (, L)

WBC . \times /L

H m % (, % %)

H m m -

MCV L(, L)

m , , k , m RDW . % (, . % . %) () . H m

I μ / L (, L)

. H m , x

IBC μ L (, μ L)

. T .

(continued)

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PART 4 ■ Erythrocyte Disorders

CA

CA S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

nttiin

n u

u e

e d

d))

A - m - m

. W m m-x m . S q x m m ?

m m

m m , ,

. W

.

m mm (AOI)?.

■ Critical Thinking Group Discussion Questions

NOTE: This is a good time to write out the answers to the

. D AOI?

Critical Review Group Discussion Questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

7. P

A.

Iron Def ciency and Iron Overload

B. GI

1. M m

C.

A. m

D. m

B.

C. m m m

8. I z

D. m

A.

B.

Iron Def ciency and Iron Def ciency Anemia

C.

D. m

2. T IDA

A.

9. T _____ .

B.

A. . .

C. x

B. . .

D.

C. . .

D. . .

3. I mm

A.

10. . A x m () _____% m

B.

m () _____ .

C. m

A.

D.

B.

C.

4. D k m

D.

A.

B.

11.

C.

A. m

D. m –

B. H m

5. F m

12. T m m , A.

m , :

B.

A. N M

C.

B. R m k

D. m

C. P B

D. W -G m

6. P

A.

13. M

B.

A. m m

C.

B. m j j m

D. m

C. m m

D. j j m m

CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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REVIEW QUESTIONS (continued)

14.

20. T m ,

A. m

m , x m k

B. m

m . P

C. m

B m m m , D. B C

. I k

m

15. I IDA,

A. m m

A. MCV , MCH , MCHC

B. m

C.

B. MCV , MCH , MCHC

D.

C. MCV , MCH , MCHC

21. A m mm /

D. MCV , MCH , MCHC m

A. mm

16. T m m _____

B.

IDA.

C. m

A. m , m

D.

B. m , m

C. m

22. T m AOI D.

_____ .

A. m , m

17. I ' m m -

B. m , m

, m m ,

C. m , m m

m :

D. m

Serum

Serum

Serum

23. L k _____ -

Iron

IBC

Ferritin

% Saturation

m .

A

D

I

I

D

A. mm k

B

D

D

D

D

B. mm

C

D

I

D

D

C. mm m

D

D

D

I

I

D. A B

18. I m m -

24. W m m m

, X

mm (AOI)?

:

A. R

Bone

% Sidero

B. I

Marrow blast in

C. E j

Serum

Serum

Serum

%

Iron

Bone

D. m mm

Iron

IBC

Ferritin

Saturation Storage

Marrow

A D I I D I D

Disorders Related to Hemoglobin Biosynthesis:

B D D D D D D D

Sideroblastic Anemia

C D I D D D D D

25. S m

D D D I I

I I

A. (m m)

B. (. . , m)

Anemia o In lammation or Anemia o Chronic

C. m

Disorders/Diseases AOI or ACD

D.

19. I ACD, m , -

, IBC m

26. A mm m

A. m , IBC

A.

B. m

B. m , IBC m /

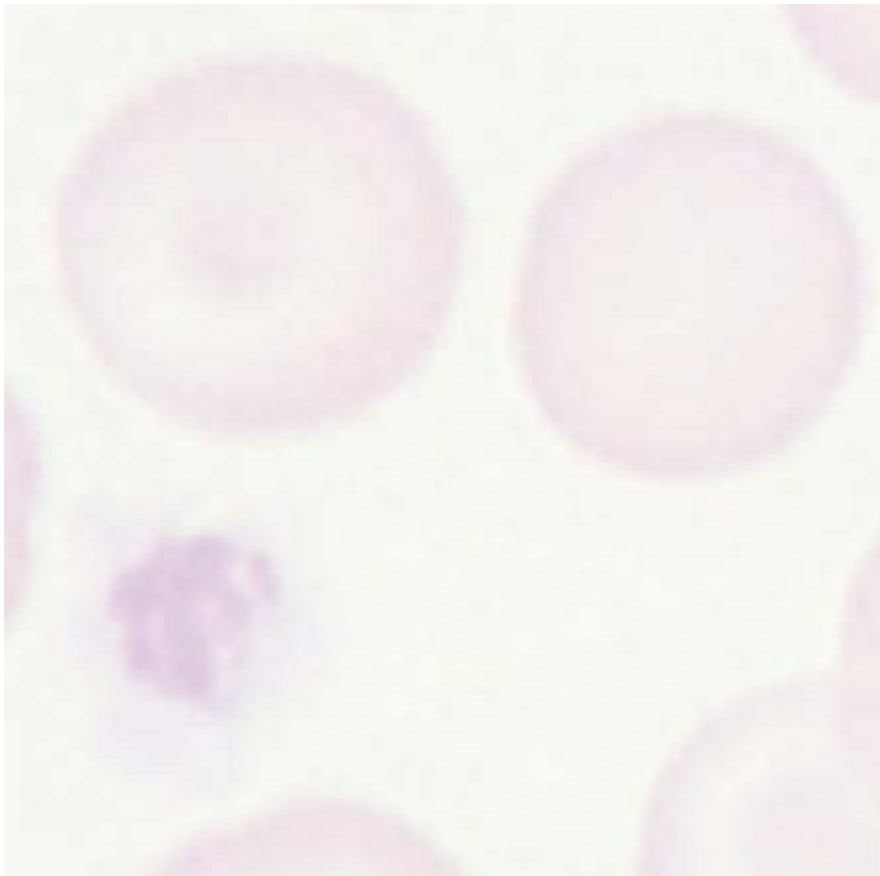
C. m

D. m

C. m m , IBC m

D. m , IBC m

(continued)



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PART 4 ■ Erythrocyte Disorders

REVIEW QUESTIONS (continued)

27. T -

*34 T

m m ?

m m k

A. H m m

() _____ q () _____.

B. F

A.

C. C m

B.

D. m

C. m

D. m

28. S m

A.

35.

B. m

A.

C. q

B. m B

D.

C. m B

Disorders Related to Hemoglobin Biosynthesis:

D. m D

Hemochromatosis

*36. T m , m -

*29. T m

, k

m m (HH)

A. '

A. m

B. ' m

B. m –

C.

C.

D. m

D.

30. P B m

*37. I . , k

A.

m

B. H z

W -G m x m -

C.

D.

A. m m m

B. m

31. T m ,

C.

m , x m k

D.

. N

. Ex m m

38. I C . , ' m

()

, km m . P B

m m

. I k

A. m

m

B. m

A. m m

C.

B. m

D. k

C.

D.

Disorders Related to Hemoglobin Biosynthesis: Globulin Synthesis

Disorders Related to Hemoglobin Biosynthesis:

Porphyria

*39. I f , m _____ .

*32. A q m

A.

A.

B.

B.

C. m

C. m m

D.

D. m

*33. W m , _____

m .

A. m

B.

C. m

D.

CHAPTER 14 ■ Disorders of Iron Metabolism and Heme Synthesis

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C zz M, M L. D m -

COMPANION RES OURCES

m : m m m RNA .

Hematology Am Soc Hematol Educ Program, : – , .

:// . . m/

C BF, . Im m z m

, Blood, (): – , .

E -

D L G. M A m , N Engl J Med (): – , m .

.

H ,

Ek z C, . T m

-

mm m, Hematol J, : – , .

.

F k PG. U m , Hematology Am Soc Hematol Educ Program, : – , .

G z . H , , Blood, ():

– , .

BIBLIOGRAPHY

G z , N m E. H m . Biochim Biophys Acta, : – , .

A m JW. T m mm /m : m m H M, . D m -

m m , Hematology Am Soc o Hematol Educ Program,

m

– , .

m : R m z - , - -

A NC. F : , Blood,

, Hematol J, (S): , .

(): - , .

H k C, . M m , A m AE, . H mm -

Haematologica, (): - , .

m , Blood, (): - , .

J O. R m .

B m R. O - m x m m , Clin Lab Sci, (): - , .

mm , Int J Lab Hematol, : - , .

B m G, . A m -

L RJ. A m , Clin Lab Sci, (): -

, Haematologica,

, .

(): - , .

M BI, . T m m -

B E, W J. T m :

, Br J Haematol, : - , .

m m m ? Blood, P KV, G k JM. P m m (): - , .

, Haematologica, (): - , .

B E, W C. H m A -

P KV. V m : Am : () -

m m ,

m m MCV, Blood, (): – , Haematologica, (): – , .

.

P EA, S SL. Anemia in the Older Adult. .U D . m.

B E, S k S. m R A , .

, T e Hematol ASH News Rep, (): , .

ML. Anemia: is it Iron De ciency? P MEDLAB

C m C. I : -

A H C , D , UAE, J , .

m , Hematology Am Soc Hematol Educ Program, : – ,

W G, G L . A m , N Engl J Med,

.

(): – , .

C m C. I - m , N Engl J Med, (): –

W G. P , m m

, .

, Eur Hemtol Assoc, (): – , .

Principles o Blood Collection and

CHAPTER

Macrocytic and Megaloblastic Anemias

15 Processing

KEY TERMS

folic acid deficiency

macrocytosis

vitamin B12 deficiency

ineffective erythropoiesis

megaloblastic anemia

intramedullary hemolysis

pernicious anemia

LEARNING OUTCOMES

Macrocytic anemias

- Describe the clinical signs and symptoms of pernicious anemia.
- Name, at least three, examples of conditions associated with non—
Summarize the laboratory assays used to confirm cobalamin
megaloblastic macrocytosis.
deficiency and state the results associated with pernicious anemia.
- Describe how DNA synthesis differs in nonmegaloblastic macrocy-

- Explain the usual management of and therapy for pernicious anemia.
- tosis from megaloblastosis.

Folic acid deficiency anemia

Megaloblastic anemias

- List three etiologic causes of folate or folic acid deficiency.

- Define the term megaloblastic anemia.

- Briefly describe the epidemiology of folate or folic acid deficiency.

Differentiate the pathophysiology and the appearance of red blood

- Explain the physiology of folic acid deficiency.

cells on peripheral blood smears from patient's with nonmegaloblastic

- Describe the body's requirements for folate and the physiologic role of folate.
- tic anemia versus megaloblastic anemia.

of folate.

- Name the two common causes and other less common causes of

Summarize the process of folic acid metabolism and explain how a megaloblastic anemia.

deficiency can result in megaloblastosis.

- Explain the cellular maturation abnormalities in the bone marrow in

- Describe the clinical signs and symptoms of folic acid deficiency.

megaloblastic anemias.

■ Name the laboratory assays used to confirm folic acid deficiency and

Assess an algorithm of clinical chemistry laboratory tests used to

state the results associated with folic acid deficiency.

distinguish megaloblastic anemias.

Case study

Pernicious anemia

Analyze the patient history, clinical signs and symptoms, and labo-

■ Describe the body's requirement for vitamin B

ratory data for the stated case studies, answer the related critical

12 and the physiologic

role of vitamin B

thinking questions, and conclude the most likely diagnosis.

12.

■ List four etiologic causes of vitamin B12 deficiency and describe two

distinguishing clinical or laboratory characteristics for each.

NOTE:

■ Briefly describe the epidemiology of pernicious anemia.

■ indicates MLT and MLS core content

■ Explain the etiology and pathophysiology, including the immune

indicates MLT (optional) and MLS advanced content

nature, of pernicious anemia.

MACROCYTIC ANEMIAS

V m m

B x . . I m , -

Macrocytosis, m

m mm m . M

m (MCV), m m

m pernicious anemia

megaloblastic anemia xm m m

m . F x m , MCV

.

m m m mm

N m m m k mm

,

. m x m RBC

m .

m DNA

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CHAPTER 15 ■ Macrocytic and Megaloblastic Anemias

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Drugs That Cause

BOX 15.1

TABLE

15.1 Megaloblastic Anemia

Mechanism

**Examples of Nonmegaloblastic Macrocytic
of Action**

Type of Medication Examples

Anemias

Modulates

Immunomodulator

Azathioprine

Am

purine

Mm

Antineoplastic agent

Thioguanine

metabolism

Am

Immunomodulator and Methotrexate

H m m

antineoplastic agent

P m m

Interferes with Antineoplastic agent

Hydroxyurea

O F m A m

pyrimidine

Anesthetic agent

Nitrous oxide

M m

synthesis

A m

Decreased

Organic compound

Isoniazid

A q m

absorption of

H m (-

Alkaloid extract

Colchicine

vitamin B

m I III)

12

D m -B k m

Increased

Inorganic compound

Sodium

O C

excretion of

nitroprusside

H m

vitamin B12

R

Destruction of Gas

Nitric oxide

S m (m ,

vitamin B12

mm)

Decreased

Hormone

Estrogen

M m G JP, . (.), Wintrobe's Clinical Hematology, absorption

Antibiotics

Ampicil in and

, P , PA: L W m & W k , :V . , of folic acid

other penicil ins

, . .

Erythromycin

Tetracycline

Antiseizure

Phenobarbital

m . MCV m

L.

Antimalarial

Quinine

Chloroquine

MEGALOBlastic ANEMIAS

Folate

Antineoplastic agent

Pemetrexed

analogue

Antimalarial

Pyrimethamine

M m z

activity

m , m m -

Antibacterial

Trimethoprim

,

Immunomodulator and Methotrexate

m . L k m—

antineoplastic

m . A m

Reference adapted from Hesdorffer CS, Longo DL. Drug-induced megal-

, ineffective erythropoiesis

blastic anemia, N Engl J Med, 373(17):1650–1651, Table 1, 2015.

m m

m .

T m **megaloblastic** m m

I z m m -

m . D - m m

m DNA . M m

, m -

k m . M m

, m m m

m z

m m (.).

intramedullary hemolysis.

D m m m -

M , m m

m B . S

q m , C (m B),

m DNA

m m

. T m - k

() m . -

m , DNA , - m

m k m

DNA. T m

. m m

- m m . O -

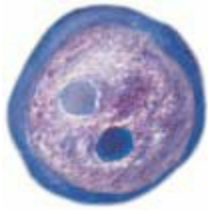
vitamin B12 olic acid de iciency,

x .

R m m -

.

m m m



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PART 4 ■ Erythrocyte Disorders

BOX 15.2

P rome ga lobla s t (Me ga lobla s tic Rubribla s t)

Exam ple s of Co nditions Co ntributing to

Cobalam in (Vitamin B12) De ciency

VITAMIN B12 DEFICIENCY

Ba s ophilic Me ga lobla s t (Me ga lobla s tic Prorubricyte)

D ()

B m m B

L k

P m

G

P olychroma tophilic Me ga lobla s t (Me ga lobla s tic Rubricyte)

I m

F m

MEDICATIONS (INHIBITORS)

H

Orthochromic Me ga lobla st (Me ga lobla stic Me ta rubricyte)

P m

MALABS ORPTION

A

G

P olychroma tophilic Me ga locyte (Me ga lobla s tic Re ticulocyte)

D m

P m

R

F m m B

Me ga locyte (Ova l Ma crocyte)

m (Im -

G ä k m)

FIGURE 15.1 M m . (R

D - m B m

m A S, P K. Anderson's Atlas o Hematology,

INTESTINAL

P , PA: L W m & W k , , O m , x m , m .)

m

S

m m (F . .). T

Diphyllbothrium latum

m B m DNA ,

m .

IMPAIRED UTILIZATION

T m . T

m II m

, m RNA,

INCREASED DEMAND

m .

P

I :

Etiology

M m

(B x .). M m m B

PERNICIOUS ANEMIA

m

m (). O

Epidemiology

m B

V m B k

. I z m B -

(IF) mm m

Diphyllobothrium latum

E . O m k m m

B m - -

. P

IF . T m

m -

m . I

. M m , k IF m m m , m m

A Am L ,

m .

. I mm m m

A

. N m

, -

m B

m m .

CHAPTER 15 ■ Macrocytic and Megaloblastic Anemias

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A m

Vitam in B12 (Cobalamin)

m

C m z

mm .

m . M m m

A x m % -

B . T m B

m m . T ,

m . T m

- m ,

m W (μ /) m

q m j . I f m m q m . T

m mm ,

m m . I k

m

m m j m mm .

x (MHC).

P m m mm

Vitam in B12 (Cobalamin) Transport

mm .

T mm

C m m -

(H m '), - m -

m

, A ' , m , m q : m, G ' , m

. I (IF)

.

. m II (C II)

. R

Phys io lo gy

IF, , z

N m m m m -

m

, m B z m

m m mm , m .

(m) . M

I , m IF m

.

x q q m V m B m z m m . A H, m

m , k m , k m m IF m z m m

m -IF m x (F . .). B IF x -

m m . I

m m .

m m . T m x m

A ' , μ

m. I m,

. m B

m -IF m x -

x m μ / . A q μ

m m m

m B ,

.

. A -

T m x m m

m μ .

q C II. C II

Die ta ry intake

Fundus re gion

FIGURE 15.2 V m B .

of vita min B12

(secretion of intrinsic factor [IF])

and folates

B12 and

folate

Stomach

(B

Liver

12)

Duodenum

(vitamin B12 + IF)

Transport

Transcobalamin

Transcobalamin II + B12

Transcobalamin II

Jejunum

(B12 + IF)

B12

Ileum

IF

Reticulocytes

PART 4 ■ Erythrocyte Disorders

m z

. M m x

. L k IF, C II, -

-IF / . T m

m ,

m

m . R C

mm .

II m m m

. C II m

Sig ns and Sym ptom s

, m m k .

T R m -

A q m m -

m - . T R

, m m m

m m . T

m . B

k ,

m m m, m m

m m x m

m .

m

I m m , -

. R

,

k . T

, mm m

m C I C III , m k,

m m -

. C I k

m . T

m m . E m

m m

z m

m .

, ·

P m k m -

. T , k , -

m m m m

Pathophys io lo gy

. A (k

P m

m), , . G

.

q .

E . P m m-

, x , , .

Vitam in B12 (Cobalamin)

C , , m I

. N m m

m (B x .), C II

m . P , ,

m .

, , m m , m , -

I m ,

. T

IF . I m j

m m m

m , -IF -

m . I , ,

(m

, m .

m) . M

V m B

m

m m . I , m m

m mm . T

. B -

IF-k -

x - m -

m .

m .

Gas tric Patho log ical Finding s

Laboratory Findings

T m :

T m m

m B , -

- z m ,

m m .

m, - .

H m x m A mm , m , m . H , m m (k m . T

m) m -

mm -

z . T

.

z MCV,

A α β

m L. T m

H⁺/K⁺-A P , z

m (MCH) %

.

. C MCV,

A mm A x -

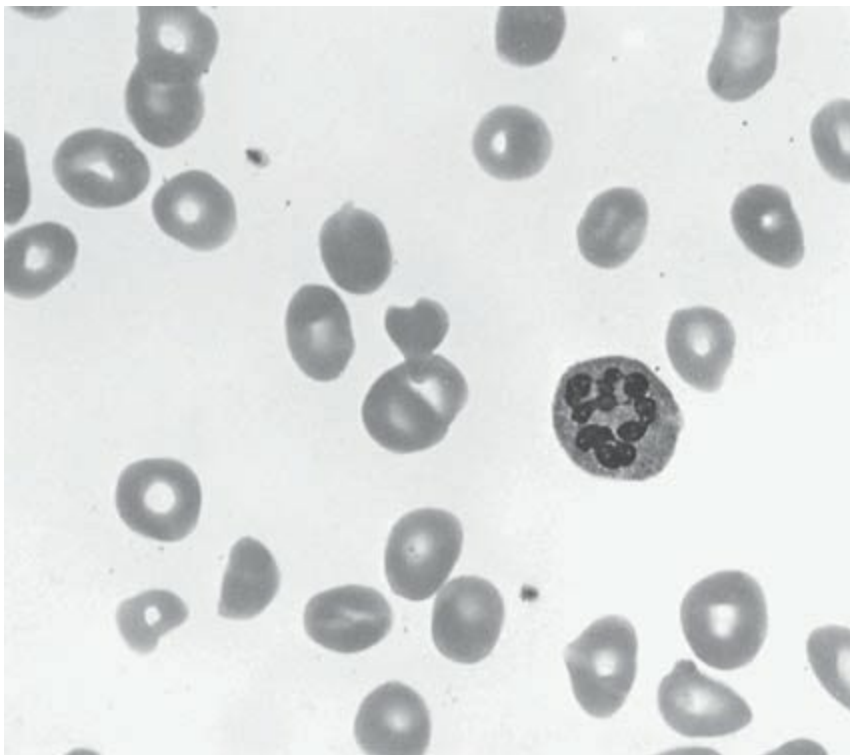
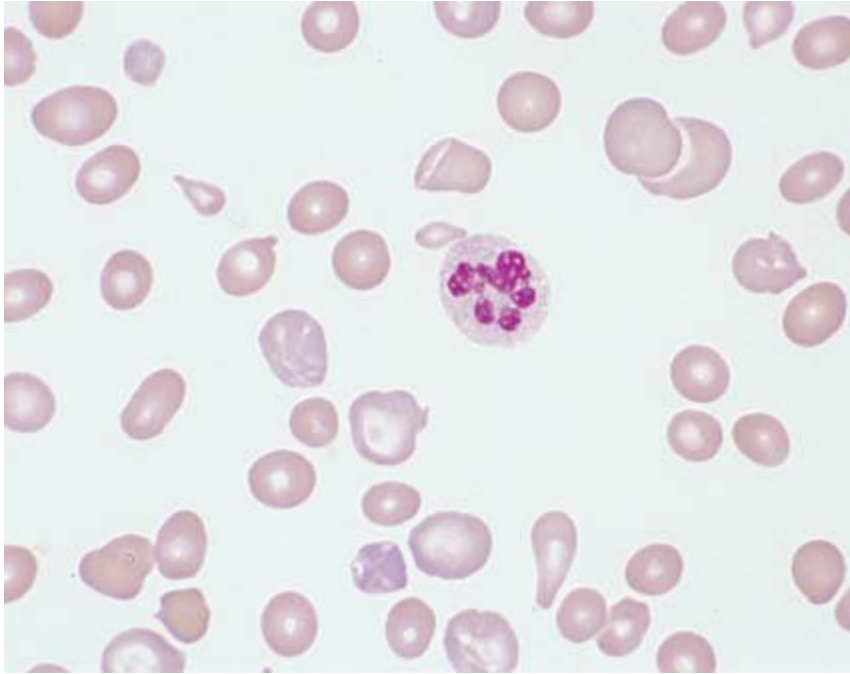
m , m MCV

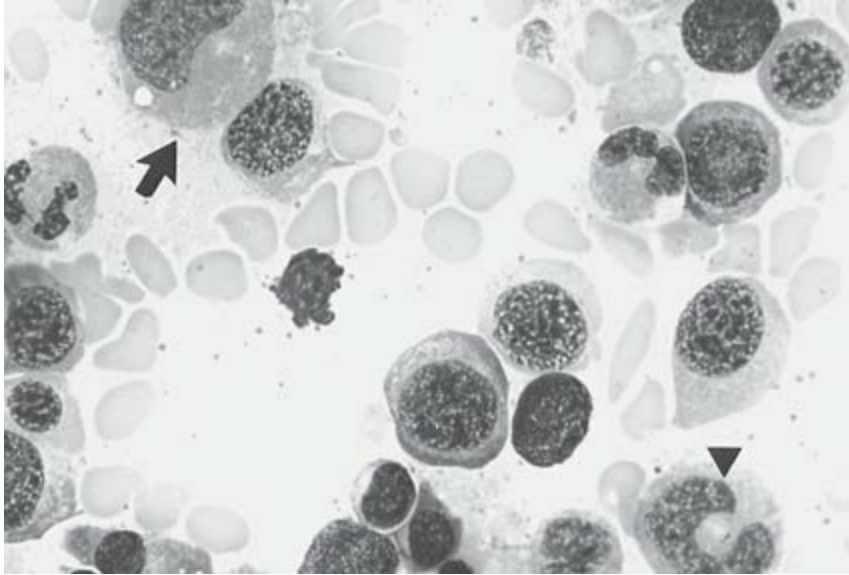
IF, ,

m . T m m

m , m

(MCHC) m . I m ,





CHAPTER 15 ■ Macrocytic and Megaloblastic Anemias

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FIGURE 15.5 B m m m -

m m m

FIGURE 15.3 P m : m

m . T k

m m .

m . N -

: m m (arrow)

m . T

(arrowhead). (R m M C KD. Clinical Laboratory

m k ,

Medicine, , P , PA: L W m & W k ,

, m .)

.

Ex m m m -

k m

m , x -

m , (F . .). E -

m m m -

, m , m . R

. A m m

, H -J -

. T -

, C m . A m -

.

k m m (m) T m (F . .) (F . .)

m

(). P

, m m -

m .

m . E

T % -

- m . N - m ,

m . S q m B m , m

mm m, . A -

IF, -

m RNA -

% .

, m

P m . I

. M m m m

m m %, m

m k

m m . M k

m m

m

C , m -

k m m m .

G m - m m . C , m -

m , m m .

T m m m -

(M:E) m : . I

, .

C m mm -

m (.

. , M A m A m). U ,

m m ' m m -

m k .

A m m m

FIGURE 15.4 M m z -

m m B ,, m , m m -

m - m z m m . (R m M C KD.

m , m , IF-k (IF

Clinical Laboratory Medicine, , P , PA: L

), (I G), .

W m & W k , , m .)

A LDH .

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PART 4 ■ Erythrocyte Disorders

TABLE

Me galo blas tic Ane m ia Te s ting*

15.2

Basic Testing

Level

Follow-Up Testing

Level

Interpretation

Vitamin B12

>400 pg/mL

Not PA

>400 pg/mL if neurologic Serum methylmalonate—

May confirm B12

symptoms

nic acid (MMA) and

deficiency

homocysteine

100–400 pg/mL

Serum methylmalonic <0.4 $\mu\text{mol/L}$

Not PA

acid (MMA)

$\geq 0.4 \mu\text{mol/L}$

PA

Optional testing IF-blocking
antibody, parietal cell antibody,
IgG, and gastrin levels
<100 pg/mL

Optional testing IF-blocking

PA

antibody, parietal cell antibody,
IgG, and gastrin levels

Folate

Low

Folate deficiency

Low or normal

RBC folate

*Presence of macrocytic red blood cells.

PA, pernicious anemia.

T mm S

mm m . A

mm B -

mm m . T LDH

IF

m -

. A -IF m

m m .

IF. A x m %

I m , m m -

m . M -

m . C

m (%) . I

m (m B) -

, m m -

m .

. L -

m -

Treatm ent and Mo nito ring Therapy

m m .

A , (HC) T m m B

m , m m .

m m j μ m A HC

B m . T m

TABLE

15.3 Vitamin B12 Assays Reflect Conditions

Normal Reference

Deficiency

Assay

Range

Negative Balance

Depleted Stores

in Tissues

Anemia

Serum cobalamin (pg/mL)

200–900

150–500

100–300

50–250

50–250

Serum MMA ($\mu\text{mol/L}$)

<0.4

<0.4

<0.4

0.4–20

1–20

Hypersegmentation of

None

None

None

1+

2+

neutrophils

MCV (fL)

80–95

80–95

80–95

90–110

100–130

Hemoglobin (g/dL)

12–15

12–15

12–15

12–15

<12

MCV, mean cell volume; MMA, methylmalonic acid; RBC, red blood cell

CHAPTER 15 ■ Macrocytic and Megaloblastic Anemias

287

m m m

Folate s

m m m . A

F , m , k μ m m B

m k . A m m

m B

m - -

. T mm -

x m % m B

, , ,

m IF.

. T m .

A m m S m m m-

(vitamin B

x . A

12)

m , m m -

q m .

. T

I , m mm m k ; m . F

k m m . T m

, k m

x m k m z

,

k . T MCV

.

, m ,

. T m

Ep ide m iolo gy

x . R -

M k

m

.

. M m m m x m

F (B x .)

m m m .

S m -

q , m , m -

m , m

m . C k m

x k .

k

m . I , -

, m m , -

NOTE: This is a good time to review the definitions of Key

z ; Terms in the Glossary and ash cards on . It is a

m , k ; x m ; good time to complete Review Questions related to preced-m .

ing content.

Laboratory Findings

FOLIC ACID DEFICIENCY ANEMIA

F m m

m m

D mm m , ,

mm (.).

k m

-

. Folic acid deficiency anemia

NOTE: This is a good time to complete the end of chapter

.

review questions.

Etiology

M m -

:

BOX 15.3

. D -

. T , z

Potential Causes of Folate Deficiency

, , . A

m m -

I q k

m - -

I m , x m , ,

m , M , x m , , m , k .

m

B m , x m , -

A m :

. I z m M () , x m , , k m m , m x

. m m

A m

288

PART 4 ■ Erythrocyte Disorders

TABLE

15.4 Fo lic Acid As s ays Re ect Conditions

Norm al Re fe re nce

De cie ncy

As s ay

Range

Ne gative Balance

De ple te d Store s

in Tis s ue s

Ane m ia

Serum folic acid (ng/mL)

5–20

<3

<3

<3

>3

RBC folic acid (ng/mL)

>200

>200

<200

<200

<200

Serum homocysteine (μmol/L)

5–15

5–15

5–15

15–250

15–250

Hypersegmentation of

None

None

None

1+

2+

neutrophils

MCV (fL)

80–95

80–95

80–95

90–110

100–130

Hemoglobin (g/dL)

12–15

12–15

12–15

12–15

<12

MCV, mean cell volume; MMA, methylmalonic acid; RBC, red blood cel

CHAP ER HIGHLIGHT S

■ N m m m B

z m . M

Macro cytic Ane mias

.

■ P m q m

■ M , m m

.

(MCV), m m

■ T m x m

m m x

m m (k m) m

m .

■ M m

z .

m m m

■ C MCV, -

m .

m , m MCV m .

■ N m m m k mm

■ P m .

.

■ T m -

■ T MCV m L.

k , .

■ R , H -

Megalo blas tic Ane mias

J , C m .

■ A m k m m

■ T m m m m

(m)

DNA .

().

■ T % -

■ R m m m m m

m . S q m B m ,

m m .

m

IF, %

■ M m m j

, m

.

m .

■ C m mm -

m .

■ T m m

m B

■ IF x m %

. H ,

m

m . M

m .

m (%) . I

, m

Pe rnicio us Ane mia

m .

■ P m mm -

IF .

Folic Acid De ciency Anem ia

■ A m -

■ F

m ,

m

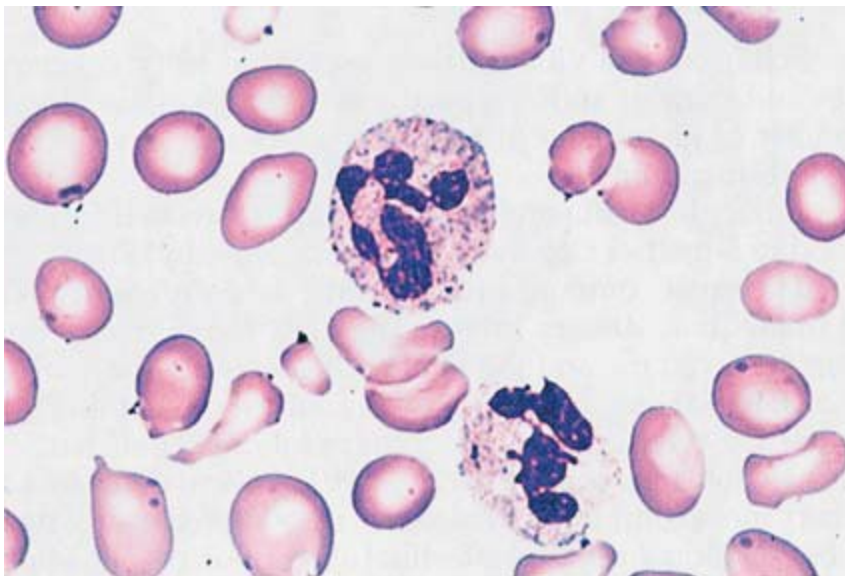
z , m

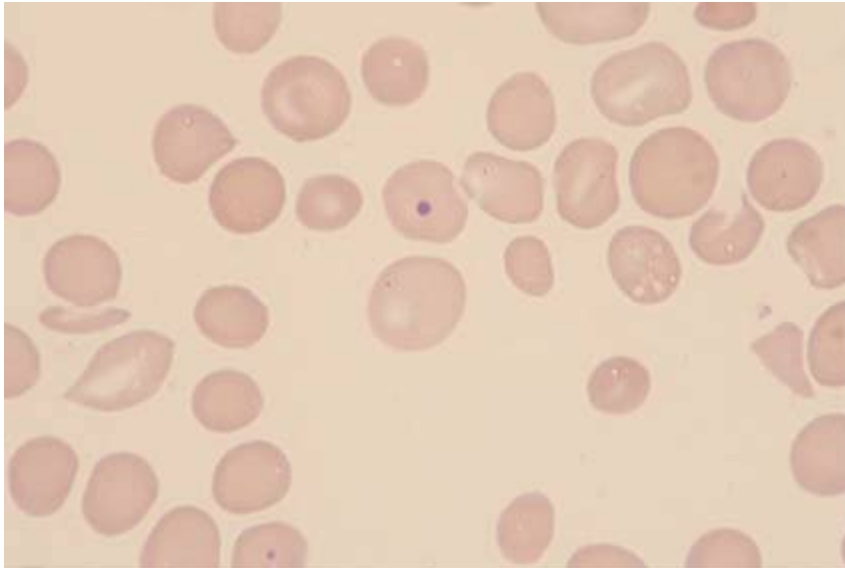
mm

k m m m

.

.





CHAPTER 15 ■ Macrocytic and Megaloblastic Anemias

289

■ F , m ,

■ F ,

m k .

k m ,

■ T m . S m

.

m m

■ F m m -

x . A q

m

m .

m mm .

■ I , m m-m m .

CASE STUDY

Cas e Study 15.1

. W

m ?

A - - m

m .

S x m m

. T CBC.

■ Laboratory Data

T :

H m . / L

H %

RBC . \times /L

WBC . \times /L

H RBC :

MCV . L

MCH .

MCHC / L

(R m R E, F JL. Pathology, , P , PA: L –R , : , m .) T m m m

k . O

, : m B , , m IBC, m , m LDH. A x m -

. T

:

V m B : m /L ()

S m : m

R : . %

S m IBC: m

S m : . m / L ()

S m LDH: , (-

)

T .

(R m A SC. Anderson's Atlas o Hematology, P , PA: W K H /L W m & ■
Critical Thinking Group Discussion Questions

W k , m , C .)

. W m m -

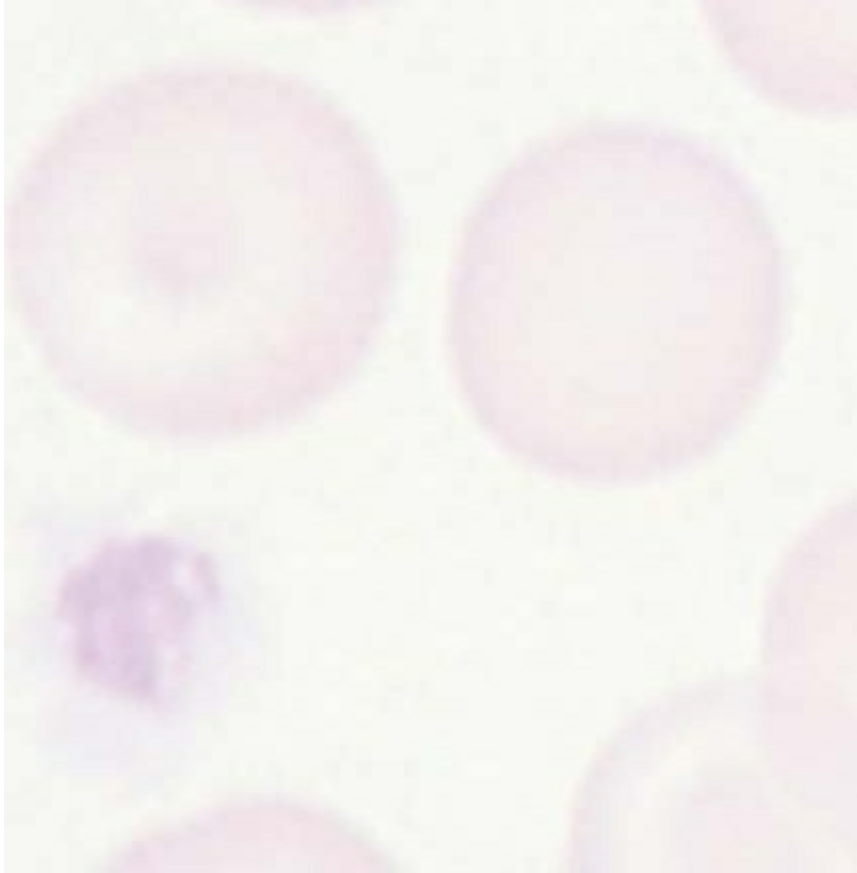
?

. W k m

Note: This is a good time to write out the answers to the

?

Critical Thinking Group Discussion Questions.



PART 4 ■ Erythrocyte Disorders

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

*8. T A

m mm

Macrocytic Anemias

A. IF

1. T m mm m m -

B. m

m

C.

A. m m

D. A C

B.

C. m

*9. A k

D. m

A. m

B. m

*2. W m m -

C.

m m ?

D.

A. O m

B. R m

10. C m m

C. H -J

A. IF

D. H m

B. C II

C. R

Megaloblastic Anemias

D.

3. M m

A. m

11. I m ,

B.

C.

A. k

D.

B. m

C. m

4. M m

D.

A. m

12. T

B. z

m

C.

A. %

D.

B. . %

C. < . %

5. I m m ,

D. x m . %

A. MCV , MCH , MCHC m

13. I m , m –

B. MCV , MCH , MCHC m

C. MCV , MCH , MCHC m

A.

D. MCV m , MCH , MCHC m

B. m

C.

*6. W

D.

m m ?

A. O m

14. I m , m B

B. H m m m

A.

C. H -J P m

B. m

D. L m

C.

D.

Pernicious Anemia

*7. W m

15. I m ,

m ?

A.

A. RBC m

B. m

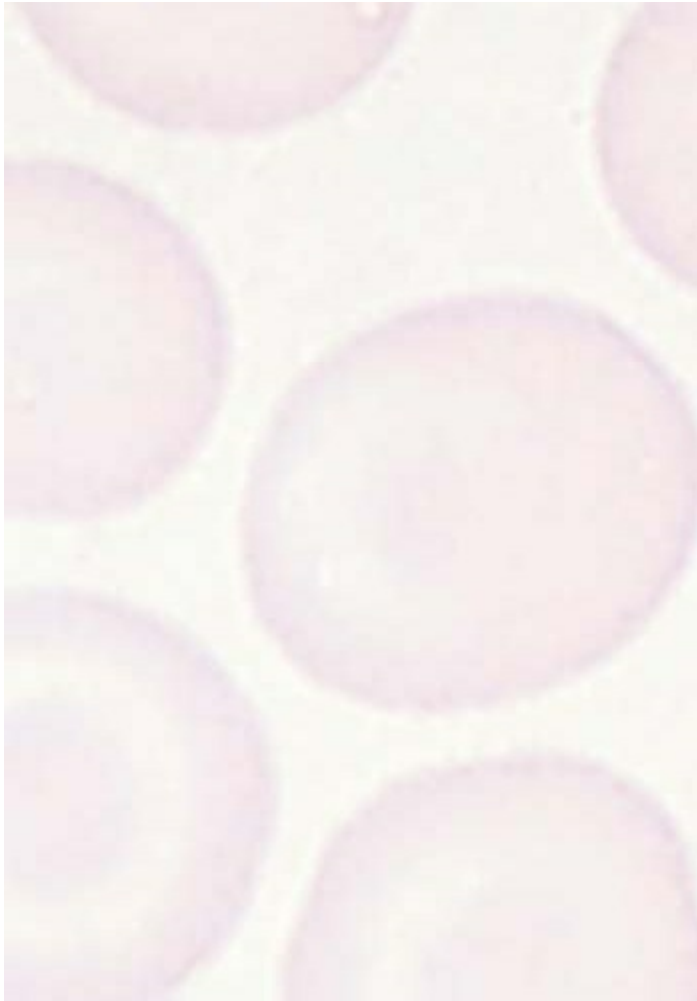
B. RBC m m

C.

C. m

D.

*D. H m MMA



CHAPTER 15 ■ Macrocytic and Megaloblastic Anemias

REVIEW QUESTIONS (continued)

16. I m , m

21. T m -

A.

m m , RBC

B. m

C.

A. m

D.

B. m z

C.

17. I m ,

A.

*22. I m , m -

B. m

, k

C.

m , m k

D.

?

A. C

18. I m , m LDH

B. C m

A.

C. P m

B. m

D. F

C.

D.

*23. F

A. m B

19. I m , j

B.

A.

C.

B. m

D. m B

C.

D.

Folic Acid Deficiency

*20. T m q m

?

A. m

B. k

*C. m

D.

COMPANION RESOURCES

BIBLIOGRAPHY

:// . . m/

A E, B B, S J. F m m : A m B

E W -

, Arch Intern Med, : ,

.

m .

E -N HM, . G m H ,

, South Med J, (): – , (

-

m . m . *mj* . m F ,).

.

G JP, . (E). Wintrobe's Clinical Hematology, , P , PA: L W m & W k , .

H CS, L DL. D - m m , N Engl J Med, (): , – .

M M, B -P S. L m B

, Labmedicine, (): – , .

Principles o Blood Collection and

CHAPTER

Hemolytic Anemias

16 Processing

KEY TERMS

acquired hemolytic anemia

glucose-6-phosphate dehydrogenase

isotimmune hemolytic anemia

autoimmune hemolytic

(G6PD)

methemoglobin reductase deficiency

anemia

hemoglobinuria

paroxysmal cold hemoglobinuria

complement

hemolytic anemias

paroxysmal nocturnal

extravascular hemolysis

intravascular hemolysis

hemoglobinuria

extrinsic hemolytic anemias

intrinsic hemolytic anemia

pyruvate kinase deficiency

LEARNING OUTCOMES

Hemolytic anemias

- Describe hemolytic disease of the fetus and newborn as an example

- Define the term hemolytic anemia.

of isoimmune hemolytic anemia.

- Name at least three categories of intrinsic versus extrinsic hemo—

Compare immediate and delayed types of hemolytic transfusion

lytic anemia

reactions.

- Name two medical conditions that can be the cause of hemolysis.

The role of complement in hemolytic anemia

Describe the physiology and typical laboratory findings in hemolytic

anemia.

- Discuss the action of complement in producing hemolysis.

Complement-mediated disease

Inherited hemolytic anemias

- Characterize the etiology of paroxysmal nocturnal hemoglobinuria.

- Name two categories of inherited hemolytic disorders
- Explain the physiology of paroxysmal nocturnal hemoglobinuria.

Explain the basis of structural membrane defects.

- Describe the clinical signs and symptoms, laboratory findings, and
- Name and discuss various types or varieties of membrane defects.

treatment protocol of PNH.

Explain the consequences of erythrocytic enzyme deficiency in two

- Briefly describe the characteristics of cold agglutinin disease.
- defects.

Case studies

Acquired Hemolytic anemias

Analyze the patient history, clinical signs and symptoms, and laboratory data for the stated case studies, answer the related critical

- Name and briefly explain three categories of acquired hemolytic
- thinking questions, and conclude the most likely diagnosis.

anemia.

- Name four mechanisms of drug-induced hemolytic anemia.

NOTE:

- Discuss immune mechanism related to acquired hemolytic
- anemia.

- indicates MLT and MLS core content

indicates MLT (optional) and MLS advanced content

- Compare various types of autoimmune hemolytic anemia (AIHA).

HEMOLYTIC ANEMIAS

mm (m),

mm, zm. O

Tmm hemolytic anemias -

zm

mm -

- Iq

m.

- Ix

Hmm

- Imm

mmxm -

Immm

mm. I

m. W

292

293

m

THE ROLE OF COMPLEMENT IN

m , m

HEMOLYTIC ANEMIA

.

M m m m ,

Complement m m m-m m , m -

. T -

, , . T m m

m , m m , -

m m -

m . H m m m m

m m - .

m - , -

A m m (F . .) m

.

m m :

Classical

Lection

Alternative

B

Ab

B

3

H

1q

20

MBL

"Tick-over"

D

bacteria

Initial

bacteria

Ba

Bb 3

C3 Convertase

1r

Ab

1q

B

3

1s

3a

D

B 3b

ba cte ria

1r

4

C1 Esterase

1q

2

Ba

1s

Bb 3b

3a

2b

P

4a

P

2a

3b

4b

Bb 3b

C

C

3 Conve rta s e

3 Conve rta s e

P

2a

C

4b

5

5 Conve rta s e

3b

3b

Bb 3b

3b

3b

C5 Convertase

6

5a

7

5b

MEMBRANE ATTACK COMPLEX

6

8

5b

7

6 5b

8 7

9

6 5b

8 7

MAC

6 5b

8 7

FIGURE 16.1 C m m .

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PART 4 ■ Erythrocyte Disorders

■ C mm G (I G) mm M (I M) BOX 16.1

mm .

■ M - -

-

Com plem entary Reg ulatory Prote ins

.

CD () x

■ A

(GPI) m mm m m ,

m m C -

C . T .

m m m m k

■ T m C C

m x (MAC).

.

CD [m m (MCP)]

■ P m k k C

m m

m .

C .

A m C -

CD GPI-m m

. F m , m m m

k C C m

m m

MAC.

m m k m x (MAC) q -

F I m m . I m .

, C .

T m m m m

F H

C m . A m m -

. I C -

m m

. I m I.

. S m -

m m I-m C

m m -

.

m m; m

$f_X, CC.$

Activation of Complement

$CCC.T$

$q_m - C, C, C, C, C,$

$N_m, mmm -$

$C, C, C, C - x_m$

$m.Cmm$

.

$mm - m -$

$Cmq; x -$

$m.Cmm - mm$

$Cmj qm -$

m paroxysmal nocturnal hemoglobinuria

$m.$

PNH mmm (HUS)

$Tmj:$

$m/$

mmm

.R

mm

. Am m m

m m . S m m

. M m k m x (MAC)

(B x .) k m-m .

T m m k m x (MAC) q m

A m m

m x m m m

m

m . W m

C , x m m m .

, C , C , C , C m MAC.

B C m m

T C C C C m x m z C m

MAC m m m

(), m m

.

k ,

A m m , z m -

x . B m x C , **osmotic**

m m m

cytolytic reaction . T -

m x. T C

z m . B z m

j m m m . A

m z m , m C

m m f

; , m m m .

x m

m m . I , m

Clas s ic Pathw ay

, . T q

x m (N +)

T m m m j

H O m ,

m m -m mm . T

.

CHAPTER 16 ■ Hemolytic Anemias

295

Alternative Pathw ay

BOX 16.2

T m

q . H , ,

m -

Exam ple s of Inherited Hem olytic Ane mias

, m m m -

- - .

STRUCTURAL MEMBRANE DEFECTS

A

H

Manno s e-Binding Lectin Pathw ay

H

M - m m m m-H m

, (),

H x

m C q. M - , R

m mm m,

ERYTHROCYTIC ENZYME DEFECTS

m m

G PD

.

G

H x k

Hemolytic Anemia : Membrane Disruption

P k

H m -

m m . T m m-

DEFECTS OF THE HEMOGLOBIN MOLECULE

H C

H S-C

■ I m (m

H S-S (k m)

m)

T m

■ A q m

(x m m)

F

m m. T m

x

x m

m

, ,

- . T m

(.).

q

m

Inhe rited Hem olytic Anem ia

. F x m m ,

Et io logy

m m k

. C m m m

I m m m m-m

, z m , m -

. C - - m

m . B x .

x m m m .

S m m m (

F . . B) m m -

S t ructura l Mem bra ne De fe ct s

m . S , m

P m m m

k , , , . . R

m x m , . T m m

m j m m

TABLE

16.1 Comparison of Intravascular and Extravascular Hemolysis

Intravascular

Extravascular

Site of destruction of

Within blood vessels

Spleen or liver

erythrocytes

Mechanism

Activation of complement IgM or IgG

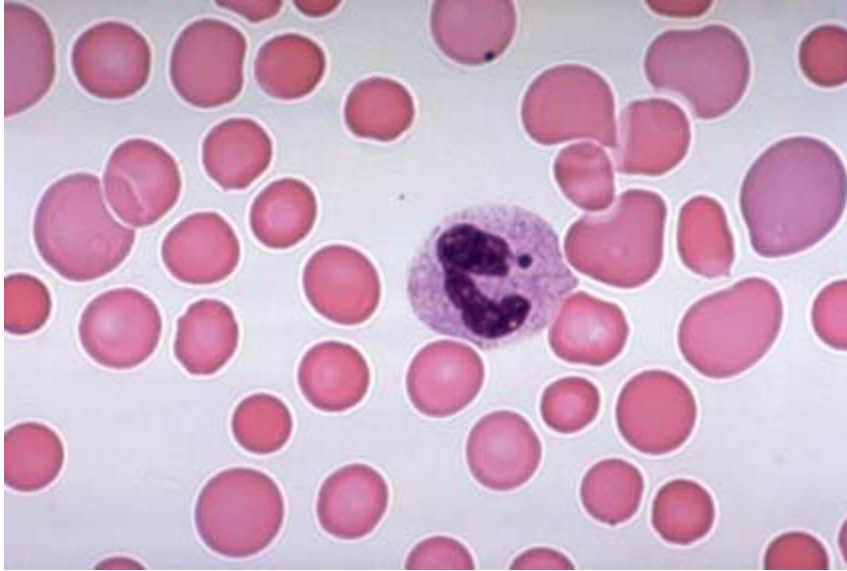
Cell-mediated phagocytosis of IgM-or IgG-coated cells

Laboratory findings

Hemoglobinuria direct antiglobulin test

Positive direct antiglobulin test erythrocytes

Hemosiderinuria



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PART 4 ■ Erythrocyte Disorders

x CO m

N E . I

. A k m j k

. M m

m m k m m . M

m - m m -

m j m m

m . A m m m m , m

.

HS m m m -

■ A m x

, , m m .

■ C m m m m

T m -

■ C m m

m m k .

M m m m

T m k k m , m - k k

m , m m

m m . G -

m m . T

k

m m m j m

m m . I

m m

m k k -

m m m m m

m m m m -

m .

, x , m m H m x , . L m m -

. I HS, m m ,

. M m mm

- - m ,

q k , , m . S

m m m ' ,

x . T m m

k . C m m

m m

x m m

m m ; , m m

. I m x -

m m . A

m m m m , m

.

.

m k m m .

Ex m k

S m m m

m (N +), x m m

, k (HPP),

m .

, m . A -

C , -

x .

, m j ,

. S m m—

Here dit a ry S phe ro cytos is

m , m , j ,

H (HS) (F . .) -

m . O m

m m m m m j -

m . H m m -

m m ; m

m . P m m

mm m m m

. T m m

(MCV) m . T m -

m (MCH) m , m

m (MCHC)

%. S MCHC.

T m % . I m

, m HS

-

AIHA. A m , m -

m , . P m

m m

q HS.

S m m m

. S -

m j

m . S .

FIGURE 16.2 H . T

S m k - -

m m m ,

, (). (R m

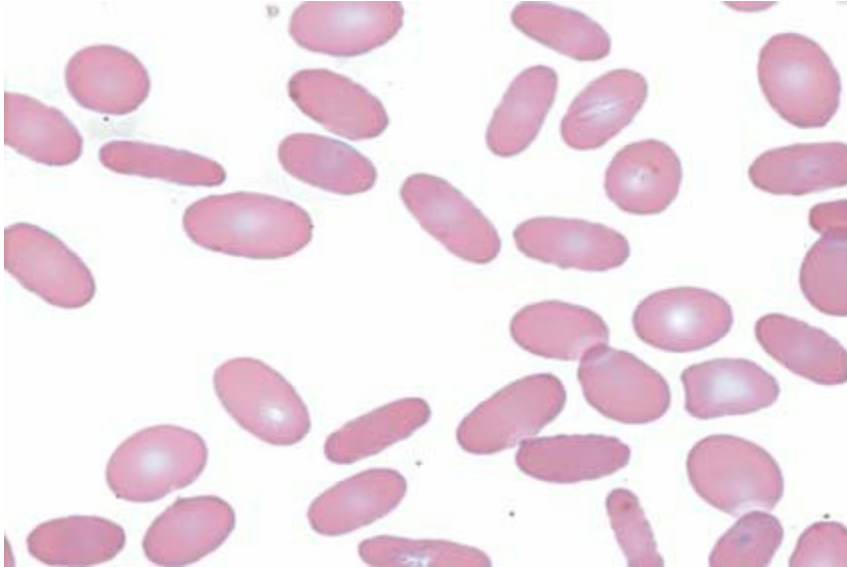
. S m

R E, F JL. Pathology, , P , PA: L

k (. %

W m & W k , , m .)

m).



CHAPTER 16 ■ Hemolytic Anemias

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Here dit a ry Elliptocyto s is

M k , k-F . m mm -

m m m .

z -

S m m m -

, m ,

. O k m , m mm .

m m m

H (HE) -

m

HE, HPP, m m k -

m , . O -

. T m j HE- , m m . , m m m

, m m

k . T q m -

m m ,

m z

k .

m m m k ,

C m . A m -

m - - -

(%) mm HE -

. m x m . HE m

m m . I m m m

(m) m . I m z

x , . I ,

m, m m m .

m m m (N +).

T z m m m .

M m . N m N HE , x m m .

m j

H , k m -

m -

m j .

: mm HE, , -

I m m , m m .

m .

T m m S HE q m , m .

: m HE

Here dit a ry Pyrop oikilocyto s is

m HS. T

x m % % HE m

HPP m ,

E . T m m -

mm HE, m k . I m -

HS.

m

S m HE q M

m k . A m

. T m -

m , z

, m m

m x m . MCV

m m

m L m -

. H m m .

m . T m m

T m m m

k m m .

HE () Dis orde rs of Eryt hrocyte Hydrat io n % . A

m m k

D

m, q . I ,

m .

m m m m .

H m m m m

m m .

A m m ,

’, ’

-

k .

P m

x m , m () -

m , m

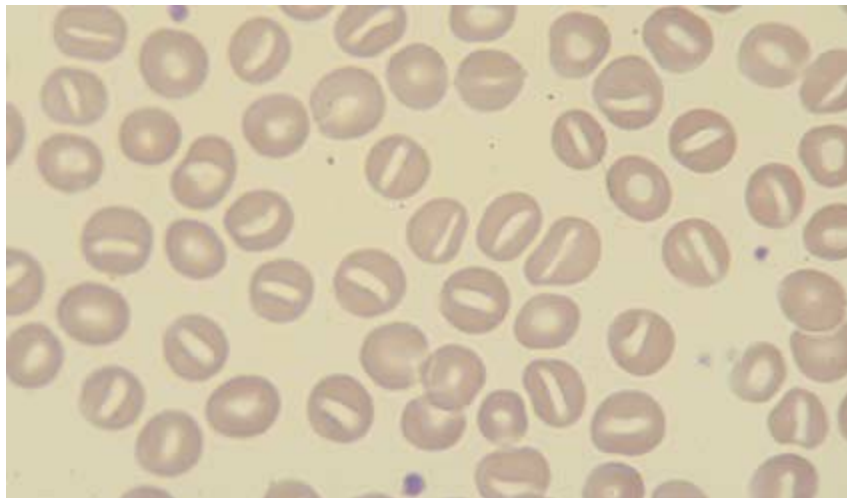
m . T x m

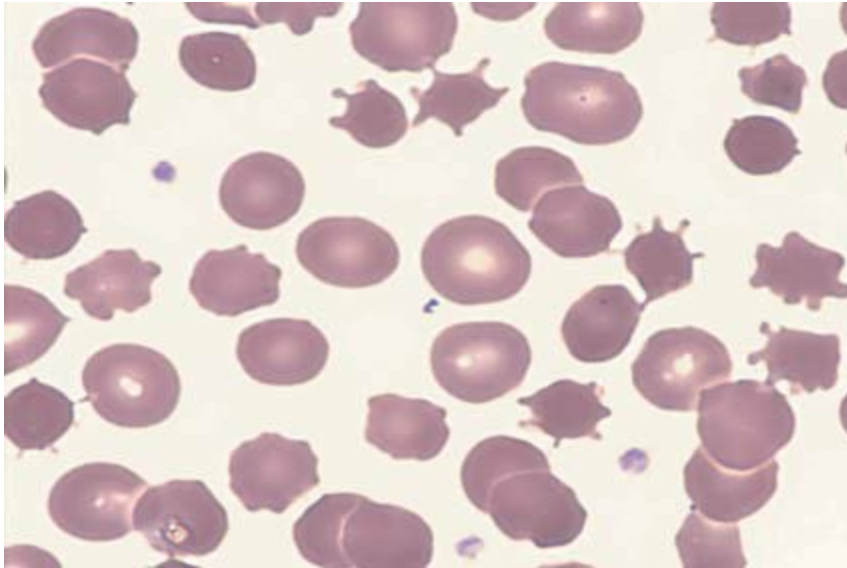
m .

Here ditary Xerocytos is

FIGURE 16.3 O (). (R m A SC. Anderson’s Atlas o Hematology, P , PA: W

H x (HX) m , m m-K H /L W m & W k , C , m , m .) m . I , m





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PART 4 ■ Erythrocyte Disorders

q m .

m MCHC (%

T K+ x N + x,

%).

N + . T .

A m m m . B

T MCHC

m . P m

. W MCHC %,

% % m . O m -

m m

m . A m -

.

(A P).

T HX -

S m .

m m m I , R -

m x . R

m . T k R m x -

m m m m x .

m m .

P m m , -

Rhnull Disease

m , m , z m .

R , R m , -

T m m , -

m , m m

. A m m

m . T m ,

.

, R -H m

C z m m -

L -W (LW) m

, - m m m . I

. R m m K⁺

k m HX

m k

.

m N -

K-A P m . T m m m

He re ditary S to m a to cyto s is (He red ita ry

K⁺ k . T m

Hydrocytos is)

m . , m

H m (F . .)

/ L m

m , m , -

. M -

, HS, .

m . H m F .

$T_{mm}m, m(N^+),$

Acanthocytes

$m(K^+), NK.$

$A(F..)$

$T_{mm}m, m_j.$

m_{mmmm}

A -

$m.B$

$:mm.$

m_x

$A_{mmm}-$

,

$mmz-$

$m-$

$B(B), m.M$

km

$mm \times qm$

$m-k.T-$

$m.A$

$.TMCHC.TMCV_m$

m m

x -

.

, m ,

FIGURE 16.4 S m . (R m A SC.

FIGURE 16.5 H . (R m Anderson's Atlas o Hematology, P , PA: W K

A SC. Anderson's Atlas o Hematology, P , PA:

H /L W m & W k , C , W K H /L W m & W k , C

m .)

, m .)

CHAPTER 16 ■ Hemolytic Anemias

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M m m ,

m . C

m m m . MCV, MCH, MCHC,

m m

m m .

m m . I m z m

, x k , - -

S pu r Ce ll He m o lyt ic Ane m ia

m , PK, m

T m - m m m m . M z m . A

m m m m

m x m %

m .

% . H m m

m m . I m , m / L.

Glucos e -6-Pho s pha te De hydro gen a s e

R % %, Glucose-6-phosphate dehydrogenase (G6PD de ciency), C - - m

m mm z m ,

. M mm .

x ,

O -

, , . D

, k , M L ,

m q m

m (. . , x).

x m % Am B k m ,

m , q , q

Ne uroa ca nt hocytos is

m m

N (NA)

-B k .

-

T X-k z m , G PD ,

. C , NA z

m . S m M E

m . T

x m G PD ,

k ,

x m , % m K J % m

m m , m j

B .

m m .

M z m -

NA x

m . , m

m m . R. I z

G PD-I m .

. R .

A M , m m , m

G PD . G PD m

NOTE: This is a good time to review the definitions of Key

m . F x m z -

Terms found in the Glossary and ash cards on

. It

m . I m , x

is also a good time to complete the Review Questions related

m . I m

to the preceding content.

m (z), -

x . O

m z m ;

Eryth ro cyt ic Enzym e Me ta bo lis m Defe cts

G PD .

L k m m , z m T , k , m-m m . D -

z m . T

m m m

x m

m m (CNSHA). CNSHA

q m . T z m

G PD z :

m m, -

z m , , -

Glucose-6-phosphate + NADP G6PD

→ 6-phosphogluconate + NADPH

m m. H m z m

x ,

T -

,

(-). N m - -

.

(NADPH)

T m

m . A x m -

, x z , m

. G - - (G PD)

NADPH m

. P k (PK)

m x m .

. M m

L q -

T z m .

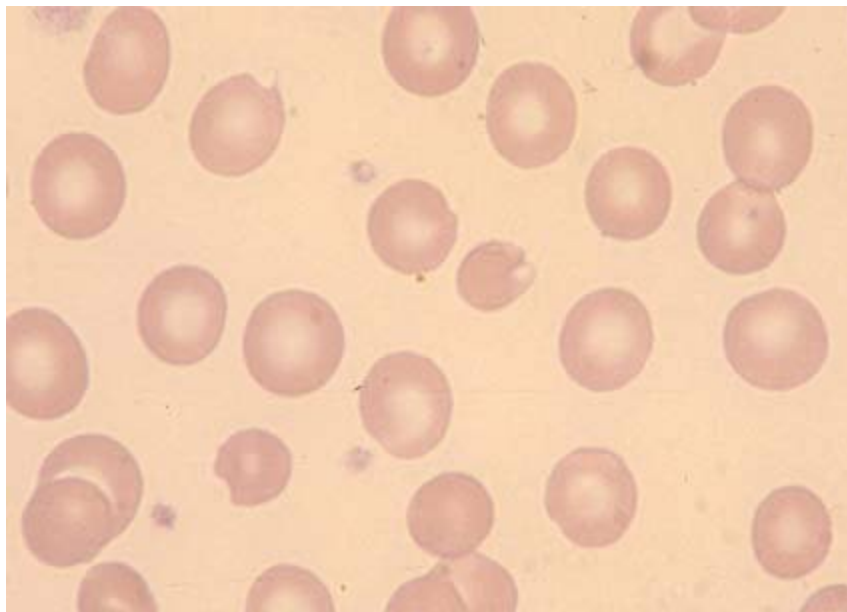
G PD, m

S m m m m ,

, H z

m

m H z . H z



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PART 4 ■ Erythrocyte Disorders

D

PK z m . T

z m :

PEP + AD P + Mg²⁺ PK Enzyme

→ Pyruvate + ATP

+

Pyruvate + NAD

H + H⁺ LDH Enzy me

Lactate + NAD

→

(UV Fluor escence)

(No Fluor escence)

T NADH

m - (UV) ,

NAD .

FIGURE 16.6 G - - . (R m A SC. Anderson's Atlas o Hematology, P , PA:
Methe m o glo bin Re ducta s e De cie ncy

W K H /L W m & W k , C

, m .)

H m x z m

m m . A x m %

m m m -

, W -

m . H z m NADH-

(F . .).

m m

m m . A z m ,

Pyruva te Kin as e

NADH , m -

Pyruvate kinase PK def ciency %

m j m M -

. T m mm

, x x

z m z m G PD

. T m m methemo—

PK.

globin reductase de ciency m m -

PK m . T P

x (C

Am q PK , x

).

. T z q

% . % N Am , E ,

Acquired Hem olytic Ane mia

A . A A Am -

q z . m m m-Et io logy

m A W .

Acquired hemolytic anemia

T m PK-LR PK. PK -

m .

Em -M .

S x m

M k m x

q m m B x . .

A P. A

A m j x m

PK q A P

, m m

k ,

m m , m m mm

, m m . T

.

q m -

m m .

Che m ica ls a nd Ven om s

PKI m m x -

m , A P , m

m . F ,

. L A P

-

, - (DPG),

x . T m m ,

, , . m m-

. L m , ,

m .

A P

I G PD x .

m .

I , , -

V m m m k m

, z

m

PK .

m (DIC).

P m PK

m m , m

Phys ica l Agen ts

m (-

H m .

). P , -DPG

B m x , m z m k.

m m m . T

CHAPTER 16 ■ Hemolytic Anemias

301

m . m j

BOX 16.3

m

m **march anemia**, -

m . D m

Exam ple s of Agents and Co nditio ns

As sociate d w ith Acquired He mo lytic

- m .

Ane mias

O x m m

CHEMICALS, DRUGS, VENOMS

■ T m m (P)

A

■ H m m m (HUS)

C (W)

■ HELLP m (H m , E L z m

L m

, L P)

N (m)

N z

In fe ct iou s Microo rga nis m s

P

S m m m (.) P

. T

R

z (. ., m) . M m S m

m j m m m

INFECTIOUS MICROORGANISMS

m

B : Clostridium ., , E. coli O157:H7,

m , Plasmodium falciparum.

I m , Clostridium perfringens,

P z : Leishmania, m (Plasmodium .),

m x m . I , x m

x m

Bartonella.

IMMUNE MECHANISMS (ANTIBODIES)

A m m m

A mm m -

. Escherichia coli O157:H7

C m

m m j k I

N Am . T

P x m m

m k m

A mm m m-

- . L k -

D

, m , q

I

E. coli O157:H7. S

S

E. coli O157:H7 m m

L m (CLL, m ,

m m , m j

m m ,)

. T m m m m

H m (HDFN)

, m .

I m

S x , , m m

P x m m

m j .

PHYSICAL AGENTS

S

Immune Mechanisms (Antibodies)

Aqmm **TRAUMATIC AND MICROANGIOPATHIC HEMOLYTIC**

mmmm . I ,

ANEMIAS

-

Dm

mmmm , ,

E. coli O157:H7

mm . Tmmmm

Hmmm

, ,

P

-(B x .).

Tmm

Autoimmune Hemolytic Anemias

Autoimmune hemolytic anemia (AIHA)

m , q

mm -

m .

, , q

m . T

Microa n gio pa th ic Re d Ce ll De s t ru ction

k . D m AIHA m—

DIC x m m m m .

mm m

DIC m

m m B , , m ,

m m . T m

.

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TABLE

**16.2 Repre s entative Micro organis m s As s o ciate d w ith Hem olytic
Anem ia Bacte ria**

Paras ite s

Virus e s

Bartonel a bacilliformis

Babesia microti

Cytomegalovirus

Borrelia recurrentis

Babesia divergens

Epstein-Barr

Clostridium perfringens

Leishmania species

Escherichia coli O157

Plasmodium falciparum

Haemophilus influenzae

Trypanosoma brucei gambiense

Mycobacterium tuberculosis

T. brucei rhodesiense

Mycoplasma pneumoniae

Neisseria meningitidis

Salmonella typhi

Streptococcus species

Vibrio cholerae

AIHA m

. A R

m I G m m m

-U, -LW, -K , Jk F .

I M m . A

C m .

m m .

I m m x ,

A mm m m m

F mm m

m m

F

, . P m -

m m m . T , z

, .

, , m

m

Warm -Type Autoimmune Hemolytic Anemia

.

I m-AIHA (.), I G

M m . P

m m x . T -

m q x , , -

m m AIHA m

m , . T

x m

.

T m-AIHA

z m . I -

BOX 16.4

AIHA,

. I AIHA,

Clas s i cation of Im mune He mo lytic Anem ia

m .

AUTOIMMUNE HEMOLYTIC ANEMIAS

Cold-Type Autoim m un e He m o lytic Ane m ia

A mI AIHA - (. . ,

A -

, CAD),

A m- -

I M . C -

IS OIMMUNE HEMOLYTIC ANEMIAS

H m (HDFN)

m m . T -I. T

R m

°C m m

ABO m

m .

DRUG-INDUCED HEMOLYTIC ANEMIA

Is o im m une He m olyt ic Ane m ia

A mm m x m m

A m m

H m (HDFN), I

x m isoimmune hemolytic anemia, m x -

N mm mm

RBC m . F

m m

m k m , m

k m x .

CHAPTER 16 ■ Hemolytic Anemias

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TABLE

16.3 Compariso n o f Warm and Cold Autoimmune Hemolytic Ane mia

Warm AIHA

Co ld AIHA

Optimal temperature of reactivity

37°C

4°C

Immunoglobulin class

IgG

IgM

Complement activation

±

+

Site of hemolysis

Extravascular

Intravascular

T

m m .

: m k m ,

I RBC m

, m -

, m

m .

, -

z m . L

Et io logy

x ,

T m m HDFN

q j .

m I G

B m x m

I I G . A -A -B

m -

m -

m.

m (I M) , q I G m m

HDFN ABO m

Dia gno s tic Eva lua t ion

m . A -A -B

A

. H

HDFN: T

-A, -B I G O m

ABO

m HDFN.



■ R

Ep ide m iolo gy

■ S ; -

T HDFN m ABO -

Am ()

m . T m q m



S m

ABO m m O



D

A B, A.



P m

U , R -D



F m K -B k (m

m q m m HDFN.



)

A -D x m % -

ABO HDFN. S m m -

T m -

m m mm z D ,

' m

q HDFN -D

m .

.

Preve nt io n

S igns an d Sym ptom s

I , R I G m U

A I G, mm

S . S m , HDFN

, m m

-D m , m m -

.

m -D

O m m -

m . A R - m

,

R I G, R

. H m

k , D -

m m z -

m .

. B m , m -

Hem olyt ic Tra ns fu s ion Re a ctions

- .

m RBC , , ,

Im m e dia te Tran s fus io n Re a ction s

m -

T m mm m

. I RBC m ,

ABO – m

x m m , m

. I - x m

m

, m

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PART 4 ■ Erythrocyte Disorders

. A m m

M

.

m -

A m

m m .

mm . I m-O m m -

x

m m

- ,

.

m m , m , k , m .

Q , HUS. A

O m -

HUS m m

m m z -

m m , m , , -

. M m extravascular hemolysis

, - DIC . Q ,

intravascular hemolysis. I -

, m

m m f , -

q - , -

m ,

, m.

m . A ,

T m -

mm m m m

DIC . O m -

m m ,

k q q

m k m m .

(q q m

). P

Delayed Hemolytic Reaction

- , , , A m m m m . P

. I mm ,

k

x . T

. T m m m -

RBC

mm -

RBC (),

,

m m m RBC

m

m m. I

m m m x m

m m , x

() .

m . M I G –

D m -

x , m .

HUS. P m - -

(PAI-)

Drug -Ind uce d Im m u ne Hem olyt ic An em ia

HUS.

D - mm m m (DIIHA) m mm , x , Me dica l Co nditions

. DIIHA m

V m m

m . AIHA

m m . O x m-m m k DIIHA. Imm m m k m (CLL).

m m m

(. . ,), m , m .

Pathophys io lo gy

- x :

T m .

. D - , x m ,

A m , m m

. I

z . M (%

%) m x , m

.

m m .

. D - , x m ,

T m m m m .

T m m m z -

RBC ' m m

.

.

H m m j W - m m , m m m : m . T

. S m

m . A m

RBC m m . T m RBC . I

m z , m m

I G , -

m m

RBC , m

. T m

z , x RBC .

k , m x , -

O , m m m . T

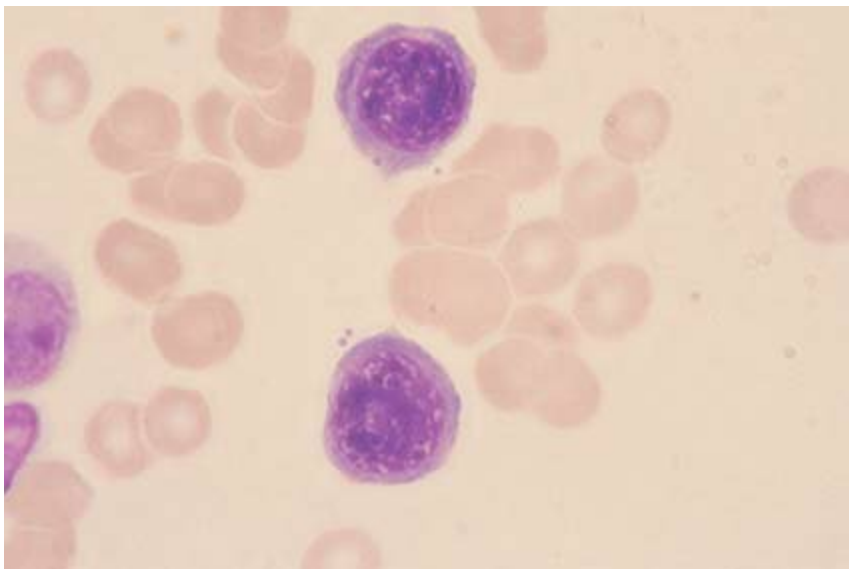
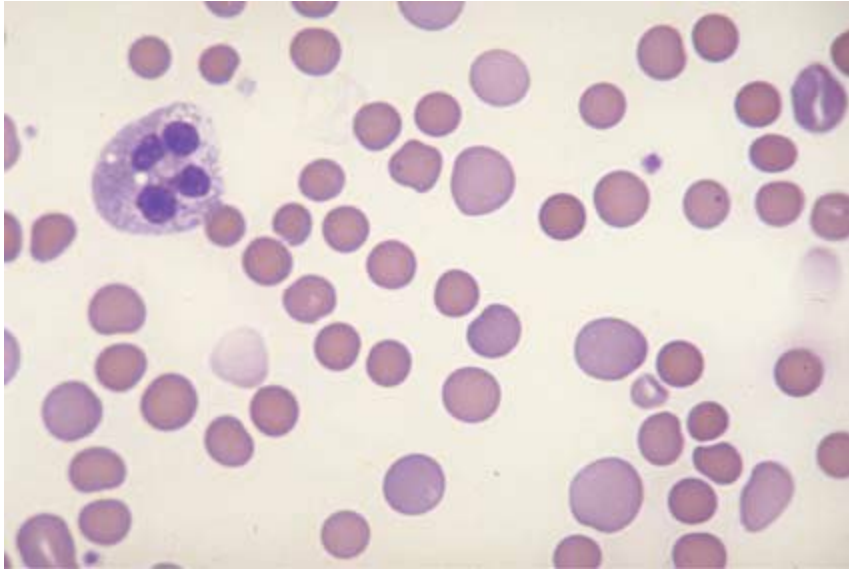
. M m

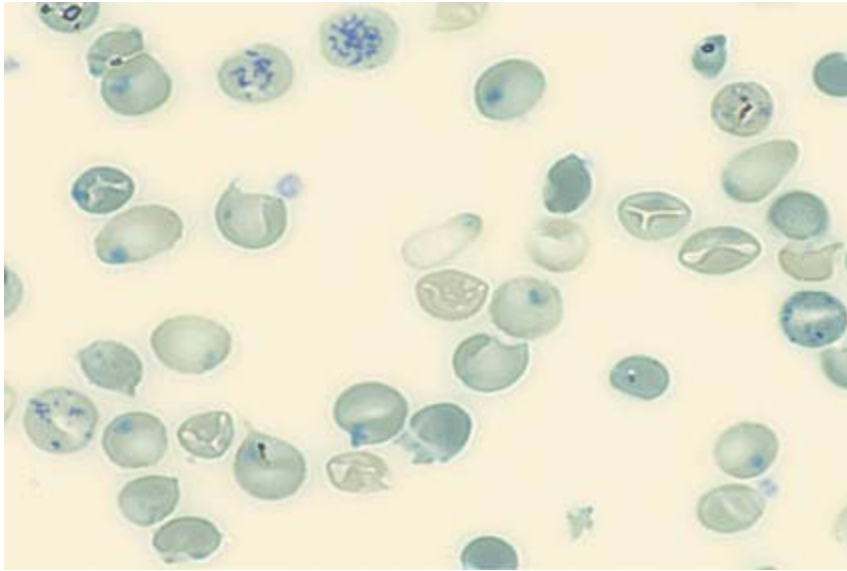
m ,

m . B . A

m m.

m





CHAPTER 16 ■ Hemolytic Anemias

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j , m ,

j

, x m.

W , m -

j x

j . T

j m , j

m .

S m m m -

. I , m

m -

. T m m x m

m . M

FIGURE 16.8 P m m ().

m m x k ,

(R m A SC. Anderson's Atlas o Hematology,

m k m .

P , PA: W K H /L W m & W k , C , m .) I m m , m . R m

m m

C m -

m m m

j ()

m . I m m mm . B , m

x m

m m m -

k , m x

z m m (MPS),

(hemoglobinuria).

x m / L. S m ,

x m ,

Diagnostic Tests

. H

MPS m x m

H m z -

m

m m

. R -

m .

. F

T m m x

mm m ;

m , H , . B

m

m x m m

k

(F . .), m k -

-

m m m . O m

.

, , m , -

S

m , , m (F .).

A (AHG) ()

T (C)

O ()

m . A m

C m m

x

B m

.

D m G PD

H z (F . .)

FIGURE 16.7 S . (R m A SC.

Anderson's Atlas o Hematology, P , PA: W K

FIGURE 16.9 H z . (R m A SC. Anderson's H /L W m & W k , C , Atlas o Hematology, P , PA: W K H /

m .)

L W m & W k , C , m .)

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P m

m - -

BOX 16.5

m .

. B x . , ,

m m -

Differential Testing in Hemolytic Anemias

m m

A ,

.

B m x m

C

NOTE: This is a good time to complete Review Questions

G PD

related to the preceding content.

M j ()

L

COMPLEMENT-MEDIATED DISEASE

T m

x m m (PNH),

m m . W m m

m m m (HUS) m -

, m m ,

m m (HUS),

m , m . C -

(CAD), CD . C

m PNH m

m m -m .

m m .

x m .

PNH x m m ,

m , m . I m

Paroxys m al No cturnal Hem o glo binuria

z m (x m)

- () (m -

Et io logy

). S m PNH m

PNH q m m m m m x m m -

m .

GPI, m

m z

Ep ide m iolo gy

m m .

- m

PNH , q ,

m . A x m % % -

m x m m -

m m k m .

m q m m

T m (, X-m m , PIGA. T PIGA ,). M .

m x m , q

S - m m .

GPI . T

, m m ,

Pa th ophys io logy

CD CD . T m m

M m PIGA -

m

Clinical Manife s tatio ns o f

Typical Profile of Quantitative

TABLE

16.5 Complement-Mediated Diseases

TABLE

16.4 LABORATORY Findings in

Hemolytic Anemias

PNH

Hemolysis, Thrombosis

Test

Result

HUS (typical)

Hemolysis, thrombocytopenia,
renal failure

Hemoglobin, hematocrit, RBC

Decreased

HUS (atypical)

Hemolysis (extravascular),
count

lar), thrombocytopenia,

Serum haptoglobin

Decreased

thrombosis, renal failure

Red blood cell survival (^{51}Cr)

Decreased

CAD

Hemolysis (extravascular and

Lactic dehydrogenase (LDH)

Increased

intravascular)

isoenzymes (LD1 and LD2)

TTP

Hemolysis, thrombocytopenia,

Bilirubin (total)

Usually increased

thrombosis

Antiglobulin test

Positive or negative

Congenital CD59 deficiency

Hemolysis

CHAPTER 16 ■ Hemolytic Anemias

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. T q k

RBC WBC . Im ,

m m

m , , m GPI . A GPI- -

- m -

m m m-

.

. T m m , H - m m -

CD CD ,

PNH.

m m -m . T x

I PNH . % z (,

m m .

), %

D m , x m

, .

m x (NO), m

m m . NO

Tre a tm en t

m . R

m , , NO

. A m

m , -

. E z m , m S ,

, m .

m k m m -

T m m

m . I FDA—

PNH k . S m

m , m q

m x

, k m PNH .

. P m PIGA

E z m - - , m z , m -

m ,

C .

m m

m .

Paroxys m al Cold Hem og lobinuria

Clinical Signs and Symptoms

Paroxysmal cold hemoglobinuria

AIHA. I - m -

■ PNH

m . I m x

. I m

. E

m

- , I G

. M m -

m m . T , D -

m m m -m

L -P ,

m .

m

C m m , m -

m m m m . T

, , m -

m .

.

T PNH m ,

Co ld Ag glutinin Dis e as e

-

I m CAD,

- . PNH . P

mm , m , MAC m-x m .

, m m m

. I m j ,

La boratory Fin din gs

C m m m

M m m -

q m .

/ L. P m m

CAD m .

m , m

P m CAD B-m -

. T m -

m -

(-) (.

m m m -

. m/). F m m m

m m m , m z m m ,

mm m

- m . S

. H m , x

CAD m

- m m m -

m m ,

, m

H k ' m m , . M

m .

m x m -

T m mm

CAD.

PNH . A

, m m

NOTE: This is a good time to complete end of the chapter

m GPI—

Review Questions.

m k GPI



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PART 4 ■ Erythrocyte Disorders

CHAPTER HIGHLIGHTS

mm . A

mm .

■ T mm m m m

■ L k m m ,

m

z m .

. W m

■ T q m m -

m , m .

m .

■ H m

■ A mm m -

m m . T

m- . O

m m m -

m

(m m) q

, m , x m -

m

m , x m m .

(x m m). F -

■ P m m

-

m , H , . B m

m m. T m x

x m m

m

. O m

, , .

m , , m ,

■ I m m

m , , m

m m , z m ,

m .

CASE STUDIES

Cas e Study 16.1

A - - m

. A CBC

m .

■ Laboratory Data

T m CBC :

H m . / L

H %

RBC . × /L

WBC . × /L

■ Critical Thinking Group Discussion Questions

T RBC :

. W m

MCV . L

?

MCH .

MCHC / L

. W m ’

m ?

m ,

m , m . P

. D m m

m m . F

.

CBC , ’ m -

m . T

Cas e Study 16.2

:

A - - m m

m . m / L

m . S m k . O

R . %

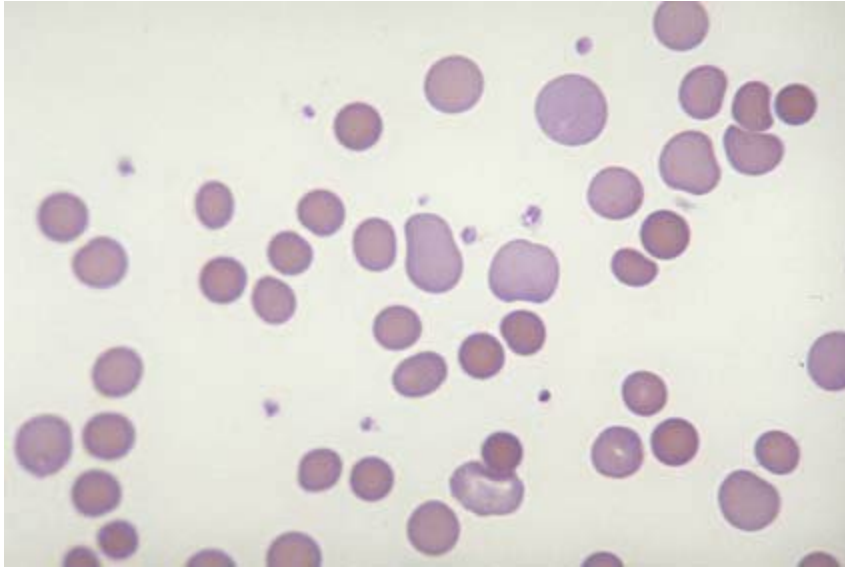
k ,

N AHG

k m k . O -

I m

x m , . T m



CHAPTER 16 ■ Hemolytic Anemias

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C

C A

A S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

m CBC, m ,

.

■ Laboratory Data

T :

H m . / L

H % SI

RBC . \times /L

MCV L

MCH .

MCHC / L

WBC . \times /L.

R m A SC. Anderson's Atlas o Hematology, P -

m m m m ;

, PA: W K H /L W m & W k , C , m .

, m m , ,

, m / WBC,

x m .

■ Laboratory Data

m m .

T :

m . m / L, '

H m . / L

k .

H % SI

T m . T

RBC . \times /L

: ,

MCV . L

AHG, m . T

MCH .

:

MCHC / L

R %

T m , m , D AHG +

, + m , m -

I AHG +

/ WBC . T m . m /

S m

L. T O- . T AHG

T k q m

(+).

. A (m m

F ,

RBC) m m

R m m

AHG

, AHG

.

m , m .

T m O- .

■ Critical Thinking Group Discussion Questions

H . T

. W m

- ¯. A

?

' - ¯. T

m ' m D- ,

. W m ?

C- , ¯- , E- , - . T -

' D- , C- , ¯- ,

. W m m ' -

E- , - .

?

■ Critical Thinking Group Discussion Questions

Cas e Study 16.3

. I q m ?

A , ,

j . T m ' m -

. W m ?

Omm (I G)

. W ' ?

. T . N -

Cas e Study 16.4

m . T

A - - m m

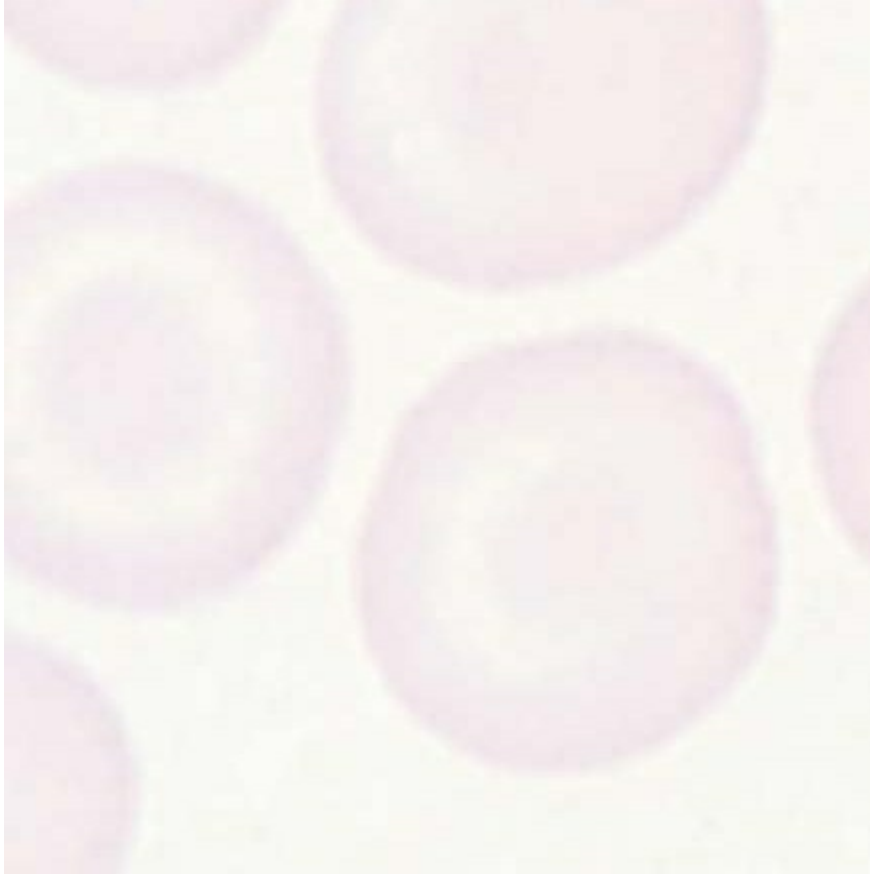
: CBC, m -

m . P

, R , m .

x m m -

(continued)



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CA

CA S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

,

m . T m

. T

m . S m

m k . H

q . T m ,

CBC, , .

%.

■ Critical Thinking Group Discussion Questions

■ Laboratory Data

. W ' ?

T m :

H m . / L

. W RPI?

H m %

RBC . \times /L

. F m ?

WBC . \times /L

T k m-NOTE: This is a good time to write out the answers to the (%), k

Critical Thinking Group Discussion Questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

7. T m ()

.

A. m m

1. H m

B. z m

A. m m

C. m m

B. m m

D. m

C.

D. m

8. P k (PK) ()

.

2. I m

A. m m

A. RBC

B. z m

B. RBC

C. m m

3. Ex m

D. m

A. RBC

B. RBC

9. H

A. -

4. W m

B. m

?

C. m mm m

A. R

m m N E

B. k

D. m , m

C. S m

D. U j

10. H

A. -

5. G PD ()

.

B. m

A. m m

C. m mm m

B. z m

m m N E

C. m m

D. m , m

D. m

11. H k (HPP)

6. H () A. -

.

B. m

A. m m

C. m mm m

B. z m

m m N E

C. m m

D. mm

D. m

CHAPTER 16 ■ Hemolytic Anemias

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REVIEW QUESTIONS (continued)

12. H m

19. W z m m m m ?

A. -

A. G - - (G PD)

B. m

B. P k (PK)

C. m mm m

C. NADH-m m

m m N E

D. H x k

D. m , m

20. A q m m

13. H x

A. m

A. -

B. m

B. m

C.

C. m mm m

D.

m m N E

D. m , m

*21. T m m

m m m

14. H z m -

A. Pasteurella tularensis

m

B. E. coli O157-H7

A. G PD

C. Staphylococcus aureus

B. m

D. Clostridium botulinum

C.

D. m m

*22. I m-mm m m (AIHA)

A. I M, -I,

15. A m m %

B. R m q

Am B k m m G PD

C. I M

D.

A.

B. m q

*23. I - AIHA

C. q

A. I M, -I,

D. q

B. R m q

C. I M

16. W m mm z m

D.

m m?

*24. I mm m m

A. G - - (G PD)

A. I M, -I,

B. P k (PK)

B. R m q

C. M m

C. I M

D. H x k

D.

17. W m mm z m

25. T

m m

m m?

A. m

A. G - - (G PD)

B. m

B. P k (PK)

C.

C. M m

D.

D. H x k

26. W

18. W -

m m ?

G PD z m ?

A. I m

A. H z m

B. I m

B. R

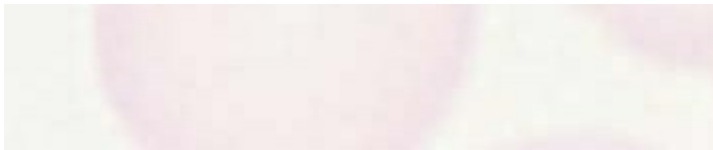
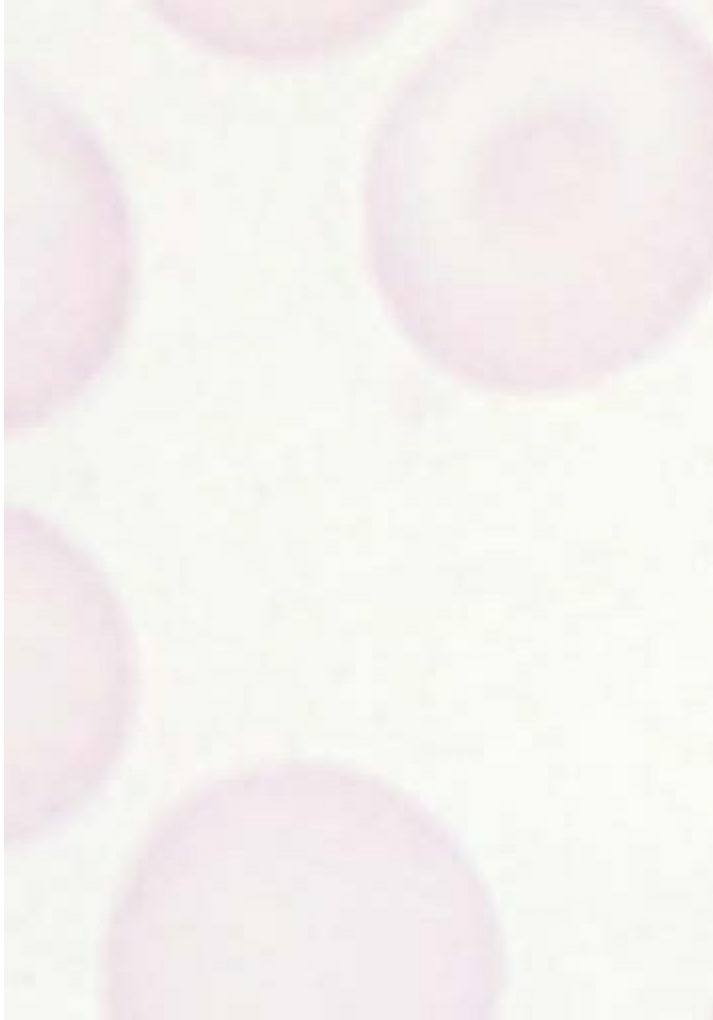
C. I , m

C. H m m

D. O m

D. A

(continued)



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PART 4 ■ Erythrocyte Disorders

REVIEW QUESTIONS (continued)

27. W m

29. P x m m

m ?

A. D m k m

A. m

B. I

B. m

C. I m

C.

D. D

D.

*28. P x m m x -

30. T PNH ()

m m .

A. m

A.

B.

B. m

C. m m

C.

D. A B

D. z m

G M, . S m -

COMPANION RES OURCES

m - m -

:// . . m/

m , Blood, (): – , .

G PG. D m m m-E W -

m m , Am Soc o Hematology Annual Meeting,

m .

Hematology 2015, O , FL, : – .

H

H m P, . N x m m -

-

, N Engl J Med, (): – , .

.

H A, P JR. M m m m

m , Am Soc o Hematology Annual

Meeting, Hematology 2015, O , FL, : – .

BIBLIOGRAPHY

K GK, . S m EPO

m m ,

A RH. Q : A m m Hematol J, (S): , .

m , Ann Intern Med, (): – , .

L MA, . A m A WU, . L -

x m m : B m m -

C m , J Hematol, (S): , .

m C k m

B P, . A k

m m , Blood,

m m (G) PR-PK

(): – , .

, Hematol J, (S): , .

M M, . C m

B k RL. P x m m , Lab Med, (): –

, .

m m , Haematologica, (): – ,

B M, D m R (.). Q m m

.

m , CAP ODAY, (): , .

M J N, . P x m m

B k RA. C m m m m , Am Soc o Hematology

m , m m

Annual Meeting, Hematology 2015, O , FL, : – .

x m m , Hematol J, (S): , C P, . E m m . R

.

, Hematol J, (S): , .

M z Y, . M z G PD

D M, M DC. H m : I , J Hematol, (S): , .

M m , , , Am Soc o Hematology O MG, H LM. A mm m m -

Annual Meeting, Hematology 2015, O , FL, : – .

, Lab Med, (): – , .

D PM, C z L. P k

P AD, F NV, Sk VB. T RHU-EPO m

m m , Haematologica, (): – , .

, Hematol J, (S): , .

F LMR, . D

Sm LJ. P x m m , Clin Lab Sci, m - m , Hematol J, (S): , .

(): – , .

CHAPTER

Hemoglobinopathies and T alassemias

17

KEY TERMS

ca rrie r

s ickle ce ll dis e a s e

va soocclusion

h e m o g l o b i n o p a t h i e s

th a l a s s e m i a s

h e r e d i t a r y

tra it

LEARNING OUTCOMES

Hem og lobin defe cts

- Briefly describe the value of the techniques of hemoglobin electro-

- Describe the common denominator in hemoglobinopathies.

phoresis and deoxyribonucleic acid (DNA) analysis.

- Name the three major categories of classification of hemoglobin

- Explain the process of prenatal diagnosis of SCD.

defects.

- Delineate the general management of SCD.

- List the components and percentage of normal adult hemoglobin.

Sickle cell syndromes

- Compare the disease state and trait condition of a hemoglobinopathy.

- Describe the conditions of sickle β thalassemia, sickle-C (SC), and

Sickle cell disease

sickle cell trait.

- Describe the etiology of sickle cell disease (SCD).

Thalassemia

- Explain the epidemiology of SCD.

Analyze the structure of the hemoglobin molecule in SCD and relate

- Compare the conditions of α and β thalassemias.

it to the pathophysiology of the disease.

- Outline the laboratory findings in thalassemia.

- Describe the clinical signs and symptoms of SCD.

Other hemoglobinopathies

- Briefly explain the symptoms of SCD in children.

Compare the prevalence of hemoglobins C, SC, D, E, and H.

- Describe the symptoms of SCD associated with pregnancy.
- Describe the general characteristics of hemoglobin (Hb) C disease,
- Discuss the clinical manifestations of SCD in adults.

Hb SC disease, Hb D disease, Hb E disease, Hb H disease, methemoglobinemia, and unstable hemoglobins.

- Characterize the general signs and symptoms in the categories of hemoglobinemia, and unstable hemoglobins.

pain, pulmonary complications, and stroke associated with SCD.

- Describe the persistence of fetal hemoglobin.
- Identify globin chain defects causing SCD, hemoglobin C disease, and hemoglobin E disease.

Case studies

- Outline laboratory findings that are typical of SCD.
- Analyze the patient history, clinical signs and symptoms, and laboratory data for the stated case studies, answer the related critical questions, and explain the mechanism of traditional and novel pharmaceutical therapies for SCD.

thinking questions, and conclude the most likely diagnosis.

■ Recognize and identify major clinical signs and symptoms and abnormal laboratory tests results including peripheral blood smear picture that are typically associated with homo and hetero condi—

NOTE:

tions of HgS, HbC, HbD, and HbE and compound heterozygous condi-

■ indicates MLT and MLS core content

tions involving HbS and other variant hemoglobins.

indicates MLT (optional) and MLS advanced content

I m m m , m -

m m

(C). U m , m

m . T

m (m A) (F . .)

q m k

m

m m m

m . T -

hemoglobinopathies

z . N m

m .

313

314

PART 4 ■ Erythrocyte Disorders

β 2

β 1

BOX 17.1

Percentage of Eastern Mediterranean Region

with an Abnormal Hemoglobin*

Proportion

Country of Population

>10%

Cyprus (17%), Bahrain (13%)

6%–10%

Iraq, Morocco, Oman, Qatar, Saudi

Arabia, Sudan, Syria, Yemen

4%–6%

Iran, Kuwait, Lebanon, Libya, Pakistan,

Tunisia, United Arab Emirates

α

<4%

Afghanistan, Ethiopia, Egypt, Jordan

2

$\alpha 1$

FIGURE 17.1 S m . (R m

*

P CM. Pathophysiology Concepts o Altered Health States,

T m m z -

m , x m , G PD, m m . D

, P , PA: L W m & W k , ,

m A M .

m .)

HEMOGLOBIN DEFEC S

De mo g raphics

SCD β thalassemias m mm m

A m m m sickle m . T “m ”

cell disease (SCD) m m , m .

x m M (B x .) -

P m m m z (S/S) S A S A C .

z (S/A) trait. T k m

T m m

m C, E, D, SC, SE,

z -

SD . I m S,

Plasmodium alciparum.

m m m m

T k m q -

x

C A . T m m j

m . T m

k M . T m mm

k x

N Am , m S m z m -

m . T M E ,

m m m

S A , O α m

m x . A

m .

m S m

SCD mm m -

m

m m S -S A , S Am , C ,

. I m m C, m

C Am , S A , I , M

m m x

k , G , I . I U

m .

S , , , m -

A m m m m A . T

q m m A

A Am , ,

F . .

H Am . A m Am ,

m m

A Am , k .

m

B A H -

-- .

m U.S. ,

m x m -

.

Norma l he moglobin

Glu

Be ta cha in

T m -

k m

m , .

He moglobin S

Va l

Be ta cha in

Etiolo gy

FIGURE 17.2 C m m k m m -

H m , x m , SCD, -

. (G , m ; V , .)

m q

CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

315

m m . I m

% -

BOX 17.2

m , m j ,

m . H , m

m m

Exam ple s of Se lecte d He mo glo binopathies

m z .

ABNORMAL MOLECULAR STRUCTURE

D m

H SS (k)

m z .

H SA (k)

H m m m m -

H C

. A x m % m m-

RATE OF SYNTHESIS

m m m z m .

β T m

A m m

α T m

, m -

COMBINATION OF TWO MOLECULAR ALTERATIONS

m

OR A MOLECULAR ABNORMALITY AND SYNTHESIS IS

.

DEFECT

Dis e a s e Ve rs us Tra it

H S-H C

H S- β m

I m m ,

-

m . A m -

z m

z , m

NOTE: This is a good time to review the definitions of the Key

m . I SCD, m

Terms in the Glossary and ash cards on

. Also,

m . A -

a good time to complete Review Questions related to the

z m m m . A

preceding content.

m

C .

SICKLE CELL DISEASE

Abno rm a l He m o glo bin Mo le cule s

S k (SCD) m m

A m m m

m , x m , m , m m j : k m .

. A m m m -

T z

m m ,

H S, m , m -

x m , SCD

k -

. A m

m .

m m -

SCD (H SS), m mm m m -

, x m , m

, x k

. D m m m

m (C). O k

, x m , H E- β

m k . C mm

m

H SC β m .

P , m

M m m

m ,

. T m j m (m -

m . A m U

) m β -m .

S SCD.

A m , - DNA

m

Etiolo gy

m m -

m . S x m m -

T k m m .

m j

H S m H A

B x . .

(GA G)

N m m mm x β

: H A (% %), H A (% %), H A

m m ..T m

(% %), m (H F) (%).

m z (), x

T m j H A. ,

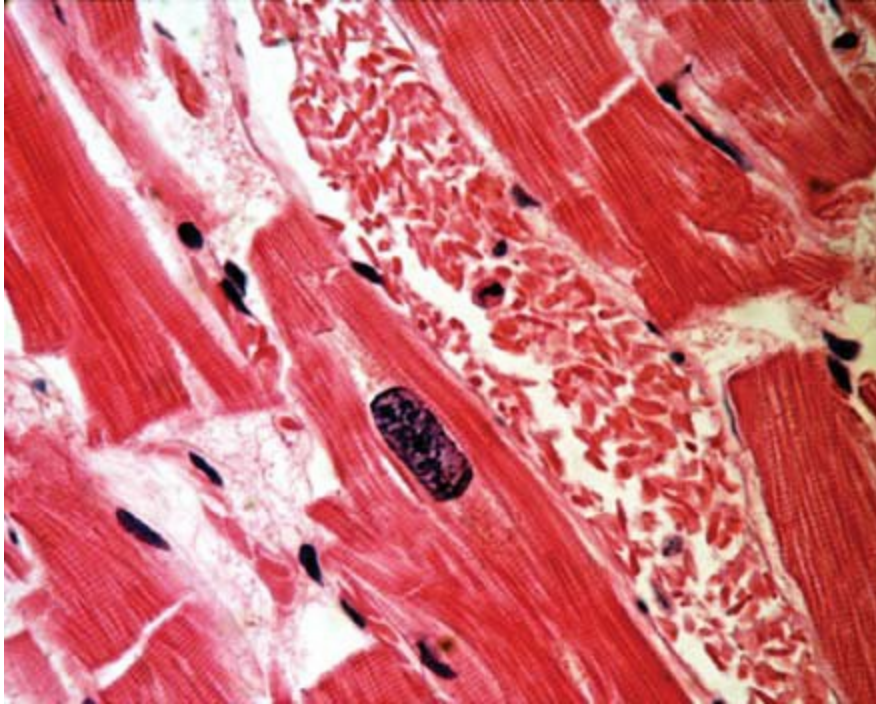
k . T m z m -

m m

m m .

.

(RBC) m x m m .



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PART 4 ■ Erythrocyte Disorders

Epidemiology

Thalassemia

α, β -

SCD αα ββ A -

αα, β -

, M, C, k .

S C Am, A, E I -

B m -

. T k carrier

m m , m P. alciparum m , k x m

z . T

m m f

m

. W k m

m m.

m , m k

H SC m A

m . T

Am , SC β m (S β m) k

, A Am . A ,

x . T k x m k m

m mm U S .

x . T

SCD, m z m SCD, m mm

.

m m . M

W k x , -

, Am SCD.

m . R k k

T x SCD m

RBC m m m . T

. T m -

m , m . I ,

SCD m m ,

.

A Am

. F H SC , m

Vaso occlu sion

m m .

T k -

m m vasoocclusion (F . .

Pathophys io lo gy

.). S k -

m m -

S ickling

m .

W H S x , m m z

T m m -

k . S q m

k ,

x ' k . T -

.

m m SCD m m

m , x z m **Clinical Signs and Symptom s** m . I z

m H SS m , m

A

m m m z m.

m k . A m P m z H S

, k k

x m x

. T , m m m -

. S k m x , H,

. O

, - , -

m , , H C, H O—

A . S k H A, H F (%), H J, α m .

W k , q

m m (MCHC) m m x . D x m S x m -

A x m S.

R , m

SCD . I

m z x m S

m m m -

m . I

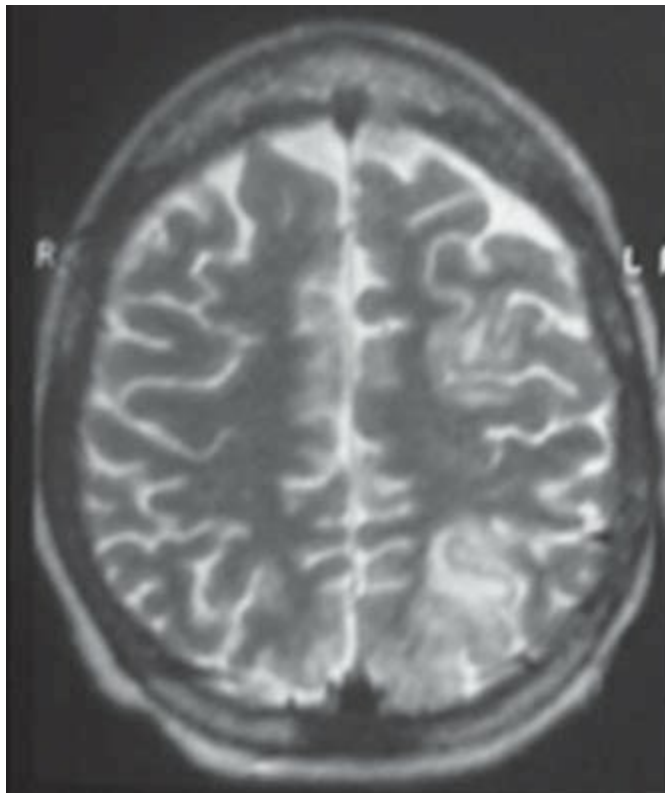
x , m

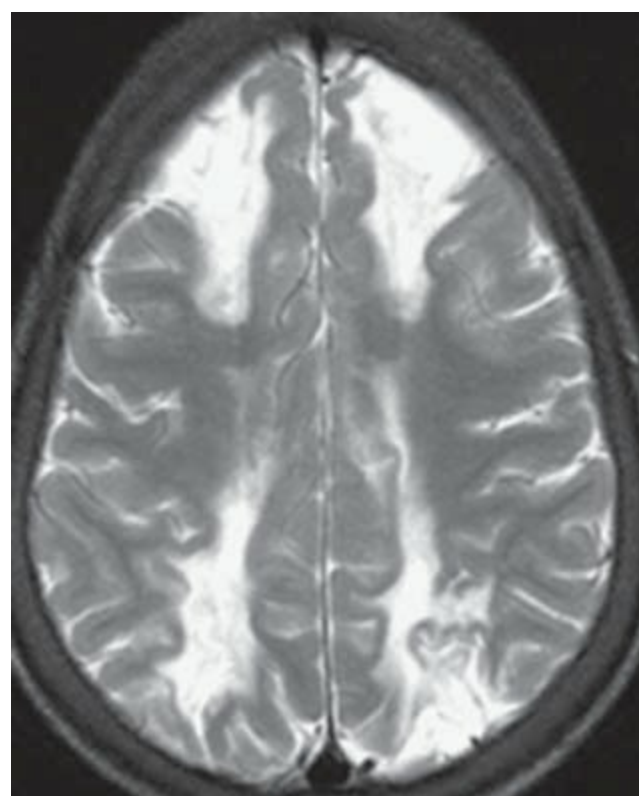
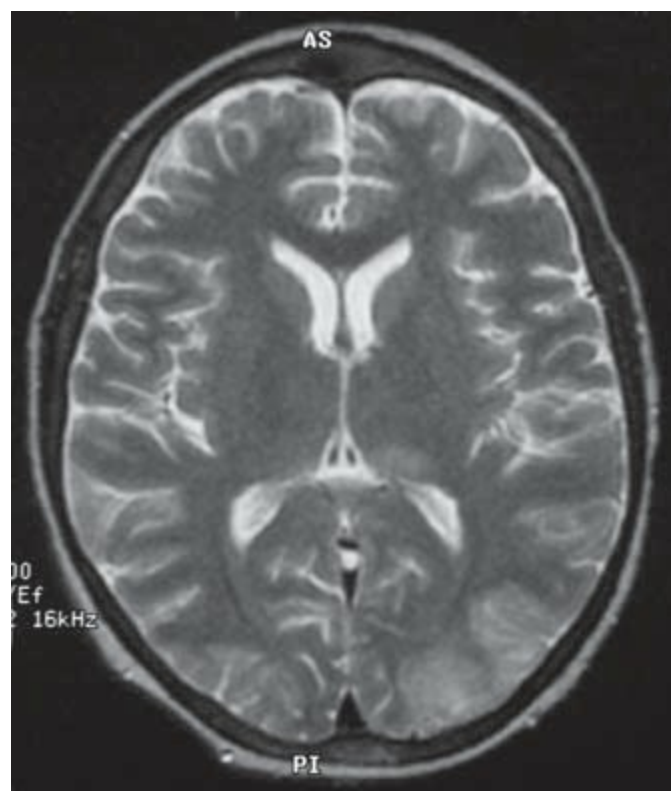
.

T m m m **FIGURE 17.3** V k . (F m B k m m m AP, P. Practical
Cardiovascular Pathology, P , PA:

m x .

L W m & W k , .)





CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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A

B

C

FIGURE 17.4 C . A. “S ” m k m . B. B

k m m m . **C. L m k m .** (F m N DG, H MA: Rogers’ Handbook o Pediatric Intensive Care, , P , PA: L W m & W k , .) m m ,

m

m , k , ,

m , m . T -

j , m .

q

k .

Sym ptom s Asso cia te d w it h Pre gna ncy

T SCD. M

I , m

m .

m m %

H , % % % %

m %.

.

Clinical Ma nife s t a tions in Ad ult s

Sym ptom s in Children

SS m z m m ,

I SCD, -

m % %. R

m . T

H F k m k

m ,

, , . I

k m ,

, m m -

m m Streptococcus pneumoniae Haemophilus

. T m -

in uenzae . I m q m .

(.).

A m , -

S m m

, m -

m . V

m . C m

q m m .

T m mm m

m m m .

Causes of Death Among Children

A m

TABLE

17.1 **with SCD**

SCD. H

m , m

Caus e

Pe rce ntage of To tal De aths

, m , -

m . T

Infection

44

m % %

Splenic sequestration

16

, m m % %. R m -

Sudden, unexpected death

14

. L m .

Cerebrovascular accident

12

F SCD

. A

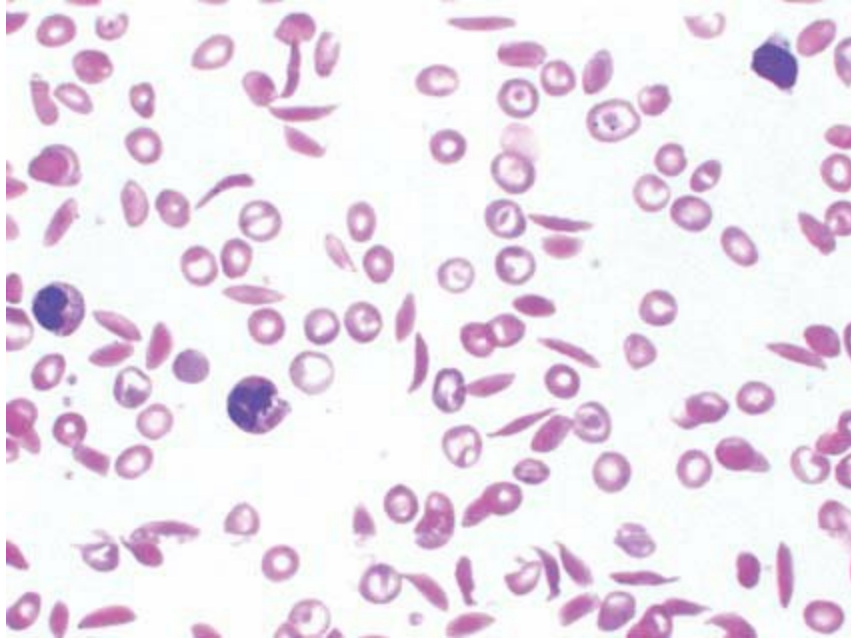
Congestive heart failure

7

Miscellaneous

7

, m , k .



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PART 4 ■ Erythrocyte Disorders

P m m m k

. R k

H F. P SCD m

. / L

(. \times /L) k.

General Signs and Symptoms

Pain

P k m k SCD

m m m . A

, ,

m q m SCD mm -

m m m

FIGURE 17.5 S k m . S k (straight arrows)

. H - m

(curved arrows) . (R m R

SCD,

R, S DS. Rubin's Pathology: Clinicopathologic Foundations o

m k .

Medicine, , P , PA: L W m & W k ,

, m .)

Pu lm o na ry Com plica t ion s

T mm SCD

, m , m ,

z . I -

. H -J m

m m m (-

m . I

) m

, k () m -

SCD z

$m(F \cdot \cdot B \times \cdot)$.

$k m. T$

$L m$

$m \cdot$

$(\% \%), m$

$S t r o k e$

$m m (M C V) L; -$

$m; j m m m ;$

$- S C D k$

$m m x ;$

$\cdot B H S m$

$m (L D H); m -$

$m, z m (A S); \cdot$

$m m ,$

$, , k \cdot$

Special Laboratory Testing

$C S C D -$

m

$O - -$

$x m m$

(IEF) - m q m -

. -

(HPLC). H m HPLC

SCD k

, , m . G

. B k

DNA m , k m k D .

m m

H , D ,

.

m mm z -

. m

k k. P x m -

BOX 17.3

m k

m k . R

m - m m

Com m on Laboratory S ig ns o f Hem olys is in

m H F k k S CD

m .

R (m)

Laboratory Tes ting

U j m

I

I m (. / L), m -

D m

, ,

D m m x

(WBC) , , × /L

I m m m m

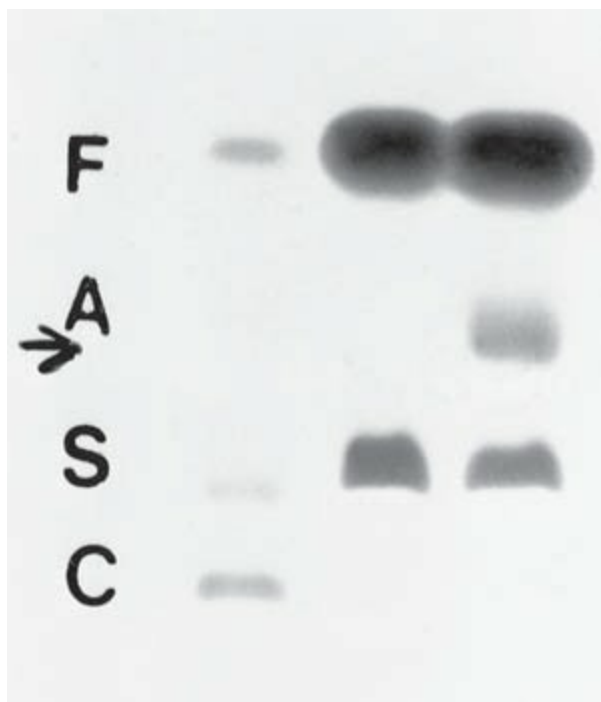
mm . T m

E LDH

m m , -

M AS

k , m . R m m



CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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D m m -

m m

, m m m -

DNA m (PCR).

, m ,

.

Pre na ta l Dia gnos is

P m m m

Hemoglobin Electrophoresis is

q SCD. A DNA m

A, L P -

m, m

m m SCD

m k . T -

m (F . .). T m

DNA

k m -

m k .

m q ,

k m .

Screening of Newborns for Sickle Cell Disease

I SCD, m % %

and Carriers

H S, % % H F, m m H A .

T m H F. T -

% H F % H A m m .

High-Pressure Liquid Chromatography

H F m α - γ - . D

E k H

m , β - -

m - x HPLC m

γ - . I m m

m q H A H F

, x m % - α γ -

m . T m m-

% β - .

m - -

S SCD m

m m m

D C m . SCD (H SS)

.

A Am U

S m

DNA Ana lysis

. W m -

W -

m j -

m , m

m , SCD

m m . T

- m . E

m

SCD

DNA .

k

T

. A m m

m m , , m j

, . V SCD

.

S x m k -

SCD. A k

m β β S ,

m H F H A H S.

T m H A H S

α m β .

S m

. A

k m -

, m

. C m

m .

B -

m m ,

. A m

, m

FIGURE 17.6 H m

m m . B m k

– H . . **Le lane.** A m x H C, m

H S, H F. **Middle lane.** C m k m m (. . , -

m H S H F, H A (m

k , , m). S m H S– β m H S HBFH). **Right lane.**

m k m

C m k H S, H F, H A. (R m M C KD. Clinical Laboratory k m m . S m m

Medicine, , P , PA: L W m & W k ,

-

, m .)

- .

PART 4 ■ Erythrocyte Disorders

Ex m m m -

■ N x . A m

m .

m m SCD.

I U S , m - m D x , m - IEF HPLC

x ,

q m m m . m x m k . T -

k m m .

%

■ N . T m

SCD. T - IEF H A, S, C

U S . N k -

m H F m m .

N , W A .

M k

m H S

In fe ct iou s Dis e a s es

m .

T m m .

V m , z A,

Managem ent o f S ickle Cell Dis e as e

H. in uenzae mm z . I , -

m mm -

T m m SCD :

q .

. M m

Blood Tran s fus io n

.

B x m m m . T m j -

. D m

SCD

; m , -

■ m x -

■ k m m

Tre a t m e nt

B m

. H m . / L

SCD. H , f , -

m m m

k , .

()

C m m SCD m -

. A -

. I m m

. A m

, m

. A m m

. G

. A m x

m , x ,

. S q

P m x, Haemophilus in uenzae , m -

. P ()

, , x m ,

Ex m

m m , -

, m m, m ,

. m , q

, m,

, q x . Ex

j m . P -

m m m ,

x m

k ,

q .

m , , -

m, , q -

Drug The ra py

.

D x , .

Ex m m : H x , , m -

■ G . G m H F m m SCD -

. F m

m m . A m

m z x m S. T x m m

x m . H x

H F.

m SCD m

■ B . B m

m . I

, m m H F

m , x

.

m q z

■ C m z . T - - m -

.

m ,

T x m m m k m.

m m , m m

CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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. B m ,

m . A m m m m k m - m

- m m

.

, , .

I , - m x

Hem atop oie t ic S te m Cell

SCD .

H m m (HSC)-

H x m m m

m m m -

q m q .

, x m ,

H , m x m m-SCD, β m .

, m

m , m

Pre ve nt io n

H F . B -

G m SCD.

,

W m SA (), -

m k m

m

m m .

k z DNA m m .

Ex m H F

Nove l Pha rm ace ut ica l The ra pie s

- m m - . S -

C , x FDA—

m x

SCD. S m

m

m SCD m

. M m - k - -

. N :

m m

m . R

■ m

SCD ,

■ m

H S m z .

■ x

■ - mm .

N x ; - mm

SICKLE CELL SYNDROMES:

, x m , ; -

PA HOGENESIS AND NEW APPROACHES

. B m x

SCD m z . T

SCD, k

SCD q H S

q SCD. I m k

. T -

m m m m

H S .

m m .

.

Bon e Ma rrow Tran s pla nt

Sickle b Thalass em ia

B m m SCD

E . C

T k m β

m m (. . ,

m m m

k , m ,) z : k β m (F . .). T -

HLA-m -

m

. A x m %

m k M . P

TABLE

17.2 Various Clinical States Associated with the Presence of Hb S

Clinical Severity

Genotype

Percentage Hb S

Percentage Non-S Hb

(Scale 0–4)

Other Clinical Features

SS

80–98

2–15 (fetal)

3–4

10%–20% reticulocytosis; rare

splenomegaly

S β thalassemia

60–90

10–30

1–3

Splenomegaly; microcytosis;

4+ target cells

SC

50

50 (hgb C)

1–3

Splenomegaly, 4+ target cells

AS sickle trait

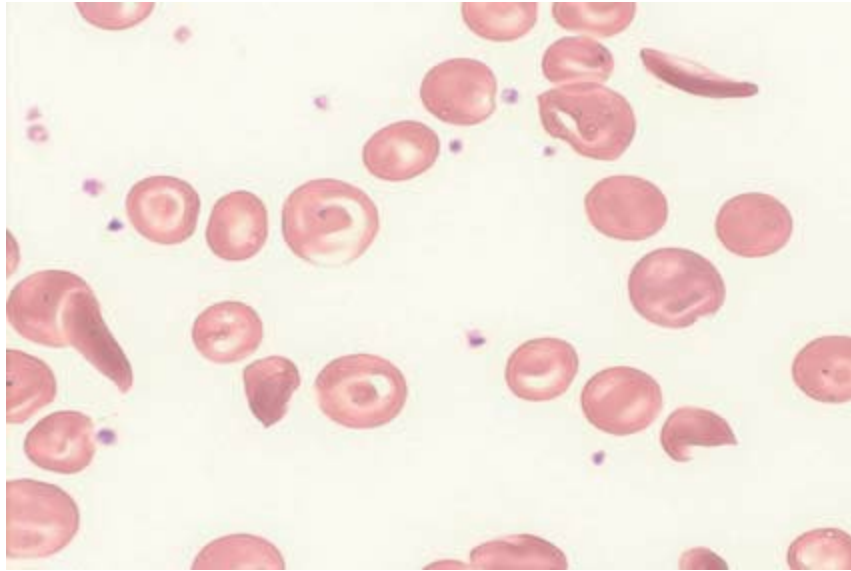
30–40

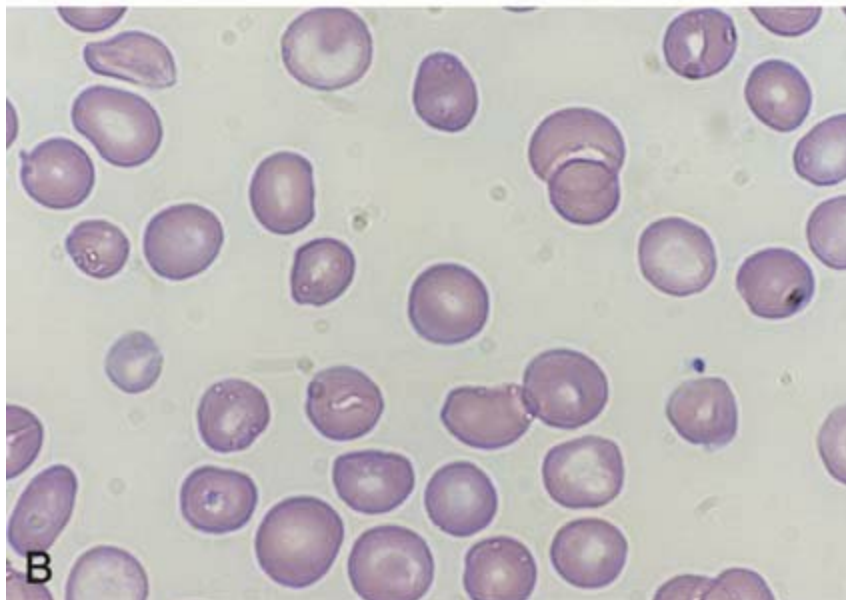
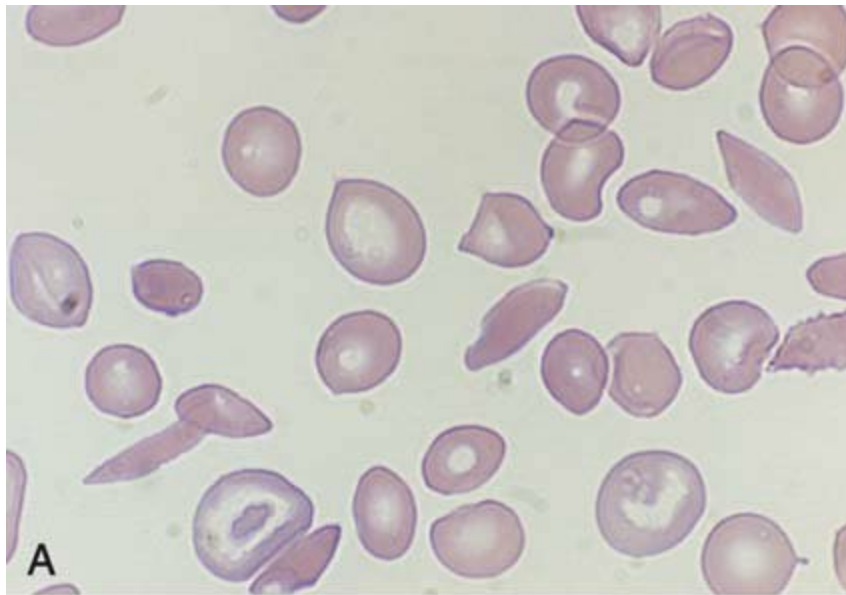
60–70 (hgb A)

0

Normal morphology; no

splenomegaly





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FIGURE 17.7 H S/ β m . (R m A SC.

Anderson's Atlas o Hematology, P , PA: W K

H /L W m & W k , C , m .)

m m m . S m

% .

P H A (S β -

m) SS . T

S β + m m k m m H A

x m m .

S β m x m

m m . T

m m , m -

FIGURE 17.8 H SS SC. **A.** S k m . **B.** H SC -

m , , , , k

. (R m G JP, . Wintrobe's Clinical Hematology,

. H m % %

, P , PA: L W m & W k , ,

m S % % (F). T

m .)

m SS . S m m

q m .

m m .

H S/ β m SCD. T

m k m m.

m , m mm .

C m mm , m

m m , m

(B x .).

S ickle -C Dise ase

I H SC , H S C,

H A m H F.

BOX 17.4

T m

SCD x : -

, m ,

Abnorm alities Re porte d w ith Sickle Cell Trait

m m m

AS S OCIATIONS WITH S ICKLE CELL TRAIT VERY

. M m %

LIKELY

m / L. S k

S

m , x m %

H

m .

H m

T m .

B

T m z SC (F . .)

m mm m SS ,

AS S OCIATION WITH S ICKLE CELL TRAIT POSS IBLE

q H C % H S. T -

P m m m

m SC . T

C m q

SC AS (. . , k

R

) . F , SC ,

P

m

A

H C. S , SC

I k x (

% H S S A . P

m m)

SC x m m m

CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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S ickle Cell Trait

m m

A x m % k A m z

.

H S. T , H S A. S k

I m m ;

-

m m q

m m m , P. alciparum,

z m m . G

m . S k m m m . S k

m m . T α

, -

m , ζ ,

$m_k \cdot L_x$

$m_m \cdot m_m -$

$m_k(AS) m$

$m, , \alpha-q.$

$m_H A. AS_k$

$O m_m, , -$

$SS \cdot A, AS -$

$m - \alpha m_m \cdot T \gamma$

z_k

$m_m \cdot I m$

$x.$

$\alpha \beta m,$

$m_m,$

$m.$

$m, k,$

$A m$

$m_k \cdot E$

m

$\% \% m$

$q \cdot O -$

k H S. C

m m k

m . T

m , (

,), , ,

. A m m

.

. A m q

, f

NOTE: This is a good time to complete Review Questions

mRNA

related to the preceding content.

. A m m

x

. A m m

HALASSEMIA

m

; mRNA m -

De mo g raphics

. A ,

T m m, q m m

m , -

m x x .

Pathophys io lo gy

A mm m . I , H E- β -

T m z

m H H m j

m . W , H E- β m

m m m . I α m ,

m q m . T

α - -

H E % m

m H H

S A . I N Am ,

m . T m β m

. α T m ,

α - , -

, z m

β -

. H H, H H- (CS),

. S RNA m m

m z α m m -

m β m

. C H H

RNA . T f RNA

m -

.

m H H H H-CS. H m z

α m , , m mm b **Thalas s e mia**

.

β T m m mm - -

Etiolo gy

. M m β m

m , , m -

T m m

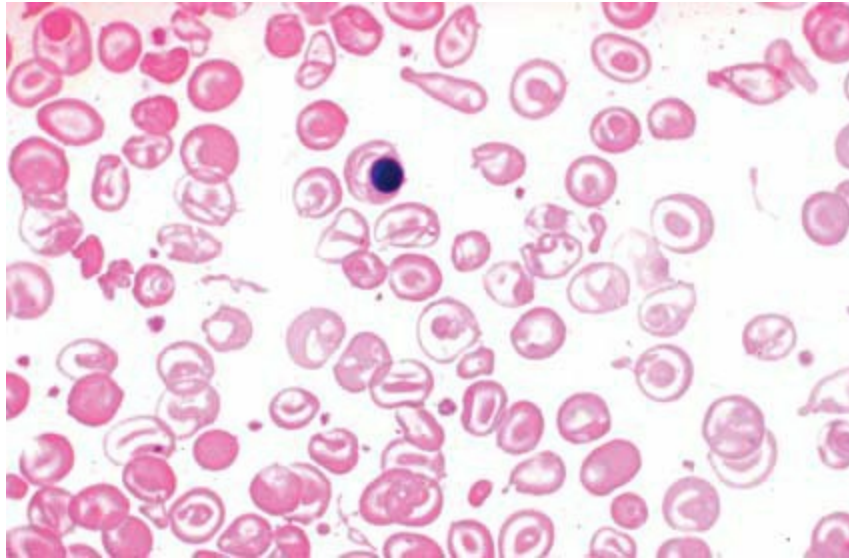
β - . M

. T

m β -k

m (. ., H S H C) m

β - ,



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x m γ - ,

, .

I k m x m

α - β m β - α m m , β m , m -

m ().

I

.

A m m β β -m

, . I

m m , q q α

β z , : α : β

FIGURE 17.9 T m . T

m m , k ,

. I β m , β

(arrows). (R m R R, S DS.

; , x α .

Rubin's Pathology: Clinicopathologic Foundations o Medicine,

T β

, P , PA: L W m & W k , ,

m ,

m .)

, m .

T x α

, m m m . M m

La boratory Fin din gs

- m ,

I β m , m

. P -

m , m , . T m

m

/ L m z

. T m m .

. P m , -

β T m m (.). T

k , m , , m , m -

m (F . .). E

. I m z β m , m m

m m . T

m m

(MCV, MCH, MCHC) . I

m γ - β - m

, (RDW)

. I , m m-

.

m . S m m

O

m m .

m (% %), m , m -

A β m -

, m , -

, m m z β m

- (IBC). S m

m -

, m k m .

m , mm z ,

S x m -

. P -

m -

m q

m .

m m m m ,

A β m -

. A m -

A m .

H m H F

. A m

H A. A m m z β

m m .

m m H A, H A ,

TABLE

17.3 b **Thalassemia Types**

Phenotype

Clinical Descriptions

Thalassemia minor

Mild anemia, microcytosis, abnormal erythrocyte morphology, splenomegaly

(Thalassemia trait)

Thalassemia intermedia

Moderate anemia and ineffective erythropoiesis, microcytosis, abnormal erythrocyte morphology, splenomegaly, iron overload, not transfusion dependent Thalassemia major

Severe anemia caused by ineffective erythropoiesis, transfusion dependent, organ

(Cooley's anemia)

damage (heart, liver, etc.) secondary to iron overload, extramedullary erythropoiesis, hepatosplenomegaly

CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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H F. T H A

x m m

β - .

. PCR m m

H z β m m k

. U m m

m m m .

m . PGD x HLA -

O MCV,

m . T

H A , m .

m m m

. mm z SCD,

mk , m .

m m . S m

m .

Pre natal Dia gno s is

P β m

Ne w born S cre en ing

m

H , m

DNA PCR m -

β m m j . I

. L , m

U S , m α -m m m -

, H H H H C S H E

. T m m -

m z .

m - m

m

- - (β/α) .

x m

B , m

m U S .

m m - k

I m , m

. P m (PGD)

m m HPLC IE

m z ,

- q m k m -

m . I H H

, q

H B ’

S um mary o f S elected Labo ratory

TABLE

17.4

x q - -

Findings in Hem og lobino pathies

. P m

Dis orde r

Te s t

Re s ult

m .

I m , m

SCD

Hemoglobin

Decreased

q -

PCV

Decreased

m . S -

m β

Erythrocyte count

Decreased

m m j m hereditary m -

Hb S

Signi cantly

(HPFH), H E β m m H EE, H H

increased

m α m . G m m

Hb A

Decreased

Hb F

Increased

.

Sickle cell trait

Hemoglobin

Normal

Tre a t m e nt

PCV

Normal

M m m -

Erythrocyte count

Normal

m . T m Hb S

Increased

m m

m

Hb A

Slightly decreased

x . I m ,

Hb F

Normal or slightly

q

increased

m m -

Homozygous β

Hemoglobin

Decreased

m. m m, m

thalassemia

. T

PCV

Decreased

:

Erythrocyte count

Decreased

■ (N Am

Hb S

Negative

)

■ x m

Hb A

Signi cantly

■ x ()

decreased

Hb F

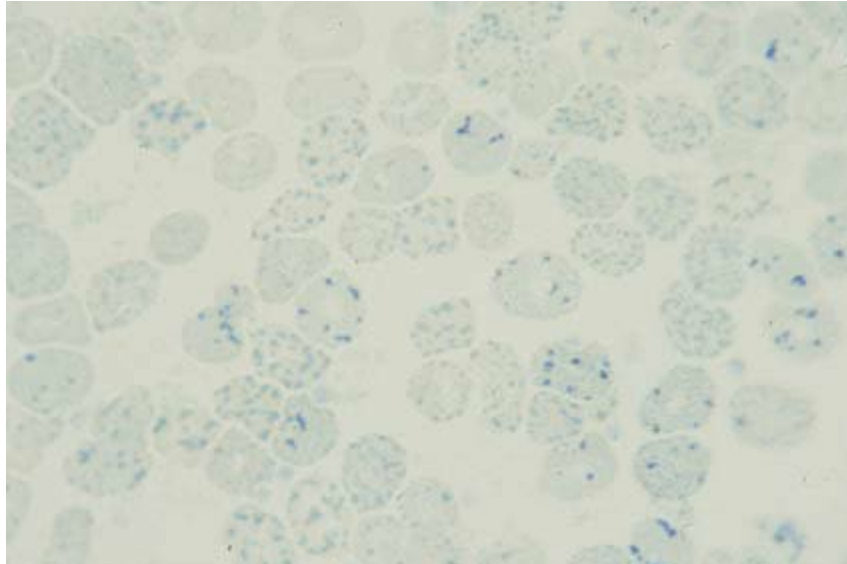
Increased

T m x m

m

PCV, packed cell volume.

' q .



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B m m HLAm m z -

β m . H m mm ,

m HLA-m ,

. C

.

S k m m . S m m m β - (m

) ' m , mm

m

.

FIGURE 17.10 α T m (-) H H .

A m

(R m A SC. Anderson's Atlas o Hematology, m ' -

P , PA: W K H /L W m & W k , C , m .) H F— m m

. S

H F m ' ,

m β . T m z -

m .

m m α m .

I m , P α -m (α m -) m .

m α . T m α - β - -

S -

x β .

. U , m -

T H H .

m

H H, m m β ,

.

f m x (\times f H A).

E H H H H

Preve nt io n

(F . .).

C S

T

m z β m m

m m m H H

%.

m

. H H , m -

a **Thalas s e mia**

m m m q

m S A . A

I β m , m

m x . C , -

m , m j α m

, m x -

m α -m -

. H m m / L.

m m . α T m

R (MCV, MCH, MCHC) . T

m m

m % %. H m

:

m H H (% %), m

. S (α)

m H B , m m

. α T m (α)

H A .

. H H (α)

α m m

. H H B (α)

q m α - : H H -

m m ,

I , m -

H H m . O

α . T m α

H H m z

q m α m

.

m ; ,

H H B

m m . H m -

. T k α -m

, m m ,

. A

m m .

(F . .).

H m , m

, mRNA

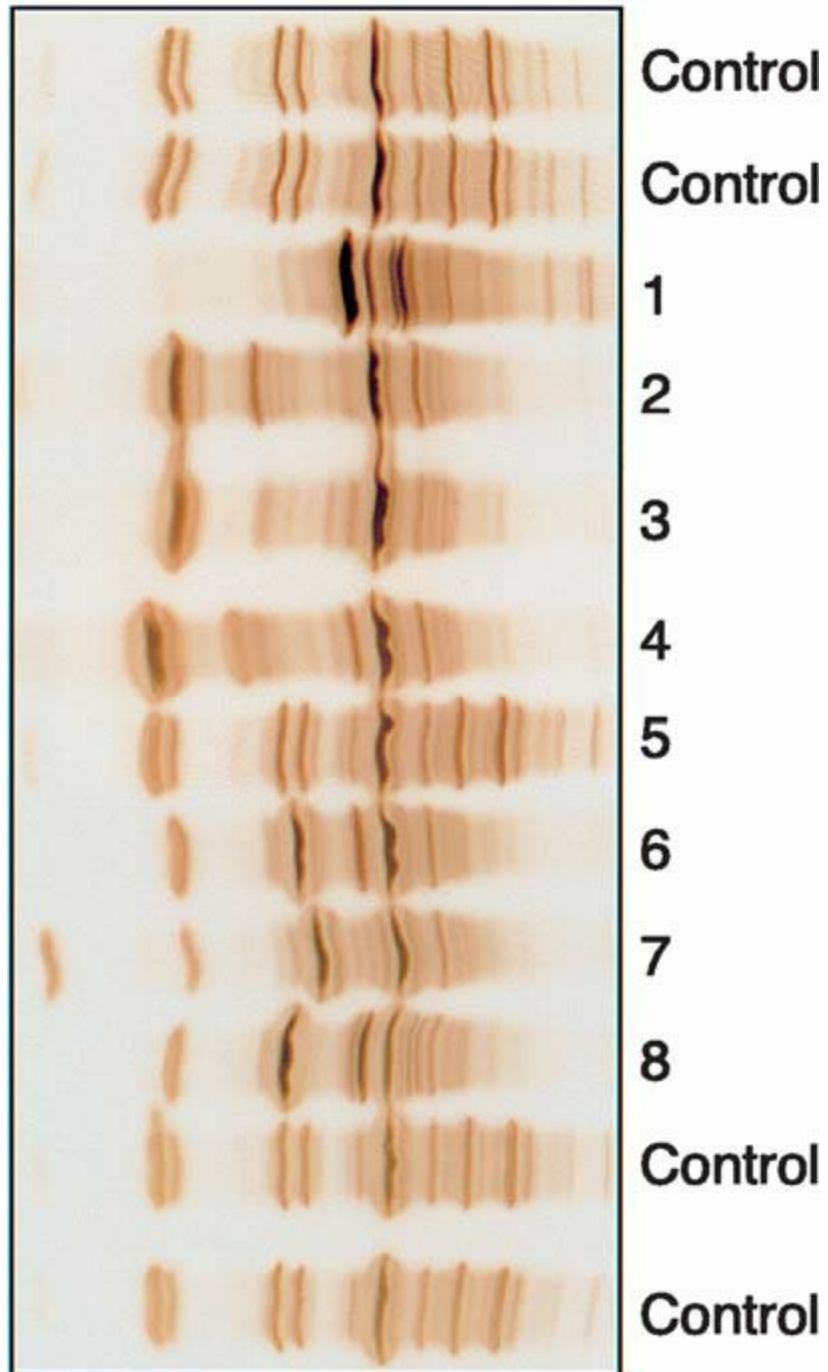
m . I m m m m-NOTE: This is a good time to complete Review Questions

m α , -

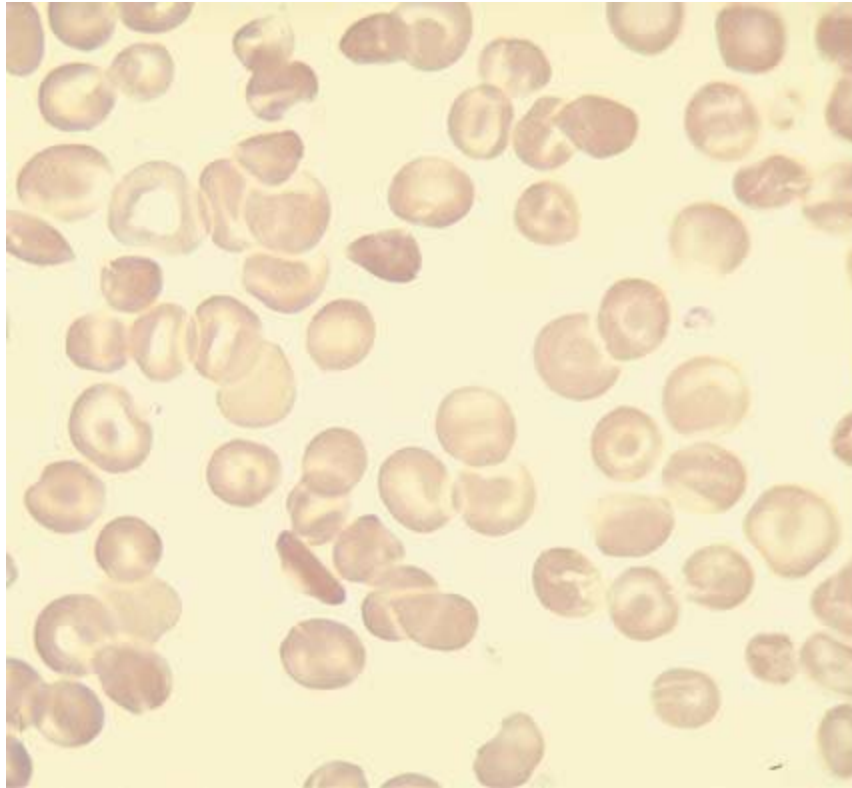
related to the preceding content.

m x - m ,

— C A₂ SGA JN +



CM877749-13



CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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FIGURE 17.12 H C . C : M .

D : H x , -

k; m m m ; m

. C : H C C . (R

m A S C. Anderson's Atlas o Hematology, P ,

PA: W K H /L W m & W k , C , m .) m m , m m m . H C m m .

L m m .

He mo g lo bin S C Dis eas e

T m H

FIGURE 17.11 IEF x m mm m

S m H C m -

. 1, H F . T H B -

. T m SCD,

, α -m ; 2, H O-A ; 3, H E ; 4, H C ; 5, H C-S-G, J, N; 6, H D- H C k

P j ; 7, H G-P ; 8, H S . M -

H S.

m m C m m m m SCD.

m m . (R m M C KD. Clinical

L x m m

Laboratory Medicine, , P , PA: L W m

, , ,

& W k , , m .)

(F . .).

He mo g lo bin D Dis eas e

O HER HEMOGLOBINOPATHIES

H D . P m z He mo g lo bin C Dis eas e z m m . S m m

x m m . H D

$T_{mm} -$

$m_m H S H G k -$

$H S (k) . H C m$

$H_m H A H.$

$H A m ,$

$, m \times m$

He mo g lo bin E Dis e as e

$m (NH) m \beta . T x$

$H S ; , m$

$T m q$

.

$S A . H E / \beta m$

$D x m C m m . I m T , q (F . .) . E m$

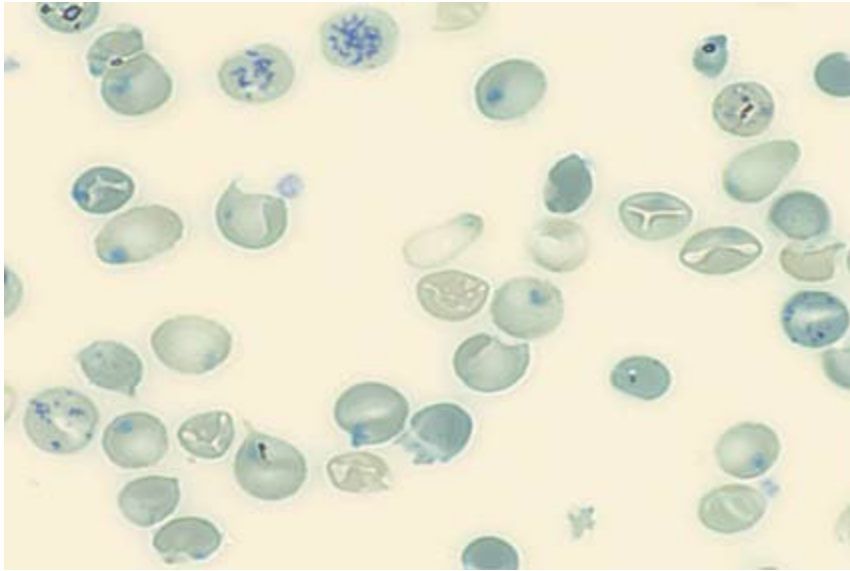
$H E m \% . H E m$

$m m m , m \%$

$m z (E/E) z m (A/E)$

$m x m . C$

$m z m \alpha$



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β m . H E

m m β

m .

C . I m

m m (A/E) m m (E/E) -

m (E/ β).

I m z , m m m m .

m . I

H E m m

m , (,

H) z m -

. I U S , m H E

FIGURE 17.13 H z . C . (R m m -

A SC. Anderson's Atlas o Hematology, P , PA:

m - k (. .,

W K H /L W m & W k , C

S A).

, m .)

He mo g lo bin H Dis eas e

m m . P q

H H m m m .

m m m x

T m q m

m m x m m

α - (α -m).

% %. T m m m m -

T m m

m x . I

S A , M ,

m x m

M E . B x mm -

m x . Ex H M

m S A ,

- - -

H H U S

(G PD) , m

.

m m m q k m H H m k H

. I -

m . T m m

m m , x m m H H . A .

x m . T

m m , x

Uns table He mo glo bins

H H , RDW

U m m

MCV . A

m k

m x m , , k , m

m . T

m m . M m

% % m

m m .

m . S m m

I m m m

.

H z . A

, m , m m m

Methe mo glo binem ia

, m . H m -

M m m

m m

m m . C

x z . H z -

m m m q x , (F . .) α - β - m -

H M , NADH-m m (

. m m , H B H H,

). .

m .

H M m . I m -

m m

HEREDITARY PERSISTENCE OF FETAL

hemoglobin.

HEMOGLOBIN

NADH-dependent

, $k_{RH}F(m)$ -

.

$m \cdot T \times \% m$ -

P_{mm} -

$\cdot T_m$

$m \cdot m \%$

H F.

CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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■ $C_{mm}HSC$

NOTE: This is a good time to complete end of chapter

β -m (S β -m).

Review Questions.

■ $IUS, m\%A$

Am.

■ A

CHAPTER HIGHLIGHTS

metals. As

, the

Hemoglobin Defects

.

■ Defects -

■ As a result of the mutation, the

the

Sickle cell disease

■ Pathophysiology -

.

the

■ The most common types are C, E, D, SC, SE, and SD.

Thalassemia

■ In S, the T chain is deficient

■

the T chain

x

the

the -

.

k

T m m

x m S m z m



m -

m m -

, f mRNA,

m m

x , ,

x .

.

■ A m S m

C m m : m j

m



m . T

m .

.

■ β T m m mm -

■ I m m C, m m

. M β -

m x

k β - ,

m .

x m

■ H m m m m -

γ - , ,

. A x m % m m-

.

z

α m z m .

■

α . α m

■ T mm m m

-

m m

m .

.

■ I m ,

m . A

m z

Other Hem og lobino pathies

m -

z , m m -

■ H C m

. A z m

H S (k) .

m m .

■ H E q

S A . I m T , q

S ickle Cell Dise ase (Sickle Ce ll Anem ia)

H E m %.

■ M m m

■ SCD z H S, m , m m .

m

■ U m m

k m

m k -

.

m

■ SCD (H SS), m mm m m -

m m .

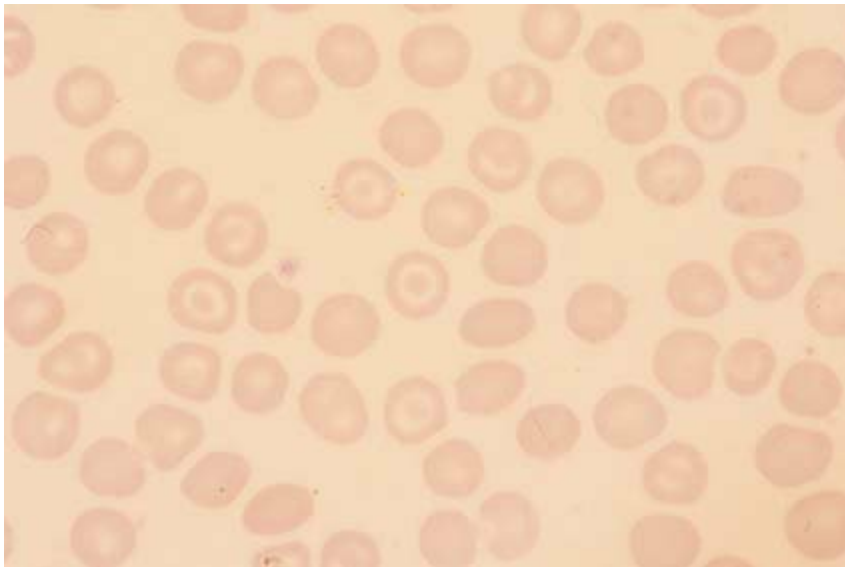
, x k

■ H H F

m . O k m z m m -

k .

.



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PART 4 ■ Erythrocyte Disorders

CASE STUDIES

Cas e S tudy 17.1

. W k ?

A - - k m m

. W ' ?

. S CBC

.

Cas e S tudy 17.2

■ Laboratory Data

T - - L x -

T :

z , m ,

m . T

H m . / L

E m SCD x -

H %

k . O m , -

RBC . \times /L

: CBC, ,

WBC . \times /L

m , m , H S -

T RBC :

, , , .

MCV L

MCH .

■ Laboratory Data

T :

MCHC / L

H m . / L

T ' m m -

H %

m , m ; , m m

RBC . \times /L

() m .

T RBC :

A m m

m m . T -

MCV L

m . A m

MCH

m RBC

MCHC / L

m .

T WBC . \times /L. T

O , -

m m , m , m -

k , k -

, k , m ,

, m . T

H -J , m , m

:

k RBC (), (RBC) S k

m / WBC . T

H S

m m ,

H : H A % , H F % , H S %

. \times /L.

' m . m / L.

H m m H S % H

F %. T H S . A

,

m . T m .

■ Critical Thinking Group Discussion Questions

. W ' ?

. W ?

. Ex -

m.

Cas e S tudy 17.3

■ Critical Thinking Group Discussion Questions

A - - m G k -

. W m

S F . S

' m m ?

m . S

CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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C

C A

A S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

■ Laboratory Data

$z \beta m . H ,$

$H m . / L$

“ m ” k -

$H m \%$

. T :

$RBC . \times /L$

CBC, , m .

WBC . \times /L

T m , -

■ Laboratory Data

k - , m , -

T :

m . T . %.

H m . / L

H %

■ Critical Thinking Group Discussion Questions

RBC . \times /L

. W ?

H WBC . \times /L. T

. W ?

m m ,

+ m , m (). H

. W ?

RBC :

MCV L

. H ' ?

MCH .

MCHC / L

. W , m AS -

m m ?

T m

m . T m

. W m m ?

H A (, . %; m , %

%) F (, %; m , %).

T m .

Cas e Study 17.5

O

A - - m z

.

m kk N . O x

■ Critical Thinking Group Discussion Questions

, x m

. Ex m m

. S

.

x

m E C U S

. W k m ?

K m , N .

A , -

. I k m , , x -

m ?

, m

q , . S

Cas e Study 17.4

m

K m .

A - - I m m I K m , x m m m m m x m .

. A C B C

T . S

.

j m ,

k . S

■ Laboratory Data

m m

RBC . \times /L

. S . H m -

H m %

m ,

H m . / L

. S . S

WBC . \times /L

x k .

P \times /L

MCV L

P x m . S

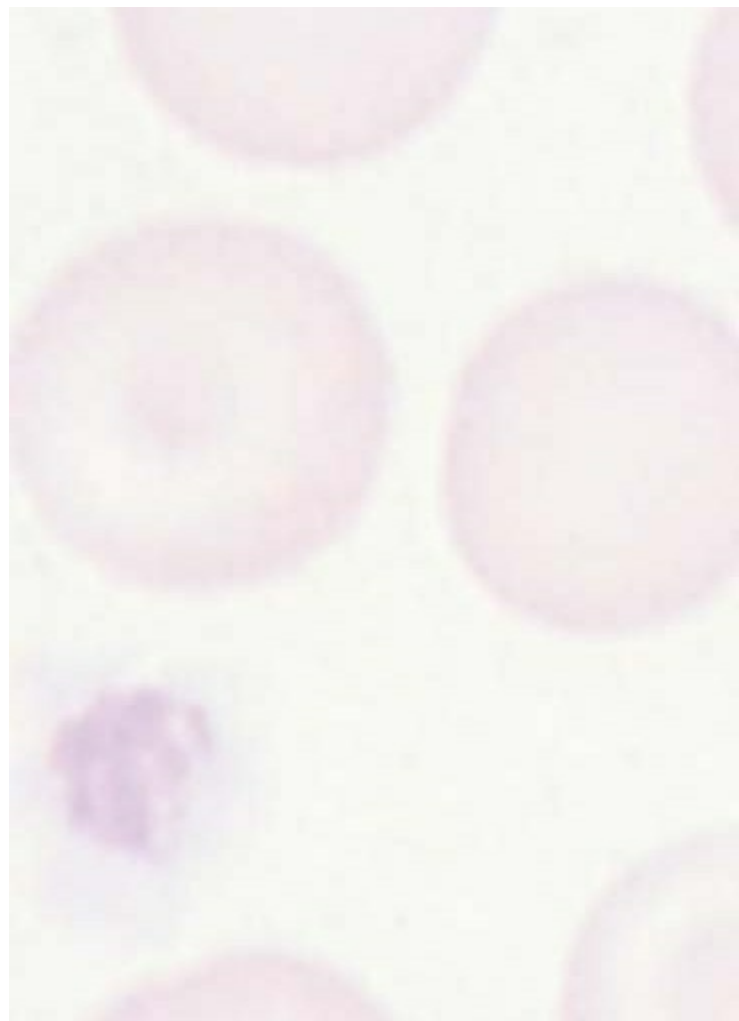
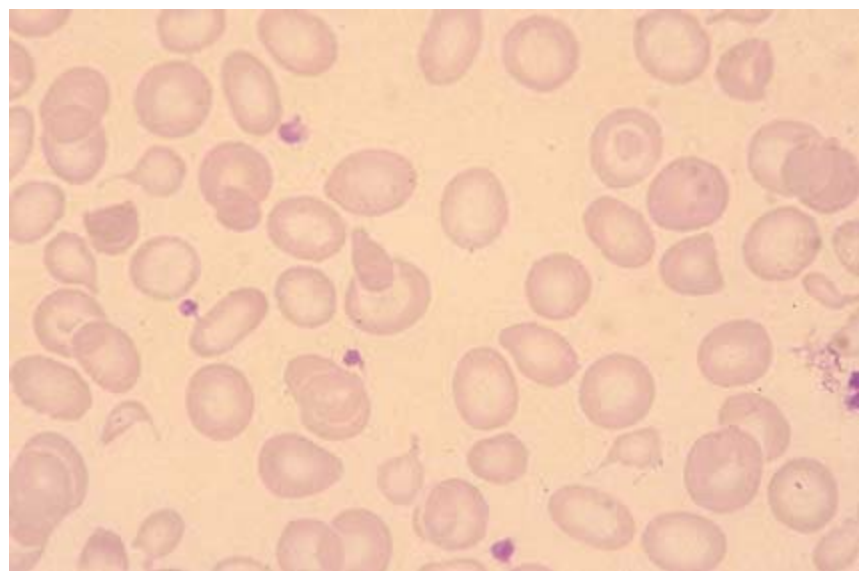
MCH

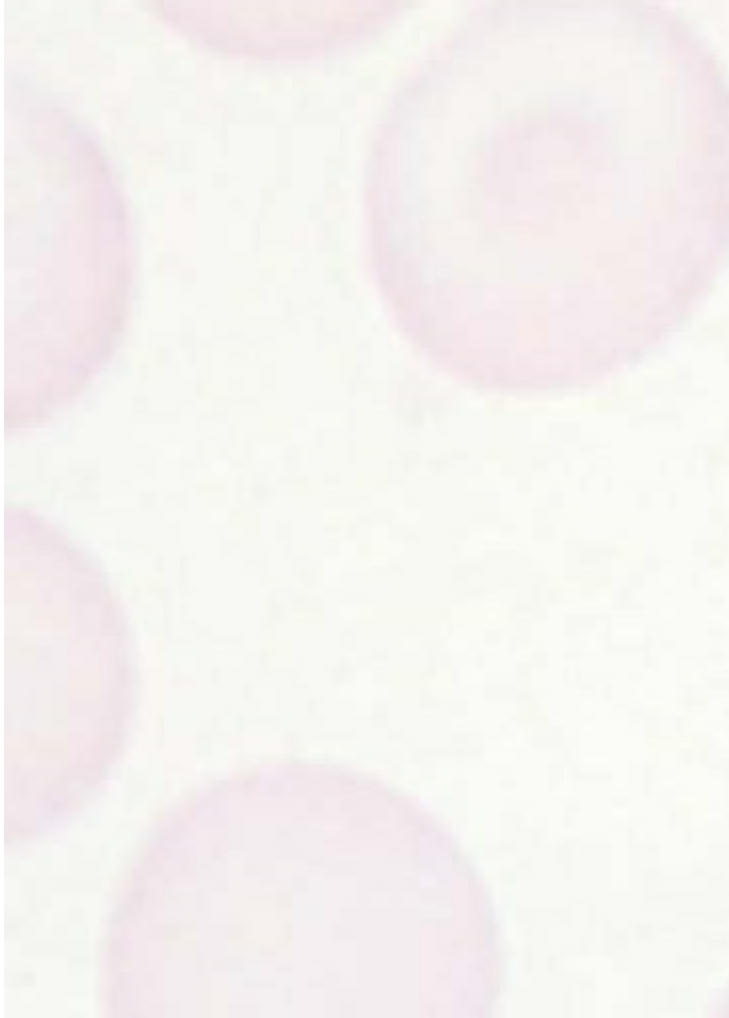
, , m . H

MCHC / L

.

(continued)





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PART 4 ■ Erythrocyte Disorders

CA

CA S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

■ Leukocyte Differential

MCH

%

MCHC / L

% m

%

S m m

% m

m . U m .

% m

T m + m -

, + , + .

U + .

■ Critical Thinking Group Discussion Questions

. W ?

. D

m ?

. W ?

(R m A SC. A ' A o Hematology, P -

. W ?

, PA: W K H /L W m & W k ,

, m .)

. T m A . H

■ Critical Thinking Group Discussion Questions

k ?

. W ' m

m ?

Cas e Study 17.6

. W - ?

A - - T m

m m . T m

. W m m ?

m , m , RBC ; m ;

; .

. W m ?

■ Laboratory Data

H m %

NOTE: This is a good time to write out answers to the

H m . / L

Critical Thinking Group Discussion Questions.

MCV L

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

3. N m m m-1. T mm m m : H A (% %), H A (% %), H A

(% %), H F (%).

A. m m

A.

B. m

B. F

C. m

D. q m

4. I m ,

A. z m m

2. H m

B. z m m

A. m m

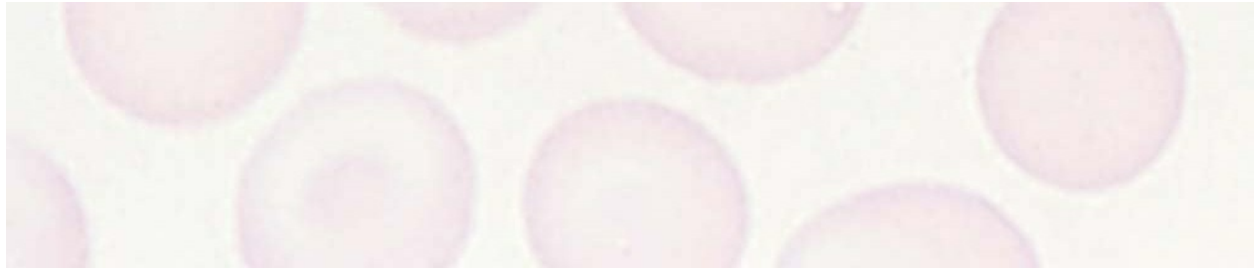
C. m z m m

B. m

D. m z m m

C. m

D.



CHAPTER 17 ■ Hemoglobinopathies and Thalassemias

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REVIEW QUESTIONS (continued)

5. I k ,

12. T m z

A. (GA G)

A. m m q m

B. m

m

x m

B.

m

C. m C.

D. A B

D. k m m

6. I_k, m

*13. $H_m z \beta m$

A. m

A. $m m$

B. $m m m$

B. $m m$

C. q

C. $m m$

D. $m m$

D. $-m$

*7. $O_m m m m$

*14. $I_{\alpha-m}, \alpha, m$

?

A. k

A. $H A$

B. k

B. $H A$

C. αm

C. $H H$

D. $H SC$

D. H F A

8. I k

*15. W m k m m j

, m m x

q m

A.

?

B.

A. I

C.

B. C x

D. m

C. P m

D. H

9. W m k Am -

z H S?

*16. T m -

A. %

α m

B. %

A. m m , m

C. %

B. m , m

D. M %

C. m , m

D. m , m

*10. W k

k ?

*17. T m

A. I H x .

' z β m

B. Ex m .

C. Ex m x

A. H A m

.

B. H A

D. S k .

C. H A

D. H F

11. T m mm m k

?

*18. D x m C

A. A

A.

B. O

B.

C. S k

C. m

D. A

D. A C

(continued)

PART 4 ■ Erythrocyte Disorders

REVIEW QUESTIONS (continued)

19. T H E m

*20. M m

A. S A

A. m m

B. C

B. m m

C. V m

C. m

D. N Am

D.

D L G. M m , N Engl J Med, (): – , **COMPANION RES OURCES**

.

:// . . m/

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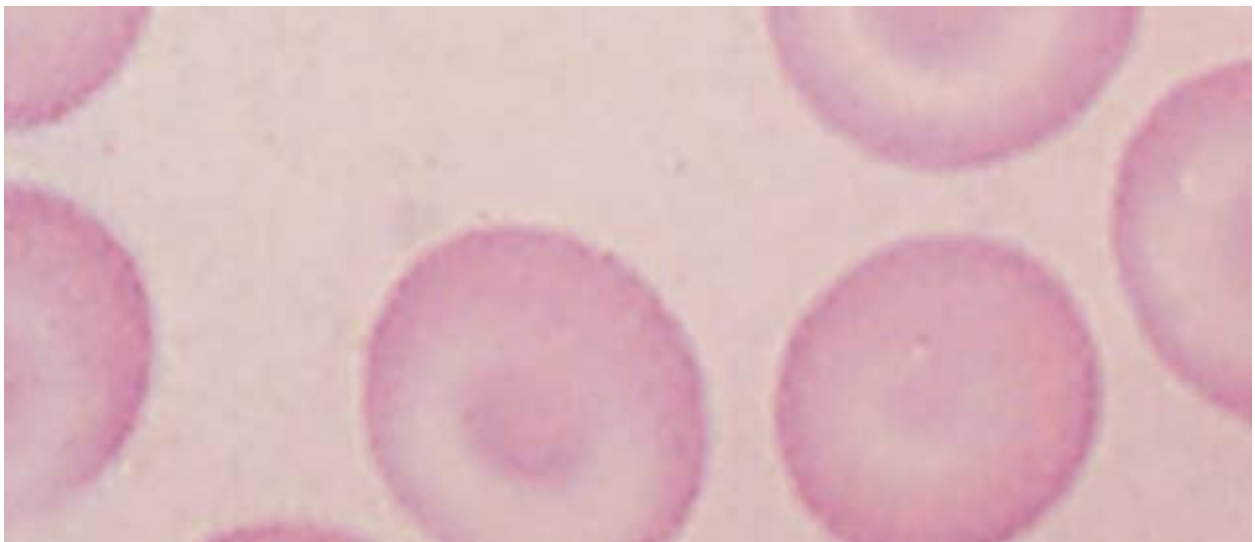
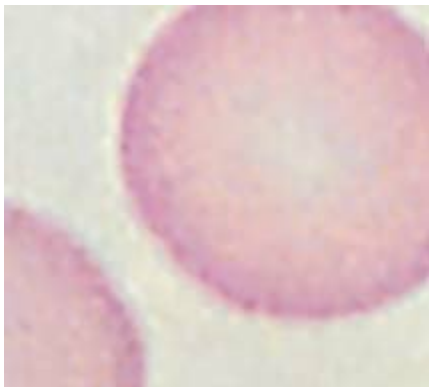
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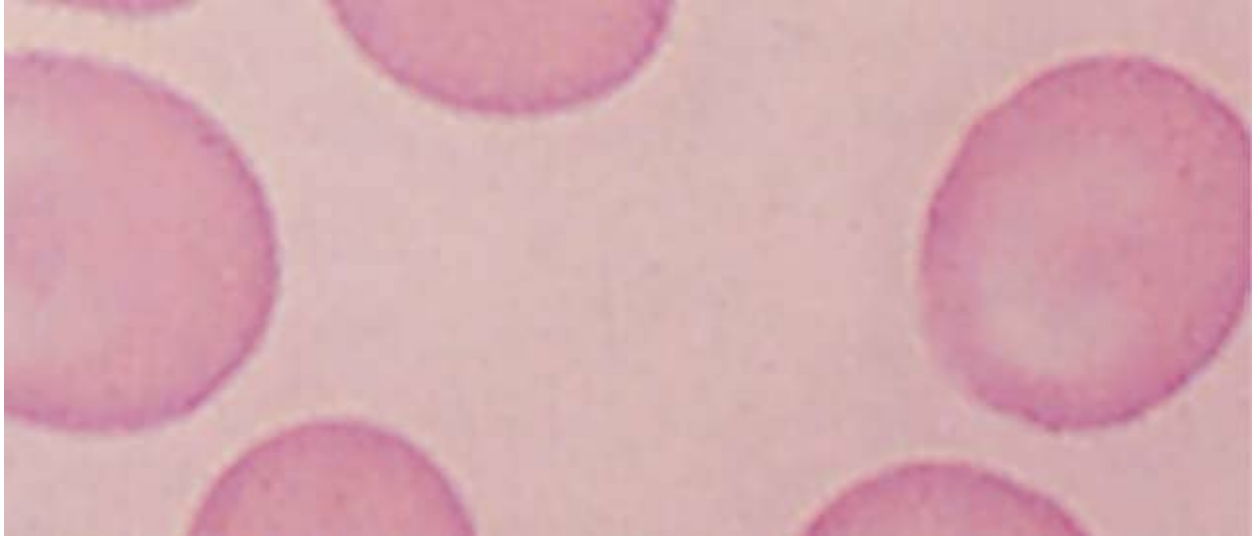
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PART FIVE

Nonmalignant Leukocyte Disorders

Disorders of Granulocytes and

CHAPTER

Principles of Blood Collection

18 Monocytes

KEY TERMS

Alder-Reilly anomaly

hyposegmentation

myeloperoxidase (MPO) deficiency

Chédiak-Higashi syndrome

Lazareukocyte syndrome

neutropenia

chemokinesis

Leukocyte adhesion deficiency (LAD)

Niemann-Pick disease

chronic granulomatous disease

leukocytopenia

Pelger-Huet anomaly

Döhle bodies

leukocytosis

reactive neutrophilia

Ehrlichia

lysosomal storage diseases

Seal-blue histiocytes

eosinophilia

May-Hegglin anomaly

Tay-Sachs disease

Gaucher's disease

mucopolysaccharidosis)

toxic granulation

hypersegmentation

myeloblasts

LEARNING OUTCOMES

Granulopoietic alterations : quantitative

■ Describe the appearance of cells with the following cytoplasmic

disorders

abnormalities: toxic granulation, Döhle bodies, Ehrlichia, and

■ Define the terms leukocytosis and leukocytopenia.

vacuoles.

■ Explain quantitative neutrophil responses in normal and nonmalignant

■ Recognize cellular alterations when stained blood films and digital

nant conditions.

images of Chédiak-Higashi syndrome, Alder-Reilly inclusions, and

- List examples of general conditions that can cause leukocytosis.

Ehrlichia are observed.

Summarize the pathophysiology of granulopoietic alterations.

- Recognize May-Hegglin anomaly on stained blood films and digital

- Describe at least one representative condition in which an

images.

increase in neutrophils, eosinophils, basophils, or monocytes can

- Abnormalities of mature granulocytes in body fluids.

be found.

Describe abnormal inclusions that can be seen in granulocytes

Assess the steps in the workup of a differential diagnosis in

obtained from body fluids.

neutrophilia.

Describe the methods for recognizing leukemoid reactions versus

Inherited functional abnormalities

leukemia, and leukoerythroblastosis.

Assess and summarize the clinical and laboratory characteristics of

- List examples of common conditions that can cause neutropenia.

May-Hegglin anomaly, Chédiak-Higashi Syndrome, and Alder-Reilly

Compare the conditions of transient and congenital neutropenia.

anomaly.

- List at least one representative condition in which a decrease in neutrophils, eosinophils, basophils, or monocytes can be found.

Granulopoietic alterations : qualitative disorders

Describe the characteristics of lazy leukocyte syndrome and leuko-

Morphological abnormalities of mature

cyte adhesion deficiency.

granulocytes

Compare chronic granulomatous disease, myeloperoxidase (MPO)

- Identify nuclear alterations such as hypersegmentation, Pelger—deficiency, and other defects in microbicidal activity of neutrophils
- Huët anomaly, pseudo—Pelger-Huët anomaly and pyknotic forms on
- and monocytes.

stained blood films and digital images.

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Monocyte-macrophage disorders

Cases studies

- Describe the general characteristics of lysosomal storage disorders.

nalyze the patient history, clinical signs and symptoms, and labora—
Identify and differentiate the defects found in Gaucher’s disease and
tory data for the stated nonmalignant disorders of granulocytes and
Niemann-Pick disease.

monocytes case studies, answer the related critical thinking ques—
Compare the etiology and laboratory ndings in miscellaneous lyso—
tions, and conclude the most likely diagnosis.
somal storage disorders.

NOTE:

■ indicates MLT and MLS core content

indicates MLT (optional) and MLS advanced content

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CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

337

Leukocytic Increases or Decreases

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TABLE

18.1 and Examples of Related Disorders

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Neutrophilia

Neutrophilia

BGP .

In ammatory conditions

W m ,

Infection

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Physical stimuli (e.g., heat or cold)

” “ ” z

Surgery

m mm . S

Burns

m -

Stress

x j

Some drugs and hormones

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Some types of leukemia

z x m .

Eosinophilia

Active al ergic disorders (e.g., asthma

N

and hay fever)

“reactive neutrophilia.” C

Dermatoses

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Nonparasitic infections

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Some forms of leukemia

WBC \times /L m

Parasitic infections (nonprotozoan)

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Basophilia

Ulcerative colitis

m m k m

Hyperlipidemia

, m , -

Smal pox and chickenpox

k k (LAP) , m

Chronic sinusitis

.

Chronic myelogenous (myelocytic)

A

leukemia

k -

Polycythemia vera

. L k

Monocytosis

Infections (e.g., tuberculosis and bacte—

m m

rial endocarditis)

m / .

Fever of unknown origin

In ammatory bowel disease

Neu troph ilia Wo rkup

Rheumatoid arthritis

Hematological disorders (e.g., hemolytic

A k

anemia)

m x m . A

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Neutropenia

Bone marrow injury or in ltration

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Starvation

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Anorexia nervosa

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Cyclic neutropenia

I

Increased destruction or utilization

mm , x m -

Entrapment in the spleen

. A m (CBC) k

Eosinopenia

Glucocorticosteroid hormones

m (ESR) (

Acute bacterial or viral inflammation

C) . O -

Basopenia

Hormones (e.g., corticotropin and

, m x m m

progesterone)

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During ovulation

Thyrotoxicosis

Eosinophilia

Monocytopenia

No known conditions

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PART 5 ■ Nonmalignant Leukocyte Disorders

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CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

339

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NOTE: This is a good time to review the de nitions of the Key

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Terms in the Glossary and ash cards on

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Y m , A , W E . B m -

a good time to complete Review Questions related to the

m m

preceding content.

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m m , m z , k .

MORPHOLOGICAL ABNORMALITIES OF

Eosinopenia

MAJORITY GRANULOCYTES

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m mm .

Nuclear Abnormalities

Basopenia

T m m , -

Hyperegranulation

, m m -

Hypersegmentation m q m

. P x m .

m m

TABLE

18.2 Com parative Morpholo gical Characte ris tics o f Granulocyte Abno rmalities Clas s i cation

Nam e of Abnormality

Major Characte ris tic

As s ociate d Conditio n

Nuclear

Hypersegmentation

Segmented neutrophils with >5 lobes

De ciencias of vitamin B12 or folic

Abnormality

or nuclear segments

acid with abnormal y enlarged, oval-shaped erythrocytes

Pseudohypersegmentation may be

seen in old segmented neutrophils.

Pelger-Huët anomaly

Mature neutrophils with heavy

Abnormal nucleic acid metabolism,

chromatin clumping.

although the specific abnormality is unknown.

Nuclear shape may resemble a

Function of the cells are considered

dumbbell (pince-nez form) or a

to be normal.

pair of eyeglasses.

Pyknotic nucleus

A condensation of a neutrophil

Artifact of EDTA anticoagulated blood

nucleus

that has been stored for ____ hours.

Distinguish from similar-looking

nucleated red blood cells, e.g.,

metarubricyte or normoblast.

Cytoplasmic

Toxic granulation

Prominent dark granulation,

May represent the precipitation of

Abnormality

either none or heavy, can be observed

ribosomal protein (RNA) caused by

in band and segmented neutrophils or metabolic toxicity within the cells
monocytes.

Most frequently associated with

infectious states. It may be seen in

conditions such as burns and malignant disorders or as the result of drug.

(continued)

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PART 5 ■ Nonmalignant Leukocyte Disorders

TABLE 18.2

Comparative Morphological Characteristics of Granulocyte Abnormalities (continued) Classification

Name of Abnormality

Major Characteristics

Associated Condition

Döhle bodies

Single or multiple, light-blue-staining

Represent aggregates of rough

inclusions on Wright-stained blood

endoplasmic reticulum (RNA) and

smears

may be associated with a variety

of conditions such as viral infections, burns, or certain drugs. Döhle

body-like inclusions may be seen in

May-Hegglin anomaly.

Ehrlichia

Ehrlichia are small, gram-negative

bacteria that primarily invade leukocytes. Ehrlichia typically appear

as minute, round bacteria (cocci),

ranging from 1 to 3 μm in diameter.

In the leukocytes, Ehrlichia divide to

form vacuole-bound colonies known

as morulae, the Latin word for mulberry, referring to the mulberry-like

clustering of the dividing organisms.

The formation of morulae is a defining characteristic of this group of

bacterial pathogens.

Vacuoles

Circular membrane-enclosed

Number and/or size of vacuoles

structures in the cytoplasm

increases as a cell ages. The contents of vacuoles consist of water and soluble waste products of the cell.

Inherited

May-Hegglin anomaly

Presence of Döhle body-like inclu—

50% of patients do not have symp—

Functional

sions in neutrophils, eosinophils,

toms; others have manifested abnor—

Abnormalities

and monocytes. Abnormal y large

mal bleeding tendencies. The cause

and poorly granulated platelets and

of the hemostatic defect is unclear,

thrombocytopenia (a decreased num—

but it is proportionate to the degree of

ber of platelets) frequently coexist in

thrombocytopenia.

this condition.

Chédiak-Higashi syndrome Characterized by very large granules.

Neutrophils display impaired chemo—

These gigantic, peroxidase-positive
taxis and delayed killing of ingested

deposits represent abnormal lyso—

bacteria. Patients with this disorder

somal development in neutrophils

suffer from frequent infections, which

and other leukocytes, such as

suggests that neutrophils with this

monocytes and lymphocytes

defect are not efficient bactericidal

cells.

Alder-Reilly inclusions

Purple-red particles are precipitated

Alder-Reilly granules are most com—

mucopolysaccharides seen primar—

monly seen in patients with Hurler's,

ily in neutrophils, eosinophils, and

Hunter's, and Maroteaux-Lamy types

basophils.

of genetic mucopolysaccharidosis.

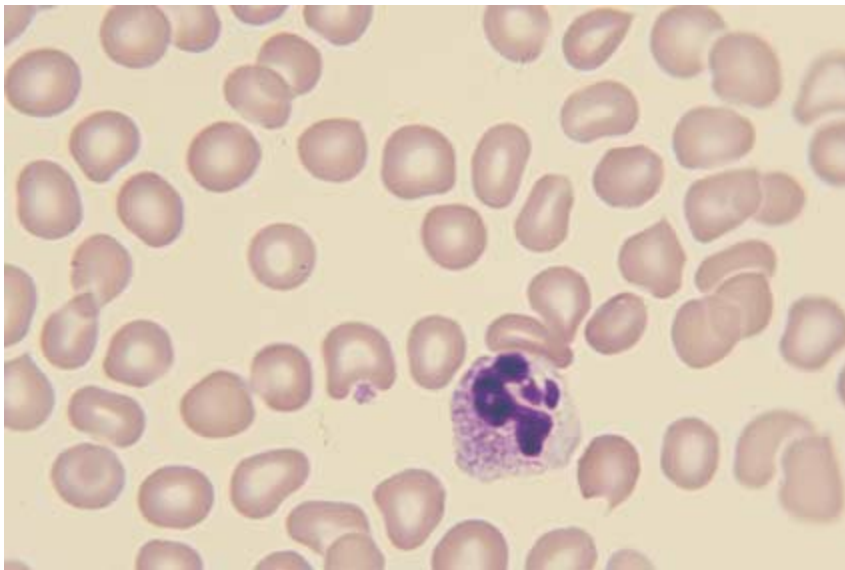
Chronic granulomatous
disease

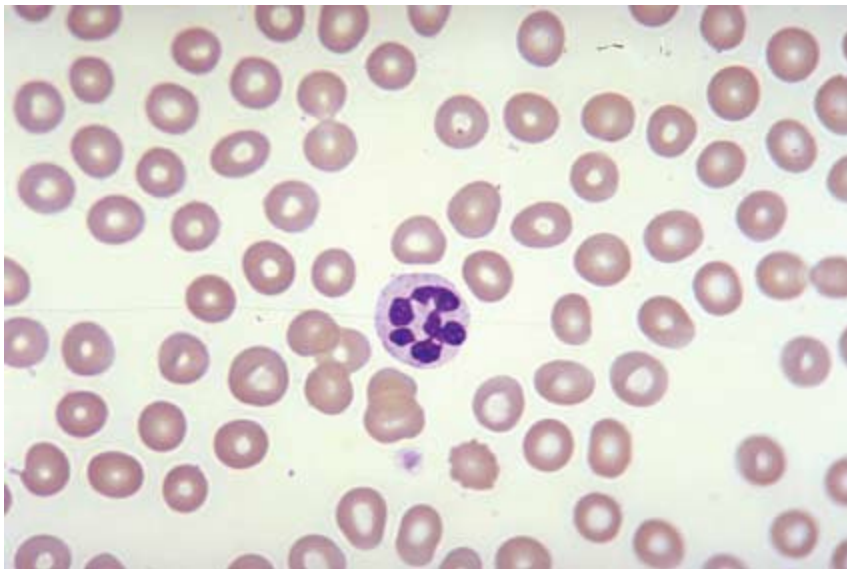
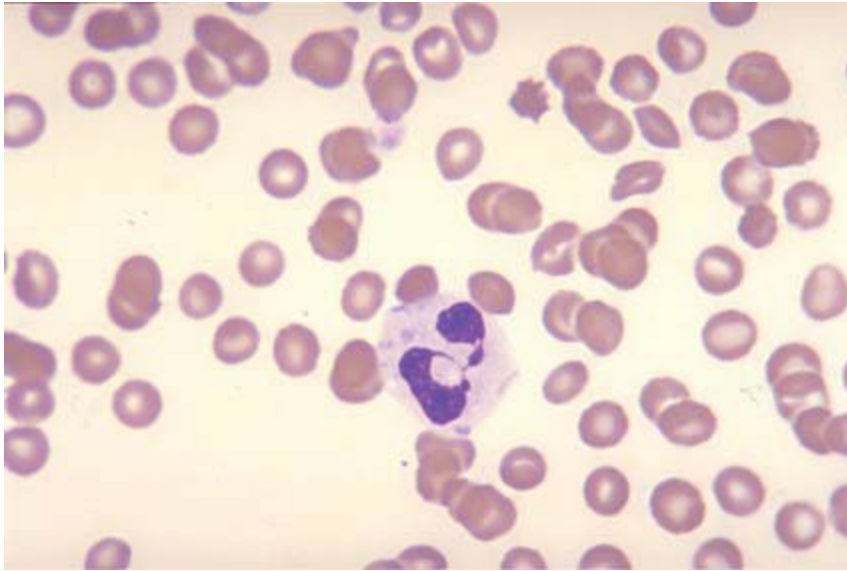
Myeloperoxidase

deficiency

Leukocyte adhesion

deficiency





CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

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Pyknotic Nucleus

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Toxic Gran ula t io n

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Toxic granulation m k Anderson's Atlas o Hematology, P , PA: W K

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Pelger-Huët anomaly q , m

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Ehrlichia

I U S , m Ehrlichia z m - . T

FIGURE 18.2 P -H ë m m ,

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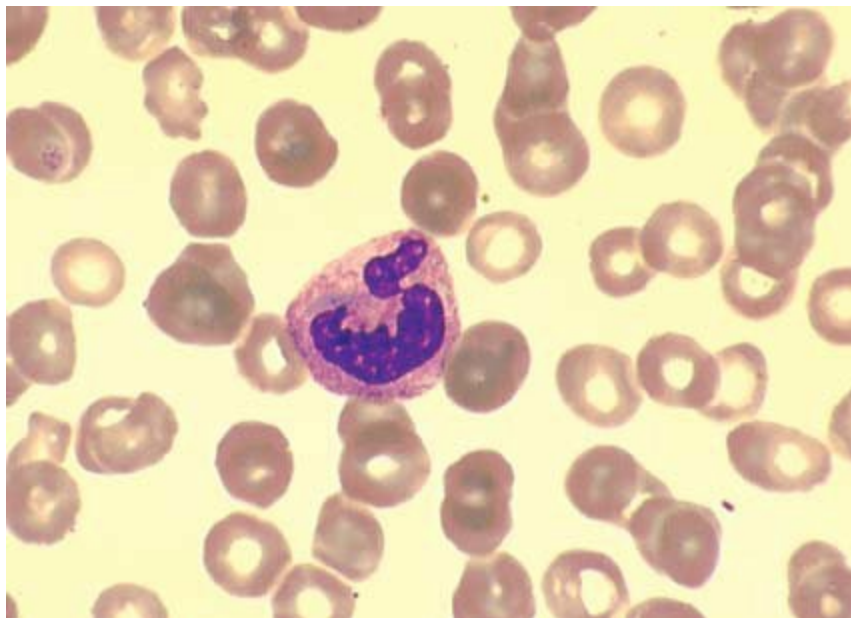
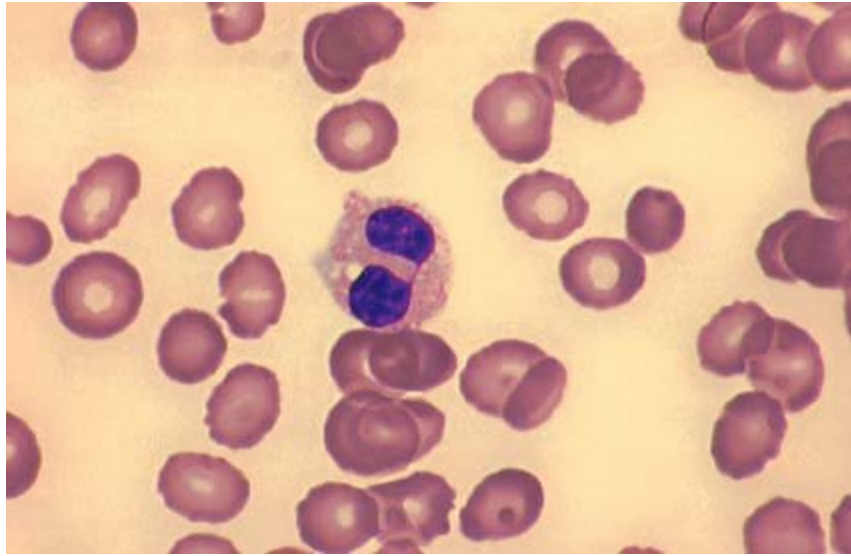
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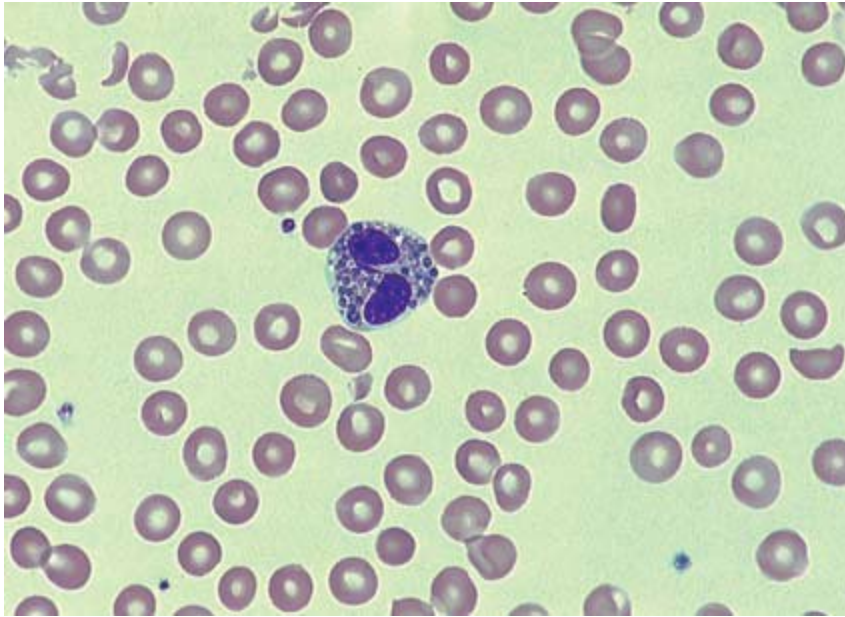
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Anderson's Atlas o Hematology, P , PA: W K

L W m & W k , , m .)

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PART 5 ■ Nonmalignant Leukocyte Disorders

FIGURE 18.4 Dö . (R m A SC.

Anderson's Atlas o Hematology, P , PA: W K

FIGURE 18.5 Ehrlichia cha eensis m -

H /L W m & W k , , m .) (DH). N m , , m . R m k , -

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m , ×. (R m M C KD. Clinical Ehrlichia cha eensis, Ehrlichia ewingii, -

Laboratory Medicine, , P , PA: L W m m x m m Ehrlichia phagocytophila.

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Chédiak-Higashi syndrome

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INHERITED FUNCTIONAL

ABNORMALITIES

May-Hegglin Anomaly

May-Hegglin anomaly

FIGURE 18.6 M-H m

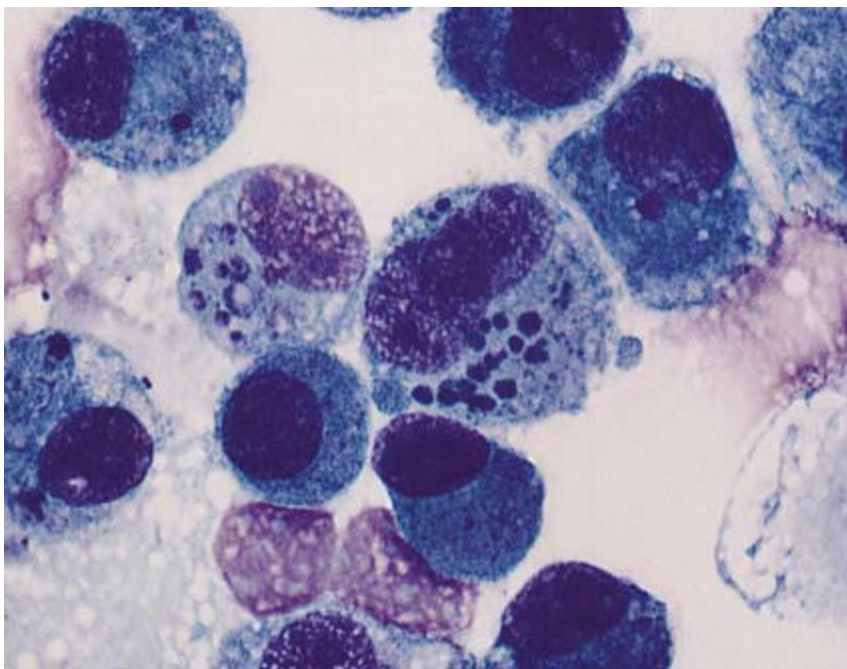
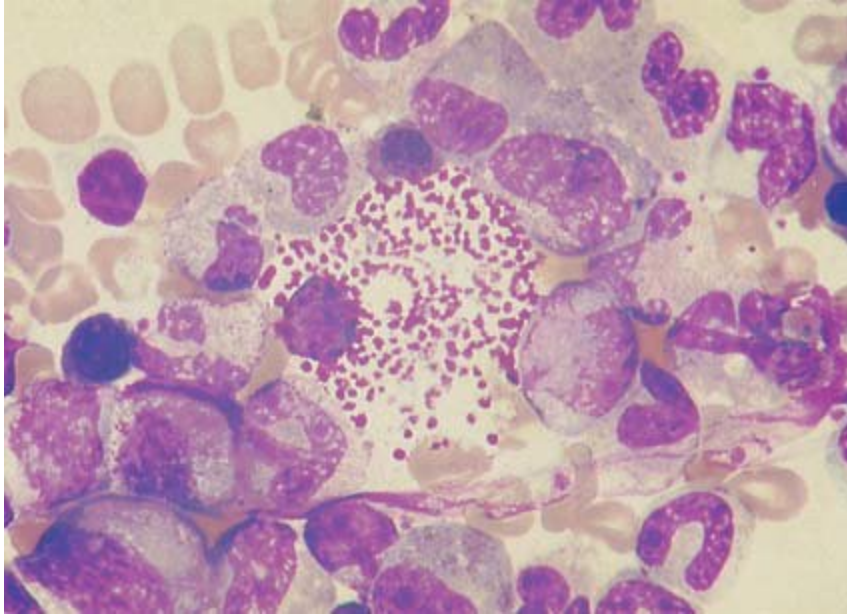
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M C KD. Clinical Laboratory Medicine, , P , m

PA: L W m & W k , , m .)



CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

343

GRANULOPOIE IC AL ERA IONS:

QUALITATIVE DISORDERS

Defective Leukocyte Motility and Chemotaxis

Defective Motility

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FIGURE 18.7 C é k-H m .

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, P , PA: L W m & W k , ,

Lazy Leukocyte Syndrome

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Lazy leukocyte syndrome

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Allder-Reilly Inclusions

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Alder Reilly inclusions -

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Leuko cyte Adhe s io n De cie ncy

Leukocyte adhesion de ciency (LAD) m -

NOTE: This is a good time to complete Review Questions

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related to preceding content.

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PA: L W m & W k , , m .) E B z . LAD-

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PART 5 ■ Nonmalignant Leukocyte Disorders

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Myeloperoxidase Deficiency (MPO Deficiency)

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Myeloperoxidase (MPO) deficiency (A -G m-

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I m CGD, X-k

m m , m -

Lyso so mal S torage Dis e as e s

m . T

m . R CGD

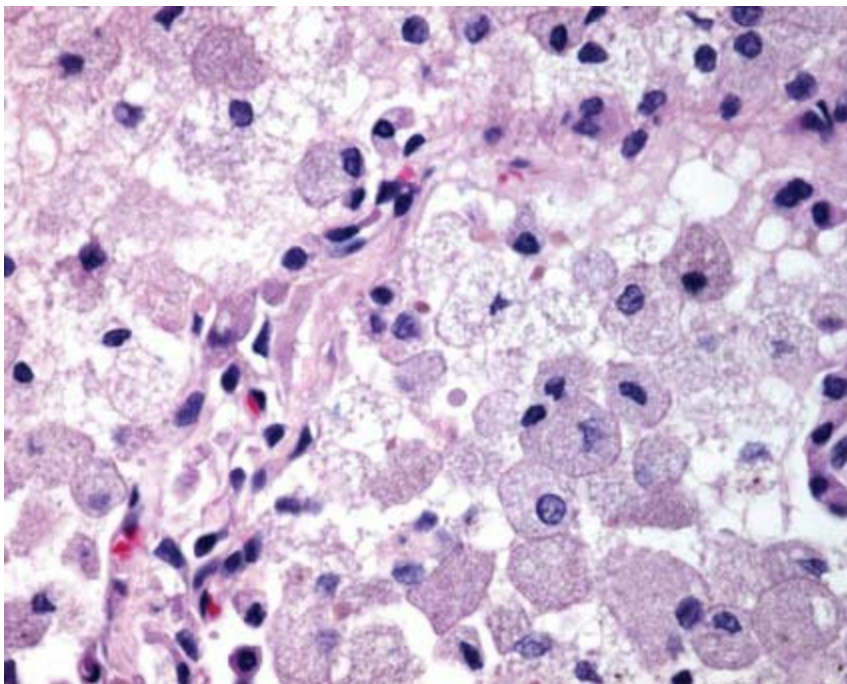
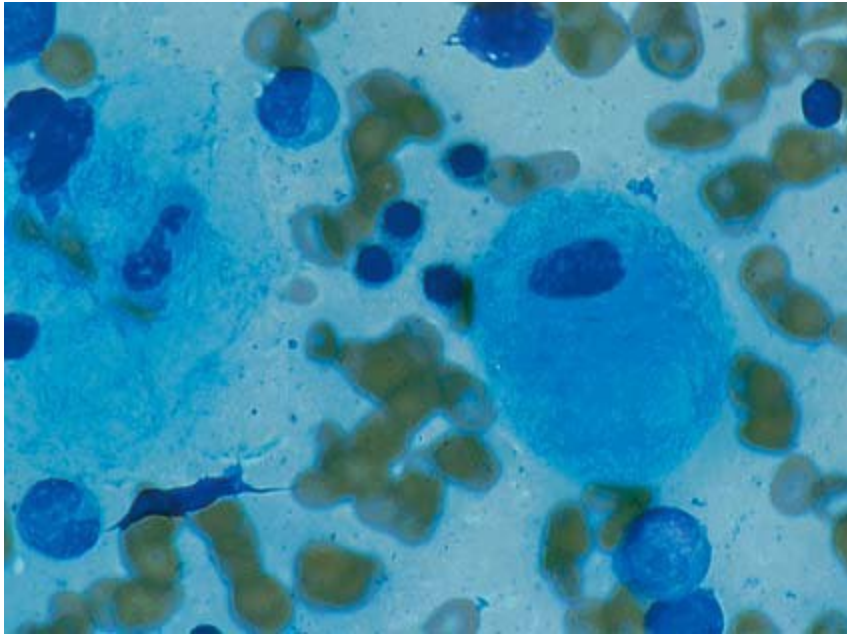
L m m m

k - - -

m . Lysosomal storage dis-

(G PD).

eases m .



CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

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T

$N_m - P_k$;

$. D z m , ,$

$' .$

$, -$

$T z m$

$, m$

$m m -$

$. T -$

$, m . S m m$

$m m : -$

$m (F . .). T$

$m (GAG) (m [MPS])$

$, P k' , m G '$

$.$

$; , m m -$

$M m$

$. B m \times m m$

$m , A -R m -$

$z m \mu m z m -$

$. D m m$

m.

G', N m -P k, S - -

. I MPS, m m m -

Mis ce llane ous S to rage Dis e as es

m .

Tay-Sachs Dis e a s e

Gaucher's Dis eas e

Tay-Sachs disease -

.

Gaucher's disease, , -

I m mm m -

m m. G' m

m m . C

q ,

m -S

m . I m , m

. O m -S -

m ; , m

. S m m -

m .

, , m

T β- , m.

z m m m -

-S .

, m . A z m -

T m m m-

, m (m)

m A k z (E) (F . .). G ’

J . T m

. T G ’ ,

m mm F -C mk m.

m Q , O O Am mm

T m , ,

P , C j L .

m m. T -

M HEXA -S .

k -

T HEXA m k

m m .

z m - x m A,

. T z m

Niem ann-Pick Dis eas e

m k GM

Niemann-Pick disease m G ’

m m m.

FIGURE 18.10 H m m

FIGURE 18.9 G ’ . B m m -

m-N m -P k .

m m . (R m

(R m C P . Color Atlas and ext o Pulmonary

M C KD. Clinical Laboratory Medicine, , P , Pathology, P , PA: L W m & W
k , , PA: L W m & W k , , m .) m .) 346

PART 5 ■ Nonmalignant Leukocyte Disorders

. I -S ,

■ N , -

m m GM - .

, mm , m . E

m , ,

Glyco s am ino glyca ns (Form e rly Ca lle d

m . B

Mu copo lys acch arido s es)

m , m x, k x,

Mucopolysaccharidosis -

.

m . T

■ M , -

m : H ' m (MPS I-H),

, k , mm

H -S m (MPS I-H/S), S ' m

(MPS I-S), m m . B

m .

m m , MPS I

■ D k m

.

m

P MPS I

.

m m

■ N m m j

, m m -

. E

.

. B

M IDUA MPS I. T IDUA

m , .

z m

k m

Morpho log ical Abno rm alities o f Mature

GAG . GAG m , **Granulocyte s**

m . M

IDUA m m

■ A m m , -

IDUA z m . T k IDUA z m

, m

m GAG ,

.

m . T m GAG z

■ H m m q -

m , m

m m

. R GAG m

m . T q

-

m B x

m m m m .

m , - .

P m m m

S e a -Blue His tiocytosis

.

Sea-blue histiocytosis m

■ P -H ë m m .

, m m

A m m , -

- -G ’

, m m m

. S - -

m , m . H m m -

S k S . S -

P -H ë m m .

mm

■ x m k m m m (MDS, , ,

C). A q

m m .

m - , MDS

■ Dö m , - -

m mm m

W - m . T

E .

m ,

m m m . Dö

- k m M -H m .

NOTE: This is a good time to complete the end of chapter

■ Ehrlichia m , G m-m

Review Questions.

k . I k , Ehrlichia

m - k m ,

CHAP ER HIGHLIGH S

m - k m .

Granulopo ie tic Alte rations : Quantitative

Inhe rited Functio nal Abno rm alities

Disorders

■ Myeloid -

■ Dqk -

z Dö - k

k .

, , m . Am

■ Am

m

, , . A

(m) q x

mm .

.

CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

347

■ Cék-Hm . Imkmmz . N -

.

mm x k

■ A

.

.

■ A -R - -

m . T

Mono cyte-Macro phage Dis orde rs

m x .

■ Q m -m

■ C m

m . Ex m

m .

Histoplasma capsulatum.

■ I , m

m .

Granulopo ie tic Alte rations : Qualitative

■ M G ’

Dis o rders

N m -P k .

■ M m m -

■ D m m x

G ’ , N m -P k, -

q . L k m m m

.

z k m -

■ I m m , m k

m m m .

m .

■ M -S -

■ D m

m (m m).

m , m x

■ S - m -

, m .

, m m

N m m x

- -G ' .

CASE STUDIES

Cas e Study 18.1

■ Critical Thinking Group Discussion Questions

. W m ' -

T -m - N x

?

k m

x q . T

. C m (Enterobius vermicularis) ?

. P x m

- m . H m-

. W m ?

. °F. T m

.

Cas e Study 18.2

A - - m m m

■ Laboratory Data

m m

T m

m . S

m . H k . × /L. T

mm

k :

. P x m

N %

q .

S m %

N m m . T m-L m %

°F (. °C). A m

M %

.

E %

■ Laboratory Data

A m k . A T m m -

x m .

m . T k × /L. T

k m :

S m %

B m %

(continued)

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PART 5 ■ Nonmalignant Leukocyte Disorders

C

C A

A S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

L m %

■ Laboratory Data

M %

H m m m ,

E %

k . × /L. T

:

T m x

k .

B - %

S m %

■ Critical Thinking Group Discussion Questions

. F m -

L m %

?

M %

E %

. W m ? I

■ Critical Thinking Group Discussion Questions

m ?

. W m

?

. W ?

Cas e Study 18.3

. W ?

A - - m k -

. I ?

m f

. P x m

Cas e Study 18.5

m

A - - m m

m . A m , ,

.

.

S S A , -

m .

■ Laboratory Data

T ' m m m .

■ Laboratory Data

H , k . × /L,

m , m ,

k :

(RBC) . T k -

S m %

. × /L. T

N %

:

E %

B %

L m %

S m %

M m k,

L m %

. M .

L - m T ' , m m .

m k , m . T

G m- ,

■ Critical Thinking Group Discussion Questions

Pseudomonas .

. W m q -

' m ?

■ Critical Thinking Group Discussion Questions

. W k, ?

. W - m

k ?

. D

k ?

. I m ?

. W m x '

Cas e Study 18.6

k ?

A - - k

Cas e Study 18.4

. H x m

A -m - k m

m m . A S A m

. R -

.

.

CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

349

CA

CA S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

■ Laboratory Data

■ Leukocyte Differential

RBC . \times /L

B %

H m %

S m %

H m . / L

L m %

WBC . \times /L

M %

P \times /L

P . RBC m -

MCV L

m m . R

MCH

m . T x -

MCHC / L

.

■ Leukocyte Differential

B %

■ Follow-Up

S m %

T x , ' m m k

E %

m m , m

M %

$^{\circ}\text{F}$ (. $^{\circ}\text{C}$). A m -

L m %

S A . S Borrelia

burgdor eri, Ehrlichia cha eensis, HGE

D

m (PCR)

m ; + m

m . T

m ; + x

m .

k .

A - m x m

■ Laboratory Data

m m . T

RBC . \times /L

m : m . A

H m . / L

. T x -

H m %

m m .

WBC . \times /L

P . \times /L

■ Critical Thinking Group Discussion Questions

. W

■ Leukocyte Differential

m ?

B %

S m %

. W -

L m %

?

M %

P . RBC m -

. I m k m m m .

?

S q m -

. W ?

([IFA]) Ehrlichia

equi . T m

Cas e Study 18.7

(:). F m ,

A - - k m m IFA E. equi I G (:), m . T

I M m : . I M I G

. P x m

E. cha eensis .

m °F (. °C). A m ,

■ Critical Thinking Group Discussion Questions

, S A . O -

. W

. T q

?

m .

. I m HGE?

■ Laboratory Data

RBC . × /L

. I HGE m mm ?

H m . / L

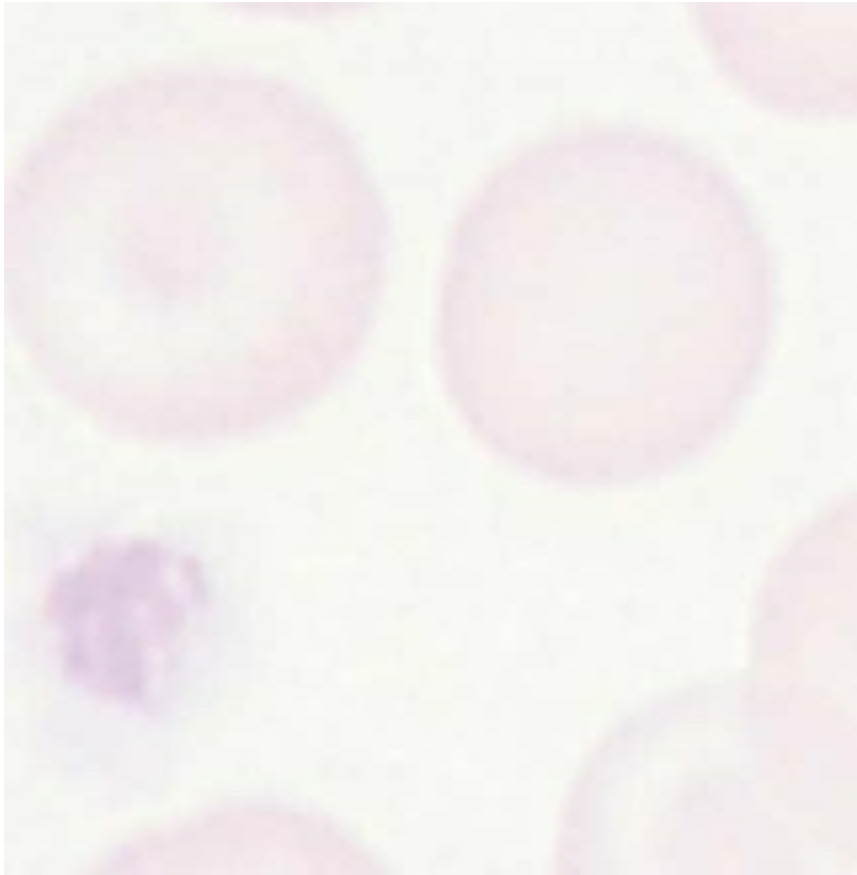
H m %

WBC . × /L

NOTE: This is a good time to write out the answers to the

P . × /L

Critical Thinking Group Discussion Questions.



PART 5 ■ Nonmalignant Leukocyte Disorders

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

Morphological Abnormalities of Mature Granulocytes

8. P – P -H ÷ m m

Granulopoietic Alterations: Quantitative

1. L k

A.

A. m m mm

B. k m

m ' m m

C. m

B. m z m

D. m

m - m m

C. m m m m -

9. W P -H ÷ m ?

D.

A. D k - k RNA

B. F m m

2. N

C. F m

. S .

D. P m

A. S

B. B

10. P -H ë m m

C. S

A. m B

D. A

B. q

*3. A -

k m m m k m

C. m m

A. k k (LAP)

B. m (ESR)

D.

C. m

D.

*11. P -H ã m m -

4. C -L _____

mm .

A. m CD

A. m

B. m

B.

C. m

C.

D. B C

D.

12. W

5. M

m ?

A.

A. D k - k RNA

B. k

B. F m m

C. m

C. F m

D.

D. P m

6. N

13. H m m

A. m j

A. m B

B.

C. z

B. q

D.

C. m m

*7. C z

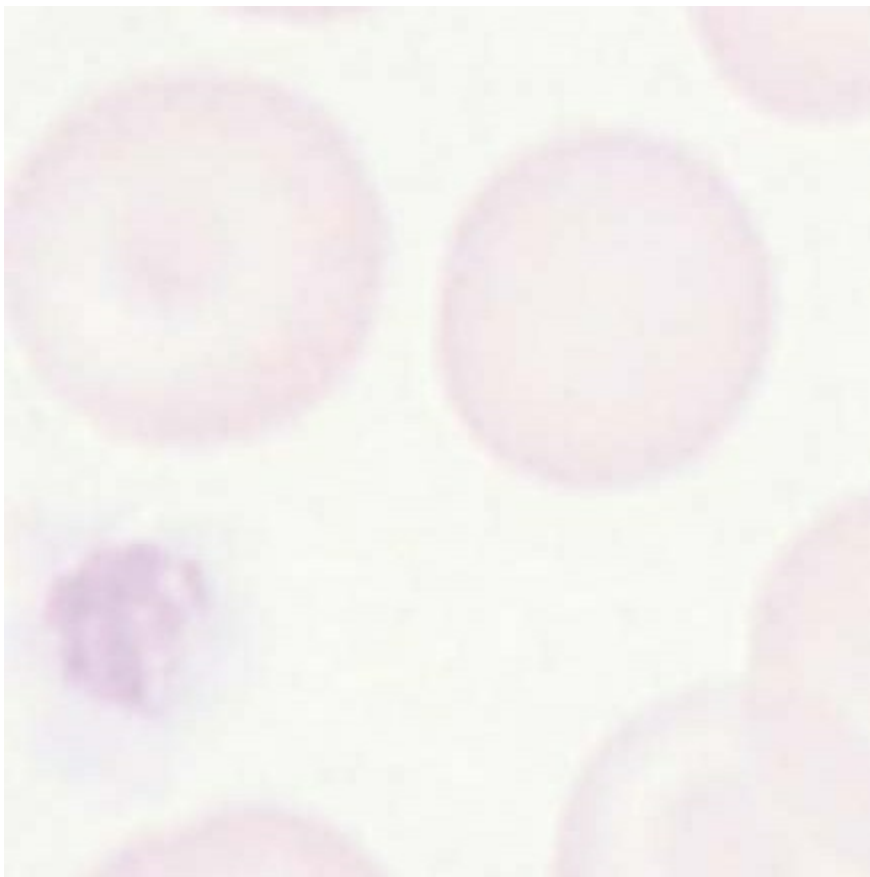
A. m

D.

B. k

C.

D.



CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

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REVIEW QUESTIONS (continued)

14. W Dö

21. W C é k-

?

H m ?

A. G x -

A. G x -

B. P m

B. P m

C. D k-m

C. Dö – k

D. S m -

D. S m -

15. Dö m

22. C é k-H m m

A. m B

A. m B

B. q

B. q

C. m m

C. m m

D.

D.

16. W x 23. C é k-H m ?

A.

A. D k - k RNA

B. m -m

B. F m m

C. m

C. F m

D.

D. P m

24. C é k-H m m 17. I U S , m

Ehrlichia

A.

A. E. chaffeensis

B. m m

B. E. ewingii

C. m m

C. E. phagocytophila (m)

D. k

D.

*25. W A -R

18. E m _____.

?

A. m q

A. G x -

B. k

B. P m

C.

(m)

D.

C. Dö – k

D. S m - –

*19. W M -H

m ?

*26. C m A -R m

A. G x -

m

B. P m

A. Dö

C. Dö – k

B. x

D. S m - –

C. A

D.

*20. M -H m m -

*27. T m

A. m

m

B. m

A. A -R m

C. x

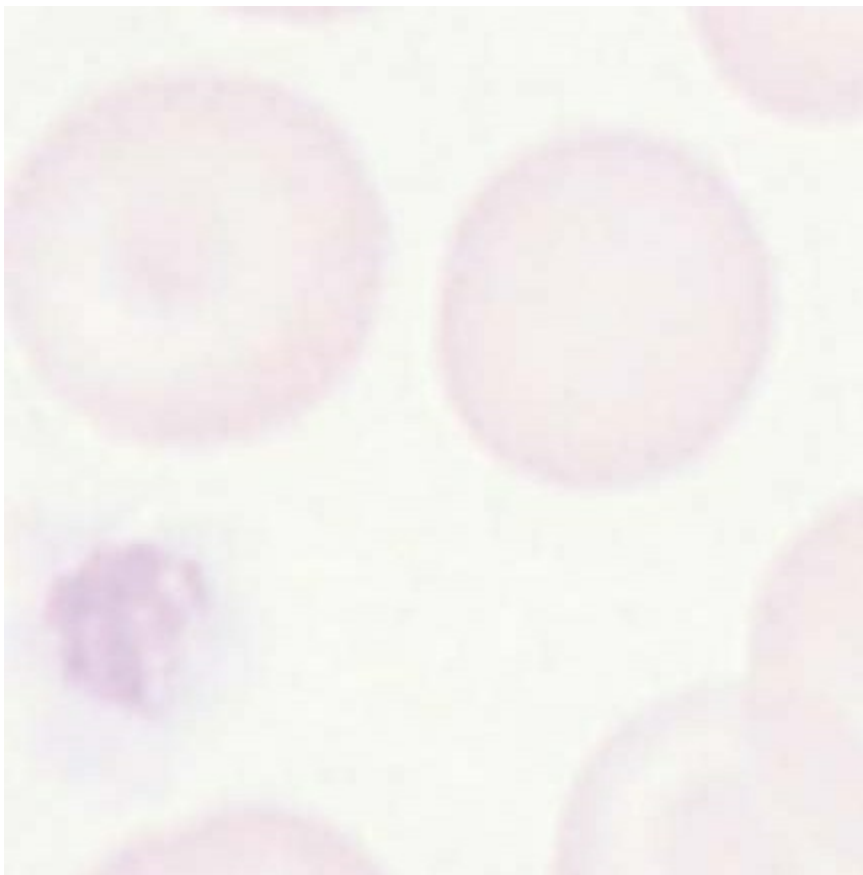
B. P -H ã m

D. m

C. M -H m

D. C é k-H m

(continued)





PART 5 ■ Nonmalignant Leukocyte Disorders

REVIEW QUESTIONS (continued)

Inherited Functional Abnormalities

*35. B

28. C m

• , -

?

A.

A. x

B.

B. Ehrlichia m

C. m

C. I m

D.

D. H m

29. L z k m

*36. B

A.

• , m

B. m -m

?

C. m

A. S

D.

B. I m

C. I

Monocyte Macrophage Disorders

D. I

30. G ’

*37. B

A.

., -

B. m -m

?

C. m

A. x

D.

B. Dö

C. M

*31. N m -P k

D. L m

A.

B. m -m

*38. B

C. m

. , k

D.

?

A. C é k-H m

*32. G ’

B. G ’

A. k m

C. L m k m

B.

D. I m

C. β -

D.

*39. B

. , m

Case Studies

?

*33. B

A.

., k ?

B. m

A. H m m

C. m

B. B

D. m

C. A

D. P

*34. B

., k ?

A. I m

B. A mm

C. C

D. A

CHAPTER 18 ■ Disorders of Granulocytes and Monocytes

353

G B, S k C, C S, . L mm -

COMPANION RESOURCES

: , , , rends Immunol, ():

— , .

:// . . m/

H m R, . (.) Hematology: Basic Principles and Practice,

, S . L , MO: E C L , : .

E W -

K SJ. A m m m -

m .

m k . I : B JA, D DH (.).

H ,

T e Phagocytic Cell in Host Resistance, N Y k: R P ,

-

: .

.

K k JA (). Leukocyte Di erential Counting, Re erence Leukocyte

(WBC) Di erential Count (Proportional) and Evaluation o

BIBLIOGRAPHY

Instrumental Methods; Approved Standard, , W ,

PA: C L S I (CLSI), A JW, H OP, R SO, . L k k . IV. T

:H –A .

, m -

K DB, : C k m

m j , J Clin Invest, : – , .

WDR1 Blood, .

B kk JS, . H m U M

M k EK, . Medical Parasitology, P , PA: S , U S , JAMA, (): – , .

: .

B J, . I m , Lab Med, O’C BH. A Color Atlas and Instruction Manual o
Peripheral Blood (): – , .

Morphology, B m , MD: W m & W k , .

B E. G ’ , N Engl J Med, (): – , .

S RB. G k m . I : C D C P (CDC). Human Ehrlichiosis K k J (). Laboratory
Hematology, E , UK: C -

in the United States (. .). R O , .

L , : – .

D AM J . M m . I : S k JL (.).

S GD, C HI, J CJ. D

Fundamentals o Clinical Hematology, H , MD: H &

x m , Med Sci Sports Exerc, R , : – .

(): – , .

Principles of Blood Collection and

CHAPTER

Disorders of Lymphocytes

19 Processing

KEY TERMS

acquired immunodeficiency

infectious mononucleosis

lymphoproliferative

syndrome

leukopenia

reactive lymphocytosis

cytomegalovirus

lymphocytopenia

seronegativity

Epstein-Barr virus

lymphocytosis

systemic lupus erythematosus

LEARNING OUTCOMES

Characteristics of lymphocytes

Describe the etiology, epidemiology, clinical signs and symptoms,

- State the normal relative value reference range for lymphocytes in and laboratory data for Bordetella pertussis (whooping cough) in an adult.

Describe the etiology, epidemiology, clinical signs and symptoms,

- State the absolute number of lymphocytes using the total leukocyte count and the relative number of lymphocytes.

Lym pho cyto pe nia

- Explain the difference between an absolute lymphocyte count and a relative lymphocyte count.

- Define and explain the term lymphocytopenia.

Explain the pathophysiology and laboratory features in transient

- Name several disorders of lymphocytes frequently encountered in the hematology laboratory.

lymphocytopenia and pathological lymphocytopenia and congenital qualitative disorders of lymphocytes.

Lym pho cyto s is

Im m une dis orders as s ociated w ith

- Describe why lymphocytosis occurs in infants and children up to

lympho cytopenia

10 years of age.

- Name major immune deficiencies associated with T cells or B cells.

- Name at least three nonmalignant conditions associated with an absolute lymphocytosis.

Describe the pathophysiology of early-phase and late-phase acquired immunodeficiency syndrome (HIV/AIDS).

- Name at least three malignant conditions associated with lymphocytosis.

Assess the hematology laboratory patient data, including serologi-

Disorders associated with lymphocytosis

cal markers in HIV/AIDS.

- Describe the etiology, epidemiology, clinical signs and symptoms,

Describe the etiology, epidemiology, clinical signs and symptoms, and laboratory data for infectious mononucleosis.

and laboratory findings of systemic lupus erythematosus.

- Describe and recognize the appearance of lymphocytes associated

Cases studies

with infectious mononucleosis.

Correlate the heterophil antibody test to a diagnosis of infectious

Analyze the patient history, clinical signs and symptoms, and laboratory data for infectious mononucleosis.

ratory data for the stated case studies, answer the related critical

Assess and correlate traditional antibody titers found in infectious

thinking questions, and conclude the most likely diagnosis.

mononucleosis with EBV viral antigens.

Describe the etiology, epidemiology, clinical signs and symptoms,

NOTE:

and laboratory data for cytomegalovirus.

■ indicates MLT and MLS core content

Describe the etiology, epidemiology, clinical signs and symptoms,

indicates MLT (optional) and MLS advanced content

and laboratory data for toxoplasmosis.

CHARACTERISTICS OF LYMPHOCYTES

$\times 10^9/L$. A

m lymphocytopenia. W

T m m

m m

% %. T m

, lymphocytosis.

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CHAPTER 19 ■ Disorders of Lymphocytes

TABLE

19.1 Exam ple s of Nonmalignant Dis o rde rs o f Lym pho cyte s**Dis orde r****Etiology****Laboratory Data**

Viral disorders

Infectious mononucleosis

EBV

Lymphocytosis

Variant lymphocytes

Increased titer of heterophil antibodies

Reactive lymphocytosis

Coxsackie group

Lymphocytosis

Negative for heterophil antibodies

CMV infection

Herpes group—CMV

Slight lymphocytosis

Variant lymphocytes

Negative for heterophil antibodies

Positive for ANA, RA, and CMV nonspecific antibodies

AIDS

HIV

Leukopenia

Lymphocytopenia

Abnormality of T-cell subsets

Bacterial disorders

Whooping cough

Bordetella pertussis

Significant lymphocytosis

Rare lymphoblasts

All antibodies negative

Parasitic disorders

Toxoplasmosis

Toxoplasma gondii

Variant lymphocytes

Negative for heterophil antibodies

Positive for Toxoplasma antibodies

Autoimmune disorders

Systemic lupus

Autoimmune

Positive LE cell preparation

erythematosus

Positive for ANA antibodies

Lymphocyte subset abnormalities

ANA, antinuclear antibodies; RA, rheumatoid factor antibodies; CMV, cytomegalovirus; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

ciency virus.

D m (.) q

m m

. M -

m . T m

m m .

m m m

Ex m infectious mononucleosis,

m

cytomegalovirus (CMV) , q mm -

.

m (AIDS). B

L m mm

m . T -

mm . I -

x m ,

, m

, m m .

m

I , - (mm -

. A (. . , m ,

) m

, m , CMV

m m

,

m (.).

reactive lymphocytosis)

. S m (. . , Bordetella pertussis -

[])

LYMPHOCY OSIS

. P (. . , x m)

. D (. . , p-m -

Lymphocytosis m -

)

x m , m -

. U mm (. . ,

× /L. T

m x)

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PART 5 ■ Nonmalignant Leukocyte Disorders

TABLE

19.2 Causes of Lymphocytosis

Lymphocytosis Associated with Atypical Lymphocytes

% of White Cells That Are Atypical Lymphocytes

Lymphocytosis Associated with

>20%

<20%

Small Mature Lymphocytes

Infectious mononucleosis

Mumps,* varicella,* rubeola, rubella, atypical Reactive lymphocytosis

Infectious hepatitis

pneumonia, herpes simplex, herpes zoster,

Pertussis

Posttransfusion syndrome

roseola infantum, influenza,* other viral il -

CMV infection

nesses, tuberculosis,* rickettsialpox, brucel—

pn-Aminosalicylic acid (PAS)

losis,* toxoplasmosis,* radiation

Phenytoin (Dilantin) and mephenytoin

(Mesantoin) hypersensitivity

Agranulocytosis

Lead intoxication

Stress

Leukemias and lymphomas*

*Higher counts of atypical lymphocytes are occasionally found.

M m

Epidemiology

. L m k m (m)

A EBV m m

. T k m m m

,

. W m' m m

m

. C

. U , -

m EBV x -

k .

T q seronegativity %

DISORDERS ASSOCIATED WITH

, m

LYMPHOCYTOSIS

m , %

Infectious Mononucleosis

. A m x , -

mm

I m , ,

. I W , m x

- m lymphoproliferative Epstein—

EBV . A x m -

Barr virus (EBV). EBV B k' m m , x ; m m m m

(

A ; m ; -

). A x m % m -

m m , , x.

.

I k k

Et io logy

. EBV m m mm m—

EBV

, m m j m

m . EBV m . I m

mm m m . I mm -

% ' x ,

, EBV m

m k m q k .

% %. B m mm

EBV m DNA . I m -

mm m m -

, B m ,

k m

m

m . I m -

m x m -

m – k -

. O

m m CMV

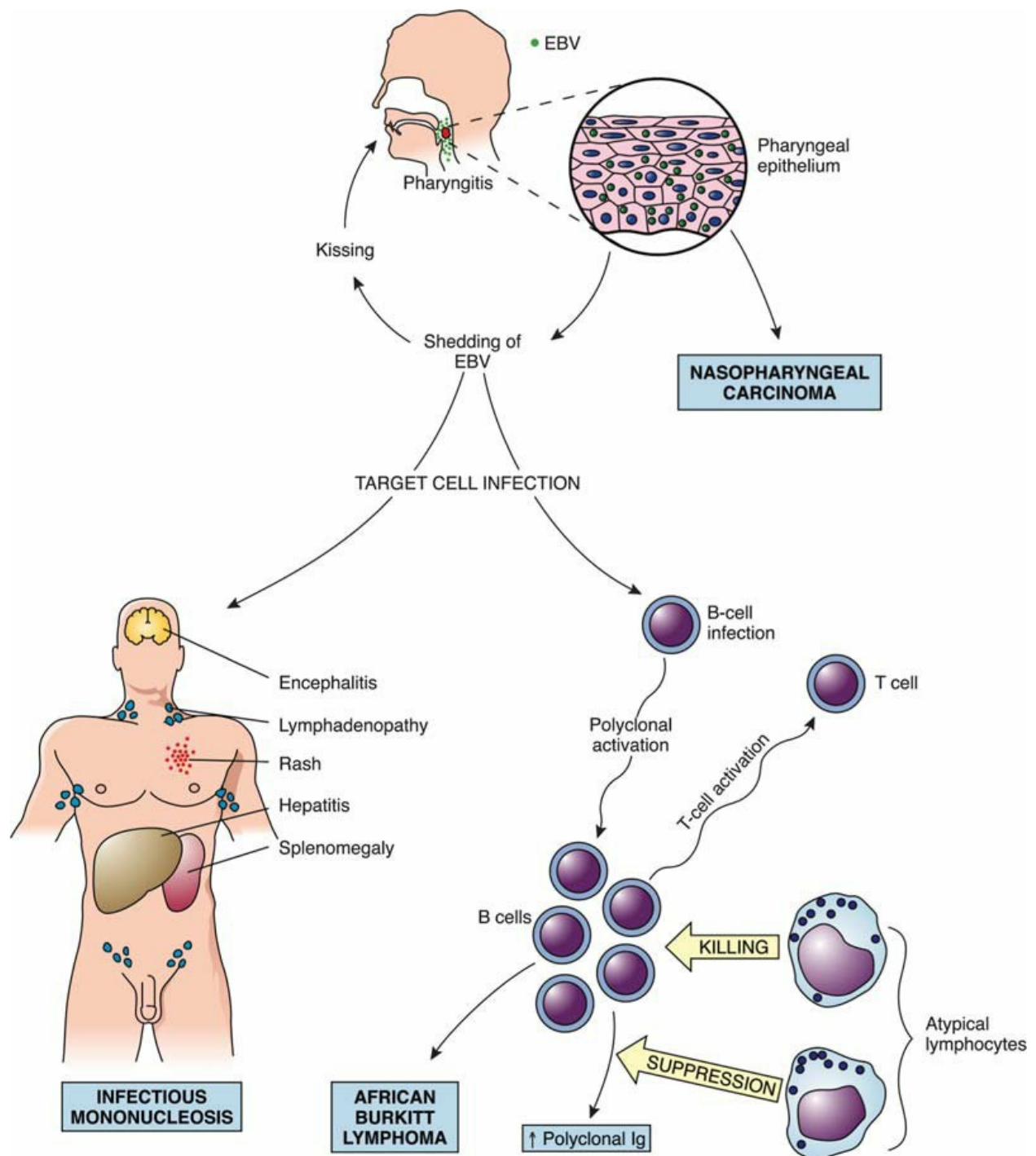
m B m

EBV.

m m

A x m m-x (F . .).

. R



CHAPTER 19 ■ Disorders of Lymphocytes

FIGURE 19.1 R EBV m , m , B k ' m m . EBV

m

. I m , m , m .

I mm m x , EBV m . EBV B

m , . T B m m -

, k B mm . S m B

m mm m m B k ' m m . (R m R E, F JL.

Pathology, , P , PA: L W m & W k , , m .) m EBV-

, m m q -

m , m

z m , m m . A

.

m , m -

I , m -

.

m q / , (F . .). I

T m

mm , EBV

m ; , m % %. A , k . C m

m .

x m , m , , , -

m . S m %

Clinical S igns an d Sym p tom s

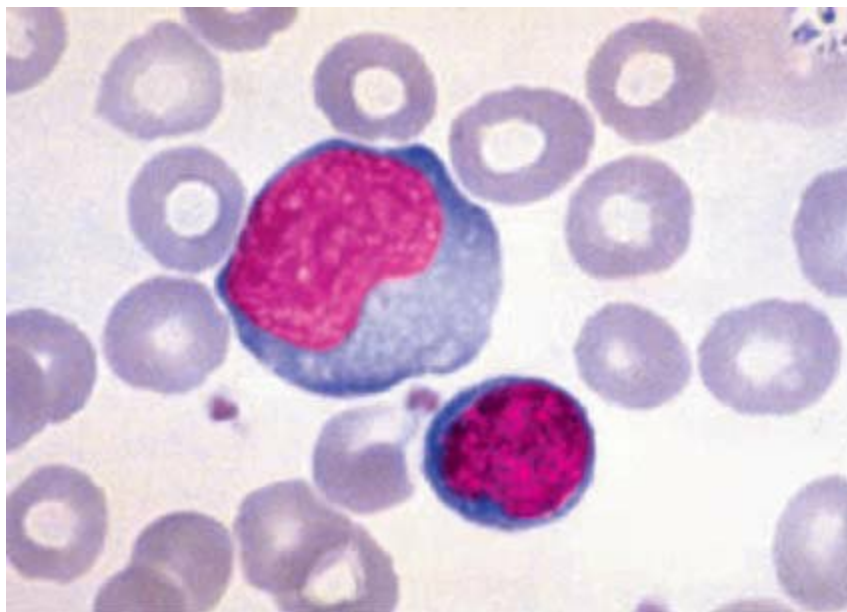
,

T m j

. W , , m -

m m . I

. J q , m mm



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PART 5 ■ Nonmalignant Leukocyte Disorders

800

He nke e t a l.

700

He a lth e t a l.

Da vids on

n

o

i

600

t

a

l

u

p

o

500

p

0

0

0

,

0

400

0

1

r

e

p

300

s

e

s

La rge "a typica l"

Norma l

Ca

200

lymphocyte

lymphocyte

100

FIGURE 19.3 T m . T “ -

” m . (R m M C H. T e Nature o Disease Pathology or the Health Pro essions,
P , PA: 5

10

15

20

25

30

35

40

L W m & W k , , m .)

Ag e (ye ars)

FIGURE 19.2 I m

m m mm .

.

H m m

(), :

m . A m

-

m m m .

m . R

B m m EBV-

. T

m CMV-

m , EBV m

.

. A m

W , % %

m m

m

m m .

. T

La boratory Da t a

L m **Descriptive Feature s of the m . H m**

Clas s ic Dow ney Clas s i cation of

k m

TABLE

19.3 Lymphocytes Seen in Infectious

× /L x m ;

Mononucle os is

% m leukopenia. A k m -

Type I

, m

Nucleus

May be irregularly shaped

m .

m m % %, %

Cytoplasm

Usually many cytoplasmic vacuoles

% m . m

Dark blue (basophilic)

(F . .) x m

Type II

m m

Nucleus

Chromatin is coarse and clumped

m . I ,

D C (.)

Cytoplasm

Increased amount

m . E -B m -

Dark blue (basophilic) around the periphery

CD .

or in a radial pattern

I m m m -

A few cytoplasmic vacuoles

, m

Type III*

m f m k . T m

. T

Nucleus

Nucleoli usual y visible

m EBV -

Enlarged in size

. H m -

Cytoplasm

Dark blue (basophilic)

. T m

*This cell resembles an immature lymphocyte.

CHAPTER 19 ■ Disorders of Lymphocytes

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Acute

FIGURE 19.4 A -

Incuba tion

illne ss

Conva le sce nce

EBV. (VCA, ; HA,

; EA, ; EBNA,

IgG VCA

EBV .) (S : H W, H

IgG EA

G. S m . I : E M,

EBNA

A B (.). T e Epstein-Barr Virus. B ,

r

e

G m : S -V , : - ;

t

Ti

HA

mk B, S J. E -B -

IgM VCA

m . I : G S, B J,

B k N (.). In ectious Diseases, P ,

PA: WB S , : – .)

0

1

2

3

4

5

6

7

Months

m %

Cyto me galovirus Infe ctio n

m

.

Et io logy

F

T -

m , m m mm CMV

m EBV .

. I , CMV

C EBV

. I ,

x m m , -

.

, mm .

H m CMV m m m—

EBV-B m x

. T z m

(F . .). I

: m x I, m x II, -

EBV x (VCA), z , EBV, CMV. A -

(EA), (NA), -

, DNA

. A mm

DNA x m

M (I M) G (I G) EBV

. T

. EBV- (.)

m m

mm , m -

.

m

A m -

(F . .). T m m

,

m m EBV-

. T q m ,

.

m m , m

A -I

m . T m x . T mm m m .

-

m .

Ep ide m iolo gy

NOTE: This is a good time to review the de nitions of Key Terms

CMV q m m in the Glossary and ash cards for . Also, a good time

. m CMV q -

to complete Review Questions related to preceding content.

m x (m , TABLE

19.4 Characteris tic Antibo dy Fo rmatio n in Infectio us Mononucleo s is

VCA IgM

VCA IgG

EA-D

EA-R

EBNA IgG

He te rophil

No previous exposure

—

—

—

—

—

—

Recent (acute) infection

+

+

±

—

—

+

Past infection (convalescent) period

—

+

—

—

+

—

Reactivation of latent infection

±

+

+

±

+

±

VCA, viral capsid antigen; EA-D, early antigen (diffuse); EA-R, early antigen (restricted); EBNA, Epstein-Barr nuclear antigen; IgG,

immunoglobulin G.

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PART 5 ■ Nonmalignant Leukocyte Disorders

, , ,)

m , m , m , m -

. T m k m q

, m m .

CMV , , mm -

. I m

. I m

.

m m m m -

CMV , , m k. F

m , , , m , z m , . I m , m m m -

q CMV , m j

CMV .

m .

T m

I m , q CMV

k CMV.

m , m , m ,

A

.

k m m

- q CMV m

, , m

m - k m

. T x , ,

k j .

m

T CMV m m

. D ,

m m -

. B , m -

m , j , m , -

x m m CMV.

m . C ,

B CMV , m q CMV m m , , ,

m m

mm , q m -

(CID).

. A CMV m j

m m AIDS.

La boratory Da t a

CMV x z

I CMV , m x m

. I , CMV m

k . A

m U

m m % m -

S . P m m CMV

mm . C m m m

m .

m . A m

H m-m -

k

k m .

x CMV. N m m m

T x q

k m , -

m () ,

m

m m .

. T CMV

m % %.

Clinical Signs an d Sym p tom s

A , , m -

A q CMV m m -

m m m -

. I m j

CMV-I M CMV-I G

, CMV m m . O , -

. S m

m , - , m - k m I M m .

. T m m , ,

D CMV-I M m -

m , m . L m -

. F - ,

m m . I

, -

mm m -

m . A

. V m x m

, EBV, oxoplasma

CMV ;

, () -

m .

m . T (ANA),

P x q ,

m (RA),

m CMV,

.

x

mL / k m .

Toxoplas m os is

H x CMV -

m . I q m

Et io logy

CMV , , T m m oxoplasma gondii x m -

m , , G -B é

. oxoplasma z C .

CHAPTER 19 ■ Disorders of Lymphocytes

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Ep ide m iolo gy

C x m

x m m

m m m m . M

m . . gondii N A

m m m-

, m , z . I

m , m . I

m %

m

, m

. H m mm

m , m m

m . T m

, , -

, k . T (%) q m .

P m k

La boratory Da t a

m , % .

T

B m F . D m

m . A m

. A

m m . T

m m , , -

m -

. F -

oxoplasma . A

m , , ; q k

m k ,

m ; m k m

,

m .

m m

T z - m x m -

m .

z

B . gondii f , m

k . P k

m . D

mm

, , m .

.

A m mm , m , m

Re active Lym pho cytos is

. m

R m , m-k -

, .

m . N -

m U S

Et io logy

. gondii -

R m ,

, m . I -

m m C x k .

m , m U

S q m oxoplasma -

Ep ide m iolo gy

-m . A x m % m

q m

R m m -

.

m . C m mm m .

C q , x x -

A m m

m . / , .

.

Clinical S igns an d Sym p tom s

Clinical S igns an d Sym p tom s

I ,

A . S m m m m . A z m , , m m ,

. A

m m , , -

, m z m

, . S m m m m

m.

m m m m m . T

I q , m m q m

. L m

. T m m -

m m , .

m , , , m -

C m m -

, x m . A m x m

m . . gondii

. I , m , m

m mm m-

, m .

m ,

x m . T

La bora tory Dat a

H k -H k m m

L k m z -

. R x m

. I m m

mm AIDS. P m

k × /L. I m

m m mm .

m m m , m

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PART 5 ■ Nonmalignant Leukocyte Disorders

m m m . T

LYMPHOCY OPENIA

m m -

m m j m , m , m -

L m . × /L

m . T m

m . × /L m

- . N m . A

. A m mm

m . R EBV

m ,

.

m .

T k

m

k . A m m , , m

. P

m .

x m

I m m -

, m , -

, k m \times /L,

, m . T

m m - m . O

m mm ,

k m m .

(. . , x), x .

Bo rd e te lla Pe rtu ssis (Ha em op h ilu s

Pe rtu s s is) **Infectio n**

IMMUNE DISORDERS ASSOCIA ED WI H

LYMPHOCY OPENIA

Et io logy

W B. pertussis, -

Imm m m m mm m

.

m q . T

- B- . A q mm -

(AIDS) m -

Ep ide m iolo gy

m x m . H k '

T m mm z .

m k m

C . Ex m mm

Clinical Signs an d Sym p tom s

- B-m B x . .

F k , m m **Acquired Im munode ciency Syndro m e** m . C , m m

(HIV/ AIDS)

m k .

A mT m mm (HIV) (F . .)

m .

m acquired immunode ciency

La boratory Da t a

T k

BOX 19.1

$\times /L, m$

$\times /L. T m$

Majo r Imm une De ciencias As s ociated w ith

$\times /L. T k m m j$

T-Cell and B-Ce ll Diso rde rs

m

m m

Ex m -C B-C D

x m .

-C D

L m m k C

. L m

D G ' m (m)

m - m (LPF) m

A q

B. pertussis. T m z

A q mm m

m m m ,

H k ' ,

m m m

C m k m

.

S m m

O m , m m

B-C D

m , m . A C

m

B ' mm m

.

A q

A mm

M m m

NOTE: This is a good time to complete Review Questions

A q Imm S m (HIV/AIDS)

related to preceding content.



CHAPTER 19 ■ Disorders of Lymphocytes

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Trans membrane

Inne r me mbra ne

B m m x CD

prote in

HIV .

Core

A m m

Oute r

CD , m RNA

e nve lope

CD , m CD . T , Vira l , m m , m

RNA

m . I ,

m m

CD , , m f , m m

HIV . T -

m AIDS—

m k A m .

Ep ide m iology

Enzyme

A U.S. C D C

P , , m . m -

HIV

FIGURE 19.5 HIV . (R m Stedman's Medical U S , , (. %) Dictionary, , B m , MA: L W m & W k ,

.

, m .)

HIV

. W , . m

syndrome (AIDS). A HIV z ,

HIV-m

HIV . A m

m .

HIV . I , . m

AIDS, m

Et iolo gy

AIDS m .

A ,

E -S A

m , m

HIV/AIDS, S S A , E

m . D k m, E C A , L Am -

, m , m - m

HIV AIDS.

I (H LV-I), , H LV-II

R A Am

.

x m m

I , P I P

. T HIV (%) mm m x m m -

m x m (MSM), -

. T m m -

- k x (%),

,

j (%),

AIDS. T Am m D .

x m - -m x -

R G m ,

j (%).

H LV-III. I , G m m -

HIV m m m , m

, k

AIDS. W m -

m . W HIV -

LAV H LV-III m , -

, m %

mm m HIV,

m m , m

m m m

, . S m -

k AIDS.

, HIV

I HIV- , AIDS—

m % m -

, HIV (HIV-) . I -

HIV U S .

m , m

P HIV m m mm

. T HIV- , -

HIV m

, HIV- .

AIDS U S . M -

HIV /

AIDS m m m / .

m . T , ,

Clinical S igns an d Sym p tom s

CD m m m-

. M , m %

Ea rly Sta ge s

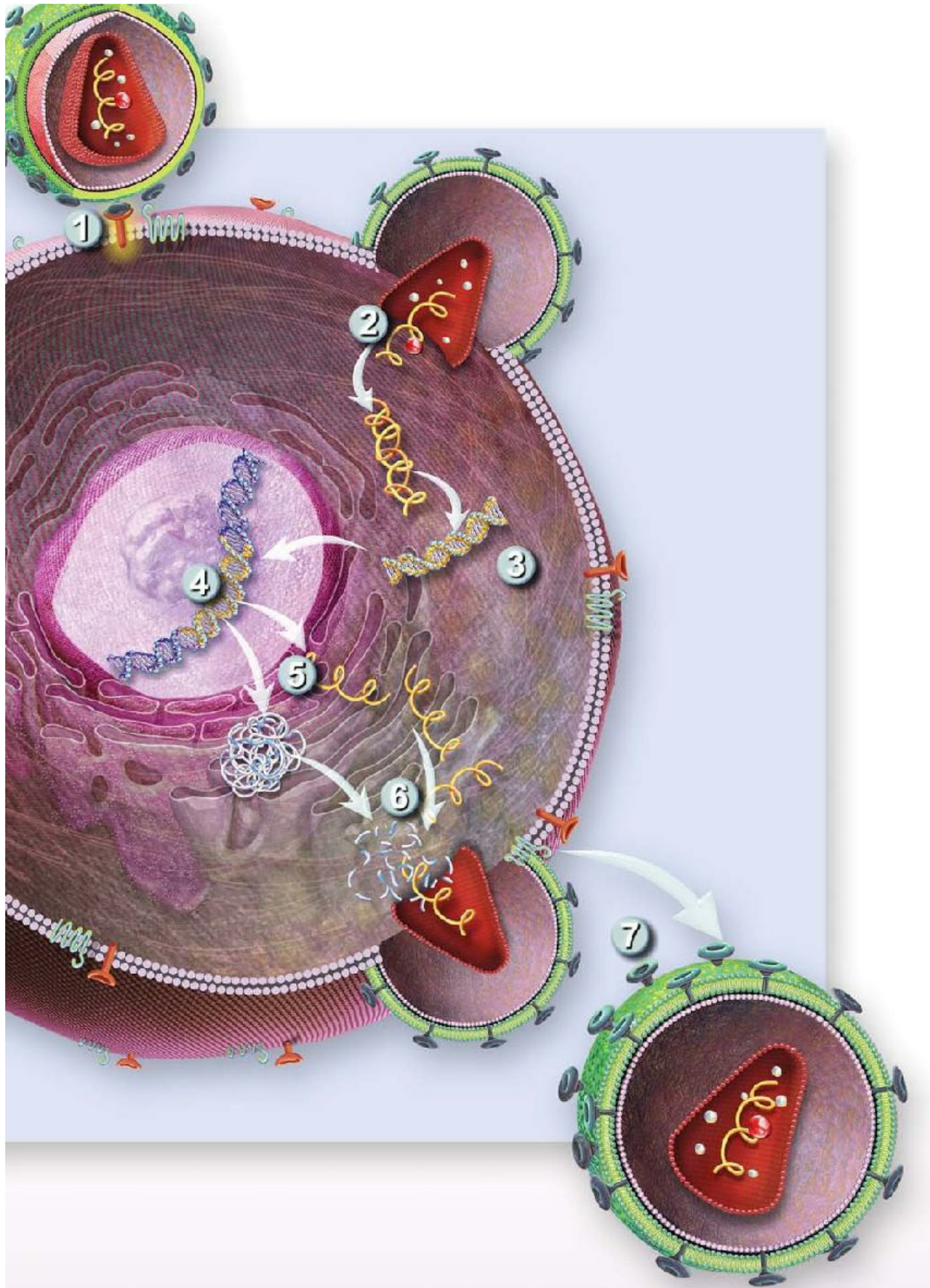
m , m , k ,

T HIV-m x m m CD

m m m m

HIV. I , x m %

(F . .). ,



①

②

③

④

⑤

⑥

⑦

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PART 5 ■ Nonmalignant Leukocyte Disorders

HIV

HIV virion (virus particle)

Lifec ycle

HIV binds to the T-cell.

Viral RNA is released into
the host cell.

Reverse transcriptase
converts viral RNA into
viral DNA.

Viral DNA enters the T-cell's nucleus and inserts
itself into the T-cell's DNA.

Viral RNA

Reverse

The T-cell begins to make

transcripts

copies of the HIV

components.

Protease (an enzyme)

helps create new virus

particles.

The new HIV virion (virus

particle) is released from

the T-cell.

Viral DNA

T-cell

Viral RNA

New HIV virion

(virus particle)

HIV proteins

FIGURE 19.6 HIV .

CHAPTER 19 ■ Disorders of Lymphocytes

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HIV-m m m

. O m -

m , m .

m , m B-mA k m x

(HBLV) m (HHV-),

, m - k - k

HIV-m

, m , k . N m-HIV .

m . T m m

HIV m

La bora tory Dat a

HIV—

L HIV- - -

. T k m

m m m .

q , .

S k

F HIV -

m .

m m m , m m

I m m m m- .

AIDS, m m m

m m .

La te Pha s e

B k m x AIDS

U HIV , m

. T m m

mm , AIDS j m -

. T mm m

. F m q HIV—

m - (CD +)

, -

m . T m (

. A m

- - m [. ., CD +:CD +])

AIDS . T ,

m m AIDS. N m ,

k ’

: x . : m x . I

m . I , , m AIDS, . : . - m -

, -

, /

. HIV m /

/ x . T

m . T m

CD + , , m

.

CD + . T m x

C m m HIV—

m m . A

x m , , m

, m :

. T m AIDS -

HIV/AIDS

z m

. I -AIDS , m-

. L Pneumocystis carinii m -

m m

m k AIDS. O , x m ,

.

CMV, , q m x .

T CD +

C Histoplasma capsulatum -

mm . I m

z q . T

m AIDS. T

m q m HIV/AIDS -

m HHV-B ,

, **Kaposi's sarcoma**,

CD + . I m m

m . M B—

HIV- , HHV-m ,

m m m mm z

m mm m m

HIV/AIDS k HIV/AIDS.

.

B m m q , k m -

z B-

. E m

m . L m m

m m - m-HIV- m m (C). A m

m m mm m z

m , m -

.

k m (PWM), x

.

Dis e a s e Progres s ion

O m AIDS -

A HIV

m m .

AIDS ,

. O ;

Se rolo gica l Ma rke rs

x m , k m x -

De tection of Vira l Antig e n

HIV m

F , m

AIDS m q k . S m

mm m . Imm -

mm m m

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PART 5 ■ Nonmalignant Leukocyte Disorders

HIV. S m z ,

α , α -m ,

m , m x

β -m . R k - -

k HIV- .

k - .

T m q -

m

Sys te mic Lupus Erythe mato s us

HIV- . A

x m m -

Et io logy, Signs , an d Sym p tom s

k . W z m

Systemic lupus erythematosus (SLE) m

mm m HIV- -

mm

m DNA m ,

. SLE m m -

() k m

, m mm j ,

() . T

, - . SLE

, ,

m m

-

m m .

m m W . T -

m m mm m

q

m .

W -

C m m , , m -

q m - z m mm

, (j), (mm

- . A

j), m (-

. A

). D

SLE-k m . T m

z , m , z . I

.

, , ,

I

z

.

m m .

T

D m q

m m

mm m x

m. T

k . A k

m q

, m

mm m x , -

m . A

HIV- –

,

.

, m .

Antib odies to HIV-1

La boratory Da t a

A HIV-x -

T SLE m . T

m k m -

, , LE ,

. B , m - ,

, ,

-

m m .

.

I HIV

LE Cell Pre pa ra t io n

% % ,

T LE

CMV, EBV, A B, . gondii

. A LE m m

mm (-) m x

m

. C , m m

m . T

HIV k

m m .

. I ,

m -

Ant ibo dy Te s t s

, m . I A DNA mm m m m , -

m . ANA

. - ,

SLE

m

LE

.

NA , , q -

. ANA m

Ot he r Im m un e Cha nge s

.

A mm . T -

m

mm m

(DNA RNA) ()

CHAPTER 19 ■ Disorders of Lymphocytes

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m

CHAP ER HIGHLIGH S

m .

S m

Characte ris tics of Lym pho cyte s

- DNA (DNA DS-DNA).

H DS-DNA m SLE -

■ T m m %

. S m

%, . . × /L.

m - DNA (SS-DNA).

■ A m

ANA m q

m m m .

mm (RIA). I q ,

■ A m

DS-DNA . A

m m m .

q m . :

m m-

Lym pho cyto s is

m .

■ L m m -

A ANA m

x m , m -

SLE, , ,

. × /L.

, m

■ I , m

. A ANA

m

m SLE

m . M -

. O , -Sm,

m , m -

% % SLE -

k m (m), k m

m k SLE. P - SLE

m m , W m' m m .

.

L m x m -

Dis o rders As s ociated w ith Lymphocyto s is

m m . T -

I m E -B .

m



W B. pertussis,

m m



m mm

. T

.

m .

■ x m z

. gondii.

Lym p hocyte Subs e t s

D m m j mm -

Imm une Dis o rde rs As s ociate d w ith

SLE. Am - , k ,

Lym pho cyto pe nia

, z - -

Imm m

. Am B ,

■

q () q () m -

m m

m q . T

, I G, q m

- B- .

mm m x

H m mm (HIV/AIDS)/AIDS -

k .



m x m k m . T

m m .

■ S m m - -

NOTE: This is a good time to complete end of chapter

.

Review Questions.

SLE.

CASE STUDIES

Cas e Study 19.1

■ Laboratory Data

T m m -

A m m m x m

m ; , k

, , -

. × /L. T m

. A x m

m %. M m

f ,

m (%) , -

m (m) , m ,

m. T

. A m , ,

m . T m

m .

.

(continued)

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PART 5 ■ Nonmalignant Leukocyte Disorders

CASE STUDIES (continued)

T m ' -

Cas e Study 19.3

. A - m

.

A - - k

m

■ Critical Thinking Group Discussion Questions

. P x m -

. W ' m ? I m . T

m ?

m .

. W m ?

■ Laboratory Data

T m m

. I m

m ; , k . ×

, ?

/L. T m

%; m m m .

. D .

■ Critical Thinking Group Discussion Questions

. W m

. A k m ?

?

Cas e Study 19.2

. W ?

A - - k m m

. W m m ?

. T x m f -

Case Study 19.4

x . T

A - - m m

.

k

P x m

x k . O -

, m ,

x m , j

m (m). T

k , m k

m , m -

k . T

m , , m

m . T -

, .

: m , -

, m .

■ Laboratory Data

T ' m , x k -

■ Laboratory Data

. T k × /L, %

T m m m ,

k m . M m -

k . × /L. T

m m . T m

: m %, %, mm -

%, m %, %. T

. T m ,

m , k ,

m -

m . T m

. T m

m . A -

q .

m . A ANA

; .

■ Critical Thinking Group Discussion Questions

. W ?

■ Critical Thinking Group Discussion Questions

. W m ?

. W m k -

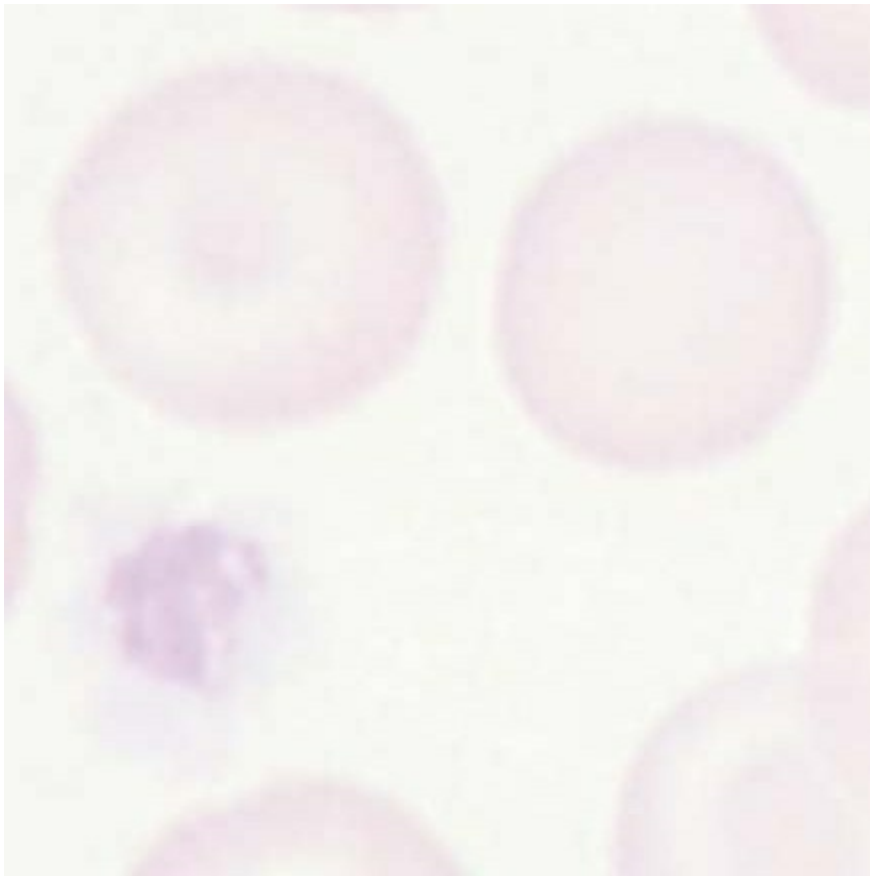
?

. W ANA ?

. W m m m ?

NOTE: This is a good time to write out the answers to the
Critical Thinking Group Discussion Questions.

. W x ?



CHAPTER 19 ■ Disorders of Lymphocytes

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REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

10. W z

m ?

Characteristics o Lymphocytes

A. E : EBV

1. L m m

B. A -

A. k

C. A A

B. m

D. N

C.

m

*11. T m

D. m

z

A. m

2. R x

B. :

A. m

C. ' m

B. m

D.

3. C x

A. m

*12. EBV m ?

B. m

A. CD

B. CD

Lymphocytosis

C. CD

D. CR

4. Ex m m m

13. O m x m , -

A. m

() m

B. m (CMV)

_____ .

C. q mm m (AIDS)

A. -

D.

B. BC. m

Disorders Associated with Lymphocytosis

D. m

5. I m

A. m

14. T EBV

B. m

A.

B. B

6. W

C. m

A. m

D. m

B. m

15. W z

7. x m

m ?

A. m

A. A

B. m

B. L k

C. L m \times /L

Immune Disorders Associated with Lymphocytopenia

D. L m m

*8. m _____

AIDS.

*16. W CMV

A.

?

B.

A. E : m

C.

B. L m

D.

C. A

D. B A B

*9. Imm

A. m

*17. AIDS

B. m

A. m

B. CMV

C. HIV—

D. EBV

(continued)



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PART 5 ■ Nonmalignant Leukocyte Disorders

REVIEW QUESTIONS (continued)

*18. W z () 21. A C . , ' m -

x m ?

A. S m m m m m

A. \times /L

B. O m

B. \times /L

C. E :

C. \times /L

D. A

D. \times /L

*19. W LE -

*22. A C . , x -

SLE?

m k

A. R m

A.

B. ANA

B.

C. C m m x

C.

D. A Sm

D.

*20. A C . , ' m

*23. A C . , x

A. k

A. m m

B. k

B.

C.

C.

D.

D.

Morbidity and Mortality Weekly Report, 2000, 100, 1000-1000.

COMPANION RESOURCES

Sm, .

:// . . m/

CDCP. Mother-to-Child (Perinatal) HIV Transmission and Prevention, 2000.

EW -

J, .

m .

F m AF, H SM. T I E m , Immunol Allergy H ,
Clin North Am, (): – , .

-

F GR. E -B . I : B M (.). extbook o Human

.

Virology, L , MA: PSG P , : – .

H SM. T x m , Br Med J, : – , .

K JS, . C m , J In ect Dis,

: – , .

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L E , H W. E -B : -

, Lab Manag, : – , .

A AK, L m AH, P S. Cellular and Molecular Immunology,

M GE (.). Principles and Practices o In ectious Disease, ,

, P , PA: E (S), .

N Y k: W , .

B KA. N m m , Clin Lab Sci, M , KL. B m , Am A Imm , (): – , .

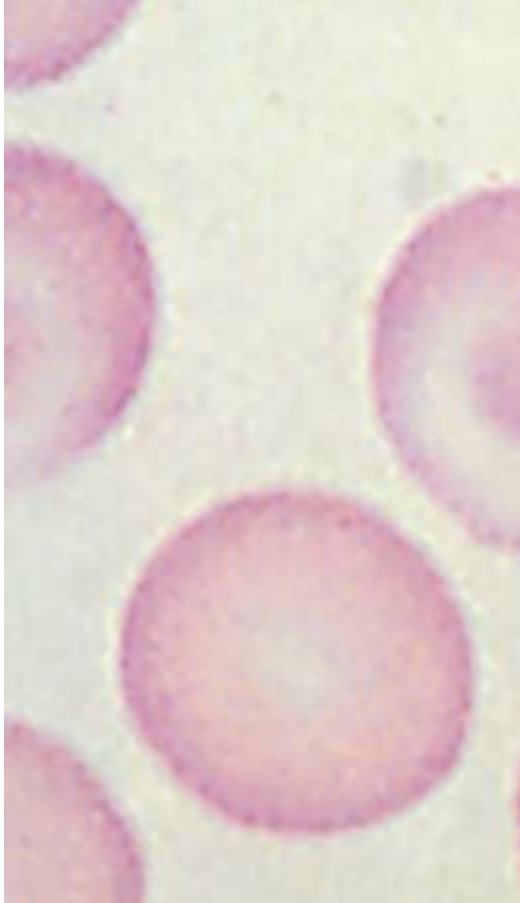
AAI A C Imm , B , MA. A , .

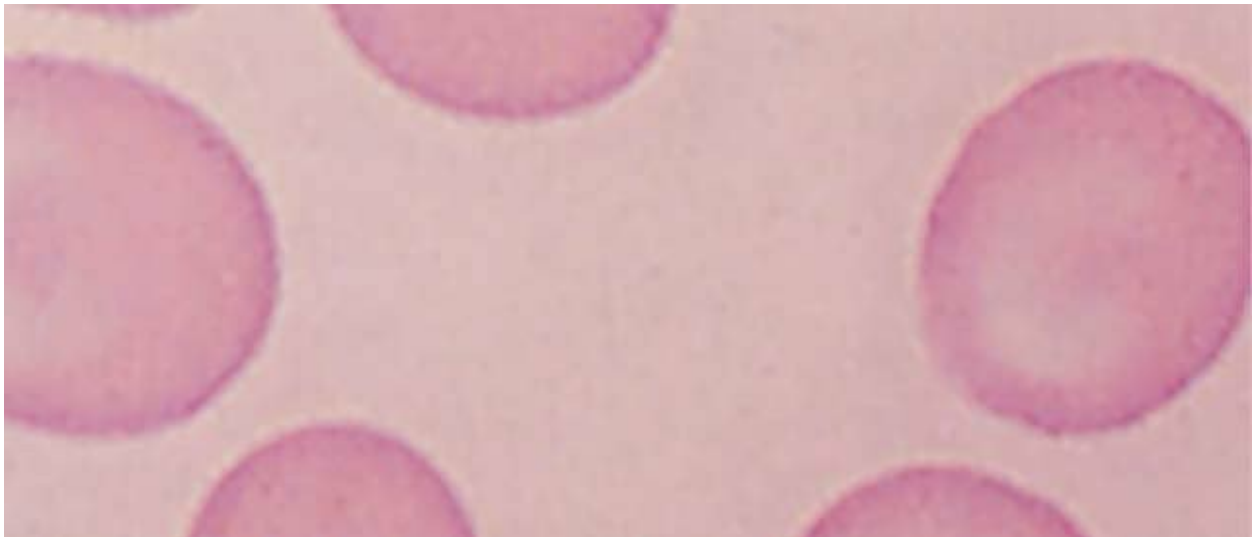
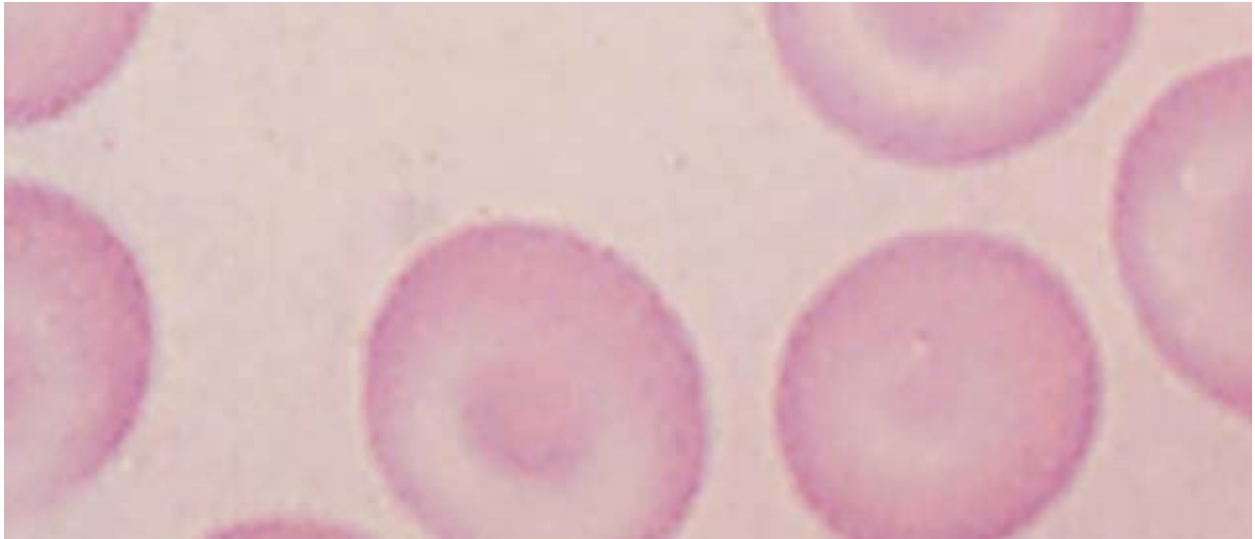
CDC P.P

WHO z UNC ' F . HIV/

HIV —U S , — ,

AIDS- . R J , .





PART SIX

Neoplastic Disorders

Characteristics of Leukemias,

CHAPTER

20

Principles of Blood Collection

Lymphomas, and Myelomas

KEY TERMS

acute

lymphadenopathy

tumor-suppressing genes

aneuploidy

lymphomas

vaccine

chronic

myelomas

World Health Organization (WHO)

French-American-British (FAB)

neoplasms

classification

classification

oncogenes

le u k e m i a

p r o t o o n c o g e n e s

LEARNING OUTCOMES

Com paris on of leukem ias , lympho mas , and

- Name one genetic defect that is correlated with an increased inci-

myelo mas

dence of leukemia.

- De ne and differentiate the terms neoplasm and malignant.
 - Explain the signi cance of the discovery of the human T-cel leuke-
 - Compare the characteristics of leukemia, lymphoma, and myeloma.
- mia virus (HTLV) family and describe associated disorders.
- Describe the role of protooncogenes and oncogenes in leukemias

Form s of leuke mia

and lymphomas.

- De ne and compare the terms acute and chronic leukemia.

Dem og raphic distributio n o f le uke mia and

- Differentiate between acute and chronic myeloid and lymphoid leukemias based on clinical and hematologic ndings.

lympho mas

- Describe the variations in the incidence of leukemia in different eth-

Clas s i cations of leuke mias

nic and racial groups.

- List the traditional forms of the major types of leukemias.
- Correlate patient age to the overall incidence of various leukemias
- Compare the FAB and WHO staging systems for leukemias.

(hematopoietic neoplasms).

Prognosis and treatment

- Describe the overall differences between the incidences of leukemia in female and male patients.
- Compare the early treatment of leukemias and lymphomas with current therapy.

Leukemia vaccines

Factors related to the occurrence of leukemia

- Explain the role of vaccines in treatment and/or prevention of leukemias.

Describe the effects of ionizing radiation on the incidence of leukemia.

Name one chemical that is correlated with an increased incidence

NOTE:

of leukemia.

- indicates MLT and MLS core content

Name several occupations that are associated with a higher-than—

indicates MLT (optional) and MLS advanced content
normal risk of hematological malignancies.

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372

PART 6 ■ Neoplastic Disorders

**COMPARISON OF LEUKEMIAS,
m m / , LYMPHOMAS, AND MYELOMAS**

k . C k m

m m , m m m

Leukemia, lymphomas, myelomas -

m / , -

, neoplasms.

k m x m

T m m

m . T

m . V

m m k m , m

m ,

m , -

, m , m , m .

m m m

L k m , k ,

.

m . L m m m m -

C m MDS MPN .

m m, m m

. T m m m H k ' m—

Note: This is a good time to review the definitions of Key

m -H k ' m m . M m m

Terms found in the Glossary and ash cards on

m . I m m , ,

and also a good time to complete Review Questions related

m m m m

to the preceding content.

(.).

FORMS OF LEUKEMIA

CLASSIFICATIONS OF LEUKEMIAS

T m m k m m H , V z -

A V k m

Table 1. H

Table 1. m -

Table 1. m k m

Table 1. m m m , k , lymph-m m -

adenopathy. V k m

.

, m . N , m mm -

m m -

Table 1. T French-American-British (FAB) -

k m m m x .

k m m

T m m , m , m - World Health Organization (WHO) classification m.
T FAB m m .

acute chronic. A k m -

T WHO m m m

z m m , m mm

.

TABLE

20.1 Comparative Features of Leukemias and Lymphomas

Leukemias

Lymphomas

Myelomas

Basic characteristic

Overproduction of various

Solid malignant tumors of the lymph

Overproduction of

types of immature or mature

nodes

plasma cells in the

leukocytes in the bone marrow—

bone marrow with

row and/or peripheral blood,

concurrent production

in most types of leukemias

of abnormal proteins

Cell type

Usually involves leukocytes

Lymphocyte is the distinctive cell type.

Plasma cells

of the myelogenous or lymph—

Reed-Sternberg cells are diagnostic of

phagocytic cell types

Hodgkin's-type lymphoma.

Site of malignant cells

Malignant cells freely travel—

Malignant cells are initially confined

Plasma cells form a

pass the blood-brain barrier

to the organs containing mononuclear

mass or tumor that is

phagocyte cells such as the lymph nodes, located in the bone

spleen, liver, and bone marrow.

marrow.

Notes

Lymphomas can spill over into the

circulating blood and present a leukemic—

appearing picture on a peripheral blood

smear.

CHAPTER 20 ■ Characteristics of Leukemias, Lymphomas, and Myelomas

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French-American-British Classification

m). S m q m

U FAB m, k m

.

k :

■ M

FAC ORS RELA ED O HE

■ M

OCCURRENCE OF LEUKEMIA

■ L m

W m

L k m q

m , m j -

m m m m m -

k k m

. I k m

k , x m , m -

x -

, m , m -

m . L k m m

m , m k m (CLL).

- , m m

M mm m k k m

m -

(m), , .

m m .

O m k

A m j k m

, j m

, m m -

k , x .

k m m m (

.). T

World Health Organization Classification

. G mm

T () WHO -

. O x

m m : m , m-

. E m x

, / . C

. C m x

m m m mm -

. G m

,

. V

m , m m .

. S

P m , x m , m k -

m , m m m / k m , k m

NOTE: This is a good time to complete Review Questions for

m , m

related preceding content.

m , m m m -

m , x m , m m , m -

/m m , m

Genetic and Immunological Factors

m , m () B- /NK m ,

M m k m ; H k ' m m , / m .

m m m mm .

T m m m

S m m m :

. W m

m m ,

, m x ,

.

Potential Pre dis pos ing Factors

TABLE

20.2 for the De velopment of

Le ukem ias and Lym phom as

PROGNOSIS AND REA MEN

Be nze ne , Hydro carbons , and

U k m m m m .

Che m icals

Hair Dye s

R

. T , -

Environmental

Ionizing radiation, insecticides,

,

herbicides, and fungicides

.

Drugs

Alkylating agents and chloramphenicol

M , , m

Viruses

Herpesvirus (EBV)

m m

m m k m

Human immunodeficiency virus (HIV)

m m . I , m

Human T-cell leukemia virus (HTLV-I)

m , Genetic

Down's syndrome

x m , m k m (CML). E

syndromes

Fanconi's anemia

m q m m

m . T m m m

Hematologic

Myelodysplastic syndromes

(C

conditions

374

PART 6 ■ Neoplastic Disorders

■ ranslocations m mm DNA

Oncogenes Fo rm ed by S o matic

k m . A m

TABLE

20.3 Mutation of Normal Genetic Loci

m m k m

m m . T k

Oncoge ne

Dis orde r

— x m ,

m

Abl

Chronic myelogenous leukemia

m .

Myc

Burkitt's lymphoma

■ Deletions m m . T

Ras type

Variety of tumors

m k

k, x m , m .

■ Inversions m m

q

, . T

m m k

()

. B

.

m ,

■ Addition m x m m

x x m m . T m m m .

. T m

T k -

m .

. O m E m , m m x m m k DNA m m .

m m m . T , -

C DNA m

m . I

m . F x m ,

m , m m

FL , -KI , RAS

m . O

mm m k m (AML).

m , m x -

. O x m

Oncoge ne s

% % m m

m m

A oncogene m

. M , m

f -

m ,

. C m

(.). E k m

. C - m -

.

:

. T m x -

Tum o r-S u ppre s s ing Ge ne s

m z m

A

m m .

. T tumor-suppressing genes -

. S m m ’

m . W

m DNA.

, k

. P m mm m’

m m , z m .

m -

. S m m

. T , m -

m

m

.

m m

.

R -

T m m -

. A ,

m . I k m

, m .

x -

M . C

. N m -

, m

j . D

z , m DNA

m m

. T m m -

- mm

m m m .

m . Ex m

Protoon coge nes

. β -I

. m

Protooncogenes m

. m

. N -

, , k

N m m -

k . T m

m m . I m

CHAPTER 20 ■ Characteristics of Leukemias, Lymphomas, and Myelomas

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-

Occupations Related to a Higher-

- ,

TABLE

20.4 Than-Ave rage Risk o f Malig nancy

in Hem atologically Re late d S ite s

m . G m

Occupation

S ite

. I m

-

Chemist

Lymphatic system

m m m ,

Radiologist

Bone marrow

m m m

Rubber industry worker

Blood

k .

A m x m m -

Woodworker

Lymphatic system

,

. S m -

m , k m . B z

m

, m ,

m m m

m , - . I m . T m m - -

m k , m , -

m m

, , , . C m m .

x m k k

Im m un olo gica l S urve illa nce

m k m (AML) m

Imm mm m

k m (ALL). T j -

m . A -

- - k m

x x x m

m m . .

mm j ,

P m

m mm

mm , x m , ,

. T m m m m

k , m m

m m

ALL.

m m .

A k k m m-m m **Occupational Expos ure**

m m m-m

m . T k m -

I z x m -

m m

k m . H ,

.

M m C , I ,

m - k m . T H m

Genetic Abnormalitie s and As s o ciatio ns

--

k m . B m ,

C m m k m m m q k m . S -

. T m m -

k m ,

k m m q .

k m m ,

. M k m

Environmental Exposure

mm

S

Tkmmx-

km (

,xm,,x-,

mm)km,k.

C,.Ex-

Tkm-

mmm

kmmm

kkm,xk

m,mm

km.

,q,-mm

Exk

mmmm

km.Jmm

mmk

kkm,

.

x .

Dow n's Syn drom e

Che m ical and Drug Expos ure

D ' m (DS) m mm m

O x m

aneuploidy. C DS, m , k m . P x

m m , mm mm z k

m , m ,

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PART 6 ■ Neoplastic Disorders

k m . DS

A RNA DNA

ALL m k k m

m m . S m -

(AMKL). DS m -

, EBV m ,

(MD), k m m AMKL.

B k ' m m

T k m D '

m , .

m m

V , m m m -

m m . C

. MD DS-AMKL m

m m -

q m x X-k GA A

.

, GA A . T

m x

Eps te in-Ba rr Virus

(GA A) m GA A

T DNA-EBV B k ' m m

m m m .

z k

C DS m m k ALL

m m . B k ' m m

. T

- z m q A

m k k m -

q . I m ,

. MD, -

E U S ,

z mm m k

% % B k ' m m -

, % % D ' ,

m EBV. Am

m . M MD ,

W , EBV -

k m m . A x m %

m m .

MD AMKL .

Hum a n T-Ce ll Le uke m ia Virus e s

Ge n et ic Abno rm a lities

T m m H L V m (H L V-I)

T m mm CML

. S , m

m ALL k P m -

m m m m , DNA () m

m m , (;). I

- k m m m . H L V RNA m

% % k m . O , m-

() k m .

m m m

H L V-I m

, (;), , (;).

m - k

O m m deletions inerm . C U S ,

sions (m DNA -

. HIV q mm -

m m) mm .

m (AIDS). T

H L V-I H L V-II -

Viral Agents

z z -

mm .

C , x m , E -B (EBV), HIV . R

m m (“m ”)

, - RNA z m ,

U S . I A , k B k ’

, RNA DNA.

m m , m m k m .

T m

I m - m m / k m -

DNA RNA— , m .

(H LV-) - m

k m . M J C .

Se condary Caus es of Leukem ias

T mm U S .

C

Vira l Cha racte ris t ics

m k

T m m

, AML, . D k

(, m x)

, x m , m m , m mm (HIV). O

x , x m , -

, k k k m . T

m , k m m -

k m m m . T m f .

:

S AML m :

. T q , EBV

■ W m (. . ,

. T ,

)

x m , HIV

■ W (. . , F ' m)

CHAPTER 20 ■ Characteristics of Leukemias, Lymphomas, and Myelomas

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■ W m m m

Es tim ated Ranked

■ W k m ,

He m atopoie tic Neo plastic

m

TABLE

20.5 (A) Diagno ses Annually in the

AML x % %

United S tate s and (B) De aths

H k ' , -H k ' m m , m

Annually in the United States

m m , k -

. A -

Type of Cancer

New Cases

- AML m

m m m II -

(A)

(. . , x).

Lymphoma

$\pm 60,000$

S m m

Non-Hodgkin's lymphoma

$\pm 53,000$

m k m . T -

Hodgkin's lymphoma

$\pm 7,500$

m k m (AML) m

m , x m , m

Leukemias

$\pm 30,500$

k m , .

Acute myeloid leukemia

$\pm 10,500$

A - m k m Chronic lymphocytic leukemia

$\pm 7,000$

Chronic myeloid leukemia

$\pm 4,250$

. T k m -

m k . T

Acute lymphoblastic leukemia

$\pm 3,700$

k m m m

Other types of leukemias

$\pm 5,000$

Multiple myeloma

$\pm 14,500$

.

(B)

Lymphoma

$\pm 25,000$

DEMOGRAPHIC DISTRIBUTION OF LEUKEMIA AND LYMPHOMAS

Non-Hodgkin's lymphoma

$\pm 24,000$

Hodgkin's disease

$\pm 1,500$

L k m , m m , m m m -

Leukemias

$\pm 22,000$

m % U S .

Acute myeloid leukemia

$\pm 8,000$

L m m m q . T m j

m m -H k ' (

Chronic lymphocytic leukemia

$\pm 4,500$

. A, B).

Other types of leukemias

$\pm 7,000$

T k m

Chronic myeloid leukemia

$\pm 2,000$

, , , ,

Acute lymphoblastic leukemia

$\pm 1,500$

. A m k m ,

m m , m m

Multiple myeloma

$\pm 11,000$

, m m

. T m m , m m .

■ T m

Ethnic Origin and Race

■ T m

A k m ,

S I , -

C () k m J C .

m -

I W , CLL m %

k m (ALL). M m AML.

k m , m A , . ALL

C k m .

m mm m W m

O ALL,

A Am A Am . M m

. P m

mm m A Am m

k CLL k m . T

W .

m k m , m , A

k m m - .

k m . L k m (

A m k

m) m .

m m . P .

L k m m mm

T k

. T

m.

k m (.) mO ,

m m - m

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PART 6 ■ Neoplastic Disorders

Age -Adjus te d Invas ive Cance r Incide nce Rate s and 95% Co n de nce

TABLE

**20.6 Intervals for Ages 0–19 by International Classi cation of Childho od
Cancer (ICCC) Gro up and S ubgroup and Age, United State s***

Age

Cance r Type s

0–14

0–19

All ICCC groups combined

148.4

165.4

I. Leukemias, myeloproliferative, and myelodysplastic diseases

47.1

42.7

(a) Lymphoid leukemias

36.0

31.0

(b) Acute myeloid leukemias

6.9

7.3

(c) Chronic myeloproliferative diseases

1.3

1.7

(d) Myelodysplastic syndrome and other myeloproliferative diseases

1.4

1.4

(e) Unspecified and other specified leukemias

1.4

1.3

II. Lymphomas and reticuloendothelial neoplasms

16.2

25.2

(a) Hodgkin's lymphomas

5.8

12.6

(b) Non-Hodgkin's lymphomas (except Burkitt's lymphoma)

6.3

8.9

(c) Burkitt's lymphoma

2.5

2.4

(d) Miscellaneous lymphoreticular neoplasms

1.3

1.1

(e) Unspecified lymphomas

~

0.3

*Rates are per 1,000,000 persons and are age adjusted to the 2000 US standard population (19 age groups; Census P25–1130).

Adapted from Centers for Disease Control and Prevention (CDC).
www.cdc.gov, retrieved on January 10, 2010.

, ALL. T

mm -

ALL m

MRD m -

.

m m m

, m

Gender

.

M m k m m q

Am m m , NOTE: This is a good time to complete end of chapter x m m .
ALL

Review Questions

m mm . AML

q m .

T m m

CLL, m : m : . A

CHAP ER HIGHLIGHT S

U.S. Cancer Statistics C

D C P , m m

Co mparis on o f Leukem ias , Lym pho mas , and

m -H k ' m m

Mye lo m as

H k ' m m . A m m

m m m .

■ L k m

M m k m m m .

z mm

m k m -

/ .

LEUKEMIA VACCINES

■ L m m m k m , m m

m m

T m j k m -

m .

m m - m ,

q . k m ,

Form s o f Le uke mia

m m (MRD) .

R U.S. N I

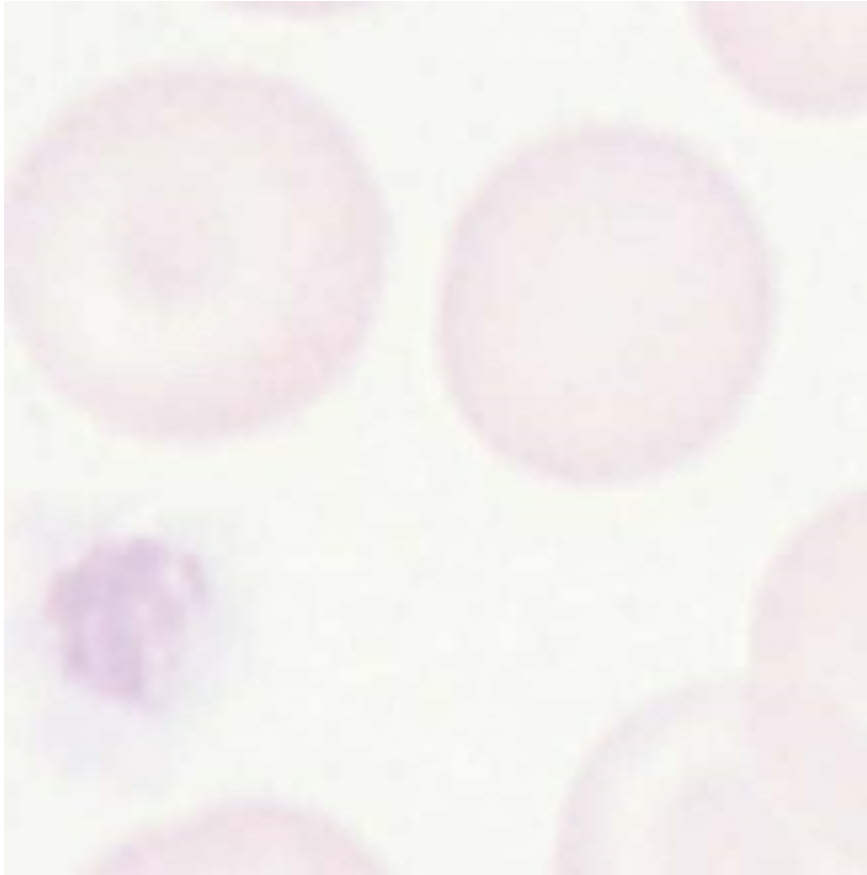
■ A k m z m m H **vaccine** , m mm m

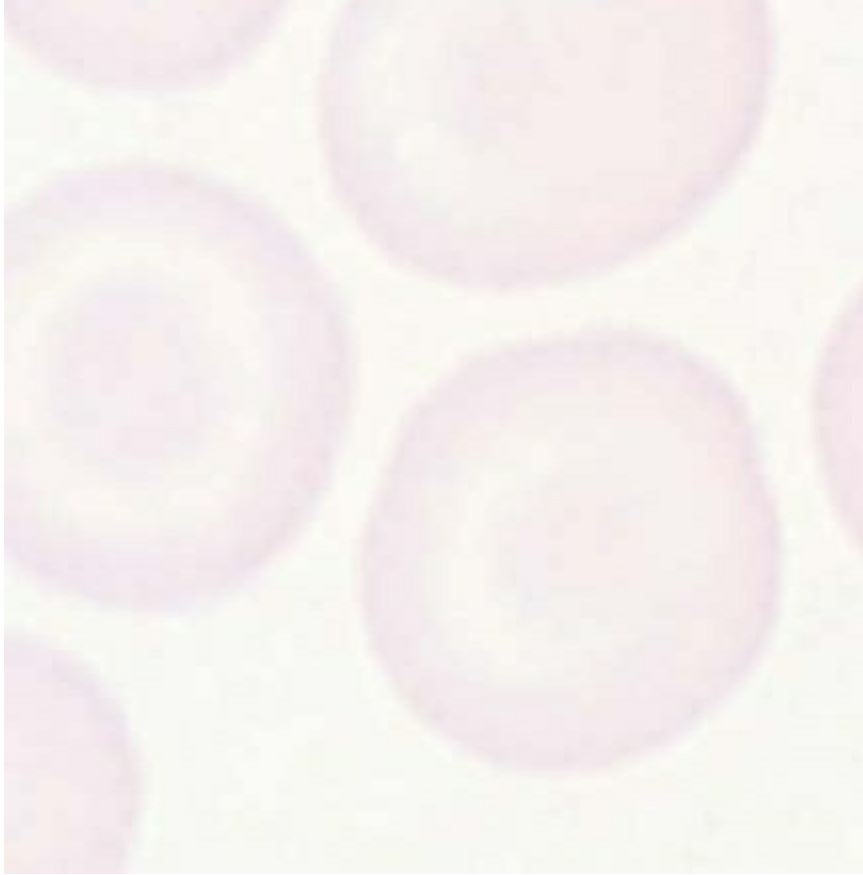
k m CML

m / ,

m m . m mm

k .





CHAPTER 20 ■ Characteristics of Leukemias, Lymphomas, and Myelomas

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■ C k m m m ,

■ T k m m F -

m m m m /

Am -B W

, k

H O z .

m x m m .

■ T m **Pro gno s is and Tre atme nt** m k m , m

■ U k m m m m .

m , -

■ S m q -

m m m

.

.

Factors Related to the Occurrence of Leukemia

Classi catio ns o f Le ukem ias

■ T k m m m

■ T mm k m -

.

: m -

■ T , m

, m , m .

x , .

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

Factors Related to the Occurrence o Leukemia

Comparison o Leukemias, Lymphomas, and Myelomas

5. T k m

A. z

1. A k m

B.

A. k

C. m x z

B. , m m m

D.

C. m -

D. A C

6. HIV

A. k m

2. D m m m m

B. Séz m

A.

C. AIDS

B. m m m

D. k m

C. m

D. -

*7. C - m

Forms o Leukemia

A. x -

m z

3. A k m

m

A. m m k m

B. ' m-DNA

B. m mm k

C. mm m' z

m

m

C. k -

D.

D. m m k m

8. T k m

A. S J

B. Am k Am

Classifications of Leukemias

C. m m

4. A FAB WHO -

m

D. m m

A. FAB m m

B. WHO m m

C. FAB m k m

D. WHO m k m

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PART 6 ■ Neoplastic Disorders

M S, Iz S, C JD. I m , -

COMPANION RESOURCES

m , m m k m D m , Blood, (): – , .

:// . . m/

M N D . . -m . /

M RW. T mm m k E W -

k m . I : L DL (.). Cancer Epidemiology in the USA

m .

and USSR. B , MD: N C I , : H ,

– .

-

N G. S m q m m

.

k m m m : , Am J Med Genet, : –

, .

S W, . L k m U m **BIBLIOGRAPHY**

N : – , JAMA, (): – ,

.

C MP, W m IL, P k CY. C m :

S SH, . (.). WHO Classi ication o umours o

, Lab Med, (): – , .

Haematopoietic and Lymphoid issues, L , F : IARC, .

D T G, . V k m k m W S, . M m k O k R N

m m ? Leuk Res, (): – , .

L , JAMA, (): – , .

CHAPTER

Acute Leukemias

21

KEY TERMS

Au e r ro d s

e ryth ro le u ke m ia

m icro RNA

a zurophilic gra nule s

Fre n ch -Am e rica n -British (FAB)

n e xt g e n e ra tio n s e qu e n cin g

b la s ts

cla s s i ca tio n

re la p s e

ch lo ro m a

le u ko s ta s is

s te m ce lls

cyto g e n e tic a n a lys is

megakaryoblastic leukemia

World Health Organization (WHO)

LEARNING OUTCOMES

General characteristics of acute leukemias

Discuss treatment options and strategies in AML, including induction and consolidation therapy.

- Name three examples of conditions that are considered to be genetic lesions.

Define and describe characteristics of a relapse.

- Describe the fundamental characteristics of blood and bone marrow
- Discuss the purpose, advantages, and concerns related to allogeneic hematopoietic-cell transplantation.

- List and describe basic characteristics of classifications recognized

Summarize various types of new therapies in AML.

by the French-American-British (FAB) and World Health Organization

Acute lymphoblastic leukemia

(WHO) systems.

- Discuss the epidemiology of acute lymphoblastic leukemia (ALL) in
- Discuss general prognostic factors and the importance of monitoring minimal residual disease on the survival of acute leukemia

the United States.

patients.

- Summarize the pathogenesis of ALL.

- Name and briefly describe the FAB classification of ALL.

Acute myeloid leukemias

Using FAB and WHO criteria, describe subtypes of B lineage, early B

- Discuss the concept of clonal heterogeneity in acute myeloid leukemia—precursors, “common” CALLA (CD10) positive, pre-B, T-cell lineage, leukemia (AML).

and early T precursor (pro-T, pre-T, cortical-T, medullary-T) and pre-

- Coordinate factors related to epidemiology and long-term survival precursor lymphoid neoplasms

of AML patients.

List cytogenetic and molecular abnormalities commonly associated

Discuss the importance of cytogenetic and molecular analysis to with the major acute leukemic subtypes.

identification and treatment of AML.

- Describe the clinical symptoms, laboratory findings, and special

Describe the 2016 World Health Organization classification revision—identification techniques in ALL.

sions related to AML.

- Summarize treatment strategies and prognosis in ALL.

Interpret findings from immunophenotypic, cytogenetic, and molecu-

Life-threatening emergencies

lar findings, and apply to criteria used by WHO.

- Name and describe life-threatening emergencies.
- Discuss factors associated with the prognosis in AML.
- Explain the importance of microRNAs.

Future trends

- Describe the utility of monoclonal antibodies in differentiating
- Discuss future trends in the treatment of leukemia.

between various leukemias.

- Explain the chromosomal alterations that may be observed in vari-

Case studies

ous AML.

Analyze the patient history, clinical signs and symptoms, and labo-

- Summarize and apply the diagnostic blood and bone marrow laboratory data for the stated case studies, answer the related critical thinking questions, and conclude the most likely diagnosis.

Define the reactivity of leukemic cells with various cytochemical stains: Sudan black B stain, myeloperoxidase (MPO), and the peri—

NOTE:












odic acid–Schiff test.

■ indicates MLT and MLS core content

Compare the two common esterase procedures in terms of their

indicates MLT (optional) and MLS advanced content

purposes.

MORPHOLOGY	CLASSIFICATION	NUCLEUS	NUCLEOLUS	CHROMATIN	CYTOPLASM
	L1 ACUTE LYMPHOBLASTIC (principally pediatric)	Uniformly round, small	Single, indistinct	Slightly reticulated with perinucleolar clumping	Scant, blue
	L2 LYMPHOBLASTIC (principally adult)	Irregular	Single to several, indistinct	Fine	Moderate, pale
	L3 BURKITT-TYPE	Round to oval	Two to five	Coarse with clear parachromatin	Moderate blue, prominently vacuolated
	M0 MYELOBLASTIC (minimally differentiated)	Round to oval	Single to multiple, distinct	Fine to coarse	Scant, non-granulated
	M1 MYELOBLASTIC (without maturation)	Round to oval	Single to multiple, distinct	Fine	Scant, variably granulated
	M2 MYELOBLASTIC (with maturation)	Round to oval	Single to multiple, distinct	Fine	Moderate azurophilic granules with or without Auer rods
	M3 MYELOCYTIC	Round to indented to lobed, "cottage-loaf"	Single to multiple, (granules may obscure)	Fine	Prominent azurophilic granules and/or multiple Auer rods
	M4 MYELOMONOBLASTIC (biphasic M1 and M5)	Round to indented, folded	Single to multiple, distinct	Fine	Moderate, blue to gray, may be granulated
	M5 MONOBLASTIC	Round to indented, folded	Single to multiple, distinct	Variable, lacy orropy	Scant to moderate, gray-blue, dustlike lavender granules
	M6 ERYTHROBLASTIC	Single to bizarre multinucleated, multilobed	Single to multiple, distinct	Open "megaloblastoid"	Abundant, red to blue
	M7 MEGAKARYOBLASTIC	Round to oval	Single to multiple, distinct	Slightly to moderately reticulated	Scant to moderate, gray-blue, with blebbing

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PART 6 ■ Neoplastic Disorders

GENERAL CHARACTERISTICS OF ACUTE

French-American-British Classification

LEUKEMIAS

I, F, Am, B

m m m ,

E k m q . I F -Am -B (FAB) m m m , m (F . .). T FAB m -

m m m , m -

W -

m , m ,

m m .

k m , , m S , FAB (.) m . A , k m m j —AML

k m z blasts

ALL — .

mm k m .

T FAB m AML (M

M) ALL (L L). T

French-American-British and World Health

m m k m

Organization Categories

m . C

k m k m

A k m

. I ,

m . A mm % m f

French-American-British system (FAB) World

kin.

Health Organization (WHO) kin

FAB mitosis %

World Health Organization (WHO) Classification

mitosis /

Immunofluorescence FAB

mitosis WHO mitosis

mitosis, mitosis -

% .

mitosis

FIGURE 21.1 Akin : FAB . (Rosenberg, F. J. L. Pathology, , P, PA: Lippincott & Wilkins, , mitosis.)

CHAPTER 21 ■ Acute Leukemias

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TABLE

21.1 The FAB Classification System

Subtype Name

Cellular Characteristics

M0 myeloid

Undifferentiated blasts, AML—not otherwise categorized

M1 myeloid

Blasts and promyelocytes predominate with minimal maturation of myeloid cells

M2 myeloid

Myeloid cells demonstrate maturation beyond the blast and promyelocyte stage

M3 promyelocytic

Promyelocytes predominate in the bone marrow

M4 myelomonocytic

Both myeloid and monocytic cells are present to the extent of at least 20% of the total leukocytes M4eos myelomonocytic Both myeloid and monocytic cells are present to the extent of at least 20% of the total leukocytes with eosinophilia M5 monocytic

Most cells are monocytic; two subtypes (a and b) are recognized, one characterized by large blasts in bone marrow and peripheral blood, the other (differentiated type) by monoblasts, promonocytes, and monocytes M6 erythroleukemia

Also known as Di Guglielmo's syndrome; megaloblastoid development—abnormal proliferation of both erythroid and granulocytic precursors; may include abnormal megakaryocytic and monocytic proliferations. The presence of micromegakaryocytes is closely associated with erythroleukemia.

M7 megakaryocytic

Large and small megakaryoblasts with a high nuclear-cytoplasmic ratio; pale, agranular cytoplasm

L1 homogeneous

One population of cells within the case; small cells predominant; nuclear

shape is regular with an occasional cleft; chromatin pattern is homogeneous and nucleoli are rarely visible; cytoplasm is moderately basophilic L2 heterogeneous

Large cells with an irregular nuclear shape; clefts in the nucleus are common; one or more large nucleoli are visible; cytoplasm varies in color L3 Burkitt's lymphoma type

Cells are large and homogeneous in size; nuclear shape is round or oval; one to three prominent nucleoli; cytoplasm is deeply basophilic with vacuoles often prominent AML, acute myeloid leukemia.

AML. T W H

, k m ,

O z (WHO) (B x .)

.

m —m , m , mm -

T m m -

, , —

k m m x m m k

.

. B m m ,

k m

m .

Pro gno s tic Facto rs

M m m q

N % k m m

- - m - - (R -PCR)

. T m m

m k m - -

k m m . N

m .

x m % %

C m m

m . T

P

CD P- , x ,

m . AML -

■ R

m FL 3

■ R

m .

P , , x -

, , mm

m - . D - , NOTE: This is a good time to review the de nitions for the k , x

Key Terms found in the Glossary and

PART 6 ■ Neoplastic Disorders

BOX 21.1

World Health Organization (WHO) Classification* of Acute Leukemias and Related Neoplasms ACUTE MYELOID LEUKEMIA WITH RECURRENT

AML M (M)

GENETIC ABNORMALITIES

AML M (M)

P-AML (;)(q ;q); RUNX1—

AML M /M (M)

RUNX1 1

AML M (M)

AML ()(. q) (;)(. ;q); CBEB—

P M (M)

MYH11

AML M , /M (M)

AML (;)(;q); MLL 3-MLL

AML M M (M)

AML (;)(;q); DEK-NUP214

A k m

AML () (q q .) (;) (q ; q .) ; RPN1-EVI1

A m m

(NEW m GA A2

m RPN1 , q

MYELOID S ARCOMA

Mye loid Prolife rations Re late d to Dow n's

MECOM(EVI) q . .

Syndrome

AML (m k) (;) (; q) ; RBM15—

m m

MKL1

M k m D ' m

E : AML m NPM1

E : AML m CEBPA

Blas tic Plas m acytoid De ndritic Ce ll Ne oplas m

AML m m -

Acute Leuke mias o f Ambig uo us Line age

m m AML -

A k m

.

P m x k m

A P m L k m (APL) (;)

(;)(q ;q .); BCR-ABL1

(q ;q); PML-RARA

M x k m (; q); MLL

AML WITH MYELOYDYSPLASIA-RELATED CHANGE

THERAPY-RELATED MYELOID NEOPLASMS

M x k m , B-m , NOS

AML, NOT OTHERWISE SPECIFIED AML-NOS

M x k m , -m , NOS

U AML (M)

P : k (NK) m

AML m m (M)

k m / m m

AML m (M)

S : F K. CSP 2015 Short Course—Leukemia Classification 2016:

What, When, “Who,” CSP , SD , CA,

.

* T W H O z . Classi cation o umors o the Hematopoietic and Lymphoid issues, , L , F

.

ACU E MYELOID LEUKEMIAS

m

k m .

Clo nal He te ro ge ne ity

Epidem io logy

AML -

z m k m

AML m mm k m -

q m m -

m , U S

m m m - ,

(F . .). C AML -

, .

m

M AML z -

— -

m

m .

. Next-generation

M % AML x -

sequencing (NGS) m q -

m m . R m

x k m m .

. P

V relapse

AML, x ,

. C m -

- m

m m mm

m .

CHAPTER 21 ■ Acute Leukemias

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Example of Chromosomal

TABLE

21.2 Translocation: t(9q+;22q-)

Code Meaning

t

The translocation of chromosomal material from

one chromosome to another nonhomologous
chromosome

9;22

The numbers of the 22 pairs of autosomal chromosomes or pair of sex
chromosomes that are

involved in the translocation

Q

The lower portion (arm) of a chromosome

involved in the translocation

P

The upper portion (arm) of a chromosome

\pm

The chromosome that gained the extra chromosomal material followed by the
chromosome that

lost the chromosomal material; these symbols

may be absent

A m m m m m **FIGURE 21.2** A m k m . I m m q -

mm k m . T m m m .

m m m -

A m -

. T m (m) .

m m -

(S m m , ×.)

m m .

C k m

Cyto gene tic Analys is in AML

m m m -

AML. S m

C m

FAB . T

AML ALL %

m

k m k m

m

m m m . C m m -

m AML ALL m -

. Imm

k m

Examples of Chrom os o mal

m , , m m .

Trans lo cations Co ns is tently

TABLE

21.3

N'

Associate d w ith He matolo gical

k m

Malignant Dis e as e

m , m -

m (m m)

Dis orde r

Trans location

m m m .

CML, ALL, AML

t(9;22) (q34;q11)

S P m m , AML (FAB M2)

t(8;21) (q22;q22)

m m m m -

(m m , -

AML (FAB M3)

t(15;17) (q22;q21)

, ,)

Burkitt's lymphoma,

t(8;14) (q24;q32)

m k m m m , AML, ALL,

ALL (FAB M3)

t(8;22) (q24;q11)

-H k ' m m . A m m

t(2;8) (p11;q24)

m (k)

m m m

Lymphoma (follicular)

t(14;18) (q32;q21)

k m . T m

CLL, multiple myeloma

t(11;14) (q13;q32)

m m m m k -

T-cel leukemia/lymphoma

t(8;14) (q24;q11)

m (.).

t(10;14) (q23;q11)

T k

m () . Ex m m m -

CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; AML,

m

acute myeloid leukemia; FAB, French-American-British classification; CLL,

chronic lymphocytic leukemia.

m m . .

386

PART 6 ■ Neoplastic Disorders

C m

Frequency of Recurrent Gene

TABLE

21.4

mm

Mutations in Adults with AML

(DNM 3A, ASXL1, IDH2, E 2) -

k m m m -

Mutation

% Frequency

AML. A k m m

NPM1

25–35

m x m -

CEBPA

6–10

, . C m

m m

RUNX1

5–15

k m

FLT3-ITD

±20

m . M m

NRAS

±15

AML — m -

DNMT3A

18–22

m m -

m . C m

ASXL1

5–17

AML -

TET2

7–25

m AML. NPM1AML k m

Modi ed from Döhner H, Weisdorf DJ , Bloom eld CD. Acute myeloid leu—
AML .

kemia, N Engl J Med, 12(373):1140, 2015.

NPM m m

.

m m m

k k m **WHO Clas s i catio n Revis io ns** . A x m -

T WHO (B x .)

-

m x,

- (A RA). A RA m

m FAB AML

(;)

AML (B x .)

m k m .

(B x .).

NOTE: This is a good time to review questions related to the

Genetic Differences

preceding content.

AML -

, M

Molecular Analysis

k -

. I

NGS AML , M j AML

q (. .)

m ,

. NGS

m q x k

■ FL 3 (Fm - k k)

m .

■ C - (CBF) m x

TABLE

21.5 Eight Functional Categories of Genes Commonly Mutated in Acute Myeloid Leukemia Functional Category Mutations Examples of Mutated Genes

1. Class III signaling genes

Tyrosine kinase receptor gene (FLT3)

2. Myeloid transcription factors

RUNX1

3. Nucleocytoplasmic shuttling protein

Nucleophosmin (NPM1)

4. Spliceosome-complex genes for regulation of RNA processing splicing

SRSF 2

5. Cohesin-complex genes for accurate chromosome segregation and transcriptional

STAG 2

regulation

6. Chromatin modification regulation

ASXL1

7. DNA methylation

TET 2

8. Myeloid transcriptional regulations

TP53 (a tumor suppressor gene)

Modified from The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia, N Engl J Med, 368:2059–2074, 2013.

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m . D CBF m x, (;)

BOX 21.2

(q ;q) () (q / (,) (;q) , -

AML . T

m j AML m-New Acute Myeloid Leukemia Subtypes—2016

m -

k m .

■ AML RUNX1 m ()

I WHO , m

■ AML BCR-ABL 1 m ()

() (q q .) (;) (q ;q .) -

■ AML CEBPA m

m GA A

■ F m AML/MDS (m)

m q .

■ N m

P AML NPM (m)

m (B % m .

m AML CEBPA m m

S : F K. CSP 2015 Short Course—Leukemia Classi cation

. AML CEBPA m

2016: What, When, “Who,” C S P , S D , AML m m -

CA, .

m . T m AML

. AML RUNX m

AML BCR-ABL

-

T F L3 III k .

. T k

U , FL x m -

BCR-ABL .

m . F L k

k m m

Key Labo rato ry Finding s

m m . F L k m %

AML.

A m . A m

T CBF m x m x -

m m m m k -

m m m

m . A k -

, m m m m

k . T m

k m .

BOX 21.3

AML z m ,

, , , m

, m m

Ne w Acute Myelo id Leukem ia Criteria

m m. A k

m mm m

. B CEBPA m q AML

m k AML. T M:E AML

CEBPA m .

.

. R AML-MRC m -

M m m m

.

m , x -

. I NPM1 m , AML

m m k m m ,

NPM1 m (m).

, m

. I CEBPA m , AML

AML.

CEBPA m (m).

I AML, m

. I MDS MDS-

(. .).

, AML-MDS

NPM1, CEBPA m .

. B m

- m . N -

PROGNOSIS OF AML

k m ,

m / m MDS.

A AML , AML

. A m m

% %

m-m :

% %

. F m MDS/AML

. T m m

. F m m m

. O -

m m

. F m MDS/AML -

m .

m m ,

I AML z m m -

F ' m ,

— (;)(q ;q); (;) (q ;q) (q)—

. I m ,

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PART 6 ■ Neoplastic Disorders

TABLE

21.6 Laborato ry Inform ation

Morphology of blood cells

% blasts, % dysplastic cells

Flow cytometry

To confirm myeloid lineage: CD33+, CD13+, myeloperoxidase (MPO) +

Cytogenetics

Karyotypic subtypes

Molecular mutations

FLT 3-analysis required for all cases of suspected AML, NPM1, CEBPA, RUNX1, BCR-ABL 1, KIT, (selected cases)

other prognostic factors.

AML, MDS, MPD, etc.

AML, MDS, MPD, etc.

AML, MDS, MPD, etc.

ALL, AML.

.

PML - AML

NOTE: This is a good time to complete Review Questions

AML, NPM1, etc.

related to the preceding content.

FLT-3, IDH, DNMT3A, etc.

.

Characteristics of AML Subgroups

MicroRNAs

AML zmm -

MicroRNA x, m -

mm -

m, m

. Tqm

AML. MRNA - - -

fk. TM

RNA m

m (F. .). T

zmm mRNA

qMM -

-

m .

.

Mmmm

R, mRNA

km. Mm -

mm

k m . T M

m m , x m ,

erythroleukemias m -

m m . M RNA

m . A m m , TABLE

21.7 Characteris tics o f Dys plas ia

Cytoplas m ic Morphology Exam ple s

Ce llular Line age

Nucle ar Morpholo gy Exam ple s o f Fe ature s

of Fe ature s

Erythroid

Unequal binucleation

Ring sideroblasts

Multinucleation

Coarse stippling

Internuclear bridging

Incomplete hemoglobinization

Karyorrhexis/pyknosis

Vacuoles

Nuclear budding/lobulation

Granulocytic

Giant cells

Nuclear/cytoplasm asynchrony

Hypersegmentation

Hypogranularity

Coarsely condensed chromatin with long strand

Pseudo Chédiak-Higashi granulation

connections between lobes

Auer bodies

Hyposegmentation (pseudo Peyer-Huët)

Vacuoles

Megakaryocytic

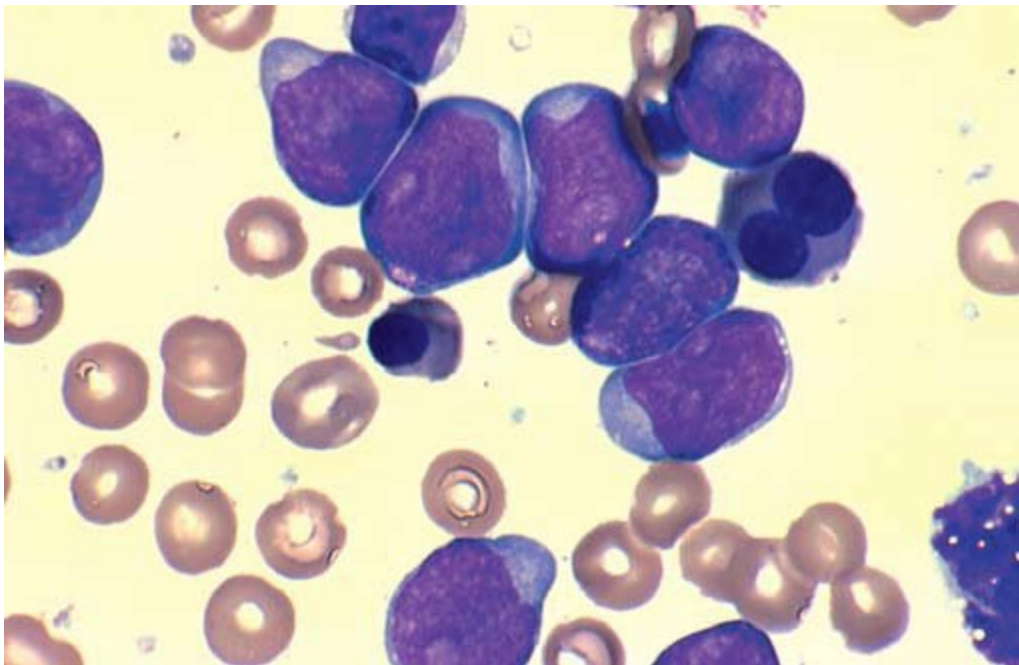
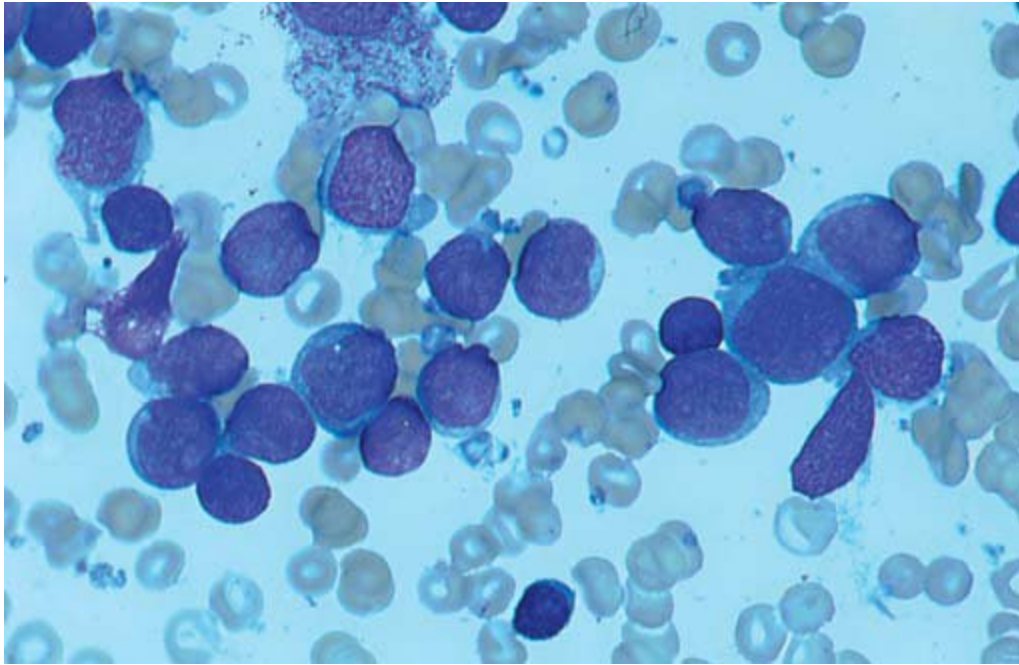
Micromegakaryocytes

Giant platelets

Hypolobulation

Hypogranular

CD34+



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m mm k m

m , m -

m . T m : m

FAB M : . T m m .

m .

Clinical S igns an d Sym ptom s

FAB M z

m m . T m

, , , .

P x m m , m; m m m-

; ; . A

m m , m , m -

FIGURE 21.3 A m k m M . (R m

; , x m % x

M C KD. Clinical Laboratory Medicine, , P , m m .

PA: L W m & W k , , m .) C m AML

m ALL. O z m m

m m k M

m m

. M m k k -

AML. I m ,

m . M

q z m MPO

k m (.).

. T m chlo-

FAB M0

roma. I m , m AML.

FAB M m WHO AML

z (NOS) (F . .). T

La bora tory Dat a

m (x m %

A m m x m

AML)

% AML . L k m

. P

, k

m m k m

× /L.

m . AML, m m -

T m x m

, m . T m

mm , m mm

m mm m km k m . I

/ . Imm

m m mm m -

. E m z

, k m k m (FAB M).

m , , m . T m

T m k m .

(F . .) m m %

Am m m k m , m m

% m . T -

m mm . W

m m , - Auer

, , AML -

, FAB M x m % .

M M m j .

T mm FAB M FAB M .

Acute Mye lo id Le ukem ia (FAB M1)

T WHO m m -

k m m . T m k m

Examples of Monoclonal Antibody

TABLE

21.8 Classification of Acute Leukemias

Myeloid lineage

CD13, CD3, CD15, MPO, CD117

T-cell lineage

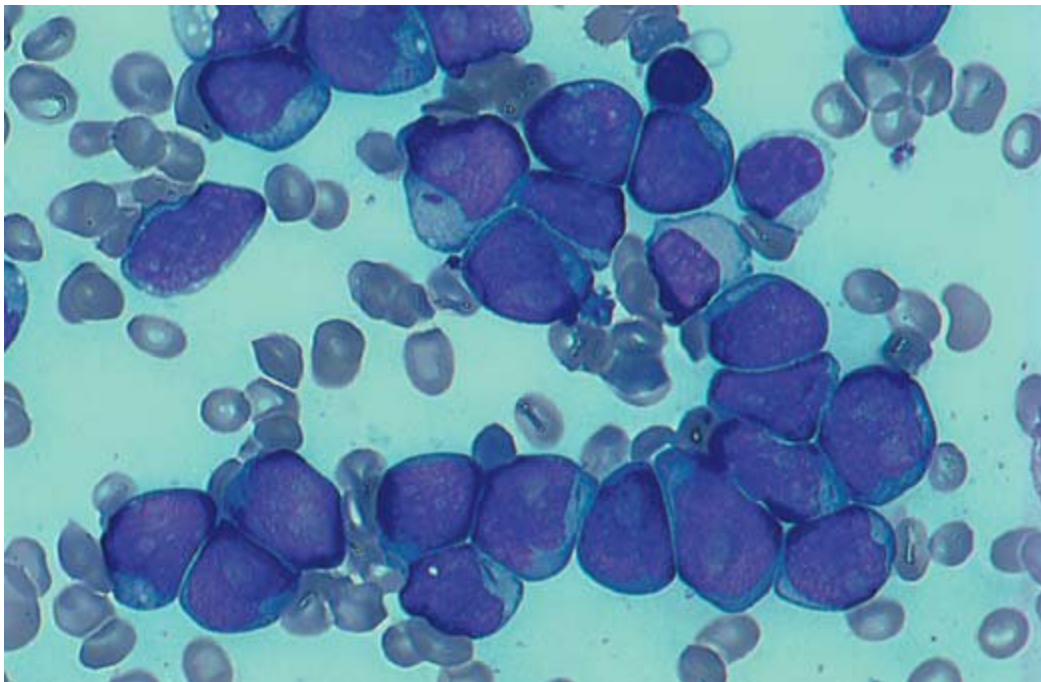
CD2, CD3, CD5, CD7

B-cell lineage

CD19, CD20, CD22, CD79a

FIGURE 21.4 A myeloid leukemia. (Reprinted from Clinical Laboratory Medicine, P, Megakaryoblastic CD41, CD61

PA: L W m & W k , , m .)



PART 6 ■ Neoplastic Disorders

m. Auer rods m -

Acute Pro mye lo cytic Leukem ia (FAB M3)

. T m

m . G , , m (

I m k m (APL), m

). S m

, m x -

m m m

m m . T x m m : m : .

. D , m m m

S A R A m

. A m

m z A R A - m , m

m (q P - H ã m). A m m %, -

m . M

- %. T m

% .

m m m - -

,

Acute Myeloid Leukemia (FAB M2)

mmmk -

m (CML).

TWHO mm

km m . T FAB M m k m

Clinical Signs and Symptoms

m - . T m

F m m , m -

; , x m %

, mm . H m , -

. T x m

m , m q . M

m : m . . . S . m .

m k m

, ,

Clinical Signs and Symptoms

k .

H m m , x ,

, mm m -

La bora tory Dat a

m . H m , m , m

L m m FAB M

q .

. A m m m

La boratory Da t a

. k m -

k k . L k q .

A m m m .

P m m (F . .).

L k mm ,

T m m , m , k x × /L.

. C , z

M m m

m. T

(F . .). T

m - m -

m m m .

. T m ,

T m m -

k . M m

m z m - azurophilic granules. A mm m m

m . M

m A . A m

.

(DIC) (C) mm APL.

C , M z -

m m ,

PML

α (RAR α).

M () m -

k m (PML-M) (F . .)

% % PML. I m PML-M m k m

m

PML.

M , m m m

, m -

. O x A . T

PML/RAR α

PML m % AML-M

PML/RAR . T

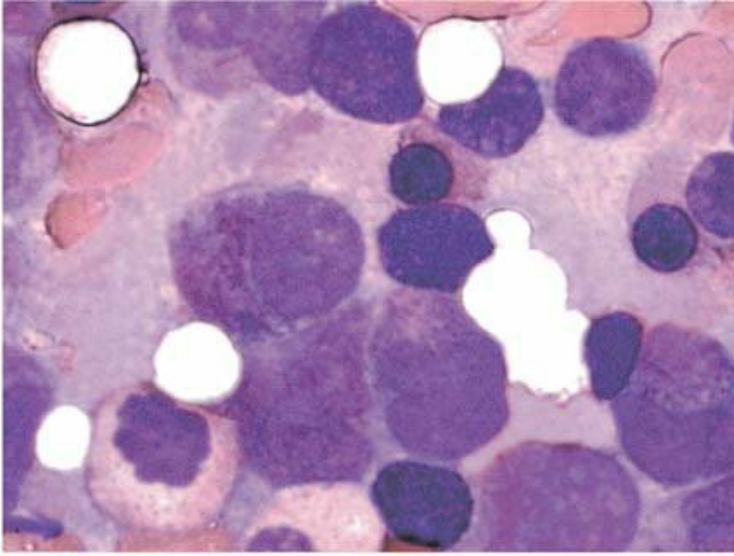
FIGURE 21.5 A m k m M m

A . (R m M C KD. Clinical Laboratory PML/RAR

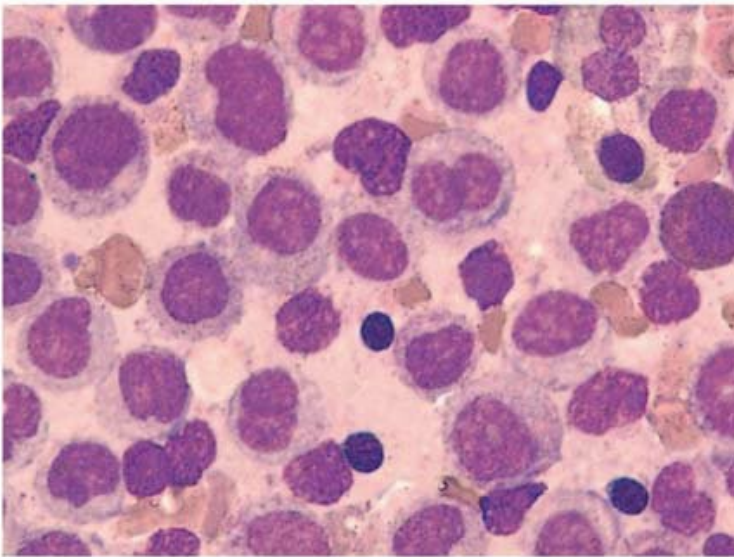
Medicine, , P , PA: L W m & W k , m m

, m .)

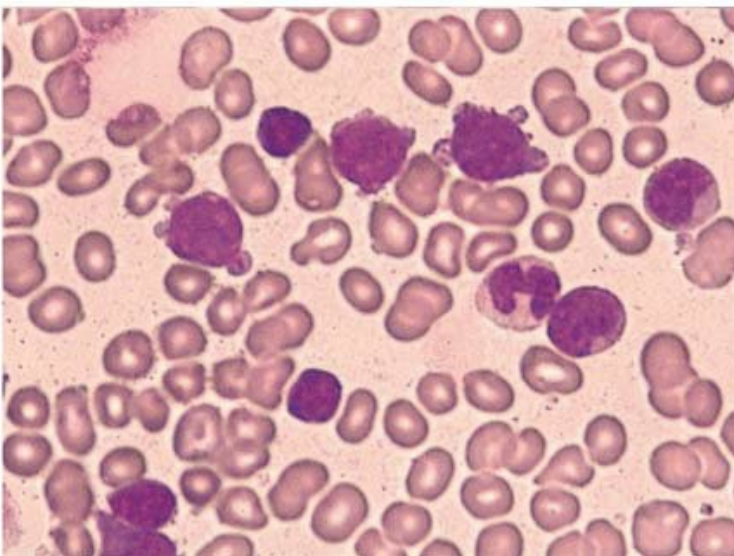
- - .



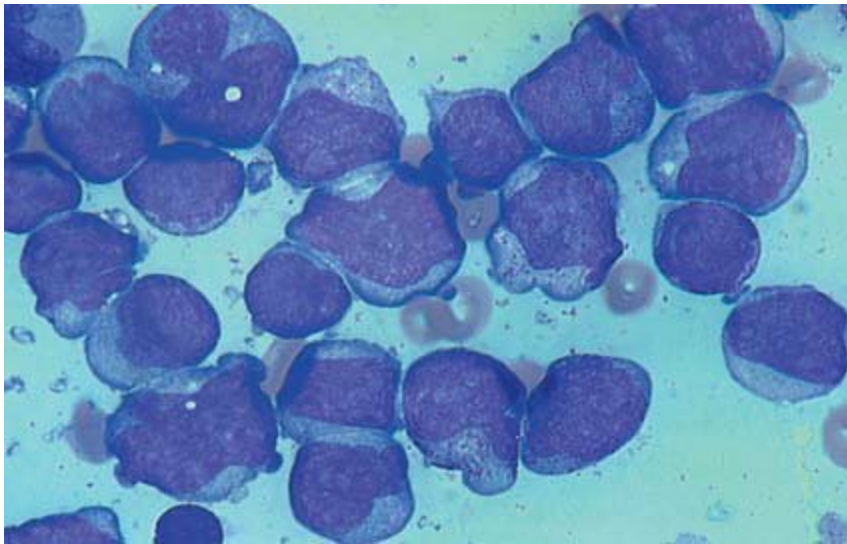
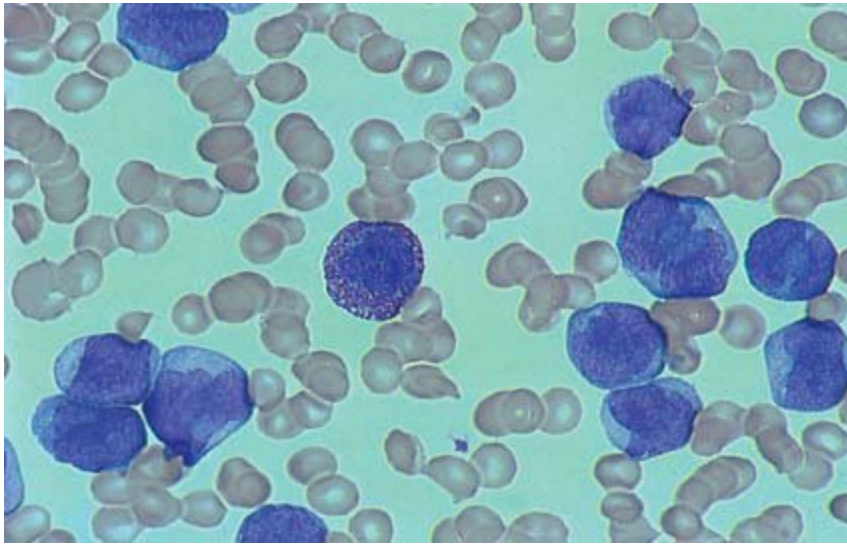
A



B



C



CHAPTER 21 ■ Acute Leukemias

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FIGURE 21.7 A m k m M (

m k m). (R m M C KD.

Clinical Laboratory Medicine, , P , PA: L

W m & W k , , m .)

J m m k m (JMML)

m

% m m . m JMML x m .

Clinical S igns an d Sym ptom s

S m m m k m m

m k m . F , ,

m mm . P m .

H m m

. G k m

m .

P FAB M FAB M k m ALL (-

m -) k (

x k) k k m . **Leukostasis** -

, -

k m . T m mm

. S m m k ,

m m , .

La boratory Da t a

I FAB M (F . .),

m . A m m

FIGURE 21.6 A m FAB M . S A. C

M . B. M (M). C. H .

(R m G JP, . Wintrobe's Clinical Hematology,

, P , PA: L W m & W k , ,

m .)

Acute Mye lo m onocytic Le uke mia (FAB M4)

T WHO m m m k m .

O m k m mm -

. T q

. A m m

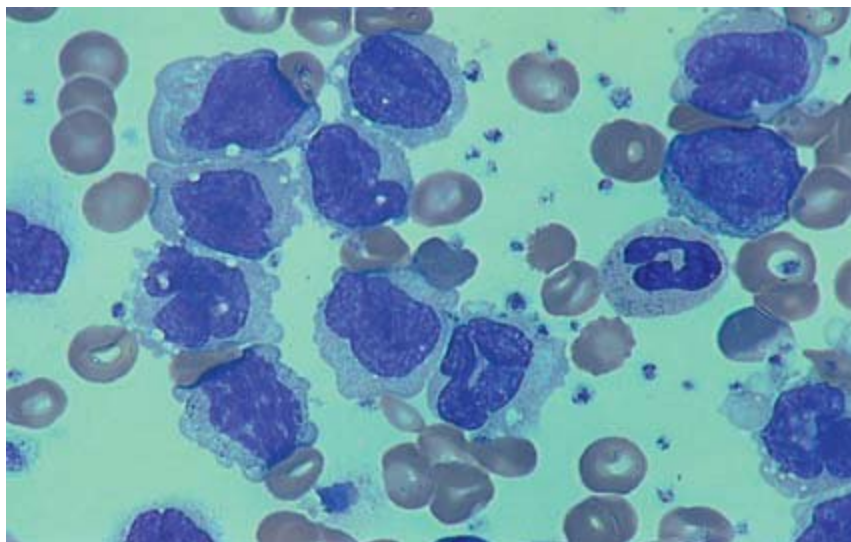
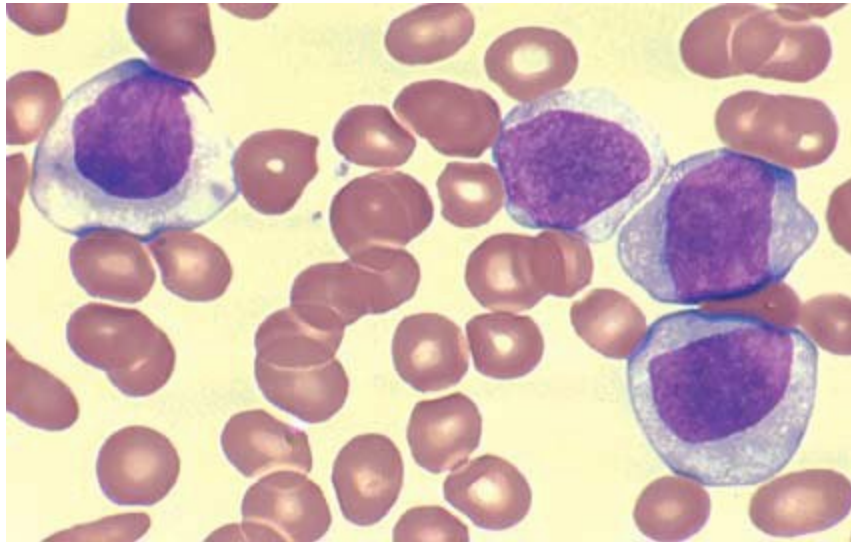
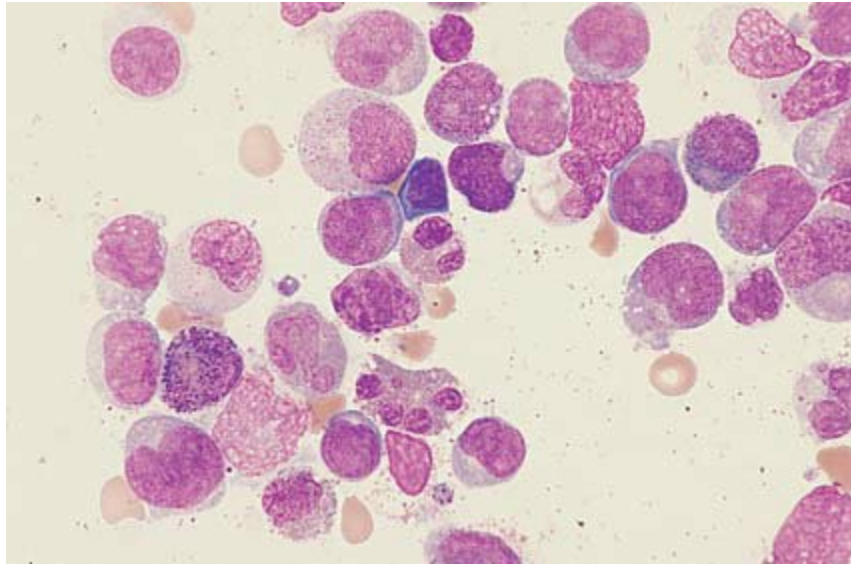
k m m m m m -

m m m m .

T m : m . : . M m

FIGURE 21.8 A m k m M . (R m m m k m m, M C KD. Clinical Laboratory
Medicine, , P , x m m .

PA: L W m & W k , , m .)



PART 6 ■ Neoplastic Disorders

. T k m k -

k . T k x

× /L. I m , m

x × /L .

O m , m m-

, x m % m

m . T m

m . T m

m . A m . P m

m . A

m , q

-P -H ë m m . T m

FIGURE 21.10 A m k m M . (R m

. E

M C KD. Clinical Laboratory Medicine, , P ,

. DIC .

PA: L W m & W k , , m .) A FAB M FABM E . A m -

(F . .) m m

m .

x m q (m m)

, m m k m ; ;

Acute Monocytic Le uke mia (FAB M5)

k . E m m

mm . Ex m m m

P m k m mm

.

% k m . m , FAB M (F . .)

FAB M (F . .), . T

La boratory Da t a

WHO m m k m (FAB M)

A m k m , m m-m k m (FAB M), .

. T k

T FAB M m m mm

m × /L. P m

(m ,); FAB M m k -

(F . .) m x

m (m ,

m . M m %

). T m : m . : M A

% . B q m

m x m . : M B m. B

m - m , m k m , mm . T

x , m m -

m m m

.

. A mm m

.

Clinical Signs an d Sym p tom s

T m k m m , -

Erythro le uke mia (FAB M6)

m . m -

m m k m , T WHO m FAB M M -

, m m . P

k m . T m k m ,

m m D G m ' m , -

mm

(F . .). A

FIGURE 21.9 W - m m , m , m -

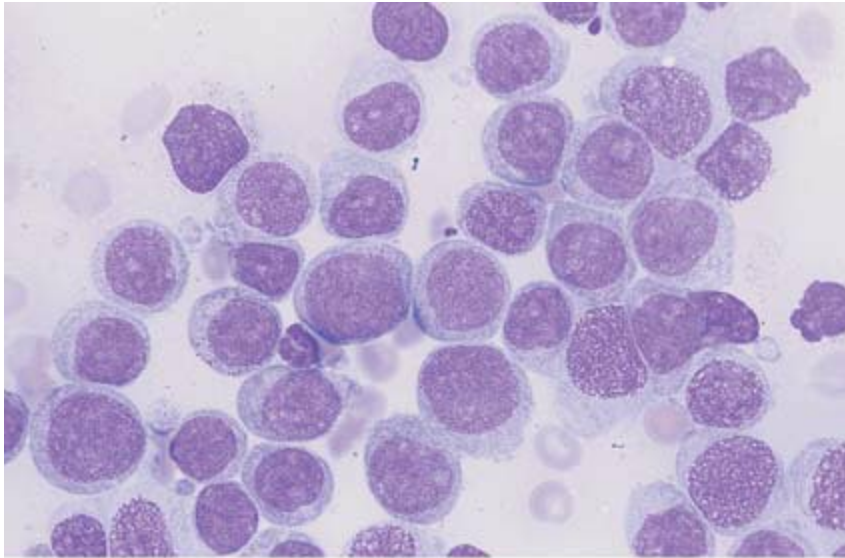
. (R m G JP, . Wintrobe's Clinical Hematology,

FIGURE 21.11 A m k m M . (R m

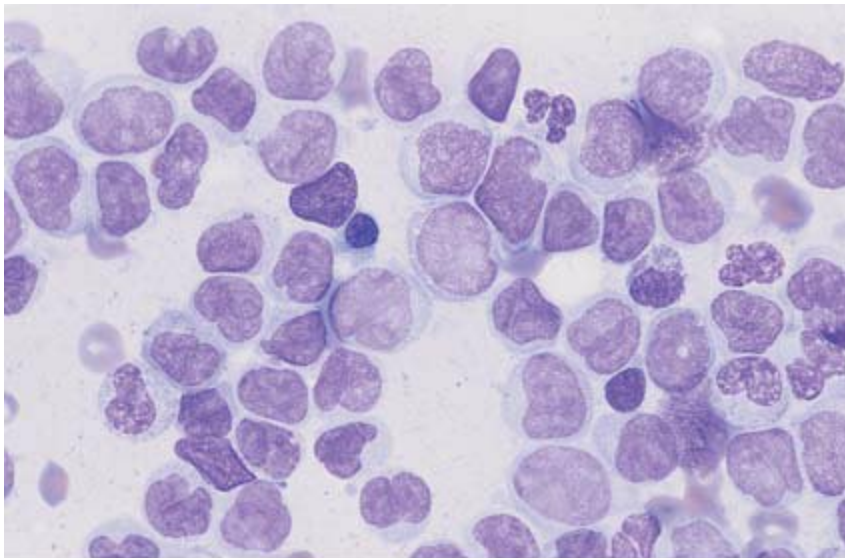
, P , PA: L W m & W k , ,

M C KD. Clinical Laboratory Medicine, , P , m .)

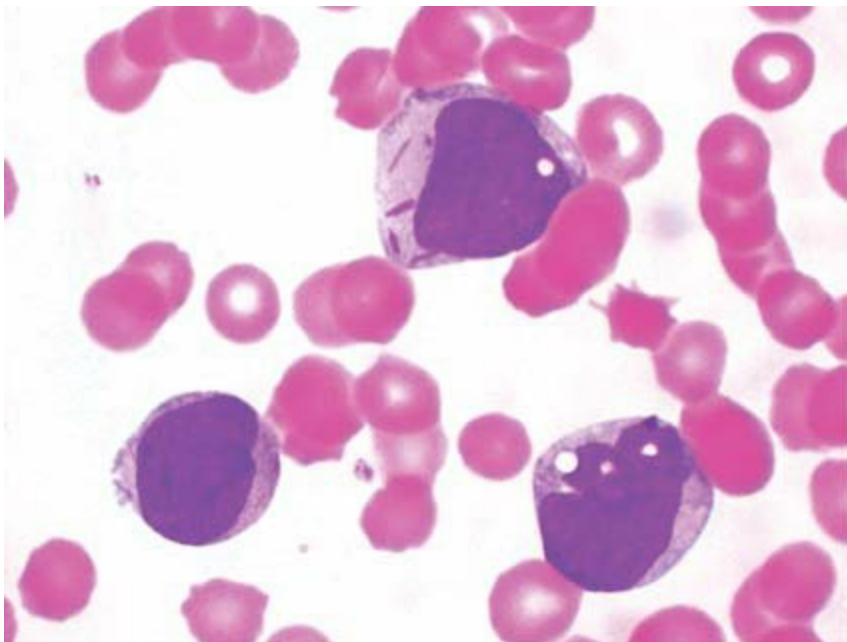
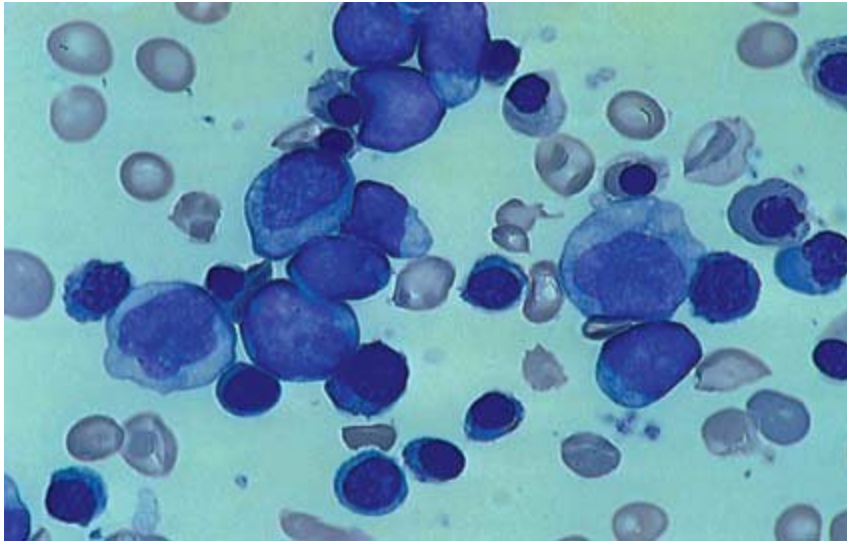
PA: L W m & W k , , m .)



A



B



CHAPTER 21 ■ Acute Leukemias

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FIGURE 21.12 Acute myeloid leukemia (FAB M1). **A.** FAB M1. **B.** FAB M1. (Reprinted from J. P. Wintrobe's Clinical Hematology, 12th ed., Philadelphia: Lippincott Williams & Wilkins, 2001.)

-

Selected Examples of Unusual Forms

. T m ; -

, m . T

Eosinophilic Leukemia

m : m . . . T

E k m , -

m .

m CML. I

k m , . D

Clinical Signs and Symptoms

.

A m m m m m .

m k m . S m -

H m , m , m

m , m -

q .

k , m (CNS) m .

m m

m . L k k

Laboratory Data

$\times /L \text{ m x . A m -}$

B m

$\times /L. \text{ O -}$

m . E

m , m % k

m

. T m m

- m . S m

; , m

x .

. T m -

B m m A (F . .).

m , m m m . I

P m m m

m k m , m %

m .

T m M:E . I m

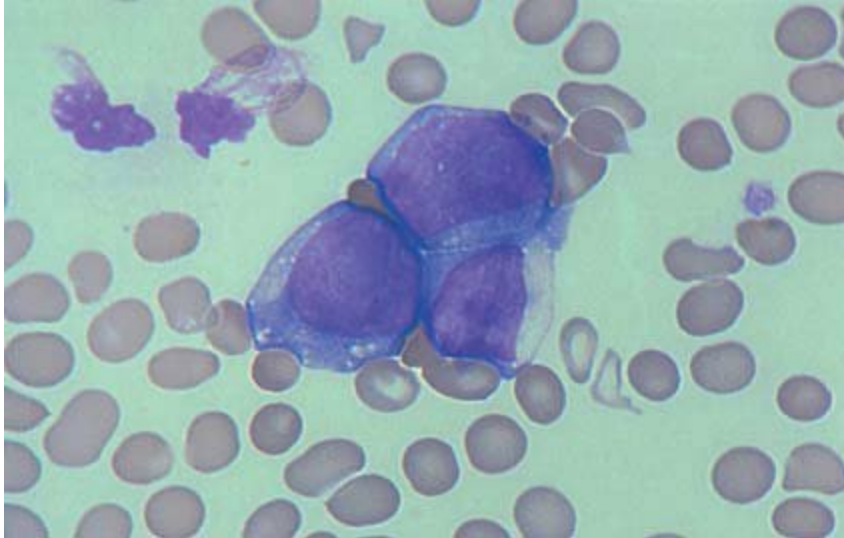
k m , f

m m k m .

FIGURE 21.14 A m k m . A

m (arrow). (R m R R, S DS. Rubin's

FIGURE 21.13 A m k m M . (R m Pathology: Clinicopathologic Foundations o Medicine, , M C KD. Clinical Laboratory Medicine, , P , P , PA: L W m & W k , , PA: L W m & W k , , m .) m .)



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PART 6 ■ Neoplastic Disorders

. A m -

m m k m .

TABLE

21.9 Sudan Black B Re actions

Ba s op hilic Le ukem ia

Cell Type

Re actio n

B k m (m k m) m k m . F q , m Positive reactions

m k . P m Granulocytic cel s

Become increasingly posi—

k m x k , k -

(neutrophils and

tive (sudanophilic) as they

$x \times /L$. P m

eosinophils)

mature

m % .

Myeloblasts

May have a few, smal gran-

Acute Megakaryoblastic Leukemia FAB M7

ules in the Golgi region

Promyelocyte

Increased granulation

T WHO m megakaryoblastic leukemia. I

Neutrophilic myelocytes

Granules concentrated near

m k m , % m

the nucleus or rim of the

m k (F . .). A m k -

cytoplasm

k m . I

x m % % AML.

Metamyelocytes,

Strongly positive

bands, and segmented

Clinical Signs and Symptoms

neutrophils

O m q x . R

Eosinophils at all stages

Granules react positively at

.

the periphery of the granule

Laboratory Data

Monocytes and

May have granules scattered

precursors

over the entire cell

C , m .

D m Variable reactions

. T q m m m -

Basophils

m AML. Imm

Negative reactions

m k x m

(sudanophobic)

: CD CD . B -

Lymphocytes and

MPO .

lymphocytic precursors

P , .

Megakaryocytes and

thrombocytes (platelets)

NOTE: This is a good time to complete Review Questions
related to the preceding content.

Erythrocytes

Erythroblasts may display a few granules that

Cyto chemical Staining

represent mitochondrial

V m

phospholipid components

m . A m mm

, m m (

TABLE 21.10 Myeloperoxidase Reactions

. – . , B x .).

Cell Type

Reaction

Positive reactions

Neutrophilic granulocytes Strongly positive
except blast forms

Eosinophils

Positive

Monocytes except blast

Positive, but reaction is faint
forms

with few granules

Negative reactions

Basophils

Lymphocytic cell series

FIGURE 21.15 Akin M. (R m M C

Plasma cell series

KD. Clinical Laboratory Medicine, , P , PA:

L W m & W k , , m .)

Erythrocytic cell series

CHAPTER 21 ■ Acute Leukemias

TABLE 21.11 **Periodic Acid–Schiff (PAS) Reactions****Cell Type****Reaction**

Positive reactions*

Neutrophilic granulocytes except blast forms

Strongly positive

Megakaryoblasts in malignant or proliferative disease; both Strongly positive blasts and megakaryocytes are strongly positive

Erythrocytes in erythroleukemia (FAB M6)

Strongly to moderately positive

Variable reactions

Eosinophils, basophils

Granules are negative; cytoplasm may contain faintly positive (PAS) granules

Monocytes

Faint pink cytoplasm, with or without granules

Lymphocytes

May contain a few pink or red granules

Thrombocytes

Intense pink or red

Megakaryocytes

Diffuse pink or red; may have coarse red granules

Lymphoblasts (leukemic)

30%–40% may show strong coarse or block-like positivity

Negative reaction

Erythrocytic series

Negative

Myeloblasts and monoblasts

Faint diffuse reaction may be occasionally observed

*Appear as a diffuse pink or large red aggregate of particles.

REA MEN OP IONS

m m m k m -

, k . T

G AML

m m m

m . R m

m k . T

k m

m m , BCR-ABL

m k k m

m % P -

(.). O , m j

(P +) CML % % (P +) ALL.

TABLE 21.12 **Cyto che m ical Re actio ns in S e le cte d Le uke m ias**

FAB Clas s

SB

MPO

PAS

NASDCA

(α) NA (7.6)

Acid Phos

M1 myeloid

+

+

—

—

—

—

M2 myeloid

	++
	++
	—
	+
	—
	—
M4 myelomonocytic	
	±
	±
	—
	±
	++
	—
M5 monocytic	
	±
	±
	++
	—
	++
	—

M6 erythrocytic

—

—

+

+

+++

—

M7 megakaryocytic

—

(+)*

+

+

+++

—

L1 or L2 (T-ALL)

—

—

±

—

—

(++)†

*Test is performed on unfixed preparation.

†The reactivity must be focal or Golgi in nature.

FAB, French-American-British Cooperative Group Classification; SB, Sudan black B; MPO, myeloperoxidase; PAS, periodic acid–Schiff; NASDCA, naphthol AS-D

chloroacetate esterase; (α) NA, alpha-naphthyl acetate esterase; Acid Phos, acid phosphatase.

Note: Negative reactions for all tests, except for the PAS, indicate common acute lymphoblastic leukemia, pre-B or B-cell lymphoblastic leukemia, or unclassified acute lymphoblastic leukemia.

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AML -

BOX 21.4

. I k k m

m . T

m x

Esterase Staining Reactions

.

T m j -

NAPHTHOL AS -D CHLOROACETATE ESTERASE

k m m q

POSITIVE REACTIONS *

. R m m

P m

% m

M

m mm

M m

.

B m

S m m

NEGATIVE REACTIONS

(B x .). C m (

M ()

MDR1) x m m-M (m) m k P- . T m

P m m †

k m (. . ,) m m m , x -

ALPHA-NAPHTHYL ESTERASE (PH 7.6)

MDR1 -

POSITIVE REACTIONS

m m

M

.

H

O m m

S m ()

chemotherapy m

M m ()

MRP, m - -

M k

; mE

x; LRP, .

NEGATIVE REACTION (WITH SODIUM FLUORIDE

A m -

IINCUBATION)

m AML m

M

k ,

. P III m

*R m m m .

†

P- (. . ,) O k .

.

TABLE 21.13 Newer Agents in Development for Treatment of AML

Drug Class

Drug Actions

Epigenetic modifiers

Hypomethylating agents, IDH1 inhibitor, IDH2 inhibitor, bromodomain inhibitors,

LSD1 inhibitor, histone deacetylase inhibitors

Tyrosine kinase inhibitors

FLT3 inhibitors (first and second generation), KIT inhibitors

Cell-cycle and signaling inhibitors

MDM2 inhibitor, PLK inhibitor, aurora kinase inhibitors, cyclin-dependent

kinase inhibitors, phosphatidylinositol 3-kinase inhibitor, PIM kinase inhibitor,

Hedgehog-pathway inhibitors, mTor inhibitors

Nuclear export inhibitor

XPO 1 (also called CRM1 inhibitor)

Antibody-based therapies

Antibody-drug conjugates, bispecific antibodies, stem-cell targeting, CXCR4 targeting, immune checkpoint blockade, chimeric antigen receptor T cells

Cytotoxic agents

Quinolone derivative, new drug formulation CPX-351, nucleoside analogues

Other agents

B-cell CLL-lymphoma 2 protein inhibitor, immunomodulatory drug for example,

lenalidomide, aminopeptidase inhibitor, retinoic acid, CXCR4 antagonist, E-selectin antagonist, homoharringtonine derivative Modified from Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia, N Engl J Med, 12(373):1148–1149, 2015.

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. W mm

BOX 21.5

m k m k , k

m , - -

.

Adverse Prognostic Factors

FACTORS USED TO PREDICT A POOR RESPONSE TO

Relapse

INDUCTION CHEMOTHERAPY

A AML m j m . D -

U k

m AML

A >

. A m m ,

S AML

, ,

Ex

, m j m . A

F m

, m j

P k > , × /L

.

FACTORS USED TO PREDICT A RELAPSE

P — (;), U k

(;), () m — m m

A >

x m %

D m

q . F

F m

m

P k > , × /L

- , mm

F m

m - (-) x m

AML, m k m .

m m , -

m HLA-m

HLA-m

Chem othe rapy

. P q -

m

C x m k m

% m . m APL

. m AML

m AML.

. L - %

% m m .

Alloge ne ic Hem atopoie tic-Cell

M AML ALL m ,

x .

Trans plantation

T :

A m m HLA-m

m . T m j m

.

m m

P m AML m k m -

m .

m , x m %

% .

In duct io n Pha s e of Th e ra py

P m m -

I m m

m m m

-

k m m .

mm k m - - -

C -

k m . A m -

m m . A m

k x

% %

m m

. I , m-

.

% %.

I AML m

Tra ns p la nt a tion Te chnique s

(, A -C)

C m

(). P

k m f mm

AML m m ,

m m . N m -

(HIDAC)

m f mm

m m , m x , .

j . A

O m , m

m m

AML .

m ,

m x m . F

Con solida t ion The ra py

m k

S m

m

m m

mm . k

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PART 6 ■ Neoplastic Disorders

m

A AML

x .

m m CD ,

-j

Dono r Graft a nd Ce ll S ou rce Opt ion s

(-CD CD). CD -

A HLA-m . G m

.

HLA-m m . S

, m HLA-m

NOTE: This is a good time to complete Review Questions

q m .

related to this content.

Com plica tions of Allot ra ns p la nt a tion

A m - , k m -

, - , m , **ACU E LYMPHOBLAS IC LEUKEMIA** m . L m k

Epidem io logy

GVHD

. N k m

I U S , ALL m mm -

m

, % m

m .

. I , -

A GVHD m j z

U S .

. Ex mm

ALL m , k

m k , x m , E -B m -

. P ALL m

k m .

(m : m , %: %). T

ALL .

New Therapie s

T . /m H , .

/m , . /m k .

N m m AML(.) -

Pathoge ne s is

k ,

DNA m , x , -

S D ' m -

x m , m -

k ALL, m

, k m m - .

z . S q q

I m

ALL

k , -

(.).

m m z m IDH IDH ,

M m -

q m AML -

, ARID B, CEBPE, GA A1,

. N m x

IKZF , k ALL -

.

ALL . R m-m PAX5

**Pro po s ed Se quential Acquis itio n o f Ge netic Alteratio ns Co
ntributing to Pathoge nes is TABLE 21.14 of ALL**

S tage

Im pacte d Ce lls

Com me nts

Predisposition

Hematopoietic stem cells

Common variants—IKZF1, CEBPE, ARID5B

Created by either common inherited variants or,

Rare mutations PAX5, ETV6, TP53

rarely, deleterious germ-line mutations.

Initiating Translocation

Lymphoid progenitor

Self-renewal, developmental arrest,

Lesions are acquired.

epigenetic reprogramming, proliferation

Secondary Mutations

Pro-B or pre-B cell

Tumor suppressors, Kinase-Ras-P13K

Along with structural genetic alterations

signaling, lymphoid signaling, tran—

contribute to an arrest in lymphoid development

transcriptional coactivators, chromatin

and disruption of multiple cellular pathways

remodeling

Diagnosis

Lymphoblasts

Modified from Hunger SP, Mullighan CG, Acute lymphoblastic leukemia in children, N Engl J Med, 373(16), 1541–1552, 2015.



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TABLE

21.15 Morphological Classification and Characteristics of ALL

FAB Type

Morphological Classification and Characteristics

Size of Blasts

Nuclear Shape

Nucleoli

Cytoplasm

L1

Small

Indistinct

Scant

Invisible

L2

Large, heterogeneous

Indented, prominent

Large, abundant

Moderately clefted

L3

Large

Regular oval to round

Prominent, basophilic

Prominent, vacuoles

ALL, acute lymphocytic leukemia; FAB, French-American-British Cooperative Group Classification.

Evans ALL. Ann

Px

ALL, k, m, G, x, m.

m, mm, L, m

m % . L k m m -

Classi catio ns o f Acute Lym pho blastic

Leukem ia

k m q mm . L k m

m (F . .). N

T FAB ALL

m

■ L ()

k .

■ L ()

■ L (k m B k ')

Laboratory Data

m m)

I ALL, m m m m –

T m -

m . T -

, z , m ,

k % % ,

m m (.). A

$k\ m \times /L. L$

-

$\% x\ m\ k\ k -$

$m . ALL$

$m \times /L. I\ x\ m$

$k\ m\ m$

$\% k .$

$m\ m .$

$P\ m\ (F . .)\ m -$

$ALL\ m - B- . I$

$\% . I$

$- m\ k\ m\ (-ALL)$

$, m$

$m\ m\ m\ m -$

$\% m , m , m . T$

$m , - m .$

m , A

$I , m\ \% ALL\ L .$

$m\ m. T$

$-ALL\ \% ALL .$

-ALL % % ALL. T

m ALL B m .

T WHO m FAB

L L B m k -

m / m m m (B-ALL)

m k m / m mm (- ALL), . P Bm k m (B-ALL) m m-mm B- . P -ALL

m m mm - .

Clinical Signs and Symptom s

T m m ALL m

k . S m m , , -

, , , m . B j

FIGURE 21.16 L k m

m m m m

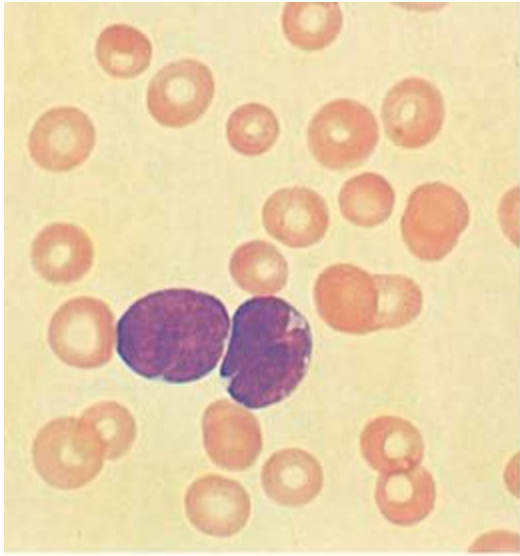
ALL. (R m m W, J E. T e Wills Eye

mm . P x m , ,

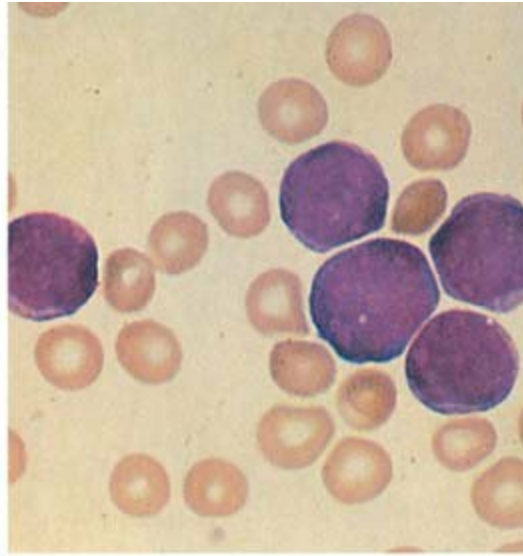
Hospital Atlas o Clinical Ophthalmology, , P , PA:

k m .

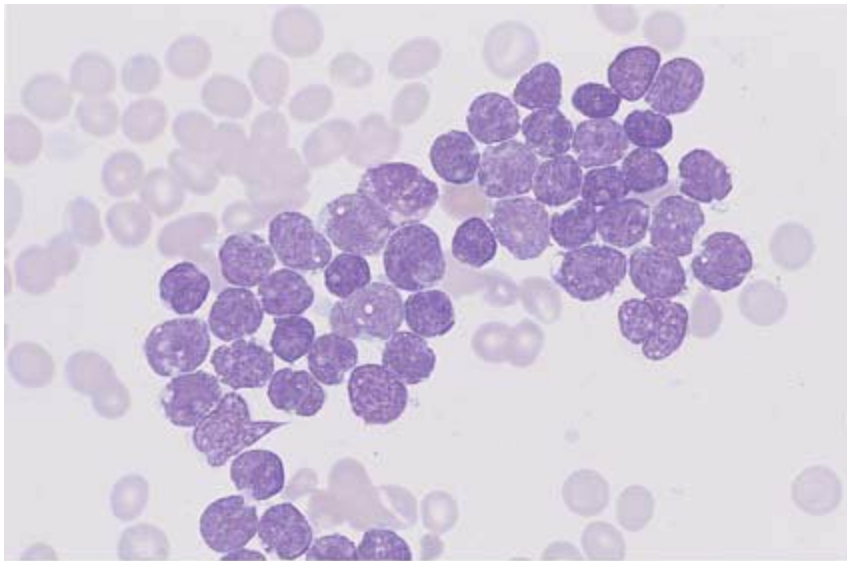
L W m & W k , , m .)



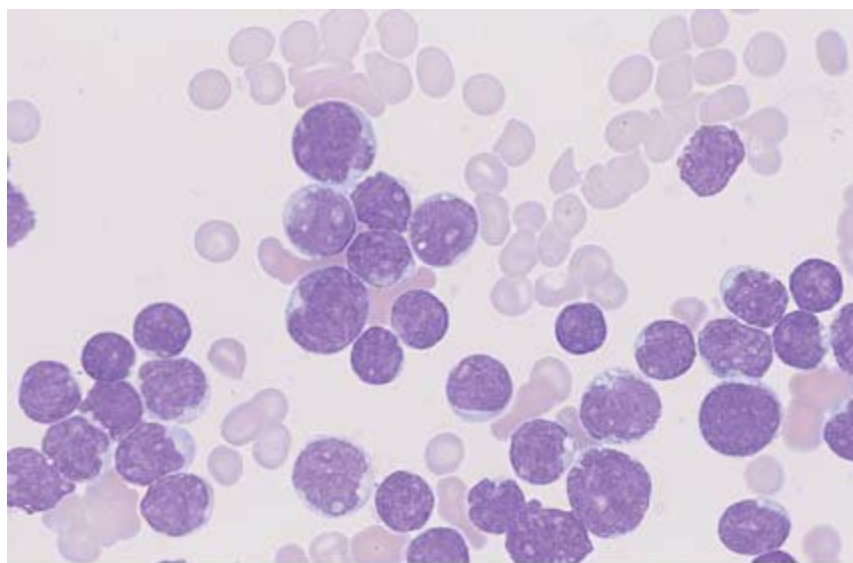
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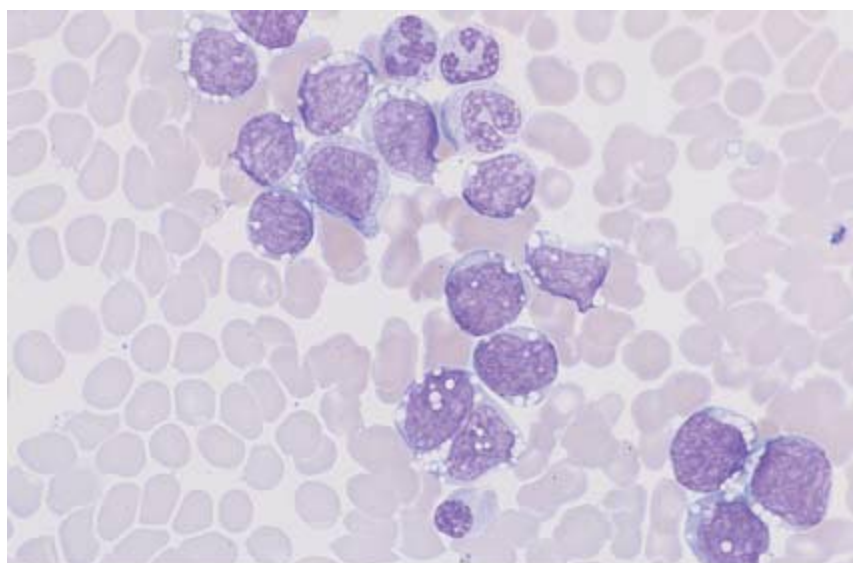
B



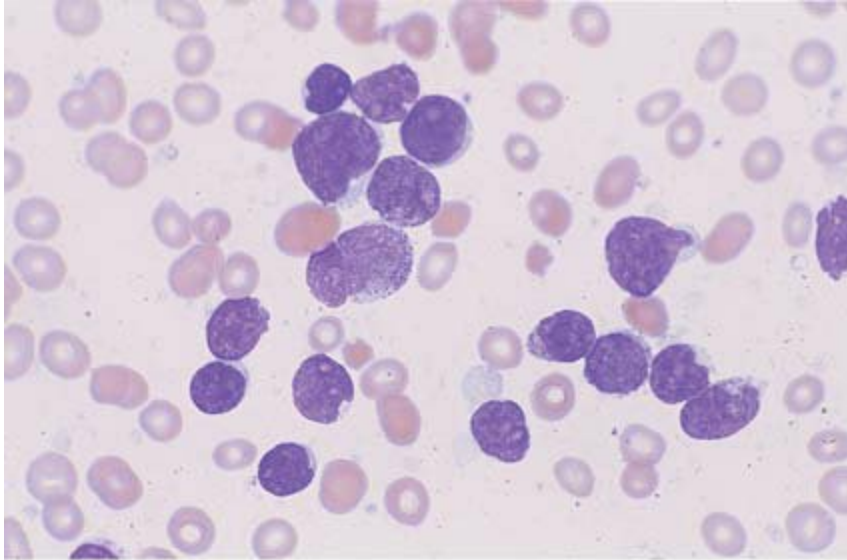
A



B



C



D

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PART 6 ■ Neoplastic Disorders

FIGURE 21.18 A k m . A. L m k m .

B. L m k m , . (R m G C G JP, . Wintrobe's Clinical Hematology, , P , PA: L W m & W k , ,

m .)

k m m m . I FAB

L (F . .), m m

FIGURE 21.17 A m k m . I m q m m .

mm k m . T m

E m m k

m m . B -

m k m . T m ,

(m) m m

,

m . (S m m , ×.)

m m k m .

- m . T -

P m m k m -

. I ,

m m



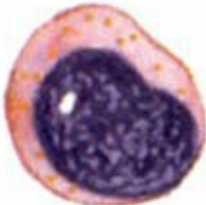




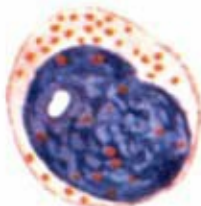
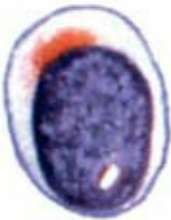


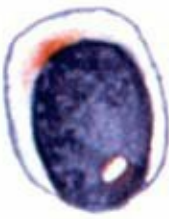

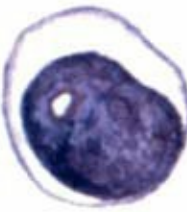

m , m mm -

m . I , ALL (F . .)

m m m

FIGURE 21.19 A m k m (ALL). **A.** FAB L . **B.** FAB L . **C.** FAB L . **D.** ALL
-

m , m . (R m G JP, . Wintrobe's Clinical Hematology, , P , PA: L W m & W
k , , m .)

Reaction	LYMPHOID	MYELOID	MONOCYTOID
MPO			
SBB	—		
PAS			
ANAE			
ANAE/NaF			
CAE	—		—

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m , m m -

DNA. (+)

.

m m

m j ALL - B- ,

S pecial Ide nti cation Te chnique s

x m B-ALL,

m m AML. -

S m k , m m

m m m m

mm , x m

CML . I -

ALL (F . .). D -

, m k

x m ,

m .

m m k

T mm ALL (ALLA) -

m (.). m x -

m % ALL. CD

() z m z

x

ALL.

Cyto ge ne tic Analys is in Acute

Lym pho blas tic Leukem ia

A m k m

m k . S ALL

(;), (;), (;), (;), (;), P m -

m . G m m m m

, , . A m k-L L ALL, (;) mm L ALL

B k ' m m m m -

m m .

P m m m k -

m ; m k m m , (;), (;), (;) .

Treatm ent

T m m m ALL

m x m

m

m . R ,

-m x . m

m P (P) m m

ALL. P m m m m

m m . G ,

m m m BCR

m m m j x -

m k m ABL m m m

. A P m m

CML, x m % % ALL

m P m m .

F - - ABL k

m - P m m -

ALL. I m m , -

- k , -C x

FIGURE 21.20 A m , m , m -

(m) , , m (x) , k m : m . MPO, m x ; SBB, x m (-CVAD) m ALL

S k B; PAS, -S ; ANAE, -

(;) m m m

(); N F, m

; CAE, . (R m R E, m m m .

F JL. Pathology, , P , PA: L W m Imm , m m , & W k , , m .)

m m z

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PART 6 ■ Neoplastic Disorders

TABLE 21.16 **Im m uno lo g ical Marke rs in Acute Lym pho blas tic Le uke m ia Type**

FAB

TdT

CALLA

CD7

CD19

HLA-DR

Slg

Precursor B-cel ALL

L1, L2

+

0

0

+

0 +

Common ALL

L1, L2

+

+

0

+

+

0

Pre-B-cel ALL

L1, L2

+

+

0

+

+

0

B-cel ALL

L3

0

0*

0

+

+

+

T-cell ALL

L1, L2

+

0*

+

0

0

0

Null cel ALL

L1, L2

+

0

0

0

+

0

*Some cases are positive.

TdT, terminal deoxynucleotidyl transferase; FAB, French-American-British Cooperative Group Classification; CALLA, common ALL antigen; CD, cluster designation; Slg, surface immunoglobulin; +, positive; 0, negative.

CD CD B—

x m . T - m

ALL x m.

m x m % %.

I ALL m AML, m m k.

I ALL m x

PROGNOSIS OF ALL

m m k . M

, , , -

T m m ALL

, l - , m , m x .

m . F

U k AML, m ALL

k m m m

(.).

m CNS. C m

I, %, ,

k m

% . A % %

CNS.

ALL m , %

P m ALL :

% m x m m .

m -

m ALL

m m -

m .

- k m m ,

m . T m

NOTE: This is a good time to complete Review Questions

-m k -

related to the preceding content.

m x . M m

TABLE 21.17 **Pro g no s tic Facto rs in Pe diatric ALL**

Favorable Factor

Adverse Factor

Age

1 to <10 y

<1 y or ≥ 10 y

Gender

Female

Male

Race or ethnic group

White, Asian

Black, Hispanic, Native American

Initial WBC count

$<50.0 \times 10^9/L$

Equal or $\geq 50.0 \times 10^9/L$

Immunophenotype

B-cell lineage

T-cell lineage

Cytogenetic features

ETV 6-RUNX1, hyperdiploidy, favorable

BCR-ABL1, MLL rearrangements, hypodiploidy

chromosome trisomies

Genomic features

ERG deletions

IKZF1 deletion or mutations, Philadelphia

chromosome-like ALL with kinase gene alterations

Modified from Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children, N Engl J Med, 373(16), 1541–1552, 2015.

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Mono clonal Antibodies

Imm m m

k m q -

Im m un oph en ot yp ing

.

Imm m m

T m CD

k m

(B) CD () m m m k . P

q . I m m

B x , CD , CD Bm k , m z

, m . I , CD

m m -

.

k m m m

I , CD , , CD x

(.).

mm -

D ,

m . R

m z -

m m m m .

m m x m

A - CD - m

k m . T m j ALL x

- m B-ALL .

m B-m

; m m x .

B-Lin ea ge Marke rs

T mm m m m k

CD m q CD

m . R

B-ALL. CD m j

- k m m -

B-ALL -ALL. CD

, f .

m B-ALL.

T-Line a ge Ma rke rs

CD - ALL. O -

Mono clonal Antibo die s Us ed

—CD , CD , CD , CD —

TABLE 21.18 in **Le uke m ia and Lym pho m a**

.

Identi catio n

B-Ce ll Ma rke rs

Clus te r

Ce ll Type

B-m (F . .) -B,

-B, m B- . T m k CD1a

T

-B CD . T m B -

CD2

T

mm (SI). T

CD3

T

mm ALL

CD4

T

. B

B-m

CD5

T

CD CD , m ALL -

CD7

T

. T CD m k m -

CD8

T

m B-m k m (B-CLL),

CD10

B

B-m m , B-k m x ALL, -CLL

Séz ' m , AML, CML .

CD11b

M/G

CD11c

M/G precursors

Other S urface Mem brane Marke rs

CD13

G/M (most G, some M)

O m m m k

CD14

M, some G

m m , m m , m CD19

B

B-m . T HLA-DR m k

CD20

B

.

CD21

B

CD22

B

LIFE-THREATENING EMERGENCIES

CD25

T

CD33

G

T m mm - m

k m

CD41

Megakaryocytes and platelets

CD42b

Megakaryocytes and platelets

I

B

CD45

Various cells

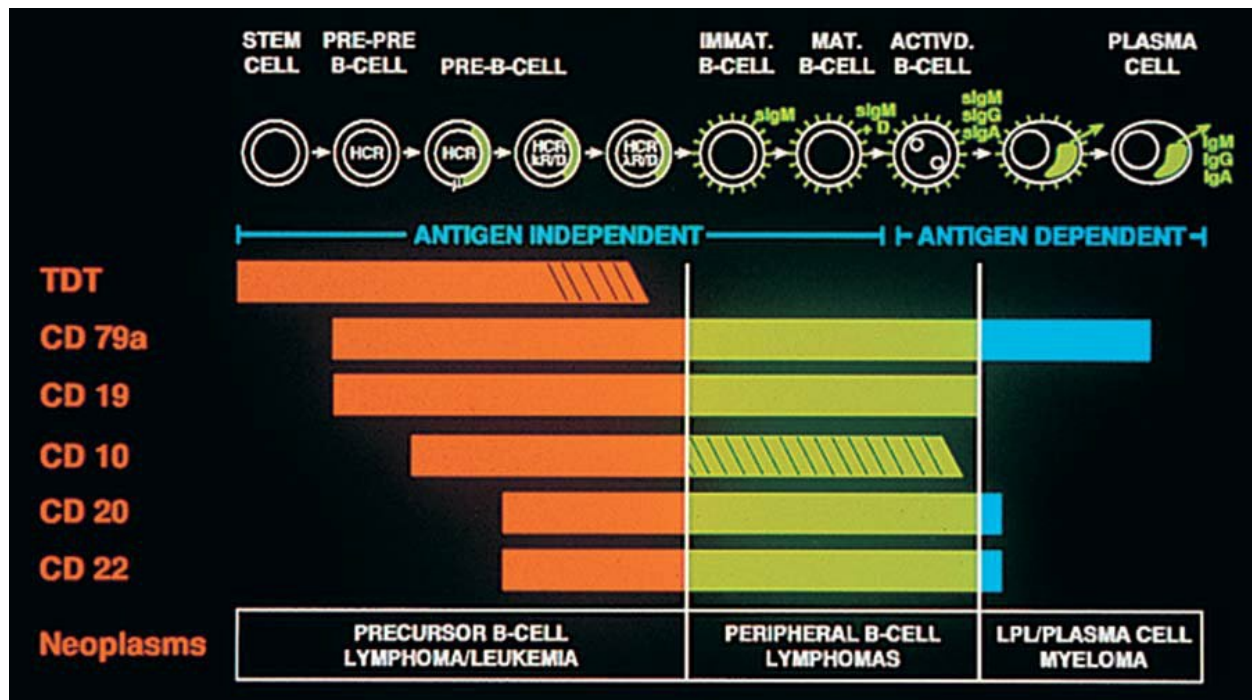
L k m

CD, cluster designation; T, T lymphocyte; B, B lymphocyte; M, monocytes;

M m

G, granulocytes.

H k



404

PART 6 ■ Neoplastic Disorders

FIGURE 21.21 B-m . (F m J ES, . umours o Haematopoietic and Lymphoid issues. L , F : IARC, : . W m m E S, J MD. Chie , Hematopathology Section, Lab o Pathology, B , MD: N C I , .) **Infectio n**

m . M m m

m ,

C m k -

.

m m m . W

m m , k .

Hype rle ukocyto s is

Ble e ding

P , m mm

m AML , \times /L

B q , -

, k . T

k m , DIC, -

k m k -

m (m K). A

m . T -

m k m m .

q x , , ,

A \times /L k m m .

.

DIC (C) mm

Stem Cell Trans plant

AML. L k m

DIC. DIC m

ALL m . P

kinetics,

).

,

exam, exam, exam

Leukemic Infiltration of Organs

met

met (PBSC). PBSC met

Intermet

quadrant ALL.

met -

Chemotherapy

. Treatment

CNS, exam, exam, exam -

met

met (exam)

met.

exam % AML.

Metabolic Abnormalities

FUTURE TRENDS: VACCINES

M m m -

V , AML, -

m k m . m m

. A ,

m m

m (PR) W m m -

CHAPTER 21 ■ Acute Leukemias

405

(W), mm

k m k m -

k m m

m m m .

AML . T

■ NGS m m mm k q m

m m m -

.

■ T WHO m

.

m m x, -

T

m FAB

m MDS, AML, CML . T -

AML.

m “ j ” M

■ A AML m mm m

m k , GM-CSF

, m

(m m). B M m m

m k m

mm m . T

.

mm m m k z

■ AML z m , k MDS, AML, CML -

, , , -

.

m , m m

m m. A k -

m mm

CHAP ER HIGHLIGH S

m m k AML. T M:E

AML .

General Characteris tics o f Acute Leukem ias

■ A AML , AML

% %

■ E k m q .

% %

M , m -

. T m m

m m , m m ,

.

m , k m , -

■ M RNA x ,

, m

m m , -

m .

m AML. R , m RNA

■ T k m z

m m -

mm k

m m

m .

m m .

■ A k m

■ AML z m

m .

mm m

■ FAB m -

m . T M

W -

m . T q M M

m m

AML (M M); ALL

m . M m-

(L L).

m m k m . M

■ T W H O z (WHO)

m k m

m —m , m -

. T M

, mm , , —

k m , m

.

m m m -

■ M m m q

k . M m k

R -PCR m -

k m .

k m -

■ V m

m .

m . A m

Acute Mye lo id Le ukem ias

mm , m

m .

■ AML -

■ G AML

z m k m

m .

q m m -

R m k m

m m m - ,

m

, .

k k m . O ,

■ AML m mm k m .

m j m

■ C m

m m k m , -

AML ALL %

k .

406

PART 6 ■ Neoplastic Disorders

■ A AML m j m . D -

■ I ALL, m m m m –

m AML

m . T

.

$k \%, \%$,

■ A m m HLA-m

$k m \times /L$.

.

$L \% x m k$

P m AML m k -

$k m \times /L$. I

m - m , x m

$x m \% k$

$\% \%$.

.

■ N m m AML

■ S m k x m -

ALL. z m z

k , DNA DNA.

m , x ,

(+) m m

$x m$, m -

m j ALL -

, k m m - .

B- , x m B-ALL,

■ A AML -

m m

m m CD ,

AML.

- j -

■ T ALL A m %

(-CD CD). CD

ALL. CD x

.

ALL.

■ A m k m

Acute Lym pho blas tic Leukem ia

m k .

■ T m m m ALL

■ I U S , ALL m mm

m x m

, % m

m

.

m .

■ ALL m , k

■ , % . A % %

ALL m ,

. P ALL m

% % m x -

.

m m . m ALL

■ T ALL

m

. T

.

H , m W ,

B k .

■ S D ' m -

Life-Thre atening Em erge ncie s

k ALL, m

z .

■ T m mm - m -

■ S q q -

k m , ,

ALL .

k m , m m ,

■ T FAB ALL L (), k .

L (), L (-

■ ALL m .

k m B k ' m m).

■ ALL m - B- .

Future Trends : Vaccine s

■ T WHO m FAB

L L B-m -

■ V , AML, -

k m / m m m (B-ALL)

. A ,

- m k m / m

m (PR)

m m (- ALL), .

W m m - (W),

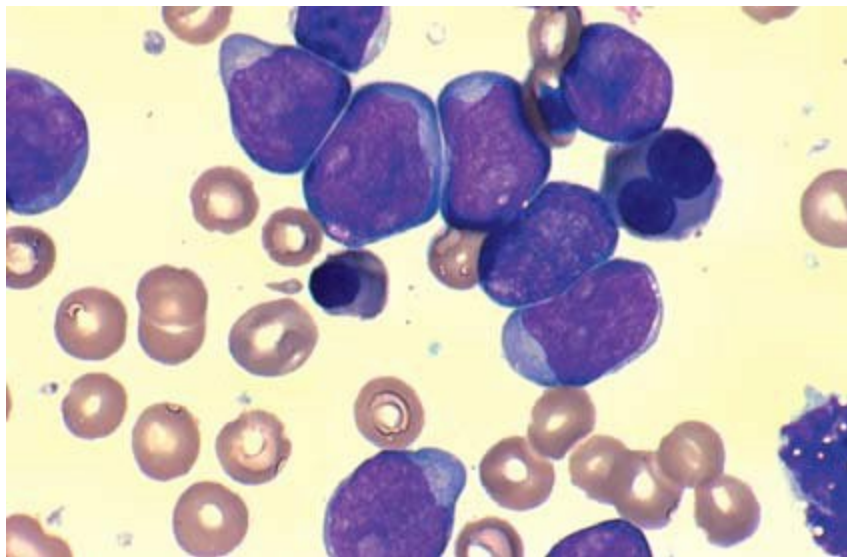
■ P B-ALL m m mm -

mm k m m

B- . P -ALL m

m mm - .

AML .



CHAPTER 21 ■ Acute Leukemias

407

CASE STUDIES

Cas e Study 21.1

■ Laboratory Data

T m .

A - - m

T k × /L. D

x k . H

k m :

m k

k m x—

B %

.

P m %

M %

P x m m m m

M m %

. H m , m -

S m %

. T m

L m %

(CBC) .

T .

■ Laboratory Data

A m m . T -

m

m .

. T k \times /L.

F - m m

T k

S k B NASDCA

:

m . N

B %

- (m

P m %

), PAS, .

M %

M m %

B %

S m %

L m %

A m .

m m

m . T

m .

F - m m

S k B,

m m k . T PAS

R m M C KD. Clinical Laboratory

. A m

Medicine, , P , PA: L W m &

.

W k , , m .

■ Critical Thinking Group Discussion Questions

■ Critical Thinking Group Discussion Questions

. W m ?

. W m ?

. W m

. W ?

?

. D m

. W ?

k m ?

Cas e Study 21.2

Cas e S tudy 21.3

A - - , A - - m m . H -

. S -

D ' m m .

m ,

P x m -

m k . P x m

m D ' m ,

m , -

m m m , m . N m -

m , m . H

m . T

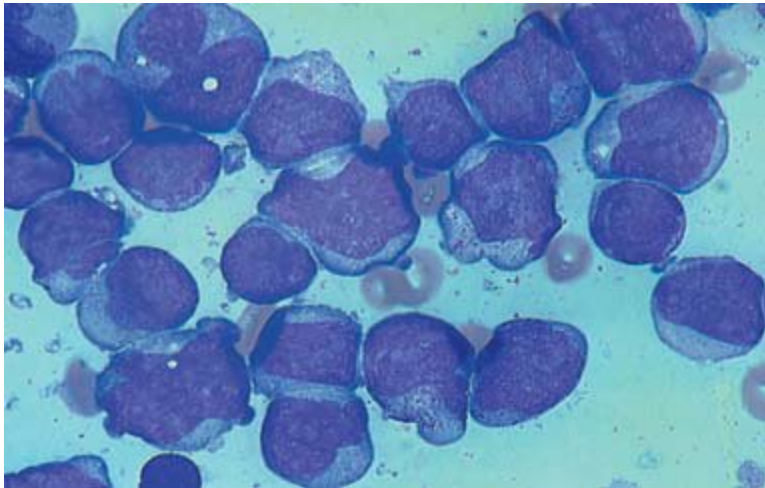
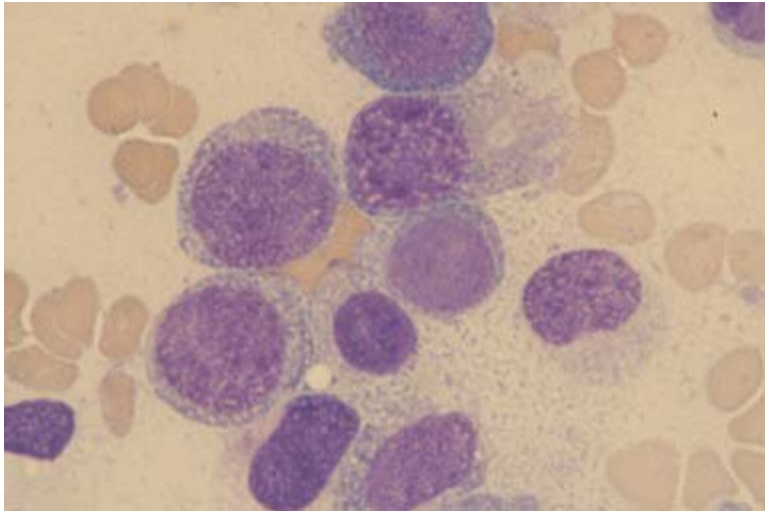
m f CBC. O

CBC .

, m m

q m .

(continued)



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PART 6 ■ Neoplastic Disorders

C

C A

A S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

T ' CBC . A x m k .

■ Laboratory Data

B m

m m . T k ×

/L. D k :

B %

P m %

M %

M m %

R m A SC. Anderson's Atlas o Hematol—

B %

ogy, P , PA: W K H /L

S m %

W m & W k , C , m .

M %

P m %

■ Laboratory Data

L m %

T CBC m

m . T k \times /L.

P m T k : m . T

m m .

B m %

P m %

M %

L m %

T .

S q m

. × /L. B m m -

mm m m . N

. T m

: S k B, ;

NASDCA, m

m j ; - ,

R m M C KD. Clinical Laboratory

, m ;

Medicine, , P , PA: L W m &

PAS , .

W k , , m .

■ Critical Thinking Group Discussion Questions

■ Critical Thinking Group Discussion Questions

. W m k m ?

. O m ,

?

. W x m -

?

. W ?

Cas e Study 21.5

. W k m ?

A - - k k m -

Cas e Study 21.4

m m m

A - - m k m

. T m

x -

, ,

m k. H x q

m .

k . T m m -

P x m -

k

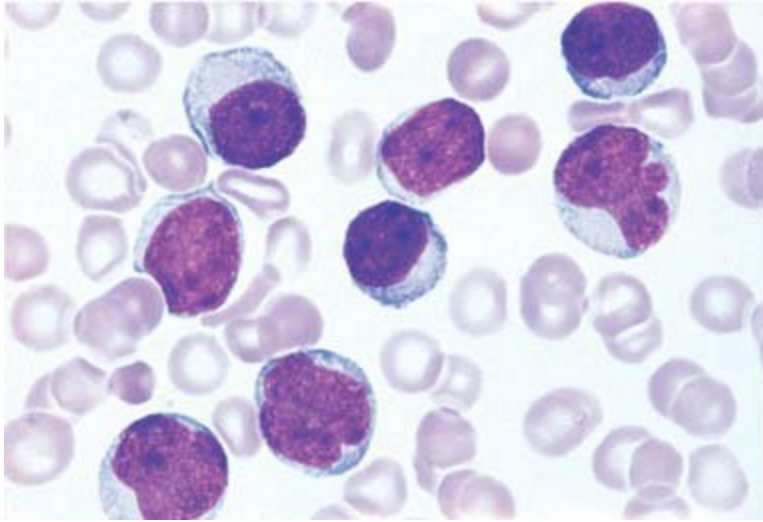
. T m m m m . T

. H m k m

m m . T

. P x m m .

m m S A CBC.



CHAPTER 21 ■ Acute Leukemias

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CA

CA S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

nttiin

n u

u e

e d

d))

P x m

. A CBC, ,

.

■ Laboratory Data

RBC . \times /L

H m %

H m . / L

WBC . \times /L

P \times /L

■ Leukocyte Differential

R m R R, S DS. Rubin's Pathology:

S m %

Clinicopathologic Foundations o Medicine, , P -

L m %

, PA: L W m & W k , ,

M %

m .

D

■ Laboratory Data

m ; + m

T ' m

m . T m . T

m . T k

.

. × /L. L k

A - m x m m

m :

. T m m mm -

B m %

, m . S q mm -

P m %

L m %

N

T -

HLA-DR

m . T

CD (CALLA)

, A -

CD

m. m (m)

CD

.

T - m .

S q m x m

PAS m m .

■ Critical Thinking Group Discussion Questions

T S k B m

. B , m k

. A mm

?

m x mm ALL m k

.

. W - m

x m ?

■ Critical Thinking Group Discussion Questions

. W m ?

. W mm -

?

. W ?

. W ?

Cas e Study 21.6

A - - m

. S m j , j

, . N . S -

NOTE: This is a good time to write out the answers to the

x mm z .

Critical Thinking Group Discussion Questions.

PART 6 ■ Neoplastic Disorders

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

8. A m x m m m

1. W

A. FAB M

k m ?

B. FAB M

A. R m m m m k -

C. FAB M

D. FAB M

B. B mm k

m

9. B m m

C. L k

A. FAB M

D. A

B. FAB M

C. FAB M

2. M m k m

D. FAB M

FAB

A. M

10. M m m -

B. M

m

C. M

A. FAB M

D. L

B. FAB M

C. FAB M

3. M k m FAB

D. FAB M

A. M

B. M

11. M , m , m m -

C. M

D. L

A. FAB M

B. FAB M

4. M m k m -

C. FAB M

FAB

D. FAB M

A. M

12. Imm k -

B. M

m

C. M

A. FAB M

D. L

B. FAB M

C. FAB M

5. L m () k m

FAB

D. FAB M

A. M

*13. A m m

B. M

m . W -

C. M

m m k m ?

D. L

Cytochemical Reactions in Selected Leukemias

6. C FAB M

(α) NA

A. k m m

SB

MPO

PAS

NASDCA

(.)

A P

A.

+

—

—

—

—

B. k m m

B.

++

++

—

+

—

—

C.

±

±

—

±

++

—

C. k m m

D.

\pm

\pm

++

—

++

—

D. k m m k

SB, S k B; MPO, m x ; PAS, -

—S ; NASDCA, AS-D -

; (α) NA, - ;

7. T FAB M

A P , .

A. m

N : N , x

B. .

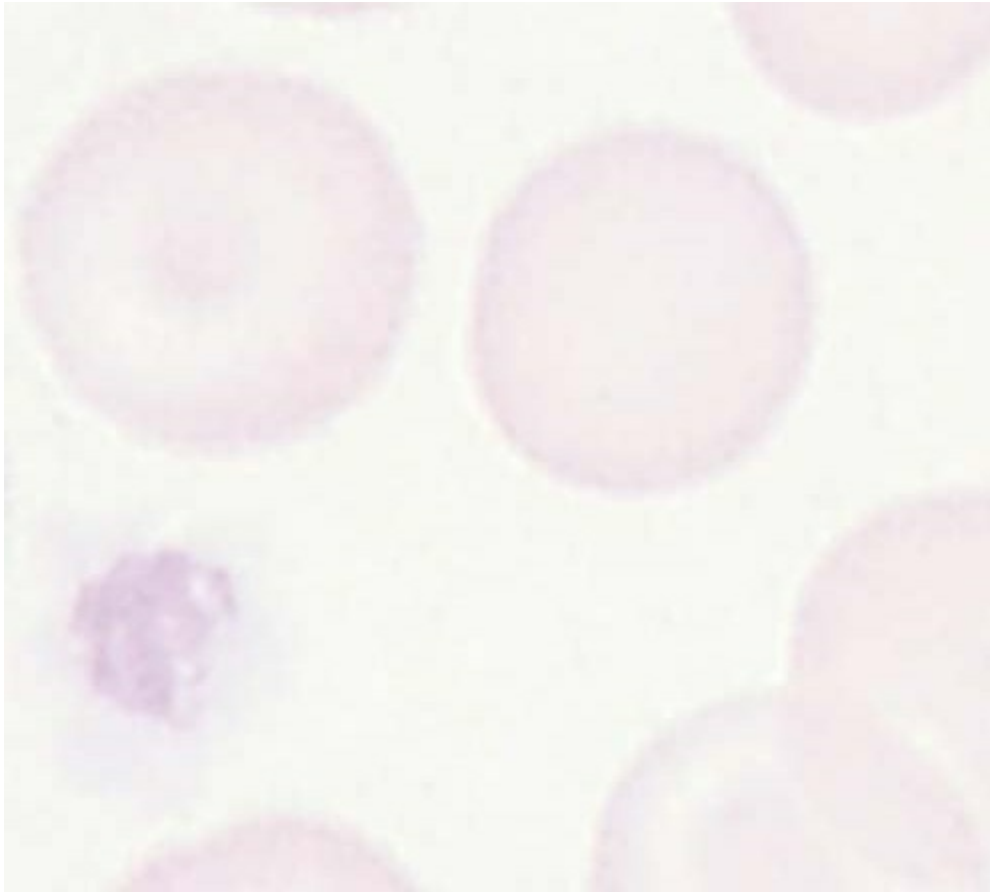
PAS, mm m k m ,

C. m -

-B B-m k m ,

D. A C

m k m .





CHAPTER 21 ■ Acute Leukemias

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REVIEW QUESTIONS (continued)

*14. T m -

*18. T M:E k m

m m

A.

. W m m -

B. m

k m ?

C.

Cytochemical Reactions

D.

P

(α) NA

19. A

R

SB

MPO

PAS

NASDCA

(.)

A P

A. m m

A.

+

+

-

-

-

-

B. x

B.

++

++

-

+

-

-

C. LAP

C.

±

±

—

±

++

—

D. m

D.

±

±

++

—

++

—

*20. O m m m k m m

SB, S k B; MPO, m x ; PAS, -

m k m

—S ; NASDCA, AS-D -

A. x

; (α) NA, - ;

B. k

A P , .

C.

N : N , x

D. m x

PAS, mm m k m ,

-B B-m k m ,

*21. W

m k m .

S B k B ?

A. L m

*15. T m -

B. M

m m

C. M

. W m -

D. E

k m ?

*22. W m m -

Cytochemical Reactions in Selected Leukemias

x ?

SB

MPO

PAS

NASDCA (α) NA (.)

A P

A. , x m

A.

+

+

—

—

—

—

B. B

B.

++

++

—

+

C. L m

C.

—

—

±

±

—

±

++

—

D. M

D.

±

±

++

—

++

—

*23. W m

SB, S k B; MPO, m x ; PAS, -

m m

–S ; NASDCA, AS-D -

m m ?

; (α) NA, - ;

A. S B k B

A P , .

B. LAP

C. N

*16. W m m m D. A

z m m z ?

*24. W m

A. P B

m ?

B. S B k B

A. S B k B

C. P –S (PAS)

B. LAP

D. M x

C. P S (PAS)

D. A

17. M (-)

25. L k m B k ' m m -

A. m k m m

B. m k m

A. FAB M

C. m

B. FAB L

D. m k m

C. FAB L

D. FAB L

(continued)



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PART 6 ■ Neoplastic Disorders

REVIEW QUESTIONS (continued)

26. C m k m

*32. W m

A. FAB M

- B-ALL?

B. FAB L

Cytochemical Reactions

C. FAB L

D. FAB L

(α) NA

SB

MPO

PAS

NASDCA

(.)

A P

27. O

A.

±

±

—

±

B.

++

—

—

—

+

+

+++

—

A. FAB M

C.

—

(+)a

+

+

+++

—

B. FAB L

D.

—

—

±

—

—

(++)b

C. FAB L

D. FAB L

a m x .

bT m G .

28. C m

SB, S k B; MPO, m x ; PAS, -

A. FAB M

—S ; NASDCA, AS-D -

B. FAB M

; (α) NA, - ;

C. FAB M

A P , .

D. FAB M

33. P AML

29. A mm ALL

A.

A. j

B. A

B. m A

C. P m m

C. k

D.

D. k m

34. W m -

*30. A m x m . W

k m mm -

m k m ,

m -

m ?

k m ?

A. REAL

Immunological Markers

B. WHO

C. FAB

d

CALLA

CD7

CD19

HLA DR

Slg

D. RYE

A.

+

+

+

B.

a

+

+

+

*35. A m ’

C.

+

a

+

m m

—

D.

+

+

m m . Imm

aS m .

CD CD . W

m

, m x ; CALLA,

x ?

mm ALL ; CD, ; S ,

A. (,) (q ; q .)

mm ; +, ; , .

B. (q) ()

C. q

*31. S B-m k () m m

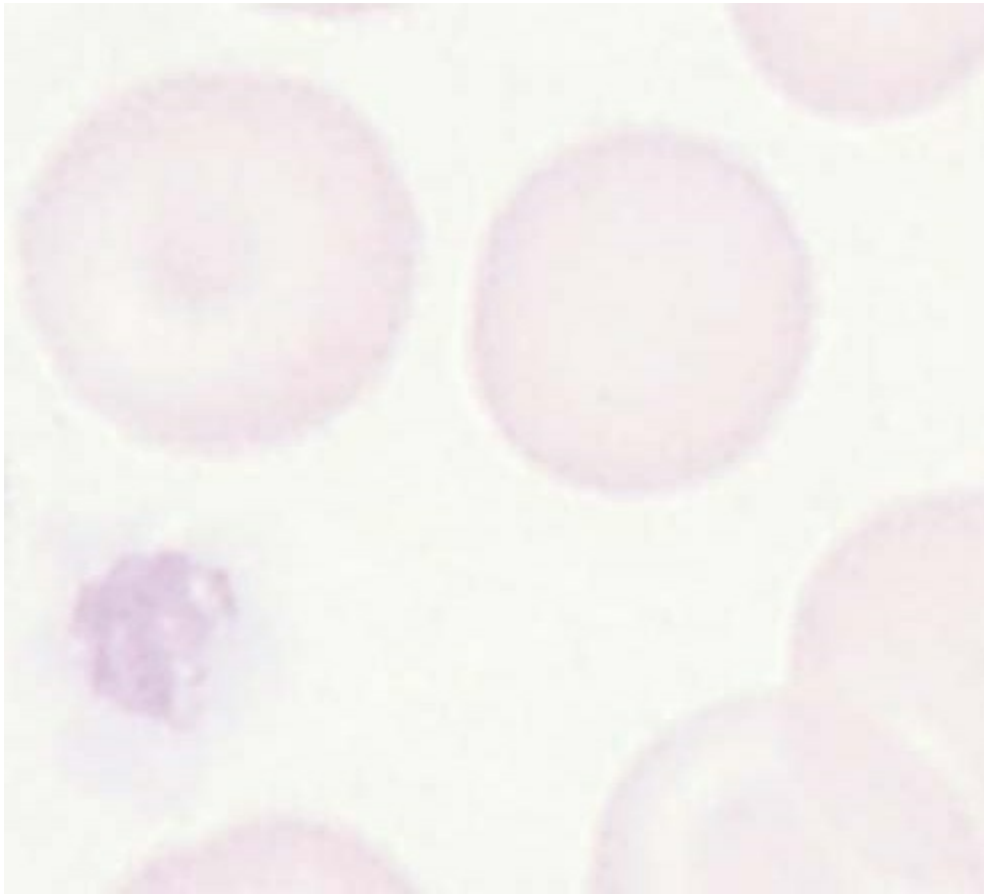
D. () q

A. CD

B. CD

C. CD

D.



CHAPTER 21 ■ Acute Leukemias

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REVIEW QUESTIONS (continued)

*36. A - - m

*39. A k CD , CD , CD

m m

(CALLA), CD . U WHO ,

. \times L : m

mm

%, \times /L,

ALL?

. \times /L. S m %

A. -B

m m ;

B. mm ALL

m k A . W

C. -B ALL

m k m m k ?

D. - ALL

A. m (;) (q :q)

B. m k m

*40. ALL P NO

C. m k m

?

D. m (;) (q :q)

A. m

B. mm

*37. A m ALL

C. m

P m m . W

D. m

m k m ?

A.

*41. P D S m

B.

ALL ?

C. ' m m

A. P m m

D. z

B. GA A m

C. q

*38. A k m

D.

W H O z :

A. WBC

B.

C. > % - m

D. m

C U, S J. m k **COMPANION RES OURCES**

m m ,

Haematologica, (): – , .

:// . . m/

C x CV, . C z -

- m k m . Blood, (): – , E -

.

m .

V ACH, . R m -

H ,

FL j m m k m , Haematologica,

(): – , .

.

Dö H, W DJ, B m CD. A m k m , N Engl J Med, (): – , .

Dö K, Dö H. M z m -

BIBLIOGRAPHY

k m , Haematologica, (): , .

F C, . A

B U, . A m m m

m m m k m , m m Blood, (): – , .

m k m , Haematologica, (): – , .

F K, CSP 2015 Short Course—Leukemia Classi cation 2016: What,

B A, . U m FAB k m When, “Who”, C S P , S D , CA, .

, Am J Med echnol, (): – , .

G RC. T m k m m (*H LV*) C N, . P m m BCRABL

m : , , Can Res Suppl, : – , .

m k m (ALL) BG F, . M P , (:), MLL

(CD +) ALL, Hematol J, (S): , .

m D x m -

C zz G, B A. M m

k m , Hematol J, (S): , .

m k m -

H DC, . D k m m m q m , x , Blood, Haematologica, (): - , .

: - , .

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PART 6 ■ Neoplastic Disorders

H , CE. A L m L k m (ALL). . m.

M CG, . BCR-ABL m k m -

. . R N m , .

z Ik , Nature, : - , .

H SP, M CG. A m k m , M BL, . Ex -

N Engl J Med, (): - , .

m k m , Hematol J,

I I, . I Ik DNA (S): , .

P - m k m -

O'B S. A k m , ASH News, (): , .

z m , Haematologica,

O RJ. A k m mm m : m -

(): - , .

, Arch Pathol Lab Med, : - , .

J S, . P CD x -

R m MD, . M RNA m k m , B-m k m , Blood, Hematologist: ASH News Rep, (): , .

(): - , .

R JC. B - - m m m : K LM, G DG. G m k m . Annu Rev , Blood, : - , .

Genomics Hum Genet, : - , .

S m C, . T x

K k AK, R k . T -

m m (PRAME) m k

(S R)

m k m , Haematologica, : - , .

m , Hematol J, (S): , S k MA. m m k -

.

m : , Haematologica, K k FP, . C - k m k m : (): - , .

m ? Haematologica, (), - , .

S FJ , L k, AW. S -

L Z, . G x - k m - m k m , Haematologica, m k m , Blood, (): - (): - , .

, .

S RM. O AML : ? Blood, (): , .

L -C F, . P m z W Z, C Z. A m k m : m GIMEMA LAM P m

, Blood, : - , .

m k m , Hematologica, (): - , .

W m DA. A m m k m ? N Engl L B, . A m k m , N Engl J Med, J Med, : , .

(): – , .

W k KP, . A m k k m (M) , M J, . R k Mayo Clin Proc, : – , .

m m k m , W BJ, . A m - x -

Haematologica, (): – , .

m k m : k , Blood,

M AJ, . FL k m m

(): – , .

m m

Y M, . K m j -

FL m

- P

m k m , Blood, : – , .

m m - m k m

M P, M KR. T k m , Hosp Phys, (): – , m - m m , Haematologica, (): – , .

.

Lymphoid and Plasma Cell

CHAPTER

22 Neoplasms

KEY WORDS

a n g i o g e n e s i s

c y t o g e n e t i c s

m u l t i p l e m y e l o m a

a n e u p l o i d y

h a i r y c e l l l e u k e m i a

n ä i v e B c e l l s

a p o p t o s i s

H o d g k i n d i s e a s e

n o n -H o d g k i n l y m p h o m a

B - c e l l p r o l y m p h o c y t i c l e u k e m i a s

h y p e r v i s c o s i t y

p l a s m a c e l l l e u k e m i a

c h e m o t h e r a p y

l e u k e m i a s

R e e d -S t e r n b e r g c e l l s

c h r o n i c l y m p h o c y t i c l e u k e m i a

lymphomas

Waldenström macroglobulinemia

clonal

minimal residual disease

cryoglobulins

multisystemic lymphoid tissue

LEARNING OUTCOMES

Leukemias and lymphomas

- Discuss the epidemiology, clinical signs and symptoms, laboratory

- Compare the characteristics of leukemias and lymphomas.

characteristics, and prognosis of Sézary syndrome.

- Describe the types of specimens and methods of analysis used to

Discuss the laboratory characteristics, including phenotyping, of study leukemias and lymphomas.

T-cell large granular lymphocytic leukemia (LGL).

Discuss the etiology and laboratory characteristics of adult T-cell

Chronic lymphocytosis

leukemia/lymphoma (HTLV-1).

- Name various categories with benign or malignant conditions that

Lymphomas

produce chronic lymphocytosis.

- Explain general characteristics of a malignant lymphoma.

Chronic lymphocyte leukemia/ small

- Name factors known to be risk factors in the development of lympho-

cytic lymphoma

phoid neoplasms.

- Describe the two contemporary lymphoma classification systems,
- Describe the diagnostic features, including clinical symptoms and including how subtypes are classified.

laboratory data, of chronic lymphocytic leukemia (CLL).

- Identify the epidemiologic characteristics of lymphomas.
 - Explain the usefulness of chromosome analysis and molecular analysis in the diagnosis and prognosis of CLL.
- Summarize the pathophysiology of most lymphomas.

- Describe the laboratory analysis of lymphoid neoplasms.
- Describe the features associated with aggressive forms of CLL.

Summarize and compare disorders that represent the leukemic

- Explain the systems to stage disease and progress.

phase of non-Hodgkin lymphomas: follicular lymphoma, mantle cell

Monoclonal B-cell lymphocytosis (MBL)

lymphoma (MCL), marginal zone B-cell lymphoma, lymphoplasmacytic lymphoma (LPL), diffuse large B-cell lymphoma, not otherwise

Compare the differences between low count and high count

specified (DLBCL, NOS), and Burkitt's lymphoma.

in MBL

- Explain the etiology, epidemiology, laboratory characteristics, and

B-cell prolymphocytic leukemia (B-PLL)

prognosis of Hodgkin disease.

Discuss the epidemiology, clinical signs and symptoms, laboratory

Plasma cell dyscrasias

characteristics and treatment of B-PLL.

- Name disorders based on proliferation of plasma cells and abnormal production of immunoglobulins.

Hairy cell leukemia

- Describe the general characteristics and laboratory data in multiple

- Discuss the epidemiology, clinical signs and symptoms, laboratory myeloma.

characteristics and treatment of hairy cell leukemia (HCL).

Identify recent changes to the diagnostic criteria for multiple

- Describe laboratory findings seen in the variant form of HCL.

myeloma with specific biomarkers.

Compare laboratory characteristics of multiple myeloma,

T-cell and NK-cell neoplasms

Waldenström's macroglobulinemia, plasma cell leukemia (PCL),

Discuss the epidemiology, clinical signs and symptoms, laboratory heavy chain disease, and monoclonal gammopathy of undetermined characteristics and treatment of T-cell prolymphocytic leukemia. significance (MGUS).

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Case studies

NOTE:

Analyze the patient history, clinical signs and symptoms, and laboratory

■ indicates MLT and MLS core content

ratory data for the stated case studies; answer the related critical

indicates MLT (optional) and MLS advanced content

thinking questions; and conclude the most likely diagnosis.

LEUKEMIAS VERSUS LYMPHOMAS

NOTE: This is a good time to review the definitions of the Key

Terms in the Glossary and flash cards on

.

The terminology includes the various

or some leukemias and lymphomas that are of lymphoreticular origin. Lymphoid neoplasms classify conditions based on

the distribution of disease in leukemic or lymphoid. The

CHRONIC LYMPHOCYTOSIS

neoplastic cells of leukemic or lymphoid have an intimate

relationship. Frequently, the neoplastic cells of these two disorders are various conditions that produce chronic lymphoid

disorders related (Table 22.1).

lymphocytosis (Box 22.1). These conditions can be benign

Leukemias represent overproliferation or accumulation

or malignant involve either T or B lymphocytes. The

of hematopoietic cells in the circulating blood and/or bone

malignant conditions that must be considered in the differential

diagnosis. During the progression of disease in some lymphoid

disorders, the diagnosis of chronic lymphocytic leukemia (CLL)

phases, the malignant cells may spill into the blood circulation—

the malignant B-cell disorders as well as spillover

of T cells. This spillover may produce leukemic phase of the

leukemic phase of some cases of non-Hodgkin lymphoma

disease. Transitions to leukemic phase are rare in disorders

(NHLs).

such as Hodgkin disease but are not uncommon in the well-differentiated non-Hodgkin lymphocytic lymphomas. In the

Chronic Lymphocytic Leukemia (CLL)/ Small

laboratory, bone marrow and peripheral blood specimens are

Lymphocytic Lymphoma (SLL)

studied by microscopic examination, flow cytometry, cyto—

Chemical leukemias are generally characterized by the pres—

ence of leukocytosis with an increase in the number of

The frequency of examination of bone marrow and periph—

lymphocytes, lymphocytosis, on peripheral blood film.

Peripheral blood involvement depends on the lymphoma subtype.

For example, lymphoproliferative disorders

Lymphomas are heterogeneous group of closely related

(Table 22.2) are characterized by accumulation of

disorders that are characterized by the overproliferation of

lymphocytes.

One or more types of cells of the lymphoid system such as

Both CLL and SLL are neoplasms composed of small B

lymphoreticular stem cells, lymphocytes, reticular cells,

lymphocytes in the peripheral blood, bone marrow, spleen,

n histiocytes. Lymphoproliferative disorders that
 involve lymphoid organs or tissues, including lymphomas.
 Immunoblastic or proliferative centers in tissue inf—
 Study of lymphoid tissue disorders is performed
 testes. In contrast to CLL, SLL is used or nonleukemic
 by obtaining specimen for biopsy or fine-needle spi—
 patients with the tissue morphology and immunopheno—
 type of CLL.

examination, flow cytometry, cytochemical staining, and
 chromosome analysis.

Relationship of Leukemias and

BOX 22.1

TABLE

22.1 Lymphomas

Leukemia Type

Solid Tumor Counterspart

Examples of World Health Organization of

Mature B-Cell Neoplasms

Stem cell leukemia

Lymphoma, undifferentiated

Chronic lymphocytic leukemia / small lymphocytic leukemia

Acute lymphoblastic

Lymphoma, poorly differentiated leukemia

B-cell prolymphocytic leukemia

differentiated; lymphocytic

Hairy cell leukemia

Chronic lymphocytic

Lymphoma, well differentiated leukemia

Plasma cell dyscrasia

differentiated; lymphocytic

Monoclonal gammopathy of undetermined significance

(MGUS)

Monocytic leukemia

Reticulum cell sarcoma

Primary myeloid leukemia

Acute myelogenous

Chloroma granulocytic leukemia

Hemochromatosis

Burkitt lymphoma /leukemia

Plasma cell leukemia

Myeloma

CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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apoptosis. The antiapoptotic Bcl2 gene is reported to be

Classification of

TABLE

22.2

overexpressed in 65% to 70% of B-cell CLLs.

Lymphoproliferative Disorders

Cytogenetics

Type

Alternative Names

CLL is heterogeneous at the clinical, cellular, and molecular

Acute lymphoblastic leukemia

levels. Chromosomal alterations occur in approximately 80%

of CLL cases; these alterations include the 13q deletion, the

Chronic lymphocytic leukemia

11q deletion, trisomy of chromosome 12, and the 17p deletion—

B cell

tion. The high rate of recurrence of the same chromosomal

abnormalities suggests that these abnormalities affect

T cell

consequently.

Prolymphocytic leukemia

Cytogenetic studies have demonstrated clonal chromosomal abnormalities in about 80% of patients with B-cell CLL

Hairy cell leukemia

Leukemic

by fluorescence in situ hybridization (FISH) testing. Based on

reticuloendotheliosis

gene expression profiling of 18 genes using microarray tech—

Plasma cell leukemia

Leukemic phase of

nology, five distinct cytogenetically defined CLL subtypes

multiple myeloma

have been identified. The most consistent finding is an extra

chromosome 12 (trisomy 12), which is present in approximately

Sézary syndrome

Leukemic phase of

tely 50% of patients. A translocation of chromosomes

mycosis fungoides

8 and 14 is also associated with B-cell CLL. Chromosome

Non-Hodgkin lymphoma

benign lymphomas can be found in CLL and T-cell leukemia—

in. A variety of chromosomal abnormalities are found, the

Large granular lymphocytosis*

most consistent being trisomy 7. In non-B types,

Reactive lymphocytosis*

translocation of chromosomes 9 and 22 may be observed.

*These disorders usually have a benign clinical course.

Immunologically, B cells display the classic surface immunoglobulin (SIg) receptor. In addition, B cells can be identified by monoclonal antibodies expressing CD19, CD20 or

Epidemiology

CD24, and CD5 markers.

CLL is the most common form of leukemia in adults in

Molecular genetics has two major applications in the field—

Western countries, but it is very rare in Eastern countries.

Diagnosis of chronic lymphoid malignancies:

CLL/SLL accounts for about 7% of NHLs in biopsies.

■ Demonstration of the clonal nature of population of

The median age of onset is 65 years. This is for the leukemic

lymphoid cells

is rare before age 20 and uncommon before age 50. But it is

■ Detection of pathogenetically important rearrangements,

now recognized even in younger persons. More lesions

for example, clonal Ig or CR gene rearrangements, than

the lesions (1.5 to 2.1:1) expected by the disorder.

are useful in diagnosis of CLL

CLL has the highest genetic predisposition of all hematologic

neoplasms. A family predisposition can be observed—

Molecular genetics detection of genomic rearrangements

present in 5% to 10% of patients with CLL. The overall risk

is not only assist with the diagnosis but can also provide

is two to seven times greater in first-degree relatives of CLL

important prognostic information. Many of these rearrangements—

patients.

can detect molecular markers for the detection of low

levels of residual disease.

Etiology

Molecular Genetics

Classic CLL is usually B-cell isor er. Mature B-cell neoplasms are clonal proliferations of B cells at various stages of

Variable-Region Genes

differentiation ranging from naïve B cells to terminally

New knowledge regarding the biology of CLL has emerged—
cells. Mature B-cell neoplasms (Box 22.1) comprise one
subset that represents 50% to 70% of CLL cases and
more than 90% of lymphoid neoplasms worldwide.

immunoglobulin variable-region gene (IgVH) hypermutation—
B-CLL is biologically and clinically heterogeneous hetero-
tation. The IgVH mutation status is important in determining—
tologic malignancy characterized by gradually progressive
impairing the prognosis of patients with CLL.

accumulation of morphologically mature B lymphocytes in
Patients with untreated CLL have better prognosis than
the blood, bone marrow, and lymphatic tissues. More than
those with untreated CLL, unlike those with low stage.

90% of CLL cells are nondividing and rest in G0 or G1 of

Cases can be divided into two subgroups on the basis of the
the cell cycle. These cells are characterized as CD5+ CD19+

presence or absence of somatic mutations in the specific

CD23⁺ monoclonal B cells.

immunoglobulin heavy chain variable-region (IgVH) genes

An excess of B cells is more likely to be result of excessive

use by the leukemic cells. The mutation status of the *ig*—

apoptosis and regulation of cell cycle control then on

immunoglobulin variable (V) gene segments allows for differential

increase proliferation rate. CLL cells are very resistant to

treatment between untreated CLL, with low or high

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PART 6 ■ Neoplastic Disorders

risk of disease progression, respectively. Mutations in these

involving the delicate homeostasis between proliferation

genes are so closely linked to the clinical courses.

and apoptosis promotes cell survival by inhibiting

Patients whose leukemic B cells express IgVH have better

cell death. miRNAs are major direct negative regulators of

survival rate (e.g., 24-year survival) compared with patients

the Bcl2 antiapoptotic protein and indirect activators of the

who lack such mutations (e.g., 6-to 8-year survival).

intrinsic apoptotic programme leading to apoptotic peptide

The leukemic cells represent patients with or without IgVH

clonal rearrangement.

mutations are frequently have cytogenetic changes that

Myeloid cell leukemia-1 (Mcl-1) is a anti-apoptotic e-

regulator of poor clinical outcome (e.g., 11q22-23 deletion,

overexpression of the Bcl2 protein family. Increased Mcl-1 expression is

17p deletion, trisomy 12, or p53 dysfunction). Patients with

associated with failure to achieve remission after treatment

biologically significant numbers of IgVH mutations are re-

lated with unresponsive to chlorambucil in patients with CLL.

frequently have chromosomal changes associated with benign

Mcl-1 expression may be useful in predicting poor response

course of the disease (e.g., 13q14 deletion).

to chemotherapy.

Note that patients with lymphadenopathy, splenomegaly,

Zeta-Chitin-Associated Protein 70

pancytopenia with atypical lymphocyte population

Another signalling-associated molecule, the zeta-chitin-

may have a requirement in gene that is crucial to cell-

ssociate protein 70 (ZAP-70), was recently discovered to
 induce apoptosis. The protein encoded by the FAS cell surface
 is preferentially expressed in the CLL subgroup without IgVH
 the receptor gene (FAS) is a member of the TNF-receptor
 family that has poor outcomes. ZAP-70, an enzyme and—
 superfamily. This receptor contains the domain that is
 highly expressed in lymphocytes, is critical for the activation—
 been shown to play a central role in the physiological regulation—
 of cells by antigen. The expression of this gene
 in precursor cells is high. So the expression of FAS has
 gene in CLL cells is surprising but has been confirmed by
 been implicated in the pathogenesis of various lymphomas
 research studies. Inappropriate expression of ZAP-70 in CLL
 indicates a defect in the immune system such as autoimmunity
 after the action of another protein tyrosine kinase, Syk,
 lymphoproliferative syndrome (ALPS). ALPS is associated with
 autoimmunity in B lymphocytes.
 B-lymphocyte survival due to dysregulation of the
 FAS apoptotic pathway.
 Thymidine Kinase

Another new finding is the correlation of the survival

Self-sufficiency in Growth

orthotopic kinase with IgVH gene uterine stroma

Self-sufficiency in growth demonstrates that normal cells

also with disease progression. DNA microarray has

require growth stimuli compared to cancer cells that re-

construct that CLL exhibits characteristic gene expression

capable of generating their own growth signals without having—

profile closely related to ordinary B cells and independent of

ing to rely on antigens in the surrounding environment in

the presence of IgVH mutations.

or extrinsically proliferate.

CD38

Stimulation of Angiogenesis and Dissemination

Expression of CD38, a membrane protein that marks cells—

This is characteristic of the malignant lymphomas of

involvement in tumor progression through signaling activity,

patients with CLL. Patients show high degree of tissue

often correlates with the presence of IgVH mutations. CD38

neovascularization.

surface expression on the malignant cell is now viewed as an

independent marker of patient's clinical outcome. CD38+

Staging and Prognosis

B-CLL patients are characterized by a prevalence of

CLL has a variable clinical course. Clinical staging systems

stage, lesser responsiveness to **chemotherapy**, a shorter

(R in Binet) or assessing prognosis in CLL were equal—

survival times than CD38-negative patients.

developed in the early 1980s, based on easily obtainable biological

clinical parameters.

Micro RNA

Testing classification is

MicroRNA (miRNA) expression profiles can be used to distinguish normal B cells from malignant B cells in CLL patients.

0 Bone marrow and blood lymphocytosis

A unique microRNA signature is associated with prognostic

I Lymphocytosis with enlarged nodes

predicts disease progression in CLL. Mutations in miRNA

II Lymphocytosis with enlarged spleen or liver or both

transcripts are common and have functional importance.

III Lymphocytosis with no

miRNAs regulate the expression of protein-coding genes

IV Lymphocytosis with thrombocytopenia

in contrast to oncogenes, tumor suppressors, or both.

Most patients with CLL now have Rai stage 0 or I disease

Alterations in CLL include the following:

diagnosis. Patients with early-stage disease are heterogeneous group: approximately 30% to 50% will have accelerated disease

- Evolution of apoptosis

progression, in the interim they live or recede in post-

- Selective deficiency in growth

sometimes never require therapy. Prognosis is roughly related to the

- Stimulation of angiogenesis in disease progression

extent of organ infiltration at the time of diagnosis.

Evolution of apoptosis is associated with overexpression

At present, in addition to Binet stages, the utilization of

of the anti-apoptotic protein bcl2. BCL2 is responsible for

many of the VH genes, molecular markers such as ZAP-70 and

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CD38, and chromosomal aberrations 11q-, 13q-, 17p-, and

These characteristics can identify patients with early-stage

+12 relapse to predict survival. The average survival times

increase who are at high risk of early relapse progression.

For untreated patients relative to each stage follows: 0,

Poor-risk patients are characterized by a median clinical

150 months; I, 101 months; II, 71 months; III, 19 months;

stage, short lymphocyte doubling time, untreated in IV, 19 months. Early patients, treated untreated,

immunoglobulin heavy chain gene (IgVH) status, distinct genomic aberrations—

survive for 3 to 5 years on average. The principal cause of

relapses, ZAP70 and CD38 expression, an elevated serum

beta-2-microglobulin level is in fact, although 25% of CLL patients have

high beta-2-microglobulin levels.

Relapses are unrelated to the disease because of relapse. The

high risk of relapse in patients with CLL is the result of

Clinical Signs and Symptoms

Intermittent (intermittent) immunodeficiency caused by suppression—

The typical patient with CLL is asymptomatic, with the exception of immunoglobulin synthesis that leads to hypogammaglobulinemia.

Relapse is usually discovered at the time of routine physical

examination. Autoimmune disease may also develop;

examination. The disease is typically suggested by abnormal
autoimmune hemolytic anemia develops in approximately
fifteen percent of complete blood count (CBC) or
one third of patients.

the evaluation of unrelated illness. Common symptoms
Since the introduction of clinical stages, there has been
include fatigue, low-grade fever, and night sweats. Other
continuous effort to identify new prognostic factors in CLL
symptoms include weakness, fatigue, anorexia, and weight
(table 22.3). Patients with evidence of lymphadenopathy
loss. Physical examination usually reveals cervical lymph
prognostic value include the
supraventricular conduction system. Hepatosplenomegaly is also frequently present.

■ Number of lymphocytes in the peripheral blood

■ Degree of bone marrow infiltration

Laboratory Data

■ Proportion of abnormal lymphoid cells in the peripheral

Normal bone marrow elements get crowded out because of
blood

the excessive lymphoid production and packing of the re-

■ Lymphocyte doubling time

roughly proportional to lymphocytes. This infiltration by the

■ Immunoglobulin heavy chain variable-region gene utilization

leukemic clone results in neutrophil, thrombocytopenia, and

thrombocytopenia

neutropenia.

■ Cytogenetic abnormalities assessed by fluorescent in situ

Although leukocytosis may be observed, it is less pronounced—

hybridization

noted in chronic myelogenous leukemia. Overall

■ Z-chain-associated protein kinase-70 protein expression

leukocyte counts can range from 30 to $200 \times 10^9/L$. In one

third of patients, the total leukocyte count is greater than

$100 \times 10^9/L$.

Factors with Prognostic

Absolute lymphocytosis is useful in defining. The International

TABLE

22.3 Significance in Chronic

International Workshop on CLL report requires that the lympho-

Lymphocytic Leukemia

cytosis must be present or at least 3 months. In addition,
the International Workshop allows for diagnosis of CLL

Factor

Low Risk

High Risk

with lower lymphocyte count in patients with cytopenias

Clinical stage

or disease-related symptoms. In the absence of extramedullary tissue involvement, there
must be $\geq 5 \times 10^9/L$ monoclonal—

Binet

A

B, C

monoclonal lymphocytes with CLL phenotype in the peripheral

Rai

0

I, II, III, IV

blood.

Bone marrow

Peripheral blood smears (Figs. 22.1 and 22.2) can only

infiltration

exhibit up to 80% or 90% monoclonal lymphocytes. Many of these

Biopsy

Nondiffuse

Diffuse pattern

cells have no overture look because of the hypercondense pattern

nuclear chromatin pattern. An occasional large lymphoblast

Aspirate

<80% lymphocytes

>80% lymphocytes

may be noted. Some cells are highly characteristic. Both the granulocytes and the platelets are normal. It is important

WBC ($\times 10^9/L$)

<50

>50

to differentiate between CLL and other benign or malignant

Prolymphocytes <10

>10

causes only lymphocytosis (Box 22.2).

in peripheral

Other ill to severe immunologic dysfunction type—

blood (%)

reflects the disease. Serum electrophoresis studies usually show

Lymphocyte

<12 mo

>12 mo

hypogammaglobulinemia Genetic studies can include

doubling time

microarray analysis (see Fig. 22.3).

β 2-microglobulin

Normal

Increased

Treatment Options

CD38

<30%

>30%

Previously, patients with CLL were only treated or palliative

expression

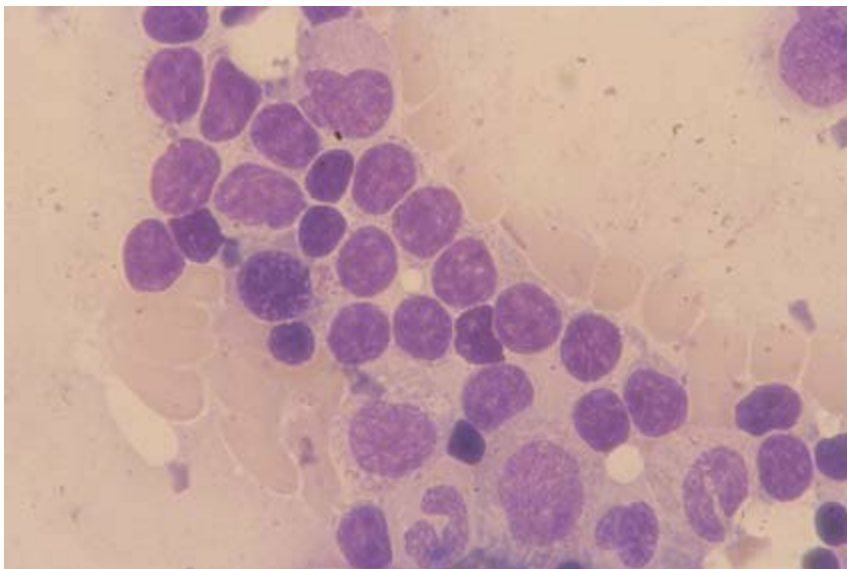
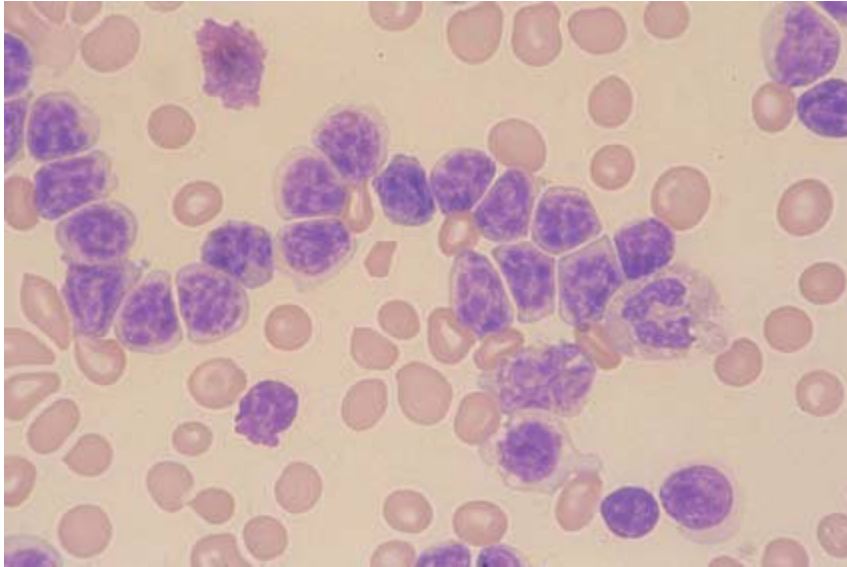
reasons, but numerous new treatment options are now available—

IgVH genes

Mutated

Unmutated

ble or the treatment of CLL.



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PART 6 ■ Neoplastic Disorders

BOX 22.2

Differential Diagnosis of Chronic

Lymphocytic Leukemia

BENIGN CAUSES

-Cell Associated

- Bacterial, e.g., B
- Viral, e.g., infectious mononucleosis

B-Cell Associated

- Persistent polyclonal B-cell lymphocytosis
- Hyperreactive liver splenomegaly

MALIGNANT B CELL

- Monoclonal B-cell lymphocytosis
- Prolymphocytic leukemia (PLL)
- Hairy cell leukemia

Leukic phase of non-Hodgkin lymphoma

FIGURE 22.1 Chronic lymphocytic leukemia. Mature cells pre-



dominate in the chronic leukemia. In this blood smear, typical

Follicular lymphoma

increase in the number of small cells is seen. (Stained with—

Mantle cell lymphoma

magnification 1,000×.)

Marginal zone lymphoma

Lymphoplastic lymphoma

Diffuse large cell lymphoma

Decision to Treat

MALIGNANT T CELL

The decision to treat patients depends on the stage of the

Prolymphocytic leukemia

in series, the presence of symptoms, and the disease activity-



Adult-onset leukemia/lymphoma

ity. Only patients in Rai III or Binet C stages should



Sézary syndrome

be treated. Patients in early stages of CLL should only be



Large granular lymphocytic leukemia

treated if symptoms associated with the disease occur (e.g.,



threatening complications of spleen or liver enlargement

Reference: Greer JP, et al. (eds.), Wintrobe's Clinical Hematology, 13th ed., in lymphos that can produce compression of the larynx 2014:1903.

blood in vessels). High disease activity is effectively lymphocyte doubling time of less than 6 months or by rapidly growing lymphoma. High disease activity is also an indication to treat in the early stages of the disease.

cyclophosphamide. This produces response in up to 70%

of patients but does not improve survival. Complete remissions are rare, and partial remissions are of short duration.

Originally, the first-line treatment of CLL in the 1960s consisted of

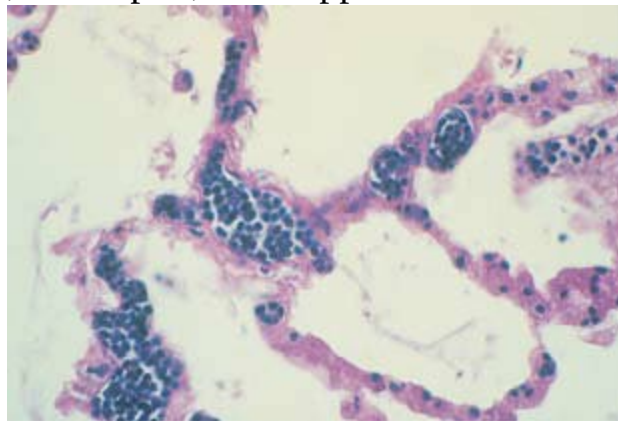
Nucleoside (purine) analogues, for example, fludarabine,

systemic alkylating agents, for example, chlorambucil or

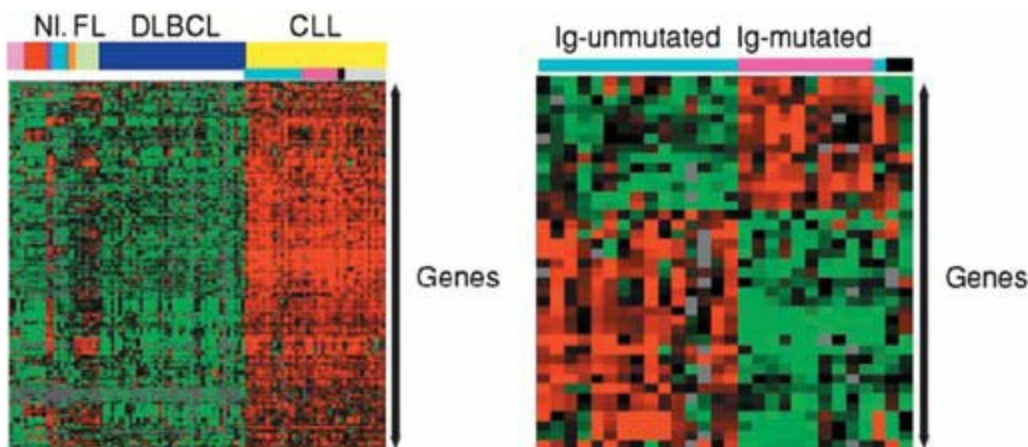
pentostatin, and cyclophosphamide, which were popular in the 1980s

FIGURE 22.2 Two slides showing chronic lymphocytic leukemia. (Reprinted from Anderson S, Poulsen K.

Anderson's Atlas of Hematology, Philadelphia, PA: Lippincott Williams &



Wilkins, 2003, with permission.)



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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(MRD) negativity in the detection of MRD up to 20% of patients with relapse. Regimens consisting of combinations of rituximab with monoclonal antibodies (e.g., rituximab, lenalidomide) are highly promising

treatments to achieve complete molecular remission.

Newer Treatments

Initial treatment of CLL patients requires assessment using the guidelines of the International Workshop on Chronic Lymphocytic Leukemia or “cutpoint series.” After clinical evaluation of fitness, patients are required to have FISH analysis for 17p13 deletion (17p-) and trisomy 12 (12+) or 17p13 deletion (17p-) and trisomy 12 (12+).

A

P53. Additional considerations include response to previous treatment or patients with relapsed/refractory CLL.

Newer treatments include the use of monoclonal antibodies such as bendamustine. treatment of CLL patients

has changed significantly over the past few years with the advent of the B-cell receptor (BCR) signaling antagonists or relapse series. In 2014, the Food and Drug Administration (FDA) approved two novel oral kinase inhibitors for patients with relapsed or refractory CLL. These inhibitors are:

- Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib

B

- Phosphoinositide-3 kinase (PI3K) inhibitor, idelalisib

FIGURE 22.3 A. Hyperleukocytosis **le** . **B.** Microarray analysis in chronic lymphocytic leukemia

phocytic leukemia (CLL) **le**; the expression of Allo gene ic He m a top oie tic S
te m Ce ll

C247 signature genes **right**. Di erenti tion o CLL p tients into

Trans p la nta tion

those with or without ut tions o the Ig V gene by the expres—

sions o 56 genes n Ig I unoglobulins. (Reprinte ro Greer

Allogeneic he topoietic ste cell tr nspl nt tion

JP, et l. Wintrobe's Clinical Hematology, 11th e, Phil elphi, PA:

(lloHSC) is the only potenti lly cur tive tre t ent v il ble

Lippincott Willi s & Wilkins, 2004, with per ission.)

or p tients with CLL. Myelo bl tive high-dose che other py,

with subsequent utologous or llogeneic ste cell tr nspl nt

(SC), is n option or young n physic lly f t p tients with

e onstr te higher response r tes th n lkyl ting gents to

rel pse ise se, but this is not n option or the jority o

provi e or longer progression-free surviv l. re t ent with

CLL p tients.

purine n logues lone oes not ppe r to i prove surviv l.

T e in verse re ctions to purine n logues re yelo—

Micro RNA

suppression n ly phocytopeni .

Purine analogues in combination with other cytotoxic

MicroRNA may potentially be used in therapy for CLL. In the
rugs (alkylators) were introduced as treatment strategy in
future, patient-specific therapeutic drugs may be designed
the 1990s. An estimate 35% of patients achieve complete
for CLL patients harboring abnormalities in miRNA expres—
sion.

sion in their lymphoid cells. miRNAs are potential targets

More recently, uracil, cyclophosphamide, ritux—

therapy as well, silencing overexpression of

in B (FCR) combination therapy has produced the largest pro—

miRNAs in inducing expression or silencing miRNAs in

portion of complete responses ever reported in CLL patients.

Cancer cells may contribute to selective tumor killing. Loss of

FCR became the new “gold standard” for CLL therapy.

miRNA expression in CLL patients may selectively suppress

CD52+ is expressed by normal B lymphocytes in

proapoptotic pathways, providing such ligand with

lost CLL cells. Anti-CD52 has been used primarily in

survival benefit.

patients who have achieved remission, in some cases, subsequent consolidation therapy. This humanized monoclonal antibody has produced significant responses in patients with relapsed or refractory CLL. Relapsed CLL has become common, and the use of monoclonal antibodies is increasing.

Minimal Residual Disease

Monoclonal antibodies have been used to detect minimal residual disease (MRD) in CLL. Relapsed CLL has become common, and the use of monoclonal antibodies is increasing.

PCR-based flow cytometry-based assays are used to detect MRD in CLL. Relapsed CLL has become common, and the use of monoclonal antibodies is increasing.

with relapsed or refractory CLL. Relapsed CLL has become common, and the use of monoclonal antibodies is increasing.

assess MRD in CLL. Relapsed CLL has become common, and the use of monoclonal antibodies is increasing.

the use of monoclonal antibodies is increasing.

or quantification by PCR.

monoclonal antibodies were achieved.

Monoclonal antibodies have been used to detect MRD in CLL. Relapsed CLL has become common, and the use of monoclonal antibodies is increasing.

tion than cytotoxic chemotherapy. They play a significant role in the treatment of minimal residual disease related to preceding content.

NOTE: This is a good time to complete Review Questions

significant role in the treatment of minimal residual disease related to preceding content.

related to preceding content.

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Monoclonal B-Cell Lymphocytosis

P53, may be treated with ibrutinib or venetoclax. Eligible patients may be considered for allogeneic bone marrow transplant.

patients may be considered for allogeneic bone marrow transplant.

Monoclonal B-cell lymphocytosis (MBL) is now known to
transplant.

prevalently occurs in CLL/SLL. Although the newly
updated World Health Organization (WHO) will retain

Hairy Cell Leukemia (HCL)

the current criteria for MBL, it does emphasize that the

“low count” MBL peripheral blood leukocyte count of less

Hairy cell Leukemia (HCL) is an uncommon chronic lympho—

than $0.5 \times 10^9/L$ must be distinguished from “high count”

proliferative disorder of the B-lymphocyte type. This is true

MBL.

B-cell lymphoma is diagnosed based on clinical features,

“Low count” MBL has significant differences from CLL

morphology, in phenotyping.

with extremely limited, if any, chance of progression. “High

count” MBL needs to be monitored. It resembles the pheno—

Cytogenetics

typical epigenetic/molecular features of Richter's.

BRAF V600E is the genetic lesion found in most cases

of hairy cell leukemia but not in HCL variant or other small

B-Cell Prolymphocytic Leukemias

B-cell lymphoid neoplasms. Recently, mutations in MAP2K1 that encodes MEK1, which is downstream of BRAF, have been reported in a subset of HCL-v variants in the neoplasms with poor prognosis. To distinguish it from the majority of HCL that use IGHV4-34 and that, like HCL-v, CLL, it requires more than 55% of circulating lymphoid cells lack BRAF V600E mutations.

to have the morphology of prolymphocyte.

Epidemiology

Epidemiology

HCL is much more common in males than in females. It usually—

B-PLLs represent a small fraction of less than 2% of lymphoid

lymphocytes patients older than 30 years of age.

leukemia.

Transformation of leukemia occurs in older adults with

Clinical Signs and Symptoms

emerged in the 1960s (age 69 or B cell). Males have higher

incidence of PLL leukemia.

Initial patient symptoms include fatigue, night sweats, leukocytopenia, thrombocytopenia, splenomegaly, and bone marrow fibrosis—

Clinical Signs and Symptoms

Splenomegaly. Pancytopenia is common. Bleeding tendency can be

present. The bone marrow may become fibrotic; therefore,

B-PLLs present with splenomegaly and lymphocytosis.

Bone marrow aspirates frequently are unsuccessful (dry

B-PLLs are typically aggressive, but subset of patients may

present with intermediate lymphocyte positive effect on

exhibit an indolent phase of variable length.

Bone marrow fibrosis.

Laboratory Findings

B-PLL can be distinguished from other B-cell leukemias—

Laboratory Findings

is by evaluation of blood cell morphology, immunophenotype—

Diagnosis of HCL includes morphologic appearance on the

immunotyping, molecular genetics.

lymphocytes, cytochemical staining, immunocytology—

Prolymphocytic leukemia is characterized by large number

of characteristic lymphocytes.

Presence of lymphocytes with the features of prolymphocytes—

HCL is so named because of the presence of ne, hairy—
phocytes in the peripheral blood. B-PLL lymphocytes have
like, irregular cytoplasmic projections that are characteristic
round nucleus, overtly abundant cytoplasm, and distinct
olymphocytes (Fig. 22.4) in this disease. Cytoplasmic pro-
“punch-out” nucleolus. The prominent central nucleolus
jections are not always obvious in HCL and in some cases
allows PLL to be distinguished from CLL/SLL. The total leu-
(e.g., relatively in other lymphoid neoplasms or reactive
kocyte count is greater than $100 \times 10^9/L$. Prolymphocytes
cells) are not specific for HCL.

must exceed 55% of lymphoid cells in the peripheral blood.

Morphologically, HCLs are large with overtly large

Most patients have disease of B-cell rather than T-cell

nuclei. So often, the slate-blue cytoplasm is vacuolated.

origin. B-cell PLL is positive for pan-B-cell markers, CD20,

The nucleus is frequently oval or slightly cleaved

CD19, CD22, and FMC7. In addition, CD24 is positive, CD5

be convoluted with homogeneous chromatin pattern. The

is variable, and CD23 is negative. The cells display strong SIg.

cytochemical features of HCL include strong cytoplasmic -

Cytogenetics reveal t(8;14), del 17p but no t(11;14). Molecular

transfection that is not inhibited by thymidine or retinoids—

genetics indicate CMYC and P53 mutation. Distinguishing

resistant cytoplasmic (RAP) stain. RAP positivity can

PLL resemble cell lymphoma (MCL) with leukemic

variability with disease progression. In addition, following inter-

conversion requires excluding t(11;14).

Immunotherapy, enzyme activity in the hairy cell may be RAP

negative.

Treatment

The immunologic markers include CD19+, CD20+,

First-line therapy for patients with B-PLL is combination

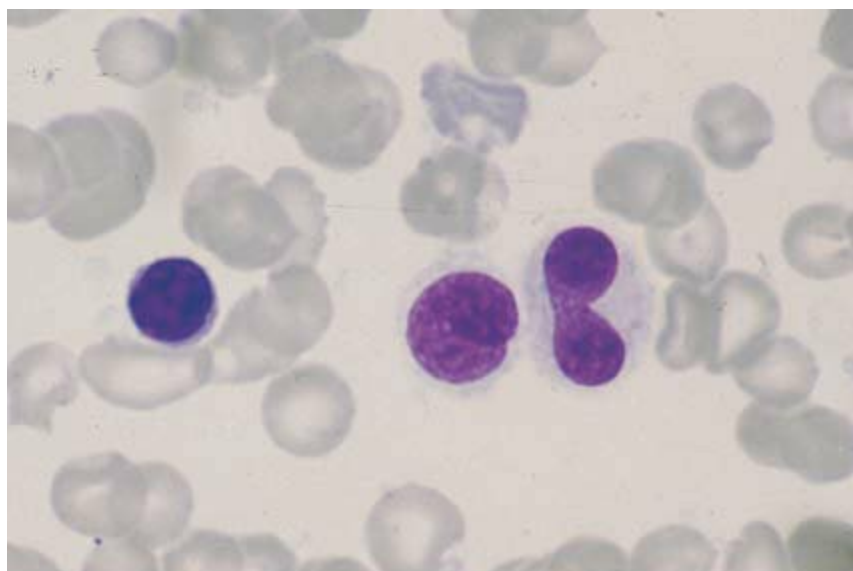
CD22+, CD24+, and CD25+ reactivity to the monoclonal

α-purine nucleoside deaminase antibody. Patients

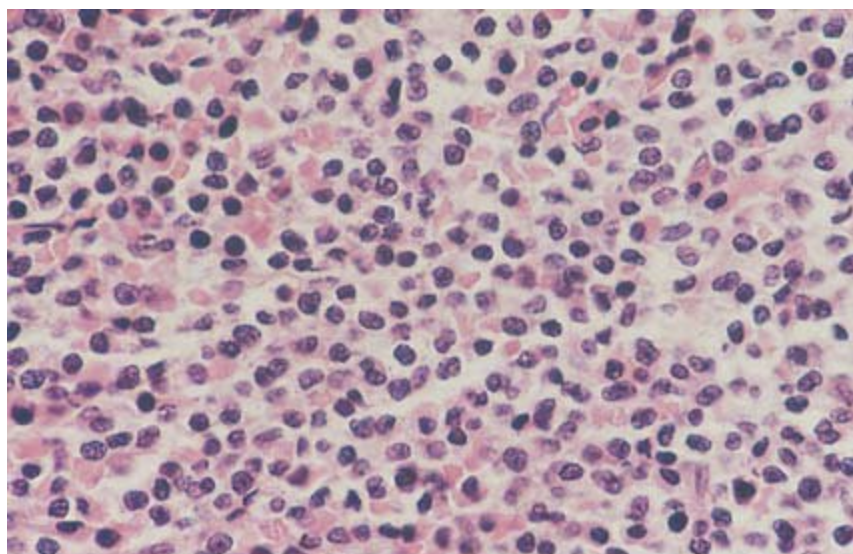
with antibody that recognizes the interleukin-2 (α) receptor. In

with B-PLL, especially patients with abnormalities of

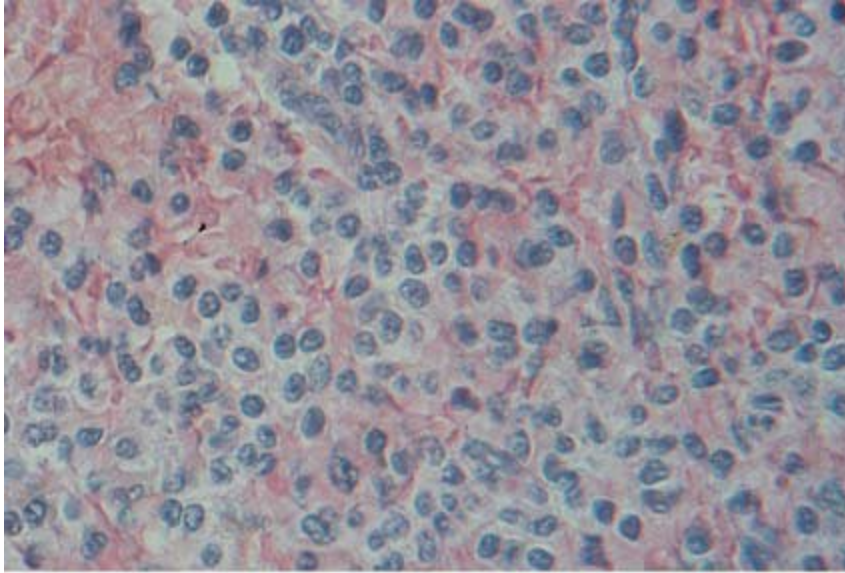
addition, the cells display strong SIg.



A



B



C

CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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FIGURE 22.4 Hairy cell leukemia. **A.** Peripheral

blood. **B.** Bone marrow (H&E stain). **C.** Spleen

(H&E stain). (Reprinted from Hines et al.

Blood Principles and Practice of Hematology, 2nd ed.,

Philadelphia, PA: Lippincott Williams & Wilkins,

2003, with permission.)

Treatment and Prognosis

T-CELL AND NK-CELL NEOPLASMS

The clinical course of HCL is more benign than many other

of leukocytes. Patients frequently live longer than expected

Mature B-cell neoplasms are derived from mature or post-germinal center B cells. The greatest risk of relapse is relapse.

thymic cells. Because NK cells are closely related to

In most cases, HCL is controllable with treatment of chemotherapy and immunophenotypic abnormalities

therapy, including chlorambucil, purine nucleoside analogs.

with cells, these two classes of neoplasms are considered

In small clinical trials, 70% of HCL patients whose disease is

together (Tables 22.4 and 22.5).

were resistant to conventional chemotherapy experience

relapse when treated with anti-CD22 recombination therapy

T-Cell Prolymphocytic Leukemia

notoxin (BL22). Recently, it was demonstrated that short

or long course of venetoclax, an inhibitor of BCR signaling

-cell prolymphocytic leukemias (c-PLLs) are associated with

threonine kinase with V600E mutation (BRAF V600E), with

poor prognosis. Distinction from CLL diagnosis requires

highly effective in patients with relapsed or refractory HCL.

more than 55% of circulating lymphoid cells to have the

morphology of prolymphocyte.

Hairy Cell Leukemia Variant

Epidemiology

A variant of HCL, hairy cell leukemia variant (vHCL),

was discovered in 1980. In addition, much rarer variants,

-cell PLL is rare (counts or less than 2% of lymphoid

Japanese variant neoplastic variant, exist. vHCL is more

leukemia. This form of leukemia occurs in older adults with

aggressive type of HCL with distinct morphologic

features in the 60s (age 61 or older). Most have

characteristics that are typical of HCL.

higher incidence of these leukemias.

vHCL cells resemble the typical HCL cell with

Clinical Signs and Symptoms

central round nucleus, prominent nucleoli, larger nucleocytoplasmic ratio, abundant basophilic cytoplasm with vacuoles

-cell PLL patients exhibit symptomatic splenomegaly

and cytoplasmic projections. vHCL may present with

lymphocytosis. -PLL patients more frequently experience

morphologic features intermediate between hairy cells and

lymphoproliferative, hepatomegaly, and skin lesions than B-PLL.

prolymphocytes.

Different diagnosis of vHCL or typical HCL can be

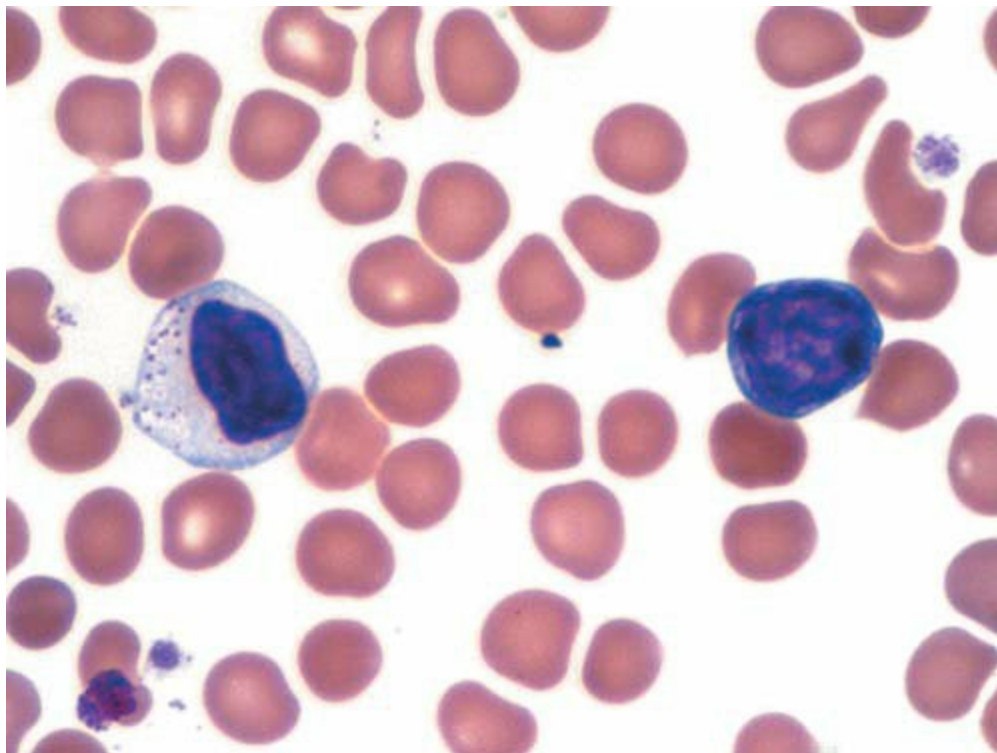
Laboratory Findings

by cytochemical staining (RAP) in unopheno-

-cell and B-PLLs can be distinguished from each other

typing using flow cytometry. vHCL has poor prognosis.

from other B cell leukemias by evaluation of blood





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PART 6 ■ Neoplastic Disorders

Immunohistochemical Features

TABLE

22.4 of Selected T-Cell Neoplasms

Surface Membrane Markers

Type of Neoplasm

CD3

CD4

CD5

CD8

T-PLL

+

	±
	—
	—/+
T-LGL	
	+
	—
	—
	+
Mycosis fungoides/	
	+
	+
	+
	—
Sézary syndrome	
T-PLL, T-cell prolymphocytic leukemia/lymphoma; T-LGL, T-cell large granular lymphocytic leukemia; +, >90%; ±, >50%; —/+, <50%; —, <10%.	
Source: Handin RI, et al. (eds.). Blood: Principles and Practice of Hematology, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2003.	
FIGURE 22.5 T-cell large granular lymphocytic leukemia. Bone marrow core biopsy reveals lymphoid aggregate with pleomorphic cell morphology (Fig. 22.5), immunophenotyping, n	

in the center in interstitial lymphoid infiltration in the junction.

molecular genetics.

Immunohistochemical stains identify the lymphoid aggregates—

Prolymphocytic leukemia is characterized by large

cells in the interstitial infiltrates -LGLs. Hematoxylin and eosin, 20×

number of small lymphocytes with scant cytoplasm

signification. (From Sun. Flow Cytometry, Immunohistochemistry,

and Molecular Genetics of Hematologic Neoplasms, 2nd ed.,

the features of prolymphocytes in the periphery—

Philadelphia, PA: Lippincott Williams & Wilkins, 2012.)

peripheral blood. Cells of -PLL often have prominent nucleolus

and are small in size with convoluted nuclear outlines.

-PLL in peripheral blood exhibits small to medium, round

Prognosis and Treatment

or irregular nuclei resembling Sézary cells. Prominent nucle-

-cell PLL is typically aggressive, but subset of patients with

oligo blasts only in small proportion of cases, but cytoplasm—

exhibits prominent phase of variable length. -PLL patients

with blebbing is common.

have median survival of 7.5 months.

Leukocytosis can exceed $100 \times 10^9/L$. Prolymphocytes

First-line therapy for patients with T-PLL is le tuzumab.

must exceed 55% of lymphoid cells in the peripheral blood.

Eligible patients may be considered for allogeneic bone marrow transplantation in cases of T-cell PLL, the immunophenotypes are CD2+,

strong translocation.

CD3+, CD5+, CD7 +(very strong), and CD52 +(very strong).

Most patients are CD4+. Negative results are observed for

Sézary Syndrome and Mycosis Fungoides

, CD1, and CD25. Cytogenetic abnormalities include

Inv 14, t(14;14), t(x;14), and Iso 8q complex. Molecular

The leukemic phase of cutaneous T-cell lymphoma (CTCL)

genetics reveal mutations CL1, MCP1, and JAK3, and

mycosis fungoides (MF) (Fig. 22.6). It is the most common

SA 5b.

variant of CTCLs.

Genetic Features and Epstein-

TABLE

22.5 Barr Virus Status in Selected

T-Cell Neoplasms

Genetic

Type of Neoplasia

Abnormality

EBV Status

T-PLL

inv 14, trisomy 8q

—

T-LGL

None known

—

Mycosis fungoides/

None known

—

Sézary syndrome

FIGURE 22.6 Mycosis fungoides (cutaneous T-cell lymphoma).

EBV, Epstein-Barr virus; T-PLL, T-cell prolymphocytic leukemia/lymphoma;

Lesions have characteristic “scurvy,” poorly defined patches

T-LGL, T-cell large granular lymphocytic leukemia.

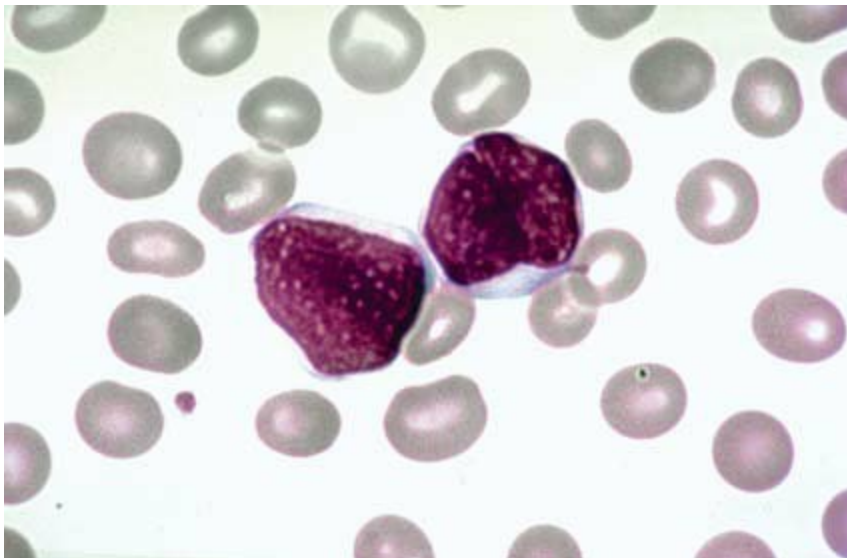
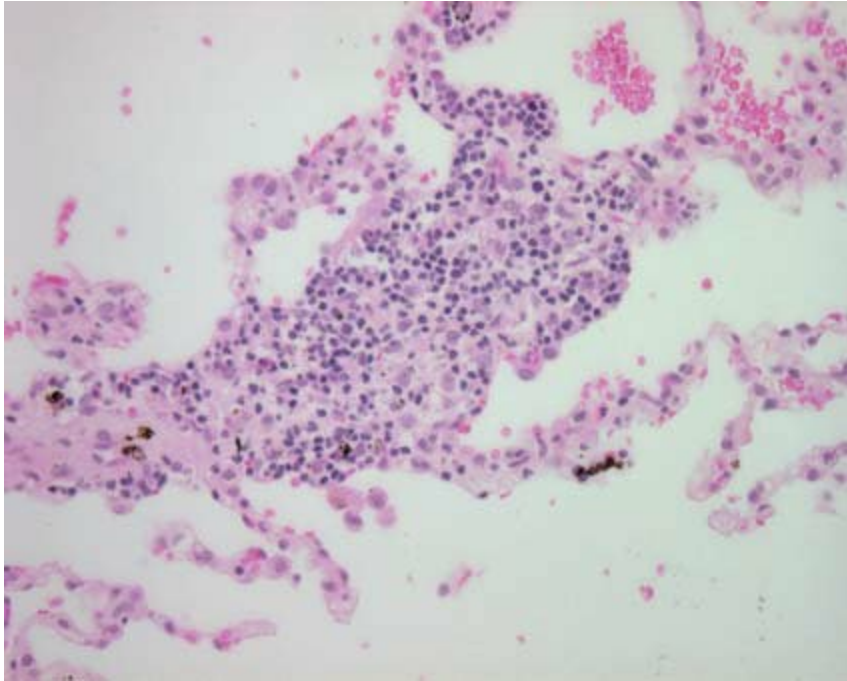
Lesions are in typical location. (Reprinted from Goodheart

HP. Goodheart’s Photoguide of Common Skin Disorders, 2nd ed.,

Source: Handin RI, et al. (eds.). Blood: Principles and Practice of

Hematology, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2003.

Philadelphia, PA: Lippincott Williams & Wilkins, 2003, with permission.)



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

FIGURE 22.8 Sézary cells. No circulating neoplastic T-helper cells with irregular nuclei and thin rim of cytoplasm are seen.

(Reprinted from Rubin E, Farber JL. Pathology, 3rd ed., Philadelphia, PA: Lippincott Williams & Wilkins, 1999, with permission.)

FIGURE 22.7 Mycosis fungoides with ocular interstitial infiltration

by small lymphocytes. (Reprinted from Cagle P. Color Atlas and

text of Pulmonary Pathology, Philadelphia, PA: Lippincott Williams

& Wilkins, 2005, with permission.)

& Wilkins, 2005, with permission.)

T-Cell Large Granular Lymphocytic

Leukemia (LGL)

T-cell LGL has presentation that is similar to CLL but is

Epidemiology

composed of mature cells. It is important to distinguish

The annual incidence of CLL in the United States is esti—

mated by the presence of abnormal

about 7.7 cases per million persons from 2001 to 2005.

phenotype or evidence of clonality. In addition, LGL usu-

ally affects between 40 and 60 years old most frequently

be distinguished from indolent chronic lymphoproliferation

icte with skin lesions th t progress to the tu or st ge.

isor ers o NK cells n ggressive NK-cell leuke i .

Clinical S igns an d Sym p tom s

La bora tory Fea ture s

T e jority o MF p tients present with e rly-st ge ise se

In LGL, p tients exhibit ne i , neutropeni , n thro -

or li ite p tches. E ch st ge o MF is ch r cterize by the

bocytopeni . A con ition o ne i c n be rel te to bone

egree o skin involve ent by p tches (-st ge). -st ges

rrow inf ltr tion or con ition o pl si o re bloo cell

r nge ro 1 with involve ent o less th n 10% o bo y sur-

(RBC) precursors. Con itions o neutropeni n thro -

ce; 2 is gre ter th n 10% o bo y involve ent by p tches

bocytopeni c n be ssoci te with i une estruction,

or pl ques. A v nce -st ge MF inclu es p tients with tu ors

splenic sequestr tion, or bone rrow inf ltr tion by lig-

(3) n erythro er (4) with or without Séz ry syn ro e

n nt cells.

s well s p tients with bloo , no l, bone rrow, or viscer l

T e peripher l bloo (Fig. 22.9) e onstr tes o est

ise se.

ly phocytosis with ture, clu pe nucle r chro tin.

Neopl stic cells o -LGL re ly phs CD2+, 3+, CD4-,

La boratory Fin din gs

CD5+, CD7+, CD8+, CD16+, CD56 +/-, CD57 +/-, n usu—

Séz ry syn ro e is ef ne s erythro er gre ter th n

lly CD56-n CD57+.

80% n leuke ic bloo involve ent gre ter th n 1,000

o circul ting typic l ly phocytes. In peripher l bloo ,

Adult T-Cell Leukemia/ Lymphoma

the ise se is ch r cterize by the presence o bnor—

A ult -cell leuke i /ly pho (A LL) is peripher l

l circul ting ly phocytes, Séz ry cells. A Séz ry cell

-cell neopl s c use by hu n -cell ly photropic

(Figs. 22.7 n 22.8) is typic lly the size o s ll ly pho—

virus-1 (H LV-1). T ere re our subtypes o the ise se:

cyte n h s rk-st ining, clu pe , nucle r chro tin

cute, chronic, ly pho tous, n s ol ering.

p ttern. T e istinctive ol e , groove-like chro tin p ttern is escribe s cerebri or .

M ture ly phocytes in

Ep ide m iolo gy

Sézary is a lymphocyte phenotype with reactivity for CD2, CD3,

ALL is most prevalent in southwestern Japan, central

CD4, and CD5.

African, and the Caribbean basin.

Prognosis is

Etiology

CCs, of which mycosis fungoides and Sézary syndrome

ALL is caused by retrovirus spread by carriers of the virus.

are the most commonly encountered, are currently not

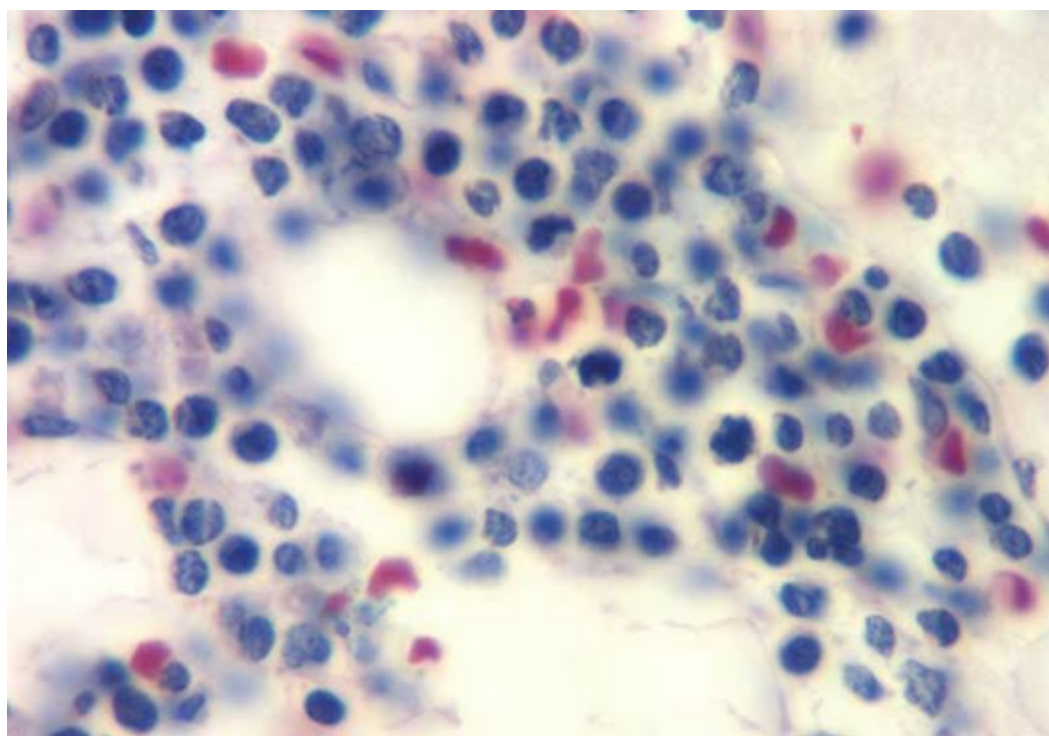
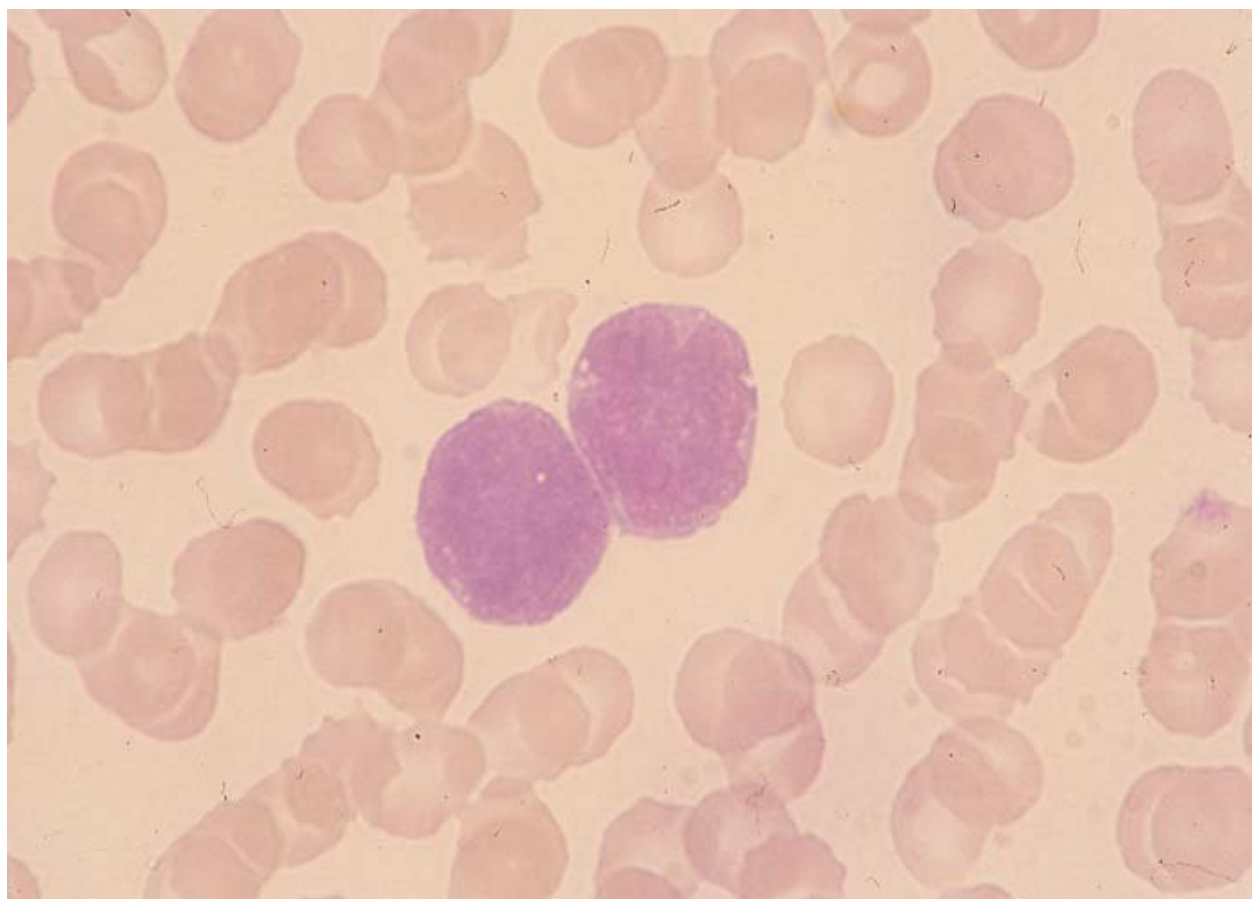
There is less than 5% chance of contracting ALL. The virally

curable in most patients. Skin-directed therapy is extremely

oncogene protein, c-myc, represses cell cycle-activating proteins that

are active in patients with early, skin-limited disease.

results in persistent clonal proliferation of infected cells.



PART 6 ■ Neoplastic Disorders

FIGURE 22.9 Non-Hodgkin lymphoma

B-cell origin is usually B-cell lymphoma.

(From Anderson SC. Anderson's Atlas of Hematology, Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2003.)

Laboratory Characteristics

LYMPHOMAS

Circulating tumor cells in the peripheral blood are not detectable in the presence of the bone marrow. In the Mantle cell lymphoma expresses itself superiorly to the peripheral blood (Fig. 22.10), tumor cells have hyperlobate lymphoid nodes. It is characterized by the infiltration of non-nuclei, sometimes with cloverleaf shape. Neoplastic cells of lymphocytes destruction of the normal architecture are CD4⁺ but often lack CD7. Cells are CD25⁺ and positive to the node. This results in the invasion and destruction of the or FoxP3, phenotype characteristic of regulatory cells. lymphoid capsule and subcapsular sinuses in the infiltrate

tion of the pericapsular tract by large numbers of the cells that

destroy the architecture of the lymph nodes. Eventually,

NOTE: This is a good time to complete Review Questions

this disorder progresses to involve the lymphoid tissues of the

related to the preceding content.

gastrointestinal tract.

Factors Associated with Lymphoid

Neoplasms

Acquired inherited genetic factors can be associated with

the development of lymphoid neoplasms (Box 22.3). Acquired

genetic factors such as alterations of protooncogenes or

tumor suppressor genes have been associated with develop—

ment of lymphoid neoplasms. Additionally, targets of

genetic germline genes that influence apoptosis. An example

is the Bcl-2 gene on chromosome 18 in follicular lymphoma.

Because of decreased apoptosis, lymphocytes accumulate

within lymph nodes. Low-grade follicular lymphomas

to rise to cell persistence not uncontrolled cell proliferation.

Inherited genetic factors associated with inherited immunodeficiency, such as Wiskott-Aldrich syndrome, are associated—

with an increased incidence of malignant lymphoma.

In addition, environmental factors such as Epstein-Barr

FIGURE 22.10 A B-cell leukemia/lymphoma (ALL). This is—

virus (EBV) is directly associated with the onset of Burkitt's

leukemia is characterized by proliferation of lymphocytes

lymphoma prevalent in African boys, so-called endemic

(here, in the bone marrow) with extremely irregular, knobby nuclei

Burkitt's lymphoma, and Hodgkin lymphoma. An inset—

(arrows). The mitotic rate among the lymphoid cells is characteristically high (arrowheads). (Reprinted from Strayer DS, Rubin E.

tious agent associated with NHL is *Helicobacter pylori*. This

Rubin's Pathology, 7th ed., Philadelphia, PA: Lippincott Williams &

is prevalent in the high incidence of gastric lymphoma

Wilkins, 2014, with permission.)

mucosa-associated lymphoid tissue (MALT).

CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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types. Rare B-cell lymphomas include Burkitt's lymphoma

BOX 22.3

lycosis unguis, variant of Sézary syndrome.

The two contemporary lymphoclassification systems are the Revised European-American Lymphoma

Exam ple s of Lympho id Ne oplasm s

(REAL) Cl ssif c tion n the Worl He lth Org niz tion

(WHO) Cl ssif c tion o u ours o the H e topoietic

B-CELL NEOPLAS MS

n Ly phoi issues, ourth e ition. T ere re pproxi—

Precursor B-cell neopl s

tely 30 i erent ly pho bnor lities, inclu ing

■ Precursor B-ly phobl stic leuke i /ly pho (pre—

entities ef ne by co bin tion o orphologic l, i un—

cursor B-cell cute ly phobl stic leuke i)

ophenotypic l, genetic, n clinic l e tures (see bles 22.6

n 22.7). T e i port nce o the v rious e tures i ers

M ture (peripher l) B-cell neopl s s with leuke ic

epen ing on the i erent types o ly pho s.

present tion

T e REAL n WHO cl ssif c tions inclu e Ho gkin is-

■ Chronic ly phocytic leuke i /B-cell SLL

e se n NHL. T e ter Ho gkin ly pho is pre erre

■ B-cell proly phocytic leuke i

bec use o the origin o the Reed-Sternberg cell in l ost ll

■ Hairy cell leukemia

cases of Hodgkin disease and the B lymphocytes. The WHO

■ Plasma cell neoplasms

classification also includes plasma cell neoplasms within the

category of B-cell neoplasms. Separate sections of the WHO

Mature (peripheral) B-cell neoplasms include

classification include immunodeficiency-associated lympho-

■ Diffuse large B-cell lymphoma

proliferative disorders, cutaneous T-cell leukemia, hairy-

■ Burkitt lymphoma/leukemia

plastic syndromes, and chronic lymphoproliferative disorders.

Within the group of NHLs, lymphoblastic neoplasms are

T-AND NK-CELL NEOPLASMS

separate from B-cell neoplasms. Both the

Precursor T-cell neoplasms

and B-cell neoplasms are subclassified into those

■ Precursor T-lymphoblastic leukemia/lymphoma (pre—

thymic)

cursor T-cell acute lymphoblastic leukemia)

■ Pre- or in utero infection or leukemic transformation

Mature (peripheral) -cell neoplasms with leukemic

- Primarily extr-nodal

presentation

- Predominantly lymph node-based entities

- -cell prolymphocytic leukemia

As variants re-emerge in the topography, changes will

- Adult -cell lymphoma/leukemia (H LV 1+)

be incorporated into the WHO classification system. Precise

- Mycosis fungoides/Sézary syndrome

immunologic subclassification is important to influence effective therapy.

HODGKIN LYMPHOMA (HODGKIN DISEASE)

- Nodular lymphocyte predominant Hodgkin lymphoma

Epidemiology

- Classical Hodgkin lymphoma

Lymphomas have been described in all races and ethnic

groups. Lymphoma is the third most common occurrence

Classifications

concern children following leukemia survivors.

Hodgkin lymphoma or Hodgkin's disease, most common

Diagnosis and subclassification of lymphomas have changed

15% of children. Three subtypes of Hodgkin's lymphoma exist in children. Typically, the major subtypes of Hodgkin's lymphoma exist in children. The subtype, Nodular sclerosis Hodgkin's lymphoma, is the most common subtype of Hodgkin's lymphoma in children (NS accounts for 70% of children who are diagnosed with Hodgkin's lymphoma).

TABLE

22.6 Immunohistochemical Features of Selected B-Cell Neoplasms

Surface Membrane

Marker

Type of Neoplasm

SIg

CD5

CD10

CD23

CD43

Chronic lymphocytic leukemia

+

+

—

+

	+
Hairy cell leukemia	

	+
	—
	—
	—
	+

Burkitt lymphoma	
	+
	—
	+
	—
	—

SIg, surface immunoglobulin.

Source: Handin RI, *et al.* (eds.), Blood: Principles and Practice of Hematology, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2003:75.

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PART 6 ■ Neoplastic Disorders

TABLE

22.7 Genetic Features of Selected B-Cell Neoplasms

Type of Neoplas m

Chrom os om al Abnorm ality

Ig Ge ne s

Chronic lymphocytic leukemia

Trisomy 12; 13q

R, U (50%), M (50%)

Hairy cel leukemia

None known

R, M

Burkitt lymphoma

t(8;14)m t(2;8)

R, M

t(8;22); c-myc; EBV +/-

Lymphoplasmacytic lymphoma

t(9;14)

R, M

del 6 (q23)

Diffuse large B-cell lymphoma

t(8;14), 3q

R, M

BCL2, myc, BCL6

Ig, immunoglobulin genes; R, rearranged; M, mutated, U, unmutated; EBV, Epstein-Barr virus.

Source: Handin RI, *et al.* (eds.). Blood: Principles and Practice of Hematology, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2003:75.

lymphoma. A second, more cellular type (MC), is associated with changes in variable-region genes that can be translated into only a few in children younger than 10. The third protein because they preserve the correct recombination. subtype, lymphocyte precursor (LP) is also more common. Cells with out-of-frame V(D)J rearrangements are by population in younger children.

apoptosis. In mature B cells, the expression of an antigen receptor is also essential for cellular survival because of its use

Non-Hodgkin lymphoma (NHL) is more common deletion of the receptor in vivo leads to rapid apoptosis.

in boys than girls. Based on the way that the tumors

Most mature B-cell lymphomas, including Hodgkin lymphoma -

behavior, the subtypes of non-Hodgkin lymphoma

lymphomas, develop as result of malignant transformation of

immature in children in contrast to those that develop

ger in l center or postger in l center B cells. Nor l B-cell
in ults. T e two in subtypes o tu ors th t
velop ent n B-cell tu ors epen on the expression o
ect chil ren re the highly ggressive (high-gr e)
ntigen receptor or surviv l n growth o the B cells, with
tu ors: Burkitt ly pho , non-Burkitt ly pho ,
the exception o Ree -Sternberg cells in cl ssic Ho gkin isn ly phobl stic ly pho
. Although NHL types
e se. T e ger in l center h s centr l role in both nor l
o tu ors re ore co on in ults, they re
B-cell i erenti tion n the genesis o B-cell tu ors. T e
so eti es seen in chil ren. Pe i tric NHL exists s
benef t o the ger in l center re ction is th t the e ense
inter e i te-gr e tu ors: ollicul r l rge-cell ly -
g inst p thogens outweighs the incre se risk o lign nt
pho , i use s ll cle ve cell ly pho , ntle
tr ns or tion o the respon ing cells.
cell ly pho , peripher l cell ly pho , i unobl stic i use l rge cell ly pho , n n pl
stic
Ki-1 l rge-cell ly pho (CD30+).

Laboratory Analys is : Im munophenotyping ,

Genotyping, and Karyotyping

Pathophysiology

Lymphoid neoplasms vary in clinical presentation and laboratory characteristics such as morphologic cell appearance,

Although the etiology of osteolysis is unknown, the

immunophenotypes, and genotypes. Immunophenotype

potential role of virus in the pathogenesis of lymphoma is

analysis uses antibodies of variable specificity to detect cell—

strongly suspected. In humans, the development of B cells

in culture in cell suspensions or in frozen or preserved bone marrow is initiated by the expression of genes or

in bone tissue sections of lymph node or soft lesions

the variable regions of the heavy chain of antibody—

or surgical biopsy. Flow cytometry is the primary tool

used in B-cell progenitors, especially by process called V(D)

use or phenotyping of NHL and leukemia is that these

junctions. In this process, the DNA located between

between one or more segments of normal lymphocyte development

the rearranging gene elements is deleted or the chromosomal bands distinguished or re-

active proliferations of

some (or sometimes inverted). Distinct gene rearrangements

lymphocytes.

equip each B cell with individual molecular markers.

The expression of the B cell antigen receptor on the

Non-Hodgkin Lymphomas

survival of B cells is critically important for the development

of B cells. In development, the cells go through

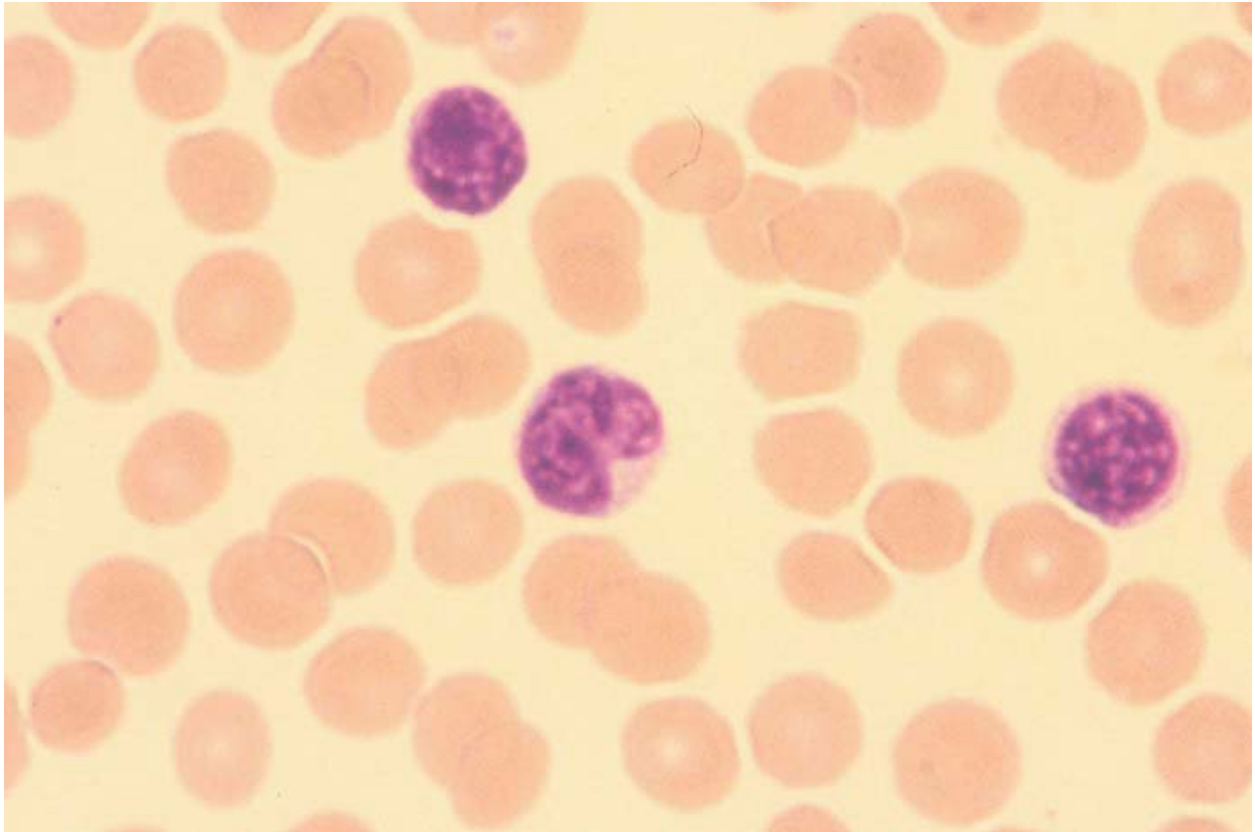
four distinct subtypes of non-Hodgkin

non-Hodgkin lymphoma: V(D)J rearrangements in which the

Lymphoma (NHL) in children include three primary

types of surviving cells: those that have acquired the

primary cells: diffuse large B-cell lymphoma (DLBCL), Burkitt's



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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lymphoma, non-Hodgkin's lymphoma, one sub—

ISFN. Immunophenotyping used to confirm presence of

type classification precursor NHL: lymphoblastic lymphoma.

lymphoma monoclonal lymphoma confirm the subtype

Aggressive, high-grade disease represents the majority of

of lymphoma CD10+. Most FL cases arise because of t(14;18)

NHL cases in children comparable with prevalence of

involving the BCL-2 gene that leads to overexpression of low-n inter e i te-gr e ly pho s in ults. There is BCL-2 protein in ly phocytes. BCL-2 protein inhibits in i—signif c nt overl p between NHL in ults n pe i trics, vi u l cell e th, poptosis, llowing ollicle center cells to but there re unique ch r cteristics in chil ren. LBCL with ccu ul te n pro uce ly ph enop thy.

IRF4 re rr nge ent th t occurs ost co only in chil ren n young ults is new provision entity in 2016 WHO

Progn os is

revision. T is ly pho is consi ere ore ggressive th n

Me i n surviv l is 7 to 9 ye rs, i in olent. Ch r cteristic lly, other pe i tric types o ly pho .

low r te o progression is observe , but it is ore o en ssoci te with prior or synchronous overt ly pho s th t

Leukem ic Phas e o f No n-Ho dgkin

require ition l clinic l ssess ent.

Lym pho mas

Follicula r Lym ph om a

Va ria nts of FL

In situ follicular lymphoma (FL) has been redefined in situ

Peipertic FL is a definite entity in 2016 WHO classification

follicular neoplasia (ISFN) with the same diagnostic criteria

but now classic peipertic-type FL becomes a separate entity in the revised 2016 WHO classification. FC is one of the

subtypes occur in adults. It is not associated with large high

grade of transformation B-cell lymphoma neoplasia

proliferative follicles frequently with prominent blastoid follicles in the United States.

Follicular center cells rather than classic centroblasts (or centrocytes). Bcl2 rearrangements must not be present, but there

Clinical Signs and Symptoms

may be subtle PCL2 protein expression. Peipertic FL also

Generalize lymphadenopathy

Lacks Bcl6 and MYC rearrangements. Nearly all cases

localize and only require excision.

Laboratory Characteristics

GI tract FC is not a variant. Duodenal-type FL has

Most variant subtypes (Fig. 22.11) have bone marrow involvement overt low-grade FL but is distinct from

may involve enteric nodes also have peripheral blood

other GI tract FL has many features that overlap with

involve . In the bone marrow, replacement of the topoietic
ISFN as well as some features resembling extranodal
precursors by bone marrow lymphocytes with abundant
perinuclear zone lymphoid . These patients have an excellent
clear cytoplasm “fried egg” appearance is observable. Cells
outcome.

in the peripheral blood bone marrow usually have very
irregular nuclear outline and deep indentation (cleavage) of the
Mantle Cell Lymphoma
nuclear membrane (butt cells).

In situ MCL is now called in situ mantle cell neoplasia
Flow cytometry demonstrates populations of B cells
(ISMCN). Distinction of MCL from other small lymphoid
with FL phenotype in about half of lymphomas with
B-cell neoplasia is important. This lymphoid subtype has

FIGURE 22.11 Non-Hodgkin lymphomas

of B-cell origin— follicular lymphoid laboratory findings: cell types, bone marrow origin
of

the lymphoma in the follicle, small cleaved

follicle center cell (centrocytes), scant cytoplasm ; large follicle center cell
(centroblasts),

b sophilic cytopl s ; n ollicul r p ttern.

I unophenotype: bcl-2, CD10, CD20,

n sIg positive; CD23 +/-; n CD5 neg -

tive. Fro An erson SC. Anderson's Atlas o

Hematology, Phil elphi , PA: Wolters Kluwer

He lth/Lippincott Willi s & Wilkins, 2003.

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PART 6 ■ Neoplastic Disorders

low r te o progress ch r cterize by the presence o cyclin

T ree types o rgin l zone ly pho h ve been

D1+ cells ost typic lly in the inner ntle zones o ollicles

i entif e :

in ly phoi tissues. P tient i gnosis is o en oun inci en—

1. Nodal marginal zone lymphoma, monocytoid B-cell lym—

t lly n h s low r te o progression.

phoma, occurs within the ly ph no es n ccounts or

Pa thop hys iology

bout two percent o ll B-cell ly pho s.

2. Splenic marginal zone lymphoma occurs ost o en in

MCL cl ssic lly h s been recognize s n ggressive but

the spleen and blood. It has been associated with Hepatitis

incurable small B-cell lymphoma that develops in the

C. This is only lymphomas about 1% of all B-cell

subtypes of B cells. two types of clinically important

lymphomas.

variants that develop along two very different pathways have

3. Extranodal marginal zone lymphoma or mucosa—

been recognized.

associated lymphoid tissue (MALT). This is the most

Classic MCL is usually composed of IGHV-unmutated

common or origin lymphoma that occurs

originally in B cells that usually express SOX11

outside the lymph nodes, such as the stomach, small intestine—

typically involves lymph nodes and other extranodal sites.

stomach, salivary gland, thyroid, eyes, and lungs. MALT lymphoma—

Acquisition of additional molecular/cytogenetic abnormalities—

phosphorylation into aggressive and nonaggressive. This is

the case for even more aggressive blastoid or pleomorphic lymphomas about 9% of all B-cell lymphomas.

plasma cell MCL. A second type of MCL develops from IGHV

unmutated SOX11-B cells that leads to leukemic nonnodal MCL

Risk Factors

that usually involves the peripheral blood, bone marrow, and spleen. These cases are frequently clinically indolent. A sec-

- Most MALT lymphomas arise in mucosal sites evolutionarily benignity, often involving P53, and occur in organically lymphoid structures.

less to very aggressive disease.

- Risk factors have been identified or development of

MALT. These factors are:

Clinical Signs and Symptoms

H. pylori (stomach)

Patients frequently present with symptoms associated with

C. jejuni (intestine)

disease involving multiple lymph node groups, bone marrow, *C. psittaci* (orbit)

marrow, peripheral blood, spleen, liver, and gastrointestinal

Hepatitis C, autoimmune-Sjögren syndrome (salivary

glands)

thyroid.

Autoimmune-Histiotosis's thyroiditis (thyroid)

Laboratory Characteristics

B. burgdorferi (skin)

Medium-sized lymphoid cells with irregular nuclear outlines

Laboratory Characteristics

derive from the follicular mantle zone reobserve. Bone

marrow, peripheral blood, spleen, and gastrointestinal tract

Immunophenotyping of B cells is CD19+, CD20+, and

frequently involve.

be CD43+ but usually not the other antigens expressed by

MCL is characterized by the immunophenotype

small B-cell lymphomas. Surface Ig usually IgM is present.

CD19+, CD20+, CD5+, CD23-, FMC-7+, and sIg (strongly

Chromosomal translocations are t(11;18), t(14;18), and

positive).

t(1;14).

Almost all cases are positive for cyclin D1 by immunohistochemistry. Cyclin D1 is involved in the regulation process

of cells from the G1 to S phase of the cell cycle. Overexpression

NOTE: This is a good time to complete Review Questions

of cyclin D1 in MCL is usually the result of t(11;14), which

related to the preceding content.

involves the Bcl-1 gene. The Bcl-1 translocation is thought

to lead to neoplastic transformation through the loss of cell cycle control.

Lymphoplasma cell lymphoma

This is an uncommon B-cell neoplasm composed of small

prolymphocytes

lymphocytes, plasma cells, and

Median survival is 3 to 4 years.

variable number of large lymphocytes. There can be bone marrow involvement in leukemic phase. Lymphoplasma cell

Marginal Zone B-Cell Lymphoma

lymphomas (LPLs) are associated with high levels of

Marginal zone lymphomas are group of indolent (slow—

IgM paraprotein such as Waldenström macroglobulinemia or

growing) NHL B-cell lymphomas. The occurrence of—

type II cryoglobulinemia.

Marginal zone lymphoma in adults is approximately 6% to

8% of all NHLs in the Western hemisphere. They account

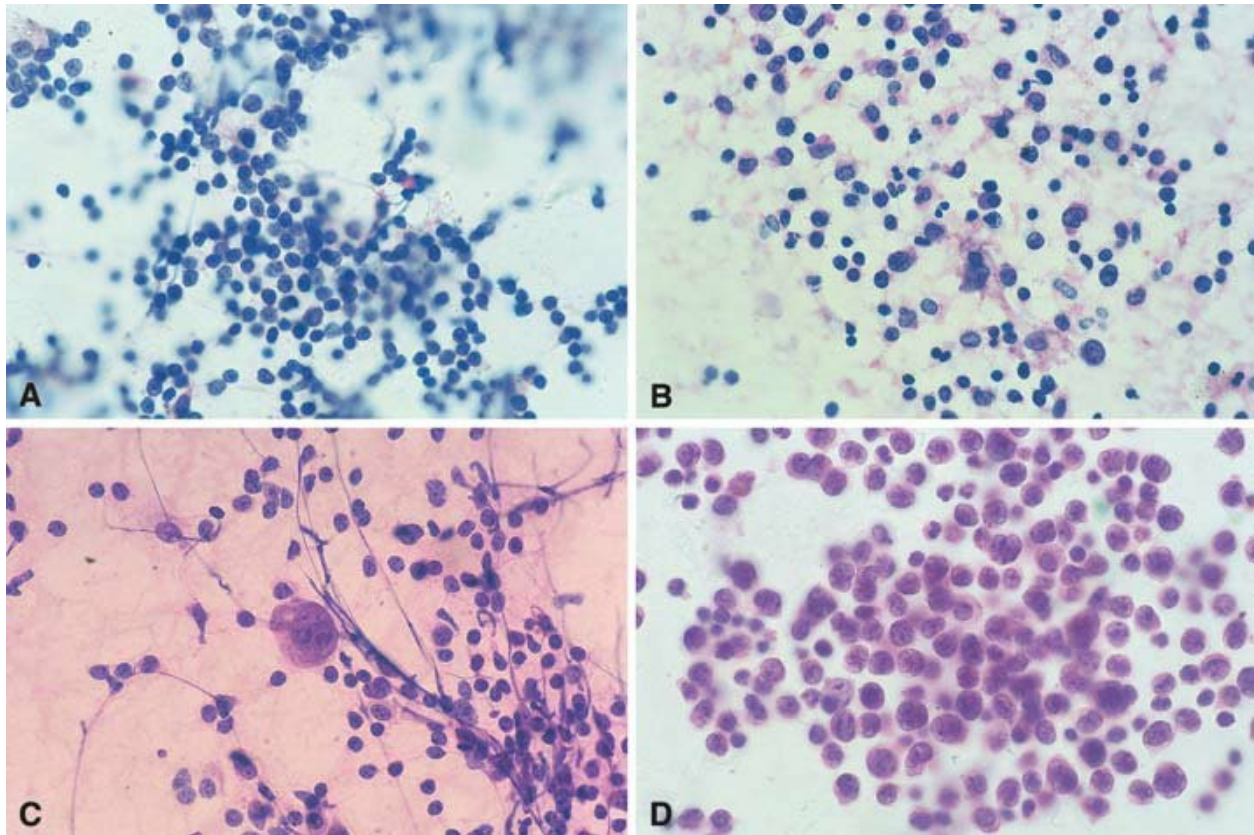
Laboratory Characteristics

or approximately 12% of all B-cell lymphomas. The median

LPLs by characteristics of the lymph node architecture.

Median age at diagnosis is 65 years.

These lymphomas transform into large cell lymphomas.



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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The higher-grade lymphomas usually produce the same Ig

Clinical Signs and Symptoms

Heavy chains and light chains of the original tumors.

Most commonly DLBCL is localized to lymph nodes.

Post-B-cell markers are CD19+ and CD20+ but are usually

CD5- and CD10-.

Laboratory Characteristics

Immunophenotypic markers are CD5+, CD10+, Bcl6, CD30+, Diffuse Large B-Cell Lymphoma, Not otherwise, and CD138+.

Specified (DLBCL, NOS)

Bone marrow involvement is rare presentation but can

DLBCL is heterogeneous group of tumors composed of large occur later in the course of the disease. Neoplastic cells are B-lymphoid cells. Some result from the transformation of significantly larger than normal lymphocytes.

lower-grade lymphoma, such as FL, SLL, or it arises de novo.

Classification

Burkitt's Lymphoma

Three different types of diffuse LBCLs can be defined based

WHO classification combines the cytologic and immunohistochemical—on gene expression subgroups:

like in (ALL-L3 of the FAB classification) with Burkitt's lymphoma. Most but not all ALL-L3 cases appear to be the

1. Germinal center, B-cell-like lymphoma that expresses

leukemic phase of Burkitt's lymphoma. High-grade lymphoma

high levels of genes characteristic of germinal center,
phollicles only has leukemic phase.

B-cell-like lymphoma germinal center B cells

2. Activated B-cell-like lymphoma, which expresses genes

Classification and Epidemiology

characteristic of itogenically activated blood B cells

3. New subgroup, type 3 diffuse LBCL, which has heteroge-

Three types of Burkitt's lymphoma (BL), high-grade NHL

neous gene expression that suggests it includes more than

lymphoma, we recognize: A endemic, sporadic,

one subtype of lymphoma

in immunodeficiency associated. Association with EBV is

100% in A endemic and 20% to 40% in other categories—

Epidemiology

ries. The DNA of EBV is found in most cases of endemic

DLBCL is the most frequent NHL lymphoma in North

one third of HIV-associated tumors.

A endemic European occur in all age groups (Fig. 22.12).

Burkitt's lymphoma represents approximately one third of all

DLBCL accounts for approximately 40% of new cases of

peripheric lymphomas occurring outside Africa. Many ultimately relapse. More than half of patients with disease LBCL reclassified into the immunoproliferative category, such as HIV virus. older than 60 years of age.

EBV+ LBCL of the elderly is now called EBV+ DLBCL. Pathogenesis is

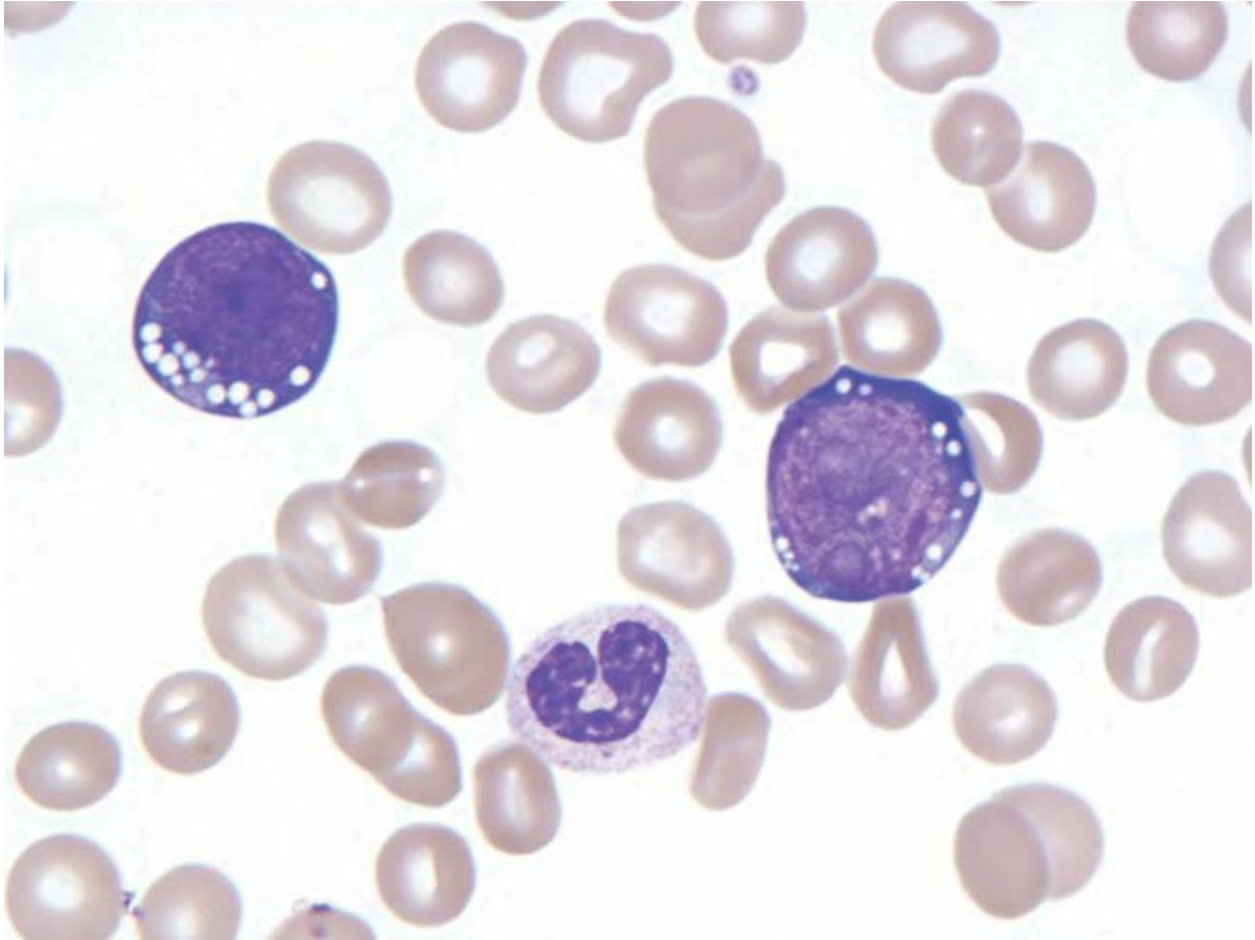
- NOS in the 2016 WHO revision. It occurs in immunodeficient patients usually 50 years of age or older. They have constitutive regulator ID3 occur in about 70% of sporadic cases with a worse prognosis than EBV negative tumors. This subtype is immunodeficiency-related BL in 40% of endemic cases.

Lymphoma is being increasingly recognized in younger patients. CF-3 promotes survival in proliferation in lymphoma patients.

cells by activating the BCR/phosphatidylinositol 3-kinase

FIGURE 22.12 Lymphomas. **A.** NonHodgkin lymphoma. **B.** Reactive arthritis in children. **C.** Mixed large and small lymphocytes in patient with Hodgkin disease. **D.** Large B-cell lymphoma. (Reprinted from Greer JP,

et l. Wintrobe's Clinical Hematology, 11th
e , Philadelphia , PA: Lippincott Williams &
Wilkins, 2004, with permission.)



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PART 6 ■ Neoplastic Disorders

FIGURE 22.13 Burkitt's lymphoma. This classic

case of Burkitt's lymphoma shows intermediate—
size cells that have round nuclei with con—
dense chromatin and multiple nucleoli. The cells

show a greater amount of cytoplasm that is

deeply basophilic with vacuoles. (Reprinted from

Pereira I, George I, Arber DA. Atlas of Peripheral

Blood, Philadelphia, PA: Lippincott Williams &

Wilkins, 2011, with permission.)

signaling pathway resulting in the expression of cyclin

in the center. Reed-Sternberg cells reflecting the

D3, which is also present in 30% of BL.

of Hodgkin disease, but less than 1% of cells in samples of

Hodgkin disease tissue are Reed-Sternberg cells.

Clinical Signs and Symptoms

Immunoglobulin gene rearrangements in Reed-Sternberg

Burkitt's lymphoma/leukemia (Fig. 22.13) can only

cells strongly suggest B-cell origin of Reed-Sternberg cells,

involves CNS, bone marrow, and peripheral blood. The

but it is imaginable that Reed-Sternberg cells could represent

an entity or the disorder classically presents with

transformation of germinal center cells with no

clinical cases in young boys in equatorial Africa.

recombination of their germ line immunoglobulin genes.

Laboratory Characteristics

Etiology

All subtypes are characterized by chromosomal rearrangements

Although the etiology of Hodgkin disease remains a question—

involve the *CMYC* oncogene, which leads to its inappropriate—

ble, it has long been suspected that the cause is an infectious

agent with long latent period.

Diagnosis

Diagnosis is based on histologic preparations and clinical -

Although little is known about the karyotypic pattern of

in the laboratory. Biopsy demonstrates the use of immunohistochemistry -

Hodgkin disease, it is clear that the involvement of specific

types of neoplastic cells with characteristic morphology. This is

chromosomes in numerical and structural abnormalities

describe the morphology of neoplastic lymphoid cells. Aneuploidy, or
evidence of the ploidy

characteristic of pleomorphic cells.

number of chromosomes, resulting from gain or loss

Distinguish Burkitt's lymphoma by the presence of *sIg*

of chromosomes or polyploidy, is characteristic of -

negative. The immunophenotype is CD19+, *sIg*+,

ture of Hodgkin disease. Hyperplasia is observed in the CD10+, and CD5-.

majority of Hodgkin disease tumors that have been

In Burkitt's lymphoma, one of three translocations is usu—

ally observed. A gain of chromosomes 1, 2, 5, 12, and 21 is

recurring numerical abnormality; structural rearrangements

normally located on chromosome 8 on either chromosome

involving chromosome 1 are frequently observed.

14, 2, or 22. These are the sites of the immunoglobulin heavy

chain in [t(8;14)], kappa light chain in [t(2;8)], and lambda light

chain in [t(8;22)] genes, respectively. In these translocations,

Hodgkin disease has a high incidence, with one peak

Myc oncogene is juxtaposed with the DNA sequence of the

occurring in the period 25 to 35 years of age—

immunoglobulin genes, resulting in the unregulated transcription

activity of the Myc gene.

Sixty percent of adults with the disease relate,

as 80% of children who have the disease.

HODGKIN DISEASE

Laboratory Findings

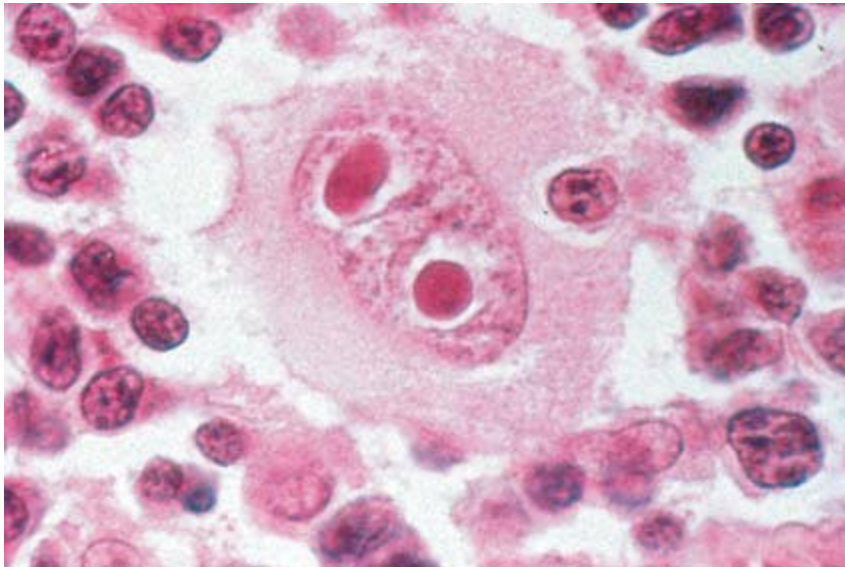
In the early stages of the disease, both the total leukocyte

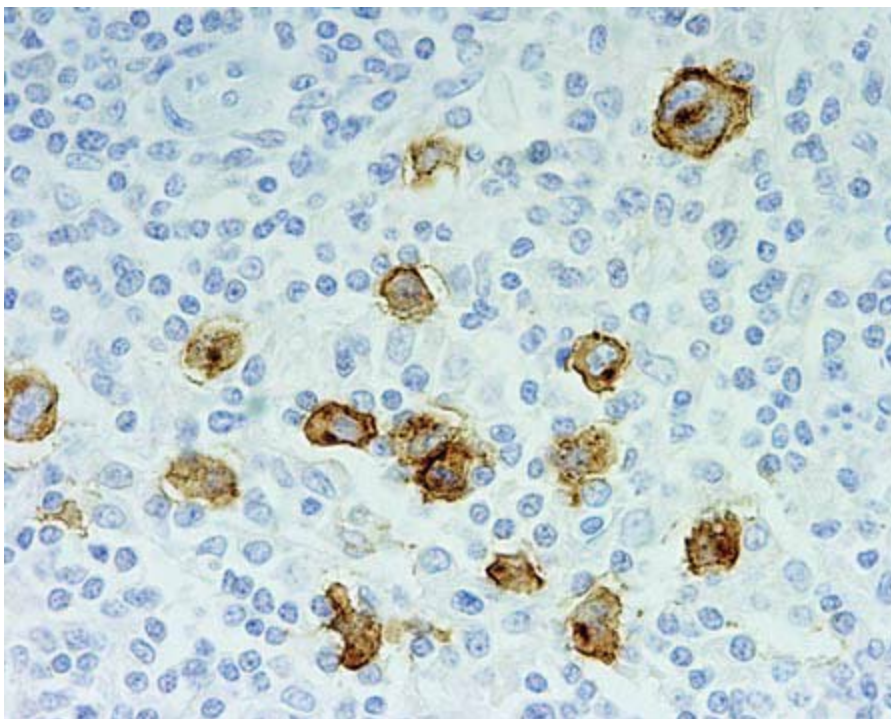
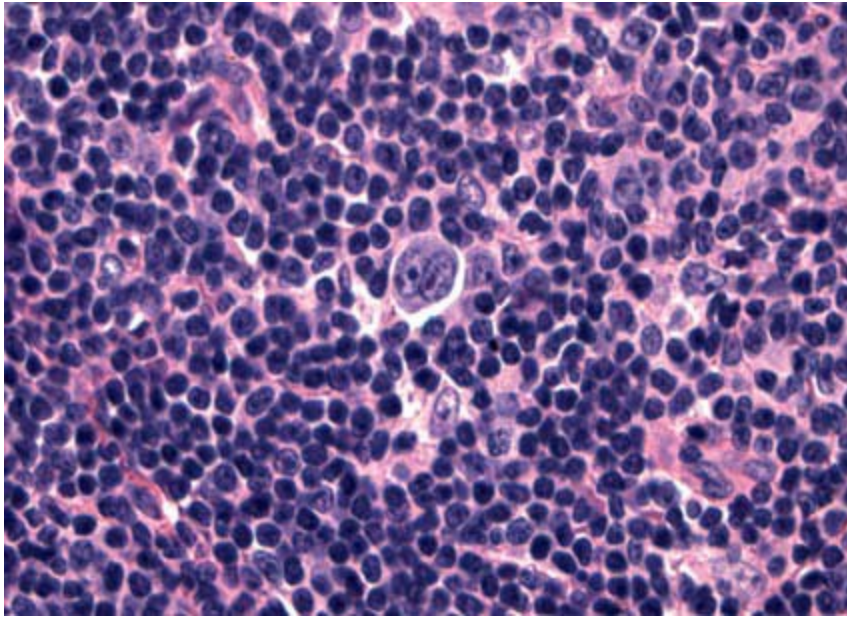
Cytogenetic studies suggest that Reed-Sternberg cells arise

count in the result of differential examination of leukocytes

from single clone, common B-cell precursor located in

from peripheral blood or not. However, in the disease





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FIGURE 22.14 Reed-Sternberg cell typical of Hodgkin's disease.

(Reprinted from Rubin E, Farber JL. Pathology, 3rd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 1999, with permission.)

FIGURE 22.16 Classical Hodgkin lymphoma. CD30-positive
vanes, neutrophils with total leukocyte counts > 15

Ree-Sternberg cells. (Cytologic illustration.) (Reprinted from
to $25 \times 10^9/L$ levels. Neutrophils, varying degrees of

Ortiz A, Fowler K, Knowles D, Weiss LM. Knowles Neoplastic
eosinophilia, monocytosis become predominant on peripheral—

Hematopathology, 3rd ed, Philadelphia, PA: Lippincott Williams &
Berlens as the disease progresses. In the latter stages of

Wilkins, 2013, with permission.)

the disorder, osteopenia develops lymphocytopenia
thrombocytopenia.

IL-2 production, and increase sensitivity to suppressor

Multinucleated giant cells or large mononuclear cell
monocytes and natural killer-suppressor cells.

variants (lymphocyte and histiocyte) are seen in Hodgkin

The cellular origin of the Ree-Sternberg cell is unknown,
disease. These cells account for about 1% of the cells in

but Ree-Sternberg cells have been shown to function as

background of inflammatory cells consisting of small lymphocytic infiltrate, histiocytes, neutrophils, eosinophils, and plasma cells in antigen-induced cell proliferation, and antigen-presenting cells in HLA-DR-restricted, antigen-specific histiocytosis is characterized by the presence of Reed-Sternberg cells.

Reed-Sternberg cells (Fig. 22.14) in the lymph nodes. The nodes in other lymphoid tissue are often infiltrated with lymphocytes, plasma cells, fibrocytes, and eosinophils. Fibrosis and necrosis are frequent findings.

Prognosis is excellent. The 5-year survival rate exceeds 98%.

Histiocytosis (Figs. 22.15 and 22.16) is considered an early stage of histiocytosis, extensive infiltration is distinct clinical entity. Diagnosis is primarily by histology sections of lymph nodes.

Histiocytosis (Figs. 22.15 and 22.16) is considered an early stage of histiocytosis, extensive infiltration is distinct clinical entity. Diagnosis is primarily by histology sections of lymph nodes.

occur in approximately one third of patients. Only, combined

Hodgkin disease is characterized by persistent effect in

combination treatment with abbreviated chemotherapy

the cellular immunity with abnormalities in lymphocytes,

limited irradiation has reduced the relapse rate, but

irradiation therapy increases the risks of second cancer.

Zevalin is the first non-rituximab conjugate (RIC)

to be approved by the U.S. FDA for the treatment of CD20—

positive relapse or refractory, low-grade, follicular, or transformed B-cell NHL, including rituximab-refractory follicular

NHL. The toxicity of this treatment is primarily hematologic.

Approximately 20% of patients require transfusions of platelets and RBCs. Severe neutropenia, thrombocytopenia, and

neuropathy occur in up to 30% of treated patients. Documented

relapses range from 3 to 5+ years following treatment.

The treatment of advanced Hodgkin disease was improved

considerably with the introduction of combination chemotherapy—initially
echlorethine, vincristine, procarbazine, and prednisone (MOPP) and later the ABV
or

doxorubicin, bleomycin, vinblastine, and procarbazine

FIGURE 22.15 Hodgkin lymphocyte-rich “classical” Hodgkin

(ABVD). Patients receiving these therapies have 20% to

ise se. The background is primarily lymphocytes with Reed-Sternberg cells. (Reprinted from Greer JP, et al. Wintrobe's Clinical Hematology, 11th ed., Philadelphia, PA: Lippincott Williams & Wilkins, 2004, with permission.)

relapse but without improvement in survival.

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Prognostic Factors

relevant structural alterations include—

1. Histologic type, tumor size, number of involved lymphatic nodules with respect to structural relevant factors.

nodes in extranodal sites, presence or absence of sys—

Analysis of DNA sequences, location of the chromosomal

translocation, erythrocyte sedimentation rate (ESR),

baseline level of the recurring structural alterations in leu—

lactate dehydrogenase (LDH), albumin, β -2-microglobulin,

key surface markers, has revealed that the genes located

on the leukine phosphatase, hemoglobin, clinical stage, and

t these sites re protooncogenes. As result o the genetic presence or bsence o involve ent o bone rrow, ut tion in uce by the chro oso l re rr nge ent, the inguin l ly ph no es, or both re prognostic in ic tors. unction o the gene is ltere , thereby converting the gene Ch r cteristics o the p tient inclu e ge, gen er, n the to n oncogene.

presence or bsence o ly phocytopeni .

2. Other biologic l e tures re i port nt prognostic ctors

NOTE: This is a good time to complete Review Questions in Ho gkin ise se n inclu e the seru level o soluble related to the preceding content.

CD30 (n ntigen expresse by Ree -Sternberg cells), the nu ber o ctiv te cytotoxic cells, n the presence or bsence o CD15 (n ntigen expresse by Ree -Sternberg cells in so e v ri nt o Ho gkin ise se).

PLASMA CELL DYSCRASIAS

Ger in l center, B-cell–like l rge cell ly pho h s the best prognosis with 60% 5-ye r p tient surviv l r te er Multiple myeloma (MM) is clon l pl s cell neopl s

anthracycline-based chemotherapy. It exhibits two oncogene associations with abnormal protein production. Plasma cell genetic events not seen in the other subgroups: t(14;18) translocation associated with MM relapse:

location of the BCL2 gene amplification of the c-rel locus

■ Solitary MM (SMM)

on chromosome 2p. Patients in the type 3 subgroup have

■ IgM monoclonal gammopathy of undetermined significance

survival rate of 39%; those in the active B-cell-like subcategory (IgM-MGUS) group have the poorest prognosis, with survival rate of 35%.

■ Non-IgM monoclonal gammopathy of undetermined significance (Non-IgM-MGUS)

Treatment

■ Light chain monoclonal gammopathy of undetermined significance

The outcome of treatment or response. Hematologically poor—significance

shows improvement over the past two decades.

■ Solitary plasmacytoma

Cure rates of more than 70% are now possible with hybrid

■ Solitary plasmacytoma with initial relapse involve ent

regimens of chlorambucil, vincristine, procarbazine,

MM evolves to clinically silent prelymphoma stage, prenisone, oxorubicin, bleomycin, and vincristine monoclonal therapy is often significant (MOPP-ABV); regimen of oxorubicin, bleomycin, vincristine, and prednisone (ABVD); or regimen of bleomycin, MM prelymphoma (MGUS) is SMM with much higher risk of progression to MM compared to MGUS. Procarbazine, prenisone (BEACOPP). Relation—Plasma cell leukemia is an increase in number of plasma cells. It has been suggested that beneficial only for patients who have cells in the peripheral blood should be considered. Have achieved partial remission or better. or multiple myeloma not separate entity.

Treatment for patients with diffuse large B-cell lymphoma (DLBCL) is cyclophosphamide, oxorubicin, vincristine, and prednisone.

Epidemiology

(CHOP). Rituximab, chimeric IgG1 monoclonal antibody against the CD20 B-cell antigen, has therapeutic activity. Multiple myeloma accounts for approximately 1% of all

in diffuse LBCL. The addition of rituximab to the CHOP types of lymphoma increases survival about 10% of the biologic response rate and increases the complete response rate and prolongs survival. MGUS is present in more than 3% of the population—event-free survival in elderly patients with immunoglobulin levels above the age of 50 years progresses to diffuse LBCLs comparable with CHOP alone without clinically or biologically significant difference in toxicity.

The onset of this disorder is between the ages of 40 and 70 years, with peak incidence in the seventh decade of life.

Characteristics of Other Forms

Myeloma is not found in children nor in infants who are less than 30 years of age.

In NHL, Reed-Sternberg cells are absent. The infiltrating plasma cell myeloma is more common in elderly patients, usually of one type or have mixed cell populations (1.4:1). It occurs twice as frequently in African American lymphocytes, histiocytes, eosinophils, and so on. African Americans in whites.

pl s cells.

Cyto ge ne tic Ana lys is

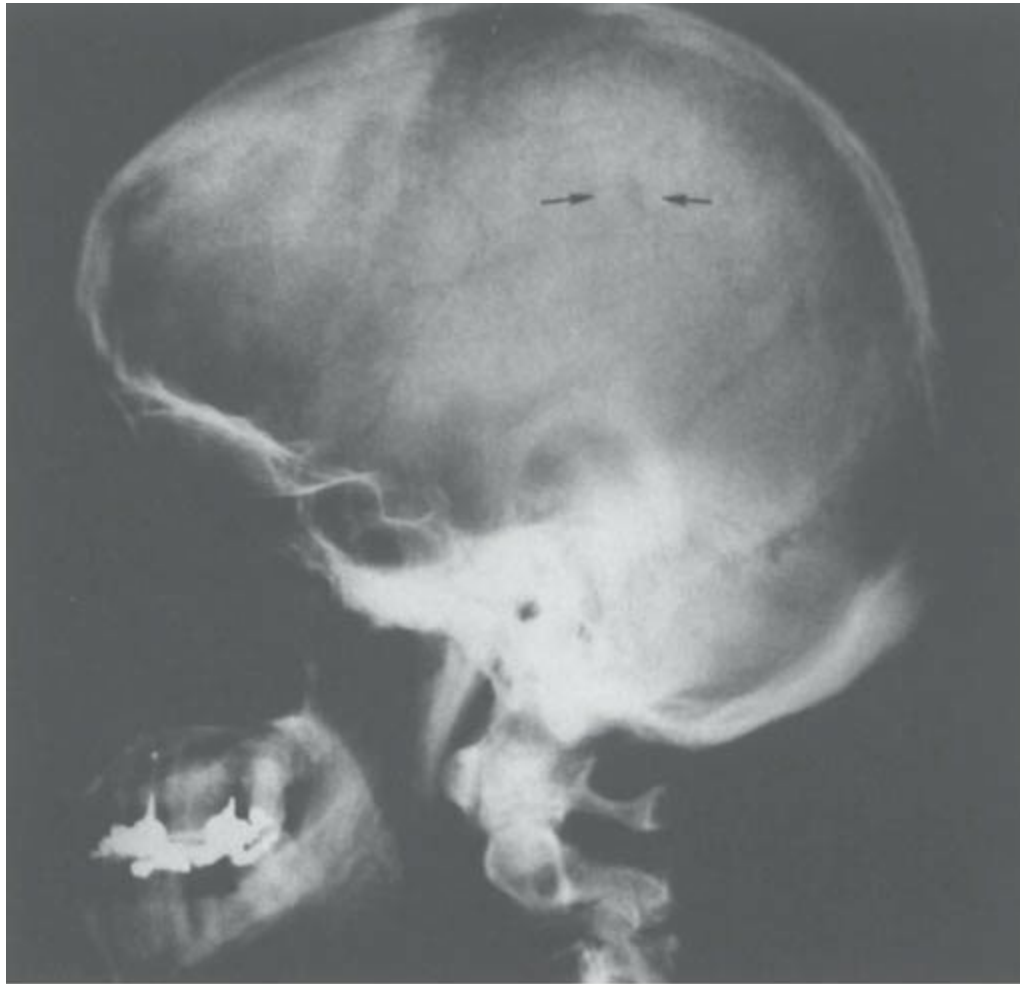
Etiolo gy

T e chro oso l no lies th t h ve been observe

In ultiple yelo , typic lly, the bone rrow is involve ,

in he tologic l lign nt ise se inclu e structur l

but the isor er y involve other tissues s well. T e



A



B

CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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FIGURE 22.17 Lesions in multiple myeloma. **A.** A single osteolytic lesion is evident in this skull radiograph; it exhibits “punch-out” appearance. **B.** Another osteolytic lesion is evident in the tibia.

etiology is unknown; however, relationship between

Several factors contribute to the immunosuppression

in the possibility of viral cause has been suggested. The

system. Multiple myeloma is a compensatory response

likelihood of genetic control in some cases is supported by
in synthesis and increase in concentration of normal Ig—
well-organized reports of 23 identical clusters with ultra-
noglobulins. As the tumor burden increases, the antibody
multiple myeloma.

response becomes more impaired in the degree of humoral

Chromosomal abnormalities are found in the stem cell

immunosuppression increases.

patients with multiple myeloma. Numerous changes in struc—

Complete activity is also efficient in patients with

tumoral abnormalities, including giant chromosomes, transloc-

multiple myeloma. As the disease progresses, granulocyto—

tions, deletions, have been associated with plasma cells.

penicillin develops result of bone marrow failure.

Multiple myeloma cells uniformly overexpress CD38.

treatment with corticosteroids results in transient -cell

sequestration, increased synthesis of immunoglobulins, and

Clinical Signs and Symptoms

decrease in neutrophil count of neutrophils. As

result of cytotoxic chemotherapy, there is variable decrease in

Multiple myeloma is historically defined by the presence of

the numbers and function of cells, B cells, and granulocytes.

an organome, specifically hypercalcemia, renal failure, anemia, and bone lesions. These
recalled the CRAB

Laboratory Data

features that can be attributed to the neoplastic process. In

2014, the International Myeloma Working Group updated

the revised definition of MM and related plasma cell disorders

the diagnostic criteria for MM to specific biomarkers or

is based on laboratory outcomes (Table 22.8). Anemia is present

the diagnosis of patients who do not have the CRAB fea-

ture. The time of diagnosis is approximately two thirds of patients.

tures. The updated definition of MM also includes—

Increased plasma volume caused by monoclonal protein co-

cally results in revision of the diagnostic criteria or the

only processes hypervolemia. The leukocyte count can be

symptomatic phase of MM, SMM.

nor, although about one third of patients have leukopenia.

Symptoms of multiple myeloma include bone pain (typi-

Relatively lymphocytosis is usually present. Sometimes, eosino-

cally in the back or chest) that is present at the time of diagno-

philia is noted. In recurrent cases in the terminal stages, platelet counts are less than two thirds of patients, weakness, fatigue. Platelet cells (Fig. 22.18) amount to 50% of the leukocytes—Weight loss and night sweats are not prominent until the leukocytes in the peripheral blood. Rouleaux formation (discussed elsewhere) is variable. Abnormal bleeding may be prominent in Chapter 7) on peripheral blood smear is common. Features. In some patients, the joint symptoms result from bleeding is common. Platelet abnormalities, impaired coagulation, renal insufficiency, hypercalcemia, or hyperglycemia. Aggregation of platelets, interference with platelet utilization. In addition to the conclusive laboratory findings, the contribution by the abnormal monoclonal protein contribute to including bone marrow examination results, approximately bleeding. Inhibitors of coagulation factors and thrombocytopenia. 90% of patients suffer from bleeding in the structure—topography marrow infiltration of platelet cells or compression of the skeleton (Fig. 22.17). Therapy also contribute to bleeding. Some patients have

International Working Group for the Diagnosis of Multiple Myeloma and Related TABLE

22.8 Plasma Cell Disorders Diagnostic Criteria*

Dis order

Laboratory Diagnos tic Crite ria

Active multiple myeloma

Clonal bone marrow plasma cells $\geq 10\%$ or biopsy-proven bony or extramedullary

plasmacytoma and any one or more of the following (CRAB)* attributed to underlying plasma cell proliferative disorder features and myeloma defining events: 1. Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically: Hypercalcaemia: serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL) Renal insufficiency: creatinine clearance <177 μ mol/L (<2 mg/dL)

Anemia: hemoglobin value of >20 g/L below the lower limit of normal, or a hemoglobin value

Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT

2. Any one or more of the following biomarkers of malignancy:

Clonal bone marrow plasma cell percentage $\geq 60\%$

Involved/uninvolved serum free light chain ratio ≥ 100

>1 focal lesions on MRI studies

Smoldering multiple myeloma

Both criteria must be met:

1. Serum monoclonal protein (IgG or IgA) ≥ 30 g/L or urinary monoclonal protein ≥ 500

mg/24 hours and/or clonal bone marrow plasma cells 10%–60%.

2. Absence of myeloma defining events or amyloidosis

Non-IgM MGUS†

All criteria must be met:

1. Serum monoclonal protein (non-Ig M) <3 g/dL

2. Clonal bone marrow plasma cells $<10\%$

3. Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) or amyloidosis that can be attributed to the plasma cell proliferative disorder IgM MGUS

All criteria must be met:

1. Serum monoclonal protein (non-Ig M) <30 g/L

2. Bone marrow lymphoplasmacytic infiltration $<10\%$

3. No evidence of anemia, constitutional symptoms, hyperviscosity, lymphadenopathy, or hepatosplenomegaly that can be attributed to the plasma cell proliferative disorder Light chain MGUS

All criteria must be met:

1. Abnormal FLC ratio (<0.26 or >1.65)

2. Increased level of the appropriate free light chain (increased κ FLC in patients with ratio

>1.65 and increased λ FLC in patients with ratio <0.26)

3. No immunoglobulin heavy chain expression on immunofluorescence

4. Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) or amyloidosis that can be attributed to the plasma cell proliferative disorder 5. Clonal bone marrow plasma cells <10%

6. Urinary monoclonal protein <500 mg/24 h

Solitary plasmacytoma

All criteria must be met:

1. Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells 2. Normal bone marrow with no evidence of clonal plasma cells 3. Normal skeletal survey and MRI (or CT) of the spine and pelvis (except for the primary solitary lesion)

4. Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) or amyloidosis that can be attributed to the plasma cell proliferative disorder

CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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Dis order

Laboratory Diagnostic Criteria

Solitary plasmacytoma with

All criteria must be met:

minimal marrow involvement‡

1. Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells 2. Clonal bone marrow plasma cells <10%

3. Normal skeletal survey and MRI (or CT) of the spine and pelvis (except for the primary solitary lesion)

4. Absence of end-organ damage, e.g., CRAB, attributable to a lymphoplasma cell

disorder.

POEMS syndrome

Both criteria must be met:

1. Polyneuropathy
2. Monoclonal plasma cell proliferative disorder

Any one of the three other major criteria:

1. sclerotic bone lesions,
2. Castleman's disease,
3. elevated levels of VEGFA

Systemic AL amyloidosis

Any one of the following six minor criteria:

1. Organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy)
2. Extravascular volume overload (edema, pleural effusion, or ascites)
3. Endocrinopathy (adrenal, thyroid, pituitary, gonadal, parathyroid, pancreatic)
4. Skin changes (hyperpigmentation, hypertrichosis, glomeruloid hemangiomas, plethora, acrocyanosis, urticaria, white nails)
5. Papilloedema
6. Thrombocytosis/polycythemia

All criteria must be met:

1. Presence of an amyloid-related systemic syndrome (e.g., renal, liver, heart, gastrointestinal tract, or peripheral nerve involvement) 2. Positive amyloid staining by Congo red in any tissue (e.g., fat aspirate, bone marrow, or organ biopsy)

3. Evidence that amyloid is light-chain-related established by direct examination of the amyloid using mass spectrometry-based proteomic analysis or immunoelectron microscopy 4. Evidence of a monoclonal plasma cell proliferative disorder (serum monoclonal protein, abnormal free light chain ratio, or clonal plasma cells in the bone marrow) Reference: International Myeloma Working Group (IMWG) Criteria for the Diagnosis of Multiple Myeloma, www.imwg.myeloma.org, October 29, 2015, based on Rajkumar SV, Dimopoulos MA, Palumbo A, *et al.* International Myeloma Working Group revised updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol* 15(12):e538–e548, 2014.

* CRAB = hypercalcemia = serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL), renal insufficiency = creatinine clearance <40 mL/min or serum creatinine >177 μ mol/L (>2 mg/dL), anemia = hemoglobin of >2 g/dL below the lower limit of normal, or a hemoglobin value <10 g/dL, bone lesions—one or more osteolytic lesions on skeletal x-ray, CT, or PET-CT.

†Monoclonal gammopathy of undetermined significance

‡Solitary plasmacytoma with 10% or more plasma cells is considered multiple myeloma.

tenancy toward thrombosis, which may be neglected

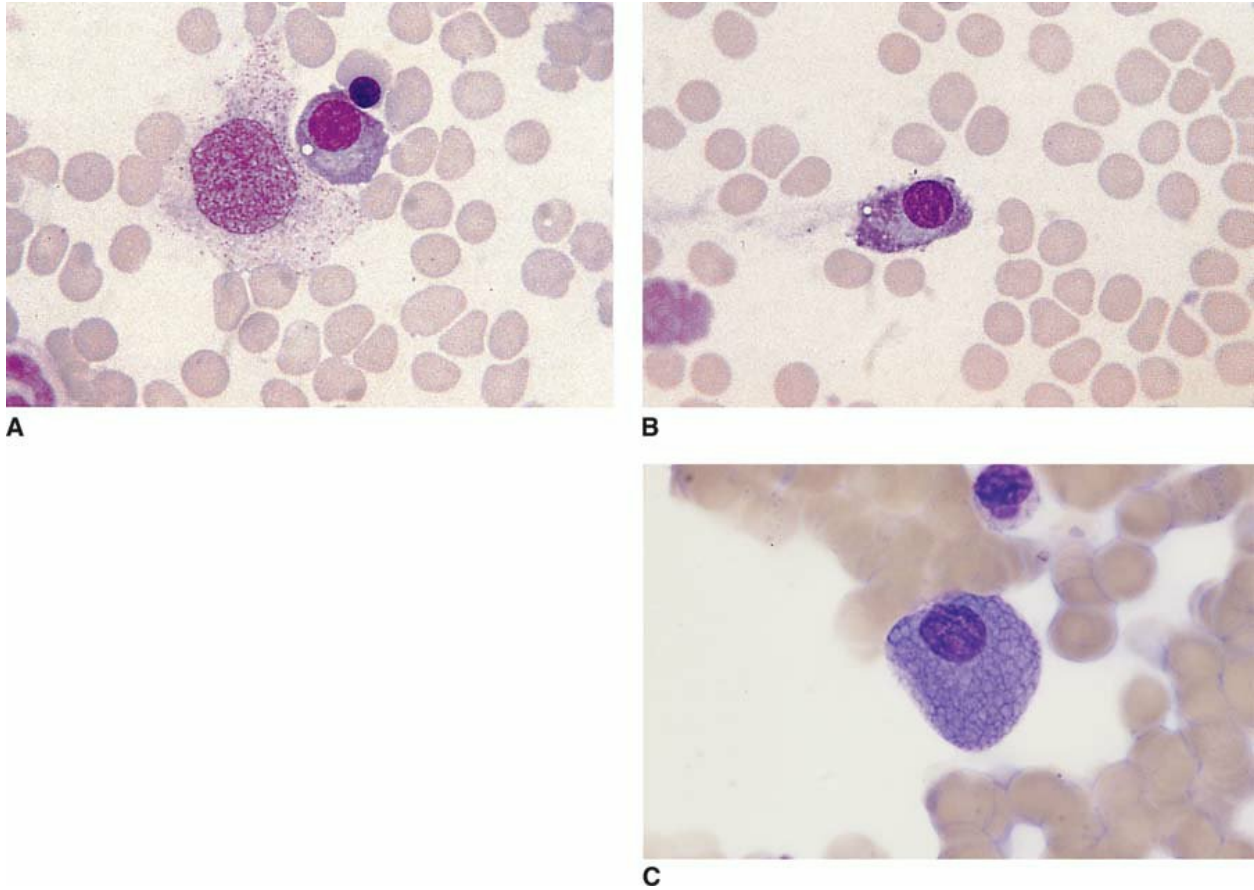
is IgG in the majority of patients. Less frequently IgA is seen,

by shortened coagulation time, increased fibrinogen, and

normal IgD is demonstrated.

increased factor VIII.

Growing clinical acceptance of serum free light chain in
 Electrophoresis of serum usually demonstrates the
 presence of a band but elevated in the urine tests or Bence-Jones pro—
 overproduction of IgM (19S) antibodies. Electrophoresis
 test in the identification of MM. Monoclonal free light chains
 (Figs. 22.19 and 22.20) of the serum or urine reveals that
 can occur either as part of an intact monoclonal immunoglobulin or as free light
 chains; hence localized
 immunoglobulin or a single product. Usually, these free light
 chains are seen in 75% of myelomas. A monoclonal serum
 kappa or lambda chains are bound to the heavy chain in
 protein is detected in 91% of patients. The type of antibody
 of the immunoglobulin. Plasma cells also produce low levels



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PART 6 ■ Neoplastic Disorders

FIGURE 22.18 Various types of plasma cells in

serum or bone marrow. A prominent perinuclear zone of cytoplasmic vacuoles is seen in **A**.

B. The cytoplasm in **C** has a reticular appearance. The

other cells in **A** are nonplasmacytic reticular cells.

late polychromatic erythroblast. (Reprinted from Mills

SE. Histology for Pathologists, 3rd ed., Philadelphia, PA:

Lippincott Williams & Wilkins, 2007, with permission.)

amount of free light chains that were secreted sooner

(kappa) or heavier (lambda).

Nephelometric/turbidimetric quantification of free kappa

and light chains has revealed that up to 20% of MM cases

have free light chain-only disease in which light chains in the

serum and/or urine are the only immunoelectrophoretically detectable

Monoclonal

protein.

Treatment

The role of therapy in both SC-eligible or SC-ineligible

Polyclonal

myeloma patients is to achieve remission, usually

response with minimal toxicity.

Multiple myeloma is incurable with conventional

therapy.

chemotherapy.

Melphalan-based high-dose chemotherapy with hematopoietic

stem cell support increases the rate of complete

ALB α_1 α_2 β

Y

recession and extends event-free survival. Many

FIGURE 22.19 Abnormal serum protein electrophoretic patterns

patients still experience relapse, and options for salvage therapy—

contrast with normal pattern. Polyclonal hyperglobulinemia—

polyrelapse.

line is, characteristic of benign reactive processes, shows broad-

Patients with myeloma who experience relapse after

baseline increase in immunoglobulins, owing to immunoglobulin

high-dose chemotherapy have few therapeutic options.

secretion by reactive plasma cells. Monoclonal gammopathy—

Thrombocytopenia, lenalidomide, and bortezomib are novel agents

therapy of unknown significance (MGUS) or plasma cell neoplasia shows

that first event rate is cyclical in treating relapse and

in relapse, or spike, owing to the homogeneity of the immunoglobulin—

reactive MM. Angiogenesis is important in tumor progression—

ulin molecules secrete by single clone of aberrant plasma cells. ALB,

sion. As in immunoglobulin, thymoma inhibits

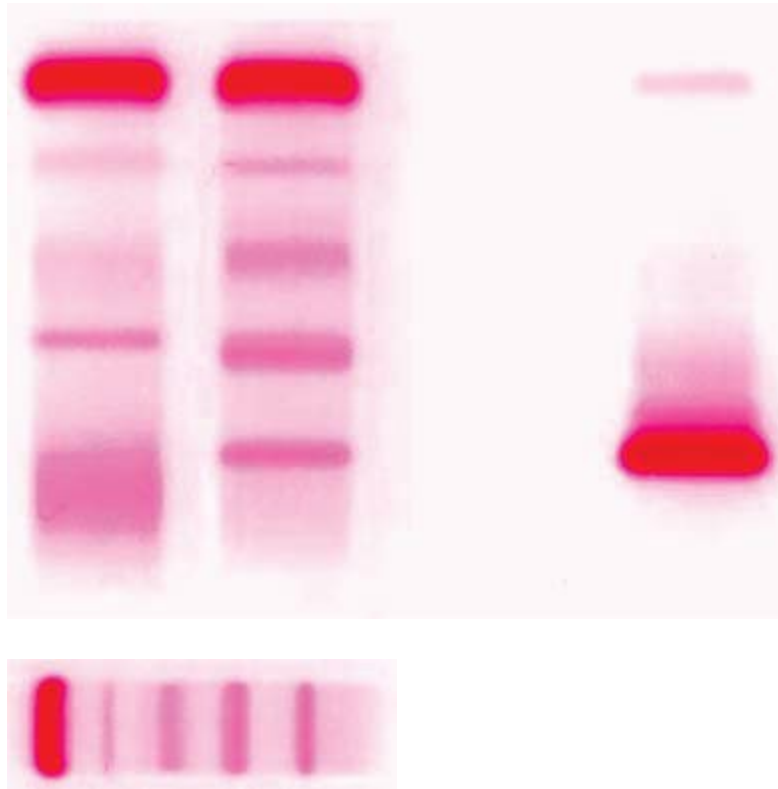
lbumin. (Reprinted from Rubin R, Strayer DS. Rubin's Pathology:

Clinicopathologic Foundations of Medicine, 5th ed, Philadelphia, PA:

angiogenesis and in cases apoptosis of cells in established

Lippincott Williams & Wilkins, 2008, with permission.)

neovascularization.



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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Battery

Normal

Patient

Blank

Pa tie nt

s e rum

s e rum

urine

Albumin

Albumin

Alpha globulins

Monoclonal

Ga mma

(“M-prote in”)

globulins

s pike

Be ta globulins

Monoclona l

Beta

prote in

globulins

(“M-prote in”)

Alpha

Gamma globulins

globulins

Serum initially

Direction of

Urine initially

Patient

placed here

protein migration

placed here

serum

in electrical field

FIGURE 22.20 Protein electrophoresis in polysclerotic. Serum or urine is placed on one end of strip of gel, across which an electrical current is applied. Most proteins have negative charge and migrate toward the positive pole at various speeds according to their molecular weight and charge. The serum on the left shows a narrow band in the γ globulin region. Serum protein has spilled into urine, which demonstrates banding. In the sclerotic patient serum on the right, the height of the banding is proportional to the amount of protein present in each band. Monoclonal protein appears as a single, narrow (monoclonal) "M-protein." (Reprinted from McConnell H. The Nature of Disease Pathology or the Health Professions, Philadelphia, PA: Lippincott Williams & Wilkins, 2007, with permission.)

Emerging Therapies

targeting tumor cells more effectively by killing

resistant to newly diagnosed relapsed disease

of CD38-negative-bearing plasma cells with antibody expression of antibody to improve understanding

dependent cell-mediated cytotoxicity (ADCC) and

immune biology and access to new drugs. When “novel”

apoptosis. Drotinutab is the most active monoclonal

antibodies, including immunomodulatory agents (IMiDs), and proteasome

inhibitors (PIs) in clinical trials.

When the first of these agents entered the field, they were con-

■ Elotuzumab is humanized anti-SLAMF-7 antibody that

specifically binds to combinations with greater efficacy.

It targets the cell surface glycoprotein CS1 on the myeloma

Reseach is now extending to patients who are experiencing—

cell death on NK cells resulting in activation of the NK cells.

improving response. Recent phase 3 drug trials suggest that combination—

It exerts anti-myeloma effect via ADCC in myeloma cell

combinations of new agents with each other or with lenalidomide or

lines in myeloma cells.

therapy produce response with more impact and prolonge

■ Nivolumab is an example of novel drug that is based on

duration of response and overall survival. Various novel

the assumption that programmed death 1 (PD-1) pathway

combination therapies are emerging or improving overall

serves as checkpoint to limit T-cell–mediated immune

responses (see Box 22.4).

PD-1–blocking antibodies have been used to

enhance immunity in solid tumors and obtain durable

clinical responses with acceptable safety profile. PD-1

IMMUNE APPROACHES

emerging represent one of the ways by which tumors

evade immunosurveillance.

A newer treatment compared to another pathway is intro-

■ Adoptive cell transfer therapy consists of chimeric anti-

body in 2000. This strategy incorporates various immune

cell receptor (CAR) cells or regulatory T-cell

approaches:

interventions.

Antibodies

Chimeric antigen receptor (CAR) cells represent

process of engineering autologous cells with CARs against

■ Durable is humanized anti-CD38 antibody. It

CD19 therapeutic strategy or evaluated by combining

cells through multiple mechanisms of action by directly

autologous cells with anti-CD CAR.

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PART 6 ■ Neoplastic Disorders

BOX 22.4

Emerging Therapies: Novel Combination Therapies in Relapsed Patients

IMMUNOMODULATORY AGENTS (IMiDs)

of histone deacetylase inhibition while initiating

1. Lenalidomide is second-generation IMiD with greater toxicity.

potency and less toxicity than thalidomide.

2. Pomalidomide is third-generation IMiD that shows

OTHER NEW TARGETS

number of beneficial pharmacologic properties with

1. KSP Inhibitors

lenalidomide.

ARRY-520 inhibits the spindle orientation mitosis

that results in cellular apoptosis.

PROTEASOME INHIBITORS (PIs)

2. AK Inhibitors

1. Bortezomib is the first to have single-agent activity in

Auresertib is selective oral AK kinase inhibitor with
combines well with many other agents.

antitumor activity in solid tumors.

2. Crizotinib is second-generation epoxypyrazole, which

3. Selective Inhibitor of Nuclear Export

induces irreversible proteasome inhibition and combines

Selinexor, also known as KP-330, is new oral novel
well with other agents.

agent. It is the first drug in new class of agents known

as Selective Inhibitor of Nuclear Export (SINE™) co-

HISTONE DEACETYLASE INHIBITORS (HDAC)

possesses. Selinexor works by inhibiting XPO1, protein

1. Vorinostat is histone deacetylase inhibitor that can be

found in the nucleus of cancer cells, which activates

combines with bortezomib.

tumor suppressors by retaining them in the nucleus of

2. Promitin is the first FDA-approved HDACi for

cancer cells. This results in apoptosis of cancer cells,

especially patients who have received at least two prior

while largely sparing normal cells. Selinexor also reduces

st n r ther pies, inclu ing bortezo ib n n i uthe levels o oncoproteins such s c-yc n bcl-2, th t

no o ul tory gents.

re uces the growth o c ncer cells.

3. Rocilinost t (ACY-1215) is selective HDAC-6 inhibitor

Re erence: Loni S, Nook AK. Novel co bin tion ppro ches or

evelope in n tte pt to preserve the clinic l e c cy

yelo , Hematology Am Soc Hematol Educ Program, 2015, 286–292.

Regul tory cell (regs) re CD4+ cells th t strongly

WALDENSTRÖM PRIMARY

inhibit ntitu or i une responses in yelo p tients.

MACROGLOBULINEMIA

(LYMPHOPLASMACYTIC LYMPHOMA)

The rape utic Vaccinatio ns

Epidem io logy

Den ritic cell (DC) v ccines re APCs th t c n sti ul te

vigorous -cell i une responses. Novel ppro ches o

T e con ition o WM h s n ge-specif c inci ence. It is

co bining DC v ccine n PD-1 ntibo y or post utolo—

ost co only oun in ol er en; the e i n ge o

gous tr nspl nt re un ergoing clinic l tri ls.

onset varies between 63 and 68 years of age. Onset is usu—

Peptide vaccines are represented by PVX-410, which

lly insidious. The incidence of WM is higher among whites.

is composed of tetrapeptide from three unique regions

Ninety percent of patients with WM have MYD88 L265P

of yellow-associated antigen XBP, CD138, and CS1.

tutions.

The goal of this vaccine is to induce immunity against

yellow cells by selectively stimulating tumor-associated

Pathophysiology

antigen-specific cytotoxic lymphocytes (PVX-410

alone in combination with lenalidomide) that re

WM is B-cell neoplasia characterized by lymphoplasma cells evolving from the treatment of solitary plasmacytoma.

proliferative disorder with infiltration of the bone marrow

monoclonal immunoglobulin M (IgM) protein. This is

Prognosis

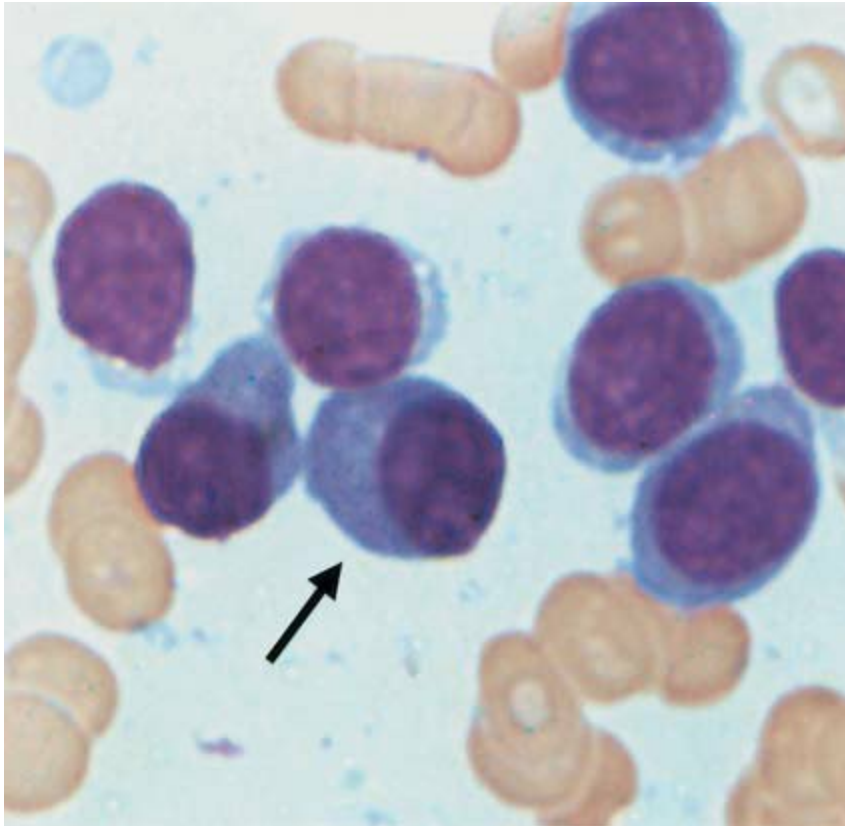
lymphoplasma cell proliferative disorder is

associated with the production of abnormally large amounts

This disorder runs a progressive course, and most patients

of globulin of the IgG or IgM type. The basic bone—

ie in 1 to 3 ye rs. T e jor c uses o e th re in ection
lity in this croglobuline i is uncontrolle proli er -
n ren l insu ciency.
tion o ly phocyte n pl s cells.



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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Clinical Signs and Symptom s

Ch r cteristic lly, bloo s ples re ecribe s h ving hyperviscosity. Detecting
onoclon l g op thies

The symptoms of WM relate to the extent of tumor infiltration—usually involves serum protein electrophoresis (SPEP) and determination of elevated IgM levels in the blood circulation. Immunoelectrophoresis (IEP) to test both serum and urine. Symptoms include weakness, fatigue attributable to anemia, Additionally, cryoglobulins can be detected in the patient's circulation. Bone pain is virtually nonexistent. About one-third of patients with WM have neurologic abnormalities. when cooled to 0°C and dissolve when heated. In most cases, The incidence of infection is twice the normal rate. Patients with monoclonal cryoglobulins are IgM or IgG. usually suffer from chronic anemia and bleeding episodes. Thrombocytopenia and hyperviscosity also contribute

Treatment and Prognosis

to the bleeding disorder.

Treatment is postponed for symptomatic patients, and pro-

Laboratory Data

gressive anemia is the most common indication for initiation of treatment. Therapeutic options include plasmapheresis

The most consistent feature of the bone marrow lymphogests, nucleoside analogues, and rituximab. Novel agents, none of whom patients is the presence of pleomorphic or, for example, vertebral, show promise as targeted therapy. B-lineage cells that are different stages of maturation, such as small noncleaving cells, are common in WM.

Lymphocytes, lymphoplasmacytic cells (but not B cells)—Prognostic factors include the patient's age, β_2 -microglobulin level, monoclonal protein level, hemoglobin level, and platelet count. The reported median survival of patients with WM ranges between 5 and 10 years. The lymphocyte-plasma cells vary morphologically from the time of diagnosis.

Typically, ranging from small lymphocytes to obvious plasma cells (Fig. 22.21). Their cytoplasm is frequently irregularly and peripherally (PAS)-positive. The

cells (Fig. 22.21). Their cytoplasm is frequently irregularly and peripherally (PAS)-positive. The

NOTE: This is a good time to complete end of the chapter

that is probably identical to the circulating cryoglobulin.

Review Questions.

The total leukocyte count is usually normal, with no absolute lymphocytosis. More moderate to severe degrees of neutrophilia

are frequently observed on peripheral blood smears as well

CHAPTER HIGHLIGHTS

secondary. The patient's platelets may be
greatly increased, and the ESR is increased. Platelet counts

Leukemias and Lymphomas

are usually normal. Bleeding caused by abnormalities in
platelet hesitancy and prothrombin time may be seen,

■ The tertiary prothrombotic disorder includes the variation of factor VIII may be
low.

secondary leukemia is a lymphoid transformation -
phoretic origin.

■ Lymphoid neoplastic conditions are based on the distribution of disease in
lymphoid. During the

progression of disease in solid lymphoid, the malignant
cells may spill into the blood circulation. This spillover
may produce leukemic phase of the disease.

■ Transitions to leukemic phase are rare in disorders such

as Hodgkin disease but are not uncommon in the well-differentiated non-Hodgkin
lymphoid lymphoid.

Chronic Lymphocytosis

■ The WHO Classification of our hematopoietic

in lymphoid diseases enhances the classification of

lymphoid neoplasms by including immunophenotypic features and genetic abnormalities to differentiate disorders.

■ Examples of disorders of the mature B-cell neoplasms classification include CLL/SLL, B-PLL, hairy cell leukemia.

FIGURE 22.21 Wälenström. Bone marrow aspirate showing

Chronic Lymphocytic Leukemia/Small

lymphoid cells with lymphoid cytomorphology.

Lymphocytic Lymphoma

(Reprinted from Greer JP, et al. Wintrobe's Clinical Hematology, 11th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2004,

■ Chronic leukemia is generally characterized by the presence of leukocytosis with an increase in the number of

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PART 6 ■ Neoplastic Disorders

lymphocytes, lymphocytosis, on peripheral

■ "Low count" MBL has significant differences from CLL blood.

with extremely low, if any, chance of progression. "High

■ Malignantly proliferative disorders are characterized by an accumulation of lymphocytes.

not typical epigenetic / molecular features of Rai stage 0.

■ CLL and SLL represent neoplasms composed of small B lymphocytes in the peripheral blood, bone marrow, spleen, and

B-Cell Prolymphocytic Leukemia

lymph nodes, along with prolymphocytes and preprolymphocytes or proliferating proliferation centers in tissue infiltrates.

■ B-PLL represents malignancy of B prolymphocytes

In contrast to CLL, SLL is the term used for nonleukemic infiltrating blood, bone marrow, and spleen.

patients with the tissue morphology and immunopheno-

■ Prolymphocytic leukemia is characterized by large number type of CLL.

of small lymphocytes with scant cytoplasm and the features of prolymphocytes in the peripheral blood.

■ CLL is the most common form of leukemia in adults in

Western countries, but it is very rare in Eastern countries. CLL and SLL account for about 7% of NHLs in

Hairy Cell Leukemia

biopsies.

- HCL is a non-chronic lymphoproliferative disorder.

- The median age at onset is 65 years.

of the B-lymphocyte type.

- More than 95% (1.5 to 2.1:1) are affected by the

- The typical B-cell lymphoma is diagnosed based on clinical—
disorder.

clinical features, morphology, and phenotyping and generally

- CLL has the highest genetic predisposition of all hematologic malignancies with curative intent.

tologic neoplasms. A family predisposition can be observed

- HCL is more common in females and usually affects patients
between 5% to 10% of patients with CLL.

older than 30 years of age.

- Classic CLL is usually B-cell disorder. It is characterized
by gradually progressive accumulation of morphologi-

T-Cell and NK-Cell Neoplasms

usually involve B lymphocytes in the blood, bone marrow,
and lymphatic tissues.

- T-PLLs are associated with poor prognosis.

■ More than 90% of CLL cells are non-dividing and rest

■ Distinction of CLL diagnosis requires more than 55%

of

circulating lymphoid cells to have the morphology of

prolymphocytes in the cell cycle.

■ Molecular genetics detection of genomic rearrangements in prolymphocytes.

They not only assist with the diagnosis but can also provide

■ Prolymphocytic leukemia is characterized by large number

of important prognostic information.

of small lymphocytes with scant cytoplasm in the

■ Alterations in CLL affect expression of apoptosis, selection

of features of prolymphocytes in the peripheral

blood. Cells of -PLL often have prominent nucleolus

in the nucleus.

in the nucleus.

of intermediate size with convoluted nuclear outlines.

■ CLL has variable clinical course. Clinical staging systems

-PLL in peripheral blood exhibits small to intermediate

in assessing prognosis in CLL were developed in the early

round or irregular nuclei resembling Sézary cells.

1980s, based on easily obtainable biologic and clinical

■ Sézary syndrome is the leukemic phase of CLL, with lymphocytosis and pruritus.

features.

■ Newer treatments with other drugs were introduced

■ In peripheral blood, the disease is characterized by the presence of abnormal circulating lymphocytes, Sézary lymphocytes, to the other drugs. This strategy appears to promote cells. A Sézary cell is typically the size of small lymphocytes or first-time-observe survival benefit but is not cytotoxic staining, clumped, nuclear chromatin considered curative.

pattern. The distinctive oval, groove-like chromatin pattern

■ In the future, patient-specific therapeutic drugs by better describe the cerebro.

design for CLL patients harboring abnormalities in

■ T-cell large granular lymphocytic leukemia (LGL) has miRNA expression in their malignant cells.

presentation that is similar to CLL but is composed of

- Myeloblastic high-grade chemotherapy, with subsequent mature cells.

autologous or allogeneic SC, is the most intensive or

- It is important to distinguish reactive lymphocytosis from another type of CLL.

by the presence of abnormal phenotypes or evidence of clonality.

Mono clonal B-Cell Lymphocytosis

- In addition, LGL must be distinguished from indolent chronic lymphoproliferation disorders of NK cells and

- MLD is now known to precede virtually all cases of aggressive NK-cell leukemia.

CLL/SLL. Although the newly updated World Health

- ALL is peripheral-cell neoplasia caused by human T-cell

Organization (WHO) will retain the current criteria for

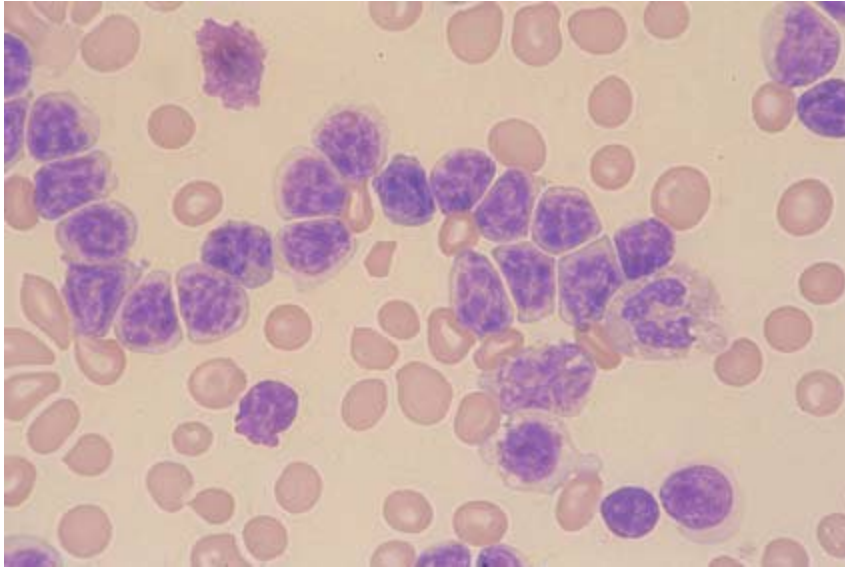
lymphotropic virus-1 (HTLV-1). There are four subtypes of

MLD, it does emphasize that the “low count” MLD peripheral—
the disease: acute, chronic, lymphoid, and smoldering.

peripheral blood leukocyte count of less than $0.5 \times 10^9/L$ must be

■ In the peripheral blood, tumor cells have hyperlobate nuclei, so called "high count" MBL.

nuclei, so called "cloverleaf" shape.



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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Lymphomas

■ Hodgkin lymphoma is characterized by the presence of Reed-Sternberg cells.

■ Lymphomas are closely related to leukemias. Initially, lymphomas are confined to the lymph nodes, but they

Plasma Cell Dyscrasias

spillover into the blood in the leukemic phase.

■ The REAL and WHO classifications include Hodgkin

■ Multiple myeloma is a distinct plasma cell disease, typi—

cally seen in NHL. The presence or absence of Reed-

Sternberg cells is critical in establishing diagnosis

■ Plasma cell leukemia is considered to be a form of—

Hodgkin.

Multiple myeloma, not separate entity; however, increase

■ Leukemic phase of NHLs can be seen in small B-cell lympho-

neutrophils of plasma cells remain in the peripheral blood

phases such as “in situ” follicular lymphoma that has

rather than in the bone marrow.

been renamed “in situ” follicular neoplasia (ISFN), MCL,

■ The outstanding laboratory characteristics include the

regional zone B-cell lymphoma with plasmacytic

presence of Bence-Jones protein in the urine and

lymphoma (LPL).

abnormal serum /or urinary electrophoretic pattern.

■ Other NHL lymphomas include diffuse large B-cell, not other-

■ Plasma cell disorders related to MM resembling MM,

wise specific (DLBCL, NOS). In addition, EBV+ LBCLs

IgM monoclonal gammopathy of undetermined significance

of the elderly is now called EBV+ DLBCL (NOS).

ence (IgM-MGUS), non-IgM monoclonal gammopathy

■ Burkitt's lymphoma in the WHO classification combines

of undetermined significance (Non-IgM-MGUS), light

chain B-cell lymphoblastic leukemia (ALL-B2 of the FAB

classification) with Burkitt's lymphoma. Most but not all

cases, solitary plasmacytoma, non-solitary plasmacytoma

ALL-B2 cases appear to be the leukemic phase of Burkitt's

with initial relapse involvement.

lymphoma. High-grade lymphomas less commonly have

lymphoma. High-grade lymphomas less commonly have

■ WM is a clonal lymphocyte-plasma cell proliferative

leukemic phase.

associated with markedly large amounts of the g

■ Three types of Burkitt's lymphoma (BL), high-grade

globulin type (IgG or IgM).

NHL lymphoma, recognize: Aromatic (endemic), spo-

■ Abnormal serum electrophoresis patterns in the pres—

ritic, in addition to efficiency associated.

ence of cryoglobulin re characteristic.

CASE STUDIES

Case Study 22.1

A 58-year-old female with a long history of rheumatoid arthritis was admitted to the hospital for elective surgery. Although the

patient has been complaining of generalized fatigue,

she suspected that it was work-related problem rather than

physical problem. Physical examination revealed that she

had both cervical and supraclavicular lymphadenopathy.

■ Laboratory Data

Her preoperative blood count revealed that her erythrocytes

and hemoglobin were within normal ranges; however, her

total leukocyte count was $26.5 \times 10^9/L$. The distribution of

(Reprinted from Anderson S, Poulsen K. Anderson's

leukocytes was as follows:

Atlas of Hematology, Philadelphia, PA: Lippincott

Williams & Wilkins, 2003, with permission.)

Segmented neutrophils 18%

Lymphocytes 75%

■ Critical Thinking Group Discussion Questions

Monocytes 1%

1. What could be the possible explanation for the leukocytosis—

So even if the lymphocytes are of the same type, cells were

toxicosis concurrent with phagocytosis?

present. The distribution of platelets was normal.

Follow-up laboratory tests include infectious mononucleosis—

2. What further testing could be done to establish

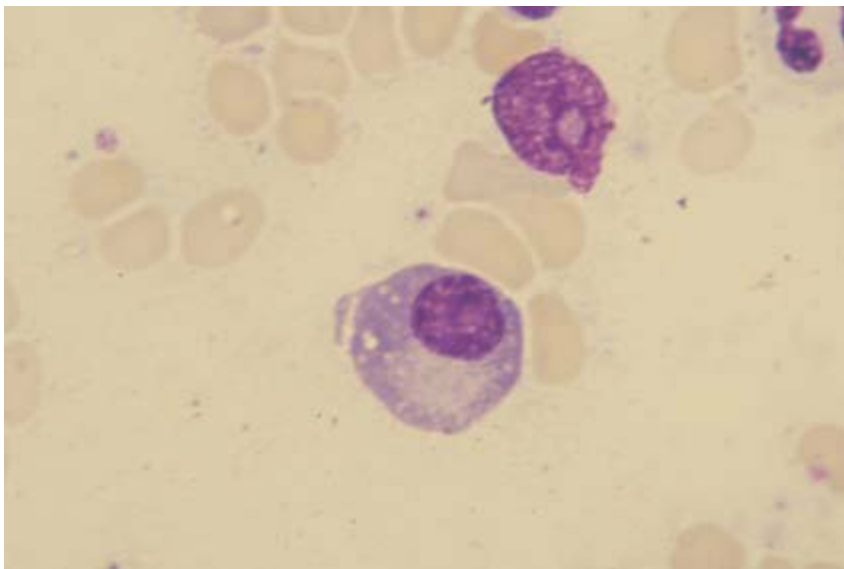
leukocytosis screen, with negative results. Bone marrow examination -

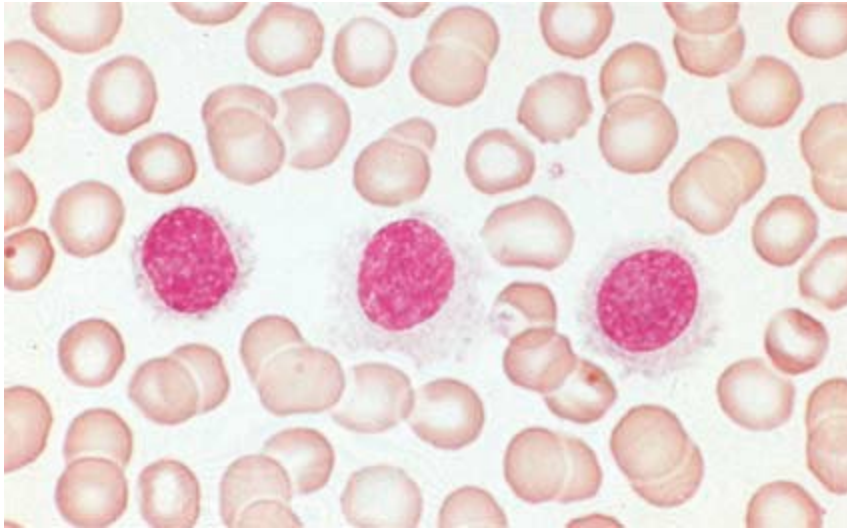
diagnosis?

Examination revealed lymphocytic infiltration of approximately 50%

of the cells in the marrow.

3. What is the patient's prognosis?





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PART 6 ■ Neoplastic Disorders

CASE STUDIES (continued)

Cas e Study 22.2

■ Laboratory Data

Hemoglobin 6.0 g/L

A 58-year-old male college professor with a history of physical—

Hematocrit 20%

clinical use of increasing fatigue and weakness. He also

total leukocyte count $4.1 \times 10^9/L$

reported pain in his lower back and legs when he walked.

Physical examination revealed that the patient had

■ Leukocyte Differential

ucous e br nes n hep tospleno eg ly. T e physi—

Pl s cells 25%

ci n or ere CBC n urin lysis. A ollow-up ppoint—

Seg ente neutrophils 26%

ent w s sche ule or the ollowing week.

I ture gr nulocytes 13%

Ly phocytes (v ri nt or s note) 36%

■ Laboratory Data

■ Other Test Results

T e CBC reve le th t the p tient h ne i . His leu—

Urin lysis positive or bloo n positive or protein

kocyte count n i erenti l count were nor l, except

Bloo culture neg tive

or roule ux (rolle coin) ppe r nce o the RBCs. T e

I unoelectrophoresis ecre se IgG

result o urin lysis w s nor l. T e p tient w s c lle ,

Co ple ent ss y ecre se

n it w s requeste th t he returns to the l bor tory or

ition l tests. T e physici n or ere the ollowing tests:

■ Critical Thinking Group Discussion Questions

ESR, kidney screening profile, liver blood profile, and

1. Why does this patient have immunocompetency?

radiographic skeletal survey, with the following results:

ESR, 50 /h; normal kidney profile; normal liver

2. Why does the patient have granulocytopenia?

profile except for increased globulin protein. The skeletal survey indicates bone lesions in various sites.

3. Could this patient have neoplasia?

Case Study 22.4

A 75-year-old woman was referred to multispecialty group practice for evaluation of chronic lymphoproliferative disorder.

■ Laboratory Data

Hemoglobin 9.0 g/L

Hematocrit 32%

RBC count $2.9 \times 10^{12}/L$

total leukocyte count $7.5 \times 10^9/L$

total platelet count $150 \times 10^{12}/L$

The peripheral blood smear reveals lymphocytic-looking

(Reprinted from Anderson S, Poulsen K. Anderson's

leukocytes with cytoplasmic projections, prominent nucle—

Atlas of Hematology, Philadelphia, PA: Lippincott

oligo, not over 11 smaller cell size.

Williams & Wilkins, 2003, with permission.)

■ Critical Thinking Group Discussion Questions

1. What follow-up laboratory tests might be ordered to assist in establishing definitive diagnosis?

2. What type of leukocyte disorder could be present?

3. What is the nature of the protein found in the urine?

4. What is the most significant laboratory finding in this disorder?

Case Study 22.3

(Reprinted from Rubin R, Strayer DS. Rubin's

A 70-year-old woman with diagnosis of rectocol—

Pathology: Clinicopathologic Foundations of

multiple myeloma with onset to the hospital

Medicine, 5th ed., Philadelphia, PA: Lippincott

because of bleeding caused by pancytopenia.

Williams & Wilkins, 2008, with permission.)



CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

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CA S

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■ Follow-Up

2. From what type of morbidity is the patient suffering?

RAP still in worse negative.

3. What other laboratory assays would be interesting

■ Critical Thinking Group Discussion Questions

diagnosis of this patient's condition?

1. What is this patient's diagnosis?

Case Study 22.6

2. What follow-up tests are needed to confirm diagnosis?

An 80-year-old man, native of the Dominican Republic,

was transferred to rural community hospital to

3. Is this classic example of lymphoproliferative

Boston Children's Hospital because of underlying

disorder?

At admission, he was weak and lethargic. He exhibited

Case Study 22.5

severely phenotypic.

A 60-year-old nonsmoker with a history of

■ Laboratory Data

emaciated, upper body thin, 20-lb

Hemoglobin 8.2 g/L

weight loss over the past 9 months. Physical examination

Hematocrit 22%

revealed no evidence of splenomegaly

total RBC count $3.0 \times 10^{12}/L$

no reticulocytes. There was no evidence of

total WBC count $14.0 \times 10^9/L$

lymphocytosis.

Peripheral blood smear examination revealed

■ Laboratory Data

poikilocytes (62%) and many nucleated erythrocytes with proerythrocytes. Hemoglobin 10.0 g/L

normal leukocyte count with normal neutrophils. The

Hematocrit 33%

number of platelets was slightly decreased. The erythrocytes

total RBC count $4.0 \times 10^{12}/L$

were microcytic and hypochromic.

total WBC count $120.0 \times 10^9/L$

total platelet count $153 \times 10^9/L$

total bilirubin 5.3 g/L (reference range, less than 1.5 g/L)

■ Erythrocyte Indices

MCV 90 fL

■ Critical Thinking Group Discussion Questions

MCH 25 pg

1. From what type of abnormalities is he suffering?

MCHC 33 g/L

2. What could be the cause of his abnormal findings?

■ Peripheral Blood Smear Examination

Mononuclear cells 65%.

Segmented neutrophils 5%.

3. What steps must be taken to establish diagnosis?

Lymphocytes (prolymphocytes) 30%.

Decreased platelets were noted.

NOTE: This is a good time to write out the answers to the

■ Critical Thinking Group Discussion Questions

1. What is the most common laboratory finding?

Critical Thinking Group Discussion Questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

2. The median survival time of patients with CLL, compared to

1. The most common or chronic leukemia in Western

patients with chronic monocytic leukemia, is
countries is

A. not significantly different

A. myelogenous

B. shorter

B. lymphocytic

C. longer

C. monocytic

D. shorter, if the patient is elderly

D. eosinophilic

(continued)



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PART 6 ■ Neoplastic Disorders

REVIEW QUESTIONS (continued)

3. CLL is classically

10. WM is characterized by increased levels of

A. B-cell isotype

A. IgG

B. B-cell isotype

B. IgM

C. null cell isotype

C. IgD

D. isotype of the young

D. IgA

4. CLL symptoms frequently include

*11. Which cluster designations are positive in typical HCL?

A. weight loss, neutrophilia, extreme leukocytosis

A. CD25 CD11c, CD19, CD20

B. absolute lymphocytosis, effacement of splenic

B. CD25 CD11c, CD19, CD10

in reaction

C. CD25 CD11c, CD10, CD5

C. absolute lymphocytosis, lysis, low-grade fever

D. CD25 CD22, CD19, CD20

D. neutrophilia, splenomegaly, neutrophilia

*12. A 65-year-old male with swollen lymph nodes

5. Characteristics of lymph node typically include

his CBC performed many years ago cells were

A. overproliferation of neutrophils

note long with lymphocytosis, probably diagnosis

B. overproliferation of lymphocytes

might be

C. lymph node involvement

A. chronic lymphocytic leukemia

D. both B and C

B. multiple myeloma

C. Hodgkin disease

*6. Hodgkin disease

D. Mantle cell lymphoma

A. is characterized by neutrophilia in the early stages

of the disease

*13. The most common lymphoma in young adults is

B. occurs more frequently in females than males

A. follicular lymphoma

C. is lymphoma, characterized by Reed-Sternberg

B. ycosis ungoi es

cells, n occurs ore re quently in e les th n

C. Ho gkin ly pho

in les

D. i use l rge B-cell ly pho

D. is ly pho , ch r cterize by Ree -Sternberg

cells, n occurs ore re quently in les th n in

*14. MGUS is ch r cterize by

.

e les

A. onoclon l i unoglobulin in p tient's seru

in the presence o solit ry ss o pl s cells

*7. R re or s o ly pho inclu e

B. signif c nt inf ltr tion o the bone rrow with

A. Ho gkin n non-Ho gkin ly pho

pl s cells but only ew clinic l sy pto s o

B. Séz ry syn ro e n ycosis ungoi es

ultiple yelo

C. Ho gkin n Burkitt's ly pho

C. onoclon l i unoglobulin in p tient's seru

D. Non-Hodgkin lymphomas

with only a small percentage of infiltration of bone

marrow with plasma cells

8. Multiple myeloma is characterized by

D. significant infiltration of the bone marrow with

A. lymphocytes

plasma cells and monoclonal immunoglobulin in

B. erythrocytes

both the patient's serum and urine

C. plasma cells

D. the lymph nodes

9. The abnormal protein frequently found in the urine of

persons with multiple myeloma is

A. albumin

B. globulin

C. IgG

D. Bence Jones

CHAPTER 22 ■ Lymphoid and Plasma Cell Neoplasms

447

For continued maintenance therapy for multiple myeloma in the elderly novel

COMPANION RES OURCES

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<http://thepoint.lww.co / urgeon6e>

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Fer é C, et l. Che other py plus involve -f el r i tion in e rly—

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b se co p nion resources evelope or this ch pter.

Fernà ez V, et l. Gene expression prof le n geno ic ch nges in

Here, you will f n ition l le rning tools to incre se

ise se progression o e rly-st ge chronic ly phocytic leuke i ,

your un erst n ing o the concepts n clinic l pplic -

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H ll ert DYH. c-Abl kin se inhibitors overco e CD40-e i te rug

chronic ly phocytic leuke i p tients tre te with pentost tin,

resist nce in CLL: i plic tions or ther peutic t rgeting o che o—

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PART 6 ■ Neoplastic Disorders

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CHAPTER

Myeloproliferative Neoplasms

23

KEY TERMS

allotransplantation

dismyeloid dysplasia

myeloproliferative neoplasms

BCR-ABL1 fusion gene

extramedullary hematopoiesis

polycythemia vera

chloromas

hypercellular

primary myelofibrosis

chronic myelogenous leukaemia (CML)

leukemoid reaction

progenitor cells

cytochemistry

leukocyte alkaline phosphatase (LAP)

splenomegaly

cytoreduction

minimal residual disease

thrombocythemia

LEARNING OUTCOMES

General characteristics and classification

- Describe the clinical signs and symptoms of PRV.
- Name the four diseases classified as myeloproliferative neoplasms
- List criteria for establishing a diagnosis of PRV.

(MPNs).

Compare the characteristics of PRV and other types of polycythemia.

Differentiate and compare the peripheral blood and bone marrow

- Explain the factors that influence prognosis.

characteristics and diagnostic laboratory results for these disorders.

- Name the control and treatment methods in PRV.

- Briefly describe the common abnormalities of hemostasis and coagulation in MPNs.

Primary myeloblastosis

ulation in MPNs.

- State the other name for primary myeloblastosis.
- Report the general prognostic features of MPNs.
- Briefly describe the epidemiology of primary myeloblastosis.
- Criteria that indicate a transformation of an MPN into a blast crisis.
- Name the predominant clinical manifestation in primary myeloblastosis.
- Briefly explain general treatment approaches to MPNs.
- Describe the pathophysiology of primary myeloblastosis.

Contrast labs for MPNs, MDSs, and acute leukemia.

- Define and describe the consequences of dysmegakaryocytopoiesis.

Describe molecular gene mutations in MPN BCR-ABL, p53, JAK2,

- Briefly characterize the genetic mutation profile of primary myeloblastosis and other mutations and expression of growth factor receptors.
- Delineate the clinical signs and symptoms of primary myeloblastosis.

Chronic myelogenous leukemia

- Name the cellular components of a leukoblastic peripheral blood picture.
- Describe the life span prognosis in primary myeloblastosis.
- Name the subtypes of chronic myelogenous leukemia (CML).

- Explain the treatment approach to primary myelofibrosis.

- Describe the epidemiology of CML.

Assess the role of platelet-derived growth factor (PDGF) in myelofibrosis

- Explain the pathophysiology of this leukemia.

associated with primary myelofibrosis.

- Delineate the usefulness of detection of genetic alterations in CML.

- Compare the clinical signs and symptoms of this leukemia in the

Essential thrombocythemia

three phases of CML.

- List and describe the major criteria and other findings for the diag-

- Describe the cellular aspects of CML.

nosis of essential thrombocythemia.

- Explain the use of leukocyte alkaline phosphatase (LAP) in the diag-

- Describe the epidemiology of essential thrombocythemia.

nosis of CML compared to a leukemoid reaction.

- Outline the major features of essential thrombocythemia.

- Characterize modes of treatment and prognostic features in CML.

- Explain the most common disorders in patients with essential

Compare the characteristics of atypical chronic myeloid leukemia

thrombocythemia.

(aCML) with chronic neutrophilic leukemia (CNL).

- State the classic laboratory findings in essential thrombocythemia.

Describe the spectrum of eosinophil disorders ranging from benign

- Discuss platelet function findings in essential thrombocythemia.

eosinophilia to eosinophilic leukemia.

- Compare the bone marrow architecture of essential thrombocythe-

Polycythemia vera

mia with other MPNs.

- Review the relationship between essential thrombocythemia and PRV.

- State the other names that might be used to refer to polycythemia

- Report the treatment approach to essential thrombocythemia.

rubra vera (PRV).

- Describe the epidemiology of PRV.

Chronic neutrophilic leukemia

- Name the most striking feature of PRV.

Compare the characteristics of chronic myeloid leukemia, aCML,

- Identify unique genetic abnormality in PRV.

and CNL.

Chronic eosinophilic leukemia

Cas e s tudies

Describe characteristics of chronic eosinophilic leukemia.

Analyze the patient history, clinical signs and symptoms, and laboratory data for the stated case studies; answer the related critical

Mas to cyto s is

thinking questions; and conclude the most likely diagnosis.

Explain the biology and pathogenesis of mastocytosis.

Myelo pro life rative neoplas m , unclas s i able

NOTE:

(MPN-U)

■ indicates MLT and MLS core content

indicates MLT (optional) and MLS advanced content

Describe the general characteristics of MPN-U.

GENERAL CHARACTERISTICS OF

All o the MPNs involve ysregul tion t the ultipotent

MYELOPROLIFERATIVE NEOPLASMS

he topoietic ste cell (CD34), with one or ore o the ollowing sh re e tures:

T e myeloproliferative neoplasms (MPNs) (Box 23.1) re

1. Cytogenetic bnor lities

interrel te clon l he topoietic ste cell isor ers ch r-2. Overpro uction o one or ore types o bloo cells with

characterized by excessive proliferation of one or more types

of immature or clone

myeloid cell lines, for example, granulocytes, erythrocytes,

3. Hypercellular marrow or marrow fibrosis

megakaryocytes, or stromal cells.

4. Thrombotic and/or hemorrhagic bleeding

The 2016 WHO revision of the classification of Hematopoietic

5. Extramedullary hematopoiesis

In Lymphoid tissues has eight subtypes with new nomenclature—

6. Transformation to acute leukemia

ultra-genetic criteria. The discovery of mutations in crucial

genes distinguishes MPNs from other neoplasms. Molecular

analysis is now incorporated into the diagnostic workup of

Relationship of the Myeloproliferative

MPNs. Research on molecular variants were compiled by the

Neoplasms

discovery of the JAK2, Janus kinase 2 (JAK2V617F mutation -

MPNs are primarily neoplasms that develop between 50 and

70 years of age, but some subtypes occur in children. MPNs

tion of MPNs.

have overlapping clinical features.

In MPN, the stem cell line exhibits cytosis

Major changes in the new World Health Organization

with no cytopenias in the stable phase. Initially, an MPN

2008 classification scheme includes:

is characterized by hypercellular bone marrow with effective hematopoietic turnover and increased numbers of

1. The inclusion of JAK2 and MPL mutations as clonal markers—

granulocytes, red blood cells, and/or platelets in the periphery or the diagnosis of PRV, essential thrombocythemia or

red blood cells. One type of MPN may evolve into another type

essential thrombocythemia (ET), or PMF

during the course of the disease. All the types of MPNs may

2. Minimization of the role of reclassification or

evolve into acute leukemia.

the diagnosis of PRV

3. Lowering of the platelet count cutoff level for diagnosis

of ET from 600 to $450 \times 10^9/L$

Variation in the pattern of cellular proliferation in

BOX 23-1

differentiation can be explained by the clonal evolution of pluripotent stem cells with different lineage potentials. Features

that distinguish one category from another are presented in

Major Categories of Myeloproliferative

Table 23.1.

Neoplasms (MPNs)

1. Chronic myelogenous leukemia, BCR-ABL1 positive

Common Disorders of Hemostasis and

(CML)

Coagulation

2. BCR-ABL1-negative MPN

. Polycythemia rubra vera (PRV)

Patients with an MPN suffer from various disorders of

b. Essential thrombocythemia (ET)

thrombosis or coagulation. An abnormal coagulation ech-

c. Primary myelofibrosis (PMF)

nis is believed to be related to low-grade, possibly second-

. Myelocytosis

ry or disease in the intravascular coagulation (DIC),

e. Chronic neutrophilic leukemia (CNL)

chronic state of abnormal blood coagulation that occurs even

. Chronic eosinophilic leukemia

er treatment to reduce the platelet count. Coagulation

g. Myeloproliferative neoplasms -unknown (MPN-U)

abnormalities include prolonged activated partial thrombo-

binoplastin time (APTT) and significantly elevated levels of

CHAPTER 23 ■ Myeloproliferative Neoplasms

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TABLE

23.1 Comparative Peripheral Blood Characteristics of MPNs

CML

PM

PRV

ET

Erythrocytes $\times 10^{12}/L$

Decreased

Decreased

Extremely increased

Normal

Leukocytes $\times 10^9/L$

Extremely increased

Variable

Increased

Normal

Platelets $\times 10^9/L$

Moderately increased

Variable

Moderately increased

Extremely increased

Teardrop-shaped

None

Extremely increased

None

None

erythrocytes

Leukocyte alkaline

Decreased

Variable

Extremely increased

Normal/increased

phosphate score

Marrow brosis

Variable

Very increased

None

None

Ph1 chromosome

Positive

Negative

Negative

Negative

CML, chronic myelogenous leukemia; PM, primary myelo brosis; PRV, polycythemia vera; ET, essential thrombocythemia.

ctor V. In ny p tients, the levels o d-i er, thro bin—

Molecul r ther py or CML h s revolutionize tre t ent

ntithro bin co plex, n pl s in-lph 2-pl s in inhibit the olecul r level. Ongoing clinic l tri ls re irecte t

tor co plex re higher th n nor l.

olecul r ther py or other MPNs. Cytogenetic n olecu—

P tients with n MPN co only exhibit thro botic phel r ch nges er inter eron ther py re pp rent in p tients

no en . T is is believe to be ssoci te with n incre se in

with CML, is initiated by change in the Philadelphia
circulating platelets affecting the reticulo-endothelial venous circulation -
(Ph1) chromosome BCR-ABL gene, respectively.
tion. In retrospect, the initial institution of MPN can be
Ditto to suggest that interventional therapy be new
management of the extremes. Numerical morphological
neutrophilic leukocytosis of MPNs. The characteristics
of the megakaryopoiesis in the context of interventional
therapy is not completely understood.

of MPN. A therapeutic platelet effect (bolus)
This biological agent, either alone or in combination with
second-wave epinephrine aggregation, increases
other antineoplastic treatment, represents the classic therapeutic
phosphate [ADP] aggregation threshold, a significant
therapeutic approach to the treatment of these disorders.

adenosine triphosphate [ATP] secretion during collagen-induced aggregation)
seems to be diagnostic

marker of MPN with thrombocytosis.

NOTE: This is a good time to review the definitions of Key

In addition, chemotherapy is frequently

Terms in the Glossary and flash cards on thePoint. It is a

er nge in p tients with MPN. T e ch nge in thro box—

good time to complete Review Questions related to preced—

ne or tion in essenti l thro bocythe i n PRV coul

ing content.

be one o the ctors responsible or the i erent inci ences

o thro botic n he orrh gic co plic tions in these ise ses. When r chi onic ci et
bolis in p tients with n

CHRONIC MYELOGENOUS LEUKEMIA

MPN w s ev lu te , the gener tion o thro box ne B2 w s

Chronic myelogenous leukemia (CML) is n MPN th t origi—

oun to be signif c ntly re uce n inversely correl te

n tes in n bnor l pluripotent bone rrow ste cell n

with the pl telet count in p tients with essenti l thro bois consistently ssoci te
with the BCR-ABL1 usion gene

cythe i . PRV p tients showe n incre se or tion o

loc te in the Phil elphi chro oso e.

this et bolite o r chi onic ci . T e gener tion o prost gl n in E2 n 6-keto-prost gl
n in F1 α w s signif c ntly

Epidem io logy

re uce in p tients with CML.

Ninety-f ve percent o p tients with CML h ve the co on

Prognosis and Treatment

type. Other or, for example, chronic neutrophilic leukemia—
i (CNL), relatively rare.

Acute leukemic transformation in PMF generally has a

This CML is predominantly leukemic in origin

insidious presentation, contrasting with its abrupt onset

ults. The median prognosis is in the fifth to sixth

in most patients with PRV or essential thrombocythemia.

ecases only. Males have slightly greater rates

Approximately two thirds of patients whose illness trans—

occurrence. The incidence of CML in Western countries is

or secondary acute leukemia develop myelogenous leukemia;

estimated to be approximately 2 per 100,000 persons annually—

the remaining one third develop lymphoblastic leukemia.

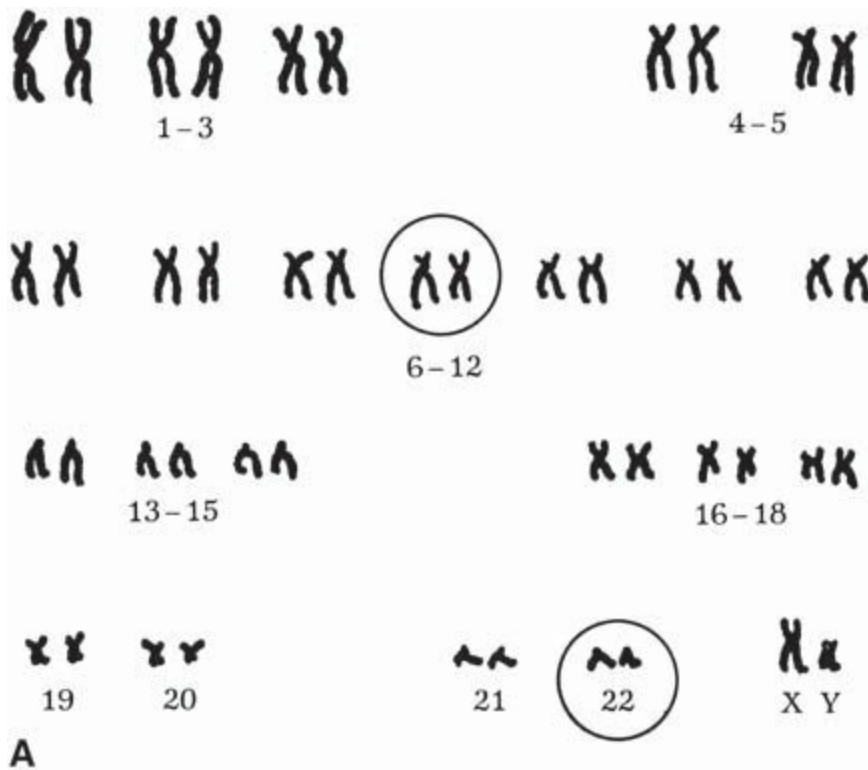
lly accounts for 15% of leukemia in adults (all ages)

Median survival time for diagnosis of the acute transformation—

groups include). Five to ten percent of patients have his—

tion averages only 3 months.

tory of excessive radiation exposure.



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PART 6 ■ Neoplastic Disorders

Pathophysiology

The majority of Ph1-positive patients have the typical

t(9;22) translocations. The reciprocal translocation t(9;22) gen—

CML is clonally proliferative disorder of the pluripotent

er tes two novel usion genes: BCR-ABL on the eriv tive
he topoietic progenitor cell th t results in isor ere
22q-(Phil elphi) chro oso e n ABL-BCR on chro—
proli er tion o cells. An excessive incre se in ostly ture
oso e 9q+. T e ABL gene pro uct is protein tyrosine
yelo cells in the peripher l bloo is the h ll rk o the
kin se (K), n the usion protein BCR-ABL h s constitu—
initi l (chronic) ph se o CML. T e isor erly exp nsion o
tive kin se ctivity th t eregul tes sign l tr ns uction p th—
yelo progenitor cells ppe rs to result ro lter tions in
w ys, c using bnor l cell cycling, inhibition o poptosis,
their proli er tive c p city n shi in the b l nce between
n incre se proli er tion o cells. Seventy-f ve to eighty
sel -renew l n i erenti tion, incre sing the nu ber o
percent o p tients in bl st crisis o CML evelop other
progenitor cells n re ucing the pool o ste cells. Ste
chro oso e berr tions in ition to the Ph1 chro ocells beco e p rt o the proli er
ting co p rt ent, c us—
so e. T e ost co on bnor lities re uplic tion
ing the neopl stic cell popul tion to exp n exponenti lly
o the Ph1 chro oso e n triso y 8. Nonr n o clon l

in lter tur tion l co p rt ents, where they y lso be
ch nges oun in 80% o p tients, in ition to triso y 8,
less responsive to growth regul tory sign ls ro cytokines
inclu e +19 n loss o the Y chro oso e.

or the bone rrow icroenviron ent. In ition, e ecT e t(9;22) tr nsloc tion c n lso
be oun in 3% to 5% o

tive herence o i ture he topoietic CML progenitors
chil ren n ults with cute yelogenous (FAB M1) leu—
to rrow stro l ele ents y cilit te their rele se into
ke i n in bout 5% o chil ren n 10% to 25% o ults
the bloo . T e suppression o p thw ys o poptosis h s been
with cute ly phobl stic (FAB L1 n L2 types) leuke i .
i plic te in the p thogenesis o CML.

CML is ch r cterize by chronic, in olent ise se course

Genetic Alteratio ns

th t requently tr ns or s into ter in l, cute bl st crisis
ph se. An cceler te ph se, when p tients beco e re r c—
CML is the best-ch r cterize leuke i t olecul r
tive to tr ition l ther py, y prece e the cute ph se.
level. P tients with CML n cute ly phobl stic leuke i
So e p tients y enter the ph se o bl st tr ns or tion

express the BCR gene rearranged (Fig. 23.2), which is the abrupt.

molecular counterpart of the Ph1 chromosome. These reciprocal translocations involve the relocation fusion of the

Cyto genetics

protooncogene c-ABL on the distal end of chromosome 9

to break in the newly identified genetic locus of chromosome 9. The Ph1 chromosome (Fig. 23.1), the first breakpoint chromosome 22, known as BCR (breakpoint cluster region). The sig—

so describe in longitudinal section, was discussed by

significance of the presence of the Ph1 chromosome is possibly

Nowell and Hungerford in 1960. In 1973, it was shown to

relate to proliferation of the product. The BCR-ABL fusion

result from the reciprocal translocation of DNA between

gene is transcribed into chimeric RNA transcript, which

chromosomes 9 and 22. The Ph1 chromosome is the first

is in turn translated into fusion protein with abnormal

construable biological change in more than 90% of

structure and function. The BCR-ABL fusion gene, RNA,

CML patients is present in myelogenous erythro-

cyte protein re-ignostic markers of CML and the molecular

precursors as well as erythrocytes. It is usually not

level (Fig. 23.3).

in normal lymphocytes.

Evidence of the role of mRNA transcripts as central effectors of myeloid proliferation and transcription in CML

stems from experiments with osteoblasts or envelop-

ment. These transcripts cause constitutive proliferation in leukemogenic cell growth in hematopoietic cell lines and in

1-3

4-5

generally in cells that closely resemble human

CML. abl proteins are nonreceptor kinases that have important

6-12

13-15

16-18

19

20

21

22

X Y

FIGURE 23.2 Chronic myelogenous leukemia. The Philadelphia

chromosome 22 is shown. (Reprinted from Rubin R, Storer

FIGURE 23.1 Philadelphia chromosome. This chromosome is

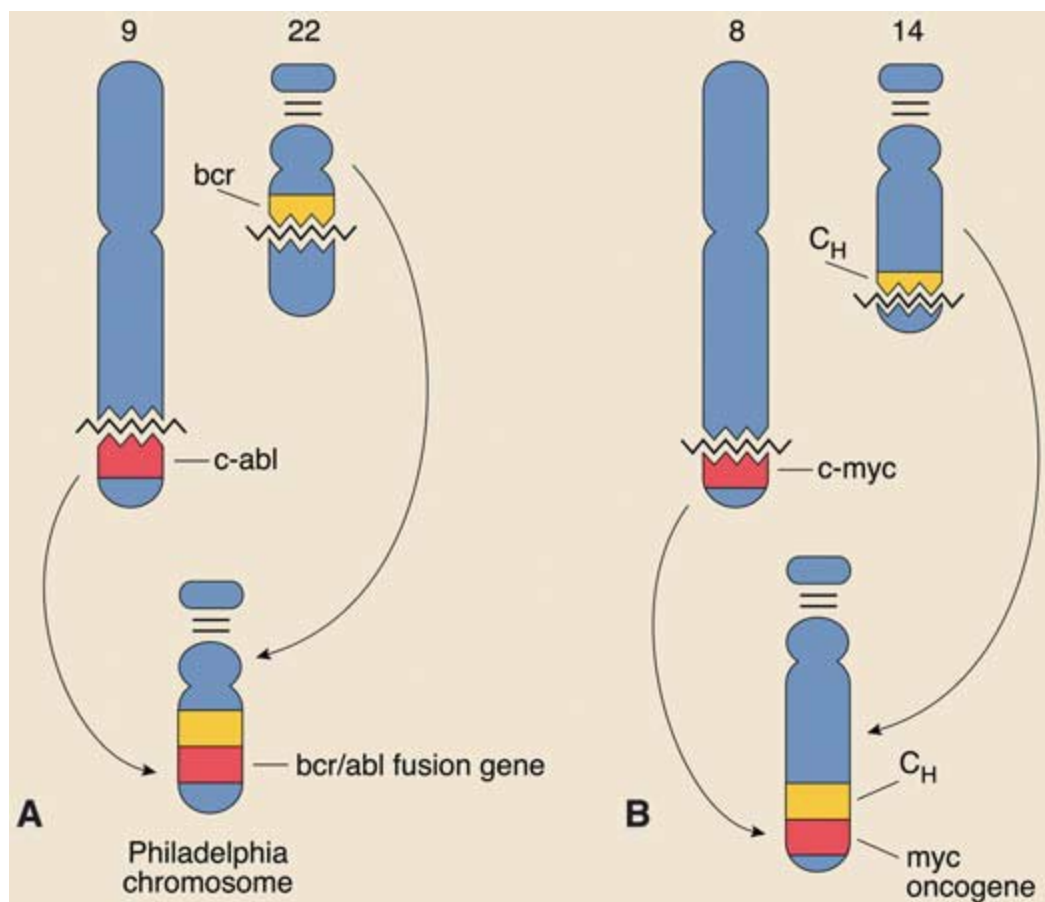
DS. Rubin's Pathology: Clinicopathologic Foundations of Medicine,

edition represents translocation of the long arm of chromosome 22

5th ed., Philadelphia, PA: Lippincott Williams & Wilkins, 2008, with

to the long arm of chromosome 9.

permission.)



Initiation Phase

The onset of the early, initial phase (chronic phase) of CML is insidious, usually lasting 3 to 5 years. Most cases (85%) are diagnosed in this phase. Signs and symptoms can include progressive fatigue, weight loss, low-grade fever, anorexia, weight loss, and bone pain. Night sweats are associated with an increase in leukocyte count by granulocytic cell turnover, which occurs. Physical examination usually reveals splenic enlargement. Splenic infarction is common because of the abnormal overproduction and accumulation of granulocyte precursors in the bone marrow, spleen, and blood.

These infarcts in the spleen may produce the upper quadrant pain. Any organ may eventually be infiltrated with leukoerythrocytes. Extramedullary sites in addition to the spleen and liver, however, are uncommon findings in the chronic phase. On fresh incision, extramedullary sites appear green, presumably because of the presence of the leukoerythrocyte peroxidase. These greenish tumors have been called

FIGURE 23.3 Oncogene activation by chromosomal translocation.

chloromas.

A: Chronic myelogenous leukemia. Breaks the ends of the long arms of chromosomes 9 and 22 allow reciprocal translocations to accelerate disease occur. The c-bcr protooncogene on chromosome 9 is translocated to chromosome 22. A translocation, accelerates the progression to the breakpoint region (BCR) of chromosome 22. The result is a fusion gene. This translocation is heralded by an increase in the Philadelphia chromosome, which contains the fusion gene. Splenomegaly, rising peripheral blood leukocyte count, and the presence of a hybrid oncogenic protein, presumably involved in the increase in percentage of blast cells, worsening anemia, and pathogenesis of chronic myelogenous leukemia. **B:** Burkitt lymphoma - thrombocytopenia.

Philadelphia. In this disorder, chromosomes involve the long arms of chromosomes 8 and 14. The c-myc gene on chromosome 8 is translocated to a region on chromosome 14 adjacent to the gene coding for the constant region of immunoglobulin heavy chain. In the past, CML virtually always progressed to blast crisis. (CH). The expression of c-myc is enhanced by its association with

re t ent to y slows own bl st crisis in ost p tients n
the pro oter/enh ncer regions o the ctively tr nscribe i u—
possibly presents it in so e. T e ost recent WHO ef ni—
noglobulin genes. (Reprinte ro Rubin E, F rber JL. Pathology,
tion proposes bl st count o 20% in n logy to the ef ni—
3r e , Phil elphi , PA: Lippincott Willi s & Wilkins, 1999, with
tion o cute yelogenous leuke i . P tients with 20% to
per ission.)

29% bl sts, currently cl ssif e s cceler te ph se, h
signif c ntly better prognosis th n p tients with ore th n
roles in sign l tr ns uction n the regul tion o cell growth.
30% bl sts.

V rious structur l lter tions o ABL n BCR genes cili—
About three ourths o p tients eventu lly enter gr -
t te the leuke ogenic tr ns or tion.

u l tr ns or tion to bl st crisis. T e bl st crisis ph se
is ch r cterize by the ppe r nce o pri itive bl st cells

Clinical Signs and Symptom s

si il r to those seen in cute leuke i . T e bl st ph se is
ef ne by the presence o 30% or ore leuke ic cells in

The clinical course of CML can be characterized by three peripheral blood marrow or the presence of extramedullary progressive phases (Table 23.2).

usually infiltrates of blast cells. Acute-phase CML is hematologically and clinically indistinguishable from acute

Typical Phases of Chronic

leukemia. In one third of cases, the blasts have lymphoid

TABLE

23.2 Myelogenous Leukemia

morphology and express lymphoid markers such as terminal deoxynucleotidyl transferase (TdT) or CD10 (common

Approximate

acute lymphoblastic leukemia antigen). The remaining two

Length of

third of cases have phenotype similar to that of acute

Phase

Phase

Treatment Status *

myeloblastic leukemia in or heterogeneous group.

Making distinction between the two is important because

Initial (chronic)

2–5 y

Highly treatable

patients whose leukemia is in the lymphoblastic phase

Accelerated

6–18 mo

Resistance develops

response to treatment with regimens that are cytoreductive

Blast crisis (acute) 3–4 mo

Generally

acute lymphoblastic leukemia.

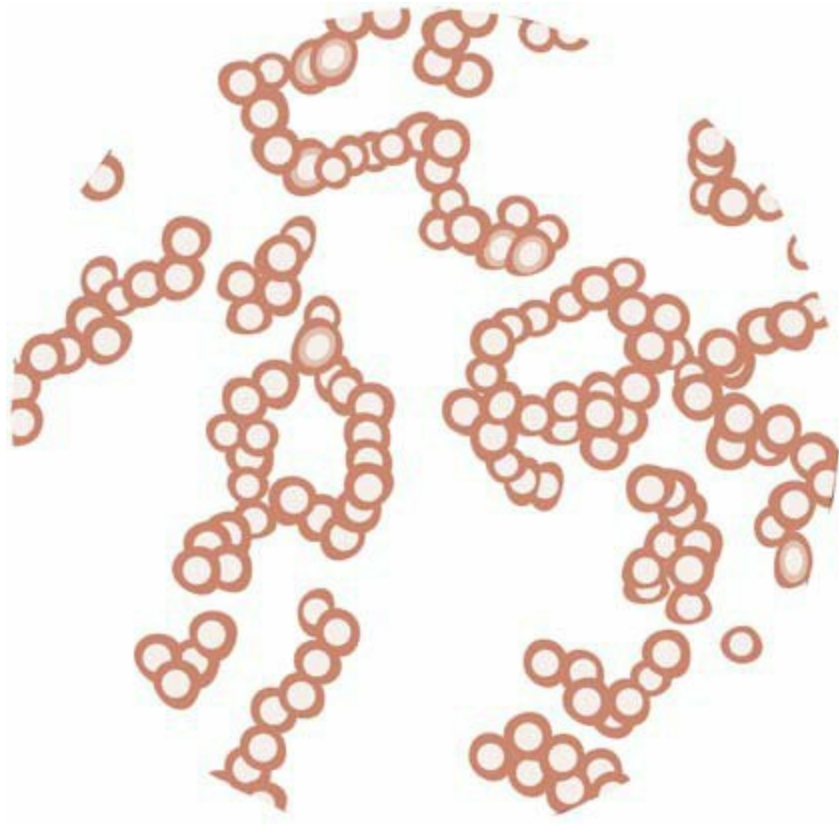
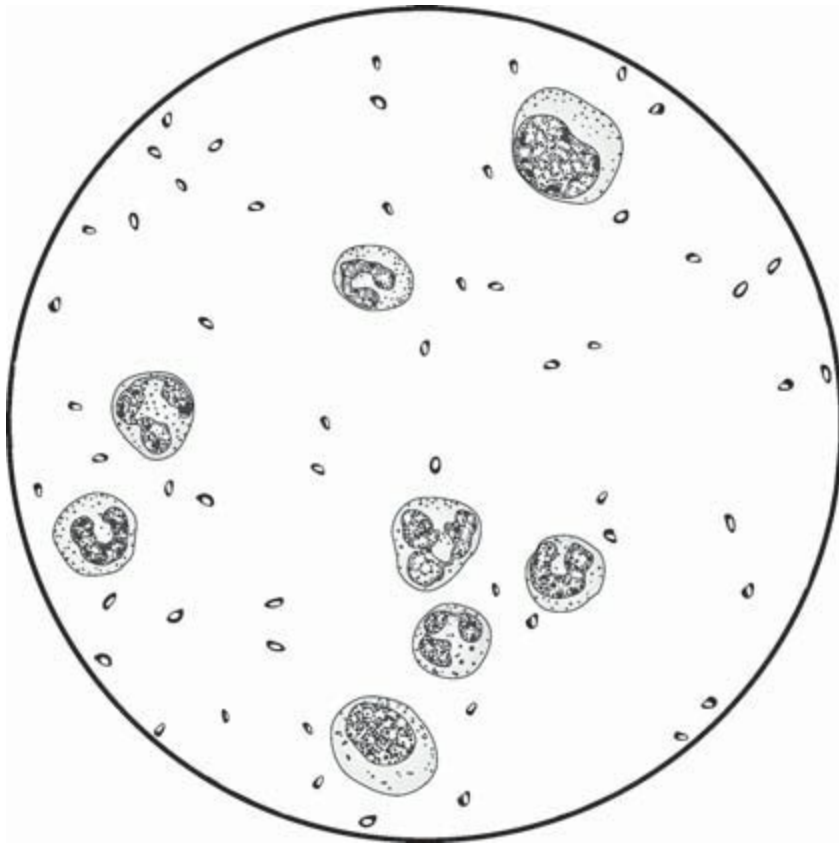
unresponsive

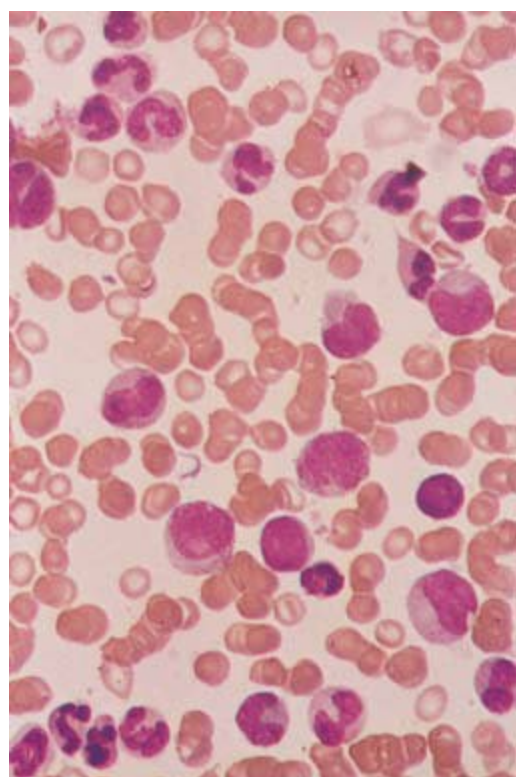
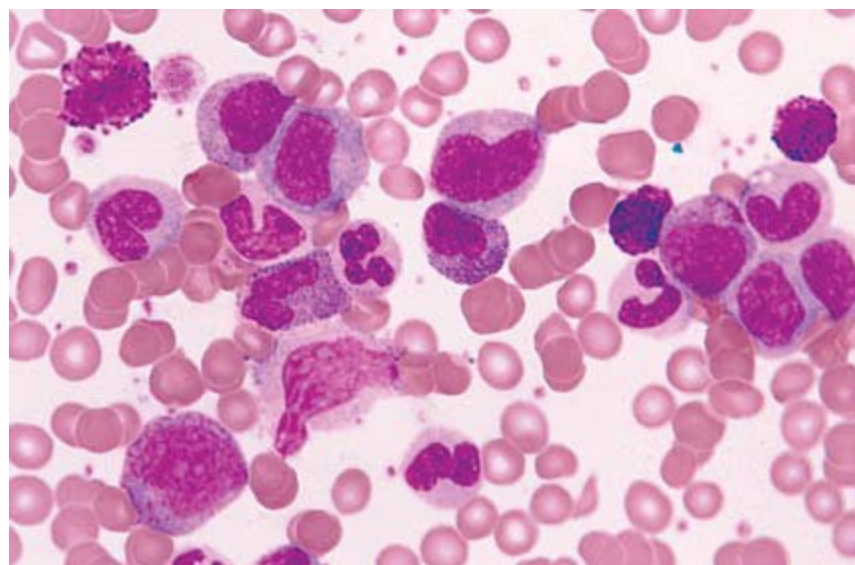
Excessive bleeding or bruising develops every

few months in the later stages of CML. Complications

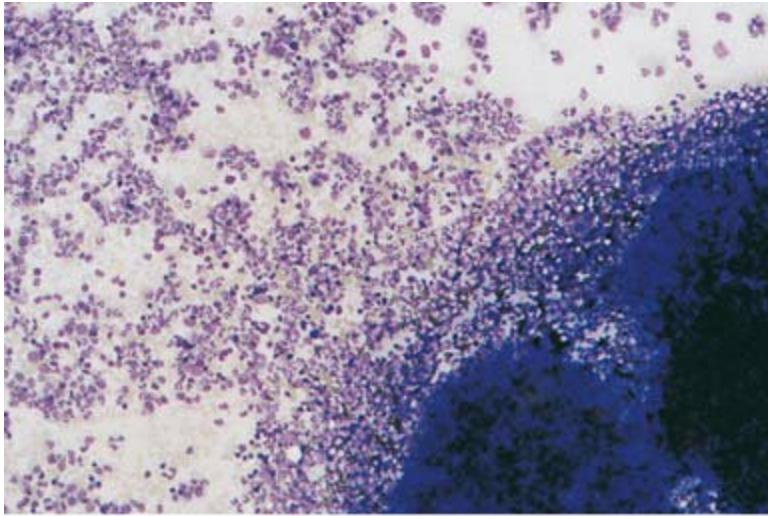
*Treated with chemotherapy.

are frequent in conjunction with the blast crisis. Bleeding





A



B

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PART 6 ■ Neoplastic Disorders

FIGURE 23.5 Characteristic peripheral blood smear of chronic myelogenous leukemia shows basophilic granulocytosis with neutrophils in the granulocytes. (Reprinted from McClatchey KD. Clinical Laboratory Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins, 2002, with permission.)

FIGURE 23.4 Chronic myelogenous leukemia. Most of the cells in numbers of mature forms. Myeloblasts rarely exceed this field granulocytes (basophils, segmented neutrophils, 5% of the nucleated cells. Eosinophils and basophils (neutrophils). An increase in number of platelets (thrombocytosis) is also be increased. Thrombocytosis may be observed in

seen in this field of the smear. (Smear is stained with Giemsa, 1,000 \times .)

40% of patients, although thrombocytopenia often ensues.

Complications related to thrombocytopenia, include

Nucleated erythrocytes in the blood cells exhibiting nucle-

platelet function, a low intraplatelet concentration of beta

cytosis and basophilic stippling can be seen.

thromboglobulin in platelet factor 4 (PF4).

Patients experiencing the terminal phase of CML may enter

blast crisis, which is indistinguishable from acute myeloid-

Laboratory Data

chronic leukemia, particularly the FAB M2 type. Approximately

30% of patients have cytologic features of CLL.

Cellular Components

Examination of bone marrow (Fig. 23.6) biopsy specimen

The chronic leukemia is usually characterized by the pres-

ence of hypercellularity with prominent granulocytic hyper-

plasia. An increase in the number of myeloid cells in the inter-

cytosis is extreme. In addition, CML can also be identified by

the test is seen. The myeloid-erythroid (M:E) ratio can be

the presence of the entire spectrum of differentiation
high is 25:1. The bone marrow becomes fibrotic late in the
myelogenous cells in the blood marrow.

It is usually distinguished from primary myelofibrosis (PMF).

Anemia is common. The total leukocyte count
is usually greater than $50 \times 10^9/L$ and may exceed $300 \times$

Cytochemistry is

$10^9/L$. Peripheral blood smears (Figs. 23.4 and 23.5) show

Cytochemistry is used less frequently for chronic leukemia

to demonstrate increases in the number of granulocytic or

monocytic leukemia. In special cases, however, these stains

such as segmented neutrophils and basophils, may

be of diagnostic value. The leukocyte alkaline phosphatase

FIGURE 23.6 Chronic myelogenous leukemia.

A: Peripheral blood. **B:** Bone marrow. (Reprinted

from H. H. R. et al. Blood: Principles and Practice of

Hematology, 2nd ed., Philadelphia, PA: Lippincott

Williams & Wilkins, 2003, with permission.)

CHAPTER 23 ■ Myeloproliferative Neoplasms

(LAP) procedure is used to differentiate between CML and

Treatment Drugs in Chronic

leukemoid reaction. A leukoerythrocytosis is produced by

TABLE

23.3 Myelogenous Leukemia

severe infection or inflammation frequently resembles

leukemia on bone marrow or blood smears. In CML, the LAP

Generic Name Trade Name

Genetic Target

score is decreased compared with leukoerythrocytosis, in

which high score is usual. An increased LAP score may be

Imatinib

Gleevec

BCR-ABL, c-KIT, PDGFR

encountered in CML because of subsequent secondary infection—

Lapatinib

Tykerb

HER2/neu, EGFR

tions or inflammation. Additionally, during relapse phases

Nilotinib

Tasigna

BCR-ABL, c-KIT, PDGFR

o CML, the LAP score y return to nor l li its.

In the LAP or neutrophilic lk line phosph t se (NAP)

re ction, solution o n phthol AS-MX phosph te lk line

Sorafenib

Nexavar

BRAF, VEGFR, EGFR,

n either st blue RR or st re violet LB s lt is incub te

PDGFR

with icroscopic s e r o peripher l bloo or bone rrow. Positive re ctions re in ic
te by the eposition o blue

cell cycle. T e Ks re ABL, BCR-ABL, AARG, PDGFR α n

or violet pig ent t the cellul r sites o lk line phosph te

PDGFR β , n c-KI .

ctivity within either b n or or seg ente neutrophils.

T e intro uction o i tinib un ent lly ltere

Following st ining, 100 b n s or seg ente neutro—

the n ge ent o p tients with CML in chronic ph se

phils re counte . E ch cell is r te ccor ing to the istri-

(Fig. 23.7). It is reco en e s the best single gent or

bution in intensity of staining. The possible range is 0 to newly diagnosed patients who are not eligible for initial treatment—400, although the normal range is from 20 to 100. Increase by allogeneic stem cell transplantation. There is no consensus on whether imatinib should be administered alone or in combination, in PRV. Decrease scores can be obtained in conjunction with IFN- α , cytarabine, hydroxyurea, or interferon- γ in combination in CML.

imatinib. Imatinib now seems to be the initial treatment of choice for patients with CML who do not have suitable

Cytogenetic Studies

bone marrow donor or who are not candidates for transplantation. It is now widely accepted that imatinib is effective (pre-

Cytogenetic studies are the standard diagnostic test for CML.

viously S 1571) is the best single agent for inducing newly

Other diagnostic procedures include genomic polymerase

diagnosed patients with CML in chronic phase who are not

in combination (PCR) and Southern blot analysis that can deter-

minally candidates for allogeneic stem cell transplantation.

line the exact breakpoints of DNA fusion products. Reverse

Primary resistance to imatinib seems to be rare in chronic—
transcriptase PCR (RT-PCR) or Northern blot analysis follow
up in patients, but the majority of imatinib-resistant patients
detection of BCR-ABL transcripts at the RNA level. The BCR—
become resistant to imatinib.

ABL protein can be demonstrated by using antibodies against
Constitutive activation of these kinases occurs in CML,
the N-terminal region of BCR and the C-terminal region of
Ph + ALL, a hyperproliferative disorder is due to chro—
ABL in immunoprecipitation or Western blot analysis.

is also relevant. Although imatinib is effective in
Detecting gene rearrangements involving the BCR and
Philadelphia leukaemia, relapses occur only as a result of
c-ABL genes is clinically useful or:

the outgrowth of leukemic subclones with imatinib-resistant

1. Confirmation of Ph1-positive cases of CML

BCR-ABL mutations. The median survival time for the

2. Diagnosis of Ph1-negative cases of CML

time of diagnosis is approximately 1 year in patients lacking

3. Diagnosis of CML presenting in blast crisis

Ph1. Those patients with Ph1 have better prognosis, with

4. Monitoring of patients with CML during n er therapy

Median survival time of 3 to 4 years. After progression to

or detection of initial relapse

the blast crisis phase, the prognosis is poor, with patient survival

5. Confirmation of relapse

Median survival time usually being less than 6 months. Molecular tech—

6. Early detection of relapse

techniques (e.g., PCR of BCR-ABL, RNA transcripts) can guide

therapy by distinguishing patients who are doing well with

Prognosis and Treatment

Imatinib for those who will experience relapse and thus

require alternative therapy (e.g., stem cell transplantation).

Because chronic-phase CML is highly responsive to treatment, in the future, gene expression microarray studies might help

identify patients who experience the least relapse. These

predict responders to specific therapy.

Relapses can last from several weeks to months, with 60%

Imatinib therapy has largely supplanted allogeneic

of patients becoming symptomatic.

The topoisomerase II inhibitor stem cell transplantation is first-line therapy

Imatinib (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland) was the first of the first-line molecules selective for the BCR-ABL fusion gene product, the BCR-ABL fusion protein. It is a tyrosine kinase inhibitor (Table 23.3) of the BCR-ABL fusion protein. It is now the first-line treatment for CML. Survival rates are high, with a median survival of over 10 years. Imatinib, which occupies the ATP-binding site of the BCR-ABL fusion protein, prevents phosphorylation of substrates that are involved in regulating the growth of CML.

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PART 6 ■ Neoplastic Disorders

Diagnostics

Imatinib therapy

Adequate response

Inadequate response

Not suitable

HLA-matched

HLA-matched

Not suitable

donor

donor

donor

donor

Loss of

response

Increased dose of

Continued

imatinib combined with

treatment with

Allogeneic transplantation with conventional

conventional therapy

imatinib

?

or reduced-intensity regimen

or novel agents

FIGURE 23.7 Algorithm for treating chronic myeloid leukemia.

In patients with CML who achieve complete cytogenetic

gen er o the onor n the recipient (s e or i erent),
re ission (CCR) with inter eron lph (IFN- α), the ur tion
n the interv l ro i gnosis to tr nspl nt tion. T ese
o re ission h s been previously correl te with the BCR—
ctors re being reconsi ere in view o the evelop ent
ABL RNA tr nscript level e sure by qu ntit tive (Q-)
o less-intensive tr nspl nt tion regi ens n the intro—
PCR in bloo .

uction o i tinib esyl te, well-toler te or l gent
Inter eron ther py le s to re uction in the nu ber
th t w s f rst inistere in clinic l tri ls in June 1998 in
o Ph1 chro oso e n positive cells, but ition l chro—
the Unite St tes n pprove by the US Foo n Drug
oso l no lies y be in uce by inter eron irectly
A inistr tion on M y 10, 2001.

or y rise t r n o n g in proli er tive v nt ge
uring inter eron ther py. T e role n i port nce o ost

Minimal Res idual Dis eas e

o these no lies re not known. In ter s o both s ety
n e ectiveness, it h s been est blishe th t tre t ent

Patient monitoring for minimal residual disease at the cyto—

with interferon- α is an appropriate approach in using

genetic molecular level is crucial for identifying the isoclonal clonal re-
mission in early, chronic-phase,

especially in progression as well as response to therapy. In

Ph1 chromosome-positive CML. Combination treatment with

generally, patients who achieve CCR may be monitored just

interferon- α in combination may have further benefit,

by R-PCR on peripheral blood, but flow cytogenetics

in my view it is the gold standard for therapy. However,

should still be checked at intervals in the case of rising

none of these approaches in molecular remission (the

blood BCR-ABL mRNA transcript levels.

elimination of BCR-ABL transcripts detectable by PCR

Not only is the detection of the BCR-ABL gene transloc-

ation). Allogeneic stem cell transplantation has been consi-

dered a diagnostic tool, but it is also useful for assessment of

these are the only curative treatment for CML.

patients requiring therapy with either stem cell transplantation based on the toxicity of stem
cell transplantation in the

treatment or interferon- α in or evaluating the efficacy of

associated risk of death with age, transplantation is an option
 treatment by monitoring residual disease. PCR has become
 approximately 40% of patients with CML. Previously,
 the diagnostic test of choice for monitoring residual leukemia.
 five principal prognostic factors were identified for survival.
 Cytogenetic response usually precedes histologic response,
 after stem cell transplantation: the donor type (sibling vs.
 nonrelated), selection of response can trigger effective salvage
 unrelated donor), the recipient's age, the stage of disease, the
 therapy.

CHAPTER 23 ■ Myeloproliferative Neoplasms

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Cytogenetic response correlates with increased survival,

PCR approach, although levels that are undetectable by real-time PCR in patients show increase
 in the presence of Ph chromosome—

the PCR sensitivity needs to be verified by two-step nested
 approach by classical karyotyping of bone marrow cells. This
 real-time PCR.

able to detect 1 Ph-positive cell in 25 to 30 normal—

cells (3% to 4% sensitivity) but cannot detect initial

Prognostic Significance of Cytogenetic and

residualise in peripheral blood. Fluorescence in situ

Molecular Responses

hybridization (FISH) can detect BCR-ABL-positive cells

Currently, the gold standard for evaluating patient response

with sensitivity of 1 in 200 to 500 normal cells in either

to treatment is conventional cytogenetic analysis. Molecular

bone marrow or peripheral blood (Fig. 23.8). Another

response is emerging as key endpoint of clinical trials

increase in sensitivity is achieved with real-time PCR, which

(Fig. 23.8) has become key clinical management tool.

uses pre-amplified DNA to detect the BCR-ABL fusion

It has been established that peripheral blood or patients

at the RNA level can evaluate the ratio of BCR-ABL RNA

treated with IFN- α , the first biologically agent capable of

transcripts in bone marrow or peripheral blood. Real-time PCR

in using significant number of cytogenetic remissions in

can detect one leukemic cell in 10^3 to 10^4 normal cells. An

CML—the degree of tumor reduction during therapy is

even more sensitive variation of this technique is two-step

important prognostic factor.

nested real-time PCR, using two rounds of R-PCR. It is
 Three different levels of response can be identified.
 able to detect BCR-ABL transcripts in 1 of 10⁶ cells without
 The hematologic response is achieved with the normal—
 quantifying the level of transcripts. Nested R-PCR is useful
 in the absence of peripheral blood counts in the presence of signs
 when other test results (including real-time PCR) become
 symptomatic. The cytogenetic responses are classified
 negative. In clinical practice, qPCR (Applied Biosystems
 on the basis of the proportion of residual Ph-positive et-
 qPCR (Foster City, California) or LightCycler (LightCycler,
 phases redefined as complete (0% of et phases), partial
 Roche Diagnostics Corp. In interim, interim) real-time
 (1% to 33%), minor (34% to 66%), or initial (67% to 99%).
 PCR is now largely replacing the nonquantitative real-time
 A major response represents the sum of the complete
 partial cytogenetic responses. Molecular remission is traditionally defined on the basis
 of the detection of residual BCR—

Leukemic Burden Response

ABL mRNA transcripts by quantitative R-PCR.

hematologic remission

Resistance in Chronic Myelogenous Leukemia

1012

Resistance has been observed in proportion of patients over

variable periods of imatinib monotherapy. Patients in the

chronic phase have responded to imatinib therapy or more

2 logs 1011

cytogenetic remission Ph positive 0%

within 2 years, but most responding patients in blast crisis year

1010

experience relatively despite continued therapy. Clinical

echenisms of imatinib resistance can be divided into two

groups: (1) reactivation of BCR-ABL with continuing open—

4 logs 109

molecular remission PCR positive

ence on BCR-ABL signaling (2) reinitiating inhibition

108

but BCR-ABL signaling is bypassed by alternative signaling

pathways. In the latter case, resistance may be overcome by the

107

evolution of the disease with occurrence of novel numeric or structural cytogenetic aberrations [e.g., trisomy 8, iso(17q)],

106

which lead to BCR-ABL–independent proliferation of leukemic cells.

105

molecular remission PCR negative

The development of imatinib resistance presents new therapeutic challenges. The fact that BCR-ABL is active in

104

many imatinib-resistant patients suggests that the chronic oncoprotein reinsertion is a target. Knowledge of

103

the mutations should permit the development of new strategies to

102

detect drug-resistant clones before clinical relapse occurs.

101

Leukemia-Specific Targets

10

Leukemia-specific targets may involve leukemia-specific

peptides that integrate -versus-leukemia. In

CML, the only unique molecule is the one encoded by the

FIGURE 23.8 Schematic illustration of therapeutic response of

CML patients on cytogenetic molecular level. (From Sergio G.

novel constructs the b2-2 or b3-e junctions in the BCR-ABL

Measuring molecular response in CML: problems and significance,

proteins. Evidence produced by mass spectrometry validates

8th EHA Congress, June 2003.)

that CML cells do express this leukemia-specific peptide

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PART 6 ■ Neoplastic Disorders

human cells can kill fresh CML cells in an HLA—

Inclusion of donor cells that are initially resistant

restricted peptide-specific manner. This makes it possible

these may be required within the first year of transplant.

to design CML-specific oligopeptides that are presented in

Newer understanding of the immune effect has led to

conjunction with HLA class I or class II molecules that they

the development of more intense conditioning or transplant in humans. Vaccination of solid tumor-

pl nt tion th t ocuses on i unosuppression o the host

ber o p tients with junction-specif c pepti es h s pro uce

r ther th n yelo bl tion. Engr ent o onor he top rti l or co plete he tologic l response in p tients

poietic cells c n t ke pl ce without necess rily er ic ting

with CML. T e v ccin te p tients envelope el ye -type

ll the host popul tion o cells. Despite the benef ts, llo-SC

hypersensitivity re ctions n CD4+ ly phocyte proli er -

is proce ure with consi er ble proce ur l-rel te ortive response. T is ppro ch to tre t ent y be v lu ble in

t lity n chronic orbi ity (e.g., chronic gr -versus-host

suppressing proli er tion o leuke i cells in p tients with

ise se or so e long-ter survivors).

ini l resi u l ise se.

When CML p tients h rbor ini l resi u l ise se, n

Chro nic Ne utro philic Le uke m ia and Atypical

ppro ch is to try to in uce i unity to ntigens known

to be overexpresse in leuke i cells. Hu n cytotoxic

Chro nic Mye lo id Le uke m ia

cells (C Ls) c n kill Ph-positive CD34+ progenitor cells.

CNL n typic l chronic yeloi leuke i (CML) re r re

The use of C-lysine directed against the Pr-1 component of myeloid leukemia is with poor prognosis. Recently, ut-proteinase 3 is also promising. Proteinase 3 is serine proteases in colony-stimulating factor 3 receptor (CSF3R) have been shown to be involved in cell differentiation and have been discovered to have high frequency in CNL and lower frequency in myeloid leukemia. It is overexpressed in myeloid leukemia. The development of CML could be caused by initially, blood reservoirs of patients with CML and CML selective deletion of Pr-specific C-lysine clone; this supports the hypothesis that both display the theory that breaking specific tolerance could be a fundamental feature of granulocytosis-pro myelocytes, myelocytes, and neutrophils or patients with initial residual neutrophils. However, there are distinct differences between the chronic phase of CML, CML, and CNL (see Table 23.4).

Allogeneic Bone Marrow Transplantation

In comparison to CML, CML and CNL have no distinct phases of disease, but they frequently demonstrate—

A long-standing universally accepted therapy for CML is the phase association with an increase in total leukocyte is allogeneic stem cell transplantation (allo-SCT). Allo-SCT count an increase in number of myeloblasts.

achieves its curative potential via the following mechanisms:

CML patients have a median survival of 25 months.

1. Myeloblast reduction induced by high-dose chemotherapy

Shorter survival time is associated with greater than

2. Allogeneic graft-versus-leukemia (GvL) effect in the

65 years of age, elderly, and high total WBC count greater

by monocytes

than $50 \times 10^9/L$ with the presence of immature granulocytes.

Major Characteristics of Chronic Myeloid Leukemia (CML), Atypical Chronic Myeloid

23.4 Leukemia (aCML), and Chronic Neutrophilic Leukemia (CNL)*

Specimen

CML (Chronic

Tested

Observation

Phase)

aCML

CNL

Blood

Total leukocyte count

Not determined

$>13 \times 10^9/L$

$>25 \times 10^9/L$

Myeloblasts

$<2\%$

$<20\%$

$<1\%$

Promyelocytes, myelocytes,

$\geq 10\%$

$\geq 10\%$

$<10\%$

metamyelocytes

Neutrophils and bands

Not determined

Not determined

$>80\%$

Basophilia

Present

Minimal or <2% of leukocytes

Not determined

Bone marrow

Granulocytic hyperplasia

Present

Present

Present

Granulocytic dysplasia

Minimal or absent

Prominent

Minimal or absent

Megakaryocytic dysplasia

Usually present

May or may not be present

Minimal or absent

Molecular

BCR-ABL or variant transcripts

Present

Absent

Absent

*Modified from 2008 WHO Diagnostic Criteria.

CHAPTER 23 ■ Myeloproliferative Neoplasms

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Chronic Eosinophilic Leukemias

o white blood cells, such as neutrophils or stem cells.

Occasionally, patients with PDGFRA-associated chronic

Among the hypereosinophilic syndromes (HESs), there is

eosinophilic leukemia develop other malignancies such as

continuum of hypereosinophilic disease. At one end of the

cutaneous leukemia or B- or T-cell cutaneous lymphoproliferative

spectrum is the eosinophilic MPN, eosinophilic leukemia—

leukemia or lymphoproliferative lymphoma.

At the other end of the spectrum are benign conditions such as parasitic infection, asthma, allergies, or drug

Chronic Eosinophilic Leukemia, Not

hypersensitivity.

Otherwise Specified (CEL-NOS)

PDGFRA-Associated Chronic Eosinophilic

This clonal MPN presents with eosinophilia not classically

Leukemia

few other neoplastic conditions. This condition lacks

PDGFRA, PDGFRB, and FGFR1 mutations.

PDGFRA-associated chronic eosinophilic leukemia is rare

CEL-NOS is defined by the following criteria :

condition. The exact incidence is unknown. Most are

likely to develop this or other leukemia than these.

- Total peripheral blood eosinophil count $\geq 1.5 \times 10^9/L$

The bone marrow is hypercellular because of eosinophilic

that persists over time

proliferation can exhibit Charcot-Leyden crystals. Bone

- Evidence of clonality or greater than 2% myeloblasts in

marrow fibrosis exists due to the release of eosinophilic basic

peripheral blood 5% to 19% myeloblasts in the bone

protein and eosinophilic cationic proteins from the eosino—

marrow

phil granulocytes.

- Total peripheral blood leukocyte count greater than $30 \times$

The presence of myeloblasts is less than the 20% threshold

$10^9/L$ with 30% to 70% eosinophils

that it is necessary to classify the conditions of cutaneous leukemia.

CEL-NOS is excluded by the presence of:

Erythrocytes and leukocytes are normal in number but
have distinctive morphologic features.

- All secondary causes of eosinophilia

New molecular and immunologic assays have enabled

- Neoplastic disorders with secondary eosinophilia

more definitive etiologic classifications, especially those cases

- Aberrant phenotypic T-cell population

associated with eosinophilic MPNs. PDGFRA-associated

- Neoplastic disorders when eosinophils represent the neoplasm—

chronic eosinophilic leukemia is characterized by mutations in the

plastic clone such as cutaneous T-cell lymphoma

PDGFRA gene. As a result of this mutation, a normal

- PDGFRA, PDGFRB, or FGFR1 mutations

protein known as FIP1-like 1/plalet-derived growth factor

- Chromosomal abnormalities: inv(16)(p13.1q22) or

translocation is synthesized.

t(16, 16)(p13.1;q22) or other diagnostic features of cutaneous

A characteristic feature of PDGFRA-associated chronic

myelogenous leukemia

eosinophilic leukemia is organogenesis by the excess

■ BCR-ABL1 fusion gene

eosinophils. Bone marrow fibrosis contributes to the pre—

ture release of eosinophils into the peripheral circulation

and subsequently deposit in various tissues. Eosinophils

NOTE: This is a good time to complete Review Questions

release substances to it in the immune response, but the

related to the preceding content.

release of excessive amounts of these substances causes

damage to one or more organs, most commonly the heart,

skin, lungs, or nervous system. Eosinophil-associated organ

POLYCYTHEMIA VERA, ESSENTIAL

can lead to heart condition known as eosinophilic

THROMBOCYTOSIS (ESSENTIAL

erythrocytosis).

THROMBOCYTHEMIA), AND PRIMARY

The criteria for diagnosing PDGFRA-associated

MYELOFIBROSIS

chronic eosinophilic leukemia is:

Polycythemia rubra vera PRV, essential thrombocytosis

■ A total peripheral blood eosinophil count of $\geq 1.5 \times 10^9/L$

or **essential thrombocythemia** (ET), in PMF were identical—

that persists over time

and is pathogenetically related to myeloproliferative disorders

■ Bone marrow hypercellularity and fibrosis

in 1951.

■ No parasitic infection, allergic reaction, or other causes of

Subsequently, PRV, ET, and PM were identified as clonal

eosinophils

disorders of multipotent hematopoietic progenitors. In 2005,

■ Elevated blood levels of vitamin B12 and tryptase

suggestive of activation in JAK2 kinase (JAK2) nonre-

■ Splenomegaly

ceptor K (JAK2V617F) was identified in most patients with

Patients with PDGFRA-associated chronic eosinophilic leukemia—

PRV and in significant proportion of patients with ET and

who are exquisitely sensitive to imatinib mesylate should

PM (table 23.5).

receive first-line therapy. Hematologic remission occurs

Subsequently, mutations in JAK-S A p th—
 within myeloid cell reversion is almost universal.
 Why in some patients with JAK2V617F-MPN suggests that
 Some patients with PDGFRA-associated chronic eosino—
 constitutive activation of this signaling pathway is uniquely—
 philic leukemias have an increased number of other types
 including these disorders. JAK2 is nonreceptor protein
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PART 6 ■ Neoplastic Disorders

TABLE

23.5 Genetic Assessment in BCR-ABL–Negative MPNs

Category

Genetic Feature *

Comments

Polycythemia rubra vera (PRV)

JAK2 100% positive

JAK2 allele burden linked to prognosis and transformation
 to acute leukemia

Essential thrombocytosis/

JAK2 60%–65% positive

Higher rate of thrombosis in J AK2 positive ET
thrombocythemia (ET)

CALR 20%–30%

Higher platelet count and higher rate of brotic transfor—
MPL 3%–5%

mation in CALR positive ET

Triple negative 5%–10%

Survival rate is longest for triple negative and shortest for
MPL negative mutated patients.

Primary myelo brosis (PMF)

J AK2 60%–65% positive

CALR+ associated with younger age, indolent disease, and

CALR 20%–25%

better survival than J AK2 positive PMF

MPL 5%–8%

Greater risk of thrombosis in J AK2+ PMF

Triple negative 5%–10%

Triple negative status linked to inferior outcome and
increased risk of transformation to acute leukemia

Mastocytosis

c-KIT frequently positive

Chronic neutrophilic leukemia (CNL)

CSF3R positive

Chronic eosinophilic leukemia (CEL)

No distinctive genotype

Myeloproliferative neoplasm—

unknown (MPN-U)

*JAK2 and CALR are usually mutually exclusive mutations.

located on the cytoplasmic inside of the cell membrane

Etiology

functions to transfer signals from the cell surface to the

nucleus. JAK2V617F mutation is an important marker to

PRV is clonal stem cell disorder characterized by hyperp—

segmented platelets with high RBC, hemoglobin, hemato—

crisis erythrocytosis, leukocytosis, and megakaryocytic

crits into those with PRV and those without this disorder.

lineages. Two distinct phenotypes exist in PRV patients: s

The V617F mutation causes the substitution of phenylalanine

the result of gene profiling in stem cells with cytokine p—

nine or variant position 617 of the Janus kinase (JAK)2

ping. The cDNA or polycythe i rubr ver -1 (PRV-1), gene (JAK2). This ut tion is o en present in PRV, E , novel he topoietic receptor, w s recently clone by vir— n IM. The olecul r b sis is uncle r, but this serves s tue o its overexpression in p tients with PRV. Northern the rese rch oun tion or evelop ent o s ll olecule blot n lysis showe th t PRV-1 is highly expresse in nor— inhibitors o JAK2.

l hu n bone rrow n to uch lesser egree in et l liver.

POLYCYTHEMIA VERA

Pathophys io lo gy

Sir Willi Osler f rst escribe polycythe i rubr ver

Although PRV is clon l he topoietic progenitor cell is- (PRV) in 1910. The clinic l escription w s th t o p tient or er with triline ge hyperpl si , the ost const nt n with engorge veins, plethor , n n elev te re bloo cell striking e ture is erythroi hyperpl si o the bone rrow. count. Leukocytosis n thro bocythe i were recognize This very slow evolution o the lign nt erythroi clone

sition lectures. In 1951, Deshek e PRV to the
leads to overexpansion of the red cells, hypervolemia,
classification of MPNs. PRV is rather common, seen
in splenomegaly red cell pooling. These consequences
the inevitable diagnosis is that of reactive erythrocytosis—
eventually cause generalized hyperplasia with subse-
quent increases in the quantity of all three cell lines.
Abnormalities in PRV erythroid progenitors re-express

Epidemiology

at the level of both the colony-forming unit, erythroid
(CFU-E), and burst-forming unit, erythroid (BFU-E),
PRV has an incidence of 2.3 per 100,000 people. The age in
which suggests multiple changes in the erythroid progeni-
togenesis is approximately 60 years, with slight le-
tters. Abnormalities in the cell compartment occur in PRV.
prevalence. Exposure to radiation, benzene, and petro-
Interleukin-3 (IL-3) stimulates multilineage hematopoiesis, but
leukemias see a to increase risk.
striking hypersensitivity of PRV BFU-E to recombinant

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IL-3 has been noted. This may be a factor in the pathogenesis of increased erythropoiesis without increased eryth—

BOX 23-2

erythropoietin concentrations.

In addition, the magnesium and zinc contents of the physiologically active erythrocytic microelements are altered.

Diagnostic Criteria for Polycythemia Vera

disturbances in erythrocytes of the peripheral venous blood.

MAJOR CRITERIA

in patients with PRV. These changes include the neoplastic

1. Hemoglobin greater than 18.5 g/L in males, 16.5 g/L

characteristic of proliferation of bone marrow cells in PRV.

in females, or other evidence of increased red cell

volume

Karyotype

2. Presence of JAK2V617F or other functionally significant

Chromosomal alterations are found in only approximately

mutations, for example, JAK2 exon 12 mutations

15% of patients, but there is no consistent chromosomal

MINOR CRITERIA

bone marrow is found in CML. A newly described gene,

1. Hypercellular bone marrow with prominent granulocytes—

PRV-1 gene, has been described in neutrophils of patients

myelocytic, erythrocytic, and megakaryocytic proliferation

with PRV, but the significance of this gene is still uncertain.

2. Decreased serum erythropoietin levels

During the first 10 years of disease, approximately one

3. Endogenous erythroid colony formation in vitro

fourth of patients demonstrate bone marrow clone; however

within 10 years, greater than three fourths of patients exhibit

a bone marrow clone. Patients with chromosomally abnormal

clone at the time of diagnosis have poorer chance of sur-

Laboratory Data

vival than those exhibiting normal karyotype in peripheral

cells. Cytogenetic results do not predict evolution of the disease—

An increase erythrocyte cell count, packed cell volume, and

hematocrit but do provide clues to the hematologic phenotype,

hemoglobin with normal erythrocytic indices recur during the disease, and
consequences of leukosuppression—

characteristic of PRV. Red cell proliferation is thought to be insensitive therapy.

penicillin-dependent endogenous erythropoietin, and hence, serum levels of erythropoietin are usually decreased. A genetic

Clinical Signs and Symptoms

Alteration of the erythropoietin receptor is thought to create the loss of regulatory function in bone marrow erythroid pro-

Plethora is the hallmark of PRV. Splenomegaly is com-

mon. Plasma levels of erythropoietin (the growth colony-forming unit assay; it occurs in more than three

thousand erythrocyte production of platelets) are elevated

in patients. Reversible, moderate hypertension is not infrequently associated with the loss of the normal negative feedback

mechanism. frequently occurs as the result of the expansion of blood volume.

An increase in total blood volume (hypervolemia) occurs in

Polycythemic values are hemoglobin approximately

PRV in disorders such as congestive heart failure, pri-

tarily 20.6 g/L, hematocrit approximately 80%,

hypercortisolism, and Cushing syndrome result

total leukocyte (white blood cell [WBC]) count of $28,000 \times$

overtransfusion of donor blood.

$10^9/L$, and platelet count of $1,400 \times 10^9/L$.

Neurologic symptoms are reported by 50% to 80% of patients with PRV, as in those with the other diseases, patients. Symptoms such as headaches, paresthesia—the red blood cell distribution width (RDW) tends to be similar, and slight alterations are frequently related to hyperviscosity rather than normal. The RDW transiently increases following administration of cytoreductive agents, corresponding except in acute patients. Other neurologic symptoms seen during the transition period from microcytes to normal blood result from associated coagulopathy.

cells. The RDW is even higher during polycythemic periods. The most serious complications are cerebral venous thrombosis during the hypofibrinolytic period. They are associated complications (vascular accidents) in the transition to with hematopoietic bone marrow failure by extramedullary hematopoiesis. PRV, sickle cell disease, sickle cell–hemoglobinopathy. RDW seems to reflect accurately the pathophysiology of PRV.

major disorders of the blood elements causing stroke.

Not all patients with a elevated red cell count have PRV.

Hemorrhagic phenomenon are frequent among patients with

Various tumors are known to result in a elevated red cell

digestive neoplasms, including gastrointestinal hemorrhages. Renal cell carcinoma is a hepatic tumor

thrombogenic, portal vein thrombosis, or thrombo-

phlebitis or creating erythrocytosis. Some produce exogenous

erythropoietin. In addition, thrombophlebitis

of the suprarenal vein. The most common secondary cause of

erythrocytosis is congenital polycythemia

is cigarette smoking. Smokers have elevated

PRV and is unrecognized.

plasma levels of carbon monoxide. Carbon monoxide is—

Severe psychotic depression is rare in patients with PRV.

plasma oxygen red cell hemoglobin, resulting in tissue—

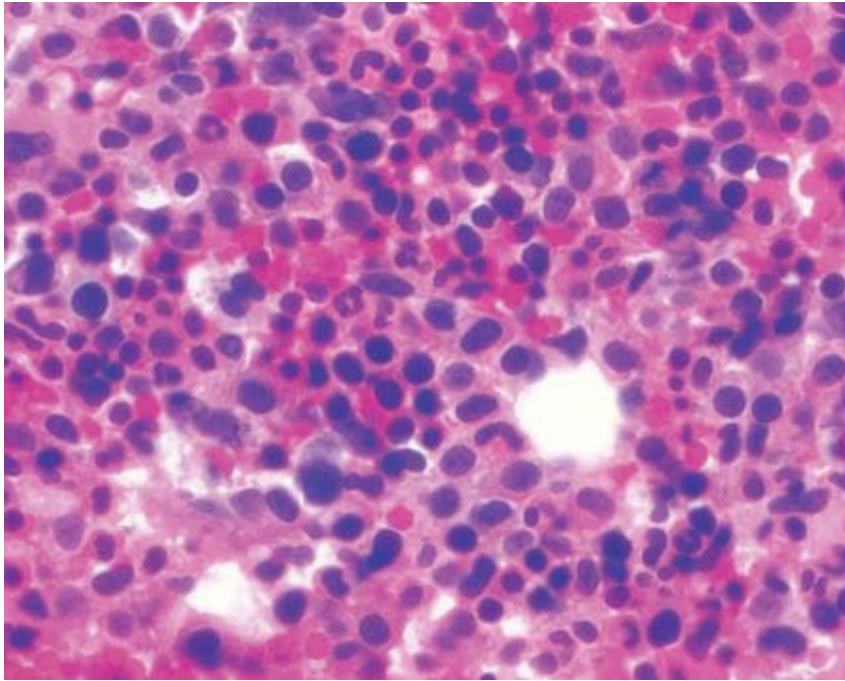
In venography—Bucher's syndrome, the

systemic hypoxia is elevated red cell production.

underlying diseases include PRV. The criterion for the diagnosis of smokers' erythrocytosis is most commonly distinguished

from PRV is presented in Box 23.2.

no true PRV by the observation that patients usually have



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PART 6 ■ Neoplastic Disorders

normal values or leukocytes in platelets. The end corpus—

Complications of PRV include thrombosis and proxi—

cul volume is usually within normal limits (patients with

clinical hemorrhage. The thrombosis seems to be related to the

PRV usually have low end corpuscul volume because of

height of the red cell volume with subsequent increase in

iron depletion of iron resulting in microcytic red cells).

blood viscosity. Whole blood viscosity pursues rather line r

Serum erythropoietin levels are normal or elevated because

rather to rise, most physicians prefer to keep the patient's
 on increase red cell production. The serum erythropoi—
 hemocrit below 45%. Increase whole blood viscosity con—
 duct level can often be used to distinguish this from PRV.
 contributes to vascular occlusions and reversible lesions, inclu—
 An elevated hemocrit above the level of 50% or greater
 indicating cerebral hypoxia and shortness
 in the absence of hypoxia is highly suggestive of the
 obstructive pulmonary disease, probably caused by circulatory ischemia, and hemocrit greater
 than 60% or even over 56%
 turbulence. Patients with blood viscosity higher than twice
 or more is consistent with an elevated red cell mass. The
 the normal value may be indicative of vascular occlu—
 oxygen saturation should be normal, and in the patient is
 sion. A correlation has been revealed among the parameters
 smoker, the carboxyhemoglobin level should also be normal.
 of red blood cell rheological properties, hemostasis, and is—
 Lymphocyte populations in patients with PRV e—
 severity.
 monitoring the CD4/CD8 ratio, only because of
 In some cases, disorders in the rheological phenomenon

decrease CD8 subpopulation. Increase lymphocyte activation—
red blood cells are triggering mechanisms in the development—
it has also been observed. Interleukin-2 (IL-2) production
of the DIC syndrome.

is significantly higher; the lymphoproliferative response both

In the chronic phase of PRV, patients with thrombocytopenia—
to phytohemagglutinin IL-2 is also greater in lympho—
proliferative complications have higher platelet counts, more
cytes for PRV patients. These observations suggest that
severe platelet aggregation effects, an increase in platelet lev—
patients also suffer from lymphoid lineage.

also about thromboglobulin and brinopeptide A compared

The bone marrow is hypercellular (Fig. 23.9) with
with patients who do not have complications. However,
increase production of all three cell lines, especially the re
thrombocytopenic complications are not predictable by
cell series. Some investigators believe that bone marrow
changes in these parameters in individual patients during the
examination is not necessary for diagnosis. Others believe
chronic disease phase.

that the bone marrow histology should be examined.
The platelet level, tissue platelet-inocultured antigen
cytogenetic analysis or the BCR-ABL mutation should be
(t-PA-Ag) is significantly increased in patients with PRV
performed. An occasional patient with CML can present
compatible with healthy individuals. In contrast, patients
with erythrocytosis, although this is distinctly unusual.
with spurious polycythemia in secondary polycythemia
exhibit significantly increased concentrations of t-PA-

Abnormalities of Hemostasis and

Ag. There is no significant difference in t-PA-Ag levels in

Coagulation

polycythemic patients with or without thrombotic
events.

Patients with PRV frequently demonstrate complex
hemorheologic disorders (high blood viscosity, etc.)

Other Laboratory Assays

reticulocytosis, intensified red blood cell aggregation, an
increase in deformability of these cells) on hemocytation
Erythropoietin excretion in the urine is increased in

isorders.

PRV, in contrast to the other kinds of polycythemia.

Recombinant erythropoietin has been used to distinguish between PRV and other forms of erythrocytosis.

Laboratory findings that support diagnosis of PRV compared

with other forms of polycythemia in the absence of

hemoglobin in the bone marrow and increased LAP

score. In addition, hyperuricemia and hyperuricosuria

are present in more than half of PRV patients that diagnosis because of excess nucleic acid excretion. The level of

uric acid parallels increases in severity of PRV as the disease

progresses.

Thrombocytosis also seems to be related to both the risk

of thrombosis and hemorrhage. The level of thrombocytosis

seems to be related, and most hematologists prefer to keep

the platelet count below 400,000. Quantitative abnormalities

of platelets also might contribute to PRV complications.

Abnormalities in platelet responsiveness to naturally occurring

FIGURE 23.9 Bone marrow clot section of PV in the proliferative

stage showing hypercellular marrow with marked erythrocytosis

and platelet inhibitors such as prostaglandins, increased

hyperplasia. (From McClatchey KD. Clinical Laboratory Medicine, levels of thrombocytes (in users of platelet aggregation), 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins, 2002.)

normal levels of naturally occurring anticoagulants



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A Summary of Significant

Differences Between

TABLE

23.6 Polycythemia Vera and Other

Types of Polycythemia

Polycythemia

Primary

Other Types

Total blood volume

Increased

Normal or

decreased

Total leukocytes

Increased

Normal

Immature red blood cells Occasional

None

cells

Platelets

Increased

Normal

LAP stain

Increased

Normal

FIGURE 23.10 A 67-year-old woman with PV showing reticulocytosis in peripheral blood smear. (Reprinted from Golub et al.,

Erythrocyte

Decreased

Normal

Weingeist A. Color Atlas of the Eye in Systemic Disease, Baltimore, MD: Lippincott Williams & Wilkins, 2001, with permission.)

Serum iron

Decreased

Normal or

increased

reducing reticulocyte production but not the expense

Erythropoietin

Decreased or

Normal or

absent

increased

Chlorambucil also produces neutropenia—

neutropenia. Neither therapeutic options.

Blood histamine

Increased

Normal

Angrelife (Agrylin, Bristol-Myers Squibb, New York) is

Unsaturated

Increased

Normal

relatively new addition to the therapeutic arsenal. Angrelife

vitamin B12-binding

is prostaglandin synthetase inhibitor that also inhibits

capacity

erythrocyte production of platelets has little effect on

Basophil count

Increased

Normal

red cell production. There is no effect on myelopoiesis.

Hyperuricemia

Present or absent

Normal

Interferon has also been used to control tumor overpro—

uction but c uses high risk o u-like sy pto s, y lgi ,

Hyperuricosuria

Present or absent

Normal

tigue, n ever.

Pro gno s is and Com plicatio ns

(proteins C n S n ntithro bin III) h ve occ sion lly

been reporte n coul lso contribute to thro basis.

T e e i n surviv l ti e or untrete sy pto tic p tients

A co p rison o the l bor tory f n ings in PRV n other

er i gnosis is 6 to 18 onths. With tre t ent, the e i n

or s o polycythe i is presente in ble 23.6.

surviv l is ore th n 10 ye rs.

Cert in prognostic ctors n tre t ent str tegies h ve

Treatm ent

n e ect on surviv l. T e clinic l course o ost p tients

is ch r cterize by low r te o cute leuke i n high

Ph le botom y

r te (pproxi tely 40%) o thro boe bolic co plic -

Pri ry control o PRV is chieve by ther peutic phlebot—

tions. Myelofibrosis develops in some patients. A high intensity (Fig. 23.10). The iron phlebotomy is to produce

the hemoglobin concentration in peripheral blood the

iron deficiency that then limits red blood cell production.

use of myelosuppressive therapy associated with

This is better performed by the removal of units of whole

increase risk of leukemic transformation.

blood by large-volume erythrocytapheresis using cell

separator. Cytapheresis produces long-lasting reduction of

red blood cell volume (microhectrit), hemoglobin, and

NOTE: This is a good time to complete Review Questions

erythrocyte counts as well as the hematocrit is appropriate

related to preceding content.

or reduction of clinical symptoms.

The evolution of PRV is probably altered by therapeutic

phlebotomy and other peptic cytoreduction, which re

PRIMARY MYELOFIBROSIS

often performed simultaneously.

The term myelofibrosis encompasses primary myelofibrosis

Chemotherapy

(PMF), postessential thrombocythemia (ET), and postpolycythemia—

The intensity of cytoreduction of the platelet count is roughly
inversely proportional (PPF-MF). PMF is characterized by systemic
thrombocytopenia. Phosphorus 32 (P32) is an effective treatment in
bone marrow fibrosis and extramedullary hematopoiesis.

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PART 6 ■ Neoplastic Disorders

Secondary myelofibrosis is caused by infiltrative disorders,
Another contributing factor is the association of
including malignancies in infections, or exposure to che-
moterapeutic agents, including the use of
alkylating agents or irradiation.

megakaryocytes, in the development of the extramedullary
hematopoietic system as well as the occurrence of periploidy

Epidemiology

(i.e., infiltration of the hematopoietic cells) largely in in-
dustrialized countries.

Patients with myelofibrosis may undergo transformation or per-

A striking variety in the presentation of megakaryocytes
and transition to PRV or may convert to CML. Approximately
the lymph type also frequently exists. Thrombocytes show

one of the patients with PRV developed myelofibrosis. giant cells with either hypertrophy of the open canalicular PMF is uncommon, with the number of new cells estimated at 1,000 to 2,000 per year in the United States or in the general population. Other remarkable features include overall rate of 2 per 100,000 worldwide. The incidence of sponge-like proliferation of the open canalicular system myelofibrosis, however, is known to be increased exposure in many of the large platelet giant cell use granulose to irritation chemicals such as benzene. of the lymphonodiosiphilic type. These abnormalities in Although there have been few reports of patients in the megakaryocytes and thrombocytes have certain unique population, the majority of patients with PMF related complications (e.g., hemorrhage and thrombosis) that in their late 50s, 60s, and 70s. It is also more common in the more often encountered out of proportion to the platelet counts white population, and even women relatively affected. in this disorder.

Pathophysiology

In addition, those neoplasms in which the dysregulation of megakaryopoiesis, which may contribute to the abnormal PMF is clonal proliferation of the multipotent progenitor cell release factors (platelet-derived growth factor and PF4) component. The bone marrow barrier is disrupted early and predominantly involve in the process of myelofibrosis. It is in the course of myelofibrosis, so that blast cells may co-exist. It has been postulated that platelet-derived growth factor and interleukin-3 cells such as colony-forming unit, granulocyte—PF4 are involved in the inhibition of the mechanisms of myelocytogenesis (CFU-GM), BFU-E, and colony-forming unit, multipotential progenitor, which triggers the bone marrow megakaryocyte (CFU-Meg) cells escape into the circulating myelofibrotic process. A relationship between the presence of blood in large numbers.

Myelofibrosis is characterized by abnormal levels of beta-2-microglobulin—Sclerosis of the bone develops in about half of patients.

Finally, PF4, and mitogenic activity in platelet-poor plasma.

However, myelofibrosis, the predominant clinical features—

pl telet extr cts h s been observe in p tients with PMF.

t tion, occurs secon rily n is not co ponent o the

bnor l clon l proli er tion. T e process o f brosis ensues

Karyotype

ro proli er tion o f brobl sts n incre se coll gen pro—

Approxi tely 40% o p tients cquire recurrent cytogenetic

uction in re ction to the bnor l clone o he topoietic

bnor lities n ne rly 80% cquire nonspecif c berr tions.

cells. Fibrosis is prob bly the result o pro uct secrete by

Sever l chro oso l bnor lities re overrepresente

eg k ryocytes.

in p tients with yelof brosis. T ese lter tions involve the

I the constituents o the he topoietic icroenviron ent

long r o chro oso e 1; onoso y n p rti l eletion

(yeloi stro) re ex ine icroscopic lly, n over ll

o chro oso es 5, 7, 9, 11, n 13; loss o Y chro oso e;

incre se, p rticul rly in so-c lle un i erenti te (pri itive

n triso y o 8, 9, n 21. P rti l triso y 1q is k ryotypic

pluripotent) n lso in tr nsition l (f brobl stic) reticul r

ch nge etect ble in unsti ul te peripher l bloo cell cul—

cells in yolk blastoids, can be observed. Unlike in tissues or bone marrow cultures, which suggests that partial transition of reticular cells as well as yolk blastoids seen in trisomy 1q is primary chromosome aberration in yolk-blastoid or an integral part of the hematopoietic microenvironment. Myelofibrosis is relevant to the pathogenesis of this disorder. Interleukin-3 is essential to play an important role in the Karyotypic changes occur as secondary events during the evolution of disease-specific yolk fibrosis. In addition, the multistep process of leukemogenesis. Therefore, changes such as evolution of leukemic fibrosis is thought to be associated with t(5;17) represent therapy-independence non—with the striking predominance of large, typically, possibly not only related to the terminal phase of leukemic disorders. Overgrown hyperpolyploid megakaryocytes, but not with Myelofibrosis is driven in osteoclasts by at least three genetic increases in precursor cells.

Mutations: JAK2V617F, CALR, and MPL. It is further diversified—Dysmegakaryocytopoiesis leading to an overproduction of affected by the acquisition of other genetic and epigenetic aberrations—

effective platelets is the most constant feature of myelofibrosis.

mutations: ASXL1, ETV6, EZH2, IDH1/2, and SRSF2.

fibrosis. Research findings imply that the significant increase in circulating progenitor cells of the megakaryocyte lineage is a hallmark of myelofibrosis.

Clinical Signs and Symptoms

may be generated by extramedullary, probably splenic

hematopoiesis. One hallmark of the megakaryopoiesis

Patients with myelofibrosis usually exhibit progressive anemia—

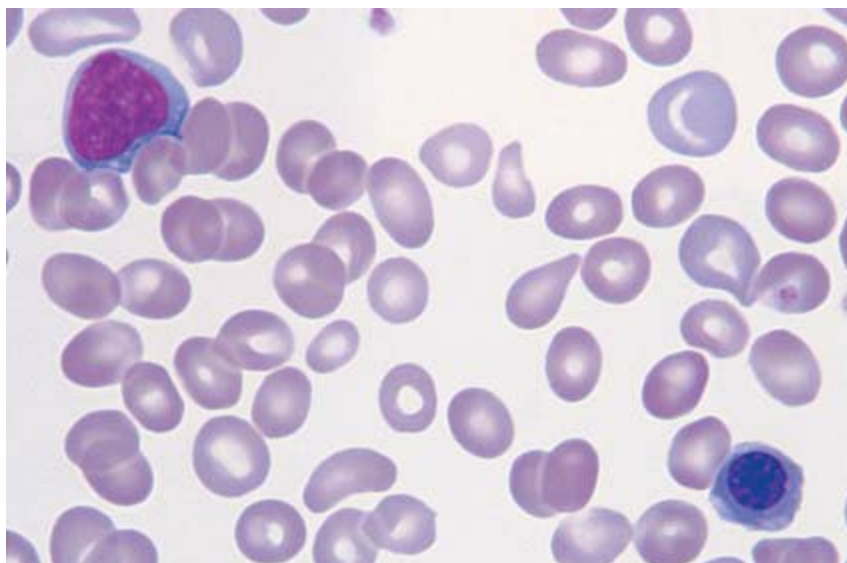
in bone marrow tissue, however, is pronounced pleomorphism, splenomegaly, and marrow fibrosis. Splenomegaly

is of the megakaryocytic cell line consisting of giant

and so-called teardrop-shaped cells by extramedullary

marrow, micro megakaryocytes, and naked (pyknotic) nuclei.

hematopoiesis. Patients may note easy bruising or bleeding



CHAPTER 23 ■ Myeloproliferative Neoplasms

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resulting from thrombocytopenia, abnormal platelet function, or both. About one third of patients have purpura.

More than 40% of patients have osteosclerosis with accompanying bone pain, anemia, leukocytosis. A small degree of joint aches, bone fullness, dyspepsia, or weight loss may be present in some patients. Portal hypertension may be evident.

In retrospect, one patient presented with breast lumps.

Excisional biopsy of the lumps revealed extramedullary hematopoiesis, similar histopathologic examination of the liver and the spleen. This type of presentation demonstrates the completely characteristic of both idiopathic conditions in the resemblance to lymphoma of the breast, although the

FIGURE 23.11 Typical peripheral blood smear of chronic pri-

marinings are too nonspecific to rule out breast carcinoma.

erythrocytosis with leukoerythroblastosis in the peripheral

blood cells. (Reprinted from McClatchey KD. Clinical Laboratory

Knowledge of the clinical history and histopathology is nec-

Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins,

essary to make the proper diagnosis. Another representative

2002, with permission.)

tion occurs in patients with cutaneous erythroid

he topoisomerase. The skin lesions present multiple patches—

Erythrocytes

ules and nodules on the trunk. Histologic examination

Milne is characterized by ineffective erythropoiesis (Fig. 23.11),

o lesion shows all three components of the he topoi—

ecreased red blood cell survival, increased hemolysis, and

etic tissue, that is, yellow, erythroid, and megakaryocytic

occur. Polychromatophils and elevated reticulocyte count

series.

in the absence of erythropoietic stress provide an important

clue to diagnosis because they significantly break down in re-

Cellular Alterations

row ultrastructure.

Histologic findings (Box 23.3) are variable and non—

uniform, but blood morphology provides the best clues

Leukocytes

to diagnosis. The leukoerythroblastic picture of the marrow. In approximately 50% of patients, the total leukocyte (WBC)

she erythrocytes, nucleated erythrocytes, neutrophils

count is increased. Most patients have total WBC counts less

than 30 × 10⁹/L, but the total WBC count can be as high as

100 × 10⁹/L. A high WBC count (neutrophilia) neutrophils

neutrophils, thrombocytosis, and polycythemia in the marrow

100 × 10⁹/L. A high WBC count (neutrophilia) neutrophils

are characteristic in the early stages. Extramedullary he-

matopoiesis, peripheral blood smear including blasts

topoiesis, peripheral cytopenias (i.e., neutropenia, leukopenia,

can create picture that can be confused with leukemia.

or thrombocytopenia), myelofibrosis, with or without

osteosclerosis, reflect the changes seen in the later stages.

Platelets

transitions among the different types of MPNs and in -

The concentration of platelets is variable, but giant splenomegaly in late leukemia or marrow failure is common.

Atypical platelets and megakaryocytes can be seen.

Thrombocytosis usually progresses to thrombocytopenia.

As myelofibrosis progresses, the entire morphologic picture

o yelophthisis (inf ltr tive yelop thy) un ol s: te r rop—

BOX 23-3

sh pe erythrocytes, nucle te erythrocytes, e rly gr nulocytic or s, biz rre pl
telets, n eg k ryocyte r g ents.

Diag no sis of Prim ary Myelo brosis*

Bo ne Ma rrow

MAJOR CRITERIA

T e bone rrow is hypocellul r n beco es f brotic with

1. Meg k ryocytic proli er tion with bnor l orphol—

n ssoci te ecre se in he topoiesis. Bone rrow spi—

ogy, usu lly cco p nie by reticulin n /or coll gen

r tion is unsucces ul in ne rly 90% o p tients bec use

f brosis

reticulin n coll gen f brosis lock in the rrow content,

2. Not eeting the criteri or other MPNs

c using ry t p. A bone rrow biopsy shows f brosis, gen—

3. Evi ence o JAK2V617F or other rel te ut tions

er lly with incre se nu bers o eg k ryocytes.

MINOR CRITERIA

1. Leukoerythrobl stosis

Pro gno s is

2. Anemia

The median survival time ranges from 4.3 to 5.0 years. In

3. Increased serum lactate dehydrogenase (LDH) levels

Patients with PMF, hemoglobin concentration, platelet

4. Splenomegaly

count, in the presence of osteosclerosis have been

*Diagnosis requires meeting all three or two minor criteria.

Identifies factors with prognostic significance. Patients

with hemoglobin concentration less than 10 g/L have

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PART 6 ■ Neoplastic Disorders

significantly shorter survival time than those with hemoglobin concentration greater than or equal to 10 g/L. A

BOX 23-4

platelet count less than $100 \times 10^9/L$ also implies significantly

shorter survival time is of prognostic significance within

the first 6 months of diagnosis. Patients with osteosclerosis,

Criteria for Diagnosis of Essential

thrombocytosis, demonstrated on radiograph of the skeleton, have

Thrombocytosis/ Essential

significantly better prognosis compared to those without

Thrombocytopenia*

osteosclerosis. The presence of osteosclerosis

1. Persistent elevation of platelets (less than $450 \times 10^9/L$)

emerges as a variable predictor in 3 to 5 years. Using

in peripheral blood

these three predictors and spleen size, prognostic scoring

2. Significant increase (hyperplasia) of megakaryocytes in

system has been designed; it categorizes patients into three

the bone marrow

prognostic groups with highly different survival times (low—

3. Not meeting criteria of other MPNs

risk group, 69 months; intermediate-risk group, 33 months;

4. Demonstration of JAK2V617F or relative uterine, or

high-risk group, 4 months).

In the absence of JAK2V617F, no evidence or relative

In addition, for thrombotic complications that

thrombocytosis, or, for example, in patients

contribute to shortened survival times are seen in approximately

one-fifth of patients.

*Diagnosis requires meeting all three or two minor criteria.

Generally, chronic phase of MF is characterized by either peripheral blood or bone marrow blast count less than 10% compared to the accelerated phase of 10% to 19% nonreactive phenotype, secondary to variety of systemic the blast phase with $\geq 20\%$. Patients in the blast phase have conditions, or they represent essential thrombocythemia—poor prognosis estimate 3 to 5 months. treatment is, primarily disorder of the bone marrow.

with induction regimens does not improve outcomes, unless it is followed eventually by the topoisomerase II inhibitor

Diagnostic Characteristics

transplantation.

The diagnosis of essential thrombocythemia is difficult

Treatment

relies on the exclusion of other myeloproliferative disorders and nonhematologic illnesses associated with an increase

Asymptomatic patients require no treatment. treatment of

concentration of platelets. Major criterion usually for

myelofibrosis can consist of periodic transfusions of platelets

ings indicate in essential thrombocythemia is represented

red blood cells, leukocytes, cytotoxic agents, and platelets

in Box 23.4.

reduction by plateletpheresis. Administration of prophylactic antibiotics may also be considered. Recombinant inter-

Epidemiology

erythropoiesis may be efficacious when used in the cell (i.e., proliferative) phase but less so when the marrow is fibrotic.

Essential or primary thrombocythemia (essential thrombocythemia or osteosclerotic. Most treatments or intervention therapy to thrombocytosis) is the least common MPN. Essential thrombocythemia—the spleen have been effective in controlling symptoms.

thrombocythemia occurs most frequently among persons in the

However, clinical improvement after irradiation is slow,

although six decades ago. Men and women equally

growth process.

ect.

Splenectomy may be appropriate in some circumstances

(e.g., massively enlarged spleen). Splenectomy in patients

Pathophysiology

with leukofibrosis is associated with nonoperative mortality rate of 13.4%, nearly orbital rate of 45.3%, and

Essential thrombocythemia is clonal disorder of utility rate of 16.3%. Almost all patients with port

potent cell origin belongs to the MPNs that include

hypertension in peripheral splenomegaly, but only about half

PRV, CML, and PMF. This disorder includes

those with thrombopenia, have experience

cutaneous hemorrhagic diathesis and thrombotic

relapsing symptoms or signs after splenectomy. There is no

events. Both thrombocytosis and platelet dysfunction can be

evidence that splenectomy does survive in myelofibrosis.

responsible for the thrombotic hemorrhagic phenomenon

Splenectomy in patients with myelofibrosis is

indicated by patients with this disease. However, qualitative platelet procedure that carries substantial risk.

Let's remember that in thrombocytosis we believe to

be the incidence of thrombotic events.

ESSENTIAL THROMBOCYTOSIS/

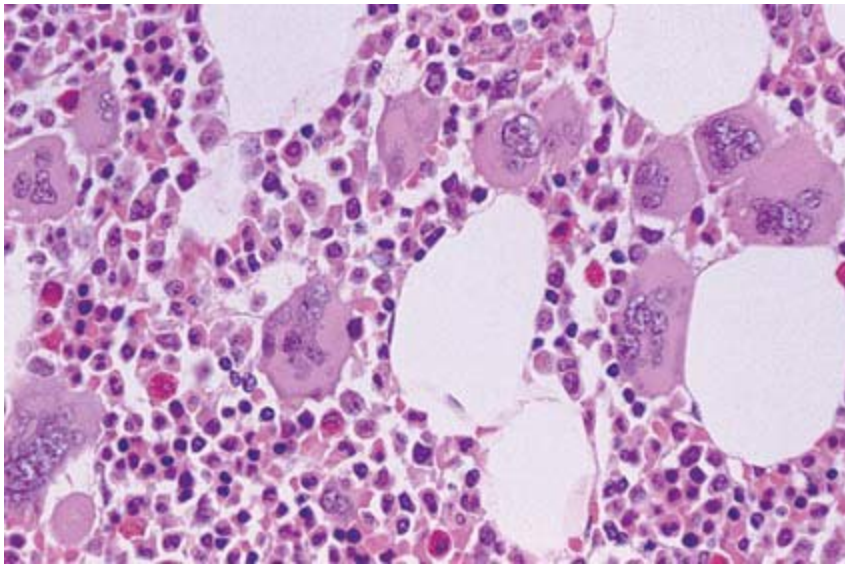
Karyotype

ESSENTIAL THROMBOCYTHEMIA

At least three-fourths of patients have normal karyotype

Essential or primary thrombocythemia is characterized by

type. The balance of patients demonstrates variable chromosomal abnormalities, with a significant increase in circulating platelets, usually in excess of $1,000 \times 10^9/L$. Elevated platelet counts may be encountered in the following conditions.



CHAPTER 23 ■ Myeloproliferative Neoplasms

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Clinical Signs and Symptoms

Thrombotic or bleeding problems are the most commonly seen disorders in patients with thrombocythemia. Patients typically manifest easy bruising, nosebleeds, or gastrointestinal bleeding. Splenomegaly is found in less than half of patients.

Neurologic limitations, however, are frequent and
are caused by obstruction of the cerebral microvasculature.
Cerebral ischemia in digital ischemia or even gangrene
relent or respond completely to reduction of platelet levels.
In addition, unexplained hemorrhage may occur.

A benign or reactive hemorrhagic or thrombotic presen-

FIGURE 23.12 Bone marrow biopsy of essential thrombocythemia
tation can be observed in subset of patients greater than 15
with megakaryocytic hyperplasia. The megakaryocytes are clustered—
to 25 years.

ing may show characteristic morphology: large size and hyperlobate nuclei. (Reprinted
from McClatchey KD. Clinical Laboratory

Laboratory Findings

Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins,
2002, with permission.)

Cellular abnormalities include

The classic laboratory finding in essential thrombocythemia
is significantly elevated peripheral platelet count.

Relationship of Thrombocythemia and PRV

The number of platelets in the circulating blood is usually
The same in events responsible for initiating thrombocythemia—

in excess of $1,000 \times 10^9/L$, with initial of $600 \times 10^9/L$.

in PRV clones are unknown. Both clonal disorders are

Plasmodium morphology reveals normal isochromosome cell;

marked by low-grade hyperproliferation of two cell lines

bleeding time is normal. In addition, pseudohyperkalemia

in cell lines plus significant stimulation of their cell

growth resulting from the preparation of serum. Potentially

line. These two disorders are differentiated by single chromosome analysis is not releasing the aggregation phase but

characteristic—the absence of an expansion of blood cell

during the erythropoietic phase of the coagulation process.

in thrombocytopenia.

Peripheral blood erythrocytes are frequently hypochromic—

The untreated cell in both disorders has pre-erythro-

cytic microcytic. Splenic trophy is present, but normal

sition to undergo transformation to either myelofibrosis or

erythrocyte morphology includes target cells, Howell-Jolly

cells leukocytes. The similarities in the natural history of

both, nucleated erythrocytes, nucleated cells. The total

these MPNs suggest that they both begin as very similar, plus—

concentration of leukocytes is elevated in about 50% of

ri-potent stem cell disorders express differently only in the patients but seldom exceeds $40 \times 10^9/L$. The LAP value is colony-forming cell level.

normal or increase. Concentrations of vitamin B12 and uric acid usually increase.

Treatment

Platelet Function

The course of the disease is rather benign and resembles that in patients with thrombocytopenia, the extent of aggregation—of PRV. It may evolve into another form of MPN in some patients. Aggregation in response to epinephrine, collagen, or ADP is significantly decreased in acute leukemia.

is usually lower than in normal controls. In order to help the pheresis has been used in a variety of clinical studies, patients with thrombocytopenia, the platelet-rich plasma primarily for its ability to remove nonclotting component, does not respond to epinephrine. The total cellular content is likely to be either plasma or cellular elements. Therapeutic plasma exchange is also significantly lower.

the pheresis is useful in certain clinical conditions, but

judicious application should be considered.

Bone Marrow

Alkylating agents and radioactive phosphorus (^{32}P) re-

Bone marrow morphology in essential thrombocythemia

effective treatments, but these agents associate with an

(Fig. 23.12) is similar to the architecture seen in PRV and

increase risk of leukemia in other neoplasms.

CML with association of extreme thrombocytosis. However, sig-

Although treatment of the symptomatic patient with plate-

nifc differences are observable between the marrow of non-

let-lowering agents or antiplatelet drugs may be indicated in

ings in MPN and those in extreme reactive thrombocytosis.

effective, the role of therapy in the symptomatic individual

These differences include the numbers of megakaryocytes,

remain highly controversial. No remarkable differences have

the presence or absence of megakaryocyte clusters, staining

been seen in the treatment of MPNs except for the evaluation—

iron, cellularity, and reticulin content.

ent of antiplatelet drug, and granulocyte. This agent seems to

In addition to increase marrow cellularity (hyperplasia),

be highly effective in controlling thrombocytosis. The relative
erythrocytic hyperplasia is striking. This conspicuous
erythrocytosis goes along with intertricular lymphoma in the
erythrocytic proliferation also includes polyploidy of
impairment of this agent on the survival time on the quality of
the nuclei, giant cells, and clusters.

Patients with MPNs have yet to be defined.

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PART 6 ■ Neoplastic Disorders

MASTOCYTOSIS

exists. The dysfunction appears to be loss of regulatory
signals that control the production of these cells.

Mast cells are effector cells of inflammatory allergic reactions. These cells secrete a
variety of vasoactive and proin-

Chronic Myelogenous Leukemia

tory elements in their granulocytes and high-nitrogen

■ CML is one of the most common forms of chronic leukemia—

IgE-binding sites.

like it. The disease course is characterized by chronic,

Mastocytosis is unique in that it is a neoplasm defined by

involvement of the bone marrow that frequently transforms into terminal—

normal expansion and accumulation of clonal stem cells
in late blast crisis phase. Accelerated phase, when
(MCs) in one or multiple organs such as the skin,
patients become refractive to traditional therapy, especially pre—
bone marrow, spleen, and gastrointestinal tract.

characterize the acute phase.

The WHO classification of stocytosis is divided into

■ The Philadelphia chromosome, Ph1, was the first aberrant
three variants: cutaneous relative skin lesions, systemic
chromosome describe in longitudinal series. It is the
stocytosis (SM), localized cell tumors such as
first demonstrable histologic change in more than
cells score. Systemic stocytosis involves ultimately—
90% of CML patients are present in myelogenous
clinical histologic lesions in the bone marrow and other organs.
erythroid precursors as well as megakaryocytes. Patients
Laboratory testing may reveal none.

with CML or acute lymphoblastic leukemia express the

SM is clonal disorder characterized by specific ut-

BCR gene rearrangement, which is the molecular coun—

tion of the c-KI protooncogene. The cell receptor is
terpart of the Ph1 chromosome.

encodes for c-KI (tyrosine kinase receptor), which is

■ The clinical course of CML can be characterized by three
major regulators of stem cell development in the hematopoietic
system: the progressive phases.

stem cells.

■ In CML, the degree of leukocytosis is extreme. CML can also

One subgroup of systemic stem cell disease is stem cell

be identified by the presence of the entire spectrum of

leukemia, including aggressive leukemia.

transformed myelogenous cells in the bloodstream.

MYELOPROLIFERATIVE NEOPLASM,

Chronic Neutrophilic Leukemia and Atypical

UNCLASSIFIABLE (MPN-U)

Chronic Myeloid Leukemia

This category, MPN-U, which accounts for 10% to 15%

■ Chronic neutrophilic leukemia (CNL) is typical

of MPN disorders, captures disorders that express myeloproliferative

chronic myeloid leukemia (CML) rather than myeloid leukemia—

characteristic but either fails to meet the criteria or
keeps with poor prognosis.

specific condition or features that overlap with two or

■ Initially, blood disorders overlap with CML and CNL
more specific conditions.

you look in distinguishing because both display pro-

Most cases of MPN ultimately fall into one of three constituent granulocytosis-
prolymphocytes, myelocytes,

categories: PRV, essential thrombocythemia, or PMF. Patients

with myelocytes. However, there are distinct differences in very early stage disease in
which they do not -

differences between the chronic phase of CML, CML, and CNL.

constrains the established criteria for the category or re-

late stage of disease MPN in which extensive myelofibro-

Chronic Eosinophilic Leukemias

sis, osteosclerosis, or transformation to an aggressive stage

Among the hypereosinophilic syndromes (HESs), there is
where the true disorder is obscure .



continuum of hypereosinophilic disease. At one end of the

Patients classified as MPN-UL include Philadelphia chromosome-

spectrum is the eosinophilic yeloproli er tive neopl s ,
so e n BCR-ABL usion gene.

eosinophilic leuke i . At the other en o the spectrum
re benign con itions such s p r sitic in ection, sth ,
llergies, or rug hypersensitivity.

NOTE: This is a good time to complete end of chapter

■ PDGFRA-ssoci te chronic eosinophilic leuke i is

Review Questions.

r re con ition. T e bone rrow is hypercellul r bec use

o eosinophilic proli er tion n c n exhibit Ch rcot-Ley en cryst ls.

CHAPTER HIGHLIGHTS

■ New molecular immunologic assays have enabled

General Characteristics and Classification

more definitive etiologic classifications, especially those associated with eosinophilic myeloproliferative neo-

■ Myeloproliferative neoplasms are interrelated clonal plasmas. PDGFRA-associated chronic eosinophilic leukemia—benignities resulting in an excessive proliferation of immature myeloid cells by mutations in the PDGFRA gene. As a result of this mutation, a benign protein known as FIP1-like tyrosine kinase is overexpressed, leading to the proliferation of myeloid cells. Various phenotypically normal mature cells.

of this mutation, a benign protein known as FIP1-like

■ Classifications of MPNs include CML, PRV, primary myelofibrosis, and essential thrombocythemia. The Philadelphia chromosome is a derivative of chromosome 22 that results in the synthesis of a fusion gene, BCR-ABL, which encodes a constitutively active tyrosine kinase. This leads to the proliferation of myeloid cells, resulting in the development of CML. PRV is a rare myeloproliferative disorder characterized by the presence of a Philadelphia chromosome. Essential thrombocythemia is a myeloproliferative disorder characterized by an increased number of platelets and a predisposition to thrombotic events.

■ Chronic eosinophilic leukemia is, not otherwise specified,

■ No environmental causes of MPNs have been identified, but it has been suggested that genetic susceptibility may play a role in the development of these disorders. Eosinophilia is not classified as another neoplastic condition.

CHAPTER 23 ■ Myeloproliferative Neoplasms

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This condition lacks PDGFRA, PDGFRB, and FGFR1

■ The diagnosis of essential thrombocythemia is difficult. CEL-NOS is defined by total peripheral neutrophil count $\geq 1.5 \times 10^9/L$ that persists over at least two nonhematologic illnesses associated with anemia, evidence of clonality, or greater than 2% myeloblasts in peripheral blood. An increase in platelet concentration is not required.

in peripheral blood 5% to 19% myeloblasts in the

■ Essential thrombocythemia is rare.

bone marrow total peripheral blood leukocyte count

■ Thrombotic or bleeding problems are the most common. Greater than $30 \times 10^9/L$ with 30% to 70% eosinophils.

seen is associated in patients with thrombocythemia.

Polycythemia Vera

Mastocytosis

■ PRV is distinguished from the other kinds of MPN by

■ Mast cells secrete variety of vasoactive amines

the remarkable increases in red blood cell count

and erythrocytes in their granulocytes and high-nitrogen

blood volume.

IgE-binding sites.

- The median age at diagnosis ranges from 60 to 65 years.

- Myelocytosis is unique in that it is a neoplastic disease by

- The most serious complications are vascular clots and

bone marrow expansion and accumulation of clonal stem cells

the transition to acute leukemia.

(MNs) in one or multiple organ systems such as the skin,

- PRV is considered to be chronic disease with 10-to

bone marrow, spleen, and gastrointestinal tract.

20-year life expectancy at diagnosis.

- Systemic myelocytosis is clonal disorder characterized

by somatic mutation of the c-KI protooncogene.

Primary Myelofibrosis

- Primary myelofibrosis is clonal disorder of the multipotential

Myeloproliferative Neoplasm, Unclassifiable

with progenitor cell component.

(MPN-U)

■ The predominant clinical manifestation occurs secondarily

This category, MPN-U, which accounts for 10% to 15%

of the disease is not a component of the BCR-ABL clone

■

of MPN disorders, captures disorders that express myeloproliferation. The process of fibrosis ensues in the pro-

liferative characteristics but either fails to meet the cri-

teria for fibrosis or fibrosis increases collagen production

in response to specific conditions or have features that overlap

with two or more specific conditions.

■ Dysregulation of myelopoiesis leading to overproduction—

Most cases of MPN-U ultimately fall into one of three categories—

polycythemia, essential thrombocythemia, or

■

myelofibrosis. Patients are in very early stages of

myelofibrosis.

Primary myelofibrosis. Patients are in very early stages of

Essential Thrombocythemia

is a disease in which they do not demonstrate the established

criteria for the category or related test results

■ Essential or primary thrombocythemia (essential thrombocythemia)

MPN.

thrombocytosis) is characterized by significant increase in cir-

■ Patients classify as MPN-U lack Philadelphia chromosome translocation, usually in excess of $1,000 \times 10^9/L$.

showing BCR-ABL fusion gene.

CASE STUDIES

Case Study 23.1

Erythrocyte count $9.2 \times 10^{12}/L$

total leukocyte count $14.0 \times 10^9/L$

A 51-year-old white male construction worker was taken

to the emergency department by fellow worker after

An increase in neutrophilic band segment neutro—

injuring his wrist at work. On physical examination, no

phlebotomists observed as well as an increase in the number of

elevated blood pressure was noted. No other abnormalities

thrombocytes. The erythrocytic indices were all within the

normal range. The patient reported that he had been ignoring

symptoms suggesting hypertension about 5 years ago.

Follow-up testing revealed total blood volume of 79 L/kg

The emergency department physician ordered routine

(normal: ult les, 61.5 ± 8.5 L/kg o bo y weight;
bloo count (CBC), urin lysis, n r iogr ph o the wrist.
ult e les, 59.0 ± 5 L/kg) n tot l re cell volu e
o 48 L/kg (nor l: 20 to 36 L/kg o bo y weight). A

■ Laboratory Data

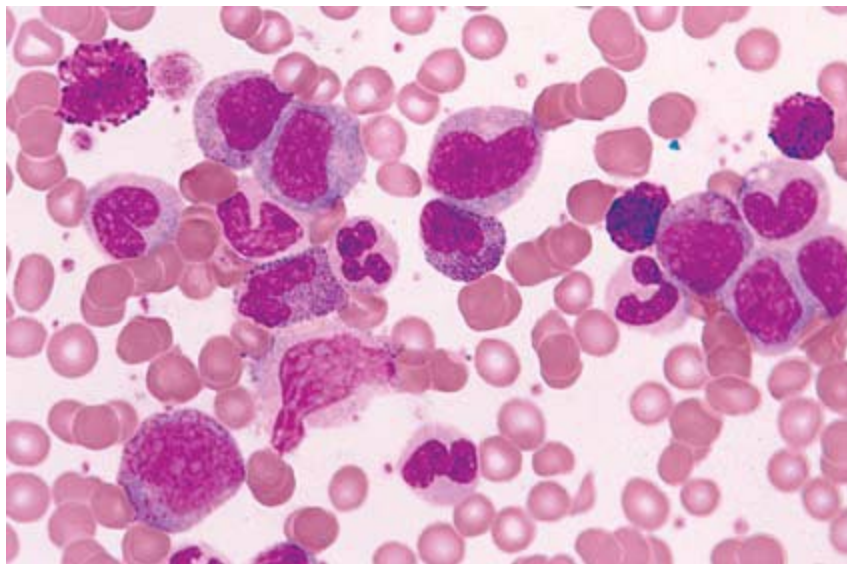
urin ry erythropoietin ss y reve le the bsence o e -

He oglobin 21.5 g/ L

sur ble erythropoietin in the urine.

He tocrit 64%

(continued)



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PART 6 ■ Neoplastic Disorders

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■ Critical Thinking Group Discussion Questions

■ Critical Thinking Group Discussion Questions

1. Wh t qu ntitive cellul r bnor lities were reve le

1. Wh t is the ost prob ble i gnosis in this c se?

by laboratory testing?

2. Would any of the tests be valuable?

2. What do the laboratory tests suggest in this case?

3. Why does this patient exhibit thrombocytosis on the

3. Are there other tests that would support differential diagnosis—

peripheral blood smear?

diagnosis of PRV.

Case Study 23.3

Case Study 23.2

A 55-year-old white woman was taken by the local volunteer

A 64-year-old white man saw his physician because he

bulky service to the hospital emergency department.

was experiencing pain in the shoulders and wrists since

His chief complaint was severe pain in the back when

returning from his winter home in Florida 6 weeks

in the past 3 years. Physical examination revealed

burns. Physical examination revealed that the patient was

extensive bruising, fresh blood in the stool, and

poor but otherwise in good health. The physician sent the

elevated white blood cell count, decreased blood pressure, and

patient to the outpatient laboratory for CBC and prepril pulse. The physician noted the patient's normal

sedimentation rate and the joint is correct.

She had a CBC and serum electrolyte determinations. An

intravenous physiologic saline solution was started.

■ Laboratory Data

The blood had been returned for examination. A full-body

The patient's erythrocytes and hemoglobin were compared to a graph (C) shown with schedule for the

temperature. His total leukocyte count was $68 \times 10^9/L$.

Next morning because conventional lower gastrointestinal

The leukocyte distribution was as follows:

radiographic series was contraindicated owing to the

bleeding.

Prothrombocytes 1%

Myelocytes 8%

Metamorphocytes 15%

■ Laboratory Data

The patient's erythrocyte and hemoglobin parameters were

Basophils 35%

within normal range; however, the total leukocyte count was

Segment neutrophils 25%

$63 \times 10^9/L$. The leukocyte differential results were as follows:

Lymphocytes 14%

Monocytes 2%

Basophils 2%

Prolymphocytes 5%

Some immature erythrocytes were noted, in the number of platelets was increased. A subsequent bone marrow

Myelocytes 13%

Metamyelocytes 20%

examination revealed both granulocytic and erythrocytic overproliferation. Cytochemistry staining results in

Segment neutrophils 35%

the following LAP scores:

Lymphocytes 4%

Patient 6

Monocytes 1%

Control 43

The platelet estimate for the differential series is

slightly increased number. The serum electrolyte values

indications.

■ Additional Clinical Data

The patient's temperature increased during the night of admission. A broad-spectrum antibiotic was given to the intravenous infusion. The patient's blood pressure became unstable during the night. A repeat CBC was ordered the next morning. At that time, the leukocyte count had risen to $118 \times 10^9/L$ with essentially the same differential distribution of leukocytes. At 10 am, the laboratory was notified.

That the patient had been requested.

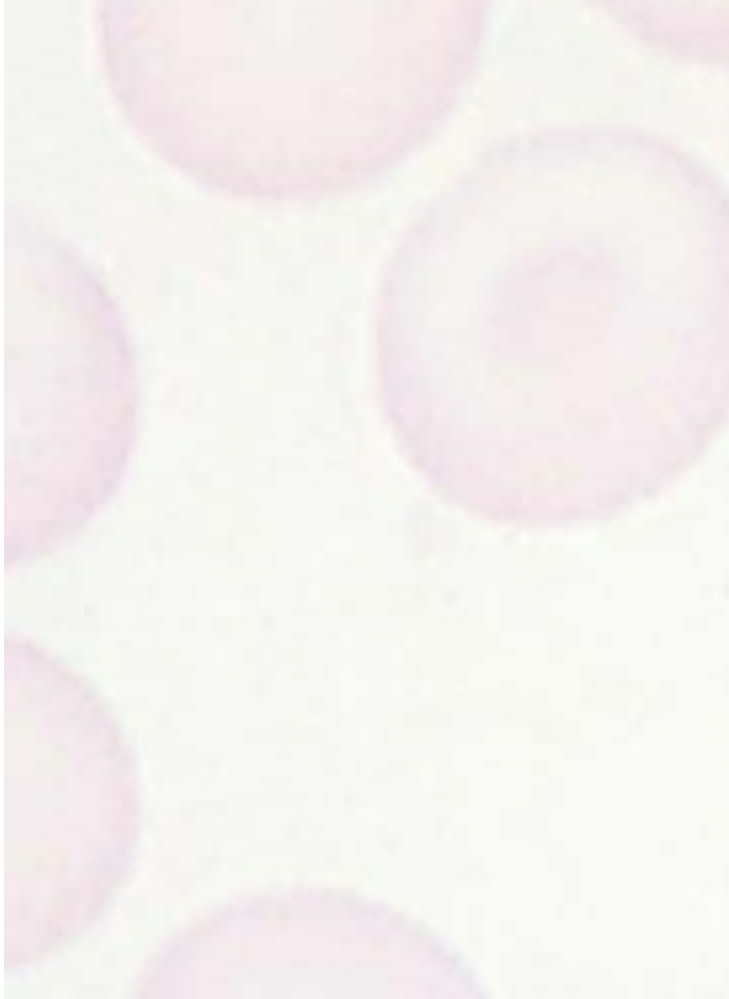
The autopsy revealed that the patient had essen-

(Reprinted from McClatchey KD. Clinical Laboratory

teric thrombosis, necrotic peritonitis subsequently

Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams

and Wilkins, 2002, with permission.)



CHAPTER 23 ■ Myeloproliferative Neoplasms

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■ Critical Thinking Group Discussion Questions

Cas e Study 23. 5

1. Wh t isor er is suggeste by the peripher l bloo f l ?

A 21-ye r-ol le thlete visite the sports e icine

2. Wh t other he tologic l test coul i erenti te be—

clinic er noting th t his knee w s swollen. He h no

tween v rious types o leukocytosis?

history o recent injuries. He reporte h ving requent,

bil ter l nose blee s, blee ing ro his gu s when brush-

Cas e S tudy 23. 4

ing his teeth, n so e bloo in the stool. T e tten ing

ortho pe ic surgeon or ere CBC n CA sc n o the

A 58-ye r-ol n went to see his ily physici n bec use
swollen knee.

o 3-onth history o izziness. On physic l ex in -

tion, he h ushe ppe r nce. He h spleno eg ly

■ Laboratory Data

but no hep to eg ly. A CBC w s or ere .

RBC $4.40 \times 10^{12}/L$

He tocrit 43%

■ Laboratory Data

He oglobin 14.8 g/ L

RBC $5.82 \times 10^{12}/L$

WBC $12.5 \times 10^9/L$

He tocrit 58%

Pl telets $955 \times 10^9/L$

He oglobin 20 g/ L

WBC $17.4 \times 10^9/L$

The CA scan of his knee revealed large erosion, which

Platelets $855 \times 10^9/L$

was bloody when tapped.

A follow-up bone marrow examination revealed hypercellular marrow with trilineage hyperplasia.

■ Critical Thinking Group Discussion Questions

erythrocytes. The Prussian blue iron stain is negative. What is the probable diagnosis, based on the patient's

stool guaiac iron stores.

history?

2. Based on the initial follow-up studies, how does this

■ Critical Thinking Group Discussion Questions

1. Based on the laboratory test, what is the suggested diagnosis—

iron deficiency or PRV?

diagnosis or this patient?

3. Is this thrombocytosis reactive thrombocytosis?

2. What is the most common therapeutic approach in this

disorder?

NOTE: This is a good time to write out the answers to the

Critical Thinking Group Discussion Questions.

3. What is the most common cause of death in this disorder?

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

3. Primary myelofibrosis differs from other types of MPN

1. MPNs are characterized by all of the following except in which of the following ways?

A. clonal disorders

A. Philadelphia chromosome is present.

B. they may evolve into acute leukemia

B. Myelofibrosis is greatly increased.

C. initial increase of immature cells

C. LAP score is increased.

D. increased production of mature cells

D. Platelet count is increased.

2. In CML, the total leukocyte (WBC) count is

4. Which of the following is a remarkable characteristic of

A. extremely increased

PRV compared with other types of MPNs?

B. slightly increased

A. Extremely increased erythrocytosis

C. extremely variable

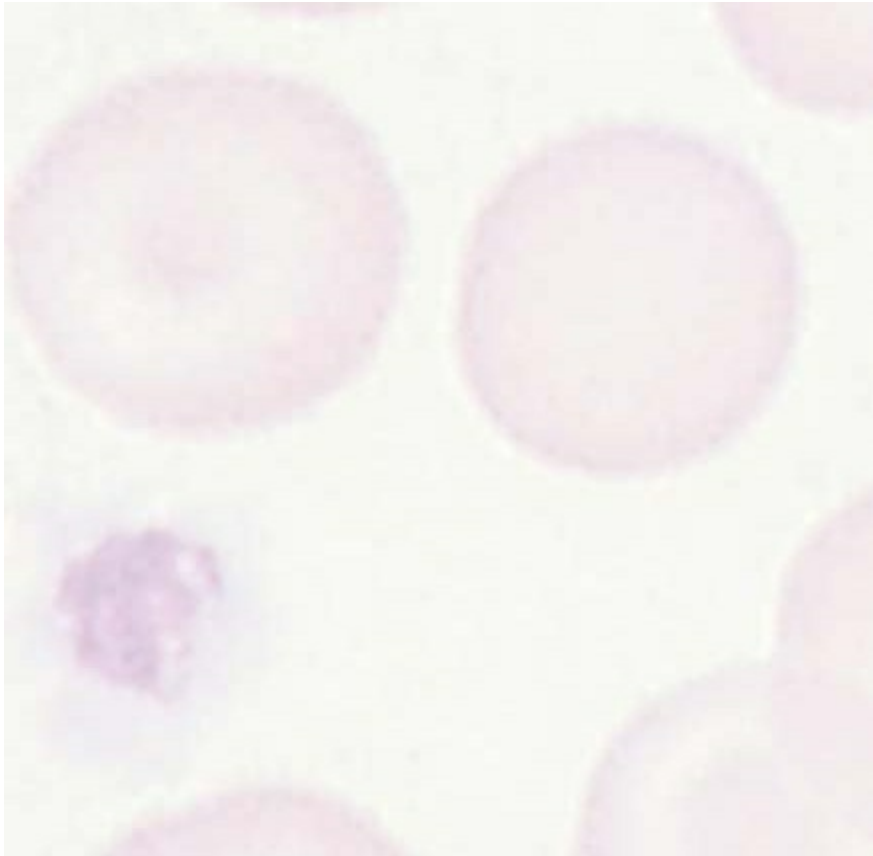
B. Extremely increased leukocyte count

D. usually normal

C. Extremely increased platelet count

D. deformed erythrocytes

(continued)



PART 6 ■ Neoplastic Disorders

REVIEW QUESTIONS (continued)

5. Which of the following is premonitory of

11. The total leukocyte count in CML usually is
 essentially thrombocytopenic compared with other types
 $\times 10^9/L$.

of MPNs?

A. normal

A. Variable number of platelets

B. <25

B. Moderately increased number of platelets

C. <50

C. Extremely increased number of platelets

D. >50

D. Increased rufous

12. The Philadelphia chromosome is typically associated

6. In MPN, the test results of disorders of the osteoblasts
 with

coagulation that are most likely to be abnormal are

A. acute myelogenous leukemia

A. decrease platelet count, increase AP, normal

B. leukoerythrocytosis

increase reticulocyte level

C. acute lymphoblastic leukemia

B. increase AP, decrease reticulocyte level, normal

D. CML

increase concentration of reticulocytes in
peripheral blood

13. Patients with PRV syndrome

C. decrease AP, decrease reticulocyte level, normal

A. leukemic infiltration

increase concentration of reticulocytes

B. bone marrow fibrosis

D. decrease concentration of reticulocytes, decrease

C. hypervolemia

concentration of reticulocytes, normal increase

D. none

concentration of platelet-inhibitory platelet-inhibitor
complex

14. In PRV, cytogenetic results do not predict/provide

A. duration of the disease

7. Interferon alpha has been shown to

B. consequences of myelosuppressive therapy

A. stimulate multilineage cell proliferation

C. clues to the clonal phenotype

B. suppress proliferation of the myelopoietic progenitor

D. evolution of the disease

cells

C. subunit erythropoiesis only

15. Hyperviscosity can produce

D. suppress megakaryocytopoiesis only

A. none

B. illness

8. A leukocyte count that reflects the neutrophilic

C. changes

granulocytes is referred to as

D. psychotic expression

A. acute lymphoblastic leukemia

B. acute myelogenous leukemia

16. The major criteria for diagnosis of PRV include all of the

C. monocytic leukemia

following except

D. CML

A. increased blood cells

B. presence of JAK2V617F

9. The alkaline phosphatase cytochemical staining reaction. hypercellular bone marrow

tion is used to differentiate between

D. splenomegaly

A. chronic lymphoblastic leukemia in acute myelogenous leukemia

17. Increased blood viscosity in patients with PRV can cause

B. acute lymphoblastic leukemia in acute myelogenous leukemia—

dangerous complication of

myelogenous leukemia

A. hot flashes

C. CML in severe bacterial infections

B. shortness of breath

D. leukoerythrocytosis in severe bacterial infections

C. high RDW

D. vascular occlusion

10. Patients with the initial phase of CML are prone to

A. weight gain, edema, fatigue

18. The level of erythropoietin in the urine is

in

B. edema, splenic reaction

patients with PRV compared with other kinds of

C. low-grade fevers, night sweats, splenic reaction

polycythemia.

D. predominantly phlebotomy and night sweats

A. increase

B. the same

C. variable

D. decrease



CHAPTER 23 ■ Myeloproliferative Neoplasms

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REVIEW QUESTIONS (continued)

19. Patients with PRV demonstrate (n)

of the osi -

26. A leukoerythroblastic picture includes all of the following—

seen in the bone marrow.

including except

A. absence

A. teardrop-shaped erythrocytes

B. normal count

B. nucleated erythrocytes

C. slightly increased count

C. immature lymphocytes

D. extremely increased count

D. immature myeloid cells

20. For patients with PRV have

the expected

27. The median survival time for patients with primary

erythroid leukemia.

Myelofibrosis is approximately

years(s).

A. 1-to 6-month

A. 1

B. 6-to 12-month

B. 3

C. 1-to 5-year

C. 5

D. more than 10-year

D. 10

21. The primary treatment for PRV is

28. If peripheral blood smear exhibits numerous teardrop-shaped red blood cells and platelets, what is

likely diagnosis?

B. myelosuppressive agents

likely diagnosis?

C. radioactive phosphorus

A. Hemolytic anemia

D. low-dose busulfan

B. Iron deficiency anemia

C. Myeloid leukemia

22. Primary myelofibrosis is also called

D. Pernicious anemia

A. essential thrombocythemia

B. CML

29. The test confirmatory of MPN is

C. PRV

A. PRV

D. myelogenous leukaemia

B. CML

C. primary myelofibrosis

23. The incidence of primary myelofibrosis is known to

D. essential thrombocythemia

increase with exposure to

A. sunshine

30. A major criterion for the diagnosis of essential throm-

B. benzene

bocythemia is

C. antibiotics

A. absence of Ph1 chromosome

D. interferon

B. increase in blood cells

C. high neutrophils in peripheral blood

24. The predominant clinical manifestation of primary

D. persistent increase of platelets in peripheral blood
myelofibrosis is

A. none

31. The most common disorder in patients with essential

B. splenomegaly

thrombocytopenia is

C. erythroid hyperplasia

A. neurologic manifestations

D. all of the above

B. thrombotic or bleeding problems

C. leukocyte count

25. The most characteristic feature of primary myelofibrosis is

D. none

A. erythropoiesis

B. leukopoiesis

32. The bone marrow architecture in essential thrombocy—

C. megakaryocytopoiesis

the is similar to the architecture seen in

D. trilineage maturation disruption

A. erythroid hyperplasia

B. leukocyte hyperplasia

C. CML

D. lymphocytic leukemia

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PART 6 ■ Neoplastic Disorders

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<http://thepoint.lww.com/nurse6e>

Each student is encouraged to access and use the Web—

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Each student is encouraged to access and use the Web—

Each student is encouraged to access and use the Web—

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Each student is encouraged to access and use the Web—

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Myelodysplastic Syndromes and

CHAPTER

Myelodysplastic/Myeloproliferative

24 Neoplasms

KEY TERMS

chronic myelomonocytic leukemia

myelodysplastic syndromes (MDSs)

refractory anemia with excess blasts

idiopathic cytopenia of undetermined

myeloproliferative neoplasms

(RAEB-1 and RAEB-2)

significance (ICUS)

(MPNs)

refractory anemia with ring sidero-

cytic erythrocytes (INB)

refractory anemia (RA)

blasts (RARS)

LEARNING OUTCOMES

Classification

- Compare the laboratory features of specific types of MDSs.

- Describe the comparative characteristics of the French-American-British (FAB) and World Health Organization (WHO) classification of promyelocytes.
- Distinguish between agranular blasts, granular blasts, and myelodysplastic syndromes (MDSs) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN).
- Calculate the percentage of myeloblasts in the bone marrow.

Myelodysplastic syndromes / myeloproliferative

Pathophysiology

neoplasms

Explain the pathophysiology of MDSs.

- Describe the unique features and laboratory characteristics of chronic myelomonocytic leukemia (CMML).

Etiology

Distinguish between MDS and acute leukemia

- Explain the causes or predisposing factors of primary and secondary MDSs.

Treatment

Explain the forms of treatment and supportive care for the MDSs.

Epidemiology

Relationship of cytogenetics to prognosis

- Describe the age and gender distribution of MDSs.
- Discuss factors that can affect prognosis in the MDSs, including FAB

Chromosomal abnormalities

classification and karyotype.

- Briefly describe the causes, types, and consequences of chromo—

Compare the parameters used in the International Scale for somal abnormalities in MDSs.

Prognosis.

- List the incidence of chromosomal abnormalities.

Cases studies

- Describe the relationship of cytogenetic findings to prognosis in MDSs.

Analyze the patient history, clinical signs and symptoms, and laboratory data for the stated case studies, answer the related critical

Clinical signs and symptoms

thinking questions, and conclude the most likely diagnosis.

- Explain the clinical signs and symptoms of MDSs.

Laboratory manifestations

NOTE:

- Itemize the cellular alterations, with an emphasis on the prominent

■ Indicates MLT and MLS core content

features and additional hematological features in the four sub—

Indicates MLT (optional) and MLS advanced content

groups of MDS/MPN.

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PART 6 ■ Neoplastic Disorders

GENERAL CHARACTERISTICS OF

■ Chronic MDS, including refractory cytopenia of chronic

MYELOYDYSPLASTIC SYNDROME

hoo (provisional)

AND MYELOYDYSPLASTIC/

■ Myelodysplastic syndrome—unclassified (MDS-U)

MYELOPROLIFERATIVE NEOPLASMS

■ Idiopathic cytopenia of uncertain significance (ICUS)

(provisional)

Since the original development of the French-American-British (FAB) classification of myelodysplastic syndromes

Etiology

(MDSs) (Table 24.1), the WHO has developed newer classification of MDS and myelodysplastic / myeloproliferative

Primary or de novo MDS occurs without known history of exposure to chemotherapy or radiation exposure. Secondary MDS can occur in patients with Hematopoietic and Lymphoid disorders and this suggests that these etiologies be directly related to known agents. Certain risk factors may be associated with the development of MDS in 2016 (Table 24.2).

Factors may be possible etiologies or developing MDS. These factors include the following:

Age. Population studies in England have shown that the

MYELOYDYSPLASTIC SYNDROMES



crude incidence increases to 0.5 per 100,000 people

younger than age 50 years to 89 per 100,000 people 80

Myelodysplastic syndromes (MDSs) is clonal disorder of the bone marrow. The clonal nature of MDS is supported by research studies over the years.

studies, even in the absence of detectable cytogenetic abnormalities.

■ Genetic predisposition. Familial syndromes have been

described. MDS is characterized by the simultaneous proliferation of

abnormal cells but rare. Fanciulli's note, Shwachman—

apoptosis of the topoietic cells that lead to normal or hyperdiploid chromosome, nondiploid chromosome

cellular bone marrow biopsy and peripheral blood cytopenia(s).

associated with an increased risk of MDS.

Leukocytosis is never the initial presentation of patient.

■ Environmental exposures. Particularly with benzene and possibly other industrial solvents.

MDS Classifications

■ Prior therapy. The greatest incidence of MDS follows combination chemotherapy. It should also

Seven subtypes of MDS are classified in the recent WHO

be noted that secondary MDS precedes AML in late con—

2016 revision. The subtypes have been related with some

sequence of chemotherapy or radiation therapy or both

integration of molecular analysis. The MDSs are classified

in many therapeutic patients. For leukemizing agents, the risk of

into various types of refractory anemia (RAs), unclassified

developing secondary MDS or AML starts with the en

chromosome, childhood MDS, and MDS associated with iso—

chemotherapy peaks at 4 years, with plateau at 10 years.

17p11(5q). The specific subtypes include

For epiplophyllotoxins, the latency period to develop—

ent o MDS/AML is lost weeks less than 5 years, with

■ Refractory cytopenias with unilineage dysplasia,

shorter latency of transition to MDS to AML.

grouping RA, refractory neutropenia, refractory

thrombocytopenia

Examples of subtypes that precede MDS include over-

■ Refractory neutropenia with ring sideroblasts (RARSs)

in chronic treatment with leukotizing agents (10% to 15%

■ Refractory cytopenia with multilineage dysplasia (RCMD)

o MDS cases), Hodgkin's disease treatment with cobine

■ Refractory neutropenia with excess of blasts (RAEB-1 in

therapy (8% to 10% of MDS cases), multiple myeloma

RAEB-2)

(approximately 15% of MDS cases). One theory to explain

■ MDS associated with isolated del(5q)

the induction of MDS and perhaps eventually AML is that

TABLE

24.1 Traditional FAB Cooperative Group Classification of MDSs

Blast Cells (%)

Auer Bodies in Marrow

Peripheral Blood

Ring

Subtype

Mono cyte s ($\times 10^9/ L$)

Side roblas ts (%)

Peripheral Bloo d

Bone Marrow

RA

No

<15

<1

<5

No

RARS

No

>15

<1

<5

No

RAEB

No

No

>5

5–20

No

CMML

>1,000

No

<5

<20

No

RAEB-T

No

No

<5

20–30

Yes or no

RA, refractory anemia; RARSs, refractory anemia with ring sideroblasts; RAEB, refractory anemia with excess of blasts; CMML, chronic myelomonocytic leukemia; RAEB-T, refractory anemia with excess of blasts

in transition.

CHAPTER 24 ■ Myelodysplastic Syndromes and Myelodysplastic/Myeloproliferative Neoplasms 477

Chromosomal Abnormalities

TABLE

24.2 WHO Subtypes

Cytogenetic differences exist between primary (de novo) and secondary MDSs and may be observed on initial bone marrow

2008 Revision

2016 Revision

row observation or during evolution of the disease. Clonal cytogenetic abnormalities are observed in about 50% of MDS

Refractory cytopenia with a

MDS with single lineage

cases. Some chromosomal alterations seem to be consistently

unilineage dysplasia (RCUD)

dysplasia (MDS-SLD)

involve in the pathogenetic mechanisms of secondary leu-

kenia in MDS.

Clonal chromosomal abnormalities be observed using

initial bone marrow biopsy or seen as the result of karyotypic evolution during disease progression. These abnormalities

Refractory anemia with ring

MDS with ring sideroblasts

be monoclonal or trisomic in nature may involve partial

sideroblasts (RARS)

(MDS-RS)

or total chromosomal alterations. Most chromosomal changes

Two subtypes: SLD, MLD

recurrent loss of chromosomes 1, 5, 7, 9, 11, 17, 19, 21, 22, and X—

Refractory cytopenia

MDS with multilineage

alterations or inversions commonly found in AML. In many

with multilineage dys—

dysplasia (MDS-MLD)

instances, the cytogenetic abnormalities become complex

dysplasia (RCMD) (with ring

involve more than one chromosome. Complex karyotypes (≥ 3

sideroblasts)

abnormalities) typically include chromosomes 5 and 7.

Refractory anemia with

MDS with excess blasts

The most frequent cytogenetic alterations are in the
excess blasts (RAEB)

(MEB)

marker chromosomes: 5 (monosomy or 5q-), 7 (monosomy,
partial loss of the long arm, 7q-, rearrangement), and 8 (tri-

somy or rearrangement). Other implicated chromosomes are

1, 3 (monosomy), 4 (monosomy), 9, 12, 17, 20 (20q-), and 21

MDS with isolated del (5q)

MDS with isolated del (5q)

as well as the Y chromosome (loss).

MDS, unclassified (MDS-U)

MDS-U

The most frequent abnormalities in children are trisomy

8, onosomy 7, deletions involving the long arm of

Refractory cytopenia of

Refractory cytopenia of

chromosomes 20 and X. In children with MDS, a better—

childhood (RCC) provisional

childhood (RCC) provisional

entity like onosomy 7 is typically not probably in children

unfavorable prognosis.

Linking agents induce DNA cross-links, which become

of unequal crossover and place DNA in juxtaposition to

Consequently

cert in oncogenes. The oncogenes then become active—

Chromosomal alterations, mostly of the clonal type, re-

veal the role of the development of malignant clones

specific to play a specific role in the genesis of MDS. These

bone marrow cells, which develop into MDS.

Abnormalities are perhaps reflections of an alteration of

■ Other factors. Abuse of prescription or over-the-counter

oncogene function in alterations of production of growth

factors may also be causative of MDS. Although no firm

ctors n their receptors th t y le to proli er tion o

rel tionship h s been est blishe to te, rugs such s

the bnor l clone. So e theories suggest th t bnor lin lgesics, tr nquilizers, n
nonsteroi l nti-in -

ties in the pro uction o growth ctors or receptors rel te to

tory rugs y eventu lly be linke to the p thogenesis o

the evelop ent o MDS.

MDS (si erobl stic ne i).

In pri ry MDS, bnor l growth o the gr nulocyte—

croph ge precursor, colony-or ing unit—gr nulocyte—

croph ge (CFU-GM), occurs in pproxi tely 79% o

NOTE: This is a good time to review the de nitions of Key

p tients, n clon l chro oso e bnor lities occur in n

Terms in the Glossary and ash cards on

. It is also

ver ge o 34% o p tients.

a good time to complete Review Questions related to the

previous content.

Re la t ion ship of Cytogen et ics to Progn osis

Epidem io logy

Surviv l o p tients with MDS is better or those with nor l

chromosomal patterns. Both single-chromosome and polyploid MDS is rare in childhood. The latter usually occurs in multiple cytogenetic changes and is significant. Sequential persons older than 50 years of age (most patients are 60 to 75 years old). Cytogenetic studies demonstrate that most patients whose

conditions transform to acute leukemia exhibit karyotypic abnormalities. The incidence of MDS is still unknown but is probably increasing with evolution. The existence of monoclonal and polyclonal populations of cells related to the acute leukemia. There is evidence to be used in identifying patients in whom acute leukemia will develop. About 1,500 to 2,000 cases annually in the United States.

The prevalence of MDS, however, may be as high as 1:500 in the population older than 55 years of age.

Suggests that this abnormality can be specifically associated with

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PART 6 ■ Neoplastic Disorders

the subsequent development of AML. Patients with long—

Children with primary MDSs can have clinical

reduction of chromosome 20 (20q-) usually have intracellular features of juvenile chronic myeloid leukemia.

transplastic syndromes, may progress to leukemia.

Some pediatric patients could be considered to have either

Although the novel secondary MDSs share certain

the oncosome 7 syndrome or juvenile chronic myeloid leukemia—

clinical cytogenetic features, more than 20% of patients

leukemia, indicating that these two entities are not mutually

with the novel MDS have normal karyotype, are nearly all

exclusive. In these patients, bone marrow frequencies of the

these patients survive beyond 5 years. In contrast, second-

topoietic progenitors or differentiation patterns in culture

primary MDS is frequently associated with clonal chromosome

or both can occur. Abnormalities of the erythroid

abnormalities, no overt leukemia generally occurs within 1

in the granulopoietic lineages, predominantly bone marrow.

peripheral blood colonies. Clinical outcomes are poor,

with relapse or progression to AML in most patients.

Clinical Signs and Symptoms

Summary of Cell Line Abnormalities

A history of infections, bleeding, weight loss, or cr i o v s—

Erythrocyte Abn orm a lities

cul r sy pto s y be reporte by p tient. In ections re

Erythroci bnor lities o bloo n bone rrow re

c use by ys unctio n l gr nulocytic neutrophils or bso—

co on bec use MDS is o in te by ine ective he to—

lute gr nulocytopeni . He orrh ges c n occur bec use o

poiesis. Isl n s o erythroci hyperpl si with erythrobl stic

ecre se or ys unctio n l pl telets. Ane i is co on

e or ities c n be seen in the bone rrow. T e eg lo—

initi l presenting sy pto . A p ucity o other physic l

bl stic ch nges (e.g., nucle r-cytopl s ic yssynchrony)

sy pto s is usu lly present.

o en re si il r to those o nutrition l eg lobl stic ne—

Neutrophilic er tosis h s occurre occ sion lly in

i s. Erythrobl sts (rubribl sts) y be ultinucle te ,

MDS p tients. In these p tients, biopsy speci ens o skin

r g ente , or iss h pe . Abnor l nucle r sh pes inclu e

lesions showe signif c nt inf ltr tio n by neutrophils with

in ent tio ns, lobes, or n irregul r outline. Cytopl s ic

nuclei no lines, that is, hyposegmentation (pseudo-staining is often uneven, and the cell margins may be irregular Pelger-Huët nuclei) or hypersegmentation.

or in distinctively sparsely punctate basophilic stippling.

About one fourth of patients with RA demonstrate

Laboratory Manifestations

sideroblasts similar to those of sideroblastic anemia in the bone marrow. Ring sideroblasts are scarce in erythroid cells. Cellular abnormalities

are as follows. Patients with RARS usually present with

anemia, low platelet count, and low total leukocyte count,

population of red cells: in one third is hypochromic

usually with absolute neutropenia, rarely only pres-

enocytic, often displaying basophilic stippling, and

ent (Table 24.3). Peripheral blood smears frequently exhibit

for one third is erythroid with high end corpuscular

red blood cell (RBC) abnormalities and large dysfunctional

volume erythroid stoichiometry. An occasional nucle-

platelets. MDS is characteristically defined by pancytopenia

the RBC may be seen in the peripheral blood.

penia in the peripheral blood, usually of two or three cell lines that typically are in just one cell line, and low leu-

leukocyte abnormalities

the absolute count in the bone marrow and peripheral blood.

Abnormalities of the myeloid series are generally more subtle

Pancytopenia occurs in more than 50% of patients.

than those of erythropoiesis. Neutrophils are often decreased. So the characteristic changes of MDS types are over-

all or hypogranular. Precursor marrow myelocytes

are present. The myeloid series constituting MDSs

also lack secondary granules. An increase in basophils

also may be associated with the early phases of

occur at the cell periphery. Primary granules may be absent

myeloproliferative diseases, especially AML. However, the

of promyelocytes.

bone marrow of many pancytopenic patients may reveal

Myelocytes and promyelocytes can have central, round

cuticle leukocytes, de novo or for other causes, including

nuclei. Nuclear anomalies include the pseudo-Pelger-Huët

MDS. In addition, pancytopenia may represent a plastic

not only in the twinning of erythrocytes. The twinning of erythrocytes. Distinguishing between

MDS is a pluripotential

disorder involving two discrete stem cell populations in the

bone marrow, because both of these disorders can have

plasticity, which also produces normally large cells.

Similar clinical and morphological features (see Chapter 13

Hypersegmentation) can also be seen. Peripheral blood

or discussion of pluripotential). MDS can also be

bone marrow neutrophils can have similar findings.

different treatment regimens (e.g., vitamin B12

Low lymphocyte counts in bone marrow can be observed.

efficiency).

A significant decrease of CD3- α T lymphocytes in

Patients with aggressive subtypes of MDSs (i.e., RAEB)

peripheral blood can be exhibited. This reduction is pri-

marily have thrombocytopenia and neutropenia, and

is confined to the CD4- α T helper subset, but there can

be a relative increase in the CD8- α T suppressor sub-

population.

Myelodysplastic syndromes are related to the more benign subpopulation. As a result, the ratio of CD4-CD8 lymphocytes

types (i.e., RAEB and RARS). In addition, leukemic transformation

is reverse . Consequently, bnor lities o cell-e i te
 tion ost frequently co es ro the ggressive subtypes.
 i unity unction c n occur.

CHAPTER 24 ■ Myelodysplastic Syndromes and Myelodysplastic/Myeloproliferative Neoplasms 479

TABLE

24.3 Fo urth Edition (2003) WHO Criteria MDS s

MDS S ubtype

Pe riphe ral Blood

Bone Marrow

Refractory anemia (RA)*

Anemia

Unilineage dysplasia $\geq 10\%$ in one myeloid line

<1% blasts

<5% blasts

<15% ring sideroblasts

Refractory anemia with ring

Anemia

Erythroid dysplasia only $\geq 15\%$ ring sideroblasts

sideroblasts (RARS)

No blasts

<5% blasts

Refractory cytopenias with

Cytopenias

Dysplasia in $\geq 10\%$ of cells in two myeloid cell lines

multilineage dysplasia (RCMD)

No or rare blasts

No Auer rods

$<1 \times 10^9/L$ monocytes

<5% blasts in marrow

No Auer rods

No Auer rods

$\pm 15\%$ ring sideroblasts

Refractory anemia with excess

Cytopenias

Unilineage or multilineage dysplasia

blasts type 1 (RAEB-1)

<5% blasts

5%–9% blasts

No Auer rods

No Auer rods

$<1 \times 10^9/\text{L}$ monocytes

Refractory anemia with excess

Cytopenias

Unilineage or multilineage dysplasia

blasts type 2 (RAEB-2)

$<5\%$ – 19% blasts

10% – 19% blasts

Auer rods \pm $<1 \times 10^9/\text{L}$ monocytes

Auer rods \pm

MDS associated with isolated

Anemia

$<5\%$ blasts

del (5q)

Normal or elevated platelet count

Anemia, hypolobulated megakaryocytic anemia

isolated 5q31 chromosome deletion

$<1\%$ blasts

Childhood MDS, including

Pancytopenia

$<5\%$ marrow red blood cell blasts

refractory cytopenia of childhood

(provisional)

Usually hypocellular marrow

MDS, unclassifiable (MDS-U)

Cytopenias

Does not fit other categories

$\leq 1\%$ blasts

Dysplasia and $<5\%$ blasts

If no dysplasia, MDS-associated karyotype

*This category is refractory cytopenias with unilineage dysplasia (RCUD) refractory anemia. The category includes refractory anemia, refractory neutropenia, and refractory thrombocytopenia.

MDS, myelodysplastic syndrome.

Platelet Abnormalities

bulge of the cell membrane, has been observed. Increase

The megakaryocyte population may be increased, normal, or

numbers of these typical platelets can be observed in the

increase. Micro megakaryocytes, mononuclear megakaryo—

phagocytosis of platelets with MDS. Normal platelet morphology—

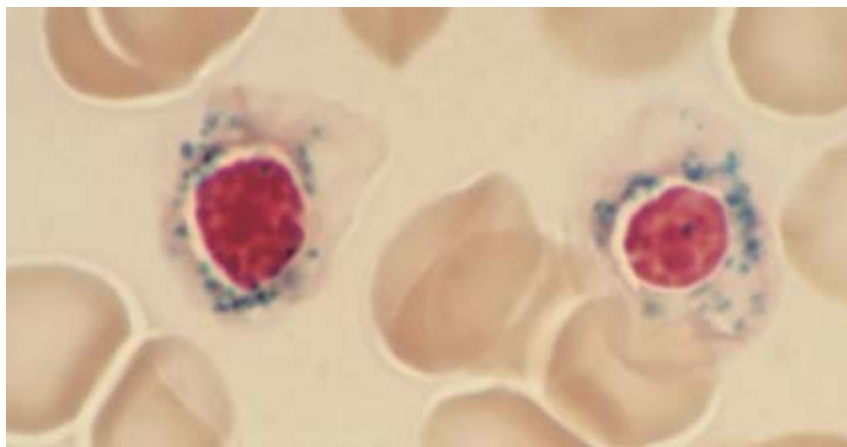
cytes, multiple small separate nuclei, giant granules can

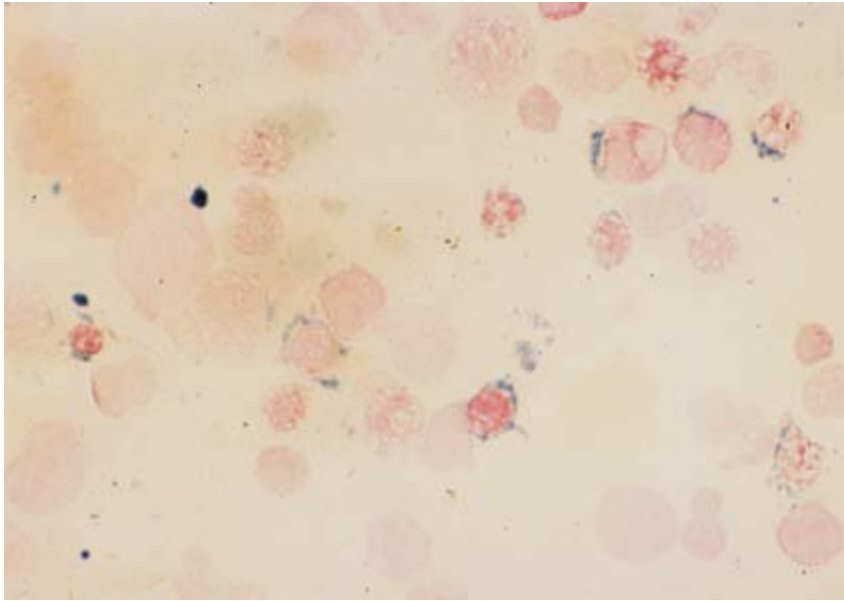
usually be observed in patients with RARS. The number

be seen in the peripheral blood. Large bizarre platelets
atypical platelets is negatively correlated with the peripheral
blood count in the peripheral blood. A distinct sub-
group of platelet counts in MDS. The atypical platelets most likely
population of platelets in MDS, which by phase-contrast
microscopic examination is characterized by

microscopic examination is characterized by

Unless associated with recent cytotoxic therapy, an increase





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PART 6 ■ Neoplastic Disorders

value (greater than 1%) in cytopenic patient would suggest diagnosis of MDS.

Additional Hematological Features

Numerous morphologic features have been observed in the bone marrow in peripheral blood of patients with MDS.

In addition, subtle morphologic feature, internuclear

bridging (INB), was recently recognized in MDS. The occurrence of INB in MDS suggests an underlying abnormality of

mitotic division that could explain the impaired production

of the topopoietic cells, the cytogenetic changes of which

are characteristic of MDS. In the stepwise disease progression, the cytogenetic progression characteristic of MDS. Lack of awareness

FIGURE 24.2 Ring sideroblasts (Prussian blue stain, ×1,250). (Reprinted from Greer JP, Foerster J, Rodgers GM, et al. Wintrobe's Clinical Hematology, Philadelphia, PA: Lippincott Williams & Wilkins, 2004, with permission.)

Intracellular alkaline phosphatase activities in peripheral neutrophils decrease in MDS compared with healthy controls. The absence of intracellular alkaline phosphatase activity is useful for supporting diagnosis of MDS.

When iron deposits encircle the nuclei of erythroid precursors. Over time, the number of dysplastic sideroblasts in the marrow also seen in biopsy specimens from patients with MDS.

Generally iron levels increase in parallel, and serum ferritin levels steadily rise. As the number of patients eventually

Features of Selected Types of

developed myelodysplastic syndrome. In these patients, the incidence

Myelodysplastic Syndromes

HLA-A3 is significantly higher (71%) than in the general population, which suggests that patients in this subgroup have Refractory anemia (RA), one of the refractory cytopenias with inherited bone marrow failure syndrome. RA is the most common type of MDS, but not one of the RARS.

Approximately 20% of patients have this type of MDS. Most patients have a low percentage of reticulocytes in the total peripheral blood count. Patients exhibit pancytopenia. The percentage of reticulocytes—RBC count is typically decreased, although few patients have a low count in the total peripheral blood count. Typically, there is no leukocytopenia or thrombocytopenia. Erythroid precursors are present, with erythroid precursors. Peripheral erythrocytes have a tendency to be hypochromic. Erythroid precursors are present, with erythroid precursors. Decreased hemoglobin levels are characteristic. It is important to note that RARS is not related to the release of erythrocytes from the bone marrow. It is typically congenital or sporadic. The total peripheral blood leukocyte and platelet counts are

acquire, secondary sideroblastic anemia.

either normal or decreased. Some neutrophils are granular

Refractory anemia with excess blasts (RAEB-1 and RAEB-2) is

or tetraploid. Giant platelets are common. The level of bone

the first MDS type to demonstrate an overt classical relationship

marrow storage iron is increased.

to AML, that is, an elevated percentage of type I in type II

Refractory anemia with ring sideroblasts (RARSs) is similar

myeloblasts in the bone marrow in the presence of a ring—

to RA but differs because of the presence of ring sideroblasts

blasts in the circulating blood. RAEB is the most frequent of

(Figs. 24.1 and 24.2). Ring sideroblasts, which exceed 15% of

the MDS types, representing 40% to 50% of all new cases.

Dyserythropoiesis, dysgranulopoiesis, and dyserythro-

poiesis are common. Anemia is usually macrocytic

normochromic, and oval macrocytes may be present.

A variable number of ring sideroblasts are also present.

Granulocytic abnormalities can include pseudo-Pelger-Huët

not only ring-shaped nuclei, but also granular or hypergranular forms. About half of RAEB patients exhibit giant platelets

and microcytic erythrocytes.

The percentage of reticulocytes in the total peripheral RBC count, white blood cell (WBC) count, and platelet

count are typically decreased. The presence of cytopenias in

FIGURE 24.1 Ring sideroblasts. The large granulules with stippled

cytopoiesis distinguishes RAEB from chronic myelogenous

iron, therefore sideroblasts, present in the bone marrow erythroblasts sur—

leukemia.

rounding the nucleus of the cells like ring. (Prussian blue stain.)

Idiopathic cytopenia of undetermined significance (ICUS).

(Reprinted from Heston in RI, Lux SE, Stossel P. Blood: Principles

and Practice of Hematology, 2nd ed., Philadelphia, PA: Lippincott

This category describes patients in whom MDS is possible

Willis & Wilkins, 2003, with permission.)

but not proven. Age-related to the topoisomeric clones given

**CHAPTER 24 ■ Myelodysplastic Syndromes and
Myelodysplastic/Myeloproliferative Neoplasms 481**

by mutations of genes that are recurrently occur. A

The median survival time for all patients with MDS is

subset of the genes are likely to initiate lesions that pro—

about 2 years. In the types of MDS with 5% to 30% or more

of expansion of a single clone.

bone marrow blasts, the risk of progression to AML is high, especially in children, and usually less than in adults.

RELATIONSHIP OF CYTOGENETICS TO

Median survival is 20 to 40 months. Progression to

PROGNOSIS

AML occurs in about 15% to 30% of patients. The percent—

age of peripheral blood bone marrow blasts is the most

MDS patients with multiple cytogenetic abnormalities have

important factor in determining survival. Complications of

shorter survival time (average, 8 months) than of patients with

bone marrow failure, including infections and hemorrhage,

single abnormalities (average, 18 months) or those with normal

cytogenetic studies.

karyotype (average, 36 months). Transformation to AML can

be observed in approximately 25% of patients with normal

karyotype, and average of 40% of patients with single abnor-

TREATMENT STRATEGIES

malities, and 50% of patients with multiple changes. Therefore, not

General treatment of MDS and MPN is RBC or platelet

transfusions. karyotype can be associated with poor prognosis.

transfusion to control bleeding. Vitamins or other
Patients with MDS and patients with AML should receive
rugs may also be given as supplement. Chemotherapy
specific karyotypes. Patients with unfavorable karyotypes
biological therapy are being tested in clinical trials. Biological
have significantly short survival times. Patients with im-
munotherapy is so effective as biological response of
karyotypes survive significantly longer but with relatively
therapy or immunotherapy. Bone marrow transplantation is
indicated in differences between patients with various diagnoses.
newer treatment approach.

Classification of patients with excess chromosomes in the choice of treatment
depends on the type of MDS
may not necessarily be based on cytogenetics than
well as the patient's general health. Still, the distinction between
MDS and AML.

colony treatment may be considered because of its effectiveness
The absence of cytogenetically normal cells in colonies
in patients in the past, but most patients with MDS are not
poor prognosis with frequent progression to AML, which is
cure with standard therapy. Participation in clinical trial

resistant to chemotherapy. Progression to AML depends not

on experimental drug may be better option.

only on chromosomal abnormalities but also on FAB subtypes MDS with no previous history of disease

type. Patients with monosomy 7, del(7q), trisomy 8, or i(17q)

(de novo presentation), treatment may take the form of one

have shorter survival times, more frequent progression to

the following:

leukemia, less response to treatment with 13-cis retinoic

acid than patients with del(20q) or t(2;11).

1. Supportive care to relieve symptoms of disease, such

One of the most widely used prognostic systems for MDS

is the International

Prognostic Scoring System

2. Another system (e.g., granulocyte colony-stimulating

(IPSS) (Table 24.4). Patients with fewer bone marrow blasts

factor [G-CSF], granulocyte-macrophage colony-stimulating factor with better cytogenetics (normal, 5q, 20q, Y) have

longer survival (GM-CSF, erythropoietin)

prolonged survival, in those with more blasts in

3. Chemotherapy (e.g., idarubicin, mitoxantrone, cytosine,

worse cytogenetics (complex or abnormalities of chromosome number)

so they have shorter survival.

4. Allogeneic bone marrow/stem cell transplantation

TABLE

24.4 International Prognostic Scoring System

Prognostic Variables

1

2

3

4

Very Good

Good

Intermediate

Poor

Very Poor

% Blasts in bone

$\leq 2\%$

$>2\%$ – 5%

5% – 10%

$>10\%$

marrow

Hemoglobin (g/dL)

≥ 10

$8 - < 10$

< 8

Platelet count ($\times 10^9/L$) ≥ 100

< 50

Cytogenetics

$-Y$, $\text{del}(11q)$ Normal, $-Y$, $\text{del}(5q)$,

$\text{del}(7q)$, $+8$, $+9$,

-7 , $\text{inv}(3)/\text{t}(3q)/\text{del}(3q)$, Complex: > 3

$\text{del}(12p)$, $\text{del}(20q)$,

$+19$, $\text{i}(17q)$, any other double including $-7/$

abnormalities

double including del(5q) single or double

del(7q), complex:

abnormalities or chromo—

independent clones

3 abnormalities

mosome 7 anomalies

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PART 6 ■ Neoplastic Disorders

Patients with secondary MDS or those who were previously diagnosed with MDS in the future will probably

BOX 24.1

receive treatment to relieve symptoms of the disease (e.g.,
neutropenia). Participation in clinical trials of chemotherapy
or biologic therapy also be an option

Categories of Myelodysplastic Syndrome/

(www.clinicaltrials.gov).

Myeloproliferative Neoplasms (MDS/ MPN)

treatment considerations must weigh the risk of therapy

■ CMML 0, 1, 2, Chronic myelomonocytic leukemia

versus the risk of problems associated with existing cytope-

■ CML, Atypical chronic myeloid leukemia

not as well as the likelihood of relapse

■ JMML, Juvenile myelomonocytic leukemia

transformation. Patients whose disease is more severe or get-

■ MDS/MPN-U, Myelodysplastic syndrome/Myeloproliferative—

ting worse or quickly relapse with chemotherapy.

leukemic neoplasms, undifferentiated

In patients with AML transforming to MDS, the clinical

■ RARS-, Refractory anemia with ringed sideroblasts

clinical responses to the standard therapy are poor. The greatly

neutrophilic

decrease in the topoisomerase in these patients is considered

responsible for their clinical picture. Leukemia-associated

inhibitory activity, which inhibits human GM progenitors,

may be responsible for the suppression of normal granul-

Pathophysiology

leucopoiesis in some patients. In addition, the profound

The pathophysiology of MDS/MPN-U involves an underlying defect of normal hematopoietic capability in these

ties in the regulation of myeloid pathways or cellular proliferation

cases may be because of multiple complex factors. Although

er tion, tur tion, n surviv l.

MDS is r re in chil ren, these represent so e o the ost i -

Isoenzy e n cytogenetic n lyses suggest th t the

f cult yscr si s to tre t. Chil ren tre te or MDS respon

p thogenesis o these clon l isor ers is ultistep pro—

poorly to convention l che other py. In re quently, chil ren

cess beginning with the est biliz tion o the ultipotent i l

y chieve re ission with intensive ther py n llogeneic

progenitor cell, c using proli er tion o ivergent clone o

bone rrow tr nspl nt tion.

genetic lly unst ble pluripotent i l ste cells th t pro uce

orphologic lly v ri ble but clon lly rel te progeny. T is

MYELOYDYSPLASTIC SYNDROMES/

type o berr tion beco es per nent when the cquisition

MYELOPROLIFERATIVE NEOPLASMS

o clon l chro oso e bnor lity exists.

(MDS/MPN)

I the cell bnor lity persists, ition l subclones with

recurrent chro oso e bnor lities e erge. T is prece es

T e MDS/MPN exist t the interph se o phenotypic lly

either failure of effective hematopoiesis or clonal transformation opposing bone marrow lineages. They are characterized by clonal expansion to acute myelogenous leukemia (AML) or by the simultaneous features of bone marrow failure or both. Hematopoiesis is dysplastic because of inefficient myelopoiesis usually with poor prognosis.

Transformation slowly expanding or so etiologically population. The combined MDS/MPN category in WHO 2016 has inclusion of blood cell precursors.

five subtypes with new molecular genetic criteria new entity, RARS-. The MDS/MPNs classification (Box 24.1)

Clinical Signs and Symptoms

includes clonal myeloid neoplasms that typically present -

Clinical symptoms are caused by complications resulting from the disease clinically, laboratory, or morphologic findings

findings that support diagnosis of MDS and other findings

that are more consistent with MPN (see Chapter 23). The

■ Cytopenias

blood picture is hybrid with at least one elevated one

- Dysplastic cells that function normally

reduce the topoiectic lineage. Patients place in this category

- Leukemic infiltration of various organ systems

category, for example, CMML, usually demonstrate hyper-

- General constitutional symptoms, such as fever, night sweats

cellular bone marrow because of proliferation of one or

more cell lines.

Etiology

Patients with refractory neutropenia with ring sideroblasts

The etiology of MDS/MPN-U is not known.

Neutrophilic dysplasia is provisionally included in this category as MDS/MPNs-U, unclear. The majority of

Incidence

these patients demonstrate mutation JAK2 V617F. The

threshold of platelets in this category has been lowered to

The incidence of MDS/MPN-U varies widely, ranging from

$600 \times 10^9/L$ to $450 \times 10^9/L$. Other laboratory characteristics

approximately 3 per 100,000 in individuals older than 60 years

include neutropenia, ring sideroblasts in the bone marrow

usually or chronic myelocytic-onocytic leukemia to

morphologically normal megakaryocytes that resemble

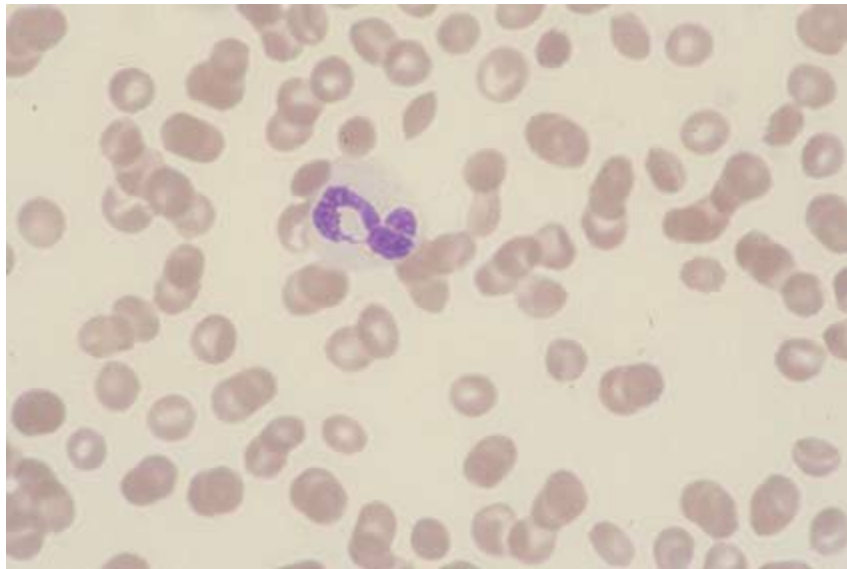
few s 0.13 per 100,000 children from birth to 14 years—

to essential thrombocythemia in primary myelofibrosis

lly or juvenile myelocytic-onocytic leukemia (JMML).

(see Chapter 23).

The incidence of MPN-U is unknown.



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Characteristics of Chronic

BOX 24.2

TABLE

24.5 Myelomonocytic Leukemia

Characteristics of Chronic Myelomonocytic

Blasts *

Leukemia

CMML-1

Peripheral blood <5%

■ Persistent peripheral blood monocytosis (greater than

Bone marrow <10%

$1 \times 10^9/L$)

CMML-2

Peripheral blood 5%–19%

■ Less than 20% myeloblasts, nonblast cells, or promonocytes—

Bone marrow 10%–19%

cytes in the blood or bone marrow

or

■ Absence of Philadelphia (Ph) chromosome or BCR-ABL1 fusion gene

Presence of Auer rods irrespective of the

■ No evidence of PDGFRA or PDGFRB mutation

percentage of promonocytes and blasts

■ Dysplasia in one or more myeloid cell lines

*Including promonocytes.

■ In the absence of dysplasia, diagnosis of CMML is supported by evidence on clonal cytogenetic

or olecul r genetic bnor lity, or onocytosis or

T is is the h ll rk o CMML. Neutrophili is co only

t le st 3 onths n exclusion o ll other c uses o

observe , with orphologic l bnor lities being present.

onocytosis.

Neutrophil precursors (pro yelocytes n yelocytes) usully ccount or less th n 10% o the leukocytes.

T e bone rrow is hypercellul r in ore th n 75% o

Chro nic Mye lo mo nocyti c Le uke mia

p tients. Gr nulocytic proli er tion c n be striking. An

Chronic myelomonocytic leukemia (CMML) is clon l he to—

incre se in erythroi precursors y be seen s well.

logic lign ncy th t is ch r cterize by e tures o both n

Genetic ssess ent or 11q23 (MLL) n NPM1 in ll

MPNs n n MDS (Box 24.2). T is or o yelo onoc ses o CMML-2 is vise . I these rkers re positive, it

cytic leuke i is uch less requent th n the cute v riety.

r ises the possibility o evolving cute yelo onocytic leu—

Di gnosis o CMML, ccor ing to the FAB cl ssif c tion

ke i . SRSF2 is nother potenti l ut tion.

criteri , istinguishes between two istinct or s, CMML-1

Cytochemical (Box 24.3) immunophenotyping of
 in CMML-2. A category of CMML-0 may exist in cases
 peripheral blood bone marrow aspirates are strongly re-
 with less than 5% blasts. One shows only an increase of
 of one. Immunophenotyping has been useful in detect-
 ing monocytes, which has no relationship to the type that
 undergoing transformation to acute leukemia. The peripheral
 transforms into AML. It is considered reactive monocy-
 blood bone marrow usually express the expected yelo-
 tosis. The other one, in addition to an increase of pure
 monocytic antigens, for example, CD13 and CD33. An
 monocytes, shows an increase of new monoblasts and pro-
 increase percentage of CD34+ cells has been associated
 monocytes. This is considered to be true CMML and usu-
 with transformation (Fig. 24.4).

usually quickly evolves into the M4 or M5 or so leukemia

CMML evolves in the age of 66 years, in the

(AML). The clinical symptoms closely resemble those of subleukemia ratio is 2.4:1.
 CMML is preceded by MDS

acute myelogenous leukemia.

of different subtype in about one fourth of patients and is

transform into acute leukemia in one month.

Pathophysiology

Dyserythropoiesis of all three cell lines is present. The percentage of reticulocytes, the total peripheral RBC count, and

the platelet count are typically decreased, although the total

peripheral WBC count may be normal or slightly decreased.

Laboratory Data

CMML includes an increase in number of cells in the peripheral blood that may be morphologically and/or functionally abnormal.

Typically, the blast percentage in the bone marrow

is always less than 20%. Laboratory findings vary

between having characteristics of MDS and MPNs. Patients

with the BCR-ABL1 fusion gene or rearrangements of

PDGFRA should not be included in this category, nor should

FIGURE 24.3 Aneuploidy with excess blasts (RAEB-1 and RAEB-2).

CMML patients with PDGFRB rearrangements be included.

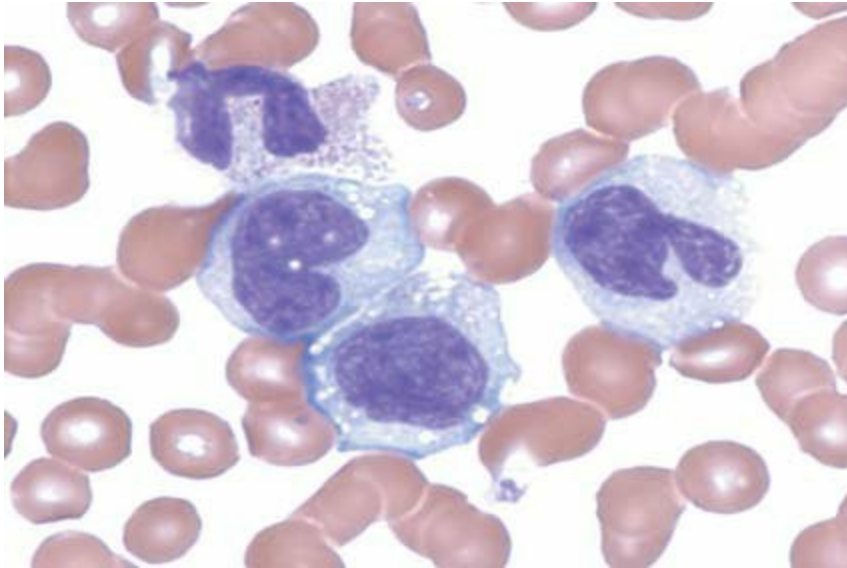
(Reprinted from Anderson SC. Anderson's Atlas of Hematology,

Peripheral blood smears (Figure 24.5) usually demonstrate

Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams &

persistent monocyte count greater than $1 \times 10^9/L$ (Fig. 24.3).

Wilkins, Copyright 2003, with permission.)



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PART 6 ■ Neoplastic Disorders

NOTE: This is a good time to complete end of chapter

BOX 24.3

Review Questions.

Cyto chemical Staining in CMML

CHAPTER HIGHLIGHTS

Alph -n phthyl cet te ester se

Alph -n phthyl butyr te ester se

Class i catio n

N phthol-ASD-chloro cet te ester se

■ Since the origin l evelop ent o the FAB cl ssif c tion

or yelo yspl stic syn ro es, the WHO h s envelope

newer cl ssif c tion o MDSs n yelo yspl stic syn-

Other Clas s i catio ns

ro e/ yeloproli er tive neopl s s.

MDS is ch r cterize by the si ult neous proli er tion

Atypic l chronic yeloi leuke i (CML), juvenile yelo-



n poptosis o he topoietic cells th t le to nor—

onocytic leuke i (JMML), n MDS/MPNS, uncl ssif ble

l or hypercellul r bone rrow n peripher l bloo

(MDS/MPNS-U) re the other, less requent cl ssif c tions in

cytopeni s.

the yelo yspl stic syn ro e/ yeloproli er tive c tegory.

1. Atypical chronic myeloid leukemia (CML, BCR-ABL1

Pathophysiology

negative). This category exhibits features of both myelo-

■ The MDSs and MDS/MPN are heterogeneous group of

myeloproliferative disorders that share the

clonal disorders of the bone marrow.

diagnosis. It is characterized by leukocytosis with

■ Analyses suggest that the pathogenesis of MDS is

involvement of neutrophils. Multilineage dysplasia is

step process beginning with the establishment of the

essential or CSF3R mutation is positive, chronic

potential stem cell, causing proliferation of divergent

myeloid leukemia should be considered.

of genetically unstable pluripotent stem cells that

2. Juvenile myelomonocytic leukemia (JMML) is

morphologically variable but clonally related progeny.

childhood. It is characterized by the proliferation of

■ Hematopoiesis is dysplastic because of inefficient

myeloid and monocytic lineages. Blasts in

proportion of slowly expanding or so-called

cytes count or less than 20% of peripheral blood cells

of blood cell precursors.

in bone marrow smears. Erythroid megakaryocytic abnormalities are frequently present. The BCR-ABL1

Etiology

etiology is absent, but mutations of genes NRAS, KRAS,

The etiology of primary MDS is unknown.

Pt PN-11, CBL, and NF1/MAPK are characteristic.



Secondary MDSs can so etiologically be related to

3. Myelodysplastic syndrome/ myeloproliferative neoplasms,



known agent.

undiscovered. This neoplasia meets the definition of

The greatest incidence of MDS follows chemotherapy—

MDS/MPNS but does not meet the criteria for CMML or



therapy in relation to therapy.

the other classification in this category.

Some predisposing factors for MDS may be genetic.

4. A provisional new category RARS- (refractory neoplasm)

■

with ringed sideroblasts in thrombocytosis) has been

Epidemiology

in 2016. Cytogenetic findings include negativity for

BCR-ABL in the presence of SF3B1 and JAK2 mutations.

■ MDS is rare in childhood. It occurs only in older individuals whose incidence increases with age. The prevalence of

MDS increases with age, reaching 1:500 in individuals older than 55

years of age.

■ It is estimated that at least 1,500 to 2,000 cases of MDS are

diagnosed annually in the United States.

Chromosomal Abnormalities

■ Chromosomal abnormalities have been observed in significant proportion of patients with MDS. Cytogenetic

differences exist between primary and secondary MDSs.

■ Chromosomal abnormalities may be clonal or trisomy—

clonal in nature and involve partial or total chromosomal

alterations. Most chromosomal changes are recurrent loss of

FIGURE 24.4 Chronic myelomonocytic leukemia (CMML).

Chromosomal alterations other than the translocations or

(Reprinted from Anderson SC. Anderson's Atlas of Hematology,

inversions.

Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams &

■ Survival of patients with MDS is better than those with non—

Wilkins, Copyright 2003, with permission.)

chromosomal patterns.

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Clinical Signs and Symptoms

MDS / MPN Disorders

■ A history of infections, bleeding, weight loss, or criovs—

Chronic Myelomonocytic Leukemia (CMML)

clinical symptoms may be reported.

■ It is clonal hematologic malignancy that is considered by

■ Anemia is common initial presenting symptom.

less frequent than the cytopenia.

■ Peripheral blood smear usually demonstrate persistent

Laboratory Manifestations

monocyte count greater than $1 \times 10^9/L$. This is the hallmark—

■ MDS is characteristically defined by pancytopenia in

rank of CMML.

the peripheral blood, usually in two or three cell lines

■ CMML develops t e i n ge o 66 ye rs, n the
th t y initi lly be in just one cell line, n low leuke—
le-e le r tio is 2.4:1.

ic bl st count in the bone rrow n peripher l bloo .

■ P ncytopeni occurs in ore th n 50% o p tients.

Other Clas s i catio ns

Treatm ent

■ Atypic l chronic yeloi leuke i (CML) exhibits
e tures o both yelo yspl stic n yeloproliferative

■ re t ent consi er tions in MDS ust weigh the risk o
isor ers t the ti e o i gnosis. It is ch r cterize by
ther py g inst the risk o proble s soci te with exist—
leukocytosis with jority o neutrophils.

ing cytopeni s s well s the likelihoo n i inence o

■ Juvenile yelo onocytic leuke i (JMML) is isor er
leuke ic tr ns or tion.

o chil hoo . It is ch r cterize by the proli er tion o

■ Chil ren tre te or MDS respon poorly to convention l
gr nulocytic n onocytic line ges.

che other py.

■ Myelodysplastic syndrome/ myeloproliferative neoplasms ,

Relationship of Cytogenetics to Prognosis

unclassifiable. This neoplasm meets the definition of

MDS/MPNs but does not meet the criteria for CMML or

■ Survival is generally good for patients with refractory
the other classification in this category.

neutrophils.

■ RARS-is new category. Cytogenetic findings include

■ Survival time for all patients with MDS is approximately
negativity for BCR-ABL in the presence of SF3B1 in
2 years.

JAK2 mutations.

CASE STUDIES

Case Study 24.1

(dysplastic) pattern with nuclear:cytoplasmic synchrony.

Many binuclear rubricytes were observed. More than 15%

An elderly white woman with history of neutropenia

of the neutrophils were sideroblasts.

Her primary concern was use of increasing shortness

of breath, dizziness, and severe fatigue. She had no history

■ Critical Thinking Group Discussion Questions

o prior treatment with drugs or exposure to lead or other

1. What resistance factors?

toxins. Her physician ordered CBC.

2. What organisms represent?

■ Laboratory Data

RBC $3.52 \times 10^{12}/L$

3. What is the most probable diagnosis in this case?

Hematocrit 37%

Hemoglobin 12.4 g/L

Cas e S tudy 24.2

WBC $8.4 \times 10^9/L$

A 26-year-old white man, with history of colitis 20 years

Platelets $275 \times 10^9/L$

Earlier, visited his physician because of fatigue. He

The peripheral blood smear demonstrate 3+ polychromatic tinging effect or
hypertension (captopril) and

philia, coarse basophilic stippling, occasional platelet abnormalities

bonic nystagmus inhibitor or glucose. He states that he

boies, occasional hyposegmented neutrophils. He usually

drinks one to two beers daily. He had colitis 1 week

cle te RBCs were seen per hun re leukocytes counted on
go n h s not elt well since then. He h s no li estyle
the peripher l bloo i erenti l. Signif c nt thro bocyte—
risk ctors. His physic l ex in tion reve le bruises in
ni with occ sion l gi nt pl telets w s lso note .
unusu l loc tions (e.g., insi e his thigh) with no report o
ny injury. He h s sc ttere petechi e.

■ Follow-Up Laboratory Data

Reticulocyte count 4.3%

■ Laboratory Data

RBC $2.47 \times 10^{12}/L$

■ Bone Marrow Examination

He tocrit 24%

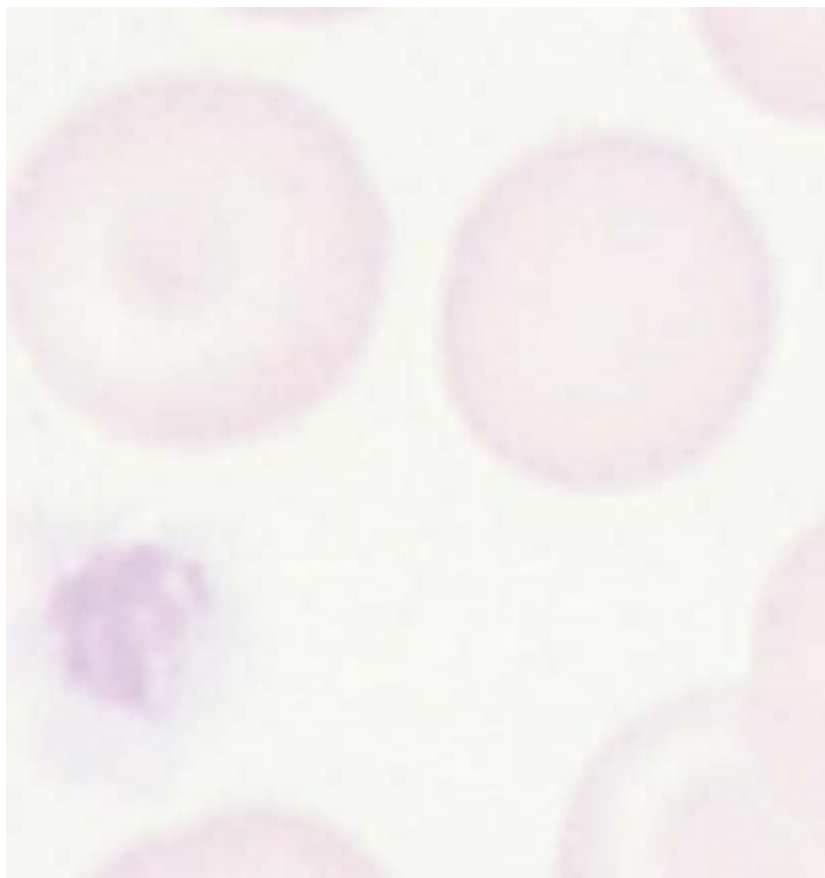
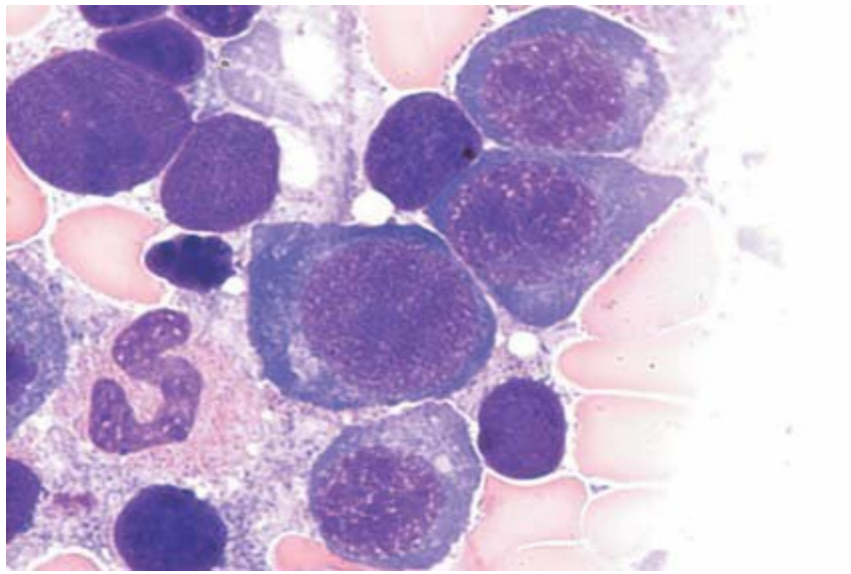
T e bone rrow w s hypercellul r with erythroi hyper—

He oglobin 7.7 g/ L

pl si . T e RBC precursors exhibite n bnor l growth

WBC $4.4 \times 10^9/L$

(continued)



PART 6 ■ Neoplastic Disorders

C

C A

A S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

Platelets $10 \times 10^9/L$

MCV 96 L

The peripheral blood smear exhibits microcytic, hypochromic red blood cells. A few hyposegmented neutrophils were observed.

■ Follow-Up Laboratory Data

Reticulocyte count 0.3%

Bone marrow examination reveals hypercellular marrow with dysplasia of the myelopoietic cells. The Prussian blue stain was positive for iron.

(Reprinted from Anderson SC. Anderson's, Atlas of Hematology,

■ Critical Thinking Group Discussion Questions

1. What is the cause of his elevated MCV?

2nd ed., Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2003. Copyright 2003, with permission.)

2. What is the significance of the hypercellular bone marrow?

NOTE: This is a good time to write out the answers to the Critical Thinking Group Discussion Questions.

3. What could be the potential cause of this patient's anemia?

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

5. The most frequent chromosomal abnormalities in children with MDSs include all of the following except

1. Patients with some type of MDS have an increased risk of

A. trisomy 8

developing

B. monosomy 7

A. acute lymphoblastic leukemia

C. deletion of long arm of chromosome 20

B. AML

D. all of the above

C. chronic lymphocytic leukemia

D. chronic myelogenous leukemia

6. The incidence of chromosomal abnormality in adults with MDSs is

with MDSs is

2. Which of the following agents has not been supported

A. 5% to 15%

by scientific research as being associated with the development of

B. 15% to 25%

development of secondary MDSs?

C. 25% to 60%

A. Alkylating agents

D. 40% to 90%

B. Organic solvents

C. Insecticides

7. The karyotype associated with high probability of

D. Both B and C

transforming to AML is

A. monosomy 5

3. An increase in incidence of MDSs is seen in

B. monosomy 7

A. males younger than 55 years of age

C. trisomy 11

B. females younger than 55 years of age

D. both A and B

C. males older than 55 years of age

D. females older than 55 years of age

8. Patients with MDSs commonly suffer from

anemia.

4. The most frequently involved chromosomes in

A. r sh

with MDSs re

B. ne i

A. 1, 5, n 7

C. visu l isturb nces

B. 3, 5, n 8

D. vertigo

C. 5, 7, n 8

D. 8, 12, n 13



CHAPTER 24 ■ Myelodysplastic Syndromes and Myelodysplastic/Myeloproliferative Neoplasms 487

REVIEW QUESTIONS (continued)

*9. An appropriate description of refractory anemia (RA) is

*11. An appropriate description of refractory anemia with

A. ne i , no bl st

excess bl sts, type 1 RAEB

B. ne i , less th n 1% bl sts

A. ne i , no bl sts

C. cytopeni , less th n 5% bl sts, no Auer ro s

B. ne i , less th n 1% bl sts

C. cytopeni , less th n 5% bl sts, no Auer ro s

*10. An ppropri te escription o re r ctory ne i with
ring si erobl ts (RARS) is

*12. In young p tients, the ther py o choice or MDSs

A. ne i , no bl sts

involves

B. ne i , less th n 1% bl sts

A. vit ins

C. cytopeni , less th n 5% bl sts, no Auer ro s

B. llogeneic bone rrow tr nspl nt tion

C. cytotoxic rugs

D. colony-sti ul ting growth ctors

Leone G. T er py-rel te leuke i n yelo yspl si : susceptibility

COMPANION RES OURCES

n incidence, Hematologica, 92(10):1389–1398, 2007.

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Malcovati L, Dell'Port MG, Pisciotta C, et al. Prognostic factors in

Each student is encouraged to access and use the Web—

the expectancy in yelowsplastic synovioses classification according

to basic companion resources available for this chapter.

to WHO criteria: basis for clinical decision making, J Clin Oncol,

Here you will find additional learning tools to increase

23(30):7594–7603, 2005.

your understanding of the concepts and clinical applica-

Mueller GJ, Bennett JM, Gosselin J, et al. Diagnosis and classification

tions of the chapter.

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Morphology of Myeloid yelowsplastic Synovioses (IWGM-MDS) consensus proposals
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we break through the impasse? Hematology Am Soc expression profiles in
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Hematol Educ Program, 2015:308–316, 2015.

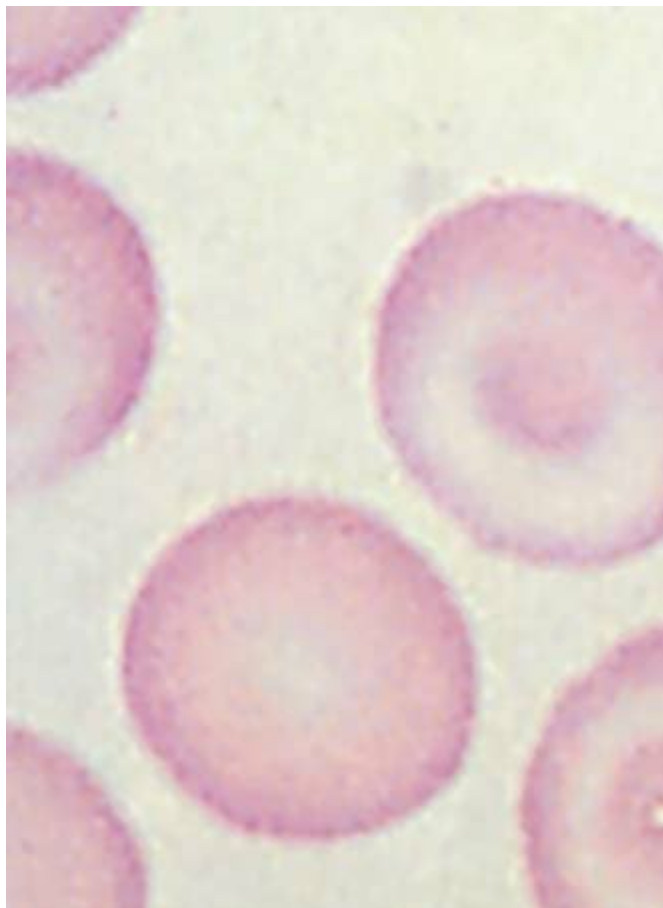
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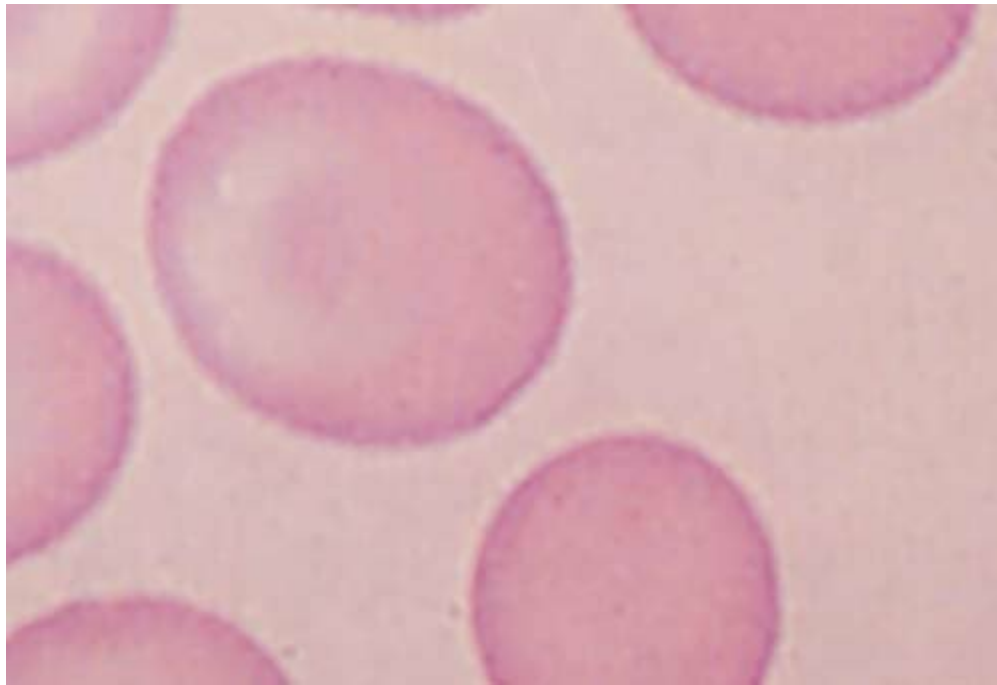
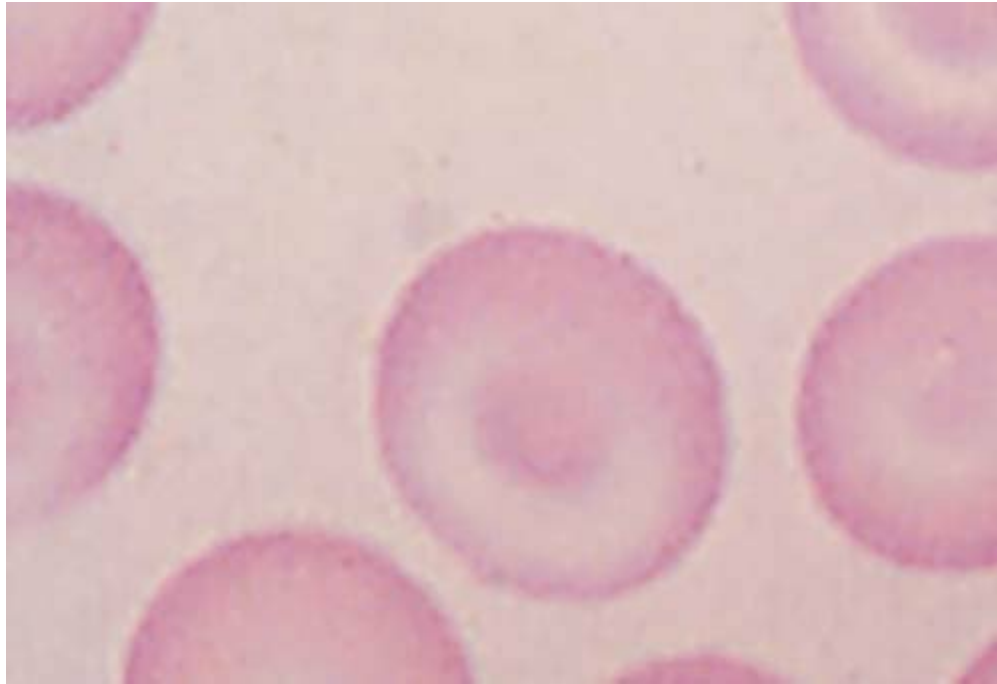
He ney ML, Gol e DW. Myelo yspl si , N Engl J Med, 340(21)

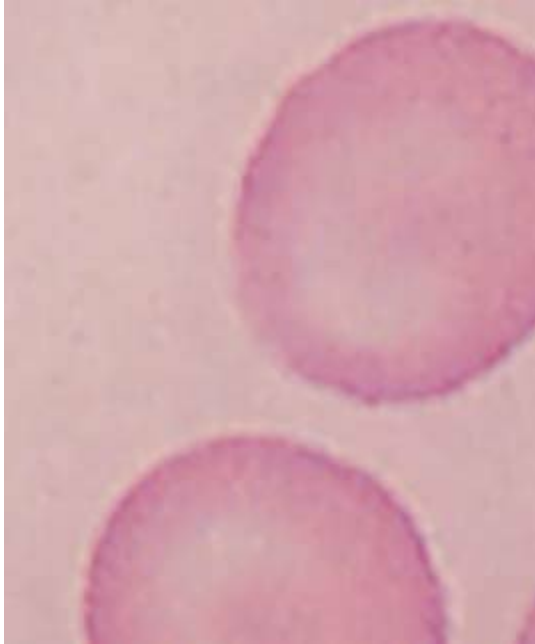
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1992.







PART SEVEN

Principles and Disorders of

Hemostasis and Thrombosis

Principles of Hemostasis and

CHAPTER

Principles of Blood Collection

25 Thrombosis: Vasculature and Platelets

KEY TERMS

aggregation

endothelium

platelet adhesion

alpha granules

extrinsic system

platelet aggregation

delta granules

glycocalyx

platelet plug

dense granules

intrinsic system

systemic circulation

endoreplication

lysozymes

thrombocytopenia

endothelial dysfunction

megakaryocyte

thrombocytosis

endothelins

mononuclear phagocytic system

vasoconstriction

LEARNING OUTCOMES

Overview of hemostasis and thrombosis

- Describe the process of formation of platelets from a megakaryocyte.
- Describe the components of bleeding and clotting mechanisms.
- List the ultrastructural components and cytoplasmic constituents of a mature platelet and describe the overall function of

Blood vasculature: structure and function

- Describe and compare the histological features of the tissues of the arteries and veins.
- Explain the life span activities of a mature platelet.
- Name the blood vessels that constitute the microcirculation and
- Explain the function of platelets in response to vascular damage.

compare their size and other features with those of arteries and

- Define generally the terms platelet adhesion and platelet
veins.

aggregation.

- Define the term vasoconstriction.

- Explain the events that take place during platelet adhesion, includ-

- Explain how vasoconstriction participates in hemostasis.

ing the substances produced.

- Describe the metabolic activity of the endothelium and its role in

- Explain the events that take place during platelet aggregation.

hemostasis.

- List substances that promote and substances that inhibit some

- Outline the general process of hemostasis in small vessels that
aspects of platelet aggregation.

contributes to the maintenance of vascular integrity.

- Briefly describe the process of platelet plug consolidation and

The megakaryocytic cell series

stabilization.

- Define the term endoreduplication and relate this process to
megakaryocytic development.

NOTE:

■ List and explain the three functions of thrombopoietin or

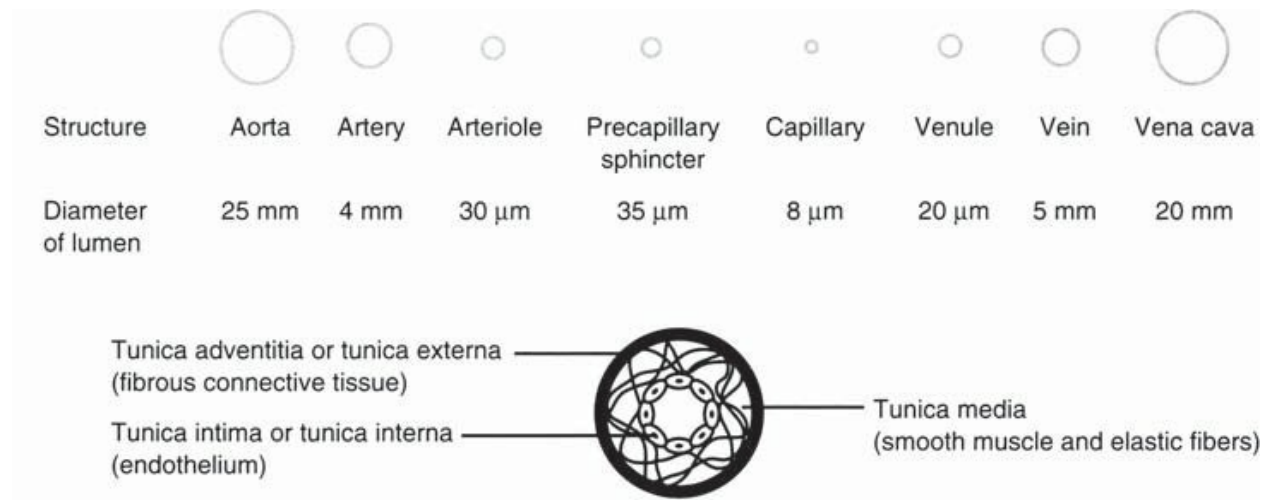
■ Indicates MLT and MLS core content

thrombopoietin-like cytokines.

Indicates MLT (optional) and MLS advanced content

■ Describe the morphological features of the mature stages of development in the megakaryocyte series.

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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

OVERVIEW OF HEMOSTASIS

consists of fibrous connective tissue that contains utononic

AND THROMBOSIS

nerve endings in the vessel wall, small networks of

blood vessels that supply nutrients to the tissues of the wall.

The intensity of circulatory homeostasis is achieved

Veins are larger and have more irregular lumen than

through the process of balancing bleeding (hemorrhage) and

arteries. In comparison with arteries, veins are relatively thin

clotting (thrombosis). Homeostasis, the resting of bleeding,

works with weaker internal clot. Elastin fibers are usually

open to several components. The major components

found only in larger veins, and there are fewer nerves distributed—

in the vascular system, platelets (thrombocytes), blood

flow to the veins than to the arteries.

coagulation factors, and fibrinolysis and ultimate tissue

repair. The other, less important, components are the co-

Arterioles and Venules

ple and the kinin system as well as serine protease inhibitors—

Arteries branch extensively to form a tree of ever-splintering

tors. Functionally, several normal processes are involved in

vessels. Arterioles are the microscopic continuation of arteries—

homeostasis following injury to small blood vessel:

ies that give off branches called arterioles, which in turn

1. Blood vessel spaces

join the capillaries. The walls become thinner as the arteri—

2. Formation of **platelet plug**

approach the capillaries, with the wall of very small

3. Contracting blood vessel, blood platelet, n

arteriole consisting only of endothelial lining and some

coagulation proteins

smooth muscle surrounded by small amount of connective

4. Development of blood clot around the injury

tissue.

5. Fibrinolytic removal of excess hemostatic material to reestablish microscopic size
veins return to venules.

establish vascular integrity

Venules connect the capillaries to the veins.

Capillaries

BLOOD VASCULATURE: STRUCTURE

The capillaries, arterioles, and venules constitute the major

AND FUNCTION

vessels of the microcirculation. As a unit, the microcirculation -

Arteries and Veins

functions as the link between the arterial and venous

circulation. Blood passes from the arterial to the venous system—

Arteries are the distributing vessels that leave the heart,

and the capillaries. Capillaries are the thinnest wall in

the veins are the collecting vessels that return to the heart.

Most numerous of the blood vessels. Sinusoids, which are

Arteries have the thickest walls of the vascular system.

Specialized types of capillaries, are found in locations such as

Although variations in the size (Fig. 25.1A) and type of vessels—

the bone marrow, spleen, and liver.

Also exist, the tissue (Fig. 25.1B) in vessel wall is divided

Capillaries are small structures consisting of supportive

into three coats or tunics. These coats are the tunica intima,

basement membrane to which single layer of endothelium

tunica media, and tunica adventitia. The tunica intima or

is tightly anchored. The basement membrane, immediately

the smooth glistening surface of endothelium that lines the

adjacent to the endothelium, is composed of loose connective

tissue (inner tubular cavity) of all blood and lymphatic vessels—

work of all fibers that support the endothelium in

cells in the heart. The simple squamous epithelium that

s barrier against particulate matter by increasing
 lines these vessels is referred to as endothelium. The tunica
 to the extravascular space. Collagen is also an important struc—
 ture consists of single layer of endothelial cells thickene
 to support the microvascular unit. Unlike the vessels
 by subendothelial connective tissue layer containing elastin fibers in venous
 systems, capillaries are composed
 of fibers. The tunica media, the thickest coat, is composed
 of only one cell layer of simple squamous epithelium, which
 contains smooth elastic fibers. The tunica intima
 performs the primary role of transport of materials between

FIGURE 25.1 Blood vasculature. **A.** Size

of vessels. **B.** tissue zones.

A

B

CHAPTER 25 ■ Principles of Hemostasis and Thrombosis: Vasculature and Platelets 491

blood tissue. Electron microscope examination of the

Endothelial Prothrombotic-

endothelium contains organelles such as mitochondria,

TABLE

25.1 Antithrombotic Balance*

ribosomes, in the endoplasmic reticulum.

Prothrombotic

Antithrombotic

VASCULATURE PHYSIOLOGY

Platelet-activating factor

Prostacyclin

Tissue factor

Thrombomodulin

The Role of Vasoconstriction in Hemostasis

von Willebrand factor

Tissue plasminogen activator

Vascular injury to large or medium-size artery or vein

Plasminogen activator

Urokinase

requires rapid surgical intervention to prevent exsanguination. When smaller vessel, such as arteriole, venule—

Inhibitor-1

Heparin-like molecules

cellule, or capillary, is injured, contraction occurs to control

Other coagulation factors

bleeding. This contraction of the blood vessel wall is called

Synthesis of factor V

vasoconstriction.

Binding of factors V, IXa, Xa

Vasoconstriction is short-lived reflex reaction of the

smooth muscle in the vessel wall produced by the sympathetic

Activation factor XII

thetich branches of the autonomic nervous system. This is not

*These various endothelial-associated factors and functions contribute

to narrowing, or stenosis, of the lumen of the blood vessel creates

to a dynamic physiological antagonism or “balance” that determines the

flow of blood in the injured vessel and surrounding vessels—

status of local hemostatic/thrombotic activity.

could rarely be sufficient to close severe capillaries.

Non-pertinent changes in endothelial structure and function

The Role of the Endothelium

tion, provoked by pathophysiological stimuli, can result in

The endothelium contains connective tissues such as collagen

localize, acute, and chronic alterations in the interactions of

genetic elements. This connective tissue matrix regulates the

endothelium with the cellular microvilli and the ability of the inner vessel wall to provide the primary

mechanism of circulating blood in the blood vessel wall. These

cellular stimuli to thrombosis following injury to blood vessels—

mechanisms can include the following:

1. The endothelium is highly active and locally is

■ Enhance permeability to (in subsequent oxidative

involve in the clotting process by producing or storing clot—

forming components of plasma lipoproteins

forming components (discussed in detail in later section of this

■ Hyperhesiveness of blood leukocytes

chapter). It is also rich with plasminogen activator, which, in

■ Functionally balances in local prothrombotic and anti—

appropriately stimulate, is released activates plasmin—

thrombotic factors, growth stimulators and inhibitors, and

gen, which ensures reabsorption of fibrin clots. Additionally,

vasoconstrictive (dilator, constrictor) substances (Fig. 25.1

the endothelium elaborates prostacyclin, which is synthe—

sis (Fig. 25.2)

size by the endothelium of prostacyclin in precursors and

strongly inhibits platelet aggregation and adhesion.

The endothelium is involved in the endothelial interactions leading to platelet activation or clot

formation of molecules such as serotonin, angiotensin, and br-

adykinin occur between the circulating blood and int-

erkinin that affect blood pressure regulation, the overent

endothelial sources. However, disrupted endothelial cells

can cross the endothelium, inhibition. With

release of prothrombotic substances that can initiate coagulation-

respect to blood coagulation, one of the basic characteristics—

tion. Collagen, in particular, initiates contact activation of

thrombocytes, and endothelium is its nonreactivity with

factor XII, thereby initiating blood coagulation.

platelets inhibit the ability to initiate surface contact activation of

The endothelium or its biologically interface between

clotting factor XII (Table 25.3).

circulating blood elements in all the various tissues of the

body. It is strategically situated to monitor systemic as well

Endothelial Vasodilators and Thrombotic-

specifically generate stimuli to potentially alter its function

25.2 Vasodilator Balance

tion listed. This process typically proceeds without

notice, contributing to normal homeostasis. The presence of

Constrictor

Dilator

unique organelle discovered by Weibel and Palé in 1964

turns out to be an important marker to identify authentic

Endothelin-I

Prostacyclin

endothelial cells. This organelle, called Weibel-Palé body

Angiotensin-II

Nitric oxide

(WPB), represents the storage granule of von Willebrand

Vasoconstrictor

Other “EDRF-like” substances

factor (vWF), molecule initiating platelet adhesion. A

Prostaglandins

receptor on leukocytes (P selectin) was found in WPB of

endothelium. Secretion of these organelles provides a paracrine

These various endothelial-generated substances contribute to the local

regulation of vascular tone through their effects on smooth muscle

or contribute to endothelium to become adhesive or platelets

contractility.

in leukocytes.

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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

in interleukin-1 (IL-1), viral infection in transmission,

TABLE

25.3 Endothelial Functions

bacterial toxins, in cholesterol in oxidatively modified lipoproteins.

Disruption of the endothelium directly contributes to our

Angiogenesis is

Synthesis of stromal components

components of hemostasis. After this event, the following

Coagulation

Vascular tone regulation

events take place:

Inflammation

Special metabolic functions*

1. Initially, rapid vasoconstriction for up to 30 minutes

Immune responses

reduces blood flow and promotes contraction of

platelets and coagulation factors.

*Transporting molecules from the vascular lumen to the subendothelium, producing angiotensin-converting enzyme, and binding lipoproteins, high—

2. In the second phase, platelets here interact with the density lipoproteins, and low-density lipoproteins.

expose subendothelial connective tissue, particularly

collagen. The aggregated platelets enhance sustained vasoconstriction by releasing thromboxane A₂ and vasoactive

amines.

ines, including serotonin and epinephrine.

3. In the third phase, coagulation is initiated through the

In 1985, ilypeptides, namely the endothelins, were

intrinsic system or the extrinsic system.

isolated and identified. The three members of the family—

4. Finally, fibrinolysis occurs following the release of tissue

endothelin-1, endothelin-2, and endothelin-3—repro—

plasminogen activators (t-PAs) from the vascular wall.

in a variety of tissues, where they act as regulators of

Fibrinolytic removal of excess hemostatic material is nec—

vasomotor tone, cell proliferation, and hormone production.

essential to reestablish vascular integrity.

Endothelin-1 is the only endothelial peptide produced in endothelial cells and is also produced in vascular smooth muscle cells. It is not stored in secretory granules within

Maintenance of Vascular Integrity

endothelial cells. Stimuli such as hypoxia, ischemia, or shear stress induce the RNA synthesis and secretion of

Vascular integrity or the resistance to vessel disruption by endothelin-1 within minutes. The half-life is approximately requires three essential factors. These factors recirculate—4 to 7 minutes.

including leukoplatelets, renocorticosteroids, and ascorbic

Endothelin-2 is produced predominantly within the kidney. A lack of these factors produces rigidity of the vessels,

neyn intestine, with smaller amounts produced in the

which makes the prone to disruption. Maintenance of vessels—

ovary, placenta, and uterus. The cells of origin are not

vascular integrity through the hemostatic process depends on

clear. Endothelin-2 has no unique physiological functions such

the events previously described. The importance of these

compared with endothelin-1.

Concentrations vary with vessel size (e.g., concentrations are lower in endothelin-3, like endothelin-1, circulates in the plasma,

likely because of vasoconstriction). The integrity of arterioles

but its source is not known. Endothelin-3 has been found in

in venules dependent on vasoconstriction, the formation of

high concentrations in the brain may regulate important

plug of platelets over the injury, in the formation

functions, such as proliferation and development in neurons

of fibrin clot. Arteries, because of their thick walls, release

endothelial cells. It is found throughout the gastrointestinal—

most resistant to bleeding; however, hemorrhage from these

infiltrates in the lungs and kidneys.

vessels is the most dangerous. Vasoconstriction is ultimately

All three endothelins bind to two types of receptors (A

important in arteries. Veins, which contain 70%

in B) on the cells of many lineages, including

of the blood volume, rupture with slight increase in

humans. Endothelin-A receptors are expressed abundantly

hypertensive pressure.

on vascular smooth muscle cells and cardiac myocytes.

These receptors initiate the vasoconstrictor action on—

NOTE: This is a good time to review the definitions of Key

thelin-1, although endothelin-B receptors also contribute

Terms in the Glossary and flash cards using

.

to this action in some vascular beds. Type B receptors re

This is also a good time to complete the Review Questions

expressed predominantly on endothelial cells and to such

related to the preceding content.

lesser extent on vascular smooth muscle cells. Endothelin-B

receptors bind endothelin-1 and endothelin-3 with similar

ity.

THROMBOPOIESIS

Endothelial Dysfunction

Manifestations, collectively termed endothelial dysfunction,

Myeloperoxidase (thrombocytes) (Fig. 25.2), and collectively

play an important role in the initiation, progression, and

active cell regulators, are the second critical component in

clinical complications of various atherosclerotic

the intensity of the atherosclerosis. These nuclear cells circu—

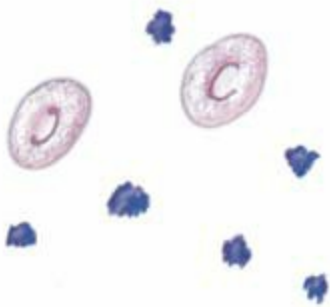
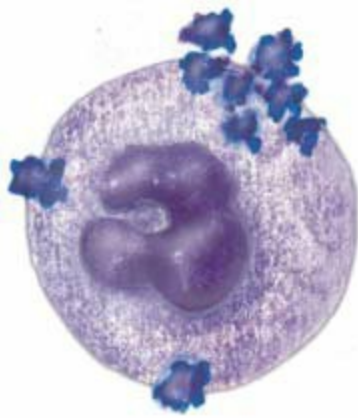
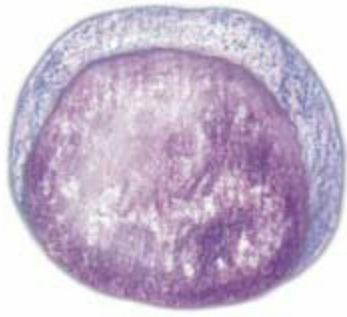
regenerative response. Various stimuli on the other hand in the peripheral blood are being produced by the

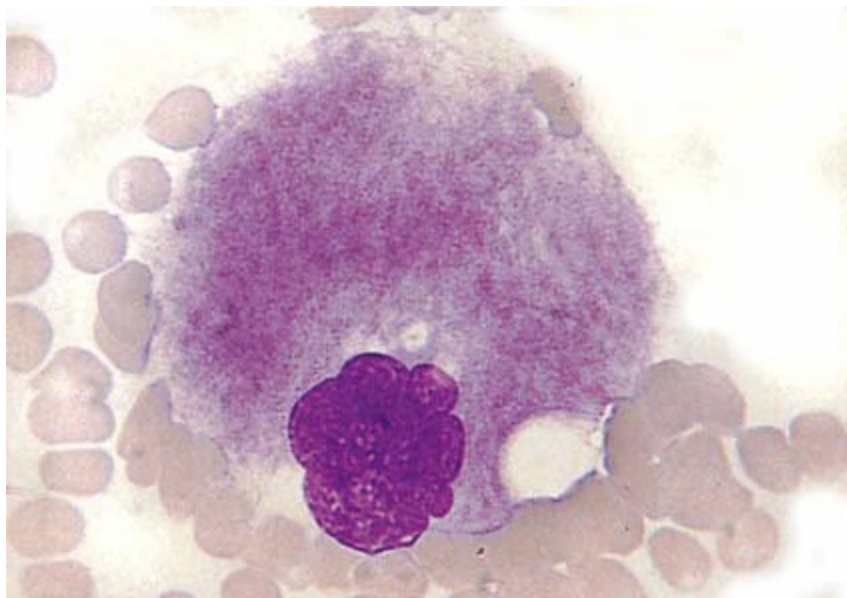
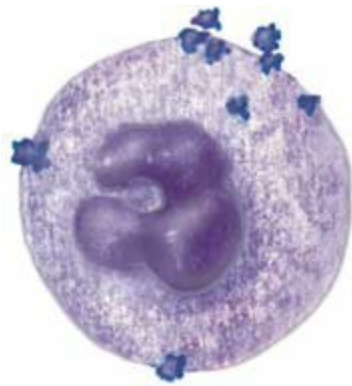
lymphocytes have been identified, including interleukin—

cytostimulating factor (CSF), the largest cells

regulatory substances such as tumor necrosis factor (TNF)

found in the bone marrow.





CHAPTER 25 ■ Principles of Hemostasis and Thrombosis: Vasculature and Platelets 493

Megakaryoblast

Promegakaryocyte

FIGURE 25.3 Granulocyte on MGG-stained normal bone marrow smear. (Reprinted from Mills SE. Histology for Pathologists, 3rd ed., Philadelphia, PA: Lippincott Williams &

Wilkins, 2007, with permission.)

size of megakaryocytes, but the sensing mechanisms that regulate platelet production have not yet been identified.

Recently, the gene for the human platelet protein was cloned and found to be expressed selectively in megakaryocytic

Megakaryocyte

cells. It was then found that antisense oligonucleotides that

block the synthesis of human platelet protein inhibit the formation of megakaryocyte colonies but not of erythroid or

granulocyte-macrophage colonies in vitro. This orphan

receptor of unknown function might be the receptor for

thrombopoietin. The protein may act synergistically with

other growth factors during the proliferation state. It is not

known whether further hormonal stimulation is necessary for

cytoplasmic maturation or platelet release.

Platelets

Megakaryocytopoiesis proceeds initially through phases

characterized by mitotic division of progenitor cells, followed

by wave of nuclear endoreduplication. Endoreduplication

is the process in which chromosomes replicate (DNA) and

FIGURE 25.2 Normal megakaryocytic series. (Reprinted from

the other events of mitosis occur without subsequent division. Anderson SC. Anderson's Atlas of Hematology, Philadelphia, PA:

fusion of the cytoplasmic membrane into a distinct boundary

Wolters Kluwer Health/Lippincott Williams & Wilkins, Copyright

cells. Recognizable megakaryocytes have ploidy values of $4n$,

2003, with permission.)

$8n$, $16n$, or $32n$. The function of megakaryocytes is

to produce, largely non-DNA-synthesizing cells to orpho-

General Characteristics of Megakaryocytic

logically identifiable megakaryocytes involves processes

Development

such as the presence of cytoplasmic organelles, the acquisition of membrane antigens and glycoproteins (GPs), and

Bone marrow megakaryocytes (Fig. 25.3) derive from

the release of platelets.

pluripotent stem cells. The sequence of development from

Thrombopoietin, the hormone thought to stimulate the

megakaryocytes to platelets is thought to progress from

proliferation and fusion of megakaryocytes, which in

the proliferation of progenitors to polyploidization, that is,

turn produce platelets, has recently been purified and cloned.

nuclear enlargement, normally to cytoplasmic tufts. Thrombopoietin activity results in several different cytomorphologies. An average of 1 to 3

kines: erythropoietin, IL-3, and granulocyte-macrophage

megakaryocytes should be observed when examining bone

colony-stimulating factor (GM-CSF). These substances have

promoted a 10× magnification. The majority of

has been shown to be able to increase megakaryocyte size, mature the MK1 stage. Quantitative estimation is one with 100×

reticular stage, ploidy.

oil immersion.

When megakaryocytes are being studied in bone marrow The Developmental Sequence of Platelets Early

promoted, their morphology must be determined from the

Development

biopsy section.

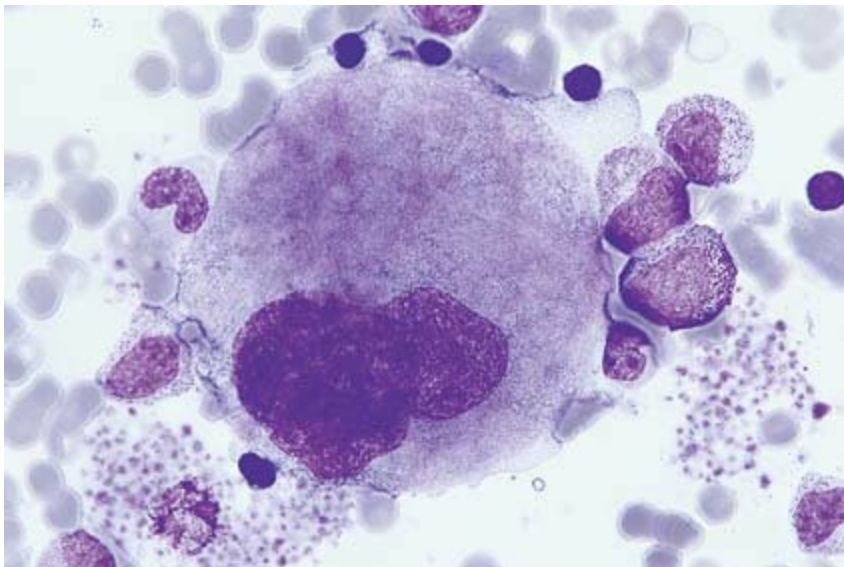
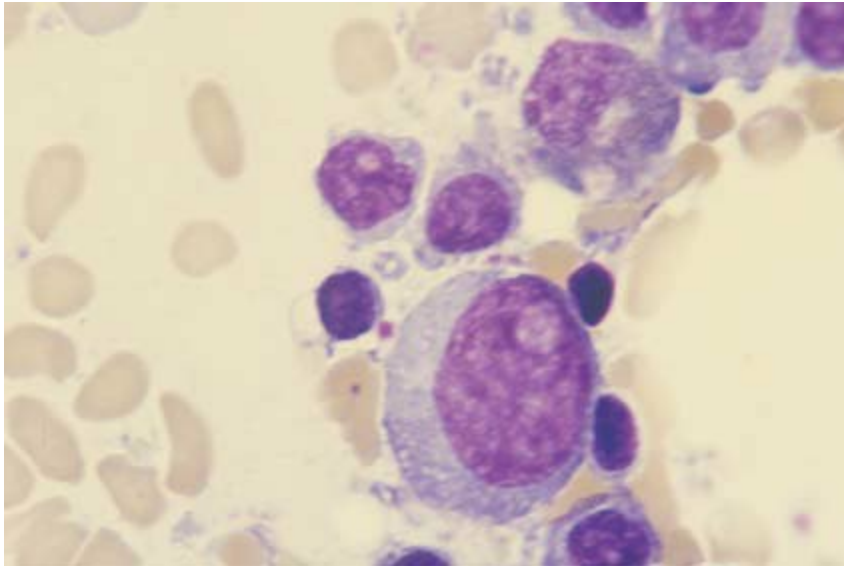
two classes of progenitors have been identified: the burst—

There appears to be a complex relationship between

forming unit-megakaryocyte (BFU-M) in the colony—

the circulating platelets in the number, ploidy, and

forming unit-megakaryocyte (CFU-M). The BFU-M is the



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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

Developmental Characteristics of

TABLE

25.4 Mature Megakaryocytic Cells

Megakaryocyte

Platelet

Size

30–160 μm

2–4 μm

Nuclear—

1:1–1:12

cytoplasmic ratio

Nucleus

Shape

Lobulated (two or more

(Anuclear)

lobes)

FIGURE 25.4 Megakaryoblast (see arrow). (Reprinted from

Chromatin color

Blue-purple

—

Anderson SC. Anderson's Atlas of Hematology, Philadelphia, PA:

Wolters Kluwer Health/Lippincott Williams & Wilkins, Copyright

Chromatin

Granular

—
2003, with permission.)

clumping

Nucleoli

Not visible

—

most primitive progenitor cell committed to erythroid—

Cytoplasm

cytoplasmic.

Color

Pinkish blue

Light-blue

The next stage of erythroid development is small,

fragments

mononuclear cell (Fig. 25.4) that expresses platelet—

Shape

Occasional pseudopods

specific phenotypic markers but is not morphologically identifiable as erythroid. These transitional cells represent

Irregular border

5% of erythroid elements. Some transitional

Amount

Abundant

in tissue eg karyocyte cells y be c p ble o cellul r

Granules

Abundant near the borders Scattered

division, but osteoblasts are nonproliferating while actively un-

derlying the cytoplasm

undergoing endocytosis.

Megakaryocytes

Mature Platelets

The final stage of megakaryocyte development is the morphologically identifiable megakaryocyte (Fig. 25.5). These

platelets have an average diameter of 2 to 4 μ , with

cells readily recognizable in the marrow because of their

younger platelets being larger than older ones. In contrast to

large sized lobulated nuclei. These cells are polyploid

megakaryocytes, platelets have no nucleus. The cytoplasm is

(Fig. 25.4).

light blue, with evenly dispersed, fine red-purple granules.

Megakaryocytes are the largest bone marrow cells, ranging—

An inactive or unstimulated platelet circulates thin,

ing up to 160 μ in size. The nucleolus (N:C) is ovoid-shaped. Its size is in the ratio can be as high as 1:12. Nucleoli are no longer visible. by the microtubular cytoskeleton beneath the cytoplasmic A distinctive feature of the erythrocyte is that it is not ebrne.

ultinucleate. The fully mature lobes of the erythrocyte
Platelets circulate in the inactive or in the flowing
she platelets to the cytoplasm on completion of their
bloodstream through endothelial-line blood vessels with—
tion. Platelet formation begins with the initial presence of
out interacting with other platelets or with the vessel wall.
pink color in the basophilic cytoplasm of the erythro—
Platelets are extremely sensitive cells and respond to in—
cyte and increase granulosity.

illustration by ordering pseudospherical structures
 retracted. Stronger structures use platelets to become sticky
 without losing their isosurface; however, changes in shape
 to an irregular sphere with spiny pseudospherical will occur with
 illustration. Transition in cellular shape is triggered by an increase in the
 level of cytoplasmic calcium. Such

changes in shape compensate by internal cellular contractions can result in the release of any of the internal organelles. A loss of visibility is associated with this change to spiny

sphere.

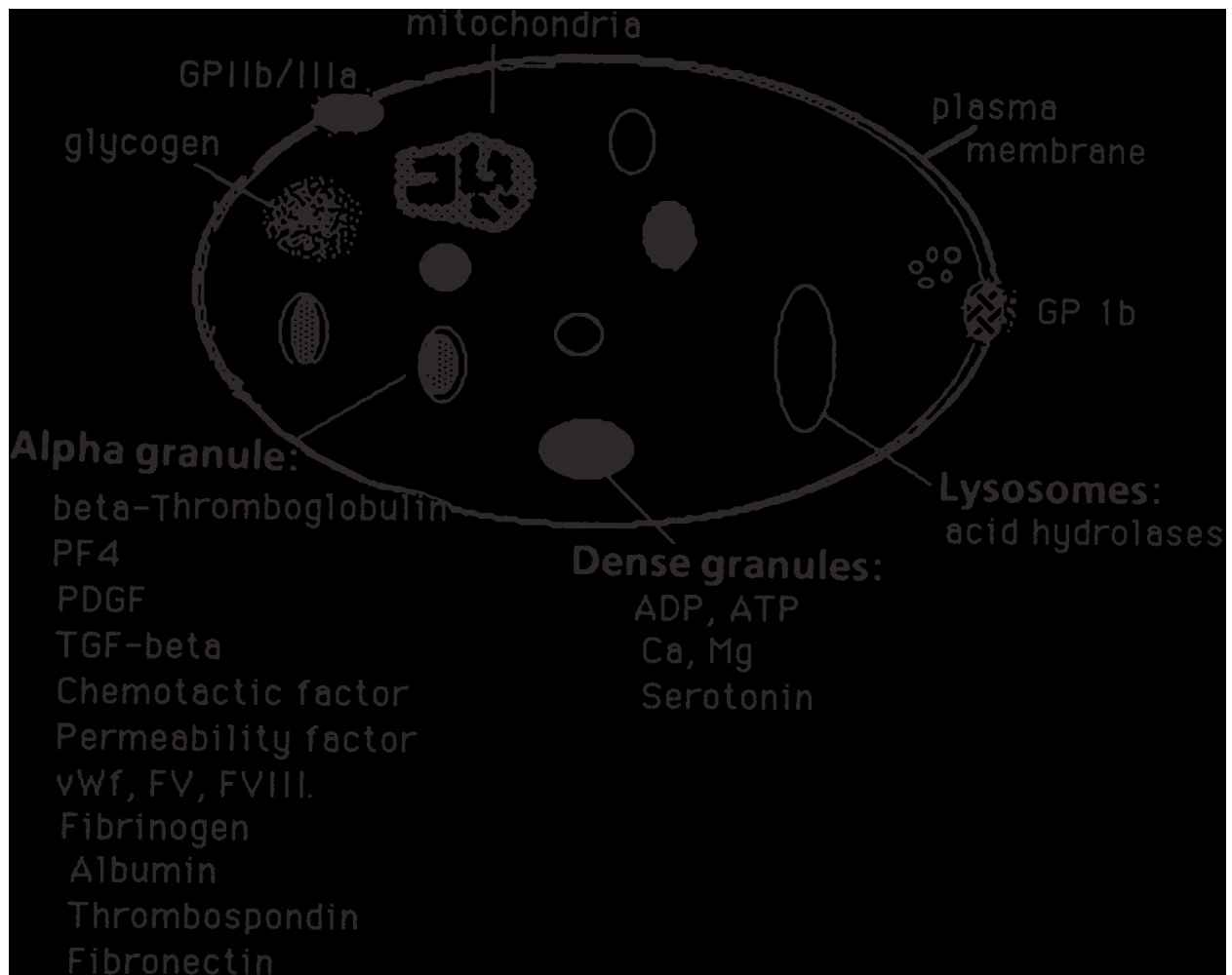
Cellular Ultrastructure of a Mature Platelet

Examination of platelet with an electron microscope reveals

variety of structures. These structures are unrelated to

FIGURE 25.5 Megakaryocyte.

the functioning of the platelet.



CHAPTER 25 ■ Principles of Hemostasis and Thrombosis: Vasculature and Platelets 495

channels of the open circulatory system and tubular systems together constitute the circulatory mechanisms of the cell.

Microfilaments and Microtubules

Directly beneath the cell membrane is a series of sube-

brne flaments microtubules that form the cellular
 cytoskeleton. In addition to providing the structure or
 maintaining the circulating disc shape of the cell, the
 cytoskeleton also maintains the position of the organelles. A
 secondary system of filaments is functional in internal organization and secretion of
 blood clotting products,
 such as fibrinogen. The filaments interact with the
 endotubular system in sequestering calcium, which initially
 causes contraction of internal organelles. These subcellular—
 internal cytoplasmic filaments make up the contractile system

FIGURE 25.6 Platelet with its inventory of granular content. (ADP,
 (sol gel zone) of the platelet.

adenosine triphosphate; ATP, adenosine triphosphate; FV, factor V;
 GPIIb, platelet surface glycoprotein IIb; PDGF, platelet-derived
 growth factor

growth factor; PF4, platelet factor 4; TGF- β , transforming growth

factor- β ; vWF, von Willebrand factor.) (Reprinted from Koopman

and van der Pluijm, 1997, p. 100.

These granules are alpha

WJ, Morel and LW. Arthritis and Allied Conditions: A textbook of

Rheumatology, 15th ed., Philadelphia, PA: Lippincott Williams &

granules, dense granules or delta granules, and lysosomes.

Wilkins, 2005, with permission.)

The platelet granules are the most abundant. Alpha granules contain heparin-neutralizing platelet factor 4 (PF4),

beta-thromboglobulin, platelet-derived growth factor, platelet-derived Glycocalyx

factor brinogen, fibronectin, vWF, and thrombospondin.

Ultrastructure examination of the platelet (Fig. 25.6) reveals

Dense bodies, and because of their presence when

the cell membrane is surrounded externally by

viewed by electron microscopy, contain serotonin, adenosine

triphosphate or glycocalyx. This glycocalyx is unique among the

phosphates (ADP), adenosine triphosphate (ATP), and calcium—

cellular components of the blood. It is composed of polysaccharides.

Lysosomes, the third type of granule, store hydrolytic

proteins and carbohydrate molecules that are related to the

enzymes. Extrusion of the contents of these storage granules

coagulation, complement, and fibrinolytic systems. The GPIIb/IIIa

requires internal cellular contraction. Secretions from the

receptors of the glycocalyx enter the membrane contact

granules are released into the open canalicular system.

reactions of platelet adherence, changes of cellular shape,

internal contraction, and aggregation.

Other Cytoplasmic Constituents

In addition to containing substantial quantities of the con—

Cytoplasmic Membrane

contractile proteins, including cofilin (thymosin),

A adjacent to the glycocalyx is the cytoplasmic membrane

cytoskeleton, in the cytoplasm of the platelet contains

whose chemical composition and physical structure.

glycogen and enzymes of the glycolytic and hexose pathways.

Extending through the plasma membrane into the

Energy and metabolic activities in cellular contraction is

interior of the platelet is an open canalicular surface—

derive from aerobic metabolism in the mitochondria

connecting system. It is this system that stores the invaginated aerobic glycolysis—
utilizing glycogen stores. The platelet is

in fact, a sponge-like portion of the cell that provides

very high-energy cell with metabolic rate 10 times that of

experimental surface to which platelet clotting factors

in erythrocyte. Based on energy availability and endogenous

are selectively sorbed. Contact activation of the membrane

constituents, the platelet is effectively equipped to fulfill the

phospholipids also generates procoagulant activity in its role of protecting the body against vascular thrombosis. The cytoplasmic membrane open channels and the endoplasmic reticulum

Platelet Kinetics, Life Span, and Normal

with the endoplasmic reticulum system that is not surface connected.

Reference Values

Although the channels penetrate the cytoplasm in its interior, they are generally in close proximity to granules. An average megakaryocyte produces about 1,000 to 2,000

platelets in other organisms. Therefore, products released by

platelets. Moreover, transit time, or the duration period of

the granules or cytoplasm can be transported to the exterior—

the megakaryocyte, is approximately 5 years.

rior environment through the channels. In addition to the

It is believed that platelets initially enter the spleen, where

over half of extracellular matrix is against the concentration

they are in or 2 years. Following this period, platelets re

turnover through the channels, phagocytosis is also

in either the circulating blood or the active splenic pool. At

likely to occur through these channels. Additionally, the

lifespan, approximately two thirds of the total number of

PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

platelets are in the systemic circulation, where the release reaction changes is compensated by series of bio—

ing one third exists pool of platelets in the spleen that

chemical reactions that occur during the process of plate—

reely exchange with the general circulation.

activation. The platelet plasma membrane is the locus of

A normal person has average of $250 \times 10^9/L$ (range of

interactions between extracellular and intracellular environ—

$150 \times 10^9/L$ to $450 \times 10^9/L$) platelets in the systemic circu—

ents. Agonists that lead to platelet activation revolve

lation. If the total platelet count is less than $150 \times 10^9/L$, it

includes nucleotide (ADP), lipids (thromboxane A₂,

is describes thrombocytopenia. If the total platelet count is

platelet-activating factor), structural protein (collagen),

greater than $450 \times 10^9/L$, it is referred to as thrombocytosis.

of proteolytic enzymes (thrombin).

Platelet turnover or effective thrombopoiesis averages $350 \times$

One of the distinct activities associated with platelet activation—

$10^9/L \pm 4.3 \times 10^9/L/y$.

ity in response to vascular injury is the continuous—

The lifespan of platelets is $9.0 \text{ ys} \pm 1 \text{ y}$. At

the end of vascular integrity by the adherence of platelets

to the endothelium, platelets rephagocytize by the

macrophages of the liver and spleen and other tissues of the

reticuloendothelial system. For the function of platelet

mononuclear phagocytic system.

platelets initially rests bleeding.

The adherence and aggregation of platelets at the sites of

CELL-BASED MECHANISM OF

vascular injury or the release of molecules involve

HEMOSTASIS: PLATELET FUNCTION

in hemostasis is wound healing and provide a barrier

surface for the assembly of coagulation enzymes that lead to

Platelets normally move freely through the lumen of blood

without adhesion. Vascular healing is promoted by stimuli—

vessels and components of the circulatory system. Maintenance

of the integrity and proliferation of endothelial cells and

of normal vascular integrity involves the nourishment of the

endothelial smooth muscle cells through the release of the into—
endothelium by some platelet constituents or the cuticle
gen, platelet-derived growth factor.

incorporation of platelets into the vessel wall. This process
requires less than 10% of the platelets normally in the cir-

Platelet Adhesion

culating blood. For hemostasis to occur, platelets not only
must be present in normal quantities but also must function

Intimal injury exposes the endothelial surface unevenly—
properly. This section discusses the hemostatic functions of
lying collagen (Fig. 25.7), platelets adhere to the subendothelial—
platelets, including platelet adherence and aggregation.

lial collagen fibers, spread pseudopods along the surface, and
clump together (aggregate). Platelet adhesion to subendothelial-

Overall Functions of Platelets

lial connective tissues, especially collagen, occurs within 1 to
2 minutes after break in the endothelium.

Following getting to the endothelium of blood vessel,

Epinephrine and serotonin promote vasoconstriction.

series of events occur, including adhesion to the injured ves-

ADP increases the adhesiveness of platelets. Considerable
self-sealing, aggregation, and secretion. Each structural
evidence indicates that the aggregation of

APTT

Intrinsic

XII

Kininogen-Precipitate-XI

IX-VIII:C

PT

Extrinsic

Platelet surface

phospholipids

VII

X

Ca²⁺

V

Prothrombin

(II)

Thrombin

TT

Fibrin

Fibrin

Fibrin

Dis rupte d tis s ue

monome r

polyme rs

XIII

polyme rs

ce ll me mbra ne s

(H-bonds)

(cova le nt

Fibrinoge n

Ca²⁺

bonds)

FIGURE 25.7 Co gul tion ech nis s. Shaded areas (“pl telet sur ce phospholipi s”) enclose the intrinsic co gul tion re ctions th t occur on the sur ce e br nes o pl telets. Dashed lines enclose the extrinsic co gu-l tion re ctions th t occur on isrupte tissue cell phospholipoprotein e br nes intru e into the circul tion.

(AP , ctiv te p rti l thro bo pl stin ti e; P , prothro bin ti e; , thro bin ti e.)

CHAPTER 25 ■ Principles of Hemostasis and Thrombosis: Vasculature and Platelets 497

platelets react by the binding of large soluble cro-

Primary wave

Secondary wave

molecules to distinct GP receptors anchored in the platelet

(a)

exposed. This increase in adhesiveness causes circulating

platelets to adhere to those already attached to the collagen.

The result is cohesive platelet mass that rapidly increases in

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size to form platelet plug.

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The transformation of the platelet from disc to sphere

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with pseudopods produces surface exposure reorganization -

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tion. Internal contraction of the platelet results in release of

n

a

(b)

granular contents of the platelet dense granules in the

Tr

lysosomal contents. This process releases the secretory

activities of other cells.

Shape change

Platelets have two sites of endothelial vascular injury in

(c)

then undergo activation and express functional GP IIb/III

receptors (also referred to as integrin α IIb

Time (minutes)

IIb β 3) or circulatinghesive ligand proteins (primarily fibrinogen). These

FIGURE 25.8 Platelet aggregation studies. Platelet aggregation studies—function of GP IIb/III receptors exhibit the recruitment of platelets involves the interaction of agonists (collagen, thrombin, ADP, and acetylcholine)—local platelets by forming fibrinogen bridges between platelets—agonists, or epinephrine) to suspension of platelet-rich platelets—process cellular platelet cohesion. Although function of (PRP); the agonist induces aggregation of platelets and allows transmission of light through the platelet component of the PRP. (a) In GP IIb/III receptors bind with other circulating adhesive molecules in platelets (including vWF, fibronectin, vitronectin)—initiates shape change that temporarily increases light transmission; then, thrombin, fibrinogen is the predominant subsequently, primary wave of platelet aggregation is recorded (signal becomes use of its relatively high concentration. The subsequent increase in light transmission) as fibrinogen binds its receptor, GPIIb/III, and begins to cross-link platelets. Unlike the other agonists, collagen is not present in these different adhesive molecules and does not induce primary wave. A secondary wave occurs among cells expressing GP IIb/III receptors. Glycoprotein IIb/

signal transduction events (resulting in platelet activation) eventually—

III is specific for platelets. Platelet recruitment depends

on the binding of GPIIb/III by fibrinogen released from

platelets exclusively on the fibrinolytic glycoprotein IIb/

platelet granules, whose contents are able to induce further aggregation—

III—dependent platelet cohesion. Glycoprotein IIb/III is

tion. (b) In storage pool release (SPD), platelet aggregation to ADP

the abundant platelet membrane protein (with approximately—

in other agonists typically shows an initial wave of aggregation, but

the aggregates subsequently dissociate because of release or absent

tely 50,000 receptors per platelet).

release of platelet granule contents. Because release of granules is

largely dependent on thromboxane, the spirin effect produces

Platelet Aggregation

similar platelet aggregation profile to that of SPD when ADP or epinephrine is used, but stronger agonists such as thrombin or collagen—

Observation of platelets by electron microscopy demonstrates the thromboxane pathway in platelet activation

strengths inhibition of organelle centralization that normally

aggregation curve. (c) Because of lack of GP IIb/III expression on the

allows platelet stimulation by collagen. Platelet aggregation—

platelet surface, platelet receptors with Glanzmann thrombosthenin system with aspirin-treated platelets shows decrease in spontaneous aggregation to all agonists except ristocetin. (From responses to collagen in absence of the secondary wave Rogers GP, Young NS. Bethesda Handbook of Clinical Hematology, aggregation (Fig. 25.8) that is normally induced by epinephrine, Philadelphia, PA: Lippincott Williams & Wilkins, 2013.)

epinephrine in low concentrations of ADP, which is reflected by the reduction in the release of (platelet) granule ADP, A variety of agents are capable of producing in vitro platelet aggregation, an energy-dependent process. These agents The biochemical platelet aggregation system is the only include particulate material such as collagen, proteolytic products of fibrinogen to monitor the effects of aspirin therapy, now enzymes such as thrombin, and biologic lines such as widely used to prevent stroke and heart attacks.

epinephrine and serotonin.

Platelet aggregation is the gold standard test to determine It is believed that bridges are formed by fibrinogen in the platelet function. Platelet aggregation in vivo is much more

presence of cell surface on platelets.

complex synaptic process than previously thought. Over

Time results in aggregation. In these aggregates reinforced

the latest evidence, it has become clear that platelet aggregation

by fibrin, they are referred to as thrombus.

represents multistephesion process involving distinct

Aggregation of platelets by the latest one pathway can

receptors andhesive ligands, with the contribution ofinhibitors by substances
such as prostaglandin E (PGE),

viaul receptor-ligand interactions to the aggregation process

enosine, and nonsteroidal anti-inflammatories

dependent on the prevailing blood flow conditions. Platelet

(e.g., aspirin). Aspirin, including aspirin-containing pro-

aggregation occurs with the end production ofthromboxane

agents such as Alka-Seltzer, induces long-lasting inhibition of

A2. It is now believed that three distinct mechanisms can ini-

effect in platelets. It is clinically detectable as prolongation

of platelet aggregation.

of bleeding time. The effects of aspirin appear to be

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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

primarily, if not exclusively, the predominant indication of

Antiplatelet Agents

prostaglandin in G/H synthesis, which catalyzes the first step in the synthesis of the prostaglandins, the conversion of arachidonic acid—

Platelets are critical in the process of hemostasis. Damage

leads to prostaglandin H

endothelial cells platelets that respond by releasing

2. Reduction of various

eicosanoids (thromboxane A

aggregating. Release of thromboxane A₂ and ADP—

2, prostaglandin E₂, and prost-

cycclin) in various tissues probably accounts for the variety of

effects promoting the process by stimulating surrounding

physiologic effects of aspirin that are the basis of its

platelets. The production of thrombin via the coagulation

therapeutic use in its toxicity.

cascade is also accelerated, stabilizing the thrombus by the

Because platelets lack the biosynthetic mechanisms

conversion of fibrinogen to fibrin.

needed to synthesize new protein, the effect is due by

The term, antiplatelet drug, is a generic term, describing
spirins cannot be representative of their lineage (proxi-
mants that decrease platelet aggregation inhibit thro-
tely 8 to 10 yrs). Therefore, treatment with spirin
bus oration. Antiplatelet drugs are osteoactive or re-
is stopped, cyclooxygenase activity recovers slowly, suc-
cessfully clots that re-constitute largely of platelets. Different
tion of platelet turnover. This explains the present pro-
cesses of antiplatelet drugs at different junctions in this
of how drug with 20-minute half-life in the systemic cir-
process. These drugs re:

culation can be effectively antiplatelet agent when

■ Aspirin

administered orally.

■ Clopidogrel

Because of the permanent nature of aspirin-in-
c-

■ Prasugrel

inhibition of platelet prostaglandin in G/H synthesis, the inhibitory

■ Dipyridole

effect of repeated doses less than 100 g is cumulative.

■ GP IIb/III nt agonists

Daily administration of 30 to 50 g of aspirin results in virtually complete suppression of platelet thromboxane biosynthesis

Aspirin

within 7 to 10 days. These changes in platelet biochemistry are associated with maximal inhibition of thromboxane production

A typical example of drug-induced dysfunction is the increased platelet aggregation, and prolongation of the bleeding time of aspirin. One or two aspirin tablets are sufficient to take account of the antithrombotic effects of aspirin.

extend the bleeding time to twice the normal value. The anti-

Qualitative platelet disorders can be attributed to the

platelet effects of aspirin are due to the inhibition of platelet aggregation, or secretion effects. Release effects are the

function. This inhibition is caused by action of nonselective,

largest group of platelet function disorders. This condition is

irreversible acetylation of platelet cyclooxygenase 1 (COX-1)

caused by properties of signal transduction -

which catalyzes the production of thromboxane and prost-

aglandins, and endothelial prostaglandins, or endothelial

the result is inhibition of thromboxane A₂

release.

synthesis, potent inhibitor of platelet aggregation and vasoconstriction. Aspirin-treated platelets will not respond to

Platelet Plug Consolidation and

enhance the release of collagen-coated surfaces but will

Stabilization

in the thrombus because of the inhibition of aggregation.

The permanently anchored platelet plug requires the

Clopidogrel

consolidation and stabilization. Fibrinogen, under the influence of

Clopidogrel is an ADP receptor antagonist that competitively

inhibits ADP binding to platelet receptors, preventing

this consolidation and stabilization. This process involves

ADP-dependent upregulation of GP IIb/III receptor, and blocks

the precipitation of polymerized fibrin around the platelet.

plification of platelet aggregation (Fig. 25.9). Clopidogrel is

The result is fibrin clot that produces an irreversible platelet

the osteoclasts use P2Y₁₂ platelet receptor inhibitor.

plug (Box 25.1).

Prasugrel

Prasugrel is in the same class as clopidogrel.

BOX 25.1

However, it exhibits more efficient platelet inhibition.

Dipyridamole

Platelet Plug Consolidation and Stabilization

The mechanism of dipyridamole is not fully understood.

- Vascular injury exposes subendothelium

stool, but it is thought to act by inhibiting endothelial release of vasoconstrictors

into platelets, thereby reducing ADP-induced aggregation.

- platelethesion

Glycoprotein IIb/IIIa Antagonists

- platelet aggregation

- platelet plug formation

GP IIb/IIIa antagonist should inhibit platelet aggregation

- consolidation of platelets

formation. However, because with the original GP

- fibrinolysis.

GP IIb/IIIa antagonist is monoclonal antibody. It has

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Age Group	Percentage
18-24	10%
25-34	15%
35-44	20%
45-54	25%
55-64	30%
65-74	35%
75-84	40%
85-94	45%
95-104	50%

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Gender	Percentage
Male	45%
Female	35%
Other	20%





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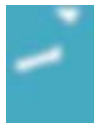
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Category	Item	Value
Category 1	Item 1.1	10
	Item 1.2	20
	Item 1.3	30
	Item 1.4	40
	Item 1.5	50
	Item 1.6	60
	Item 1.7	70
	Item 1.8	80
	Item 1.9	90
	Item 1.10	100
Category 2	Item 2.1	10
	Item 2.2	20
	Item 2.3	30
	Item 2.4	40
	Item 2.5	50
	Item 2.6	60
	Item 2.7	70
	Item 2.8	80
	Item 2.9	90
	Item 2.10	100
Category 3	Item 3.1	10
	Item 3.2	20
	Item 3.3	30
	Item 3.4	40
	Item 3.5	50
	Item 3.6	60
	Item 3.7	70
	Item 3.8	80
	Item 3.9	90
	Item 3.10	100



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CHAPTER 25 ■ Principles of Hemostasis and Thrombosis: Vasculature and Platelets 499

ADP

ADP

P GI2

A3P 5P S

Clopidogre l

MRS 2179

Ticlopidine

Cangre lor

P2Y

P2Y

1

122

12

IP

GT

G P

g

g

GT

G P

GT

GT

G P

TP

g

G

a

b

b

a

a

b

q

G

G

P LC

L b

i2

s

b

P I3

I K

IP 3 DAG

P IP2

GTP Rap1

Ca²⁺ Ca²⁺

ON

AC

P KC

Akt

Ca²⁺

P LA

ATP

cAMP

2

PC

Ara chidonic

PH P le cks trin P H

Acid

FIGURE 25.9 Platelet activation by ADP. Two receptors that can be activated by ADP have been identified in platelets. P2Y₁ and P2Y₁₂, which are coupled to different G proteins, therefore elicit different responses. G_q couples P2Y₁ receptors to the activation of PLC β .

The G_i family member, G_{i2}, couples P2Y₁₂ to the inhibition of cAMP production (via G_{i2} α) and to effector pathways that include PI3K.

n R p1B (vi G $\beta\gamma$). (Fro M r er VJ, Air WC, Bennett JS, et l. Hemostasis and Thrombosis, 6th e , Phil elphi , PA: Lippincott Willi s & Wilkins, 2012.) gents — or ex ple, eptif b ti e, nonpepti e nt gonist.

Blee ding Tim e Wit h a nd With out Asp irin

Abcixi b inhibits the f n l co on p thw y o pl telet

T e blee ing ti e test is now n in requently per or e in

ggreg tion where f brinogen bin s to GP IIb/III receptor.

vivo e sure ent o pl telet hesion n ggreg tion on

Neutr lizing ntibo ies to bcixi b c n or , there ore,

loc lly injure v scul r suben otheliu . T is test provi es

the rug c n only be use once. GP IIb/III nt gonists c n

n esti te o the integrity o the pl telet plug n thereby

c use severe blee ing, n it c n t ke over 12 hours or pl telet unction to be restore er stopping n in usion.

Ot he r Age nt s

BOX 25.2

Another ntipl telet rug is tic grelor. It is license or use with

spirin in preventing therothro botic events. T e newer P2Y₁₂

nt gonists pr sugrel n tic grelor h ve ore pre ict ble ph r-

Labo rato ry As s es s m ent o f Plate let Functio n

cokinetics n ph r co yn ics th n clopi ogrel.

Peripheral blood smear

Laboratory Assessment of Platelets

Platelet count

Petechiae

A platelet count is an essential component in the evaluation

of platelet aggregation

of the patient. Examination of the peripheral blood smear

Adenosine triphosphate (ADP)

or platelet morphology is critical because

Epinephrine

clinical clues may be obtained from evaluation of platelets—

Collagen

platelet quantity and morphology. A normal platelet count

Ristocetin

peripheral smear evaluation is basic assessment. Except for

Archi on te

extremely low platelet counts, total platelet counts reported

or automated equipment.

Platelet aggregation (release)

Platelet antibodies (IgM and IgG)

Qualitative Assessment of Platelets

Platelet surface glycoproteins (flow cytometry)

Platelet count is normal but patient has suggestive

Platelet count IV

bleeding history, assessment of platelet function should be

(BET)-thromboglobulin

conducted. Methods of evaluation (Box 25.2) include aggregometry—

Thromboxanes

aggregating agents, and light aggregation.

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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

ensures the interaction between the cell surfaces and platelet aggregation procedure is performed on a turbidimetric

platelets. Platelet adhesiveness is the process of the sticking of

biochemical aggregometer first described by Born. Changes

platelets to the vessel wall, where platelet aggregation is the

in aggregation reagents platelet-rich plasma, and

sticking or clumping of platelets to each other. The bleeding

aggregating reagents are stirred together in a cuvette. The

time reflects these aspects of platelet function.

aggregometer serves as a standardize spectrophotometer.

As the platelet count drops below $100 \times 10^9/L$, the bleeding -

As aggregation proceeds, more light passes through the
increases progressively from 3 to 8 minutes.
more than 30 minutes. A prolonged bleeding time

Epinephrine is usually used in two doses, as ADP. A

in patient with platelet count greater than $100 \times 10^9/L$

onophic curve is elicited with ADP. A biphasic curve

indicates either impaired platelet function or effect of substance usually elicited with
epinephrine. Ristocetin -

phenothiazine. Results between 8 and 11 minutes are usu—

chonic also usually induce onophic curve.

lly not clinically significant.

Lu aggregation is an extension of aggregation.

The onset of the effect of aspirin is rapid. Platelet inhibition

For more than 20 years, ristocetin cofactor (RCo) assay

is essential within 60 minutes. The effects of aspirin last for

(which ensures vWF) interfere with aggregation of platelets of the lineage of the platelet, approximately 7

lets in the presence of the antibiotic, ristocetin, and has

to 10 years. With borderline results, the aspirin tolerance test

been the only use assay or the essential

is often used and is repeated 2 hours after aspirin challenge.

of the functional activity of vWF. Recently, collagen-binding enzyme-linked immunosorbent assay (ELISA) has

Clot Retraction

been introduced as a sensitive procedure. Circulating

the contractile abilities of platelets also result in the con—

platelet vWF antigen is characteristic of generalized thrombolytic

retraction of whole clots. Clot retraction reflects the number

of platelets.

the quantity of platelets, fibrinogen concentration, fibrinolytic activity, and platelet cell volume. Because the fibrin

Platelet Adhesion

clot entraps the cellular elements of the blood, primarily

Platelet adhesion in vivo occurs as platelets attach either to

erythrocytes, the degree of clot retraction is related to the

size of the vessel wall or to each other. Methods of in vitro

extent that fibrin contracts by the volume of erythrocytes

hemolysis rely on the adherence of platelets to glass surfaces.

(hemocrit). Therefore, the smaller the hemocrit, the

The amount of adherence of platelets in blood is proportional to

greater the degree of clot retraction.

gl ss sur ce c n be e sure by counting the nu ber o

T e egree o clot retr ction is irectly proportion l to the

pl telets be ore n er exposure to gl ss be s. T e reli—

nu ber o pl telets n inversely proportion l to the he -

bility o this etho ology h s been questione ; there ore,

tocrit n the level o the bloo co gul tion ctor f brino—

use o the etho is not univers l.

gen. When clot issolution (f brinolysis) is very ctive, the

f brin clot y be issolve l ost s quickly s it is or e ,

Ant ipla te le t Ant ib ody As s ays

n clot retr ction is i p ire .

Antibo ies g inst pl telets y ppe r in the pl s o

p tients in cert in clinic l con itions, lthough it y be

Pla te le t Aggregat io n

i cult to e onstr te these ntibo ies in c ses o i une

Most pl telet ggreg tion proce ures (see Ch pter 32) re

thro bocytopeni . Av il ble techniques c n inclu e co -

b se on so e v ri tion o Born etho . Agents such s

ple ent f x tion etho s, lysis o chro iu 51-l bele

ADP, coll gen, epinephrine, sn ke veno , thro bin, n

platelets, assays of platelet-bound immunoglobulins, and ristocetin can be used to aggregate platelets. The principle of competitive inhibition assays.

The test is that platelet-rich plasma is treated with known aggregating agent. Aggregation, cloudiness or turbidity can

NOTE: This is a good time to complete the end of chapter be sure using spectrophotometer. Depending on the Review Questions.

type of aggregating agent used, curve that can be used to assess platelet function is obtained.

In vivo, platelets participate in primary hemostasis by first adhering and then aggregating at the site of injured blood vessel. Platelet aggregation is contributing factor to sub-

CHAPTER HIGHLIGHTS

cute stent thrombosis. Patients undergoing stent proce-

Blood Vasculation : Structure and Function

ure reinitiated to assess the effect of using aspirin

clopidogrel, prasugrel whose active metabolite selectively

■ Arteries have the thickest walls of the vascular system.

inhibits ADP-dependent platelet aggregation. In vitro, plate-

■ Veins are larger and have more irregular lumen than the

platelet aggregation systems use various platelet activators to in-

teries. In comparison to arteries, veins are relatively thin

tiy but not platelet function in thrombotic platelet

wall with weaker interaction.

regulation. ADP, collagen, epinephrine, ristocetin, and

■ Arterioles are the microscopic continuation of arteries.

endothelial cells release compounds to induce

Microscopically size veins are referred to as venules.

platelet aggregation.

■ Venules connect the capillaries to the veins.

CHAPTER 25 ■ Principles of Hemostasis and Thrombosis: Vasculature and Platelets 501

■ Blood passes from the arterial to the venous system via the

■ Bone marrow megakaryocytes derive from pluripotent

capillaries. Capillaries are the thinnest wall and most
potentially stem cells. The sequence of development from
nucleus of the blood vessels.

megakaryocytes to platelets is thought to progress from

■ Contraction of the blood vessel wall is called

the proliferation of progenitors to polyploidization, then
vasoconstriction.

is, nuclear endoreplication, finally to cytoplasmic

■ One of the basic characteristics of normal, intact endothelium is the formation of platelets.

thelium is its nonreactivity with platelets and inability to

■ An average megakaryocyte produces approximately 1,000
initiate surface contact activation of clotting factor XII.

to 2,000 platelets. Marrow transit time, or the duration

■ Minimal interactions leading to platelet activation or clot
period of the megakaryocyte, is approximately 5 years.

or tion occur between the circul ting bloo n int ct

■ It is believe th t pl telets initi lly enter the spleen where en otheli l sur ces. Disrupte en otheli l cells rele se they re in or 2 ys. Following this perio , pl telets re thro bopl stic subst nces th t c n initi te co gul tion. in either the circul ting bloo or the ctive splenic pool.

Coll gen, in p rticul r, initi tes cont ct ctiv tion o c-

■ At ll ti es, pproxi tely two thir s o the tot l nu - tor XII, thereby initi ting bloo co gul tion. Disruption ber o pl telets re in the syste ic circul tion, where s o the en otheliu irectly ctiv tes ll our co ponents the re ining one thir exist s pool o pl telets in the o he ost sis.

spleen th t reely exch nge with the gener l circul tion.

■ A nor l person h s n ver ge o $250 \times 10^9/L$ (r nge, 150

The Megakaryocytic Cell Series

$\times 10^9/L$ to $450 \times 10^9/L$) pl telets in the syste ic circul tion.

■ The life span of a platelet is $9 \text{ ys} \pm 1 \text{ y}$. At the

■ Mature platelets (thrombocytes), et bolic lly ctive cell en o their li e sp n, pl telets re phagocytize by the

regents, are the second critical component in the in—

liver and spleen and other tissues of the mononuclear

system of the osteoblasts.

phagocytic system.

■ These nuclear cells circulate in the peripheral blood.

■ A platelet count is an essential component in the evaluation—

being produced by the cytoplasm of bone marrow meg-

akaryocytes, the largest cells found in the bone marrow.

■ Antiproliferative drugs can interfere with the process of the osteoblasts.

■ Antiproliferative drugs can interfere with the process of the osteoblasts.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

8. All blood lymphatic vessels are lined with

1. Not all the osteoblasts depend on all of the following

A. endothelium

except

B. nerve endings

A. an intact vascular system

C. stratified epithelial cells

B. in equilibrium numbers of platelets

D. simple squamous epithelium

C. appropriate coagulation factors

D. fibrinolysis

9. Blood passes from the arterial to the venous system via

A. arterioles

2 through 6. The sequence of events following injury to

B. capillaries

small blood vessel is (2)

to (3)

to (4)

C. veins

to (5)

to (6)

.

D. arteries

A. contact between large blood vessel, blood platelets, and coagulation proteins

10. The initiating stimulus to blood coagulation following

B. formation of platelet plug

injury to blood vessel is

C. fibrinolysis to reestablish endothelial integrity

A. contraction with collagen

D. development of blood clot around the injury

B. vasoconstriction

E. blood vessel spasms (vasoconstriction)

C. stenosis

D. release of serotonin

7. Which blood vessels have the thickest walls?

A. Veins

*11. Endothelin is involved in the contraction of B. Arteries

of molecules such as

C. Cytokines

A. serotonin

D. Arterioles

B. Angiotensin

C. Bradykinin

D. All of the above

(continued)



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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

REVIEW QUESTIONS (continued)

*12. Which of the following is not correct?

18. At all times, approximately

of the total number

A. Vasoconstriction reduces blood flow in prostates

of platelets in the systemic circulation.

concentration of platelets in coagulation

A. one fourth

of the

B. one third

B. Platelets adhere to exposed endothelial connective

C. one half

tissues.

D. two thirds

C. Aggregation of platelets releases thromboxane A₂

and vasoactive amines (serotonin and epinephrine).

19. The reference range of platelets in the systemic circulation is

D. None of the above.

tion is

A. 50 to 150 × 10⁹/L

13. Which of the following is (are) true of

B. 100 to 200 × 10⁹/L

en ore uplic tion?

C. 150 to $350 \times 10^9/L$

A. Duplic tes DNA without cell ivision

D. 150 to $400 \times 10^9/L$

B. Results in cells with ploi y v lues o $4n$, $8n$, $16n$,
 n $32n$

20. T e unctions o pl telets in response to v scul r -

C. Is unique to the eg k ryocytic type o bloo cell
ge inclu e

D. All o the bove

A. inten nce o v scul r integrity by se ling inor
e ects o the en otheliu

*14. Which o the ollowing is (re) true o thro bopoietin?

B. or tion o pl telet plug

A. T ought to sti ul te the pro uction n tur -

C. pro otion o f brinolysis

tion o eg k ryocytes

D. ll o the bove

B. Is in uence by v rious cytokines, which incre se
eg k ryocyte size

21. Intravascular injury exposes the endothelial surface

C. Is influenced by various cytokines, which impact

underlying collagen, platelets (21A)

to the col—

transformation stage ploidy

lymphocytes (21B)

.

D. All of the above

A. here

B. aggregate

*15. Which of the following is not characteristic of

C. of not here

platelets?

D. both a and b

A. The presence of nucleus

B. Lifetime of 9 to 12 yrs.

22. The production of

occurs with

C. Cytoplasm is light blue with intense purple

platelet aggregation.

granules.

A. thromboxane A₂

D. Adipose shape sensitive cell.

B. cyclooxygenase

C. prostacyclin

16. The cellular ultrastructural component unique to the

D. rickettsia

platelet is the

A. cytoplasmic bridge

*23. Agents that regulate aggregating platelets include

B. glycolyx

A. collagen

C. mitochondrion

B. thrombin

D. microtubule

C. serotonin

D. illothebain

*17. Choose the incorrect statement regarding storage granules relative to the osteoblast in the platelet.

24. Examination of Wright-stained peripheral blood

A. Alpha-granules contain platelet factor 4, beta-

ser provides estimates of platelet numbers. Using
thromboglobulin, platelet-derived growth
factor (oil) immersion in the presence of erythrocytes just
prior.

touching each other, the upper limit of the number of

B. Alpha-granules contain platelet fibrinogen and von
Willebrand factor. platelets seen per field should not exceed

A. 10

C. Dense bodies contain serotonin and ADP.

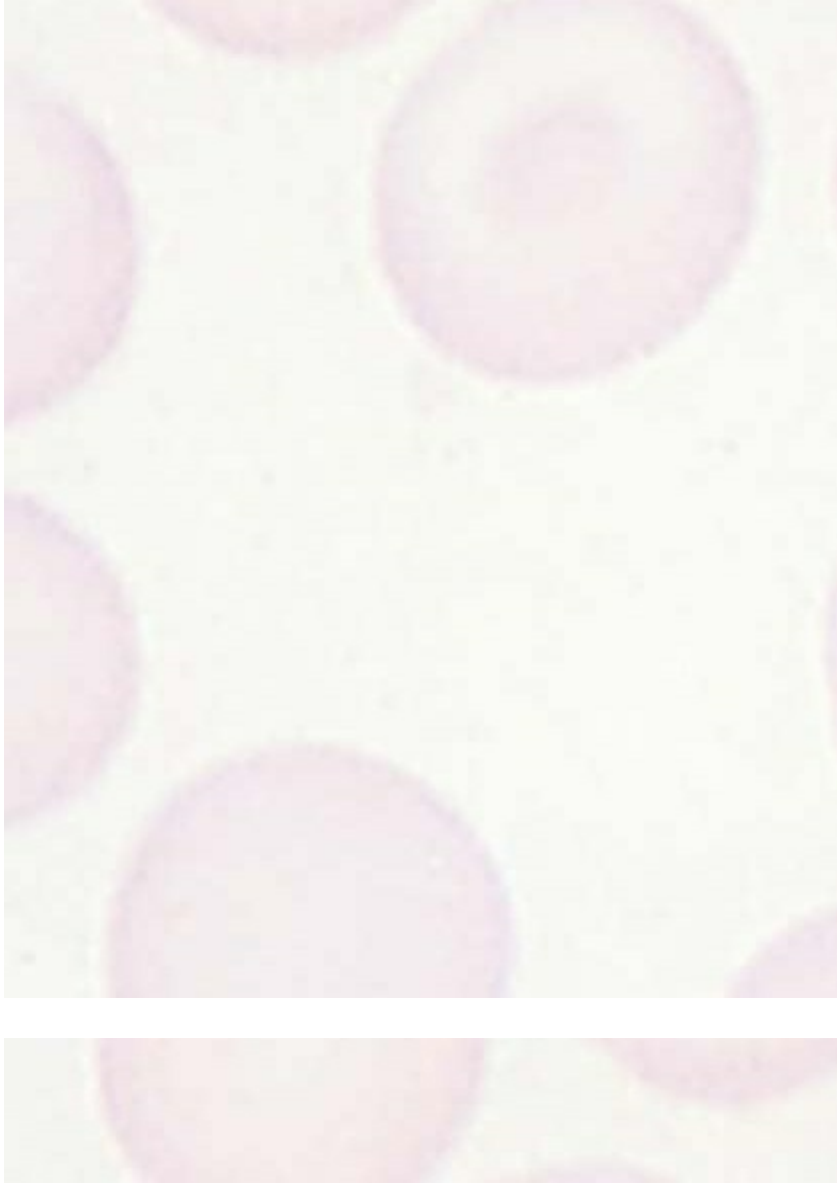
B. 15

D. Lysosomes contain cholesteryl esters, esters, and

C. 20

field in.

D. 25



CHAPTER 25 ■ Principles of Hemostasis and Thrombosis: Vasculature and Platelets 503

REVIEW QUESTIONS (continued)

25. If 10 platelets are seen per oil immersion field, what is

27. The bleeding time test measures

the approximate platelet count?

A. the ability of platelets to stick together

A. $50 \times 10^9/L$

B. platelet adhesion and aggregation on locally injured

B. $100 \times 10^9/L$

vascular endothelium

C. $150 \times 10^9/L$

C. the quantity and quality of platelets

D. $200 \times 10^9/L$

D. inhibits against platelets

26. Aspirin ingestion has the following hemostatic effect in

28. The clot retraction test is

normal person:

A. visible reaction to the activation of platelets—

A. Prolongs the bleeding time

prothrombin (thromboplastin)

B. Prolongs the clotting time

B. reaction of the quantity and quality of platelets

C. Inhibits factor VIII

and other factors

D. H s no e ect

C. e sure ent o the bility o pl telets to stick to

gl ss

D. e sure ent o the clou iness o bloo

Gr lnick HR, et l. Pl telet von Willebr n ctor, Mayo Clin Proc,

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66:634–640, 1991.

Hopkins S. Recent v nces in he ost sis & thro bosis, Adv Lab,

<http://thepoint.lww.co / urgeon6e>

15(8):42–47, 2006.

J ckson SP. T e growing co plexity o pl telet ggreg tion, Blood,

E ch stu ent is encour ge to ccess n use the web—

109(12):5087–5095, 2007.

b se co p nion resources envelope or this ch pter.

Johns CS. Pl telet unction testing: evolution or revolution? Adv Med

Here, you will f n ition l le rning tools to incre se

Lab Pro , 14(2):19–23, 2002.

your un erst n ing o the concepts n clinic l pplic -

Leung L, N ch n R. Molecul r ech nis s o pl telet ggreg tion,

tions o the ch pter.

Annu Rev Med, 37:179–186, 1986.

P trono C. Aspirin s n ntipl telet rug, N Engl J Med, 330(18):
1287–1294, 1994.

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13(9):80–90, 2004.

B bich V, et l. Selective rele se o olecules ro Weibel-P l e bo -

W gner DD, Frenette PS. T e vessel w ll n its inter ctions, Blood,
ies uring lingering kiss, Blood, 111(11):5282–5290, 2008.

111:5271–5281, 2008.

C stellone D. Will this p tient blee ? Adv Med Lab Pro , 16(4):18–25,

W rkentin E, Arnol DM. “Sp re-spleen-uxi b” or chronic I P,
2007.

Blood, 112(4):925–926, 2008.

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CHAPTER

26 Pr

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sis Vasculature and Platelets

KEY TERMS

a utoim m une he m olytic a ne m ia

im m u n e th ro m b o cyto p e n ia (ITP)

p u rp u ra

e cchym osis

im m u n e th ro m b o cyto p e n ic

s a rco id o s is

e sse ntia l a throm bia

p u rp u ra

s ys te m ic lu p u s e ryth e m a to s u s

b rin o lys is

neonatal autoimmune

(SLE)

Glanzmann's thrombasthenia

thrombocytopenia

thrombocytopenia

hematoma

petechiae

thrombocytosis

LEARNING OUTCOMES

Vascular abnormalities

Compare the hereditary defects of platelet function (with thrombo-

■ Define the terms petechiae, ecchymoses, hematoma.

cytopenia): Wiskott-Aldrich syndrome, MYH9-related thrombocyto-

■ Define the term purpura and describe various vascular conditions

penia syndromes (May-Hegglin anomaly).

that can produce this condition.

List and summarize the characteristics of primary (malignant) versus

■ Describe the effect of mechanical force on blood vessels.

secondary (reactive) thrombocytosis, including examples of disorder—

Explain the genetic basis of hereditary hemorrhagic telangiectasia

ders within each category.

(HHT).

Qualitative characteristics of platelets :

Compare the vasculitis disorders of antineutrophil cytoplasmic

thrombocytopenia/ thrombocytopenia

antibody-positive vasculitis, cryoglobulinemia, hyper-

gammaglobulinemic purpura, and Henoch-Schönlein purpura

Compare types of acquired platelet dysfunction and include hemo-

(HSP).

lytic-uremic syndrome and HELLP syndrome.

Describe the role of the vasculature in disseminated intravascular

■ Compare categories of platelet dysfunctions, including examples of coagulation (DIC).

disorders within each category.

Compare the etiology, pathophysiology, and laboratory testing for

■ Compare hereditary platelet adhesion and aggregation disorders:

bleeding disorders of perivascular tissue: Ehlers-Danlos syndromes,

Bernard-Soulier syndrome, Glanzmann's thrombasthenia, storage

pseudoxanthoma elasticum, scurvy, steroid-induced purpura, and

granule abnormalities, and von Willebrand's disease.

solar purpura (senile purpura).

- Discuss secondary aggregation disorders: hereditary storage pool

Describe purpura associated with infections and miscellaneous defect and hereditary aspirin-like defects.

causes.

Cas e s tudy

Analyze the patient history, clinical signs and symptoms, and labo-

Quantitative plate let dis o rde rs

ratory data for the stated case study, answer the related critical

- De ne thrombocytopenia, thrombocytosis, and thrombocythemia.

thinking questions, and decide the most likely diagnosis.

- De ne the terms thrombocytopenia and thrombocytosis.

- Cite at least two symptoms of thrombocytopenia.

NOTE:

Compare the characteristics of disorders of platelet production, dis-

- indicates MLT and MLS core content

orders of platelet destruction or utilization, and disorders of platelet

indicates MLT (optional) and MLS advanced content

distribution and dilution.

VASCULAR ABNORMALI IES

the microcirculation expresses itself as the condition of
purpura, which is characterized by obvious hemorrhages

Disorders of the microcirculation, atelectasis, or as a
into the skin and mucous membranes that appear as exten-

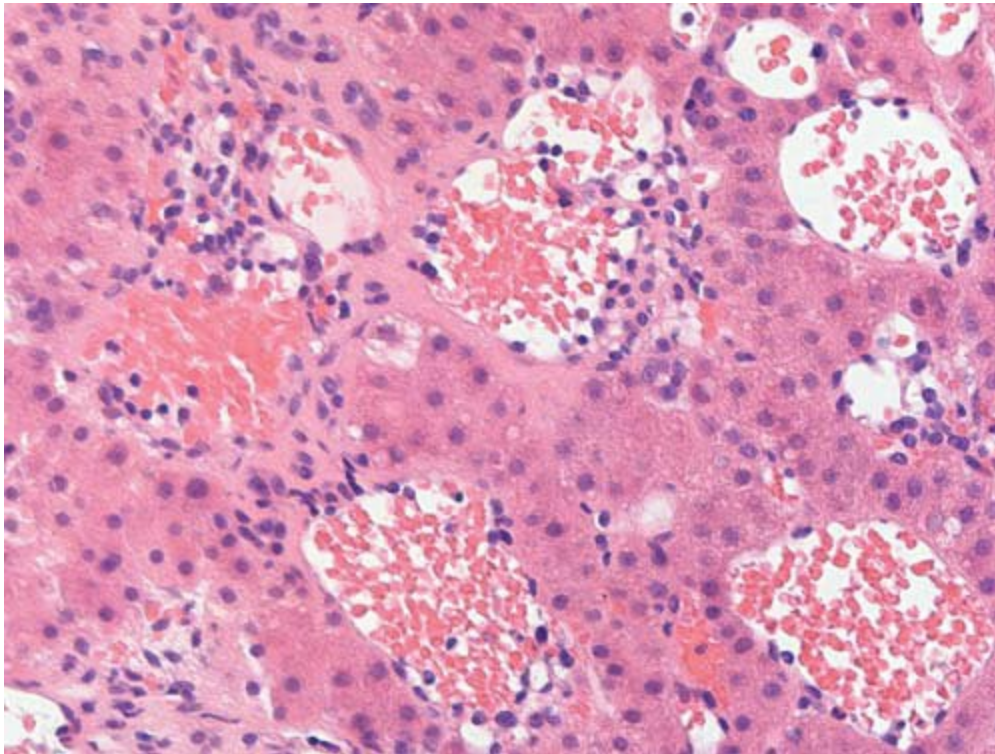
proteins may cause abnormal bleeding. Abnormal bleeding -

sive areas of re-orientation. It is a , involving the loss of red blood
cells (RBCs) from

the hemorrhagic spots on the skin and mucous membranes

504





CHAPTER 26 ■ Disorders of Primary Hemostasis and Thrombosis
Vasculature and Platelets 505

FIGURE 26.1 Petechiae. (Fro Weber JR, Ke ey JH. Health

Assessment in Nursing, 5th e , Philadelphia, PA: Lippincott Williams

FIGURE 26.3 Hereditary hemorrhagic telangiectasia. A cluster of
& Williams, 2013.)

splenic vessels is present in the hepatic parenchyma. (Biopsy

Interpretation of the Liver. Philadelphia, PA: Lippincott Williams &
Williams, 2014.)

are evident, it is called petechiae (Fig. 26.1); if the patches
of bleeding into the tissues are larger, this is referred to as
ecchymosis (Fig. 26.2).

Blood Vessel Malformations

Purpura can be produced by numerous types of vascular
conditions. These abnormalities include mechanical force,
Hereditary Hemorrhagic Telangiectasia (HHT)
genetic structural alterations, inflammation and obstruction—
Hereditary hemorrhagic telangiectasia (HHT), also known
tion of blood vessels, disorders of extracellular tissue, and
as Osler-Weber-Rendu disease or Osler-Weber-Rendu
ischaemic causes such as infection, skin diseases, and
syndrome, is caused by genetic mutations. Mutations
syndromic reasons.

in blood vessels even a small amount can result in a variety of
alterations.

Mechanical Force

An individual with HH, an autosomal dominant trait,
has a reduced amount of functional protein available in the
endothelium. Mechanical force is a frequent cause of injury. It has to
do with the blood vessels. The lack of availability of
protein has an intensity in order to damage the wall.
Functional protein is the result of a mutation associated
with the blood vessel. External pressure such as blunt trauma
damages protein or function of a effective protein that cannot
result in bruising when the force is sufficient to disrupt
its function.

vascular integrity and allows leakage of RBCs into the skin.

This functional protein deficiency is believed to result

Minor trauma experienced by anticoagulated patients or vigorous
in the characteristic lesions (Fig. 26.3), involving skin and
rough scratching can cause mechanical injury. The size of
endothelial barrier-associated histamine and other mediators
the resulting damage depends in part on the vascularity of

ing co ications. B ee ing rob e s occur ro ru ture
the region an the ensity o the surroun ing tissue.
or eakage o vascu ar a or ations. T e c inica signs
an sy to s e en on the ocation o the ru ture or
b ee ing.

T e ENG, ACVRL1, an SMAD4 genes rovi e instructions or aking roteins
that are oun in the ining o the

b oo vesse s. T ese roteins interact with growth actors
that contro b oo vesse eve o ent. T ere are three ty es
o HH : ty e 1, ty e 2, an ty e 3.

- y e 1 is cause by utations in the gene ENG.

- y e 2 is cause by utations in the gene ACVRL1.

- y e 3 is cause by an unknown utation.

Juveni e o y osis/HH syn ro e is cause by utations in the gene SMAD4.

FIGURE 26.2 Se ecte cutaneous ani estations o syste atic isOthe r Vas cu lop
a th ie s

eases. Ecchy osis (bruise). (Fro S e tzer SC, Bare BG. extbook

o Medical-Surgical Nursing, 9th e , Phi a e hia, PA: Li incott

Patients with u ti e ye o a or syste ic a y oi osis can

Wi ia s & Wi kins, 2000.)

have ight chain i unog obu in e osits in the cutaneous

PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

blood vessels. These structures are articulated by rami, and

Hypertension may lead to a globular leukoencephalopathy

which can result in a very serious trauma.

This disorder is characterized by a leukoencephalopathy—

Moya Moya disease, a chronic cerebrovascular disease. This

disorder is associated with recurrent attacks of

ischemic strokes in children and infants—

urinary. There are primary and secondary forms. Secondary

cranial hemorrhage in adults.

Hypertension in patients with underlying autoimmune disease—

Cerebral vasculopathy involves the integrity of arteries—

disorders such as Sjögren's syndrome and SLE but has been

reported, usually in arteries. This disease is characterized by

reversible infarcts in patients with chronic hepatitis C.

recurrent ischemic strokes, infarcts, with progressive

Attacks occur after prolonged standing, long walks, exer—

cognitive impairment.

cise, alcohol ingestion, or wearing tight-fitting clothes.

Vasculitis

Henoch-Schönlein purpura (HSP)

HSP, or urticarial purpura, or Schönlein-Henoch purpura.

Vasculitis means inflammation of the blood vessels, arteries—

This vasculitis disorder occurs primarily in children with a

fever, rash, or colitis. When such inflammation occurs, it

usually occurs between 4 and 11 years of age.

It causes changes in the walls of blood vessels, such as weakening—

Rash-urticarial lesions, which look like bruises, are the most

common narrowing that can progress to the point of blood

obstruction and is a sign of HSP.

blood clots.

HSP is an acute inflammatory A (IgA)-mediated—

Exacerbations in this condition include anti—

body cells characterized by a generalized vasculitis involving the

neutrophilic antibody-positive vasculitis, cryo—

vasculitis of the skin, the gastrointestinal (GI) tract, the

gastrointestinal, hyaline arterioles, and

kidneys, the joints, and, rarely, the lungs and the central—

Henoch-Schönlein purpura (HSP).

vous syste (CNS).

A bio sy o the skin, an ess co on y ki neys, can be

Ant ine ut roph il Cytop la s m ic Ant ibo dy–Pos it ive

use to e onstrate vascu itis. S ecia staining techniques

Va sculitis (ANCA)

(irect i unof uorescence) o the bio sy s eci en can be

Antineutro hi cyto as ic antibo ies (ANCAs) are ivi e

use with icrosco ic exa ination to ocu ent antibo y

into antineutro hi cyto as ic antibo y (c-ANCA) or anti—

e osits o IgA in the wa s o the arterio es o invo ve tissue.

bo y ro ucing a erinuc ear staining o ethano - xe neutro hi s (-ANCA).

ANCA-associate s a vesse vascu itis

Vas cular Obs tructio n

is observe in isor ers such as systemic lupus erythematosus

Mu ti e b ee ing isor ers are associate with ur ura

(SLE) an Wegener's granu o atosis.

associate with a thro bus, e bo us, or other occ usions

T e hu an ANCAs, escribe or the rst ti e in 1982,

cause by various actors (ab e 26.1).

are irecte against antigenic co onents ain y resent in

ri ary granu es o neutro hi s. ANCAs are sero ogic arkers o ri ary necrotizing

systemic vasculitis, articular in

Examples of Bleeding Disorders

Wegener's granulomatosis. In addition, these antibodies have

TABLE

26.1 Associated with Vascular

diagnostic interest because, in most cases, their titer is cor-

Obstruction

related with clinical activity during the disease.

Vascular

Vascular

Cryoglobulinemia

Obstruction Obstruction

Cryoglobulinemia vasculitis is associated with cryoglobu-

Category

Component

Selected Examples

in immune deposits. The cryoglobulins can reciprocate in

Thrombus

Blood clot

Disseminated intravas—

er a vesse s. Wa enströ 's ri ary acrog obu ine ia

cular coagulation

(WM) characterizes this isor er. Ninety ercent o atients

Emboli

Septic emboli

Endocarditis

with WM have MYD88 L265P utations.

Sy to s inc u e cutaneous in arcts an etechiae.

Thromboemboli

Atrial bril ation

P ate et counts are usua y nor a . Patients usua y su er

Other

Immunoglobulins Multiple myeloma,

ro chronic ane ia an b ee ing e iso es. B ee ing cause

occlusions

Waldenström's primary

by abnor a ities in ate et a hesiveness an rothro bin

macroglobulinemia

ti e (P) ay be seen, an the va ues o actor VIII ay

Plasma proteins

Cryoglobulinemia

be low. Characteristically, blood specimens are described as

Fibrin

Cryofibrinogenemia

having hyperviscosity that can contribute to the bleeding disorder.

Red blood cells

Polycythemia

The principal cause of this type of uraemia is increased

Platelets

Thrombocytosis

intracranial pressure. This condition can be observed

Fat

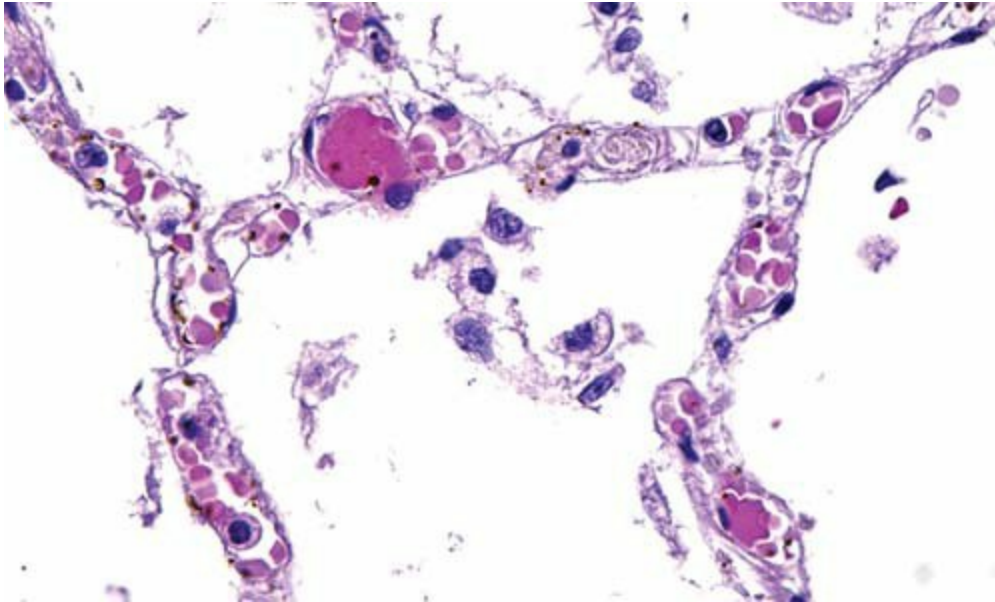
Fat emboli syndrome

around the ankles with prolonged standing and can be associated with the presence of abnormal proteins in cerebrospinal fluid—

Reference: Greer J P, *et al.* Clinical Hematology, 14th ed, Philadelphia, PA:

Lippincott Williams & Wilkins, 2016.

urine has or hyperviscosity disorders.



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scurvy, steroid-induced uric acid, and so on (senile
uric acid)

EDS are a group of rare connective tissue disorders caused
by abnormalities of collagen synthesis or processing. EDS

is due to quantitative or qualitative defects in type 3 collagen, which is particularly
abundant in the arterial wall and

intestine.

Most patients report a history of excessive bruising and

bleeding. Risk of aortic bleeding is the greatest for EDS

(type IV). Patients with this disorder are prone to arterial

aneurysms and dissections, significant bleeding from spontaneous rupture of medium-size

abdominal arteries, and

intestinal rupture. Arteria *intestina* uterine ragi ity or rupture -

FIGURE 26.4 Disseminated intravascular coagulation Histology

ture commonly arise in this type of EDS.

showing microthrombi in the lumen of the aorta and in the arteries. Arterial rupture has a peak incidence in

ies. (From Cagle P. Color Atlas and Text of Pulmonary Pathology.

Philadelphia, PA: Lippincott Williams & Wilkins, 2005.)

the third or fourth decade of life but may occur earlier.

Life expectancy is shortened with a majority of individuals dying in their 40s. Pregnancies may be complicated by

Disseminated Intravascular Coagulation (DIC)

initiated by intrauterine rupture and re-an

Initiation of disseminated intravascular coagulation (DIC)

postpartum arterial bleeding.reatments are available,

(see Chapter 28 for a discussion of this disorder)

which may include, and surgical interventions are

(Fig. 26.4) can be caused by a number of factors. Intravascular

clotting.

Endothelial damage results in the exposure of collagen and

Screening tests show no hereditary abnormalities.

base endothelium, collagen can activate coagulation cascade. Bleeding is thought to result from abnormalities in the extrinsic pathway. Factor XII has utility roles in the direct or indirect vascular collagen leading to fragility of the subcutaneous activation of coagulation.

vascular. Some patients may have abnormal bleeding times and. Regardless of the initiating event, DIC is characterized by excessive thrombin formation, conversion of fibrinogen to fibrin, and excessive consumption of platelets. DIC is a clinical syndrome that can be diagnosed by laboratory tests.

thrombin, and platelet consumption and aggregation. DIC is a clinical syndrome that can be diagnosed by laboratory tests. There are six major types of EDS. Each type has associated clinical features and laboratory findings.

and genetic defects. The vascular type of EDS IV is caused by structural defects in the $\alpha 1(\text{III})$ chain of collagen type I. Cryofibrinogenemia

III encoded by COL3A1. This type of EDS is inherited in an autosomal recessive pattern. Cryofibrinogenemia is a cause of vascular obstruction. In

this situation, cryofibrinogens, collectively as a

proteins that is involved as a reward, are involved.

Pseudoxanthoma elasticum (PXE)

This is a distinct process of cryoglobulins that recite

Pseudoxanthoma elasticum is also known as Grönblad-

stroma disease as the elastin proteins have been

Stranberg syndrome, Grönblad-Stranberg syndrome,

referred to. The lesions in associated cryoglobulinosis—

or PXE. This is an inherited connective tissue disorder

ers, such as Raynaud's phenomenon, even as the result

that results in calcification and mineralization of elastic

of blood vessels obstructing the small and medium-size

fibers, especially in the internal elastic lamina of medium-

and large blood vessels.

size arteries with subsequent rupture of the blood vessels. Elastic fibers, a component of connective tissue,

Septic Emboli

provide strength and elasticity to structures throughout

Septic emboli result from the entry of tissue into—

the body.

emia, necrosis of the skin and subcutaneous tissue, and

PXE is inherited in an autosomal recessive pattern, which

occasionally other tissues. This condition is classically associated with patients who have end-stage renal failure and are on dialysis. Most often, the parents of an individual with an autosomal recessive condition each carry one copy of the mutant gene but does not exhibit the signs and symptoms of PXE. There are several clinical types of PXE.

Classification because of Disorders of

Mutations in the ABC-C6 gene cause PXE. The ABC-

Perivascular Tissue

C6 gene provides instructions for making a transmembrane

transporter protein, MRP6 protein, also known as

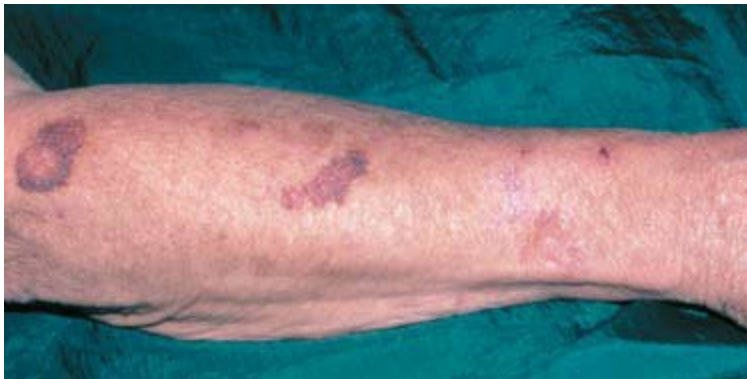
Various disorders belong to this category of biliary diseases ABC-C6 protein. MRP6 protein is primarily

located in the liver and kidneys, with small amounts in

other tissues, including the skin, stomach, blood vessels,

osteogenesis imperfecta, pseudoxanthoma elasticum,

an eyes.



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Mutations lead to absent or non-functional MRP6 protein. Some studies suggest that

the MRP6 protein stimulates the release of adenosine triphosphate (ATP) molecules.

A shortage of MRP6 may impair the release of ATP molecules.

ATP can be broken down into other molecules, including adenosine monophosphate (AMP) and pyrophosphate.

Pyrophosphate has a controlling effect on calcium and

other minerals in the body. Consequently, little pyrophosphate

is produced, and calcium and other minerals accumulate

FIGURE 26.6 Senile, or actinic, urticaria. Ecchymoses are res—
ults in elastic fibers of the skin, eyes, blood vessels, and other
tissues on the torso appear in an elderly person. (From Goodheart
tissues.

HP. Goodheart's Photoguide of Common Skin Disorders, 2nd ed.,

Mineralization of the arteries that carry blood to the

Philadelphia, PA: Lippincott Williams & Wilkins, 2003.)

heart to the rest of the body may cause other signs and sy-

ptoms of PXE. For example, patients with this condition can

as a result of loss of subcutaneous fat and changes in both

degree of narrowing of the arteries (arteriosclerosis) or a con—

stant amount and quality of collagen. Hemostasis tests are

important in the evaluation that is characterized by cracking

nor a .

an aim uring exercise because o ecrease b oo f ow to

It is i ortant to istinguish seni e ur ura ro other

the ar s an egs.

acquire b ee ing isor ers in the e er y. He ostatic ba -

ance changes with a vancing age, which ay be ue to

Scurvy

actors such as ate et activation, increase o certain c ot—

Hu ans require ietary vita in C to ro ote the e ti y

ting actor roteins, s owing o the brino ytic syste ,

hy roxy ation o roco agen. In the absence o vita in C,

an o i cation o the en othe iu an b oo f ow. T is

co agen stran s are weakene as a resu t o abnor a tri e

usua y re is oses the e er y to thro bosis rather than

he ica structures. Abnor a co agen resu ts in e ective

b ee ing.

erivascu ar su ortive tissues, re is oses a atient to ca -

i ary ragi ity, e aye woun hea ing, etechiae an ur-

Purpura As s o ciated w ith Infectio ns

ura (Fig. 26.5), ora b ee ing, an a so b ee ing in urinary

Purpura associated with direct endothelial damage by
digestive tracts.

result from physical or chemical injury to the tissue causing

Solar Purpura (Senile Purpura)

by microbial agents associated with acute alcoholism with

enteric, rickettsial diseases, Brazilian purpuric fever, rat

Patients with senile purpura have lesions that are co-

existent, hemorrhagic, *Vibrio vulnificus* infection, and

only on the hands and forearms (Fig. 26.6), without any

the parasitic infection, *Strongyloides*.

known preceding trauma. The skin in elderly people is thin

The overaction of endothelins, a vasoconstrictor, is

to increase blood pressure and vascular tone. Bacterial toxins

produce endothelialization in vessels by an endotoxin.

Purpura Related to Miscellaneous Causes

Disorders in this category can be the results of contact with—

atit, steroid use, or drug reactions. Other causes of pur-

ura can be associated with vascular fragility. Purpura

of this origin is observed in Kaposi's sarcoma and vascular

tumors.

Purura si ex haens o en in wo en an a ears
in the ti e o enstrua erio . T is ty e is ex aine with
the hor ona inf uence that is resent uring the enstrua
cyc e. I the enstruation is ain u , taking nonsteroi a
anti-inf a atory rug (NSAID) or ain re ieving a itiona y creates exce ent
environ ent or ur ura si ex.

Unusua causes o ur ura inc u e actitious or se -
i ose ur ura an re igious stig ata.

NOTE: This is a good time to review the de nitions of the
Key Terms in the Glossary and ash cards on
. It is

FIGURE 26.5 T e eggs o a 46-year-o an with scurvy. (Fro
also a good time to complete Review Questions related to
Cha e PC, Harvey RA, Ferrier DR, Biochemistry, 4th e ,
preceding content.

Phi a e hia, PA: Li incott Wi ia s & Wi kins, 2007.)

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QUAN I A IVE PLA ELE DISORDERS

be ow $100 \times 10^9/L$, c inica sy to s usua y inc u e signs
o ro onge c ot retraction such as the resence o etechiae

The normal range of circulating platelets is $150 \times 10^9/L$ to $400 \times 10^9/L$. When the quantity of platelets decreases to even—

Thrombocytopenia can be classified based on pathophysiology—

Below this range, a condition of thrombocytopenia exists.

It is divided into four different categories (Box 26.1). Various conditions in which the quantity of platelets increases, thrombocytosis is the

conditions, such as after the use of extracorporeal circulation in

result. Disorders of platelets can be classified as follows:

Cardiac bypass surgery or in a chronic liver disease, can result in—

thrombocytopenia.

■ Quantitative (thrombocytopenia or thrombocytosis)

Thrombocytopenia in itself rarely poses a threat to a patient.

■ Qualitative (thrombocytopenia or thrombocytosis)

patients, but disorders associated with it—which include

deep venous thrombosis, DIC, urinary embolism, cerebral thrombosis, myocardial infarction, an ischemic injury

Thrombocytopenia

to the lungs or brain—can result in severe morbidity and mortality. If a lesion occurs somewhere in a patient's body, a correction

is necessary. Most thrombocytopenic conditions can be classified

exists between severe thrombocytopenia and spontaneous
into major categories. These categories are as follows:
clinical bleeding. Platelets are absent or severely decreased

1. Disorders of production
2. Disorders of destruction or utilization
3. Disorders of platelet distribution and function

Disorders of Production

BOX 26.1

Decrease in production of platelets may be caused by hyperproliferation of the megakaryocytic cell line or ineffective

Pathophysiologic Classification of

thrombocytopenias caused by acquired conditions or hereditary

Thrombocytopenia

tary factors (Box 26.2). A hyperproliferative state frequently
affects other normal cell lines of the bone marrow and plate-

ARTIFACTUAL THROMBOCYTOPENIA

lets. Thrombocytopenia owing to hyperproliferation can result

■ Platelet clumping

to acquire a negative autoantibody to the bone

■ Giant platelets

marrow caused by factors such as irradiation, drugs (e.g.,

- Platelet satellitism

DECREASED PRODUCTION OF PLATELETS

BOX 26.2

- Hereditary thrombocytopoiesis
- Hypoplasia of megakaryocytes

Hereditary Platelet Function Defects

- Disorders of thrombopoiesis

INCREASED DESTRUCTION OF PLATELETS

ADHESION DEFECTS

Immunological Cause

Bernard-Soulier syndrome

Inadequate adhesion to collagen

- Idiopathic
- Secondary to drugs, infections, and other causes

AGGREGATION DEFECTS : PRIMARY

- Neonatal thrombocytopoiesis

Glanzmann's thrombasthenia

- Posttransfusion purpura

Essential thrombocythemia

Nonimmunological Cause

AGGREGATION DEFECTS : S ECONDARY

Storage ooseases

- thrombotic microangiopathies, or exa e, isAs irin-like e ects

se inate intravascular coagulation, thrombotic

Release reaction e ects

thrombocytopenic purpura, he oytic-ure ic

syndrome

ISOLATED PLATELET FACTOR III DEFICIENCY

- Damage to platelets by abnormal vascular surfaces

SEVERE COAGULATION FACTOR DEFICIENCIES

- Miscellaneous, or exa e, assive blood transfusion,

infection

ACQUIRED PLATELET DYSFUNCTIONS

Drug-induced platelet dysfunction

ABNORMAL PLATELET DISTRIBUTION OR POOLING

Anesthetics, antibiotics, cardiovascular drugs, psychotropic

- Splenic sequestration

drugs

- Hypothermia

Secondary platelet dysfunction

■ Disturbances of platelets with passive transfusion

Uremia, arthropathies, MDS, MPDs

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chemotherapeutic and other cytotoxic agents), chemicals

Isoimmune Neonatal Thrombocytopenia

(e.g., insecticides), and a cochlear implant. Infection of the bone marrow

Isoimmune neonatal thrombocytopenia results from the

by a malignant cells in the conditions of metastatic cancer, especially

immunization of a pregnant female by a platelet-specific anti-

body, and Hodgkin's disease can produce a hypercoagulable

state. The antigen is inherited by the fetus from the mother and

state. Hypercoagulation may also result from non-immune

is absent on platelets.

conditions, such as infections, unusual erythrocytosis, granulomatous disease such as sarcoidosis, and idiopathic causes.

Thrombocytopenia in Pregnancy

Ineffective thrombopoiesis may result in decreased platelet

Both immune and non-immune thrombocytopenia may

production. Thrombocytopenias of this type may be the anti-

occurring pregnancy. Pregnant women generally

estation of a nutritional disorder, such as a deficiency of vitamin B12 or folate. In these obstetric anemias caused by deficiencies of vitamin B12 or folate, the effect is inhibition of hemoglobin synthesis and increase of reticulocyte count. A decrease of approximately 10% in the reticulocyte count is typical toward the end of the trimester of pregnancy. Another disorder is iron deficiency anemia, which usually results in a decrease in red blood cell size and hemoglobin concentration. The decrease of hemoglobin concentration in pregnancy is usually due to the increase of plasma volume. Disorders of destruction or utilization of platelets may result in thrombocytopenia (HIT) or thrombocytosis. Disorders of destruction or utilization of platelets may result in thrombocytopenia (HIT) or thrombocytosis.

Disorders of destruction or utilization of platelets may result in thrombocytopenia (HIT) or thrombocytosis. Disorders of destruction or utilization of platelets may result in thrombocytopenia (HIT) or thrombocytosis. Disorders of destruction or utilization of platelets may result in thrombocytopenia (HIT) or thrombocytosis.

Disorders of destruction or utilization of platelets may result in thrombocytopenia (HIT) or thrombocytosis. Disorders of destruction or utilization of platelets may result in thrombocytopenia (HIT) or thrombocytosis.

ro a nu ber o echanis s.

He arin-in uce thro bocyto enia (HI) an associate

De s truction Ca us e d b y Im m une Mecha nis m s ,

thro botic events, re ative y co on si e e ects o he arin

Antig en s , Antib odies , or Com ple m e nt

thera y, can cause substantia orbi ity an orta ity. o

revent these co ications, it has beco e stan ar e i-

Posttrans usion Purpura

ca ractice to onitor ate et counts in atients receiving

Destruction o ate ets can occur as the resu t o inco athe arin or any exten e
erio .

ib e ate ets. A otentia y ata reaction is osttrans usion

Seru ro atients with HI contains i unog obu in

ur ura.

G (IgG) that, in the resence o s a a ounts o he arin,

activates nor a ate ets an causes the to aggregate an

Drugs

re ease the contents o their granu es, inc u ing serotonin.

Drugs or oreign substances can ro uce ate et estruction.

P ate et-activating antibo ies are s eci c not or he arin

T ese rugs inc u e quini ine, su ona i e erivatives, heroin,

but or co-existence between heparin and antithrombin, as snake venom. Such derivative reactions

factor 4 (PF4), a heparin-binding protein normally found in

involve the interaction of platelet antigens with drug antibodies -

the alpha-granules of platelets. IgG and IgM also react with

them. Moreover, reactions involve the activation of coagulation.

endothelial cells coated with PF4 (Fig. 26.7). This suggests a

Bacterial Sepsis

mechanism of antibody-mediated vascular injury that could

be responsible for thrombosis or DIC when challenged

Bacterial sepsis causes increased destruction of platelets

with heparin.

because of the attachment of platelets to bacterial antigen—

HI is the most common drug-induced thrombocytopenia—

antibody-mediated coagulation. Certain microbial antigens

exist. HI and antihemophilic syndrome (APS) are two

ways that initiate platelet clots by specific antibodies

thrombotic syndromes in which antibodies against co-

agulation factors of the microorganism. This mechanism has been reported to

coagulation factors are of fundamental importance.

cause the thrombocytopenia that frequently complicates the

In the case of APS, the antibodies are autoantibodies co-
Plasmodium falciparum type of malaria. Thrombocytopenia
are to the rug-in uce antibodies of HI. In both syn—
occurs within 1 to 3 weeks of owing vira infections (e.g.,
roses, IgG antibodies directed against positive y charge
rubea, u.s., or chicken ox), parasitic or bacteria in ecenogenous proteins, β 2-g
lycoprotein I (GPI) in APS an
tions, or hepatitis vaccination.
PF4 in HI, are of major importance.

Immune Thrombocytopenia

HI is a serious complication of the arin therapy. This
condition is also called “white clot syndrome” because it
Antibodies of either autoimmune or idiopathic origin may
poses a high risk of potentially catastrophic venous or arterial
to increase destruction of platelets. An example of
thrombosis. The mortality rate of patients with thrombosis is
an autoimmune thrombocytopenia is neonatal autoimmune
approximately 25%.
thrombocytopenia. This condition occurs in infants born to
Thrombocytopenia and thrombosis are the predominant
others with chronic idiopathic thrombocytopenia (ITP)

clinical symptoms of HIT .

Following transfusion of platelet transfusion IgG antibodies

two types of HIT exist

autoantibodies. Posttransfusion urticaria is an example of

thrombocytopenia of isoimmune origin. Posttransfusion

1. Nonimmune HIT : type I

urticaria is a rare form of isoimmune thrombocytopenia.

2. Immune HIT : type II

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Clinical Suspicion of HIT

Assess clinical probability

using 4T's scoring system

Low

Intermediate

High

(Score 0–3)

(Score 4–5)

(Score ≥ 6)

Lab testing may not be

perform immunoassay

Pe rform immunoa s s a y

ne ce s s a ry if a n

- S TOP he pa rin

- S TOP he pa rin

a lte rna te dia gnos is is

- Initia te a lte rna te

- Initia te a lte rna te

pre s e nt

a nticoa gula nt

a nticoa gula nt

pe nding te s t

pe nding te s t

If unce rta in, pe rform

re s ults

re s ults

immunoa ssa y

Ne ga tive re s ult – No

Nega tive re s ult – HIT

Ne ga tive re s ult – HIT

HIT

unlike ly

unlike ly

Pos itive re s ult – HIT

Pos itive re s ult –

P os itive re s ult –

dia gnos e d

Cons ide r S RA for

Cons ide r de gre e of

de finitive dia gnos is

pos itivity. If re s ult is

>1.0 O.D. units , HIT

is proba ble ; cons ide r

S RA for de finitive

dia gnos is

FIGURE 26.7 Patho hysio ogy o HI . wo ex anations or thro bosis in HI .
Activation o ate ets (t) by anti-ate et actor 4 (PF4)/he arin IgG antibo ies (HI
antibo ies), ea ing to or ation o rocoagu ant, ate eterive icro artic es, an neutra
ization o he arin by PF4 re ease ro activate ate ets, ea to arke increase in thro -

bin (hy ercoagu abi ity state) characterize by an increase risk o venous an
arteria thro bosis, as we as increase risk or cou arin-in uce venous i b
gangrene. However, it is a so ossib e that unique athogenetic echanis s o
erative in HI ex ain unusua thro boses, such as arteria “white c ots.” For exa
e, HI antibo ies have been shown to activate en othe iu an onocytes (ea ing to
ce sur ace tissue actor ex res sion), a though this sti u ation ay be arge y “in

irect" through poorly defined mechanisms involving platelet activation and, possibly, formation of platelet-endothelial cells. Further, aggregates of platelets and of polymorphonuclear leukocytes have been described in HIT. To what extent these cooperative interactions between platelets, platelet-endothelial cells, polymorphonuclear leukocytes, monocytes, and endothelial cells lead to arterial (or venous) thrombotic events in HIT, either in large or small vessels, remains unclear. HIT, heparin-induced thrombocytopenia. (Reference: Warkentin E.

An overview of the heparin-induced thrombocytopenia, Semin Thromb Hemostasis, 30(3):275, 2004.) **Nonimmune Heparin-Induced Thrombocytopenia** The lowest platelet counts range between $20 \text{ and } 150 \times 10^9/\text{L}$.

Nonimmune HIT is a benign disorder affecting up to 10%

$10^{12}/\text{L}$. The lowest count is reached at about 5 days after

patients receiving heparin anticoagulant therapy. The

onset of the declining platelet count. The platelet count

mechanism of action is direct interaction between heparin

begins to rise approximately 2 days after heparin therapy

and platelets.

It is discontinued and usually returns to normal within 4 to

usually, the platelet count is greater than $100.00 \times 10^9/\text{L}$.

10 days after discontinuing heparin. In rare cases, it can

Although a relapse is observed within the first 2 days of

taking up to 25 days. The heparin-induced antibody is a

heparin administration, the platelet count returns to normal

within 2 to 3 months after discontinuing heparin

even within 5 days despite continued heparin use or within administration.

2 days if heparin therapy is discontinued.

Thrombosis occurs in most patients after the platelet count increases by 30% to 50% of the normal value. The risk

Immune Heparin-Induced Thrombocytopenia

Thrombosis persists or up to 30 days after discontinuing

Aproximately 8% of patients who receive heparin therapy

heparin. Rare cases of thrombosis have been reported before

even of HI antibody but do not experience thrombocytopenia or platelet count decreases.

to enia. Another 1% to 5% of patients receiving heparin

A rare association of delayed-onset HI has been

therapy of even of HI antibody and an anti-thrombocytopenia—

observe. In these cases, thrombocytopenia began at least

to enia. At least 30% of thrombocytopenic patients even of

5 days after discontinuation of heparin therapy. Bleeding is

venous and/or arterial thrombosis.

uncommon.

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Pathophysiology

Characteristics of Immune

Immune HI is caused by an antibody that recognizes heparin-

TABLE

26.2 Thrombocytopenia

heparin bound to PF4 on the platelet surface (Fig. 26.7). The

antibody binds to the heparin-PF4 complex, which then

Newly

allows the antibody to bind the Fc receptor on the platelet.

Characteristic

Diagnosed

Chronic

Interaction with the Fc receptor activates the platelet that

results in the loss of platelets, thrombocytopenia, and platelet

Peak age

2–5 years

Adulthood

aggregation (thrombosis). As a number of cases of HI

(30–60 years)

may involve an antigen other than the PF4 complex.

History of infection

Common

Uncommon

Spontaneous remission

Common

Rare

Laboratory Data

In addition to the platelet count, three specific laboratory

studies, a ut I P is a chronic disease. I P in children

assays can be used in patients with suspected HI :

is a clinically distinct disorder and is usually acute. Among

1. Enzyme-linked immunosorbent assay (ELISA)

studies, I P is most common in young women (adolescents—

2. Platelet aggregation

about 70% of patients are 10 to 40 years old). Chronic I P is

3. Serotonin release

destructive thrombocytopenia caused by an autoantibody.

Adolescents and 80% of patients experience remissions after

The ELISA assay and serotonin release assay have sensitivity—

either corticosteroid therapy or splenectomy. Some patients

tivities more than 90%, with very high specificity for HIT. Response to other therapy; in a substantial group of patients, antibody platelet aggregation is between 50% and 80% and the disease is refractory to therapy. is very specific.

Clinical Signs and Symptoms

Increased Utilization of Platelets

Onset is often insidious. Purpura, ecchymosis, and gingival

Accelerated consumption of platelets is another cause of

bleeding are common. Hematuria and GI bleeding are less thrombocytopenia.

common, an intracerebral hemorrhage is rare. Serious

One of the most important and frequently encountered

bleeding does not occur in most patients.

or so increase consumption of platelets is immune thrombocytopenia (ITP), previously known as immune thrombocyto-

Pathophysiology

penic purpura. This antibody-mediated response, which may

be received by infection, is believed to have a devastating

The concept was that thrombocytopenia results from

effect on platelet survival. Patients suffering from acute idiopathic—

antibody-mediated destruction. There are two new
athic IP usually have a spontaneous relapse within sev—
months:

several weeks of the onset of IP. However, IP may co-occur

1. The same antibodies that mediate destruction also
other antibody-associated disorders such as systemic lupus
erythematosus (SLE). Patients with immunologic throm-

cytopenia and/or blocking their ability to release ro—
bocytopenic purpura usually demonstrate petechiae, bruise—
ments. These effects are believed to play a role.

ing, enorrhagia, and bleeding after minor trauma.

2. Ten to twenty percent of cases are not antibody-mediated.

Immune Thrombocytopenia

In acute IP, the mechanism of destruction is sug—

The new standard nomenclature, IP, replaces the ter-

gested to be either by adsorption of viral antigen onto the

immune thrombocytopenic purpura. IP is an acquired

antibody-mediated disease by antibody binding or by idiopathic disorder
characterized by isolated throm-

cytopenia of an immune complex on the surface of platelets via

thrombocytopenia (platelet count less than $100 \times 10^9/L$) and the platelet Fc (immunoglobulin) receptors. In chronic ITP, absence of any obvious initiating and/or underlying cause for the target or the autoantibody is platelet destruction of the thrombocytopenia. ITP occurs in children and adults. Platelet membrane GPs (e.g., GPIIb/IIIa, GPIb/IX, GPIa/IIa, and GPIV). It is characterized by a low platelet count, not a bone marrow, and the absence of other causes of thrombocytopenia—GPIIb/IIIa or GPIb/IX co-express. The mechanism of autoantibody. Various characteristics exist in ITP (Table 26.2). Antibody formation is unknown.

Epidemiology

Laboratory Data

ITP is a fairly rare, generally benign illness in the pediatric population. Isoantibody thrombocytopenia is the essential abnormality. Spontaneous remission. About two-thirds of children recover spontaneously. Diagnosis requires exclusion of other causes of thrombocytopenia. In adults, the incidence is approximately equal to children. Antibodies to specific platelet membrane GPs can

both genders except in the intermediate years (30 to 60 years),
be detected in outpatients, but neither these assays nor
when the disorder is more prevalent in women. ITP is clas—
sified into acute and chronic, which are often erroneously
classified by duration into newly diagnosed, persistent (3 to 12
months), relapsing, and chronic (≥ 12 months).

the diagnosis or management.

CHAPTER 26 ■ Disorders of Primary Hemostasis and Thrombosis Vasculature and Platelets 513

The American Society of Hematology has established the

Thrombotic Thrombocytopenic Purpura

Working Group for the diagnosis of TTP:

Thrombotic thrombocytopenic purpura (TTP) is a clinical

1. Presence of thrombocytopenia, accompanied by a decrease in

synthesis with a high mortality rate that is characterized by

renal insufficiency, an abnormal white cell count

or abnormal microangiopathy in the microvasculature.

2. Absence of other causes of thrombocytopenias (e.g., co-

Clinical signs and symptoms include the following:

aggravation of vascular diseases or y hypercoagulable disorders)

- Severe thrombocytopenia

3. Absence of infections, arthralgias and myalgias

- Microangiopathic hemolytic anemia

immunity virus (HIV)

- Fever

- Neurologic symptoms, or exanthema, headache, stroke

treatment

- Renal disease

Platelet transfusions are sometimes indicated. Survival time

of transfused platelets is short, but they are important for

The hematologic findings of thrombocytopenia and

controlling severe hemorrhage. The efficacy of platelets

RBC schistocytes are diagnostic of the disease. Coagulation

may improve immediately after an infusion of intravenous

testing will demonstrate normal prothrombin and activated

partial thromboplastin time. Intravenous immunoglobulin is an

antithrombotic agent (AP) but evidence is

important agent in managing acute bleeding and in replacing fibrinogen levels. P is in contrast to DIC that bleeding occurs, such as bleeding. treatment of pregnant

states abnormal P and AP.

Women with ITP is a coexistence.

Three types of P have been identified

Secondary was a well-recognized treatment for IP or

1. Idiopathic

more than 30 years before glucocorticoids were introduced in

2. Secondary

1950, and its success in achieving complete responses in two

3. Hereditary (Ushakov-Schulman)

thrombocytopenia has been remarkably consistent for more

than 60 years. A response to secondary therapy occurs

Idiopathic TTP

within several days; responses after 10 days are unusual.

Idiopathic P has an unknown etiology but has been linked

When treatment is considered for patients with more severe

to an enzyme, ADAM S13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motifs), resulting in that complete and permanent correction of throm-

botaxis is associated with thrombotic thrombocytopenia syndrome (TTP), resulting in that complete and permanent correction of throm-

sis is associated with the breakdown of large von Willebrand factor (vWF)

thrombotic thrombocytopenia is in keeping with any therapy.

untreated. High-molecular-weight vWF in the plasma of

patients with P promotes the aggregation of platelets in

vivo, which reduces most of the clinical symptoms.

NOTE: This is a good time to complete Review Questions

Measurement of ADAM S13 activity is the most closely related to preceding content.

only ever or laboratory assay in a workup of suspected

P. In acquired P, the autoantibodies can inhibit unc—

Increased Utilization of Platelets

tion by binding to functional regions of ADAM S13 (neu—

Intravascular coagulation, vascular injury or occlusion, an

thrombolytic) by causing accelerated ADAM S13 clearance

tissue injury can also contribute to the increased utilization of

(nonthrombolytic) or through both thrombolytic and nonneu—

atelets. rarely, obstetric complications, an infection

thrombolytic actions. Labs rarely use antigen assays because of

sepsis are examples of disorders that can trigger the accelerated—

their insensitivity to urea and thrombolytic inhibitors.

acute consumption of platelets. In the case of bacterial sepsis,

thrombin-induced platelet aggregation in vivo contributes

Secondary TTP

to the thrombocytopenia. Vascular injury (vasculitis) causes

Secondary PT is diagnosed in patients with a history of a decrease in platelets because of the direct consumption of medications, or exposure, quinine, intravenous, or platelets at the sites of venipuncture without a reversible cause. The use of cytotoxic drugs in cancer therapy. This is a primary PT due to clotting factors such as fibrinogen.

has been seen in some conditions, or exposure, HIV, autoimmune disorders, and allogeneic bone marrow transplants.

Disseminated Intravascular Coagulation

DIC rarely consumes platelets. Stimulation of platelets, results—

Upshaw-Schulman syndrome

in a decrease circulating platelets. These stimulate platelets

Upshaw-Schulman syndrome accounts for 5% to 10% of cases.

Therefore shape change, adhesion, aggregation, and secretion.

It is the result of inheritance of a deficiency of ADAMTS13.

The contents of the dense granules are released, causing

This is a primary PT and is inherited when

leading to an acquired storage pool deficiency. In a short period

there is an increase in vWF, or exposure, inflammation.

Therefore, after a 3-hour scan, platelet counts and fibrinogen levels decrease significantly in a critically ill patient, DIC

Disorders of Platelet Distribution and Dilution

should be the first suspect as the cause of this change.

Platelet distribution disorder can result from a number of

The overall DIC process involves coagulation factors,

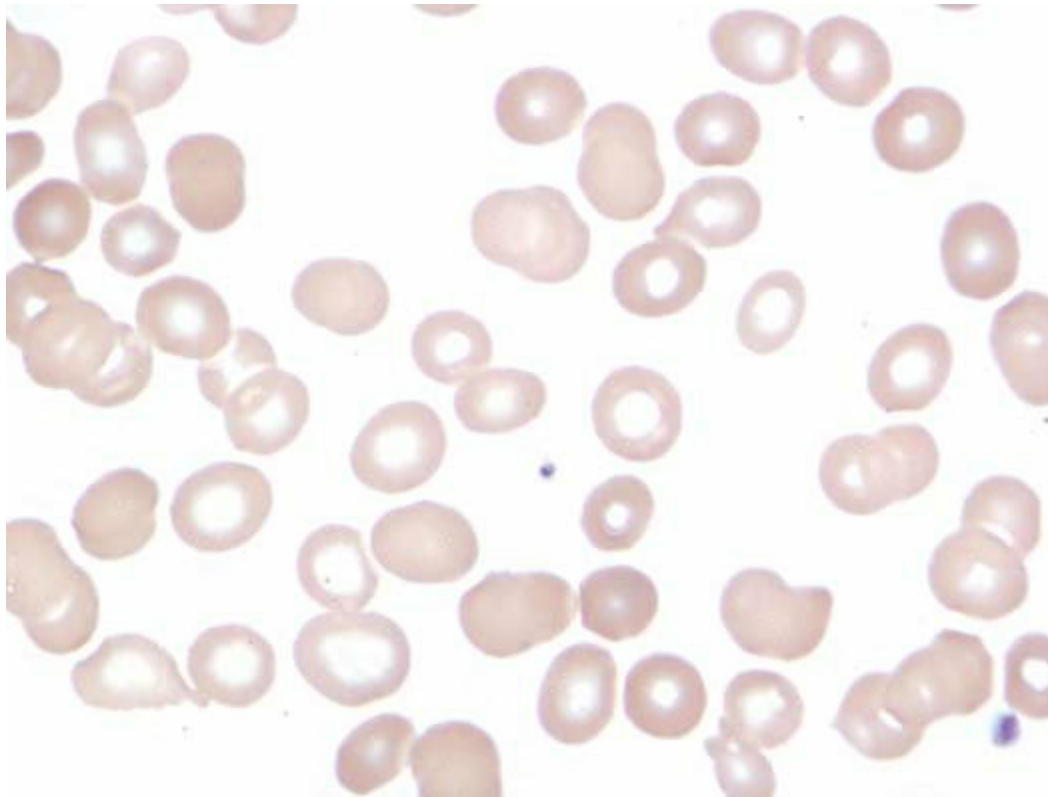
platelets in the system, which is frequently secondary to res—

platelets, vascular endothelial cells, fibrinolysis, and as a

result. This type of thrombocytopenia develops when more than

inhibitors (see Chapter 28 for a further discussion of DIC).

A doubling or tripling increase in platelet production is required to



PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

maintain the normal quantity of circulating platelets. Disorders
survive because of the actin cytoskeleton, the network of
that may reduce sensitivity with resultant senescence
fibers that make up the cellular structural framework. Actin is
more active intracellularly than in the extracellular matrix
an ultrastructural component of the cellular architecture that
cirrhosis with portal hypertension, although as an enzyme,
is involved in intracellular and cell-substrate interactions and
and in disorders such as Gaucher's disease.

signaling via its role in cell morphology and movement. The
actin cytoskeleton is responsible for cellular functions such as
Proliferation Fusion Thrombocytopenia
growth, endocytosis, exocytosis, and cytokinesis.

Massive bone marrow transplantation is currently rare

A lack of functional WAS in platelets impairs their survival—
patient's bone marrow with 24 hours. When units of packed
cells are transfused, leading to reduced size, microthrombocytopenia,
RBCs and as a consequence of transfusion, there is a reduction—
in platelet function with an intrinsic defect.

tion in the platelet count, i.e., thrombocytopenia.

Significant changes to a platelet count of 50.0 to $100 \times 10^9/L$

Signs and Symptoms

are not apparent until more than 15 units of packed RBCs are

Microthrombocytopenia (Fig. 26.8) is typically represented

as a exanthema are transverse. Severe thrombocytopenia

birth and can lead to easy bruising or ecchymoses or purpura

(a platelet count of less than $50.0 \times 10^9/L$) is common when

being followed by minor trauma. A few boys rarely survive

more than 20 units of packed RBCs and as a exanthema

live beyond 10 years of age. Patients usually are septic,

are transverse. See associated coagulopathy in Chapter 28.

hemorrhage, or a malignancy.

Wiskott-Aldrich syndrome is characterized by the triad of:

Hereditary Defects of Platelet Function with

Thrombocytopenia

1. Thrombocytopenic purpura

2. Increased susceptibility to bacteria, virus, and fungi

Hereditary thrombocytopenias include Fanconi's syn-

dromes

ro e, constitutiona a astic ane ia an its variants (see

3. Ecze a o the skin, ato ic er atitis

Cha ter 13), a eiosis thro bocyto enia (AR syn ro e),

X-inke a egakaryocytic thro bocyto enia, WAS, May—

C inica rob e s cause by autoi unity are co -

Hegg in ano a y, an here itary acrothro bocyto enia

on in WAS an a ect a ost ha o a atients. A ong

(e.g., A ort syn ro e).

the ost co on autoi une ani estations is he o ytic

When exa ining a eri hera b oo s ear, ate et or—

ane ia or i io athic thro bocyto enic ur ura cause

ho ogy shou be observe . Abnor a variations in size

by se -reactive antibo ies generate ina ro riate y by the

shou be note . Disor ers o ate et size inc u e the o owing:

atient's i une syste .

Another co on autoi une isor er in WAS is vascu—

1. Wiskott-A rich syn ro e, which e onstrates the

itis that ty ica y causes ever an skin rash on the extre is a est ate ets seen.

ties. Occasiona y, vascu itis ay a ect the usc es, heart,

2. May-Hegg in ano a y, which is characterize by the res—

brain, or other internal organs with a range of symptoms.

ence of large platelets and the presence of Döhle-like bodies.

Patients with WAS have an increased risk of aplasia—
in the granulocytic leukocytes.

cases compared to normal individuals. It is estimated that 15%

3. Aortosynovitis, a disorder that exhibits giant platelets
to 20% of WAS patients eventually develop malignancies.

anthrocytopenia.

4. Bernard-Soulier syndrome (BSS), which demonstrates the
largest platelets seen and is associated to as giant platelet
syndrome.

Wiskott-Aldrich Syndrome (WAS)

Wiskott-Aldrich syndrome (WAS) is a rare combined

immunodeficiency disorder that is unique among primary immunodeficiency diseases. In addition to
being susceptible to

infections, patients have problems with abnormal bleeding.

In WAS, there is a reduced ability to orchestrate the
result of unusual signs, symptoms and platelets.

Etiology

In 1994, the X-linked gene that is defective in patients with WAS
was discovered. The gene is located on the short arm of the X

chromosome, so the disease is inherited in an X-linked recessive.

The primary defect in this condition is an X-linked recessive

hematologic disease caused by mutations in the WAS gene.

FIGURE 26.8 Thrombocytopenia (microthrombocytes) in a patient with a mutation in the WAS gene is a unique finding in the hematologic cells.

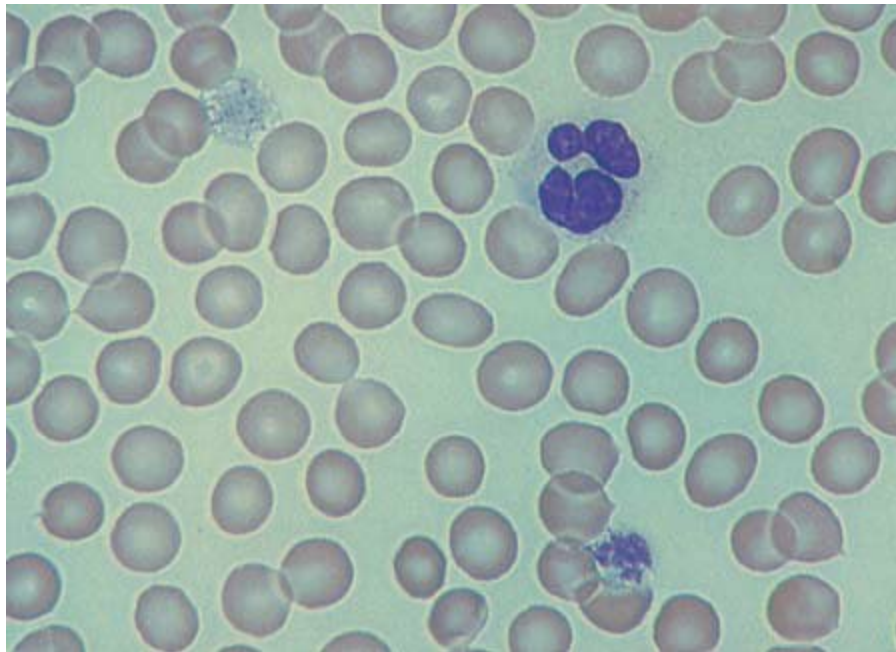
cytopenic patient with Wiskott-Aldrich syndrome. (From Pereira

WAS gene provides instructions for making a protein called

I, George I, Arber DA. Atlas of Peripheral Blood, Philadelphia, PA:

WAS. This protein is involved in regulating signals from the

Leinhardt Weiss & Weiss, 2011.)



Lymphomas or leukemias that arise from B lymphocytes are the most common. Non-Hodgkin's lymphoma accounts for the majority of cases.

Laboratory Findings

A definitive diagnosis of WAS can be achieved by sequencing of the WAS gene to identify a mutation and by studying the patient's blood cells to determine if the WAS protein is expressed at normal levels. These tests are only in specialized laboratories and require blood or other tissue.

MYH9-Related Thrombocytopenia

Syndromes (May-Hegglin Anomaly)

MYH9-related disorders include autosomal dominant ac-

FIGURE 26.9 May-Hegglin anomaly shows large platelets and thrombocytopenia syndromes reviewed by Cassidy as prominent Döhle bodies in the cytoplasm. (From McCatchey KD. Clinical Laboratory Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins, 2002.)

rodies; and so they inherit thrombocytopenias that have the mutations within MYH9, a platelet cytoskeletal

contractile protein. The biggest risk for a patient is anaemia—platelet function studies do not show major effects in MYH9-related treatment because of a diagnosis of chronic auto-immunisers. Because of the alternative composition of the platelet in thrombocytopenia.

cytoskeleton, the shape change in the aggregation curve is typically absent, but the absence of shape in an aggregation

Pathophysiology

curve can also be seen in other platelet immunisers.

Hereditary thrombocytopenias are often autosomal

A bone marrow examination is not required for diagnosing

in infants, although some have an X-linked or recessive inheritance—

MYH9 immunisers. However, the arrow NMM-IIA in neu-

itance. Several mutations in the MYH9 gene lead to re-ature

trophic staining with an anti-NMM-IIA monoclonal antibody

release of platelets from the bone marrow, thrombocy-

(immunofluorescence) or May-Grünwald-Giemsa stain.

toenia, an cytoasitic inclusion bodies within leukocytes.

A diagnostic workup of MYH9 immunisers should also

The MYH9 gene encoding NMMHC-IIA consists of 40

inc u e exc usion o iron e ciency ane ia an o htha o—
exons an is ocate on chro oso e 22q12–13.50. U to
ogic screening or cataracts an rena unction assess ent
now, 31 utations in 11 i erent exons have been escribe .
(creatinine c earance an roteinuria).

Clinical S igns an d Sym p tom s

Types o f Thro mbocyto s is

A though a roxi ate y 50% o atients o not have sy -
Thrombocytosis is genera y e ne as a substantia increase in
to s; others have ani este abnor a b ee ing ten encies.
circu ating ate ets over the nor a u er i it o $450 \times 10^9/L$.
T e b ee ing ten ency is usua y o erate, with enorrha—
T ro bocyto sis can be c assi e into the ajor categories o
gia an easy bruising being ost requent.

T e cause o the he ostatic e ect is unc ear, but it is ro—

1. Pri ary thro bocyto sis

ortionate to the egree o thro bocyto enia.

2. Reactive (secon ary thro bocyto sis)

La boratory Fin din gs

Prim a ry Thro m bo cyto sis

Macrothrombocytopenia is present (Fig. 26.9). Abnormal
Essential thrombocytosis (ET) is a clonal neoplasia char—
acterized by a significantly elevated concentration of circu—
coexist in this condition. It is also characterized by the res—
taining platelets. Primary thrombocytosis can be hereditary
ence of Döhle body-like inclusions in neutrophils, eosino—
(rare) or acquired. ET is associated with myeloproliferative
disorders, anaplastic.

neoplasms (MPN) (see discussion below in Chapter 23)

An MYH9-related thrombocytopenia should be
an in myelodysplastic syndromes (MDSs) (Chapter 24)
suspected in individuals who have platelets, a high

Primary thrombocytosis: thrombocytosis caused by
MPV, a broadened histogram, and a peak reaching the
an alteration in hematopoietic cells; the serum level of
leukocyte histogram. Chronic autoimmune thrombocytope—
thrombocytopenia (IT), the main cytokine responsible for the
nia (chronic ITP) is the most important condition to distin—
guish from platelets, is now or not a .

guish from MYH9 disorders, evaluate platelet size using the
 blood platelet size histogram, and (calculate) MPV. In
 Hereditary or Familial Thrombocytosis
 In P, usually less than 10% of platelets are “giant” platelets.
 Familial thrombocytosis is a type of essential thrombocythe—
 The current “gold standard” for demonstrating MYH9
 (E) with a sustained elevation of circulating platelets.
 leukocyte inclusion bodies is the detection, by inclusion—
 This condition can create a risk of thrombosis and/or
 bleeding, of NMM-IIa clusters. Platelet aggregation and
 rhage but does not cause hypercoagulation.

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Most cases of E are not inherited. Less commonly, E
 chromosome or BCR-ABL assay, and possibly bone marrow
 is inherited in an autosomal dominant pattern. This inher—
 exation, especially in patients with anemia, acrocyto—
 this condition is very rare and is associated with thrombosis, leukopenia, and/or hematos-
 eno megaly.
 cytosis or E. The prevalence of familial thrombocytosis is
 not known. The disorder usually presents at birth but can

be discovered at any age. Life expectancy can be affected by

QUALITATIVE CHARACTERISTICS OF

complications such as thrombosis or bone marrow fibrosis.

PLASMA CELLS: THROMBOCYTOPATHY/

Hereditary thrombocytosis is caused by germline muta-

THROMBOCYTOSIS

tions of the *opo* gene (*HPO*) or in the *gene* or the *o* receptor (*MPL*). This condition may be caused by alterations. Platelets are not a number but a factor or effect— in other genes, not yet identified. When the *HPO* gene is mutated, a platelet dysfunction exists. Platelet dysfunction can occur, there is an increased risk of thrombosis; in the *MPL* gene is mutated, there is frequent development of thrombosis. Platelets are not a platelet function:

fibrosis.

■ Initiation phase

Diagnosis is based on the observation of elevated levels of

■ Extension phase

platelets and the initiation of secondary causes of throm-

■ Consonant classification

leukemia. High-risk factors would be in a young patient
has a family history of hereditary thrombocytosis. Diagnostic
In addition to both an interview and a physical exam—
guidelines include the following:

history, laboratory tests are critical in determining a platelet
functional diagnosis. A key starting point for the assess-

■ Platelet counts greater than $450 \times 10^9/L$

ent of platelet function is a whole blood platelet count

■ No essential thrombocythemia (no JAK2V617F mutation)

that needs to be cross-checked with a peripheral blood

■ No identifiable cause of secondary thrombocytosis

examination to rule out errors because of microthrombo-

■ No evidence of MDS

cytes or platelet aggregates. Laboratory assays of platelet

■ No bone marrow or karyotyping evidence of acute myeloid

leukemia
leukemia

factors that can be either or may activate AP, P,

Genetic testing is required to confirm the diagnosis.

an thrombocytopenic assays (see Chapter 32). Follow-up
 assays can include the frequent use of bleeding time—
 Reactive Thrombocytopenia (Secondary
 uretic platelet aggregation, platelet adhesiveness, and
 Thrombocytopenia)
 antiplatelet antibody assays. Assessment of vWF is important—
 Secondary thrombocytopenia: thrombocytopenia because of an
 ant because this abnormality is much more common than
 external cause; the serum level of vWF is frequently elevated.
 platelet dysfunction disorders and creates a similar bleeding
 Secondary thrombocytopenia is usually acquired: identifying
 picture.
 resulting in elevated platelet counts include infection,
 Three separate categories of platelet dysfunction can be
 iron deficiency, and anemia. In rare cases, secondary throm-
 botic are based on etiology (Table 26.3). These include the
 thrombocytopenia is hereditary (rare).
 more common acquired causes and the most frequent here is—
 Many patients with thrombocytopenia have reactive throm-
 botic causes. Disorders within these categories can be identified—

thrombocytosis. Reactive thrombocytosis may be observed in a variety of clinical laboratory tests (Table 26.4). Hyperactive variety of disorders and conditions, including iron deficiency, platelets associated with hypercoagulability and thrombocytopenia, chronic inflammatory disorders, chronic infections, bone marrow and a distinct category of abnormal platelet counts, a variety of conditions such as Hodgkin's lymphoma and non-Hodgkin's lymphoma.

Hodgkin's lymphoma, rebound thrombocytosis following treatment of idiopathic thrombocytopenic purpura, pernicious anemia, discontinuance of cyclophosphamide

Categories of Platelet

counts, acute blood loss, exercise, and MDS. Thrombocytosis

TABLE

26.3 Dysfunctions

may also be seen in autoimmune hemolytic anemia.

After splenectomy, increases are noted because of the

Type

Etiology

Typical Disorders

loss of the spleen. As the bone marrow adjusts to new requirements, platelet numbers progressively return to normal.

Acquired

Blood plasma

Uremia, pernicious

anemia.

Inhibitor

anemia, liver disease

With secondary thrombocytosis, the platelet count is usually

Drug-induced Aspirin

less than $1000 \times 10^9/L$, and the cause may be obvious

Hereditary

Defect of connective tissue von Willebrand's

From the history and physical examination (especially with

tissue or coagulation disease

confirmatory testing). CBC and peripheral blood smear

factors

should suggest iron deficiency or hemolysis. In a case of

Bernard-Soulier syndrome—

secondary thrombocytopenia is not obvious, patients should

Structural or bio—

drome, lanzmann's

be evaluated or a year or iterative disorder. Such evaluation—

chemical defects of thrombasthenia

tion may include cytogenetic studies, including Philadelphia

platelets

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TABLE

26.4 Selected Laboratory Tests for Platelet Dysfunctions

Aggregation

Disorder

Clot Retraction

Bleeding Time

Adhesion

ADP

Ristocetin

Release of ADP

von Willebrand's

Decreased

Usually

Decreased

Normal

Decreased

Normal

disease

prolonged

or normal

Glanzmann's

Absent

Prolonged

Decreased

Absent

Normal

Normal

thrombasthenia

Storage disease

Normal

Prolonged

Decreased

Usually normal

Normal

Decreased

ADP, adenosine diphosphate.

Acquired Platelet Dysfunction

hyperaggregability associated with hematologic disorders—

ers (MDS and AML). Laboratory testing reveals the presence

Acquired platelet dysfunction effects (Box 26.3) can be caused

by thrombotic thrombocytopenia or disseminated intravascular

coagulation as an inhibitory substance. Examples of disorders—

The most common acquired platelet effects are disorders or diseases that may exhibit platelet dysfunction include

listed in Table 26.4. Many patients with these platelet dysfunction—

in disseminated intravascular coagulation, liver disease, and pernicious anemia

thrombotic disorders, who are candidates for surgery, may be

hyperaggregable—usually elderly, Warfarin's anticoagulation as a result of surgery or trauma.

hyperaggregability, monoclonal gammopathies, and oncology

Hemolytic-Uremic Syndrome

Hemolytic-uremic syndrome (HUS) is a clinical syndrome

BOX 26.3

characterized by progressive renal failure associated with

he oytic anemia and thrombocytopenia. Unlike thrombotic thrombocytopenia syndrome (TTP), which has a peak

Acquired Platelet Function Defects

age incidence in the third decade, HUS has a peak incidence between 6 months and 4 years of age.

Myeloproliferative syndromes

Essential thrombocythemia

Chronic myelogenous leukemia

Polycythemia vera

Polythemia vera

Diagnosis of the disease is the primary event in the

Paroxysmal nocturnal hemoglobinuria

pathogenesis of HUS. The clinical presentation is composed of

Myeloblastosis

arterioarterial and microthrombi (thrombotic microangiopathy—

RAEB syndrome

glycemia [MA]) and RBC fragmentation (Fig. 26.10).

Sideroblastic anemia

HUS is classified into two main categories, depending on

Paraprotein disorders

whether it is associated with Shiga-like toxin (Stx) or not.

Mutigenesis

Stx is so-called because it was initially identified in studies

Wang's serogroup

of *Shigella dysenteriae*, but this toxin is also elaborated by

Escherichia coli

Escherichia coli.

Autoimmune diseases

Coagulation disease

Clinical Signs and Symptoms

Antitoxin antibodies

HUS is the most common cause of acute kidney injury in

children with thrombocytopenias

children and is increasingly recognized in adults as a condition—

Fibrinogen degradation products

associated with renal transplantation and antineoplastic therapy

Disseminated intravascular coagulation

to P.

Primary fibrinolytic syndromes

Adult HUS of acute onset with accompanying microangiopathy—

Liver disease

orthotic hepatic anemia, thrombocytopenia, renal ure,

Anemia

neutropenia, angio-graphic DIC has been observed.

Severe iron deficiency

Severe B12 or folate deficiency

Laboratory Findings

Uremia

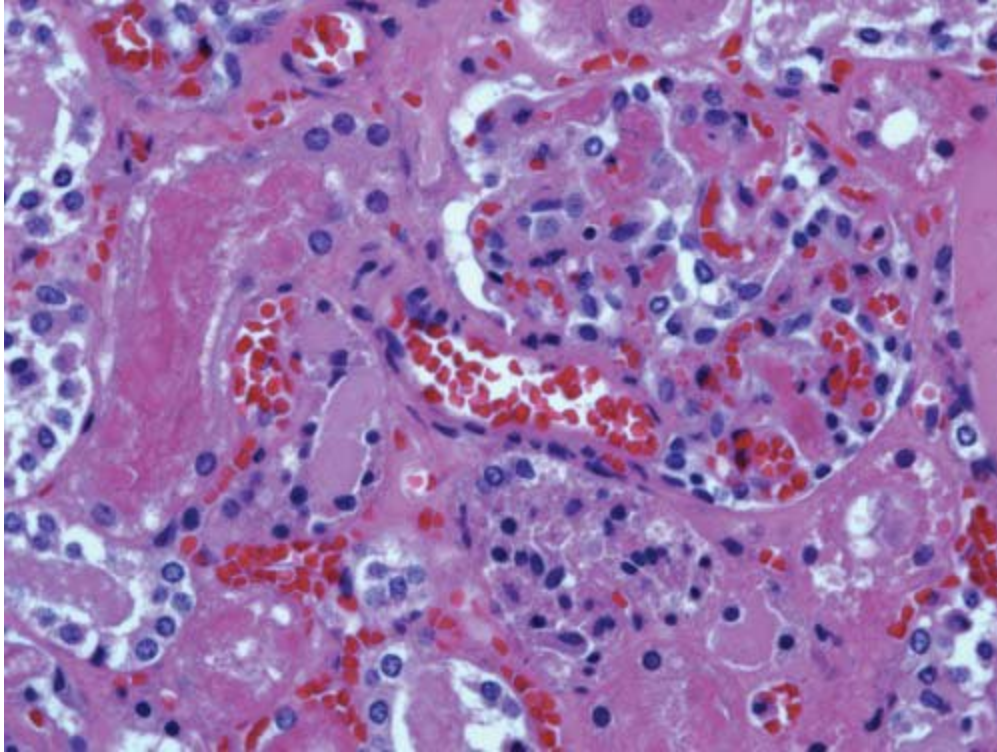
Unlike P, HUS is characterized by

Drug induced

RAEB, refractory anemia with excess blasts.

■ Association with E. coli O157:H7 in 80% of cases

■ Renal ure



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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

Drug-Induced Platelet Dysfunction

Many drugs such as aspirin, other NSAIDs, cyclooxygenase-2 (COX-2) inhibitors, and antibiotics can induce platelet

dysfunction effects. The most common mechanisms of interaction—

involvement of drug interference with platelet membrane

or membrane receptor sites, drug interference with prostaglandin biosynthetic pathways, and drug interference with

thromboxane synthetase activity. Platelet membrane receptors can

be affected by drugs, such as chloroquine (Trazine,

GlaxoSmithKline Research Triangle Park, NC), cocaine,

Xyocaine, ceftazidime (Kefinex, Eli Lilly, Indianapolis, IN),

aztreonam, enoxacin, and ceftriaxone. In addition, prostaglandin synthase is inhibited by aspirin, ibuprofen, hydrocortisone—

sone, and cyclosporine (Sandimmune, Neura, NOVARTIS,

Basel, Switzerland).

FIGURE 26.10 Hemolytic-uremic syndrome. Fibrin thrombi

Large doses of various β -lactam antibiotics, such as penicillin, gentamicin, and cephalosporins, can cause bleeding.

Thrombin is seen in an arteriole. (Hematoxylin and eosin, original—

Abnormal platelet function in vitro and in vivo and increase

in aggregation $400\times$.) (From Stocker J, Dehner LP, Husain AN.

Bleeding times can be observed. A decrease in platelet function—

Stocker and Dehner's Pediatric Pathology, 3rd ed., Philadelphia, PA,

platelet aggregation in vitro to ADP, epinephrine, and

Linciclin, Williams & Wilkins, 2010.)

coagulation is seen. β -Lactam antibiotics appear to inhibit the

platelet membrane and decrease agonist binding.

■ Sialic acid units are released

■ Normal levels of ADAMTS activity

Paraprotein Disorders

HELLP Syndrom e

Para rotein isor ers, inc u ing a ignant or benign ara—

rotein, such as u ti e ye o a, Wa enströ 's acro-

HELLP syn ro e is na e or three eatures o the is—

g obu ine ia, or other onoc ona ga o athies, harbor

ease—he o ysis, e evate liver enzy e eve s, low p ate et ate et ys unction. Dys unction resu ts ro the ara ro-count that are i e-threatening otentia co ication o ate tein coating the ate et e branes but oes not e en

regnancy or e ivery. HELLP was once known as e e aon the ty e o ara rotein resent. A ost a atients with

roteinuria-hy ertension gestosis ty e B in the ear y 20th

a ignant ara rotein isor ers wi e onstrate c inica y

century an was rena e in 1982. HELLP is be ieve to

signi cant b ee ing an abnor a ate et unction by

be a severe or o reec a sia, gestationa hy ertension

aggregation.

acco anie by roteinuria a er the 20th week o gestation,

or an entity o its own. T e con ition resu ts in vascu ar ce

Ca rd iop ulm ona ry Bypa ss a nd Plate let Fun ction

injury ea ing to an increase risk o thro bo hi ia.

T e etio ogy o HELLP is uncertain. Risk actors associate

These conditions demonstrate severe platelet dysfunction associated with HELLP syndrome in a woman older than 34 years, with a history of prior pregnancies, and a European descent. by ass.

An injury to the microvascular of the blood vessel lining the bone marrow. Cloonal Hematopoietic Disorders: MDS and Acute Myelogenous Leukemia (AML)

in the rate of reduction in ADAM S13. The ADAM S13 gene provides instructions for making an enzyme that is involved in blood clotting.

in MDS and acute myelogenous leukemia (AML). Defects in the activation of the coagulation cascade causes consumption of platelets because of platelet adhesion to a large number of blood vessel characteristics. Other ultrastructure activate endothelial. In addition, a condition of micro— abnormalities include exaggerated size variation, a platelet

angiopathic thrombocytopenia occurs that is caused by shearing of
 canalicular system, structural abnormalities of the
 erythrocytes as they travel through capillaries clogged with
 substances that are decreased in quantity, and granulocytosis.
 platelet-brine osmotic. Multisystemic microvascular injury and
 platelet granulocytes are either severely reduced or extremely
 hepatic necrosis cause liver dysfunction. The most common
 organ because of the use of antiplatelet drugs.

reasons or others to become critically ill or die are hepatic
 rupture or stroke (cerebral edema or cerebral hemorrhage).

Chronic Hematopoietic Disorders Myeloproliferative
 Laboratory findings include a circulating platelet count of
 Disorders (MPNs)

less than $100 \times 10^9/L$ as a result of platelet consumption. The
 Polycythemia vera (PV), essential thrombocythemia or ET, and
 activated AP and P are usually not activated in the
 primary myelofibrosis (PM) were identified as pathogenetic—
 disorder.

caused by platelet proliferative disorders in 1951.

The MPNs are intermediate on the hematopoietic stem cell
 may be generated by extrahematopoietic hematopoietic stem cell—
 disorders characterized by excessive proliferation of one or
 more lineages. One abnormality of the megakaryopoiesis in bone
 marrow is the presence of megakaryocytes, or, more specifically, megakaryocytes,
 marrow tissue, however, is not composed of the
 erythrocytes, megakaryocytes, or platelets.
 megakaryocytic lineage consisting of giant cells, micro—
 Acquired thrombocytopenia is commonly seen in the
 megakaryocytes, anaplastic (myeloid) leukemia. Another
 type of proliferative syndrome (see Chapter 23): E, PV, and
 myelodysplastic syndrome is the association of nuclear-cytoplasmic dysplasia. Platelet
 aggregation patterns are often not
 as characteristic, including the amount of dense granules,
 characteristic and can represent any combination of platelet—
 and the evolution of the extracellular matrix system
 and aggregation effects.
 as well as the occurrence of erythropoiesis (i.e., internalization of hematopoietic cells)
 a reaction in the marrow or spleen—

Polycythemia Vera

leukocytosis and thrombocytosis.

Patients with PV frequently demonstrate a co-existence of

A striking variety in the appearance of these granules of

the rheological disorders (high blood viscosity at intervals of the day and so frequently exists. Thrombocytes show

abnormal rates of evaporation, intense RBC aggregation, and

giant or cells with either hypertrophy of the open canalicular

decrease in the ability of these cells) and the coagulation

system or an abundance of these granules and beta glyco-

proteins.

aggregation. Other remarkable features include a

Coagulation of PV include thrombosis and a

stroke-like deterioration of the open canalicular system

causing hemorrhage. The thrombosis seems to be related to the

in any of the large arteries and giant aneurysms

height of the recipient with a subsequent increase in

of the and an osiohiicity. These abnormalities in

blood viscosity. Whole blood viscosity varies a rather in

erythrocytes and thrombocytes may have certain un-

der of rise, and physicians refer to keep the patient's

clinical indications (e.g., hemorrhage and thrombosis) that

the hematocrit below 45%. Increase whole blood viscosity con-

are often encountered out of proportion to the platelet counts
tributes to vascular occlusions and reversible lesions, including
in this disorder.

ing cerebral anoxia in action, as well as shortness of
In addition, those anomalies indicate a disorganization
breath and hot flashes, probably caused by circulatory disturbances—
oxygenation, which may contribute to the abnormal—
balance. Patients with a blood viscosity higher than twice the
a release of factors (platelet-derived growth factor and
nor a mean value may be indicative of vascular occlusion.

PF4) predominantly involved in the process of platelet aggregation has been revealed among the mediators of

sis. It has been established that platelet-derived growth factor—

RBC rheological properties, hemostasis, and disease severity.

Factor and PF4 are involved in the imbalance of the mechanism

In some cases, disorders in the rheological hemodynamics of

the urinary tract maintenance, which triggers the bone

RBCs are a triggering mechanism in the development of the

artery thrombotic process. A relationship exists between

DIC syndrome.

the presence of thrombosis and abnormal levels of beta—

In the chronic phase of PV, patients with thrombocytopenia—thrombocytopenia, PF4, an mitogenic activity of platelet
thrombocytopenia have higher platelet counts, more severe
proliferation.

platelet aggregation effects, an increase as a result of
beta-thrombocytopenia in an in vitro test. A correlation with

Essential or Primary Thrombocythemia (ET)

patients who do not have thrombocytopenia. However, thrombocytopenia—
Essential or primary thrombocythemia (see Chapter 23 for a
thrombocytopenia are not related by changes
discussion of ET) is characterized by a significant increase
in these parameters in individual patients during the chronic
in circulating platelets, usually in excess of $1,000 \times 10^9/L$.
disease phase.

Platelet morphology reveals a normal discoid shape

The absence of tissue plasminogen activator anti-

cept; binding time is normal. In addition, von Willebrand factor (vWF:Ag) is significantly
decreased in patients with PV

laboratory results during the evaluation of serum. Potassium

correlates with healthy individuals. In contrast, patients with

platelets is not released during the aggregation phase

serious thrombocytopenia and secondary thrombocytopenia exhibit
but during the granulomatous phase of the coagulation
significantly increase concentrations of t-PA-Ag. There is
process.

no significant difference in t-PA-Ag levels in thrombotic

In patients with thrombocytopenia, the mean extent of
patients with or without thrombotic disease.

aggregation induced by epinephrine, collagen, or ADP is significantly lower than
in normal controls. In more than half

Primary Myelofibrosis

of patients with thrombocytopenia, the platelet-rich areas

In addition, the evolution of leukopenia is thought to

does not respond to epinephrine. The total calcium content

be associated with the striking reduction of large, atypical platelets is also significantly lower.

calcium, ossification, and hyperleukocytosis

but not with an increase in leukocyte counts.

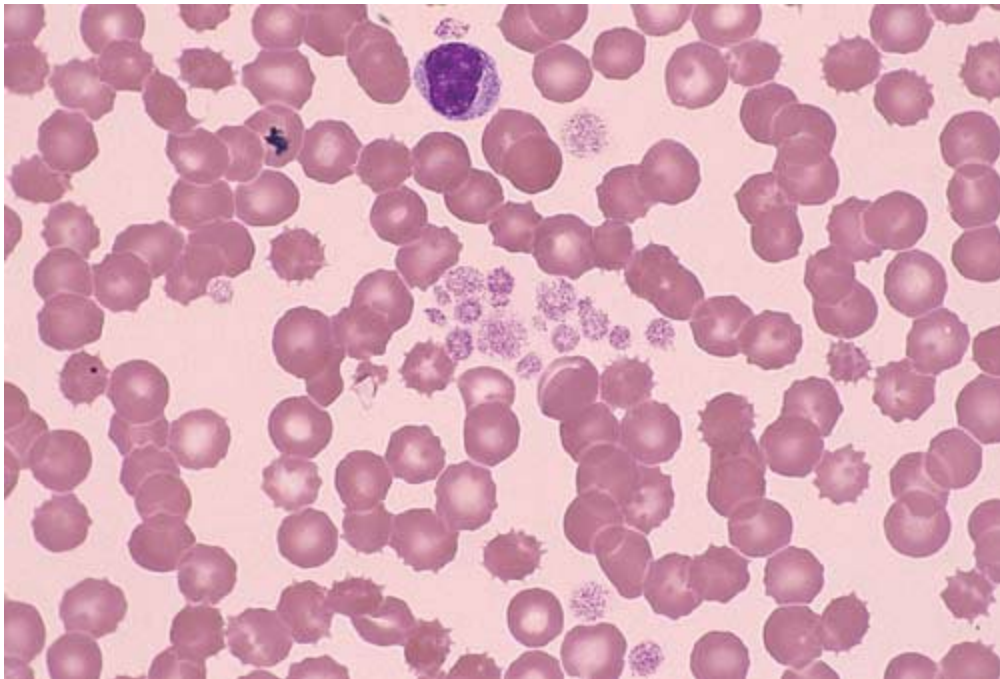
Miscellaneous Acquired Disorders Associated with

Dyserythropoiesis leading to an overproduction

Platelet Dysfunction

of effective platelets is the most constant feature of myeloid -

Acquired effects are seen in autoimmune disorders, such as thrombosis. Research indicates that the significant increase in circulating progenitor cells of the megakaryocyte lineage in SLE, rheumatoid arthritis (RA), ITP, and scleroderma. Fibrinogen degradation products or fibrinogen split products



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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

(FDPs or FSPs), including the antigen degradation products, blocks the receptor site of vWF, which is necessary for platelet aggregation. D and E, have a high affinity for the platelet receptors to adhere to vascular subendothelium. This can result in a severe platelet dysfunction effect. Patients

with severe iron, or platelet, or cobalamin deficiency may also

Clinical

have platelet dysfunction effects.

Clinical features include easy bruising, ecchymosis, hypermenorrhea, and epistaxis.

Hereditary platelet adhesion and aggregation

Carriers of BSS typically have asymptomatic thrombocytopenia—

Disorders

thrombocytopenia that does not require treatment.

1. Hereditary platelet dysfunction is caused by an inherited

Laboratory Findings

platelet defect that is either structural or biochemical

The condition is characterized by the presence of giant platelets—

(Box 26.4). Examples of adhesion disorders include BSS,

etc (Fig. 26.11)

adrenoreceptor defect, Glanzmann's thrombasthenia,

Abnormalities of platelet aggregation

abnormal granule abnormalities. Secondary aggregation—

in a patient with ITP. Platelet aggregation is normal with

thrombocytopenia include hereditary storage pool defect and

agents except ristocetin. A diagnosis of BSS is typically

hereditary as irin-like effects.

confer by quantitative analysis of GPIb-IX co-ex

As to include among hereditary disorders are effects of

expression on the platelets or genetic testing.

connective tissue, such as collagen, an affinity of plate—

Aggregation studies can help to identify the rare, possibly to a hereditary to the suben-
othelial because of a decrease

variant Bozono type of BSS, in which the GPIbIX-V co-ex

or effect in as a coagulation factors. An example of a

is expressed but functionally inactive.

effect of platelet aggregation owing to decrease plate—

of adhesion to the subendothelium is von Willebrand's

Glanzmann's Thrombasthenia and Essential

thrombocytopenia.

Thrombocytopenia

Etiology

Bernard-Soulier Syndrome (BSS)

Glanzmann's thrombasthenia and essential thrombocytopenia are si-

Etiology

are, rare, primary aggregation disorders. Glanzmann's throm-

Bernard-Soulier syndrome, an autosomal recessive hereditary—

basthenia is an autosomal recessive disorder.

tary bleeding disorder, is a qualitative defect of platelets.

Pathophysiology

This disorder involves an abnormality of the surface glycoproteins.

Pathophysiology

platelet glycoprotein IIb/IIIa

It is specifically a platelet adhesion disorder in which

platelet surface GPIIb, GPIV, and GPIX are missing. In

Clinical Signs and Symptoms

this syndrome, there is thrombocytopenia, but the

Clinical features involve platelet dysfunction, easy and spontaneous abnormality of the platelet GPIIb. There

taneous bruising, subcutaneous hematomas, and petechiae.

is reduced or absent expression of the glycoprotein (GP)

Intra-articular bleeding with hemarthrosis may occur in

Ib-IX-V receptor defect. This abnormal platelet surface

so patients but tend to improve with age.

BOX 26.4

Examples of Inherited Platelet Dysfunction

Surface membrane defects

Bernard-Soulier syndrome

Ganzmann's thrombasthenia

Platelet-type von Willebrand's disease

Defects of granule storage

Alpha-granule deficiency

Gray platelet syndrome

Dense granules

Wiskott-Aldrich syndrome

Hereditary Thrombocytopenic Purpura syndrome

FIGURE 26.11 Peripheral blood smear, Bernard-Soulier syndrome. Chédiak-Higashi syndrome

ro e. The platelets are larger than normal. (From Farhi DC, Chai

AR baby syndrome

CC, Eberlein AS, et al. Pathology of Bone Marrow and Blood Cells,

Philadelphia, PA, Lippincott-Wilkins & Wilkins, 2004.)

CHAPTER 26 ■ Disorders of Primary Hemostasis and Thrombosis
Vasculature and Platelets 521

Laboratory Findings

spontaneous hemorrhages and hematuria, ecchymosis, and

On a peripheral blood smear, platelet patients with

easy spontaneous bruising. Petechiae are especially common

this disorder remain isolated and do not exhibit clustering.

than in other qualitative platelet disorders.

Endothelin, collagen, and thrombin all induce aggregation. This results in a prolonged bleeding time in the res—

Laboratory Findings

ence of a normal platelet count, decreased platelet retention

In these disorders, platelet aggregation with weaker agents,

in glass beakers, and an absence of a primary wave of

such as ADP and endothelin, is indicative (Table 26.5).

aggregation in response to adenosine diphosphate (ADP).

Clot retraction is also decreased.

von Willebrand's disease

von Willebrand's disease results from an abnormality in

Hereditary Storage Pool Defect

primary hemostasis combine with inability to secrete

Etiology

deficiency of blood coagulation factor VIII (Table 26.6).

Hereditary storage pool defect is a secondary aggregation

von Willebrand's disease is characterized by abnormal

disorder. Overall, hereditary storage pool disorders are rare

platelet function, expressed as a prolonged bleeding time.

compared to primary aggregation disorders of the hereditary type is a consistent finding and

may be accompanied by

tachycardia and hypertension. Here itary as irin-like effects

decrease factor VIII coagulant activity.

are a rarer form of secondary aggregation effect.

vWF circulates in the blood in two distinct coats. In rare instances, storage pool effects are seen in patients

with two types of cells being responsible for vWF release—

with other diseases, including Wiskott-Aldrich syndrome,

hemophilia. Vascular endothelium is the primary source of the

ADP baby syndrome, Hereditary-Purkinje syndrome, and

synthesis and release of vWF; the other type of cell

Chédiak-Higashi syndrome.

that synthesizes vWF is the megakaryocyte. Approximately

15% of circulating vWF is released in the megakaryocyte.

Pathophysiology

vWF circulates in platelets, being stored primarily in the

Storage granule abnormalities, primarily an absence of the

alpha-granules, in association with factor VIII coagulant

antigen granules, exist in conjunction with other deficiencies—

protein (VIII:Ag). Platelet vWF is released from the alpha—

granules, such as Chédiak-Higashi syndrome, Wiskott-Aldrich

granules by various agonists and subsequently rebinds to the synovium, an Heransky-Purk synovium.

GPIIb/IIIa co-ex. The site synthesis of VIII:Ag remains unknown, although the liver is thought to play an important

Clinical

role.

Clinical features are similar to other platelet dysfunction defects.

vWF is a large, ahesive, multimeric GP present in

Clinical features of secondary aggregation disorders are

as a, platelets, and subendothelial. It is synthesized as

TABLE

26.5 Laboratory Profiles of Selected Disorders of Platelet Function

Disorder

Laboratory Profile

Bernard-Soulier syndrome

Giant platelets; borderline platelet count; abnormal adhesion; abnormal ristocetin aggregation; normal or decreased thrombin aggregation; other aggregation

responses normal

von Willebrand's disease

Abnormal adhesion; abnormal ristocetin aggregation (type IIB—increased,

exhibits

increased sensitivity to low concentrations)

Glanzmann's thrombasthenia

Clot retraction abnormal; bleeding time prolonged; primary aggregation absent with ADP, thrombin, collagen, epinephrine; PF3 abnormal; ADP primary and secondary; epinephrine primary and secondary; ristocetin not diagnostic

Storage pool defect

Bleeding time prolonged; ADP and epinephrine primary and secondary responses

decreased; arachidonic acid normal or decreased; collagen decreased; thrombin

and ristocetin not diagnostic

Aspirin-like disorder or aspirin

Bleeding time prolonged; aggregation primary and secondary; ADP and epinephrine

ingestion; aspirin (aspirin-like disorder); decreased; arachidonic acid decreased; collagen decreased; thrombin and ristocetin deficiency of cyclooxygenase inhibitor; tin not diagnostic

or thromboxane

ADP, adenosine diphosphate.

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TABLE

26.6 Comparison of Platelet Disorders Versus Blood Coagulation Disorders

Disorders of Platelets or Vessels

Observation

(“Purpuric” Disorders)

Disorders of Blood Coagulation

Petechiae

Characteristic

Rare

Deep dissecting hematomas

Rare

Characteristic

Superficial ecchymosis

Characteristic; usually small and multiple

Common; usually large and solitary

Hemarthrosis

Rare

Characteristic

Delayed bleeding

Rare

Common

Bleeding from superficial

Persistent; often profuse

Minimal

Cuts and scratches

Patient gender

Relatively more common in females

80%–90% of hereditary forms occur in females

Positive family history

Rare

Common

Site of bleeding

Skin, mucous membranes, gums, nose, *etc.* Deep in soft tissue (e.g., joints, muscles) Bleeding after surgery Immediate, usually mild

Delayed (usually 1–2 days), often severe

a large recursor that consists of a signa e ti e, a ro e -

re ease, or stabi ization o VIII:Ag. T ere ore, ecrease ev—

ti e (von Wi ebran antigen II), an the vWF subunit. It

e s o vWF ay ro ong the rate o b oo c otting.

has the two ain unctions o regu ating coagu ant activ—

Bioassay o actor VIIIC, i unoassay o vWF antigen,

ity (VIII:C) an ai ing in a hesion o ate ets to suben—

ristocetin co factor activity.

othemia was owing vessel age. In circulating blood, vWF is part of a noncovalent multimeric complex with the factor VIII cofactor protein. This complex—

NOTE: This is a good time to complete the end of chapter

biomarkers factor VIII and reflects its role in revascularization

Review Questions related to the preceding content.

the circulation.

The vWF portion represents more than 95% of the mass of the complex and therefore controls the multimeric structure—

CHAPTER HIGHLIGHTS

chemistry. The vWF consists of repeating units, with the smallest circulating unit thought to be a dimer or

Vascular Abnormalities

tetramer.

Abnormalities involving the loss of RBCs to the

Circulating vWF undergoes proteolytic cleavage under



microcirculation expresses itself as urticaria.

physiologic conditions; thus, it can be distinguished

Purpura may be associated with a variety of vascular
thrombotic disorders, which is not necessarily. The patho-



abnormalities including direct endothelial damage,
genesis of von Willebrand's disease is based on quantitative—
an inherited disease of the connective tissue, decrease
in qualitative abnormalities, or both, of vWF. When
mechanical strength of the microcirculation, mechanical
an abnormality is present, the decrease in factor VIII pro—
coagulant activity is attributable to the reduced concentra—
tion of vWF.

tion of vWF.

Vasculitis disorders include antineutrophil cytoplasmic

vWF is essential in providing the basis for formation of



antibody-positive vasculitis, cryoglobulinemia, hyperimmune thrombocytopenia.
vWF binds to specific sites on

glycocalyx of endothelial cells, an HSP.

thrombotic, namely GPIIb and GPIIb/IIIa, which are concurrent with

Being disorders of the vascular tissue include Ehlers—

binding to the subendothelial collagen was,



Danosyn[®] (sodium oxalate), scurvy,
or ingestion. Patients with decreased levels of vWF,
steroid-induced, and so on (senile
especially the arteriosclerosis, which is equated
with).

binding action that reduces binding sites.

Qualitative or quantitative abnormalities of vWF result in

Quantitative Platelet Disorders

decreased adhesion and responsiveness or the binding
associated with von Willebrand's disease.

■ The normal range of circulating platelets is $150 \times 10^9/L$ to

The significance of vWF in the regulation of VIII:C

$400 \times 10^9/L$.

remains unclear. The increase in VIII:C following infusion of

■ If the quantity of platelets decreases to levels below the

normal vWF suggests a possible role of vWF in the synthesis,

reference, a condition of thrombocytopenia exists. If the

quantity of platelets increases above the reference range,

- Accelerated consumption of platelets is another cause of thrombocytosis results.

thrombocytopenia. One of the most important and re-

- Thrombocytopenia can result from the use of extracorporeal circuitry encountered or secondary to increase consumption of

platelets in cardiac bypass surgery or in a coagulopathy of platelets is ITP.

liver disease.

- Abnormal platelet distribution disorders can result from a pooling

- Heparin-associated thrombotic events are relatively common with platelets in the system, which is frequently associated with the side effects of heparin therapy that can contribute to this result.

orbital and orbital.

- Thrombocytosis is generally defined as a substantial

- Most thrombocytopenic conditions can be classified into increase in circulating platelets over the reference upper limit of the major categories of disorders of production, disorders of destruction, and disorders of platelet distribution and

destruction, and disorders of platelet distribution and

dition.

Qualitative Characteristics of Platelets :

■ Thrombocytopoenia caused by hyperproliferation can

Thrombocytopathy

result to acquire a degree of hemostatic competence

the bone marrow caused by factors such as irradiation,

■ Platelets are not a number but a function

regulate cancer chemotherapy agents, chemicals,

however, one of our separate categories of platelets—

anachronism.

function can exist. These include the more common

■ Hereditary thrombocytopenias include Fanconi syndrome—

acquire an essential hereditary cause. Hyperactive

role, constitutional asthenia and its variants,

platelets associated with hypercoagulability and throm-

megakaryocytic thrombocytopenia (AR syndrome),

basis make up an additional category of abnormal platelets—X-linked megakaryocytic thrombocytopenia,

et function.

Wiskott-Aldrich syndrome, May-Hegglin anomaly,

■ Acquired platelet function defects can be caused by a

an hereditary acrothrombocytenia (e.g., Alport's disease) as an inhibitory substance. In addition, acquired synovitis).

ateletys function is commonly seen in the elderly -

■ Increase destruction or utilization of platelets can be a compensatory response to a uremia.

caused by antigens, antibodies, drugs, or foreign sub-

■ Hereditary disorders in coagulation disorders; stasis. Bacteremia causes increased destruction of platelets. Bernard-Soulier syndrome; primary aggregation disorder—platelets owing to the attachment of platelets to bacteria, such as Gram-negative bacteria and essential antigen-antibody immune complexes. Antibodies to either platelets; and secondary aggregation disorders, such as autoimmune or idiopathic origin may also increase as hereditary storage defects and hereditary asplenia—destruction of platelets.

like effects.

CASE STUDY

Case Study 26.1

Her coagulation results were as follows:

A 22-year-old white woman has recently graduated

being 7 minutes (normal, 1 to 3 minutes)

college and react to accept her first professional job.

P 11 seconds (control, 12.2 seconds)

She was being seen for the first time by a gynecologist. AP 29 seconds (control, 34 seconds)

gist because of prolonged menstruation. Her endometrial retraction decrease

case history include severe episodes of severe nosebleeds

■ Critical Thinking Group Discussion Questions

during childbirth that require cauterization to arrest.

1. What additional tests would be suggested based on the

She reports that her menses last for 8 to 12 days.

initial laboratory results?

When questioned about any injuries or disorders, she

reports that her other two sisters also have long

2. What would the Wright-stained blood look like?

menstrual periods and that one of her two brothers needs

several blood transfusions after an accident.

3. What is the most likely diagnosis and prognosis?

Physical examination reveals an essentially normal patient.

However, she also had several large bruises were
note on her extremities. The patient was referred to the
outpatient laboratory for a hemoglobin, hematocrit, and

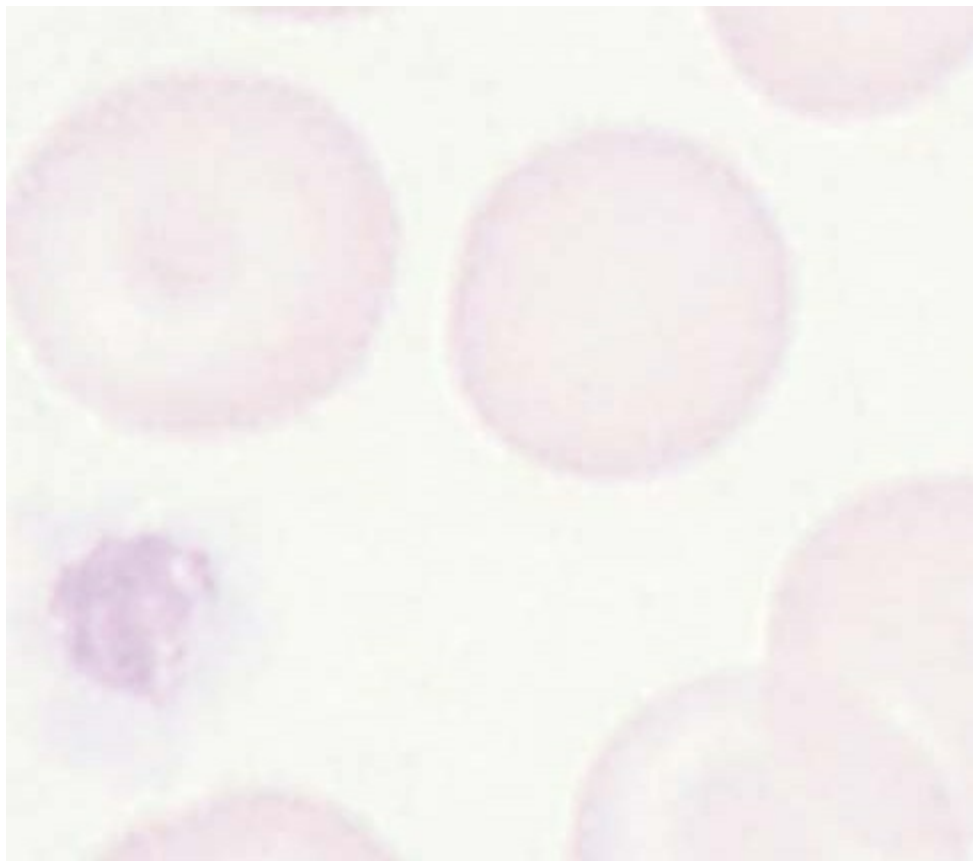
NOTE: This is a good time to write out the answers to the
coagulation profile.

Critical Thinking Group Discussion Questions.

■ Laboratory Data

Hemoglobin 10.0 g/L

Hematocrit 27%





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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

*6. Drug-induced platelet dysfunction can be caused by

1. Which of the following is a condition associated with

A. Aspirin

thrombocytopenia?

B. von Willebrand's disease

A. Direct thrombin inhibitor

C. Uremia

B. Inherited disease of the connective tissue

D. Factor V deficiency

C. Mechanical obstruction of small vessels

D. All of the above

*7. Hereditary platelet dysfunction can be caused by

A. Aspirin

2. Wiskott-Aldrich syndrome is characterized by

B. von Willebrand's disease

A. Giant platelets

C. Uremia

B. Splenomegaly

D. Factor V deficiency

C. Large ate ets

D. Absence o ate ets

*8. An exa e o an agent that oes not ro uce signi -
cant y ecrease ri ary ate et aggregation in atients

3. May-Hegg in ano a y is characterize by

su ering ro G anz ann's thro basthenia is

A. Giant ate ets

A. Co agen

B. S a est ate ets seen

B. Ristocetin

C. Large ate ets

C. ADP

D. Absence o ate ets

D. T ro bin

4. Bernar -Sou ier syn ro e is characterize by

*9. When co aring von Wi ebran 's isease an

A. Giant ate ets

G anz ann's thro basthenia, G anz ann's thro bas—

B. S a est ate ets seen

thenia wi e onstrate

C. Large platelets

A. Absent ADP

D. Absence of platelets

B. Normal clot retraction

C. Abnormal ristocetin aggregation

*5. Acquired platelet dysfunction can be caused by

D. Abnormal release of ADP

A. Aspirin

B. von Willebrand's disease

C. Uremia

D. Factor V deficiency

Arno DM. Bleeding complications in idiopathic thrombocytopenia,

COMPANION RESOURCES

American Society of Hematology Annual Meeting Educational Book,

<http://theoint.ww.com/surgeon6>

Orlando, FL, December 2015.

Aster RH, Bougie DW. Drug-induced idiopathic thrombocytopenia,

Each student is encouraged to access and use the web—

N Engl J Med, 357(6):580–587, 2007.

base companion resources available for this chapter.

Bates SM, Ginsberg JS. Deep-vein thrombosis, *N Engl*

Here you will find additional information to increase

Med, 351(3):268–277, 2004.

your understanding of the concepts and clinical—

Broberg ME. Deep thrombocytopenic purpura—the changing ther—

tions of the character.

acute and severe, *N Engl Med*, 355(16):1643–1645, 2006.

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venous thrombosis? *Clin Lab Sci*, 20(4):221–223, 2007.

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Agaitov AV, Haynes WG. Role of endothelin in cardiovascular is—

George JN, et al. Chronic idiopathic thrombocytopenic purpura, *N Engl*

ease, *J Renin Angiotensin Aldosterone Syst*, 3(1):1–15, 2002.

J Med, 331(18):1207–1215, 1994.

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27

sis: Blood Coagulation Factors

and Natural Coagulant Systems

KEY TERMS

activated clotting time

brinolysis

procoagulant

activated partial thromboplastin time

brin split products

protein C

antithrombin

natural common pathway

p ro te in S

ce ll-b a s e d co a g u la tio n

in te rn a tio n a l n o rm a lize d ra tio (INR)

p ro th ro m b in g ro u p

ce llu la r p ro te a s e s

in trin s ic p a th w a y

p ro th ro m b in tim e (PT)

co a g u la tio n in h ib ito r

kin in s ys te m

s e ru m

co m p le m e n t s ys te m

lys o s o m e s

th ro m b o m o d u lin

co n ta ct g ro u p

m ixin g s tu d y

tis s u e fa cto r

D-d im e r

n a tu ra l a n tico a g u la n t s ys te m s

w a rfa rin

extrin s ic p a th w a y

new oral anticoagulants

zymogen

factor V (Leiden)

plasminogen

kinase group

plasminogen activators

LEARNING OUTCOMES

Blood coagulation factors

Normal protective mechanisms against

- Explain the procedure for naming the coagulation factors.

thrombosis

- List the principal coagulation factors.
- Explain the effect of normal blood flow and the removal of substances from the circulation on protecting the body from thrombosis.
- Name the three groupings of coagulation factors and describe their similarities.
- Describe the activities of antithrombin as a normal body defense
- Describe the individual functional characteristics of each of the coagulation factors.

- Name the two heparin-dependent thrombin inhibitors and describe
 - Name the four basic phases of blood coagulation.
- their role as part of the natural anticoagulant system.
- Describe the sequence of events in the extrinsic pathway.
 - Describe the functions of protein C and protein S.
 - Describe the sequence of events in the intrinsic pathway.
 - Explain the activities of the cellular proteases and the role of specific
 - Describe the sequence of events in the common coagulation
- body cells in the production of coagulation factors and cofactors.
- pathway.
- Name and describe the assay techniques that can be used for the
- Compare clot-based coagulation and cell-based coagulation
- detection of fibrin split products.
- processes.
- Describe the importance of vitamin K in hemostasis.
 - Define the term fibrinolysis, and describe the major components of
- fibrinolytic system and why fibrinolysis is necessary
- NOTE:
- Name and explain the principles of the basic and global
 - indicates MLT and MLS core content

laboratory tests that are used in assessing blood coagulation indicates MLT (optional) and MLS advanced content factors.

BLOOD COAGULATION FACTORS

The formation of a clot (thrombus) usually occurs as part of the normal process of hemostasis. The soluble blood coagulation factors are critical components in the formation of a vasoconstriction and the formation of a platelet plug, but thrombus.

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Hematocytes are the principal site of the synthesis of

Common Characteristics of Coagulation

coagulation factors. However, other cells, such as endothelial

Factors

cells, also play an important role in the normal process of hemostasis and thrombosis. Classically, the coagulation cascade—Proteins that are clotting factors have four characteristics in factors have been described as reacting in a cascading sequence.

common. These characteristics are as follows:

Modifications of this sequence are now known to occur as

1. A deficiency of the factor generally produces a bleeding tendency in which the blood factors interact to or the natural blood clotting tendency interferes with the action of factor XII, resulting in thrombosis.

Factor (Fibrinolytic factor), an high-molecular weight kininogen (HMWK; Fitzgerald factor).

Basic Concepts of Blood Coagulation

2. The physical and chemical characteristics of the factor are known.

Blood coagulation is a sequential process of chemical reactions—

3. The synthesis of the factor is independent of other reactions involving as a proteins, hormones, and carbohydrates.

circulations. Most of the circulating factors (Table 27.1) that

4. The factor can be assayed in the laboratory.

participate in the coagulation process are designated by

Roman numerals. The activation of an enzymatic cascade of an understanding of the theory of coagulation

factor appears as a Roman numeral followed by the suffix -a,

and the underlying principles of laboratory work—

whereas the inactive enzymatic factors, are indicated by the
ures, it is helpful to compare the characteristics (Table 27.2)

Roman numerals. For example, factor II, prothrombin—

of various coagulation factors. Three groups of factors exist:

bin, is designated as factor II; however, in the active state,

the fibrinogen group, the prothrombin group, and the con—

it is IIa, thrombin. Nonenzymatic factors have no such

factor group.

designations. The Roman numeral designation does not

The fibrinogen group consists of factors I, V, VIII, and XIII.

indicate the sequence of reactions in the clotting process.

These factors are consumed during the process of coagulation—

For example, factor X releases factor II in the coagulation

tion. Factors V and VIII are known to decrease during blood

pathway.

storage in vitro. These factors are known to increase during

TABLE

27.1 Proteins in Blood Coagulation

Factor

Name

Alternate Terms

Coagulation Factors

I

Fibrinogen

II

Prothrombin

V

Proaccelerin

Labile factor, Ac globulin

VII

Proconvertin

Stabile factor, SPCA

VIII

AHF

AHG, antihemophilic factor A

IX

PTC

Christmas factor, antihemophilic factor B

X

Stuart factor

Stuart-Prower factor

XI

Plasma thromboplastin antecedent

PTA, antihemophilic factor C

XII

Hageman factor

Glass or contact factor

XIII

Fibrin-stabilizing factor

FSF

Others

Prekallikrein

Fletcher's factor

HMW kininogen

HMW kininogen, Fitzgerald's factor

vWF

Factor VIII–related antigen

Fibronectin

Antithrombin

Heparin cofactor II

Protein C

Protein S

SPCA, serum prothrombin conversion accelerator; AHF, antihemophilic factor; AHG, antihemophilic globulin; PTC, plasma thromboplastin component; PTA, plasma thromboplastin antecedent, FSF, fibrin-stabilizing factor; HMW, high–molecular weight kininogen; vWF, von Willebrand factor.

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TABLE

27.2 Characteristics of Coagulation Factors

Group

Characteristic

I*

II†

III‡

Molecular weight

High

Low

?

Plasma

Present

Present

Present

Serum

Absent

Present, except II

Present

Absorption (BaSO₄)

No

Yes

None or partial

Destruction

Thrombin, plasmin

Stability

Factors V, VIII unstable

Heat stable

Stable

Increase

In ammation, pregnancy, stress and fear, oral

Pregnancy, oral contraceptives

contraceptives

Decrease

Oral anticoagulants

*Group I: fibrinogen group (factors I, V, VIII, XIII).

†Group II: prothrombin group (factors II, VII, IX, X).

‡Group III: contact group (factor XI, XII, Fletcher factor, Fitzgerald factor).

pregnancy, in the presence of conditions of infection,

Factor II (Prothrombin)

and subsequent to the use of oral contraceptive drugs.

Prothrombin is a stable protein (molecular weight, 63,000).

The prothrombin group consists of factors II, VII, IX, and

In the presence of ionized calcium, prothrombin is converted

X. All these factors are essential in keeping their

to prothrombin by the enzymatic action of thromboplastin

synthesis. Vitamin K is available to the body through dietary

both extrinsic and intrinsic sources. Prothrombin has a half-

life of about 3 days with 70% consumption during clotting.

inhibited by warfarin. This group is considered to be stable

and remains well reserved in storage.

Prothrombin Ca^{2+}

+

+ extrinsic or intrinsic

The contact group consists of factors XI, XII, prekallikrein
thromboplastin → thrombin

(Fletcher factor), an HMWK (Fitzgerald factor). These
factors are involved in the intrinsic coagulation pathway.

Factor IIa (Thrombin)

They are collectively stable and are not consuming
coagulation.

Thrombin (molecular weight, 72,000) is the activated form of
prothrombin, which is normally as an inactive precursor
in the circulation. This proteolytic enzyme, which interacts
with fibrinogen, is also a potent platelet-aggregating sub-

NOTE: This is a good time to review the definitions of the
process. A large quantity of thrombin is consuming the
Key Terms in the Glossary and

. It is also a good

process of converting fibrinogen to fibrin. A unit of thrombin -
time to complete Review Questions related to the preceding
fibrin will coagulate 1 L of a standard fibrinogen solution in
content.

15 seconds at 28°C.

Fibrinogen + Thrombin → Fibrin

no ester + esters

Characteristics of Individual Factors

Each of the individual coagulation factors has some unique

properties. Thromboplastin (Formerly Factor III)

characteristics. These characteristics include the following:

Thromboplastin is the term given to any non-living

substance containing protein co-extracted from tissues.

Factor I (Fibrinogen)

These tissues can be from the brain, lung, vascular endothelium,

liver, placenta, or kidneys; these tissue types are called

Fibrinogen is a large, stable globular protein (molecular

weight, 341,000). It is the precursor of fibrin, which is

the resulting clot. When fibrinogen is exposed to thrombin,

ionized Calcium (Formerly Factor IV)

two esters split from the fibrinogen molecule, leaving a

fibrin monomer. These monomers aggregate together to

form the insoluble fibrin clot product.

The ionized calcium has replaced the factor IV.

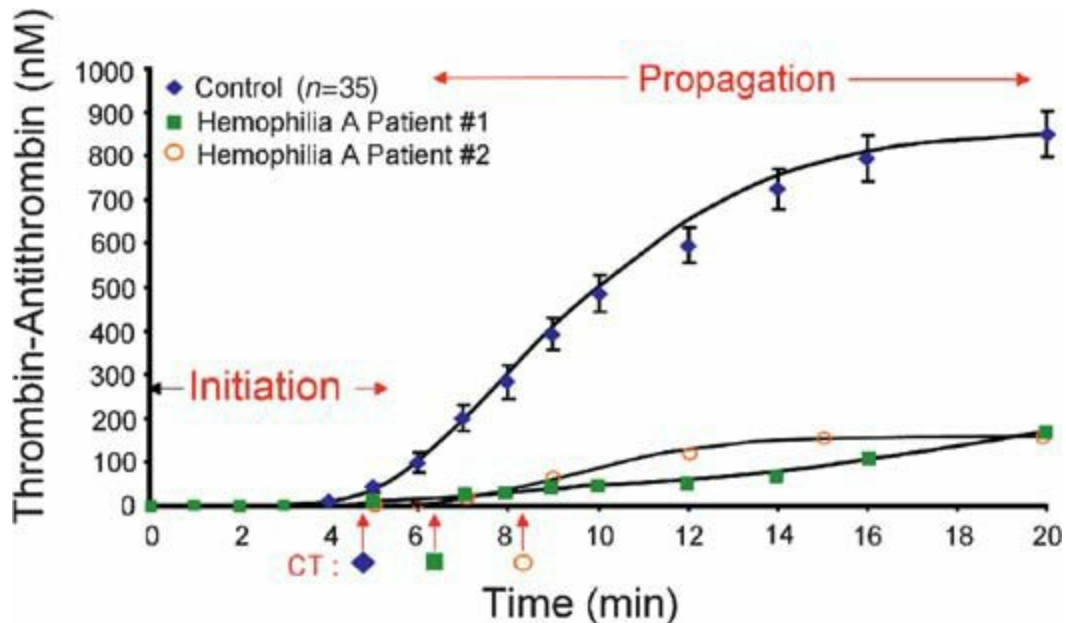
or the sodium-activated fibrin clot product.

Ionize calcium is necessary for the activation of thrombin—

as well as for the conversion of prothrombin to thrombin.

Fibrinogen + thrombin → fibrin monomers → fibrin clot

Ionize calcium is the physiologically active form of calcium



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in the human body, an onyssa amounts are needed

for blood coagulation. A calcium deficiency would not be

expressed as a coagulation dysfunction, except in cases of

passive transfusion.

Factor V (Proaccelerin)

Factor V is an extremely abundant protein. It deteriorates rapidly, having a half-

in 16 hours. Factor V is

consumed in the clotting process and is essential to the later stages of thromboastin formation.

Factor VII (Proconvertin)

Factor VII, a beta-globulin, is not an essential component of the intrinsic thromboastin-generating mechanism. It is

FIGURE 27.1 Prothrombin and factor Xa generation.

not destroyed or consumed in clotting and is found in both

(From Greer JP, et al. eds. Wintrobe's Clinical Hematology, 13th ed.,

as a plasma protein, even in serum at room temperature

Philadelphia, PA: Lippincott Williams & Wilkins, 2014.)

or up to 3 days. The action of factor VII is the activation

of tissue thromboastin and the acceleration of the prothrombinase reaction to activate factor IX in blood coagulation

tion of thrombin to prothrombin. This factor is released

is to form the intrinsic tenase complex, which efficiently activates

by vitamin K antagonists.

activates factor X to activate factor X (factor Xa). The intrinsic

tenase complex generates the second burst of factor Xa that

Factor VIII (Antithrombinophilic Factor)

results in the propagation phase of the activation of prothrombin to thrombin, an acute-phase

reactant, is consuming the
thrombin to thrombin. This is important in the hemorrhagic
clotting process and is not found in serum. Factor VIII is
a disease inherited as an autosomal recessive (Fig. 27.1). In the homozygous
extremely rare, with a 50% loss within 12 hours at 4°C in
hemophilic patients, thrombin generation can describe the bleeding
in vitro and a similar 50% loss in vivo within 8 to 12 hours after
infusion. In addition, factor VIII can be assayed by decrease
tests.

in the presence of lupus anticoagulant (LA).

Factor VIII can be subdivided into various functional

Factor X (Stuart Factor)

components. The total molecule, consisting of both a high-

This is a high-molecular weight protein that is not

of molecular weight reaction and a low-molecular weight reaction—

consuming clotting. Together with factor V, factor

tion, is described by the nomenclature VIII/vWF. Factor

X in the presence of calcium ions or the natural common

VIII/vWF consists of two major subunits. The high-molecular-

pathway through which the products of both the extrinsic and the intrinsic pathways converge to activate Factor VIII:Ag components. The overall coagulation pathway consists of the VIII:C and VIII:Ag components.

Factor VIII:C has procoagulant activity as measured by its ability to activate Factor X. The activity of Factor X is measured by its ability to activate Factor VIII.

Factor VIII/vWF interacts with Factor VIII:C and transports VIII:C in the plasma (Plasma Thromboplastin Antecedent) circulation.

Factor XI, a beta-globulin, can be found in serum because it

Factor VIII:Ag is a procoagulant antigen as measured

is on a laboratory using the clotting process. This

by immunological techniques using antibodies for Factor

Factor is essential to the intrinsic thrombin-generating

VIII:C. Factor VIII:Ag is a relative Factor VIII antigen that

mechanism.

has been identified using immunological techniques employing heterologous antibodies to VIII/vWF.

Factor XII (Hageman Factor)

Factor VIII:RCo demonstrates ristocetin cofactor activity—

Factor XII is a stable factor that is not consuming activity, which is required for the aggregation of human platelets in the coagulation process. Activation of factor XII is induced by the antibiotic ristocetin.

kininogen (with bound prekallein and factor XI) to

Factor VIII/vWF is factor VIII-vWF. Endothelial cells are negatively charged surfaces such as glass or subendothelium—known to synthesize and secrete VIII/vWF units.

in (coagulation) exposed by blood vessel injury initiates the intrinsic coagulation pathway. Surface activation of

Factor IX (Plasma Thromboplastin Component)

an artery activates factor XII to factor XIIa by exposure—

Factor IX is a stable protein factor that is neither consuming an active enzyme (protease) site. Because of its lack of activity nor destroyed by aging at 4°C for 2 weeks. Its mechanism, prekallein (activated Fletcher factor) cleaves is an essential component of the intrinsic thrombotic—

artia y activate actor XIIa o ecu es a sorbe onto the
generating syste , where it inf uences the a ount rather
suben othe iu to ro uce a ore kinetica y e ective
than the rate o thro bo astin or ation.

or o XIIa.

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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

Fa ctor XIII (Fib rin -Sta bilizing Fa ctor)

VIIa

VII

Fibrin-stabi izing actor in the resence o ionize ca ciu

Complex

T. Thrombopla stin

Ca ²⁺

ro uces a stabi ize brin c ot.

Fine ibrin c ots actor XIII +

ca ciu ions → stab e ibrin c ot

Pre ka llikre in (a ls o Know n as **Fle tcher Factor**)

X

Xa

■ Prekallikrein (PK) is a serine protease coagulation factor

Va

V

Complex

that coexists with high molecular weight kininogen. PK

Ca^{2+}

releases kallikrein which activates kinins. PK is cleaved

by F3

to release kallikrein by activated Factor XII (Hageman

Factor).

■ Patients with prekallikrein (PK) deficiency exhibit severe

prolonged activation of the intrinsic pathway (AP)

results but demonstrate no bleeding symptoms. Because

II

IIa (Thrombin)

this is an inherited disorder, any members of a family

can have a PK deficiency as was the case in the original

discovery by Kentucky family member.

Fibrinogen

Fibrin

FIGURE 27.2 Extrinsic pathway of coagulation. (PF3, platelet factor 3.)

■ High molecular weight kininogen (HMWK) is also known as Fitzgerald factor. It is a protein produced by the liver (Factor 3.)

It is involved in the early steps of the intrinsic coagulation pathway. HMWK functions as a cofactor and binds

Coagulation Pathways

with prekallikrein and Factor XI to help initiate their activation by Factor XIIa.

Initiation of clotting begins with either the extrinsic or the

■ The kininogens (low molecular weight) are also

intrinsic pathway. Factor X activation is the point of conver—

gence. Factor X can be activated by either of the two pathways

inhibit thrombin activation of platelets and stimulate ib—

an subsequent catalyzes the conversion of prothrombin to

thrombin. Thrombin activates the conversion of fibrinogen to

thrombin. Thrombin also activates the conversion of plasminogen to

thrombin.

gen activator

The Extrinsic Coagulation Pathway

The extrinsic pathway (Fig. 27.2) is initiated by the entry

of tissue thromboplastin into the circulating blood.

NOTE: This is a good time to review the definitions of the Key

throughout the course of the course

Terms in the Glossary and

. It is also a good time to

organize the course of the course. These

complete Review Questions related to preceding content.

the course of the course, the course of the course, are not a

extrinsic to the course of the course. The course of the course is not

The Plasma Clot-Based Mechanism of

necessary or activation of the extrinsic pathway because tis-

Coagulation

sue factor supplies its own cofactors.

Factor VII binds to these cofactors in the tissue

Many chemical reactions occur in the tissue, for the ini-

the course of the course is activated to factor VIIa, a potent enzyme

that stimulates the course of the course to the course of the course

the course of the course of the course of the course of the course

a stable complex of the course of the course of the course

the course of the course of the course of the course of the course

the course of the course of the course of the course of the course

seen to be largely dependent on the concentration of this—
into several sections such as the extrinsic and intrinsic pathways—
subsequently. The proteolytic cleavage of factor VIIa
ways. These pathways are not physiological pathways
by factor Xa results in inactivation of factor VIIa. Factor VII
of hemostasis but a component of the growing of factor effects an
artificially only in the extrinsic pathway. Mentions that
the focusing of laboratory assays.

enter the circulation as so-called surface or the attachment—
The initiation of the coagulation process may occur via
either an activation of factors II and V. The nature is the
one of two pathways: the extrinsic pathway and the intrinsic
conversion of fibrinogen to fibrin by thrombin.

pathway. Regardless of the initiating pathway, the two pathways converge into a
common pathway. The outcome of

The Intrinsic Coagulation Pathway

this process is the conversion of circulating into soluble coag—

The intrinsic pathway (Fig. 27.3) involves the contact activa—
tion of factors into a gelatinous fibrin clot with entrance
tion factors prekallikrein, HMWK, factor XII, and factor XI.

blood cells, a blood clot. As repair of a damaged tissue takes

These factors interact on a surface to activate factor IX to IXa. Factor IXa reacts with factor VIII, PF3, and calcium to activate factor X to Xa. In the presence of factor V, factor Xa

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proceeds in converting factor XIII to XIIIa and in converting prothrombin to thrombin. Fibrin formation occurs in three phases: initiation, propagation, and stabilization.

Initiation, thrombin, a protease enzyme, cleaves fibrinogen, releasing fibrin monomers, fibrinopeptide A, and fibrinopeptide B fragments. In the second step, the fibrin monomers

polymerize, cross-linking, and stabilization.

Initially, thrombin, a protease enzyme, cleaves fibrinogen,

XII

XIIa

Surface

which results in a fibrin monomer, fibrinopeptide A, and

HMWK

fibrinopeptide B fragments. In the second step, the fibrin

VIIa

VII

monomers spontaneously polymerize into fibrin because of

XI

XIa

Complex

T. Thromboplastin

hydrogen bonding. Finally, the fibrin monomers are linked

HMWK surface

Ca^{2+}

covalently by factor XIIIa into fibrin polymers. These poly-

IX

Ca^{2+}

form a meshy network, and the final solution is

IXa

PF3

converts to a gel when more than 25% of the fibrinogen is

converted to fibrin.

VIII

VIIIa

Factor XIII is converted to the active form, factor XIIIa, in

X

Xa

two steps. In the first step, thrombin cleaves the two

Ca^{2+}

PF3

each of the two alpha chains of factor XIII with formation of

V

VIIIa

an inactive intermediate of factor XIII. In the second

X

IIa (Thrombin)

step, calcium ions cause factor XIII to dissociate, forming

Ca^{2+}

factor XIIIa.

PF3

Fibrinogen is normally present in the plasma as a soluble

protein. Subsequent to the action of thrombin, fibrinogen

Fibrinogen

Fibrin

is transformed into fibrin, an insoluble gel. This conversion

FIGURE 27.3 Intrinsic pathway of coagulation. (PK HMWK,

of brinogen to a cross-linked polymer occurs in several stages.

reka ikrein, high-molecular weight kininogen; K₂, ka ikrein;

Factor XIIIa introduces the bonds within the polymer-

PF₃, platelet factor 3.)

erize brin network. This cross-linking makes the brin

more elastic and less susceptible to lysis by fibrinolytic agents.

activates prothrombin (factor II) to thrombin, which in turn

Fibrin or a mesh covering over the injured area, reinforcing—

converts brinogen to brin.

forces the platelet plug, and closes off the wound. After a

Strong negative charge so it is that can participate in the

short period, the clot begins to retract and becomes a firm

activation of factor XII include glass and kaolin in vitro as well

an enzyme. This retraction process is thought to be

as elastin, collagen, platelet surfaces, ka ikrein, as in, and

caused by the action of platelets trapped along with erythrocytes

high-molecular weight kininogen in vivo. Collagen exposed

erythrocytes and leukocytes in the clot. As the brin polymerizes

by blood vessel injury greatly influences the rate of reaction.

gather around the aggregate and then, the aggregates send out Factor XIIa interacts in a feedback loop to convert prekallikrein to active kallikrein. This reaction is accelerated by bradykinin. When a clot forms in a test tube, contraction of HMWK. In the absence of prekallikrein, factor retraction can be observed. The fibrin squeeze from this clot XIIa is generated slowly.

is serum.

Ionized calcium is an important role in the activation of certain coagulation factors in the intrinsic pathway.

Cell-Based (Physiologic, In Vivo)

Calcium is not required for the activation of factor XII,

Coagulation

prekallikrein, or factor XI but is necessary for the activation of factor IX by factor XIa.

Cell-based coagulation is regulated by properties of the surface. This emphasizes the importance of specific

Final Common Pathway

cellular receptors for the coagulation proteins. A cell-based

Once factor X is activated to Xa, the extrinsic and intrinsic

o e explains so e as ects o he ostasis that the c assic
athways enter a co on athway. Factor II, rothro bin,
casca e, rotein-centric o e oes not.

is activate to thro bin (actor IIa), which nor a y circu—

In contrast to the c assic theory o in vitro c ot-base
ates in the b oo as an inactive actor.

coagu ation, a ce -base in vivo o e recognizes that ce s

Fo owing the activation o actor Xa, it re ains ate et

with si i ar hos hati y serine content can ay very i -

boun an activates actor V. T e co ex o actors Xa an

erent ro es in he ostasis e en ing on their co ection o

Va on the ate et sur ace is or e near ate et-boun ac—

sur ace rece tors.

tor II o ecu es. In turn, the ate et-boun Xa/Va co ex

In a ition to rocoagu ant an anticoagu ant roteins,

c eaves actor II into thro bin, actor IIa. T e stage is acce -

nor a hysio ogic coagu ation requires two ty es o ce s

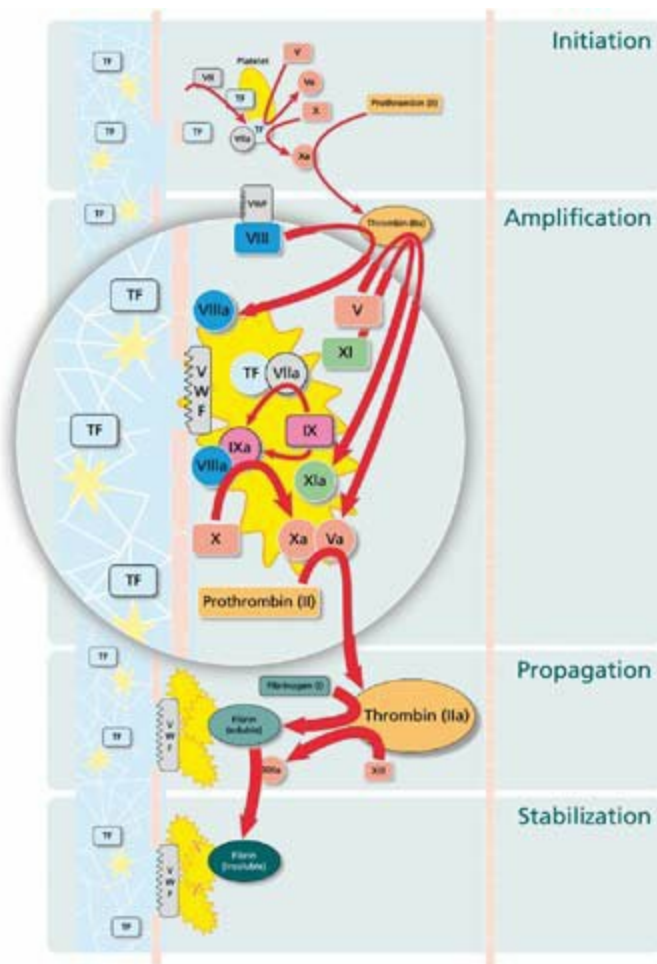
erate by actor V an ionize ca ciu .

or or ation o coagu ation co exes. issue actor (F)

co es ro a age b oo vesse ce s, an ate et actor

Fibrin Formation

is expressed by platelets. Because F-expr...
 Clotting is the visible result of the conversion of
 an activated platelet is essential for physiologic coagulation—
 fibrinogen into a stable fibrin clot. Thrombin plays a major
 role, but clotting is localized to the site of injury. Intact



TF-bearing cell

IXa

1

II

Initiation

VIIa

Xa

IIa

V, VIII plts

Plt

VIIIa

3

Propagation

2

IXa

Amplification

FIGURE 27.5 Coagulation cascade. Coagulation is thought to occur in three stages: initiation, amplification,

and propagation. The initiation stage (1) takes place on F-bearing

cells (cells such as monocytes that can bind F and present it to a

ligand), which coagulate when endothelial injury occurs and

F is exposed. The initiation stage is characterized by presentation

of F to its ligand, factor VII, and the subsequent activation of factors IX and X on the F-bearing cell. The activation of factor X

to Xa causes thrombin production and activation. Once generated,

thrombin feeds back to activate factors VIII, V, and others. The

FIGURE 27.4 Initiation, amplification, propagation, and stabilization—

the amplification stage (2) then occurs on the surface of the activated

cell of the coagulation cascade. This describes the coagulation cascade, which exposes surface molecules that act as receptors.

ity of the clotting process and illustrates the interaction between

factors and the activated factors VIIIa and IXa. The activated surface

coagulation factors and the cell surfaces of platelets in what has

allows for further thrombin production and hence the amplification

can be described as the cellular phase of the coagulation. Four sequential

of coagulation. Continued generation and activation of thrombin

and intermediate stages include initiation, amplification, propagation—

causes further positive feedback mechanisms (3) to occur that maintain, and stabilize as shown. This is the so-called coagulation cascade

and ensure the production of a stable clot, including cleavage of

products of the classic water-soluble cascade and further explains

fibrinogen to fibrin, release and activation of factor XIII or fibrin

additional aspects of the coagulation cascade are as follows:

cross-linkage, and the release of a thrombin-activatable fibrinolysis inhibitor (TAFI). (From Stoelting R, Fink P, Rath J. *Textbook of Anesthesiology*. Philadelphia: Lippincott Williams & Wilkins, 2012.)

sis inhibitor. (From Hensley FA, Gravlee GP, Martin DE. *Practical*

Stoelting's Handbook of Pharmacology and Physiology in Anesthetic

Approach to Cardiac Anesthesia, 5th ed., Philadelphia, PA: Lippincott

Practice, 3rd ed., Philadelphia, PA: Lippincott Williams & Wilkins,

Williams & Wilkins, 2012.)

2014.)

thrombin generation is not sufficient, but numerous positive

endothermic and protease inhibitors prevent clotting from

occurring. It is important to note that binthrombin with active sites.

is reacting to other parts of the body.

Thrombin that is generated in the initiation phase further

Accelerates coagulation process has the following phases

activates factor V and factor VIII, which serves as a cofactor

(Figs. 27.4 and 27.5):

thrombinase co-existence accelerates the activation of factor II by factor Xa and of factor Xa by factor IXa,

1. Initiation

respective to

2. Activation

Cleavage of fibrinogen occurs at the end of the ini—

3. Propagation

Initiation has an beginning of the next propagation phase.

4. Stabilization (Fibrinolysis)

In most clot-based coagulation assays, this is the visual endpoint of the assay which occurs with a sensitivity of 3% of the

Initiation Phase

thrombin generate .

Initiation occurs on a tissue factor (F)-bearing cell . It

begins when F factor binds to

Propagation

VIIa to activate factor IX and factor X. This activation occurs in the propagation phase of in vivo coagulation, where

factor IX by F-VIIa complex serves as the bridge between components of thrombin are generate on the platelet surface.

the extrinsic and intrinsic pathways. Factor Xa then binds

The activated enzyme complexes (tenase complex and

to factor II to form thrombin (factor IIa). Thrombin genera—

thrombinase complex on the platelet surface releases

tion through this reaction releases 3% to 5% of the total

95% or more of the total amounts of thrombin generation
thrombin generate and can be terminated by F₂ pathway
and autolysis. This ensures continuous generation
inhibitor.

of thrombin and subsequent fibrinolysis to or a large clot.

Amplification

Stabilization

In this phase, autolysis and coactors are activated to set the
thrombin generation leads to activation of factor XIII (fibrin
stage or large-scale thrombin generation. The amount of
stabilizing factor), which covalently links fibrin polymers

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and provides strength and stability to fibrin incorporate
in autolysis. In addition, thrombin activates thrombin

BOX 27.1

activatable plasminogen inhibitor (AFI) that protects the clot
from fibrinolysis.

Components of the Plasma Fibrinolytic

Implications of Cell-Based Coagulation

System

A cell-based model of coagulation allows for a more thorough understanding of how hemostasis works in vivo and

PLASMINOGEN ACTIVATORS

Endogenous

Explains the pathophysiological mechanisms behind certain

thrombotic diseases as inogen activator (t-PA)

coagulation disorders. Patients lacking factor XII, prekallikrein,

Urokinase

kininogen, or high-molecular weight kininogen do not bleed

Exogenous

abnormally. Second, patients with only trace quantities of

Streptokinase

factor XI can withstand major trauma without unusual

Acute-onset inogen streptokinase activator complex

bleeding. Patients who completely lack factor XI exhibit

(APSAC)

hemorrhagic disorders.

PLASMINOGEN INHIBITORS

A ha-2 as an inhibitor

NOTE: This is a good time to complete Review Questions

issue as inogen activator inhibitor

related to the preceding content.

Thrombin-Mediated Reactions

Plasminogen is converted to plasmin by hydrolysis to release

Numerous pathways exist that ultimately lead to the generation

of plasminogen activators. These are acting to

regulate thrombin and subsequent fibrinolysis

process gradually involves away the clot as tissue repair is taking

place following vascular injury. The categories of essential thrombin

activation, with the activated factor being regulated by the

bio-regulation of hemostasis are

mononuclear phagocytic system.

Inactive plasminogen circulates in the plasma until an

1. Procoagulant

injury occurs. The activators of plasminogen consist of

2. Coagulation inhibitor

endogenous and exogenous groups (Box 27.1). Plasminogen

3. tissue repair

activation to plasmin is the result of the activity of a number

of proteolytic enzymes. These enzymes, the kinases, are

Procoagulant

referred to as the plasminogen activators. Plasminogen activa—

Acting as a coagulant, thrombin induces platelet activators are found in various sites, such as the vascular endothelium and aggregation. In addition, thrombin activates coag—

the interstitial granules, an biological fluids. At least

from VIII to VIIIa, converts fibrinogen to fibrin, and activates

two or so tissue activators have been described: those

factor XIII to XIIIa. It converts prothrombin to thrombin via

that sequence to urokinase, a urinary activator of as—

autocatalysis.

inogen, and those sequence to urokinase. These activators

sequence to urokinase include thrombin, bacterial products

Coagulation Inhibitor

such as streptokinase or beta-hemolytic streptococci, and

These

streptokinase. Plasmin activators of plasminogen include

coagulation inhibitor activity is achieved by thrombin is the

binding of antithrombin (A₂) to inhibit serine proteases and

as a kallikrein, activate as a thrombostatin antecede -

binding to thrombolytic to activate protein C. In addition (factor XI), and activate

Hage an actor (actor XIIa).

tion, the other activity in this category is the ro otion o

It is esti ate that 1.5 i ion A ericans have a heart

en othe ia ce re ease o t-PA.

attack each year. Most are cause by c ots that cut o b oo

f ow to the heart usc e. issue-ty e as inogen activator

Tis s ue Re pa ir

(t-PA) is resent in inute quantities in the vascu ar en oT ro bin e iates tissue re
air by in ucing ce u ar che othe iu . When t-PA encounters a b oo c ot, t-PA
trans—

taxis an sti u ation o ro i eration o s ooth usc e an

or s as inogen to as in, an as in then egra es

en othe ia ce s.

the c otte brin network. As a resu t o biotechno ogy

(reco binant DNA), a synthetic tissue-ty e as inogen

Fibrino lysis

has been eve o e an is use c inica y to treat ost– yocar ia in arction an u onary
e bo i. t-PA is consi ere

Fibrin c ots are te orary structures that sea o a a age

by any to be ore s eci c an twice as e ective as stre -

area unti hea ing can take ace. Fibrino ysis is the hysi—

kinase in its own right and has several effects.

biological process that involves inhibition by

Through its synthesis of fibrin or fibrinogen, as in its enzymatic digestion of the stabilizing fibrinolytic enzymes. As

subsequent to the degradation of fibrin split products consisting

healing occurs, the cells the vessels are involved by as in.

intermediate agents X and Y, and agents D and E.

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These agents exert an antifibrinolytic (A) effect, inhibit the

4. antifibrinolytic

hemostasis system through interference with fibrinolysis

5. C1 esterase inhibitor

polymerization, and interfere with platelet aggregation.

6. Protein C inhibitor

Salicylates also act as anticoagulants in the clot.

7. Protein S inhibitor

The specificity of salicylates ensures that clot dissolution occurs

without widespread proteolysis of other proteins. Plasmin

also activates the complement system, liberates kinins and

NOTE: This is a good time to complete Review Questions

kininogen, and can hydrolyze coagulation factors V, VIII, and related to content in the preceding sections.

XII. Further clot formation is inhibited by antiplasmins and naturally occurring inhibitors, so those which prevent the activation of plasminogen. The naturally occurring inhibitors

Laboratory Assessment of Blood

include antithrombin (A₂), a heparin-2 acroglobulin inhibitor-

Coagulation Factors

Factor I, an alpha-1 antitrypsin. Plasmin is not normally found in plasma because it is neutralized by an excess of inhibitors.

Clot-based assays are often used for evaluation of patients with suspected bleeding abnormalities and to monitor anti-

Other Systems and Inhibitors

coagulant therapy. Most of these tests use citrate as an anticoagulant and the end point or a color change is the basis for clot formation.

Two other systems and protease inhibitors have an effect on

Clot-based assays based on clot formation as the end point

hemostasis and coagulation. The two adjunct systems are

are widely used in the clinical laboratory to determine the

1. Kinin system

integrity of the intrinsic or extrinsic pathways of the coagulation—

2. Coagulation system

ation system. Color-based and chromogenic assays are used

most often. Clotting assays provide an assessment of coagulation—

Kinin System

ation function, and chromogenic tests are used to measure

the level or function of specific factors.

The kinin system is activated by both the coagulation and

A wide variety of blood coagulation factors such as

brinolytic systems. Fibrinogen factor (fibrinogen) and

thrombin time and a quantitative fibrinogen concentration

Fibrinogen factor (HMWK) are also needed to enhance or

assay, can be assessed by various laboratory procedures (see

also the contact factors involved in the intrinsic system.

Chapter 32, Laboratory Manual). The two most commonly

Factor XIIa in the presence of HMWK converts fibrinogen

into fibrin. Fibrinogen is the

thrombin. Fibrinogen feeds back to accelerate the conversion—

prothrombin time (PT) and the

activated partial thromboplastin time (APTT).

sion of factor XII to XIIa, which accelerates the intrinsic system processes.
Activation of factor XII acts as the common

Prothrombin Time

path between any components of the hemostatic mechanism—

The PT is the basis for the

test, including the fibrinolytic system, the kinin system, and

international normalized ratio (INR)

used to monitor warfarin anticoagulant therapy and coagulation system.

Factor deficiencies can be a diagnostic tool for disorders

Complement System

such as liver disease.

The PT procedure evaluates the generation of thrombin

Coagulation activates cell membrane lysis of antibody and the formation of fibrin via the
extrinsic pathway

coagulate target cells. within the extrinsic pathway of coagulation—

pathway. Thromboplastin reagent is used for this assay.

Factor activation, the classic alternative pathway, can

Thromboplastin can be released by various methods: tissue

occur along with a common cytochemical pathway.

extraction of rabbit brain or lung, tissue culture, and endotoxin activates coagulation
factor by cleaving C3 into C3a

aretholysin. Tissue plasminogen activator (tPA) is a mixture of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). C3b, C1 esterase inhibitor inactivates complement and factor (F), has histamine, and calcium ions and is used to assess the role in hemostasis.

initiate clotting cascade as the P. Tissue plasminogen

Protease Inhibitors

coexists with and activates factor VII. Tissue plasminogen activator (tPA) is responsible for the attachment and activation of factors X, V, and II.

Because the fibrinolytic system is activated when the coagulation

Tissue plasminogen activator (tPA) is responsible for the attachment and activation of factors X, V, and II.

activation cascade is activated, extra fibrin is degraded and fibrinogen, and calcium are added to the blood as a result.

It is associated with the coagulation factors. Enzymes such as tissue plasminogen activator (tPA) require the fibrin clot to be dissolved.

as in an kallikrein system until they are eliminated

Reference ranges are from 10 to 13 seconds. Prolonged

by various mechanisms: liver hepatocytes, mononuclear

results can indicate a deficiency of one or more factors in the

hemostatic cells, or serine protease inhibitors present in the

extrinsic pathway: factors VII, X, V, and II or I. Prolonged

as a result. Serine protease inhibitors attach to various enzymes

values will be seen in an oral anticoagulant such as coua—
an inactivate the . Serine protease inhibitors include
rivaroxan or a coua—containing substance (e.g., rivaroxan) is

1. α -Antithrombin

ingested .

2. α -Antitrypsin

Carriers of a mutation in the α_1 -antitrypsin gene,

3. α -Macroglobulin

Prothrombin 20210, were discovered in 1996 because these

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patients demonstrate increased α_1 -antitrypsin activity. There

is no screening test for this mutation, but it can be investi—

gated using molecular techniques in clinical signs and sy —

ptoms. For example, in patients with deep vein thrombosis (DVT) or

pulmonary embolism (PE), the α_1 -antitrypsin activity is

usually elevated. In patients with liver disease, the α_1 -antitrypsin

activity is usually elevated.

Patients with liver disease are treated with warfarin. Anticoagulation is

usually initiated, but it really constitutes secondary prevention of recur—

rence. In the international normalized ratio (INR) test, the

rent PE.

Because thromboplastins are produced using different methods—

Three reagents are currently used for oral anticoagulant

tests and different sources, the sensitivity of an individual

therapy: low-intensity, therapeutic therapy (usually 1.0 to 2.0

units of thromboplastin to another can vary greatly between an

individual); moderate-intensity therapy (P ratio, approximately

within tests. Variance can even occur within a single batch

1.3 to 1.5; INR, 2.0 to 3.0); an high-intensity therapy (P

depending on the type of the reagent. The more sensitive

ratio, approximately 1.5 to 1.8; INR, 2.5 to 3.5). INR is used

the thromboplastin reagent, the longer the resulting room for patients receiving
stable, oral administration antithrombin (P); the less sensitive the reagent, the
shorter the

coagulant therapy. It does not substantially contribute to the

resulting P.

Diagnosis or the treatment of patients whose P is prolonged

to help standardize the difference in sensitivity in individual other reasons.

Various thromboplastin reagents and the effect on P assays,

Some patients do not respond to warfarin. As a result,

two approaches have been developed to standardize results.

their INR does not change as the dosage is increased. A

The first was the International Sensitivity Index (ISI) and the

hepatic cytochrome P450 is central to metabolism of drugs

second was the International Normalized Ratio INR. The

obscures resulting in clinical indications.

INR was eventually incorporated the ISI values and attempt

to make thrombin results uniformly useable.

The Activated Partial Thromboplastin Time (APTT)

The ISI is a calibration parameter that defines the

The activated partial thromboplastin time (APTT) is one of the

responsiveness of the reagent relative to a World Health

Organization (WHO) International Reference Preparation,

patients, monitor anticoagulant therapy, and as a reference—

which by definition has an ISI of 1.0. A manufacturer assigns

relative screening. A prolonged AP can be caused by a coagulation

an ISI to each commercial batch of reagent after comparing

activation factor efficiency or the presence of an inhibitor.

each batch to a “working reference” reagent preparation. This

The AP measures the integrity of the intrinsic and co-

working reference has been calibrated against international
on pathways of the coagulation cascade. The AP score—
acceptance standard reference reactions that have an ISI
measures the time require to generate thrombin an
value of 1.0. Theoretically, the more sensitive thromboplastin
brinogeners via the intrinsic and common pathways. In
has ISI less than 1.0, less sensitive reagents have an index
the AP assay, calculations and those in which that substi—
that is greater than 1.0. The ISI value is critical for calculation
tute or at least those in which are acceptable. In
of the INR, because the ISI value is the exponent in the or—
vitro, the activation of factor XII to XIIa, prekallikrein to ka-
lulin. Small errors in the ISI value affect the calculated
prekallikrein, an factor XI to XIa occurs on the negative charge
INR substantially.

guarantee. The generation of brin is the endpoint.

INR use has been recommended for monitoring oral anti-T the AP assay reflects the
activity of prekallikrein,

coagulant therapy. It is important to emphasize that the INR

HMWK, and factors XII, XI, IX, VIII, X, V, II, and I. AP

is not a new laboratory test. It is simply a mathematical ca -

may be prolonged because of a factor decrease, such as fibrin—
clotting that corrects or the variability in PT results caused
by factor I, or the presence of circulating anticoagulants.
by variable sensitivities (ISI) of the thromboplastin agents
The reference range for APTT is less than 35 seconds
used by laboratories.

(depending on the activator used). If an APTT is prolonged,
ISI

a mixing study is used to investigate the cause of the PTT patient
INR

prolonged APTT. The mixing study should include an
equal normal PT

APTT mixture Mix an APTT incubate Mix. In addition, a thrombin time and heparin anti-
Xa assay should be

PTT (thrombin time ratio) is the patient's observed PTT
considered in follow-up.

(in seconds) given by each laboratory's calculated mean

Other special or classic procedures include activate

normal PT (in seconds). A target INR range of 2.0 to 3.0 is

clotting time, Escarin clotting time, clot wave or analysis—

recommended for indications (e.g., treatment of rhabdomyolysis, fibrinolytic drugs test, mixing

study, specific factor

hyperoxia of the venous thrombosis [DV], or prevention

assays, and the various tests or inhibitors are circulating

to further clotting in patients who have had a myocardial

anticoagulants.

in action). An INR of 2.5 to 3.5 is recommended for patients

with prosthetic heart valves. When the INR is used to guide

Mixing Study

anticoagulant therapy, there are fewer bleeding events. There

A mixing study can be used in the case of a prolonged AP.

is also a trend toward fewer thrombotic complications.

A mixing study is a coagulation test used to distinguish—

The target INR for primary prevention (PE) treatment is

guish between a coagulation factor deficiency, such as factor

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VIII deficiency, an a factor inhibitor, such as a specific acquired inhibitor. The graph

is characterized, and the first an

factor VIII inhibitor or unusual anticoagulant.

second derivatives are added to the normal. The tracing

The principle of the mixing study relies on a 1:1 mixing of

against the time should reflect the whole process of clot formation as a patient's test as a. This is—

ation and clot lysis.

ing is one as an immediate and an incubate. The

In each of three graphs, three phases are distinguished: a

basic principle is that the normal plasma contributes a su-

recoagulation phase, a coagulation phase, and a postcoagulation—

cient concentration of clotting factors to “correct” or a factor

ation phase with a set of parameters.

iciency.

Possible clinical applications include

Aixing study that corrects the AP is characteristic

monitoring the course of disseminated intravascular coagulation—

of factor deficiency, an aixing study that does not correct



ulation (DIC)

the AP indicates a factor inhibitor. There are two types of

sensitivity to factor deficiencies (Factors XII, X, IX,

ixing studies: the immediate and the incubate.



VII, V, and II)

In the immediate, the AP is either immediately
potentially reflecting the severity and the prognosis of
a treating the patient as a patient or as a, with-



seems because

out further incubation. Specimens with fast-reacting factor
inhibitors will not correct the immediate.

CWA results are more accurate than standard infestation assays such as C-
reactive protein and creatinin.

Specialized Coagulation Testing

Although the CWA is inexpensive and easy to perform,
there are some disadvantages in using it. Currently

Activated Clotting Time

there are only two systems, which are able to assess the right

The activated clotting time (ACT) was developed in 1966 as

transmittance or absorbance tracings. Disadvantages include

a limitation of the classic Lee-White whole blood clot—

the possibility of a false in cases of coagulopathy as such

testing time to monitor coagulation status and heparinization

as in hyperbilirubinemia, hyperkalemia, or hemolysis. In

in immediate situations. The ACT is a point-of-care

addition, data on clinical evaluation are scarce.

where blood clotting test used to monitor high-dose heparin

therapy or treatment with bivalirudin. The dose of heparin

Factor Deficiency

or bivalirudin required in these settings is beyond the range

of a prolonged AP screen is due to a factor deficiency,

that can be measured with the AP.

in evaluating with an equivalent of normal as a

typical, where blood is collected into a tube or cartridge

with a reagent of 100% of coagulation factors present with

containing a negative charge activated coagulation activator—

replace the patient's deficient factor. The result in the AP

value (e.g., cephalin, kaolin, or glass articles) and a magnetic stir

in evaluating is shortened or corrected into the reference

bar, and the time taken for the blood to clot is then measured.

range.

The reference value for the AC ranges between 70 and 180

Correction of the AP in the mixing study suggests a

seconds. During cardiovascular bypass surgery, the desired

coagulation factor deficiency either in the intrinsic pathway

APTT range with heparin may exceed 400 to 500 seconds. The
 (factors VIII, IX, XI, and XII; high-molecular weight kininogen [HMWK]; or prekallikrein [PK]), or in the common
 APTT does not correlate well with other coagulation tests.
 ogen [HMWK]; or prekallikrein [PK]), or in the common
 pathway (also with a prolonged prothrombin time [PT])
 Ecarin Clotting Time
 such as factor II, V, and X.
 For the ecarin clotting time (ECT), venous blood
 Deficiency of factors VIII, IX, and XI will present with
 Echis carinatus snake is used to convert prothrombin to
 fibrin. A deficiency of factor XII, or prekallikrein, will
 eizothrombin, a prothrombin intermediate that is sensi—
 not increase fibrin risk but it might increase the patient's
 tive to inhibition by direct thrombin inhibitors. Ecarin also
 thrombotic risk. Additional testing, such as clotting factor
 activates the noncarboxylate prothrombin found in as a
 assays, is necessary to diagnose a specific factor deficiency.
 Heparin-treated patients. This assay is insensitive to he -
 If the prothrombin time PT is normal, this suggests an
 arin. Therefore, even so direct thrombin inhibitors can be

intrinsic pathway efficiency (VIII, IX, XI, XII, PK, HMWK).

assay even when a patient is receiving concomitant war—

If the PT is prolonged, this suggests a common pathway effi-

ciency.

ciency (fibrinogen, II, V, X).

A chromogenic variant of this assay has also been deve-

loped in which aprotinin is used as a sensitive an-

Inhibitors

thrombin generation is measured with a chromogenic

substrate. Although the EC has been used in research, the

There are three types of inhibitors:

assay has yet to be standardized and is not widely available.

1. Specific inhibitors directly against specific factors

such as factor VIII or factor V inhibitors

Clot Waveform Analysis

2. Nonspecific inhibitors such as usual anticoagulant (LA).

Principle of clot waveform analysis (CWA) is based on the tra—

3. Anticoagulants such as heparins, on a routine, abiga—

ditional AP assay. This graph illustrates the processes

trans, another direct thrombin inhibitors

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1. Specific Factor Inhibitors

Medical history needs to be reviewed. Additional

If the AP screening test is prolonged with a normal APTT,

the heparin assay (anti-Xa inhibition assay) can be used to

identify an APTT incubated, this indicates a

exclude the presence of anticoagulants.

Factor deficiency in the intrinsic or common pathway.

If the prolonged APTT screen is due to the presence of an

Chromogenic Anti-Xa

inhibitor, mixing with an equal volume of normal patient

The chromogenic anti-Xa method monitoring low—

as it will not shorten or correct the prolongation of APTT

of low molecular weight heparin (LMWH) and unfractionated

results. There is no shortening or correction in the APTT

heparin is another assay. This automated assay can replace

results. Inhibitor in the patient's plasma is present in excess

the APTT monitoring unfractionated heparin because it

antibinds to coagulation factors or protein/hydrolysis

eliminates the variability seen with APTT results.

co-exists in both the patient's as a and nor a patient

Chromogenic assays use a factor Xa substrate onto which
as a.

a chromophore has been linked. Factor Xa cleaves the chromo—

to distinguish the location of a specific factor efficiency,

chromogenic substrate, releasing a color compound that can

the AP results can be analyzed in conjunction with P

be detected with a spectrophotometer and is directly pro—

results. If the P is normal, this suggests an intrinsic pathway

proportionate to the amount of activated factor X (FXa) present.

efficiency (VIII, IX, XI, XII, PK, HMWK). If the P is pro—

When a known amount of factor Xa is added as a con—

tinge, this suggests a common pathway efficiency (brino—

taining heparin (or LMWH), the heparin enhances factor Xa

gen, II, V, X).

inhibition by antithrombin rendering less factor Xa available

If the AP Screen is prolonged, with a normal AP

to cleave the substrate. The test results in the order of a stan—

individual is of an equivalent of normal patient as a

standard curve with known amounts of heparin can be used to

but an abnormal AP incubate ix o an equa vo u e
eter ine actor Xa.

o nor a atient as a, this in icates the resence o a

So e a vantages o the anti-Xa he arin assay co are
e aye inhibitor such as s eci c actor inhibitors, ost
to the AP are

co on y actor VIII inhibitor. So e inhibitors wi e -
onstrate a e aye ty e inhibitor attern, with ti e an /or

- Una ecte by un er e b oo co ection tubes

te erature e en ence.

- Not susce tib e to inter erence ro e evate concen—

A though rare, the resence o a actor inhibitor, such as a
trations o actor VIII or brinogen ro acute-hase
actor VIII inhibitor, wi increase the risk o i e-threatening
reactions

b ee ing. T e resence o a actor inhibitor can be con r e

- Not inf uence by actor e ciencies (ossib e exce tion
by a Bethes a assay or that actor.

is A e ciency)

- No nee to estab ish an AP thera eutic range

2. Nonspecific Inhibitors

Some advantages of the anti-Xa heparin assay compared

to the AP Screen is ongoing with an AP intermediate

are to the AP are

involving an equivalent of the normal patient as a control—

ongoing with the AP incubate involving an equivalent of

■ High cost

normal patient as a control, this suggests a nonspecific inhibi-

■ Processing of specimens within 1 hour to avoid heparin

interference. The presence of low-level nonspecific inhibitors in the

neutralization of PF4

patient's sample may demonstrate an AP incubate involving

■ Questionable therapeutic range limitations

similar to the specificity of the inhibitor.

■ No safety and effectiveness data on outcomes or analog—

The presence of unusual anticoagulants, antibodies against

the therapy

protein-hormone inhibitors, will increase the risk of

thrombocytopenia. The clinical history suggests unusual anti-

Classic Assays for Coagulation Factors

coagulant (LA), further testing includes host cell base

These assays may be conducted in certain circumstances:

screening tests, host cell efficiency assays, an exclusion of the presence of inhibitors in addition to the fixing

1. One-stage quantitative assay for factors II, V, VII, and

study including an incubate AP.

X. This assay uses age serum and absorbance as a

the P. Age serum contains factors VII, IX, X, and XII.

3. Anticoagulation tests

Absorbance contains factors V, VIII, XI, and XIII.

In the AP Screen is prolonged with an AP plate

The P is conducted using specific factor—efficient as a

ix of an equivalent or a patient as a control—

with specific dilutions of patient as a. The percentage

longer with the AP incubate ix of an equivalent or

of factor activity is plotted to construct an activity curve.

control as a, anticoagulants are available.

2. One-stage quantitative assay for factors VIII, IX, XI, and

The presence of the anticoagulants such as heparin,

XII. This procedure is based on the AP assay. It is based

on arixin, abigatran, or other direct thrombin inhibition the results of patient as a

to correct specific factors can cause prolongation of both the APTT and the PT—efficient as a. The results are expressed in percent of normal. The patient's results are plotted on an activity curve.

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3. Factor VIII antibodies in hemophilia. An ELISA technique—

assays, immunologic assays, and the one thrombin technique that uses the binding of antibodies in the assay to titrate. The normal value of 200 to 400 g/L may be so high—antigen, which is subsequently detected by a decrease in liver disease or the consumption of fibrinogen human monoclonal IgG antibody with the alkaline phosphatase—owing to accelerated intravascular clotting. Fibrinogen titers using a nitrophenyl substrate system.

may be used. The normal titer of fibrinogen is 1:128 to 1:256; a titer less than 1:64 is abnormal.

NOTE: This is a good time to complete Review Questions

Thrombin Time

related to the preceding content.

The thrombin time should be measured in seconds with a routine AP. In the is routine, a heparin

New Thromboplastins

assay (anti-Xa inhibition assay; by a chromogenic assay, that can distinguish a heparin effect from a direct thrombin

The new types of thromboplastins for measuring the prothrombin inhibitor can be used.

thrombin (PT) are mixtures of those which is an excellent test determines the rate of thrombin-inhibitor

relationship human tissue factor. (F). Because the new

cleavage of fibrinogen to fibrin monomers and the subse-

thromboplastins are more sensitive (typical ISI, 1.0) than

quantification of hydrogen-bonded fibrinolytic

the traditional North American ones (ISIs, 1.8 to 3.0), the

total an insoluble fibrin clot. The normal value is less than

Patients with inherited or acquired deficiencies of

20 seconds. Routine results will be seen in the fibrinogen

coagulation factors will be much more routine with use of

concentration is less than 100 g/L. Abnormal results will

the new reagents, although normal values may change initially so be encountered in the presence of thrombin inhibitors

ally. However, the therapeutic range (in seconds) of the
for substances that interfere with fibrinolysis (e.g., heparin in patients receiving
oral anti-thrombotic agents), or high concentrations
agents is wider with the sensitive thromboplastins than with
of inhibitors that interfere with fibrinolysis
the traditional ones. The INR, however, will be the same,
of fibrinolysis such as in cases of uterine bleeding.
as with the recommended ranges of the INR or intensity of
anticoagulation.

Reptiles and Turtles

Recombinant thromboplastin has the following

This assay is similar to the thrombin time. The advantages:
one is that the clotting sequence is initiated with the snake

1. It is a human protein, not the protein of
venom enzyme, reptile, which is thrombin-like in nature
in different species.

analyzes fibrinolytic activity of the intact fibrin—

2. The method is simple, and the concentration can be readily
determined. In contrast to thrombin, which analyzes
a justly, unlike current ly available rabbit brain thrombo—

brinogen, reagent is not

astins. A just ent wi ini ize variation between i -

inhibite by he arin. T is assay is use to screen or yserent ots o the reagent;
thus, the nor a an therapeutic

brinogene ia, a coagulation isor er cause by a vari—

ranges o the P wi re ain the sa e.

ety o acquire or inherite structural abnor a ities in the

3. T e reagent is ree o contamination with noxious viruses

brinogen o ecu e. In the inherite or , a i y e bers

because it is a recombinant ro uct.

wi exhibit assay abnor a ities. In comparison, atients

4. When the ISI is a roxi ate y 1.0, the P s wi be the

with the acquire or o ys brinogene ia wi not have

sa e as those obtaine with use o the Wor Hea th

a ecte a i y e bers an wi exhibit abnor a iver

Organization reference thro bo astin. T ere ore, the P

unction tests.

ratio (P o atient/ ean nor a P) wi be the sa e as

the INR.

D-Dim e r Te s t in g

5. T e new reagents are ore sensitive to i e ciencies

The D-dimer (Fig. 27.6) is a specific fragment generated from coagulation factors that are the traditional thrombolytic agents. For patients with the osteoporosis, the D-dimer levels are elevated. Patients with the osteoporosis, the D-dimer levels are elevated. Patients with the osteoporosis, the D-dimer levels are elevated.

normal activity) will have INRs of 1.4 or less.

1. Coagulation to form the clot
2. Covalent cross-linking of fibrin by activated factor XIII

As a result of Fibrin Formation

3. Fibrinolysis to dissolve the fibrin clot into soluble

Fibrinogen Levels

fragment

Fibrinogen assays are used in detecting deficiencies of

At least three of these stages require activation of

fibrinogen and alterations in the conversion of fibrinogen

thrombin. Following the activation of a clot, the fibrinolytic

to fibrin. Fibrinogen can be quantitated by various methods—

system is activated to regulate its formation. Plasmin

o s inc u ing reci itation or enaturation etho s, tur—

egra es the brin c ot into s a er s eci c brin rag—

bi i etric or brin c ot ensity etho , coagu ab e rotein

ents o which so e or a contain the d-i er e ito e. On

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Fibrinoge n

D

E

D

BOX 27.2

Fibrin Clot

D

D

E

D

D

Conditions That Can Gene rate Fals ely

(Cros s -Linke d)

Decre ased or Fals ely Incre ase d D-Dim e r

Values

E

D

D

E

FALS ELY DECREAS ED VALUES

- Anticoagu ant ther a y

E

D-Dime r

- S a er, o er, non rogressing thro bus

D

D

Fragme nts

D

D

FALS ELY INCREAS ED VALUES

Various isease states

FIGURE 27.6 Basic structures o brinogen, cross-inke brin

■

c ot, an the d-i er.

- Postthera eutic c inica roce ures

activation of the fibrinolytic system, fibrinogen levels are decreased or a secondary elevated fibrinogen values). It is more relevant to the clotting cascade in the blood.

important to eliminate a secondary decrease values than a secondary Laboratory assay of fibrinogen has been established to help evaluate ones.

in the diagnosis of DIC and deep venous thrombosis (DVT) or pulmonary embolism (PE). The quantitative analysis of

Global Testing

quantitative fibrinogen assays are useful in the confirmation of DIC. The rapid, sensitive quantitative fibrinogen assay has

Thrombosis and hemorrhage are major contributors to morbidity—

been evidence to the existence of DVT and PE. The presence

biomarkers and mortality. Fibrinogen testing (AP, PT, and

of fibrinogen suggests that a coagulation-fibrinolytic process

PT, and) artificially coartificializes the intrinsic

is taking place but does not confirm that a thrombus has

an extrinsic coagulation pathways. These clot-based assays

are

measure initiation of clotting to the formation of a fibrin clot.

Assays used in the clinical laboratory to measure the

Move toward more rapid assays has increased and different assays can be divided into the following:

1. Point-of-care testing, particularly in surgery.

The introduction of global testing instruments addresses

1. Qualitative (semi-quantitative)

the entire hemostasis process including coagulation, fibrinolysis,

2. Quantitative

oration, and fibrinolysis. In addition, factors such as

Qualitative assays (e.g., traditional latex agglutination) are

the effect of anticoagulants and platelet function are also

analyzed in which the endpoint is detected visually.

addressed.

Because of ease of use and low cost, these methods have been

widely adopted. Because of the potential for user variability

Thrombolytic therapy, Thrombolytic treatment of

is less than ideal sensitivity, these assays are gradually

(Viscoelastic Tests)

being replaced with newer, more sensitive automated—

An automated method of the coagulation—

titative assays.

tion process in whole blood is called thrombostography.

Newer automated methods are based on optical anti-

clotting of the over time reflect the characteris-

tics and microscopical light scattering technology.

tic graph, from which the start of clot formation can be

Quantitative dilution assays may be referred to by the synonymity of the clot can be read. The method also detects

only so in thrombotic, microclot in assay,

graphical resolution of the clot due to fibrinolysis (Fig. 27.7).

thrombotic assays. In 1997, the first sensitive automated

Thrombostography is becoming an important tool in

quantitative assay was approved by the U.S. Food and Drug

detecting coagulopathies and guiding the static therapy at

Administration (the S.A. Latex D-DI assay, Diagnostica

the point of care. Additionally, it has been shown to detect

Stago, Parsippany, NJ). Now several assays are on the market

excess the fibrinolytic.

that use thrombotic technology.

Currently, two semi-automated commercial devices are

The negative predictive value of the automated assays

on the market—the thromboelastography (ROTEM analyzer,

is excellent. d-dimers are present in any patients with EM international, Munich, Germany) and the thromb—

ongoing disease processes or who have undergone an invasive astography (EG analyzer, Haeonetics Corp., Braintree, sive procedure, but these patients do not need to be supervised). Either method measures the capacity of the coagulation system to form a thrombus.

ing to DV or PE. In addition, the quantitative d-dimer assay should not be used in patients receiving anticoagulant therapy (e.g., warfarin or heparin), because they will have elevated circulating d-dimers and can generate a false positive result. However, these tests have been shown to be suitable for monitoring therapy (e.g., warfarin or heparin), because they will have elevated circulating d-dimers and can generate a false positive result.

decrease circulating d-dimers and can generate a false negative result. However, these tests have been shown to be suitable for monitoring therapy (e.g., warfarin or heparin), because they will have elevated circulating d-dimers and can generate a false positive result.

surgery, an liver transplantation, articulation in patients with low value (Box 27.2) or conditions that can generate a false positive result with low fibrinogen levels.

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FIGURE 27.7 Thrombotic Astography Patterns.

Normal

Thrombotic astography patterns associate with

R; K; MA; Angle = Normal

hypercoagulable conditions and anticoagulant use -

indications. (Froberg SM, Bittner EA, Zhao KH.

Anesthesia Review: Blasting the Boards, Philadelphia,

PA, Lippincott, Williams & Wilkins, 2016.)

Anticoagulants /thrombophilia

Factor deficiency

R; K = Prolonged

MA; Angle = Decreased

Platelet disorders

Thrombocytopenia /

Thrombocytopenia

R ~ Normal; K = Prolonged

MA = Decreased

Fibrinolysis (UK, SK, or tPA)

Presence of tPA

R ~ Normal;

MA = Continuous decrease

LY30 >7.5%; WBCL130 <97.5%

LY60 >15.0%; WBCL160 <85%

Hypocoagulation

R; K = Decreased;

MA; Angle = Increased

DIC

Stage 1

Hypocoagulable state with

secondary fibrinolysis

Stage 2

Hypocoagulable state

Although the thrombostography is able to detect

By using a “slow” fluorogenic thrombin substrate and

hypercoagulable situations, it is not frequently used to re—

continuous comparison to a simultaneous run calibrator,

thrombosis but it is used to monitor antithrombotic

thrombin generation can be monitored automatically using

therapy. It is important to the fact that thrombostog—

ateletic or as a anateletic-rich as a. An autoate

therapy does not reflect the effects of LMWHs and the new

as a-base calibrator autoate thrombography (CA) is

oral anticoagulants completely. In addition, atelectic unc—

anufactured by Diagnostica Stago (Paris, NJ).

tion either inherited or acquired will not be detected.

Activated using latex as a measure

Another shortcoming is the insensitivity to detect the effects

of coagulability (heparin, oral anticoagulants, heparins

of von Willebrand's factor, which is involved in the initiation

(fibrinolytic, direct inhibitors) and hypercoagulabilities (antithrombotic

of clotting. Factor XIII, which is mainly responsible

for fibrinolytic activity, thrombinolytic activity, proteins C

and stabilization of the fibrinogen clot, is also not adequately

an S deficiency, factor V Leiden, oral contraceptives).

is also.

In latex-rich plasma, thrombin is initiated in thrombotic

An investigation of quality control and quality assurance

bioassays, such as von Willebrand's disease, by antibodies

showed a wide variation of EM results.

blocking GPIIb-IIIa or GPIb, or by antiplatelet drugs such as

aspirin and clopidogrel. Low anticoagulant both decreases

and increases thrombin generation.

Calibrated Automated Thrombogram

A calibrated autoanalyzer thus allows a broad range of function tests of the hemostatic-thrombotic mechanism—

NOTE: This is a good time to complete Review Questions on the book.
related to the preceding content.

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NORMAL PROTECTIVE MECHANISMS

with thrombin. This coexists with any thrombotic or

AGAINST THROMBOSIS

antithrombotic activity. As is also the physiological

inhibitor of factor Xa. In addition, it is known to inhibit acIn the blood circulation, the relationship to thrombosis

factors IXa, XIa, and XIIa.

exists on the balance between procoagulant and anticoagulant factors, but in the presence of

agglutinant factors. Several important biological activities are—

however, it rarely inhibits activated serine proteases such as

and protect the body against thrombosis. These activities

activate factors II, IX, X, XI, and XII. The binding of A

include the following:

antithrombin is increased 1,000-fold or greater in the presence of heparin. Initially, A was designated heparin cofactor—

1. The normal flow of blood

throughout the body. The enhancement of the activity of A is considered to be

2. The release of activated clotting factors and articulation

the primary mechanism of heparin's anticoagulation effects.

arteria

Normally, A accounts for the majority of the thrombin

3. Natural anticoagulant systems known to be operative in

inhibitory activity in vivo. The concentration of A at the

vivo:

endothelial surface is rate limiting for inactivation of thrombin -

a. antithrombin (A)

bin and factor Xa when the plasma level of A is below

b. Heparin cofactor II (HC-II)

50%.

c. Protein C and its cofactor, protein S

A is rapidly removed from the circulation following

4. Cellular regulators

one-to-one binding with an activated coagulation factor. The

half-life of A in vivo is approximately 70 hours.

Normal Blood Flow

Heparin Cofactor

The normal flow of blood prevents the accumulation of atherogenic material. This mechanism reduces the chance of

Heparin is produced endogenously by mast cells, and heparan

sulfate synthesis.

Heparin-like molecules are found in the endothelium. Two heparin-

sulfate-dependent thrombin inhibitors are present in human

plasma: A heparin cofactor and HC-II, previously referred

Removal of Activated Clotting Factors and

to plasma heparin cofactor A. The inhibitory activity of HC-II is

Particulate Material

accelerated by heparin. The inhibition of thrombin by HC-II

Another normal mechanism against inappropriate thrombosis is not related to the activity of thrombin or fibrinogen; as such

it is the removal of the blood of activated clotting ac-

tivated thrombin-inactivated platelet aggregation and

thrombocytes. This process, along with the naturally

occurring inhibitors, limits intravascular clotting and throm-

bolysis. It does not significantly inhibit blood coagulation factors

noysis by inactivation o such actors as XIa, IXa, Xa, an
IXa, Xa, an XIa or as in.

Ila. Re ova o articu ate ateria by the ce s o the ononuc ear hagocytic syste is a
so i ortant in reventing

Protein C

the initiation o coagu ation.

Protein C an rotein S are invo ve in one o the ajor
natura anticoagu ation syste s in the bo y. De ciencia an

The Natural Antico ag ulant Sys tem s

a teration in either rotein have been c ear y associate with
re is osition to thro bosis.

T e in vivo existence o natural anticoagulant systems is essen—

Protein C, a vita in K— e en ent as a rotein synthe—

tia to revent thro bosis. T ese natura anticoagu ant sys—

size in the iver, re resents a natura anticoagu ant or e

te s inc u e A , HC-II, an protein C an its co actor, protein

in res onse to thro bin generation. Protein C circu ates

S. A an HC-II are serine rotease inhibitors. When acti—

in the b oo as a zymogen, an inactive recursor or . T e

vate , rotein C is ca ab e o egra ing activate actors V

ajority o as a rotein C exists as a two-chain zy ogen

(Va) and VIII (VIIIa) in the presence of the cofactor protein (molecular weight, 62,000) before activation. A single chain S.

or an amino beta or have also been demonstrated.

Protein C requires proteolytic cleavage to become active

Antithrombin

(Fig. 27.8). It is converted by thrombin to its enzymatically

Antithrombin (AT) is considered the major inhibitor of coagulation factors. A factor can be activated. Thrombin activates

tion. It is one of the serine proteases

protein C in the presence of the endothelial cell-associated

inhibitors that also includes heparin, C1 inhibitor—

in the cofactor thrombomodulin. This reaction con—

verts the zymogen into the serine protease, activated

inhibitor.

protein C (APC). Thrombin activation of protein C is also

It is an alpha-2 globulin glycoprotein that circulates in

enhanced by activated factor V, although considered by

the same. It is synthesized by hepatocytes, megakaryo—

efficiently.

cytes, an vascular endothelial cell. It is the principal physiologic inhibitor of thrombin that shows an irreversible major blood coagulation regulatory mechanism. APC is a natural inhibitor of thrombin by forming a stable one-to-one complex as a natural anticoagulant. Once activated, APC—in the

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IXa

TF

X

VIIa

VIIA

Platelet

Protein S

C4b-BP

Inhibits

EP CR

C4b-BP

Xa

Antithrombin

Protein S

Protein C

Activated

Va

Protein C

Inactivation

Inhibits

Heparin

Sulfate

TM

Platelet

Prothrombin

Thrombin

Thrombin

Xa

Va

Platelet

FIGURE 27.8 Schematic depiction of pathways that generate factor Xa, thrombin, and the natural anticoagulant mechanisms that regulate the activity of these enzymes. Factor X can be activated by the extrinsic pathway (factor VIIa-F) or the

intrinsic pathway (factor IXa/VIIIa-activate each other). Factor Xa binds to factor Va on activation and catalyzes the conversion of prothrombin to thrombin under physiological conditions.

Thrombin and factor Xa are inactivated by antithrombin bound to heparin sulfate or heparan sulfate associated with the vascular endothelium. Protein C is activated by thrombin bound to thrombomodulin (TM) and endothelial cell protein C receptor (EPCR). Once activated, APC functions as a potent anticoagulant by inactivating factors VIIIa and Va. Protein S enhances the binding of APC to phospholipid-containing membranes and accelerates the inactivation of factors VIIIa and Va. The complement component, C4b-binding protein (C4b-BP), or its complexes with protein S, which results in a reduction of its functional activity.

presence of its cofactor protein S (S)—regulatory cycles

Thrombomodulin (Table 27.3) is expressed in a functional

factors Va (V-Vi) and VIIIa (VIII-VIIIi). This cleavage rate—

on the surface of the vascular endothelium. Rather—

activity decreases the conversion of prothrombin to throm-

bin C activation occurs when thrombin binds to thrombo-

bin and is one of the regulatory feedback mechanisms of

thrombin. The interaction of thrombin with thrombomodulin

coagulation. Thrombin thus not only acts as a procoagulant

is characterized by the formation of a reversible, high-affinity

but also activates natural anticoagulation.

complex between thrombin and thrombomodulin. Protein

Protein C requires a second vitamin K-dependent γ -carboxyglutamate (Gla) residue for its activation. An inactive as a

tor, protein S, to function as an anticoagulant. APC is a so-
rotease inhibitor may be the major mechanism or the
believe to promote fibrinolysis by neutralizing the inhibitor
clearance of APC, but it has been demonstrated to have a
of t-PA. t-PA inhibitor (t-PA-I) functions by inhibiting t-PA,
half-life of approximately 8 minutes. Direct clearance rate
an enzyme responsible for the conversion of plasminogen to
clearance of APC cannot be excluded as an important sec-
as in.

on any mechanism.

Protein C is involved in each stage of the anticoagulant

The normal plasma concentration of protein C is 4 to

athway. This pathway can be divided into three stages:

5 µg/L. Many cases of acquired thrombotic disease (e.g.,

DVT) associate with decreased levels of protein C have

1. Protein C activation

been described in the past decade. In addition, their role—

2. Expression of APC anticoagulant activity

tion is impaired in vitamin K deficiency, liver disease, and

3. Inhibition of APC

war arin thera y.

Protein C can be activate by thro bin, but the rate

Ora anticoagu ants can re uce the eve s o rotein C an

o activation is too s ow to be hysio ogica y re evant.

rotein S. Protein C eve s ecrease ra atica y in atients

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TABLE

27.3 **Pro pe rties of Throm bo mo dulin**

Molecular weight

~74,000, single chain

Cellular location

Endothelium

Function

Accelerates protein C activation by thrombin

Mechanism

Forms 1:1 complex with thrombin; functions as a cofactor

Role of Ca²⁺ in protein C activation

Ca²⁺ is required

Role of Ca²⁺ in complex formation between thrombin and

Ca²⁺ is not required

thrombomodulin

Control of protein C activation

Thrombin can be inhibited by AT when bound to thrombomodulin

Other functions of thrombomodulin

Reduces thrombin's ability to clot fibrinogen, activate factor V,
and trigger platelet activation

Vitamin K dependent

No

AT, antithromboplastin

From Esmon CT. The Protein C Anticoagulant Pathway, Miami, FL: Baxter Healthcare, 1990.

with DIC. Patients with impaired liver function or those in

Chromogenic protein C assay involves the same venous blood

the osteoclastic activity assay also experience decrease even—

activate protein C. The quantity of APC formed is measured

as a protein C.

by its catalytic activity on a specific chromogenic substrate.

Antigenic protein C can be assessed by ELISA or Laure

Laboratory Findings

technologies, which have been well established.

The ability to detect decrease protein C activity depends on

the type of assay used (Table 27.4). Plasma is evaluated for

Factor V (Leiden)

LA before ordering the protein C assay to be sure that LA

Activated protein C resistance (APC), a hypercoagulable condition not the cause of

thrombosis. The assay concentration of

protein C is 4 µg/L.

mutation in the factor V gene (factor V [Leiden]). The vast majority

Laboratory diagnosis of protein C and protein S deficiency

individuals with a factor V APC resistance have a specific

deficiency involves functional and antigenic assays. Diagnosis

of mutation in the procoagulant factor V gene encoding

of a deficiency is best achieved using functional assays or

or a glutamine substitution for arginine in the heavy chain

screening and a combination of functional and anti-

of factor V. This is a significant change affects an APC cleavage

genic assays or confirmation and characterization of the

site on factor V such that factor V/Va is artificially resistant to

deficiency.

inactivation by APC.

Protein C Testing

The mutation slows the inactivation of activated factor

V by APC causing a hypercoagulable state. The presence

of functional protein C assays can be either clot-based or chromogenic.

Factor V (Leiden) is the most common cause of inherited

thrombophilia. It accounts for 20% to 50% of cases.

that specifically activates protein C. The resulting APC

The degree of abnormality of the APC-resistance assay

inhibits factors Va and VIIIa, thus prolonging the AP of

correlates with heterozygosity or homozygosity or the activated partial thromboplastin time (APTT) system in which the

factor V Leiden mutation; homozygous carriers have a very low

percentage in excess.

APC-resistance ratio, but the ratio of heterozygous carriers

is usually 1.5 to 1.8.

The carrier frequency of the factor V Leiden mutation

varies depending on the population. Approximately 5% of

General Assay Types for

asymptomatic white Americans of non-Hispanic ancestry

TABLE

27.4 Determination of Protein C in

are heterozygous carriers, whereas the carrier frequency

Plasma

among African Americans, Asian Americans, and Native

Antigenic

Measures amount of material

Americans is <1%, and the carrier frequency for Hispanics

present; does not measure function

is intermediate (2.5%). Homozygosity for Factor V Leiden is

much less common but may confer a substantially increase

Chromogenic

Measures some but not all functions

risk of thrombosis.

Clotting

Measures all functions of the

Factor V (Leiden) (Russell viper venom—base screen—

protein C molecule

ing test) is a sensitive functional clotting test intended for

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screening of resistance to APC in as a role in individuals

Like antigenic protein C, antigenic protein S can be evaluated—
with the factor V (Leiden) defect.

sure by ELISA or Laure technologies. More recently, a

An APC-R ratio of <2.3 suggests abnormal resistance to

the technology has been evaluated involving agglutination—

APC of hereditary origin. The reference range is 1.0–10.0.

tion of antibody-coated microtest articles. This agglutination—

DNA-base testing or the factor V Leiden mutation may be

tion is reassessed retrospectively.

useful in confirming or excluding hereditary APC-resistance,

Both total and free protein S antigens can be assessed by

a preliminary screening with the APC-resistance test.

these techniques. The measure of free (versus total) or S

protein S is one of 25% of healthy individuals treated as a.

Protein S

The association of C4b-binding protein and protein S

Protein S is another vitamin K–dependent factor as a protein

necessitates C4b-binding protein evaluation to exclude

that is an essential cofactor for APC to express an anticoagulant—

acquire free protein S deficiency. C4b-binding protein
antibody. Protein S does not require proteolytic cleavage—
can be measured by the Laurell technique or immunoassay
tion to function but it can be regulated by proteolysis.
aggregation.

Protein S circulates to C4b-binding protein (C4b-BP)
in two forms, free and bound, in a ratio of 40% free to 60%

Cellular Regulators

bound. Only the free protein occupies the functional activity. Evaluation of
C4b-binding protein (which is an

Cellular activities related to thrombosis are becoming rec—
acute-phase reactant protein) results in an acquired decrease
recognized as essential to the maintenance of hemostasis and
of free protein S.

thrombosis.

Relevant properties and functions of protein S are summa-
rized in table 27.5. Basic science research studies suggest

Cellular Proteases

that free, functional protein S forms a one-to-one complex

Phase contains, in addition to plasmin, another powerful
with APC on synthetic membrane surfaces, which increases

mechanism to initiate the activation of serine proteases on the activity of APC on the membrane surfaces aroximately the required action of cells. This mechanism consists of the 10-0 .

cellular proteases derive from the lysosomes of granulocytes

Protein S increases the rate of inactivation of factor Va

that may be traced within a thrombus. These proteases

by APC by enhancing the binding of APC to phospholipids,

block the activation or action of as in. A cellular protease

thereby stimulating the inactivation of factor Va. Protein S

of particular interest is a ha-2 as in inhibitor, which ra-

has been found within platelets, suggesting that these cells

may neutralize the fibrinolytic properties of as in.

may also be responsible for initiating coagulation by the protein S—enhance inactivation of factors Va and VIIIa by

Cells that Regulate Coagulation

APC. Similar interactions occurring in vivo may be observed

Synthesis of blood coagulation proteins was once thought to

take place on the surface of platelets, endothelial cells, and

be the domain of the hepatic cells; however, it is now known

in other tissues. An increase in APC is mediated by thrombin

that other cells are capable of synthesizing some of the coag—
necessitates an increase in protein S levels to attain axi—
umation factors and cofactors. Monocytes and macrophages
uprotein C activity.

have been demonstrated to synthesize factor VII. Platelets
and endothelial cells are now known to be the principal

Protein S Testing

components in the initiation, propagation, and suppression

The principal of a protein S functional assay is based on the
of hemostasis and thrombosis.

cofactor activity of protein S, which enhances the anticoagu—

Platelets store and release HMWK, vWF, and factor V, a

activation action of APC. This enhancement is reflected by the

of which are involved in clot formation. Endothelial cells are

prolongation of the clotting time of a system enriched with

known to synthesize vWF, factor VIII, factor V, HMWK, and

factor Va, which is a physiological substrate of APC.

protein S.

TABLE

27.5 Protein S Structure, Function, and Regulation

Protein S structure

Single chain, Mr = 69,000

Accelerates factor Va and factor VIIIa inactivation by APC (functions as a cofactor) Vitamin K dependent

Yes

Binds to membranes

Yes: forms a 1:1 complex with APC on membrane surfaces

Forms in plasma

Free and in reversible complex with C4b-binding protein

Regulation

Inactivated by thrombin; not active when complexed to C4b-binding protein

From Esmon CT. The Protein C Anticoagulant Pathway, Miami, FL: Baxter Healthcare, 1990.

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The function of protein S as a cofactor by itself is

■ Substrate for coagulation factors are critical components

believe to play a significant regulatory role in the initiation,

in the formation of a thrombus.

regulation, an suppression of hemostasis and thrombosis.

■ The fibrinogen group consists of factors I, V, VIII, and

Endothelial cells synthesize and secrete protein S and inter—

XIII. These factors are consumed during the process of
normalize this process in a dynamic manner. Once activated, the
coagulation.

APCs are formed by thrombin on the endothelial surface, it

■ The prothrombin group consists of factors II, VII, IX, and
may be in proximity to protein S (receptor), resulting in the
X. As these factors are dependent on vitamin K during
formation of an active, stable inactivator coagulation factors
their synthesis.

VIIIa and Va.

■ The contact group consists of factors XI, XII, prekallikrein (Fletcher factor), and
HMWK (Fitzgerald factor).

MODERN VIEW OF HEMOSTASIS

These factors are involved in the intrinsic coagulation
pathway.

Since the 1960s, a variety of key components of the

■ Initiation of the coagulation process may occur via one
coagulation system (e.g., protein C pathway) and other
two pathways: the extrinsic pathway and the intrinsic
hemostasis-related factors (e.g., protein Z) have been is—

athway.

covered. Historically, the intrinsic pathway was considered

■ Regardless of the initiating pathway, the two pathways converge to be the most important pathway. One major weakness of

convergence into a common pathway. The outcome of this

this pathway has been that this pathway could not correctly

process is the conversion of circulating into soluble coagula—

explain the questionable or even nonexistent role of HMWK,

transforming factors into a general brin clot with entrance

reagents, an actor XII (Hageman's factor) in the initial—

blockade, a block.

tion of coagulation.

■ As repair of a damaged tissue takes place, the clot is lysed

Recently, tissue factor and factor VIIa in the extrinsic

and the articulation is removed by the mononuclear

pathway have been demonstrated to be the major pathway

phagocytic system.

for activation of coagulation. It has also been discovered that

■ A variety of laboratory procedures are valuable in assessing—

thrombin has a more important role as an activator and

ing coagulation factors. General procedures include the

an inhibitor. Thrombin is

AP, the PT, the thrombin time, an quantitative fibrinogen concentration assay.
More specifically or classic ro-

- The key enzyme in the conversion of soluble fibrinogen

clots can be either in specific circumstances.

into fibrin

- Require for the activation of the protein C system, which

Normal Protective Mechanisms Against

thromboregulation homeostasis

Thrombosis

- Require for the activation of thrombin-activatable fibrinolysis inhibitor, which is involved in the regulation

- In the blood circulation, the relationship to thrombosis

of the fibrinolytic process

depends on the balance between procoagulant and anticoagulant factors.

The cumulative result of new discoveries in coagulation

- The normal flow of blood prevents the accumulation of

clots due to the evolution of a ternary schematic response—

procoagulant arterial.

representations of the pathways that better represent the normal

- This mechanism reduces the chance of occlusion or in vivo process of coagulation.

tion. Another natural mechanism against inappropriate

thrombosis is the removal of the blood by activating

clotting factors by the platelets. This process, along with

NOTE: This is a good time to complete end of chapter

the naturally occurring inhibitors, in the intravascular

Review Questions.

clotting and fibrinolysis by inactivation of such factors as

XIIa, IXa, Xa, and IIa.

Removal of activated platelets by the cells of the mono-

CHAPTER HIGHLIGHTS



nuclear phagocytic system is a significant in preventing

Blood Coagulation Factors

the initiation of coagulation.

■ The in vivo existence of natural anticoagulant systems

■ Being responsible for blood vessel constriction by

is essential to prevent thrombosis. These natural antico-

vasoconstriction and the formation of a platelet plug.

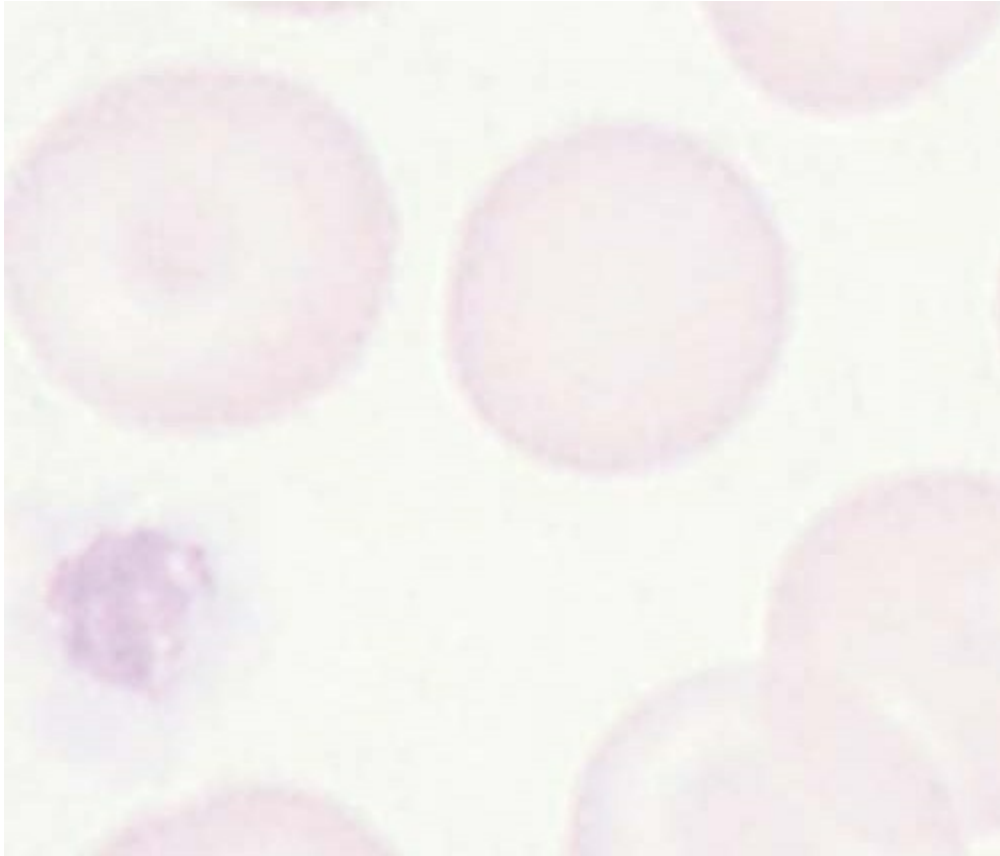
anticoagulant systems include A, Heparin-II, and protein C and

■ Formation of a clot (thrombus) usually occurs as part of

its co actor, rotein S. A an HC-II are serine rotease

the nor a rocess o he ostasis.

inhibitors.





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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

9. Systemic designation of thrombin is

1. Fibrinogen group consists of

A. III

A. factors II, VII, IX, and X

B. XII

B. factors I, V, VIII, and XIII

C. VIII

C. factors XI, XII, prekallikrein, and high-molecular-weight kininogen

D. IIa

weight kininogen

10. Systemic designation of tissue thromboplastin is

2. Prothrombin group consists of

A. III

A. factors II, VII, IX, and X

B. XII

B. factors I, V, VIII, and XIII

C. VIII

C. factors XI, XII, prekallikrein, and high-molecular-weight kininogen

D. IIa

weight kininogen

11. Synthesis or inhibition of which factor is

3. Contact group consists of

A. III

A. factors II, VII, IX, and X

B. XII

B. factors I, V, VIII, and XIII

C. VIII

C. factors XI, XII, prekallikrein, and high-molecular-weight

D. IIa

kininogen

12. Synthesis or degradation of which factor is

4. Thrombinogen group of coagulation factors is

A. III

A. known to increase during pregnancy

B. XII

B. known to increase in conditions of inflammation

C. VIII

C. known to increase subsequent to the use of oral

D. IIa

contraceptives

D. all of the above

13 through 16. Arrange the four stages of coagulation in their proper sequence.

5. The prothrombin group of coagulation factors is

A. Fibrinolysis

A. dependent on vitamin K for production

B. Formation of thrombin from prothrombin

B. considered to be stable

C. Generation of a thromboplastin

C. we reserve in store as a

D. Formation of fibrin from fibrinogen

D. all of the above

13.

6. Warfarin acts by

A. neutralizing the effects of thrombin

14.

B. interfering with fibrin polymerization

C. acting as a vitamin K antagonist

15.

D. inducing hypercoagulation

16.

7. Warfarin interferes with the normal synthesis of factor(s)

17. The extrinsic pathway of coagulation is triggered by the

A. II

entry of

into the circulation.

B. VII

A. membrane proteins (phospholipids)

C. X

B. tissue thromboplastin

D. all of the above

C. Ca^{2+}

D. factor VII

8. Vitamin K dependent coagulation factors include factor(s)

18. The intrinsic pathway of coagulation begins with the

A. II

activation of

in the early stage.

B. V

A. actor II

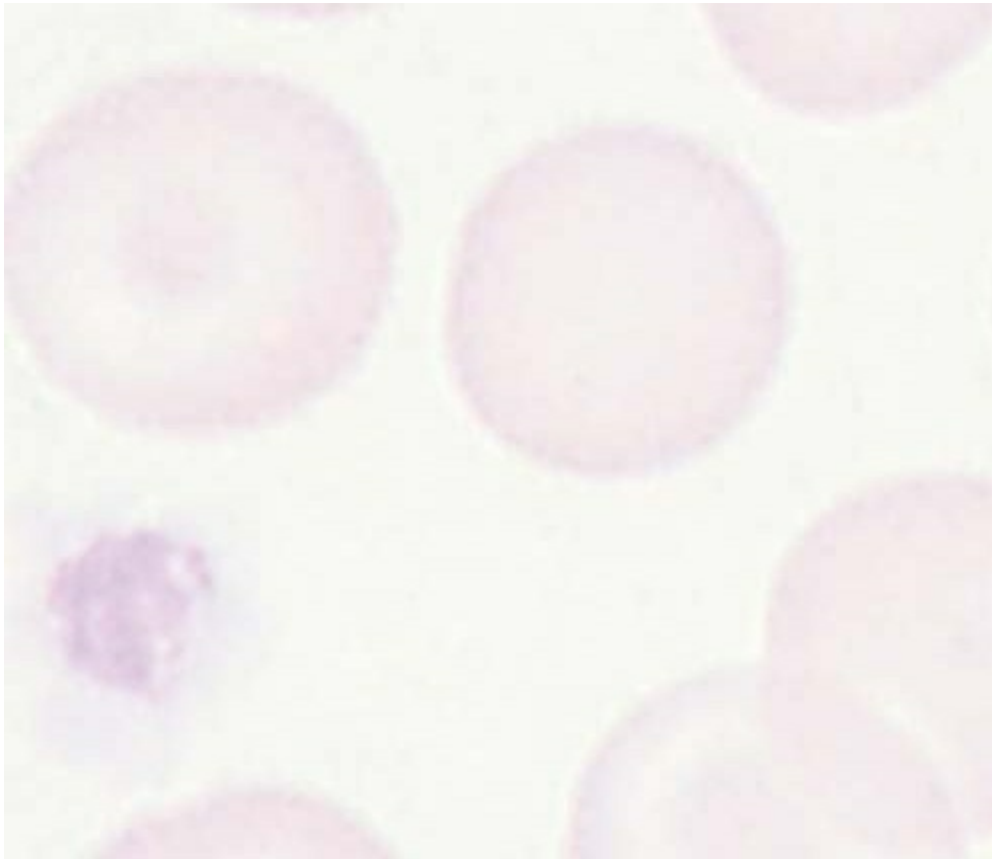
C. VIII

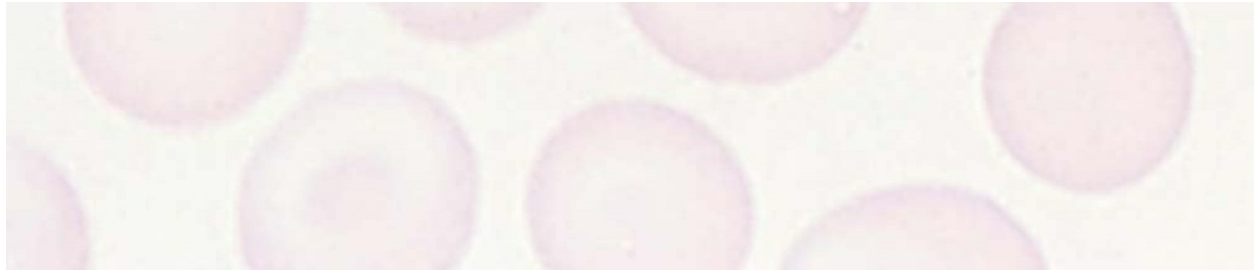
B. actor I

D. XIII

C. actor XII

D. actor V





CHAPTER 27 ■ Principles of Hemostasis and Thrombosis: Blood Coagulation Factors and Natural Coagulant Systems 547

REVIEW QUESTIONS (continued)

19. The natural pathway of the intrinsic-extrinsic

26. Neither the AP nor the P detects a deficiency of
pathway is

A. platelet factor 3

A. factor X activation

B. factor VII

B. factor II activation

C. factor VIII

C. factor I activation.

D. factor IX

D. factor XIII activation.

27. The function of thromboplastin in the prothrombin

20. Prothrombin to thrombin conversion is accelerated by

test is to rovi e

to the assay.

A. a co ex o activate actors IX an VII

A. kao in

B. actor V an ionize ca ciu

B. brinogen

C. a co ex o hos ho i i s an actor VII

C. hos ho i o rotein

D. a co ex o activate actors X an V

D. thro bin

21. Fibrinogen is converte to brin ono ers by

28. An abnor a y ro onge AP ay in icate

A. rothro bin

A. a severe e etion o brinogen

B. thro bin

B. the resence o a circu ating anticoagu ant

C. ca ciu ions

C. actor VIII e ciency

D. actor XIIIa

D. a o the above

22. The inactive plasminogen is activated to

by

29. In a clotting test, which of the following
enzymes.

tests should be performed to test the effect of the

A. thrombin

on the clotting mechanism?

B. as in

A. AP

C. as a kallikrein

B. P

D. as a thrombolytic agent

C. Fibrinogen assay

D. Thrombin time

*23. Which of the following statements are true of the fibrinolytic system?

*30. Which of the following conditions can cause an

A. Plasminogen activator and plasminogen

increase thrombin time?

B. The active enzyme of the system is as in

A. Fibrinolytic products

C. Inactive as inogen circulates in the as a unit

B. High concentrations of ionoglobulins

an injury occurs

C. Hereditary

D. All of the above

D. All of the above

24. A laboratory reagentative patient has a history

31. Hereditary inhibits the clotting of blood by neutralizing

of bleeding but has never had a bleeding episode—

the effect of

se, what test should be included in a coagulation profile? A. Thrombin

in addition to the PT, APTT, and platelet count?

B. Calcium ions

A. Lee-White clotting time

C. platelets

B. Clot retraction

D. Factor VIII

C. Bleeding time

D. Fibrinolytic products

*32. A patient has a prolonged APTT and a normal PT.

The AP is not corrected by factor VIII—deficient

*25. A patient with a severe decrease in factor X activity

as a but is corrected by factor IX—deficient as a.

would demonstrate nor a

In which factor does the patient appear to be deficient?

A. AP

A. Factor II

B. P

B. Factor V

C. thrombin time

C. Factor VIII

D. bleeding time

D. Factor IX

(continued)



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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

REVIEW QUESTIONS (continued)

33. The normal protective mechanisms against thrombosis

36. Which of the following characteristics is (are) true of

protein S?

protein S?

A. the flow of blood

A. It is a cofactor of protein C.

B. the action of antithrombin.

B. It increases the rate of inactivation of factor Va.

C. protein C and protein S

C. It enhances the binding of activated protein C to

D. all of the above

thrombin.

D. All of the above.

34. If heparin therapy is initiated in a patient, a decrease

in anticoagulant response can be caused by decreased levels of—

37. Antithrombin is the principal physiological inhibitor of

thrombin

A. thrombin

A. activated factor 3

B. factor Xa

B. activated factor 4

C. factor XIa

C. antithrombin

D. both A and B

D. factor XIII

38. Which of the following is not correct regarding cellular

35. Which of the following is (are) characteristic of protein
proteases?

C?

A. They block the activation or action of as in.

A. It is not vitamin K dependent.

B. They include a ha-2 inhibitor.

B. It is operative in response to thrombin generation.

C. They rapidly neutralize the fibrinolytic properties of

C. It inactivates factors Va and VIIIa.

as in.

D. Both B and C.

D. They participate in clot retraction.

D'Angelo A, et al. Comparison of ean nor a rothrombin time

COMPANION RESOURCES

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Disorders of Hemostasis and

Thrombosis: Blood Coagulation

CHAPTER

28 Factors, Hypercoagulable State, and
Anticoagulant Therapy

KEY TERMS

antithrombin

factor V R506Q (Leiden)

protein C

antiphospholipid syndrome

brinolysis

protein S

Christmas disease

hemophilia A

purpura

contact group

hemophilia B

thrombin

disseminated intravascular

hypercoagulable state

thrombophilia

co a g u la tio n (DIC)

lu p u s a n tico a g u la n t

LEARNING OUTCOMES

Introduction

- Detail how platelets contribute to hypercoagulation.
- Describe the relationship between hemostasis and blood coagulation.
- Compare the molecular and functional assays for diagnosis of antithrombin, protein C and protein S abnormalities.

Disorders of Defective Production

- Describe how activated protein C contributes to thrombophilia.
- Compare the mechanisms of vitamin K, severe liver disease, and
- Describe the activity of blood coagulation factors in increasing the renal disease on defective blood coagulation factor production. tendency toward thrombosis.
- Compare and contrast the X-linked disorders of secondary hemostasis: factor VII and factor IX deficiencies.
- Explain the characteristic and laboratory findings in antiphospholipid syndrome.

Describe the etiology, pathophysiology, and laboratory testing for

- Describe the relationship between impaired fibrinolysis and protein congenital and acquired von Willebrand's disorder. C, antithrombin, and plasminogen.

- Compare the laboratory findings for factor VIII and factor IX deficiencies.

- Describe the laboratory assessments that illustrate the condition of deficiencies and von Willebrand's disease.

hypercoagulation.

Give examples of uncommon coagulation factor deficiencies and describe conditions that contribute to the defective production of

Anticoagulant Therapy

blood coagulation factors.

- Discuss various categories of anticoagulant therapy.

Disorders of Destruction and Consumption

Case study

- Compare primary and secondary fibrinolysis.

Analyze the patient history, clinical signs and symptoms, and labo-

- Explain the conditions and laboratory findings in conditions of dis-

ratory data for the stated case study, answer the related critical

scenarios of disseminated intravascular coagulation (DIC) and fibrinolysis.

thinking questions, and decide the most likely diagnosis.

The hypercoagulable state (thrombophilia)

- Define the term thrombophilia.

NOTE:

- Name and compare primary states of hypercoagulability.

- indicates MLT and MLS core content
- Explain the role of vascular damage and blood flow in the hypercoagulable state.

INTRODUCTION

activities, the clotting factors are initiated to form the fibrin

clot. Fibrin formation can occur in the activity of various factors

Vascular response and platelet aggregation are responses at least 30% to 40% of normal. Being an effective fibrin

substrate for the initial phases of hemostasis. Subsequent to these

clot formation are frequently related to a coagulation factor.

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CHAPTER 28 ■ Disorders of Hemostasis and Thrombosis: Blood Coagulation Factors, Hypercoagulable State, and Anticoagulant Therapy 551

Disorders of blood coagulation factors can be grouped into

fibrin formation. For factor VIII deficiency (hemophilia A)

three categories:

an factor IX deficiency (hemophilia B), between 91% and

99% of the causative mutations can be detected by current

1. Defective production

diagnostic methods. The ability to conduct genetic testing

2. Excessive estruction

is i ortant to eter ine carrier status, renata iagnosis,

3. Patho ogica inhibition

an the ike ihoo o inhibitor eve o ent or ana hy axis

to in use coagu ation actor concentrates.

DEFEC IVE PRODUC ION

Hem oph ilia A

Vit am in K De cie ncy

Etiology

A con ition o e ective ro uction ay be re ate to a e -

He o hi ia has been use as a ara ig or un erstan ing

ciency o vita in K. T e synthesis o vita in K an e en ent

the o ecu ar atho ogica rocesses that un er ie here i—

actors can be isru te because o isease or rug thera y

tary isease. T e genetic a o he o hi ia A begins with

(e.g., ce ha os orin antibiotics). Vita in K e ciencias are

Queen Victoria in Eng an . Fro a stu y o a genetic a

a so encountere in neonates, a absor tion syn ro e, bi iary

(Fig. 28.1), it is obvious that genetica y a icte a es su er

obstruction, an atients taking ora anticoagu ants. Vita in

for hemophilia and as carriers.

Correction occurs within 2 weeks of both intake and onset—

The cloning of factor VIII facilitates the identification

of mutations that lead to hemophilia A, and inherited

factor VII deficiency. Factor VII has the shortest half-life and

factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

hemorrhage. Mutations of factor VIII coagulant activity that causes severe

Severe Liver Disease

50 mutations in the gene for factor VIII have been

Because the liver is the primary site of synthesis of most

characterized at the molecular level, and 34 in the

coagulation factors, severe liver disease can cause effective

mutations in the factor IX gene have been found to

production of coagulation factors. The liver does not synthesize the cause of theophilia B. Point mutations, in which a single base in DNA is mutated to another base, represent a number of genetic proteins.

second type of mutation that causes theophilia.

Liver disease affects many hemostasis, coagulation, and fibrinolysis. In patients with liver disease, the prothrom-

Epidemiology

bin time is noticeably prolonged, whereas the activated partial thromboplastin time is usually normal. The effects may be either

thrombotic or bleeding.

genetically homozygous or heterozygous carriers of the trait.

Conventional coagulation tests do not usually reflect the

level of factor activity ranges from 0% to 25% in persons

heterozygous for the trait and are not accurately reflected in the

homozygous for the trait and from 15% to 100% in persons

at risk of bleeding. Global coagulation assays (thrombin generation—

heterozygous for the trait. Defects of this origin may result

in, thrombocytopenia) are needed to reflect the interaction.

the decrease in production of a clotting factor, factor

VIII, or the production of functionally inactive procoagulants

Renal Disease

the clotting factor. He is a female, a sex-linked recessive

Chronic renal uremia is associated with platelet dysfunction

is observed in a few cases, occurs in 1 in 10,000 cases.

that results in bleeding. When inactive coagulation factors

are activated, bring on an interplay with renal function,

Signs and Symptoms

such as in disseminated intravascular coagulation (DIC).

Severe bleeding into the joints, hemarthrosis, and cerebral

In nephrotic syndrome, a condition of increased renal globulinuria and in arthrosis are symptoms of factor VIII deficiency.

renal impairment, associated with various conditions such as

Chronic hemarthrosis causes inflammation that can lead to

systemic lupus erythematosus (SLE), so the overall weight

of the disease is high. Bleeding into the joints can cause

proteins pass through the effective glomerulus. Coagulation

nerve conduction injury. Cerebral involvement can result in

factors II, VII, IX, X, and XII are excreted in the urine. In a few

cases, analysis, seizures, and coma that can be fatal.

tion, regulatory proteins, protein C and antithrombin, are excreted.

The loss of regulatory proteins creates a risk of thrombosis.

Pathophysiology

Classic hemophiliacs have an intact high-molecular weight

X-Linked Disorders of Secondary Hemostasis is

oiety and a deficient low-molecular weight coagulant

Factor VIII and factor IX deficiencies have an X-linked recessive

inherited pattern of inheritance. These disorders are X-linked

inherited by decrease factor VIII clotting activity in laboratory

disorders are associated with mutations in the genes or activation

assay and a normal bleeding time. Conversely, severe von

Willebrand's disease has both a decrease high-molecular weight

factor VIII and factor IX on the X chromosome. These proteins

participate as a cofactor (factor VIII) or serine protease

weight protein and a decrease low-molecular weight

(factor IX) in the intrinsic coagulation pathway resulting in

thrombosis.

thrombosis.

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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

X-linked recessive gene inheritance

Example : Hemophilia A (classic hemophilia)

Father: healthy

Mother: healthy carrier

Genetically and

,

clinically normal

hemophilia A trait

asymptomatic

Maternal chromosome

Maternal chromosome

carrier

Abnormal X

Normal X

Normal

Normal

Abnormal Normal

X

Y

X

X

Defective gene for

Normal gene for

coagulation factor VIII

coagulation factor VIII

Paternal chromosome

Child A

Child B

Normal X

Normal gene for

Female (XX)

Female (XX)

coagulation factor VIII

Healthy, carrier

Healthy, normal

chromosomes

Paternal chromosome

Child C

Child D

Normal Y

No genetic influence

Male (XY)

Male (XY)

on X chromosome

Hemophilia

Healthy, normal

chromosomes

FIGURE 28.1 X-linked recessive gene inheritance. Example: Classic hemophilia (hemophilia A). The defective gene on the X chromosome is expressed in males only because the Y chromosome contains no matching nor a gene (allele) to offset the effect of the defective X gene. (From McConne H. The Nature of Disease Pathology or the Health Professions, Philadelphia, PA: Lippincott Williams & Wilkins, 2007.)
Passive factor VIII can be therapeutically corrected **Etiology**

and the bleeding tendency reverse in most patients of von Willebrand disease involves a partial or complete deficiency of the

inhibition of factor VIII in a variety of blood products.

factor IX. Most patients deficient in factor IX have joint

One would expect that correction of the hemostatic defect

mutations; the nature of the mutation determines the event

would affect a patient at the same risk of thrombosis

of factor IX activity. More than one third of the mutations

as an unanticipated inversions, but thrombotic events in

affect critical arginine residues resulting in a dysfunctional

patients with hemophilia A are distinctly uncommon.

occurrence.

Laboratory Findings

Epidemiology

Laboratory assays reveal a strongly activated partial throm-

bin time (APTT) than hemophilia A. This is ac-

quired factor deficiency (AF), not a thrombin time (PT), and

factor deficiency is sex-linked and occurs at a rate of 1/30,000

in males. A factor assay is necessary for

diagnosis with a definitive occurrence being the usual

diagnosis (Table 28.1).

cause.

Hemophilia B

Signs and Symptoms

Factor IX deficiency is known as hemophilia B or Christmas

hemophilia B is clinically indistinguishable from hemophi-

disease.

hemophilia A can be differentiated by laboratory testing. The

CHAPTER 28 ■ Disorders of Hemostasis and Thrombosis: Blood
Coagulation Factors, Hypercoagulable State, and Anticoagulant Therapy 553

TABLE

28.1 Laboratory Test Results in Hereditary Coagulation Defects

von Willebrand's

Test

Hemophilia A

Disease

Hemophilia B

Hemophilia C

Bleeding time

Normal

Increased

Normal

Normal

Clot retraction

Normal

Normal

Normal

Normal

Platelet count

Normal

Normal

Normal

Normal

Platelet aggregation

Normal

Decreased

Normal

Normal

PT*

Normal

Normal

Variable

Normal

APTT

Increased

Increased

Increased

Increased

PT consumption

Decreased

Decreased

Decreased

Decreased

Fibrinogen

Normal

Normal

Normal

Normal

Factor VIII

Decreased

Decreased

Factor VIII:C

Decreased 2% or less

Decreased 10%–30%

Factor VIIC:Ag

Normal

Decreased

Factor VIII-vWF

Normal

Decreased or absent

Factor IX assay

Decreased

Normal

Factor XI assay

Normal

Decreased

*This test is normal when performed with human brain thromboplastin, but in a variant of the disease, the PT is prolonged if bovine brain thromboplastin is used.

This variation is produced by a molecular abnormality of factor IX that inhibits the thromboplastin-factor VII reaction of the extrinsic pathway.

PT, prothrombin time; APTT, activated partial thromboplastin time.

severity of symptoms on the type of mutation and

Etiology

the region of the gene affected. Patients with a severe form

of von Willebrand's disease may be acquired or inherited

experience the arthropathy as the most common feature.

Disorder. The congenital disorder is autosomal dominant

Laboratory Findings

in most cases. Inherited abnormalities in von Willebrand's

disease are associated with a defect of the vWF gene on

Laboratory assay reveals a prolonged activated partial thrombo-

chromosome 12, but in some patients, the coexistence of an
 abnormality (AP), nor a thrombin time (PT), and
 fibrinogen assay or plasminogen activator antigenemia—
 a normal thrombin time () A factor assay is necessary or
 sia suggests the presence of a regulatory defect or an extrinsic
 systemic abnormality. In severe cases, a large
 Autosomal Dominant Inheritance von Willebrand's
 vWF gene deletion has been identified as the basis of von
 Disease
 Willebrand's disease.

In 1926, Erik von Willebrand first described a hemorrhagic
 disorder characterized by a prolonged bleeding time and an
 autosomal inheritance pattern that distinguishes the disease

Nomenclature of the Factor VIII-von

classical hemophilia. Type I vWD has a gene mutation

TABLE 28.2

Willebrand's Factor Complex

detection rate that is approximately 65%. It is likely that in the
 majority of negative mutation detection cases, the mutations

Term

Description

are located within introns or other regulatory sequences that are not routinely analyzed.

VIII:C

Factor VIII procoagulant activity

In the early 1950s, an additional component of the is—

VIII:Cag

Antigenic expression of VIII:C

ease was identified: a deficiency of factor VIII procoagu—

vWF:Ag

Antigenic expression of vWF

ant activity (Table 28.2). These and other observations

Ristocetin cofactor

A property of vWF that pro—

distinguish von Willebrand's disease from classic factor

promotes agglutination of platelets

VIII:C deficiency (hepatoerythroblastosis). In addition, evaluation

in the presence of the antibiotic

of the tertiary structures of vWF has aided in the classification—

ristocetin

classification of the variant forms of von Willebrand's disease.

Three major types of von Willebrand's disease have been

Factor VIII-vWF complex The form in which VIII:C and

identical.

vWF usually circulate in plasma

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TABLE

28.3 Classification of von Willebrand's Disease

Type

Features

IA

All vWF multimers are present in plasma in normal relative proportion.

No evidence of intrinsic functional abnormality of vWF

Subgroups: Platelet concentration and activity may be normal, low, or discordant.

IB

All vWF multimers are present in plasma, but the larger ones are relatively decreased.

vWF has less ristocetin cofactor activity than normal

IC

All vWF multimers are present in plasma in normal relative proportion, but a structural abnormality of individual multimers is present.

vWF has less ristocetin cofactor activity than normal.

Miscellaneous: I-1, I-2, I-3, and I

Variable deficiencies of vWF:Ag in plasma and/or platelets and other abnormalities New York, undesignated types

Type

Features

IIA

Large and intermediate vWF multimers are absent in plasma and platelets.

Increased proteolysis of vWF; some variability in size of multimer present; few

cases show recessive inheritance.

IIA-1, IIA-2, and IIA-3

Subtypes demonstrate variable concentrations of plasma and/or platelet vWF:Ag.

IIB

Hyperresponsiveness to low doses of ristocetin; large vWF multimers are absent

in plasma; all multimers are present in platelets.

Increased proteolysis of vWF; few cases demonstrate recessive inheritance.

IIC and IID

Large vWF multimers are absent; unique structural abnormality of individual multimers

Decreased proteolysis of vWF

IIE

Large vWF multimers are appreciably decreased; structural abnormality of individual multimers

Recessive inheritance

Decreased proteolysis of vWF

IIF, IIG, IIH, and type B

Rare examples of a variety of abnormalities

III

Severe form of the disease; also called severe type I

vWF, von Willebrand's factor.

More than 20 distinct clinical and laboratory subtypes

Epidemiology

von Willebrand's disease have been described (Table 28.3).

von Willebrand's disease is recognized as one of the most

Three broad types of von Willebrand's disease are recognized—

congenital being disorders in humans. The exact

name. In addition, a late-onset type of von Willebrand's disease

incidence is difficult to determine because disorders are

(seu o-von Wi ebran 's isease) is cause by an abnor—
o en not c inica y recognize , but it has been esti ate to
a ate et rece tor or vWF. In a ition, acquire von
have a reva ence as high as 1% in the genera o u ation.
Wi ebran 's isease ay co icate other iseases such as
No racia or ethnic re is osition has been eter ine .
y ho ro i erative an autoi une isor ers, an ro—
Both gen ers are a ecte , but there is a higher requency o
teo ytic egra ation o vWF co icates ye o ro i erative
c inica ani estation in wo en.
isor ers. Variant or s o von Wi ebran 's isease can
be i enti e by their attens o genetic trans ission an
Pa thop hys iology
the vWF abnor a ities in the as a an the ce u ar co -
von Wi ebran 's isease is characterize by abnor a ate—
art ent. Distinguishing between various subty es o von
et unction, ex resse as a ro onge b ee ing ti e. T is is
Wi ebran 's isease is i ortant in eter ining a ro riate
a consistent n ing an ay be acco anie by ecrease
thera y (ab e 28.4).

factor VIII coagulant activity.

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Coagulation Factors, Hypercoagulable State, and Anticoagulant Therapy 555

Characteristics of Various Types of von Willebrand's

TABLE

28.4 Disease

Feature

Type I

Type IIA

Type IIB

Platelet

Type IIC

Type III

Platelet count

N

N

N or ↓

Low N or ↓

N

N

Bleeding time ↑ or N ↑

↑

↑

↑

↑

Factor VIII:C

N or ↓

N or ↓

N or ↓

N or ↓

N

N

vWF:Ag

↓

N or ↓

N or ↓

N or ↓

N

vWF:RCoF

↓

↓

N or ↓

N or ↓

RIPA

N or ↓ ↓ or absent ↑

↑

↓

Absent

vWF, von Willebrand's factor; vWF:RCoF, ristocetin cofactor; RIPA, ristocetin-induced platelet aggregation; N, normal.

vWF circulates in the blood in two distinct coats—

Qualitative or quantitative abnormalities of vWF result in

disorders, with two types of cases being responsible for vWF disorders—

decreased adhesion and are responsible for the bleeding

disorder. Vascular endothelium is the primary source of the

associated with von Willebrand's disease.

synthesis and release of vWF; the other type of case

The significance of vWF in the regulation of VIII:C

that synthesizes vWF is the megakaryocyte. Aroximately

remains unclear. The increase in VIII:C following infusion of

15% of circulating vWF is produced in the megakaryocyte.

urine vWF suggests a possible role of vWF in the synthe—

vWF circulates in platelets, being stored primarily in the
alpha granules, or stabilization of VIII:Ag. Therefore, decrease
alpha granules, in association with factor VIII coagulant
leads to vWF prolonging the rate of blood clotting.

protein (VIII:Ag). Platelet vWF is released from the alpha
granules by various agonists and subsequently binds to the
Clinical Signs and Symptoms

GP IIb/IIIa complex. The site synthesis of VIII:Ag remains
The severity of symptoms among patients with von Willebrand's
unknown, although the liver is thought to play an important
role. Disease varies greatly. Severe cases are not easily distinguish—
role.

abnormality or severe hemorrhage A, in which bleeding
vWF is a large, multimeric, glycoprotein present in
occurs into the joints and skin. Characteristically, in
as a platelet, and subendothelial. It is synthesized as

patients with von Willebrand's disease, the bleeding is usually a large recurrent that
consists of a signet ring, a role -

skin origin, with ecchymosis, epistaxis, and gastrointestinal
tissue (von Willebrand's antigen II), and the vWF subunit. It
bleeding being the most common. Bleeding associated with

has the two main functions of regulating coagulant activity

surgical procedures and oral surgery is a articular disease .

(VIII:C) and acting in ahesion of platelets to subendothelium—

Heterozygous patients may experience severe bleeding, including

hemorrhage associated with surgery. In circulating blood,

high levels of vWF, or von Willebrand factor, are associated with

vWF is associated with a noncoagulant biochemical complex with the

central nervous system hemorrhage.

factor VIII coagulant protein. This complex stabilizes

factor VIII and protects it from degradation in the circulation—

In the revised Classification of von Willebrand's Disease

classification. The vWF protein represents more than 95% of the

type I is the most common variant of von Willebrand's disease and there are
controls the biochemical

ease and appears to be based on a quantitative deficiency of

stereospecificity. The vWF consists of repeating units,

vWF. It is expressed as an autosomal dominant trait and is re—

with the standard circulating unit thought to be a dimer

is due to be caused by an inheritance of one normal allele

or tetramer.

efficient allele. Patients with severe type III disease may have

Circulating vWF undergoes proteolytic cleavage under
homozygous type I (or compound heterozygous) disease. The
physiological conditions; thus, it can be distinguished
on each basis of type I and type III disease is unclear but is
related to vWF, which is not proteolyzed. The pathogenesis of
characterized by decreased circulating levels of vWF. Factor
von Willebrand's disease is based on quantitative or qualitative—
VIII:C is decreased proportionally with respect to vWF.
qualitative abnormalities, or both, of vWF. When an abnormality
Most patients with von Willebrand's disease (50% or
is present, the decreased factor VIII coagulant activity is
more) have quantitative abnormalities and no evidence
attributable to the reduced concentration of vWF.
of a functional abnormality of vWF, which corresponds to
vWF is essential in providing the basis for formation of a
type I von Willebrand's disease and its subtypes. The genetic
normal related thrombus. vWF binds to specific sites on the
transmission of the disease is dominant, except possibly for
related, namely, GP Ib and GP IIb/IIIa, which are concurrent with
subtype I-3. Most patients have low as a level of vWF

binding to the subendothelial collagen, antigen (usually between 5% and 30% of normal) and corresponding. Patients with decreased levels of vWF, showing low levels of ristocetin cofactor activity (the assay especially the larger uterine or s, which approximate reflects the property of vWF to bind to GPIIb/IIIa and mediate bridging action that reduces long bleeding times. platelet aggregation). The factor VIII coagulant protein

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more severe in patients with acquired von Willebrand's disease.

von Willebrand's Factor

ease. Binding mucous membranes is common

TABLE

28.5 Requirements for Primary

and reflects the underlying levels of vWF activity in these

Hemostasis

in individuals. vWF activity is typically 20% or less of normal.

Activity

Interaction

Reaction

Peudovon Willebrand's Disease

Plasma vWF

Subendothelial

Platelet contact

This is a rare disorder in which patients resemble those with deposition interaction

von Willebrand's disease because of low levels or absence of with GP Ib

argely due to low vWF in the plasma. Patients with pseudovon Willebrand's disease have a platelet abnormality

Platelet vWF

Binding to GP IIB/IIIa

Platelet spreading

in which spontaneous platelet aggregation occurs. Low levels subendothelial surface

largely due to result to increase consumption—

Platelet-platelet

Platelet

ing platelet aggregation.

interaction

aggregation

vWF, von Willebrand's factor.

Increased Levels of vWF

Increases of vWF have been associated with stress,

as well as a decrease in proportion to the decrease in vWF. In

inflammation, post-surgical states, pregnancy, renal disease,

these cases, the bleeding is caused by insufficient levels of

insulin, rheumatoid disorders, scleroderma, and Raynaud

circulating vWF and factor VIII. Bleeding manifestations are

hereditary. vWF may be an indicator of vascular endothelial

cell dysfunction in patients who have a normal concentration of

factor status. Drugs such as 1-endo-8-d-arginine vasopressin—

activate vWF than in others (table 28.5).

Desmopressin (DDAVP), steroids, and hormones may also result in

In patients whose vWF shows low ristocetin cofactor

evaluate levels of vWF.

activity, except for those designated as having type B disease,

Laboratory Findings

the vWF has an abnormal tertiary structure and there is

a decrease in or absence of the arginine residues.

The following laboratory results are typical of von

Willebrand disease: type II is characterized by structural abnormal vWF.

Willebrand disease:

The circulating levels of vWF may be decreased or normal,

- Binding site: ability to interact with von

Willebrand factor (vWF) may be affected in type IIA and type IIB

- Platelet retention: typically decreased

are autosomal dominant, whereas type IIC is recessive.

- Platelet aggregation: ristocetin—abnormal

Patients with type III, the most severe form of von

- Platelet aggregation: normal with ristocetin

Willebrand disease, are likely to have a major episode of

- vWF function (ristocetin cofactor activity)

bleeding early in life because significantly decreased amounts

of vWF and VIII:C are routine. Genetically, they are thought

Quantitation of vWF antigen (vWF:Ag) can be performed

to be homozygous or doubly heterozygous. These patients

by immunoelectrophoresis. These assays measure total

probably comprise a separate group because of the typically

amounts of vWF protein, in addition to its ability to function.

recessive or a form of genetic transmission (Table 28.6).

Finally, vWF mutational analysis is useful in distinguishing between subtypes and in determining therapeutic management—

Acquired von Willebrand's Disease

ent. vWF mutational analysis uses sodium dodecyl sulfate

von Willebrand's disease is occasionally seen as an acquired

(SDS) agarose gel electrophoresis and radioimmunoassay to

condition. Associations have been made with uremia—

visualize the different molecular weight mutants.

atoms and other autoimmunizers as well as hypercoagulable disorders. The presence of a circulating antibody

to vWF may be indicated in some cases. Another mechanism

NOTE: This is a good time to review the definitions of Key

responsible for decrease in amounts of vWF in acquired states

Terms in the Glossary and flash cards on

. This is

is the adsorption of the coagulation component onto abnormal—

also a good time to complete Review Questions related to

assessment. Hemorrhagic complications are generally

the preceding content.

TABLE

28.6 Clinical Features of Various Types of von Willebrand's Disease

Type

bleeding Time

bleeding Tendency

Petechiae

Hemarthrosis *

I

Normal or increased

Mild

None

Uncommon

II

Increased

Moderate

Usually none

Uncommon

II

Increased

Often severe

Occasionally

Uncommon

*Only occurs in the most severely affected.

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with a fibrinogenemia or hypofibrinogenemia may even occur

Coagulation Factor Assays for

thrombosis.

TABLE

28.7 Autosomal Recessive Factor

When laboratory assays based on the reduction of a

Disorders

fibrin clot are performed, patients with a fibrinogenemia

may demonstrate abnormal activated partial thromboplastin

Factor

APTT

PT

TT

Fibrinogen

time (APTT), prothrombin time (PT), and thrombin time

Factor I type I

A

A

A

Absent

() assay results. All of these assays are correct in hypofibrinogenemia

including studies with normal plasma. Platelet aggregation tests are

Factor I type I

N

N

A

N

abnormal because fibrinogen is required for primary platelet

Hypofibrinogenemia

aggregation. Confirmation of the diagnosis is by antigenic

and function assays of fibrinogen.

Factor I type 2

N

N

A

Variable

Inherited fibrinogenemia must be differentiated from

Dysfibrinogenemia

acquired conditions. In type II, dysfibrinogenemia, patients

Factor II

A

A

N

N

have normal quantities of fibrinogen but abnormal coagulability.

Factor V

A

A

N

N

Genetic mutations in at least three genes encoding for the fibrinogen—

Factor VII

N

A

N

N

genetic chains (FGA, FGB, or FGG) have been identified.

In cases of dysbrinogenemia, the activated partial

Factor X

A

A

N

N

thromboplastin (AP), thrombin (P), and

Factor XI

A

N

N

N

thrombin () assays are routine. In addition, a

Factor XII

A

N

N

N

colorimetric quantitative fibrinogen assay and reticulate

Factor XIII

N

N

N

N

are abnormal.

Prekallikrein

A

N

N

N

Factor V (Labile Factor)

Fletcher factor

It is autosomal recessive, known as Owren disease

High molecular

A

N

N

N

or abnormal, can exist in mild, moderate, or severe

weight kininogen

or s. Factor V deficiency can exist in a quantitative type 1 or

qualitative type 2 or . The deficiency may be associated with

combined inherited deficiency of factor VIII, but there is no

Uncommon hereditary Clotting Deficiencies

are currently being detected in a combined deficiency.

Autosomal recessive deficiencies of the other coagulation

Laboratory testing. activated partial thromboplastin

actors are relatively rare. Examples of rare defects include

type (AP) and thrombin type (P) are rare but

factor deficiencies including factors I, II, V, VII, X, XI,

the thrombin type () is rare. Abnormalities

XII, and XIII, as well as high molecular weight kininogen

occur in one-third of patients and may be related to a

(HMWK) (Fitzgerald factor) and prekallikrein (Fletcher

deficiency of factor V inhibitor α -granules.

factor). Laboratory screening tests (table 28.7) differentiate

these coagulation factors.

Factor XIII A or Factor XIII B (Fibrin Stabilizing Factor)

These deficiencies are genetic mutations affecting either the

Fibrinogen Group

A or B chains of FXIII. A deficiency is associated with severe

In the fibrinogen group of factors I, V, VIII, and XIII, thrombocytopenia. It may be congenital or acquired in infancy

bin converts a our coagulation factors in this group. They are

inherited. Factor XIII deficiency is associated with spontaneous—

consumption clotting, and as a result, they do not exist

in serum.

Factor XIII functions as a cross-linker of fibrin; a deficiency does not affect the activated partial thromboplastin

Factor I (Fibrinogen)

Factor I (Fibrinogen) is a plasma protein that is essential for blood clotting. It is a large, soluble protein that is synthesized by the liver.

Factor I (Fibrinogen) is a plasma protein that is essential for blood clotting. It is a large, soluble protein that is synthesized by the liver.

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Factor I (Fibrinogen) is a plasma protein that is essential for blood clotting. It is a large, soluble protein that is synthesized by the liver.

deficiency. In a severe deficiency is
 deficiency but is less common than hypofibrinogenemia. Patients
 identify, followed with a mixing study to evaluate. An
 with hypofibrinogenemia are usually asymptomatic except in
 interesting laboratory finding is that a patient's blood clot
 situations of surgery or trauma.
 is so subtle in a 5-M urea solution. If clot lysis occurs in
 Production of dysfunctional coagulation factors -
 initial testing, then the test is repeated using a 1:1 mix
 fibrinogenemia (type II). Patients with hypofibrinogenemia may
 present as a neonate or as a child—
 be asymptomatic or experience a bleeding tendency
 distinguish between FXIII deficiency and a FXIII inhibitor.
 if heterozygous or the effect, or they may have a severe
 Abnormal results should be confirmed with quantitative
 bleeding tendency, if homozygous or the effect. Patients
 testing for factor XIII.

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In an inherited deficiency is identified, a functional assay is

or because of reduction of a dysfunctional enzyme, which
reference. The reference of analysis is chromogenic
is known as type II deficiency state.

assay. A low FXIII does not distinguish deficiency from

Acquired factor X deficiency is common and can be

associated with FXIII autoantibodies.

caused by

Prothrombin Group

■ Lack of vitamin K (so newborns are born with vitamin
K deficiency)

The factors in the prothrombin group (factors II, VII, IX, and
Severe liver disease

X) are vitamin K dependent in order to be functional. A

■

Buildup of abnormal proteins in the tissues and organs

of these factors contain the (gamma)-carboxyglutamic acid –

■

(acylation)

rich region, the GLA domain, that is critical for the calcium-

Use of drugs such as warfarin

binding properties of these proteins.



Factor X may be associated with a combined inheritance

Factor II (Prothrombin)

deficiency of FVII. Three genetic mutations can occur in

Factor II is a rare inherited bleeding disorder. Symptoms can be

caused by mutations in the genes VKCFD, GGCX, and VKORC1. Heterozygotes are

asymptomatic. Although it is inherited as an autosomal

recessive, it may also be acquired. Two types of Factor II deficiency

exist: type I (hypoprothrombinemia) and type II (parahemophilia).

Laboratory findings include decreased activated partial thromboplastin

time (APTT) and prothrombin time (PT), and Russell's viper venom

time (RVVT), which are prolonged. Bleeding

time (APTT) and prothrombin time (PT) are usually within the reference range.

Results of Factor X

time (APTT) and prothrombin time (PT) are usually within the reference range.

Results of Factor X

time (APTT) and prothrombin time (PT) are usually within the reference range.

assays may vary, depending on whether the deficiency is

nor a .

type I or type II. In patients with a type I deficiency, both

Another isomer of prothrombin is the prothrombin

functional antigenic factor X are decreased . In patients

20210a mutation. First reported in 1996 as a familial cause of

with a type II X deficiency, the functional level is decreased

venous thrombosis (VTE), the prothrombin 20210a

and the antigenic level varies from within the reference

mutation results in increased levels of a prothrombin -

range to a decreased level of functional

bin and a concurrent increase risk of the level of factor

factor X.

thrombosis. The exact mechanism of this isomer has not

In a factor X deficiency is the only factor deficiency, assays

been discovered . Prothrombin 20210a has an estimated prevalence of 1 in 1000-2000 in Caucasians. Patients with the prothrombin 20210a mutation have a

prevalence of 2% in whites.

nor a . In a factor X deficiency is due to vitamin K deficiency

Patients with the prothrombin 20210a mutation have a

or the presence of a vitamin K antagonist, the level of other

two-to three fold increase risk of developing thrombosis.

vita in K— and clotting factors (factor II, factor VII, Factor X, prothrombin, fibrinogen) can be inherited without factor IX, factor X, and protein C will be affected.

DNA analysis.

The contact group. As the name implies, this group requires contact with a negatively charged surface for activation.

Factor VII (Labile Factor)

These factors (factors XI, XII, prekallikrein (Fletcher factor))

This autosomal recessive factor deficiency is known as an HMWK (Fitzgerald factor)) are involved in the intrinsic—

Alexander's disease. Symptoms range from asymptomatic coagulation pathway. They are relatively stable and are to inordinately low levels. Spontaneous thrombosis not usually consuming coagulation and can be found in any age group. Low or no factor VII levels are qualitatively—
relative amounts in serum.

type I and quantitative type II. Mutations in factor VII and factor V genes or a combined V and VII deficiency are

Factor XI

LMAN1 and MCFD2.

A deficiency of factor XI is referred to as hemophilia C,
This is the only factor deficiency where only the prothrombin time is prolonged (PTA) deficiency, or
bin time (PT) is prolonged. Standard assay unfunctiona
Rosenthal syndrome, also called Miescher-Merkersson—
an idiopathic ovulation is a definitive diagnosis.

Rosenthal syndrome. This genetic defect is an autosomal
recessive trait that occurs predominantly in the Jewish
Factor X, FX (Stuart-Prower Factor)

Ashkenazi (European) heritage.

This is an autosomal recessive or acquired disorder. This is an asymptomatic or
disorder characterized by

disorder is one of the world's most rare factor deficiencies. It
easy bruising, ecchymosis, and hemorrhage in conjunction with
exists in infancy, moderate, and severe forms. Women with ac—
trauma. Bleeding manifestations in hemophilia C do not cor—
factor X deficiency may first be diagnosed when they have very
related with the factor XI level.

heavy menstrual bleeding and bleeding after childbirth. The
Laboratory screening demonstrates an abnormal activated
condition may be first noticed in newborn boys if they have

arterial thrombostatic (AP) and antithrombotic
being that lasts longer than normal arterial circulation.
binomial (P). The AP is normal in heterozygotes with a
Factor X deficiency may arise because of reduced synthesis—
deficiency. Heparin will interfere with test results. Lu
sis of the protein, which is known as type I deficiency state,
anticoagulants (LA) may interfere with test results.

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The laboratory results in this effect, as well as those of

HMWK-deficient as a fixer with antigen as a P

other hemorrhagic and von Willebrand's disease, are reversed or enhanced to a
standard curve of HMWK

sensitive in table 28.1.

versus AP. Interference occurs in these assays if antigen

is on heparin, hirudin or argatroban, an oral anticoagulant—

Factor XII (Hageman Factor)

role. Lower levels in newborns increase to adult levels by age

A unique characteristic of factor XII deficiency is that

6 months.

no characteristic tendencies are apparent. This autosomal—

a recessive is more common in Asians than other ethnic groups.

DISORDERS OF FIBRINOLYSIS AND

Factor XII, a plasma protein (glycoprotein), is a clotting

CONSUMPTION

factor. Although it is thought that factor XII is necessary for

enhance brinogen osits can result in thrombosis and a -

prothrombotic clotting, when it is deficient, other blood clot—

age to organs owing to ineffective blood flow and ischemia.

Clotting factors are also coagulate or its absence. The isor—

The fibrinolytic system serves as a protective mechanism

and is considered to be benign and is usually asymptomatic.

against excessive brinogen osits by using both brin and

A factor deficiency is usually only accidentally discovered

brinogen.

through routine blood tests.

Blood coagulation factors can be destroyed in vivo by

A factor XII deficiency may be suspected in patients

enzymatic degradation or by pathological activation of

without clinical signs or a previous history of a bleeding

coagulation with excessive utilization of the clotting factors.

is observed when the activated thromboplastin time (APTT)

Enzymatic destruction can result to bites by certain species

or thrombin (PT) is abnormal. Patients with abnormal

of snakes whose venom contains an enzyme that negates

screening test results but with no bleeding symptoms can be

brinogen to a defective brinogen. In vivo activation

screen for antiphospholipid syndrome (APS).

of coagulation by tissue thromboplastin-like arteries can

A diagnosis of factor XII deficiency can be confirmed by

to use excessive utilization of clotting factors. Conditions

a specific factor assay.

associate with this consumption of coagulation factors

Prekallikrein Deficiency

include obstetric complications, trauma, burns, frostbite

and elective surgery, shock, advanced pregnancy, septicemia,

A deficiency of this factor is also called PKK deficiency or

an intravascular hemolysis.

Fletcher factor deficiency. It is caused by homozygous or

compound heterozygous mutation in the KLKB1 gene.

Fibrinolytic activity is associated with an inhibitor

General Features of Fibrinolysis

to the clot-removing activities of plasminogen activators. No

Primary and secondary fibrinolysis are recognized as extreme

types of an abnormal bleeding tendency is noted in patients

with conditions of a variety of intravascular and extravascular

or their activities.

arteriosclerosis may have life-threatening consequences.

Arteriole activation of plasminogen to plasmin

Primary fibrinolysis is associated with conditions in which

(AP) and plasminogen generation but not

gross activation of the fibrinolytic mechanism with subsequent

arteriole (P) suggest that severe revascularization

fibrinogen and coagulation factor consumption occurs. The

deficiency has no clinically significant bleeding tendency or

important characteristic of primary fibrinolysis is that no effect on hemostasis, fibrinolysis, inflammatory responses,

endothelial erosion occurs. Primary fibrinolysis occurs

or leukocyte function.

When large amounts of plasminogen activator enter the circulatory

system as a result of trauma, surgery, or malignancies.

High Molecular Weight Kininogen Deficiency

Although the same clinical conditions may also include

High Molecular Weight Kininogen Deficiency (HMWK) deficiency -

secondary fibrinolysis or DIC, the distinction between the

deficiency is an autosomal recessive coagulation defect. HMWK

two is essential in the demonstration of fibrinolysis.

is known by a variety of names, including Fitzgerald trait,

In secondary fibrinolysis, excessive clotting and fibrinolysis—

Faujeac trait, and Williams trait. HMWK is a rare congenital—

ytic activity occurs. Increase in amounts of fibrin split (e.g.—

thrombolytic inheritance as an autosomal recessive trait. Patients

ration) products (FSPs/FDPs) and fibrinogen levels are

with HMWK deficiency do not have a hemorrhagic tendency.

detectable because of the action of thrombin on the fibrin—

lytic activity, the disorder is discovered in individuals with an iso—

gen defect. This fibrinolytic process is only caused by

activated platelets and tissue plasminogen activator (AP).

excessive clotting; therefore, it is a secondary condition.

Fitzgerald trait represents a “true” deficiency of HMWK,

Distinguishing between primary and secondary fibrinolysis

but Factor VIII is a serine protease that cleaves kininogen

(Fig. 28.8) is important in treatment.

Deficiency, in which both HMWK and low molecular weight kininogen (LMWK) are deficient. HMWK and LMWK are

Disseminated Intravascular Coagulation

both encoded by the KNG1 gene.

Laboratory findings include disseminated intravascular coagulation

Etiology

arterial thrombotic (AP) and negative uterus anti—

DIC is actually a complication or interrelationship of

coagulant (LA). A mixing study to test for deficiency uses

any diseases and does not constitute a disorder in itself. It

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1. Initiation of the intrinsic clotting cascade resulting in

Selected Characteristics

thrombin formation

TABLE

28.8 of Primary and Secondary

2. Participation as a cofactor for the conversion of prothrombin -

Fibrinolysis

ikrein to ka ikrein

3. Initiation of fibrinolysis

Laboratory

Primary

Secondary

Test

Fibrinolysis

Fibrinolysis

Regardless of the initiating event, DIC is characterized

by excess thrombin formation, conversion of fibrinogen to

Platelet count

Normal

Decreased

fibrin, and platelet consumption and aggregation. Secondary

Protamine sulfate

Negative

Positive

fibrinolysis occurs as a result of fibrin aggregation and can

test

decrease as a coagulation factors, leading to a hemorrhage—

Fibrin split products

Increased

Increased

hemorrhagic diathesis.

Fibrinogen

Decreased

Decreased

Thrombin is central to the mechanism of consumptive

coagulation. The action of thrombin on the coagulation

systems includes the following:

is also known as consumptive coagulation or fibrinolysis

1. Proteolytic cleavage of fibrinogen to fibrin monomer,

synthesizing events that may release substrates to

release fibrinogen to fibrin (fibrin monomer may

DIC include alterations in the endothelium, direct activation

or substance coexists with fibrinogen or fibrin

of fibrinogen, release of thrombolytic-like substances, and

thrombin that entraps platelets during thrombolytic action).

erythrocyte or platelet destruction. Extravascular trauma,

2. Activation of factor XIII, which stabilizes fibrin by
fibrinolytic activity, a fibrinolytic fibrinolytic activity, and
cross-linking.

(HELLP) syndrome/ectasia, advanced pregnancy, and—

3. Stimulation of platelets, resulting in decreased circulating
platelets, and platelet activation are examples of—

platelets. These stimulated platelets undergo shape change,
in situations in which tissue thromboplastin can activate
adhesion, aggregation, and secretion. The contents of the
coagulation. Infections, especially Gram-negative
bacterial pathogens are released, leading to an acquired
microorganisms, can trigger DIC by releasing endotoxins
storage deficiency. Following surgery a 3-hour span,
that exposure to agents. Stasis, shock, or tissue necrosis can
platelet counts and fibrinogen levels decrease significantly
have the same effect. Snakebites may introduce substances
in a critically ill patient, DIC should be the first suspect
that initiate coagulation by direct activation of fibrinogen to
as the cause of this change.
or fibrin.

4. Activation of factors V and VIII; however, thrombin acti—

Rebound coagulopathy may contribute to the

activation results in unstable products that have decrease

consumptive coagulopathy by releasing prothrombin that

factor V and VIII activity.

accelerate coagulation. Rebound injury may be a result of

5. Activation of protein C, which degrades factors V and

intravascular thrombosis caused by a variety of

VIII.

thrombotic products, and other clinical states. Platelet

The deposition of fibrin thrombi in the vasculature, ri—

struction also releases coagulation factors V, VIII, XII,

artery in the microvasculature, initiates fibrinolysis. T is

an XIII.

secondary fibrinolysis is responsible for the hemorrhagic

Other causes of DIC can include liver disease, y ho -

coagulation of DIC.

regenerative disorders, and renal disease. In addition, DIC can

When the fibrinolytic system is activated, plasminogen is

also be triggered by traumatic injury, hypothermia,

convert to fibrin. A ha-2 anti fibrin is the fibrinolytic
an extensive tissue damage, such as in myocardial infarction—
inhibitor uniquely designed to compete with fibrin. The ore
tion and activation. It has been associated with utilization
as in generation, the ore a ha-2 anti fibrin in the patient
surgical, obstetric, and endocrinological. Coagulation con—
sumes. This results in a vicious cycle in which increase
in fibrinogen can result.

activation leads to decrease inhibitors; this, in turn, allows
fibrinogen increase activation to continue. This is known as a
Pathophysiology

positive feedback loop that leads to a situation incoherence
The overall DIC process involves coagulation factors, platelets—
with fibrin.

platelets, vascular endothelial cells, fibrinolysis, and as a
Damage tissue, especially renal cells, releases plasmin—
inhibitors. This is a breakdown of the hemostatic mechanism—
gen activators that convert plasminogen to fibrin. Plasmin
occurs when the procoagulant factors outweigh the
is a fibrinolytic enzyme that destroys fibrin, fibrinogen, and

anticoagulant mechanisms.

coagulating factors V and VIII. Circulating as in any case to

Initiation of DIC can be caused by a number of factors. In

systemic fibrinolysis, causing increased hemorrhagic events.

vascular endothelial damage results in the exposure of co-

In the microcirculation, as in's action is primarily

against the basement membrane, coagulation can activate factor

directly against fibrin. In the circulation, the breakdown of

XII. Factor XII has multiple roles in the direct or indirect

fibrinolysis in FSPs, namely X, Y, D, and E, which inhibit

activation of coagulation including

thrombin and platelet function.

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As fibrinogen is degraded by plasmin, FSPs or .

The Role of Factor VIII

Degradation occurs whether the plasmin comes from DIC or

A very close relationship exists between factor VIII:C (pro-

thrombinogenolysis. FSPs compete with regular fibrino-

coagulant) and factor VIII:CAg (procoagulant antigen). In

genolysis or thrombolysis. This competitive bin-

DIC, it is believed that the VIII:C_{Ag} is inactivated to a lesser extent than VIII:C by enzymes released during the conversion of fibrinogen to fibrin. In this situation, patients with high FSP It is known that factor VIII:C activity is destroyed by in— even have a circulating anticoagulant behaving like heparin. I estimate amounts of fibrin, as in, an activated protein C the FSP even is high, the fibrin clotting time is significantly (aPC).

prolonged fibrinogen quantitation is slow. The second effect It is strongly suspected that the in vivo inactivation is on platelets. These sites probably coat the platelet surface, so VIII:C found in DIC is related to the degree of severe— blocking the receptor site needed for further platelet activation. ity of DIC. Furthermore, low values of factors VIII:C and When pathologic fibrinolysis occurs, not only are ac— VIII:R_{Ag} and factors VIII:C and VIII:R_{CoF} found in tors destroyed, but, through the destruction of fibrinogen, patients with irreversible shock indicate a grave clinical out— a profound anticoagulating effect inhibits secondary hemostasis

co e. Discre ancies are a so known to exist between VIII:C
an ate ets.

an VIIIR:Ag in atients with thro boe bo ic isease. Such

I the brino ytic syste is activate , it wi contribute to

ratios are use u in icators or assessing the severity o DIC.

the consu tion o any coagu ation actors. P as in, the

Current thinking in icates that ata on the actor VIII co -

ri ary roteo ytic enzy e o brino ysis, irect y attacks

ex show that the og a o a characteristic ecrease o the

an estroys the . T is beco es another or o consu -

actor VIII rocoagu ant activity in DIC or u ate in the

tive coagu o athy originating ro an entire y i erent

ast is not genera y va i .

source with the sa e en resu t.

When syste ic c otting activation begins, the bo y usu—

Th e Role of Prote in C

a y atte ts to sto it. T e two ajor inhibitor syste s o

Protein C (PC) is a ajor regu atory echanis o he ocoagu ation are antithrombin
(A) an the rotein C (PC)

stasis. In a ition, PC is now recognize as aying a crucia

an protein S (PS) syste s. T ese inhibitors are consu e

role in the pathogenesis of acute and chronic inflammation in the DIC process. Therefore, the compensatory mechanisms, or exuberant sepsis or asthma. When inflammation is often unable to stabilize the consumptive process. occurs, coagulation is a so set in motion and active participation. Coagulation factors and platelets are consumed rapidly and in enhancing inflammation.

than they can be replaced, Activators are effective, and the PC is a vitamin K-dependent serine protease that is synthesized in the mononuclear phagocytic system cannot effectively synthesize, predominantly in the liver, as a single entity overcome the activated coagulation proteins.

chain of 461 amino acids and is a natural anticoagulant protein. The conversion of PC to activated PC (aPC) is enhanced

Alternative Forms of DIC

by interaction of PC with endothelial PC receptor on the cell

Acute DIC presents in one of several forms in which a surge. Activation can also be triggered by thrombin in a patient's clotting and/or fibrinolytic system is suddenly activated at a less efficient rate and is probably not relevant in the circulate throughout the body. In essence, it is a systemic thrombotic

coagulation. The function of aPC as an anticoagulant is an—
ological process. Because two types of systems are involved,
este by its ability to inactivate two important coactors of
the clotting and/or the fibrinolytic system, several types of
the coagulation cascade: factor V/Va and factor VIII/VIIIa.

DIC can be initiated clinically:

These events are enhanced by the presence of Ca^{2+} , histone, and coagulation protein S.

1. DIC: Clotting and fibrinolysis strongly activated (post-cholesterol)

Other functions of aPC in hemostasis are maintaining
thrombolytic activity).

a functional blood. aPC has the ability to self-regulate

2. DIC: Clotting reinitiates with little or no fibrinolysis (thrombin and
thrombin-activated—
thrombolytic).

abnormal fibrinolytic inhibitor, which indirectly promotes fibrinolysis

3. Primary fibrinolysis: On fibrinolysis activated, but any
fibrinolysis. Fibrinolysis is a self-regulated because of the ability
coagulation factors consume.

of aPC to inhibit plasminogen activator inhibitor-1 (PAI-1).

In the usual case of DIC, the patient's clotting system

The inhibition of fibrinolytic activity by the PC system

the fibrinolytic system are activated. Patients are systematically activate the clearance of excess fibrin generation

causing thrombin, which, in turn, converts fibrinogen

to FPs. If APC is being consumed too rapidly, the regulatory

to fibrin. In most instances, the simultaneous generation of

ability of the PC system is sharply reduced, which results in

as in disseminated fibrin. Both the clotting and fibrin—

uncontrolled thrombosis.

fibrinolytic states are occurring at abnormally high rates. If clot

Thrombotic complications occur in patients with

thrombosis does not occur, a different form of DIC exists. In this

hereditary deficiencies of protein C (less than 60% or less of

case, the prognosis is very poor. A third type is represented

by neonatal purpura fulx in infants

by a state in which the infant reportedly has fibrinogen—

born with a homozygous PC deficiency. The studies that can

show disseminated intravascular fibrinogenolysis. Coagulation

in DIC may ultimately result in abnormal levels of PC.

Factors are regulated by the excess fibrin being generated.

Both normal and abnormal levels of PC antigen can be found,

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depending on the severity relative to the onset of DIC.

The hemorrhagic and thrombotic complications may accompany

Plasma levels of PC antigen and activity have been found to

DIC, often being elevated in the same patient. Thrombosis

may decrease in patients with DIC. Whereas thromboses

may be observed in chronic or low-grade DIC. Thrombotic

DIC patients have a decrease in PC antigen, and most a DIC

complications can include deep venous thrombosis.

Patients have a decrease even of PC activity. Monitoring

Acute DIC is severe and often life threatening. Its onset

patients reveals that PC antigen and activity decrease rapidly, and both fibrinogen and platelets may be depleted.

Progressive during the initial stages of DIC and remain at a low

Patients with chronic DIC may have intermittent

every 24 to 48 hours before gradually returning toward

normal or be recognizable only by laboratory data.

normal in nonfatal cases.

Hemorrhagic complications are also seen but are generally

less than in acute DIC.

The Role of Thrombin

Clinical manifestations of DIC include petechiae, purpura,

Mechanisms involved in DIC result in the generation of

purpura, hemorrhagic bullae, surgical wound bleeding, trauma—

thrombin in the circulating blood. Along with any feedback

in wound bleeding, venipuncture site bleeding, arterial

reactions, thrombin participates indirectly in the activation

of coagulation, an subcutaneous hematoma.

of the fibrinolytic system secondary to DIC and activates

Thrombotic thrombocytopenic purpura (TTP) is a con—

dition. The latter reaction is accelerated by the presence

of a condition that is similar to DIC (Tables 28.9 and 28.10). In

of the endothelial cell coagulation, protein S.

in addition, the iatrogenic respiratory distress syndrome, a ut

In addition to cleaving fibrinogen and releasing its

respiratory distress syndrome (ARDS), the fibrinolytic uretic

other procoagulant functions, so of the excess thrombin

syndrome (HUS), reestablishes or reestablishes, circu—

bin to protein S on the endothelial cell surface. This event

ing in the coagulation, cavernous hemangioma, and Rocky

leads to increase levels of aPC in the plasma. Once the generation of excess thrombin is decreased by the action of aPC, the coagulation process can return to normal. This negative feedback mechanism has

been demonstrated in animal models of DIC.

Laboratory Findings

In addition to the coagulation process, the fibrinolytic system can return to normal. This negative feedback mechanism has

been demonstrated in animal models of DIC.

Although the quantitative measurement of fibrinogen and

the potential to show the formation of excess thrombin and fibrinogen (FSPs) cannot distinguish between primary and secondary DIC.

Any fibrinogen assay, such as the measurement of fibrinogen in

diagnosing and monitoring these conditions. Laboratory

diagnosis of DIC requires the availability of tests that are

Clinical Signs and Symptoms

are indicative of DIC. There is no single test that can detect DIC; however, a combination of clinical and laboratory

findings is required for diagnosis but rather a combination of tests. Because

of the clinical manifestations (Box 28.1) owing to the pathophysiology—

DIC is a systemic process, values for tests are often

abnormalities associated with the syndrome. DIC may

be either acute or chronic, whether primary or secondary, cannot be used as

be acute or subacute (chronic). Chronic DIC is more common

diagnostic indicators. Sequential testing is necessary to distinguish acute DIC but is often more difficult to diagnose.

view an accurate diagnosis and effective management therapy.

Chronic DIC can convert to acute consumption in the absence

The most important consideration in the treatment of DIC is

the administration of anticoagulant therapy.

the resolution of the underlying disease or triggering event.

Either or may initially be seen with varying degrees

DIC is a disorder of blood clotting characterized by

thrombosis and hemorrhage, but bleeding is usually

hyperfibrinogenemia, a prolonged thrombin time (TT), and

an increase in fibrinogen degradation products (FDPs). Both

the presence of D-dimers.

Comparative Test Results in

BOX 28.1

Diagnosing Various Forms

TABLE

28.9 of Acute Consumptive

Significant Laboratory Findings in

Coagulation Pathway

Diss em inate d Intravascular Coag ulatio n

Clotting and

PERIPHERAL BLOOD S MEAR—FRAGMENTED RBC

Te s t

Lys is

Clotting

Lys is

P ate et count— ecrease

Fibrinogen

Decreased

Decreased

Decreased

Fibrinogen eve s— ecrease

T ro bin ti e— ro onge

Platelets

Decreased

Decreased

Decreased or

Re ti ase ti e— ro onge

normal

AP and P — prolonged

Fibrin split

Positive

Negative

Positive

Fibrin split products (FSPs)—resent

products

Ethanol gel rotation sulfate test—positive

Fibrin

Positive

Positive

Negative

Other tests—euglobulin clot lysis time, antithrombin,

monomers

coagulation factor assays, anisotropy even abnormal

D-Dimer

Positive

Negative

Negative

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Disseminated Intravascular Coagulation Compared with Thrombotic

TABLE 28.10 Thrombocytopenia Purpura

Micro pathological

Clinical Manifestations

Laboratory Abnormalities

Findings

TTP

Unexplained fever; central

Thrombocytopenia; FDP/FSP mildly

Microvascular thrombosis

nervous system dysfunction; renal

elevated in 50%; hemolytic anemia with and

with impaired fibrinolysis

failure in 11%

schistocytes fragmented red cells

DIC

Fever; hypotension; hemorrhage;

Thrombocytopenia; anemia; schistocytes

Microvascular thrombosis;

thrombosis; shock

and fragmented red cells; elevated FDP/FSP

brin deposition; active

brinolysis

TTP, thrombotic thrombocytopenia purpura; DIC, disseminated intravascular coagulation; FDP, brin degradation products; FSP, brin split products.

ests or Fibrinolysis and DIC

FSP test can distinguish between ec a sia an hy er—

Because the ani estations o brino ysis an DIC are

tension an e e a associate with regnancy.

extre e y variab e, iagnosis e en s on aboratory testing.

Coagu ation assays such as the ate et count, brinogen ev—

Note: This is a good time to complete Review Questions

e s, FSP test, actor V assay, an thro bin ti e-re ti ase test

related to preceding content.

can a be use u . Preka ikrein an antithro bin (A) have

a so been suggeste to be o rognostic va ue. T e key eature

is an e evation o circu ating brinogen-FSPs.

y ica resu ts in DIC inc u e ro onge activate ar-

HE HYPERCOAGULABLE

tia thro bo astin ti e (AP), rothro bin ti e (P),

HROMBOPHILIA

antithrombin time () and an increase in fibrinogen.

Fibrinogen levels and the total platelet count may vary,

So disorders of the hypercoagulable state, thrombophilia, are

although thrombocytopenia and a decrease in fibrinogen are

inherited from one or both parents. Examples of thrombo-

coagulopathy. The platelet count decreases earlier than fibrinogen—

thrombophilia include Factor V Leiden, Prothrombin gene

mutation, antithrombin deficiency, protein C deficiency

and tissue factor release is responsible, such as in obstetric complications

and protein S deficiency. Examples of acquired thrombo-

coagulopathy include excessive fibrinolysis with the release of

thrombophilia include antiphospholipid syndrome (APS) and

thrombotic thrombocytopenic syndrome (TTPS) occurs secondary to intravascular—

hyperhomocysteinemia.

antithrombin generation. Although the presence of FSPs is charac-

teristic, the finding has long been recognized as being

associated with hypercoagulability. It commonly occurs in

use as the sole criterion for diagnosis.

patients with DIC in severe sepsis. Recently, the occurrence

basis of the influence of infection has been recognized.

Disorders Related to Elevated Fibrin

Most of the hypercoagulable effects of infection are

Split Products

mediate by inflammatory cytokines, including IL-1, IL-6,

and tumor necrosis factor (TNF).

The normal level of serum fibrin split products (FSPs) is less

than 10 µg/L. Serum values can vary owing to exercise or

stress. Elevated urinary levels are a way of indicating a

renal disease. There is interaction between

these systems. Thrombosis and coagulation can act as

triggers for infection, and severe or systemic infection

can lead to renal dysfunction. High levels of FSPs indicate

renal dysfunction, and severe or systemic infection. Normal urinary FSP values are generally less than 0.25 µg/L.

Renal responses can trigger coagulation. A laboratory assay,

but may rise to as high as 50 µg/L in certain kidney disorders.

High-sensitivity C-reactive protein (hsCRP), a marker of

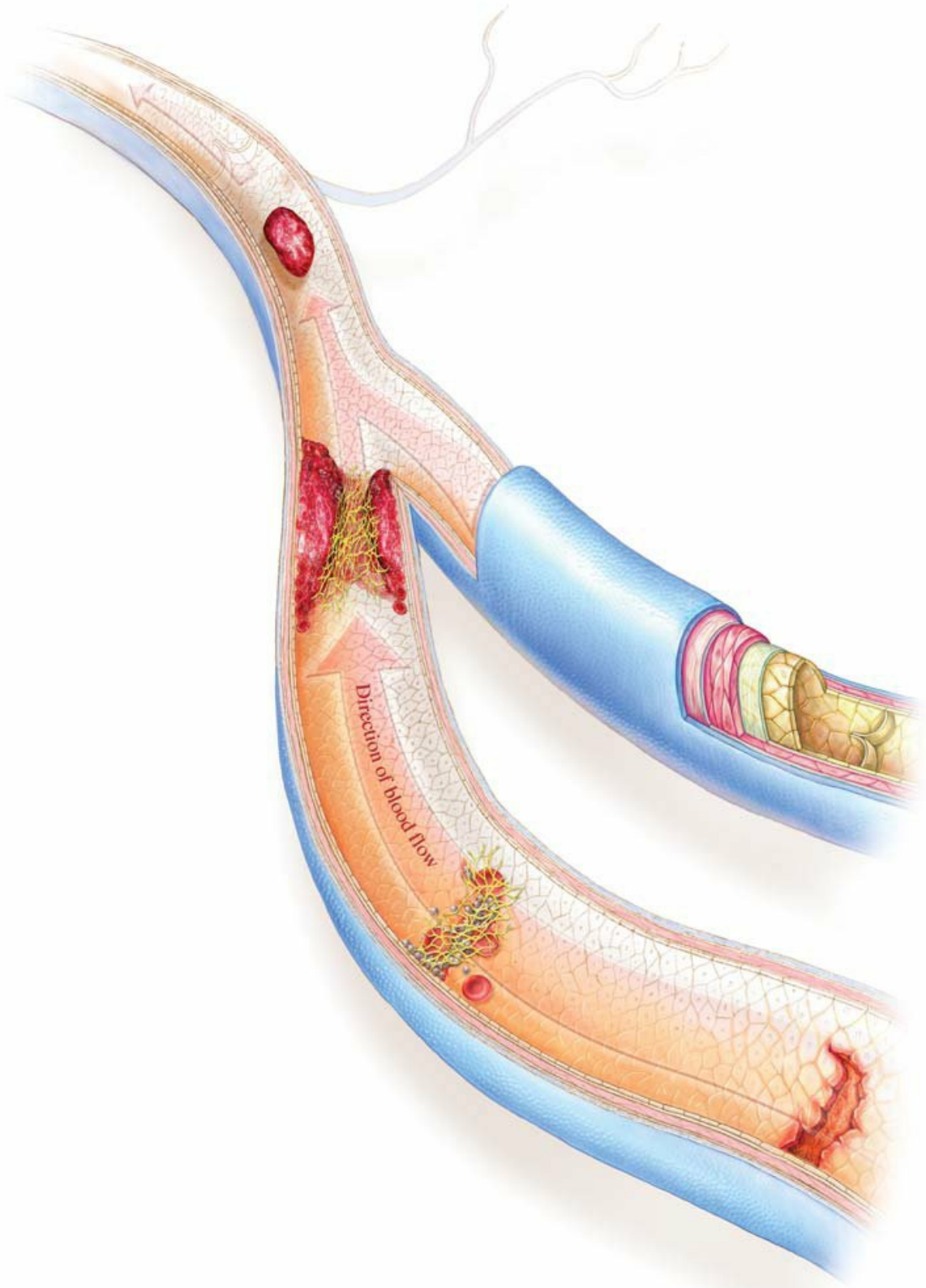
Elevated levels of fibrin split products (FSPs) can be found

in an acute thrombotic event.

In diseases of the neonate, infection, or in the DIC that these

Thrombocytopenia (Figs. 28.2 and 28.3) because coagulation conditions may generate. In cases of primary fibrinolysis, fibrinolysis is enhanced or because protective devices such as catheters can exceed $100 \mu\text{g/L}$; however, in rare cases, values of fibrinolysis are higher. An increase in the fibrinogen level can reach more than $400 \mu\text{g/L}$. These excessive and high levels of fibrinogen are referred to as the **hypercoagulable state** and return to near normal within 24 hours after the cessation of (thrombophilia).

of the disorder (e.g., sepsis). FSP levels are elevated, and thrombosis is promoted by vascular damage, by retention of fibrinogen as high as $80 \mu\text{g/L}$, in cases of chronic intra-abdominal flow, and by alterations in the blood that increase the vascular coagulation, which occurs when the placenta shows fibrinolysis clotting. A variety of high-and-low-incidence releases of thrombotic substances into the circulation. These disorders are associated with thrombosis (Box 28.2).



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thromb

Exte rn

c

T

Tunic

medi

Inte rn

Ve nous

Tu

thrombus

(e ndothe lium)

(blood clot)

lve

Clumping of:

in

blood cells

blood vessel's inner

lining

FIGURE 28.2 Formation of a venous thrombus. The illustration below shows the formation of a venous thrombus.

Acquired conditions can also contribute to a risk factor or recurrent, acute, or juvenile deep venous thrombosis or thrombophilia (Box 28.2).

occurrence in an unusual location such as a mesenteric, branchial, or cerebral vessel.

Primary States of Hypercoagulability

Secondary States of Hypercoagulability

Hypercoagulable states include various inherited and acquired clinical disorders characterized by an increased risk. Secondary hypercoagulation states may be seen in a number of thrombotic disorders.

number of heterogeneous disorders. In any of these conditions—

Primary hypercoagulable states (Table 28.11) include reactions, endocrine

activation by cytokines leads to the loss

of many rare inherited conditions that lead to disorders of the normal vessel wall
anticoagulant surface functions, with

the increase in thromboregulation. These conditions include

conversion to a proinflammatory thrombogenic phenotype.

Decrease in thromboregulation—increased activation of APC,

Important clinical syndromes associated with substantial

inherited hyperinflammation of antithrombin (AT), or even—

thrombotic events include the APS, hyperinflammation

regulation of the brane-associated as in generation.

thrombotic, hyperinflammatory syndromes, and cancer.

These inherited inhibitor disease states include AT

Hypercoagulability can be associated with systemic inflammation—

deficiency, protein C deficiency, and protein S deficiency.

ation usually leads to an increase in procoagulant functions,

These conditions should be considered in patients who have

an inhibition of fibrinolysis, and a down-regulation of the

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ARTERIAL

VENOUS

THROMBOSIS

THROMBOSIS

Hypercoagulability

F

O

Qualitative

S

Activation of coagulation

D

E

I

O

platelet

Abnormal

T

I

O

L

abnormalities

fibrinogen

A

BL

Deficient

M

E

fibrinolysis

R

O

TH

N

Platelet

Deficiency of

activation

Thrombocytosis

physiologic

AB

inhibitors

F

Deficie nt

O

clea ra nce

S

W

E

Turbule nce

I O

L

T

I

F

L

A

D

Hype rvis cos ity

S ta s is

M O

R

O

Hypertension

O

BL

N

AB

Venous

F

O

hypotonia (pregnancy)

L

S

Trauma

L

E

A

I

Erosion

T

I

W

L

Arteriosclerosis

L

A

E

M

S

R

S

Deficient

O

VE

fibrinolysis

N

AB

FIGURE 28.3 Pathophysiology of thrombosis. Factors indicated in the pathogenesis of arterial thrombosis (**left**) and venous thrombosis (**right**) are indicated. Examples of disorders leading to platelet activation and arterial throm-

basis include atherosclerosis, the hypercoagulable disorders, heparin-associated thrombocytopenia/thrombosis syndrome, thrombotic thrombocytopenic purpura, and certain antithrombotic therapies. Examples of disorders include—

including venous thrombosis in the category of deficiency of physiologic inhibitors include the inherited disorders, factor V Leiden, protein C and S deficiencies, and antithrombin deficiency. Many patients with thrombosis may have more than one of the risk factors listed. Estrogen therapy is a risk factor for venous thrombosis; its use is associated with activation of coagulation. (Source: Greer JP, et al. Wintrobe's Clinical Hematology, 13th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2014.)

BOX 28.2

BOX 28.3

Thrombotic Disorders

Acquired Risk Factors for Thrombophilia

Inherited

Advancing age

■ Factor V Leiden resistance antithrombin

Immobilization

Surgeries—High-incidence

Pregnancy

■ Prothrombin gene mutation—Low incidence

Homocysteinemia antithrombin therapy

■ Antithrombin deficiency—Low incidence

Oral contraceptives

■ Protein C deficiency—Low incidence

Maternal factors

- Protein S deficiency—Low incidence

Leukemia

Chronic inflammation

Acquire

Surgery

- Antithrombotic therapy—High incidence

Autoimmune disorders

- Hyperhomocysteinemia

Paroxysmal nocturnal hemoglobinuria (PNH)

566

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Risk factors for developing hypercoagulability include

Primary and Secondary

TABLE 28.11 Hypercoagulable States

- History of thrombosis

- Inherited and acquired thrombophilia

Primary

- Maternal age less than 35 years of age

Hypercoagulable

S e condary

- Certain e ica con itions an /or co ications o reg-

S tate s

Hype rcoag ulable S tate s

nancy an chi birth

Antithrombin II de ciency

Cancer

General Fe atures

Protein C de ciency

Pregnancy

Protein S de ciency

Oral contraceptive use

Va scula r Da m a ge a n d Blood Flow

Fibrinolytic abnormalities

Nephrotic syndrome

Vascu ar en othe ia a age ex oses circu ating b oo to suben othe ia structures that initiate thro bosis. Constriction

Hypoplasminogenemia

Myeloproliferative disorders

o b oo vesse s a itiona y creates stasis. T ro bosis can

Dysplasminogenemia

Hyperlipidemias

begin in areas of low blood flow or in situations in which the

Tissue plasminogen activator-Diabetes mellitus

viscosity of blood is increased. In patients with a high risk of

thrombotic release deficiency

thrombosis, the concentration of fibrinogen is often elevated.

Paroxysmal nocturnal

High concentrations of fibrinogen may increase aggregation

hemoglobinuria

of circulating erythrocytes, which reduces blood

viscosity. This may encourage thrombosis by decreasing the

Increased levels of

Postoperative states

blood flow at critical sites with the accumulation of activated

plasminogen

clotting factors.

Activator inhibitor

Vasculitis

Dysfibrinogenemia

APS

Platelets

Homocystinuria

Increased levels of factor VII

Stasis makes it easier for platelets to be detached from—

and fibrinogen

in blood. An increase in the number of circulating platelets

Heparin cofactor II

Anticancer drugs

may create a tendency toward thrombosis. Platelets accumulate

at the site of vascular damage, where they can furnish

substrate for the intrinsic pathway and also for

Increased levels of

Heparin thrombocytopenia

thrombin formation by absorbing activated factor X on

histidine-rich GP

as a to their surfaces. High platelet counts are usually

Obesity

favor thrombosis.

Another possibility is that a thrombotic tendency may be

three major physiologic anticoagulant systems: protein C,

cause by qualitative alterations in platelets. These alterations
antithrombin and tissue factor inhibitor.

may be caused by intrinsic platelet effects or by changes in
the surrounding plasma. Qualitative abnormalities may

Pregnancy-Associated Thrombosis

result in spontaneous aggregation, enhance sensitivity to

During pregnancy beginning at the time of conception is
aggregating agents, or increase adhesiveness.

associate with increased concentrations of coagulation factors VII, VIII, and X and
vWF. In addition, a significant change

Blood Clotting Factors

in fibrinogen is noted. Free protein S, the active, unbound

Congenital and acquired hypercoagulable states arise when

or, is decreased during pregnancy. Plasminogen activator

there is an imbalance between the anticoagulant and pro—

inhibitor (PAI)-1 levels are increased. Plasminogen

thrombotic activities occur in which the thrombotic

activator inhibitor (PAI-2) produced by the placenta increases

activities relative.

significantly during the third trimester. Thrombin generation

A tendency toward thrombophilia (abnormal thrombo—

markers, or example, prothrombin F1+2, antithrombin—

sis) may be caused by qualitative alterations in blood clot—

antithrombin complexes are also increased. It may take up to

ting factors or an increased titer of activating factors

8 weeks after delivery (postpartum) or the event of the event

that can create a tendency toward thrombosis. These factors

constituents to return to the reference range.

can contribute to thrombosis in that activating factors might

Pregnant women have an increased risk of thromboembolism—

reach critical events in the circulating blood.

is because of hypercoagulability. The condition of hypercoagulability in pregnancy is usually a favorable event

Factor V (Leiden)

women against the bleeding changes of childbirth or is the Factor V gene is an autosomal, co-dominantly inherited

carriage. Pregnant women are at a four-to five-fold increase

gene. Factor V R506Q (Leiden) mutation is the most common

risk of thromboembolism during pregnancy and the most common

on an underlying genetic cause of thrombophilia (e.g., venous

thrombophilia compared to non-pregnant women. Eighty percent

thrombosis).

of the thrombotic events in pregnancy are venous with

Factor V (Leiden) mutation results in a G-A point

an incidence of 0.49 to 1.72 per 1,000 pregnancies.

mutation that results in an Arg506-Gly substitution in the

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protein. This mutation renders factor V resistant to the activation—

by other conditions (e.g., usual anticoagulant, decrease activity of aPC and increases a defect in the natural anticoagulation

factor VIII and fibrinogen levels, oral contraceptive use, or

system. The overall effect of this mutation is an alteration in

pregnancy).

the anticoagulant properties of factor V.

Another method of testing for the mutation is by a

Factor V, like fibrin, possesses both anticoagulant and

intrinsic Russell's viper venom time (DRVV)-base test. The

hypercoagulant properties. The activated protein C-sensitive

DRVV method avoids limitations inherent in the activated

clotting time, either on factor V, transform it into an activation—

partial thromboplastin time (APTT) base method, which

activated protein C cofactor (FV_{ac}). FV_{ac} acts in unison with

requires a normal baseline APTT may be affected by
activated protein C and protein S to increase the rate of inactivation—
high concentrations of factor VIII, a natural anticoagulant, and
activation of factor VIII.

anticoagulant therapy. The DVT also eliminates the tech—
In contrast to other coagulation factors, factor V (Leiden)
nucleic acid sequence variations with factor
poses a lifelong risk of deep venous thrombosis with a greater
V—deficient state.

frequency of occurrence of thrombosis in the lower limbs than
in the chest. Fortunately, everyone who has the mutation
Genetic Testing

will not suffer a thrombotic event. Heterozygotes have a low
Single nucleotide polymorphisms (SNPs) are major contributors
(approximately 10%) to the risk, but homozygotes can
mutators to genetic variation, contributing approximately 80%
experience a 50-to 100-fold increase in risk.

of a known polymorphism. Their density in the human
Prothrombin Gene Mutation

genome is estimated to be on average 1 per 1,000 base pairs.

Activated protein C-resistant patients may be considered for
Prothrombin gene mutation (prothrombin 20210a) is the second—
factor V (Leiden) mutation by DNA PCR a indication of a
most common cause of inherited thrombophilia in the
segment of the population affected gene. General mutation
United States. It is present in about 2% of Caucasians. About
screening is not recommended. At this point, the recommend—
one percent of patients with this prothrombin gene change
mutations or testingocus primarily on individuals younger
are homozygous, which significantly increases up to 50 times
than age 50 who have a ready have an idiopathic thrombotic
higher risk of thrombosis.
event.

Rarely, the prothrombin gene change is inherited alone
Prothrombin 20210a mutation leads to an increased risk
with factor V Leiden. If a patient has a genetic prothrombin
of cerebral vascular thrombosis. Methylenetetrahydrofolate
mutation and factor V Leiden, the onset of thrombosis may
thrombolytic (M HFR) is a protein that breaks down blood clots—
be earlier in life or more severe.

tein. Deficiency of M HFR can cause hyperhomocysteinemia—

Laboratory Assessment

emia. M HFR deficiency leads to hyperhomocysteinemia

that may injure the vascular endothelium. It may also play a role

A panel of assays is required to assess hypercoagulability.

in VTE.

The activated protein C resistance test is a blood test that can

Three assays can be performed simultaneously by analyzing—

be used to detect factor V Leiden. Functional screening tests

using genomic DNA in heterozygous heterozygotes

include the following:

using polymerase chain reaction (PCR).

■ Prothrombin time (PT)

The three most common assays used to investigate a

■ Activated partial thromboplastin time (APTT)

genetic predisposition to thrombosis are

■ Lupus anticoagulation (LA) screening

1. Factor V (Leiden)

■ Factor VIII and fibrinogen (factor I) assays

2. Prothrombin 20210a mutation

■ aPC assay

3. Methylene tetrahydrofolate reductase enzyme (MTHFR)

■ Protein C and Protein S assays

■ Dimer screening test

Circulating Inhibitors

In addition, acute-phase reactants (e.g., C-reactive protein) are three different types of inhibitors:

protein [CRP]) may be assayed.

1. Inhibitors directly against specific factors such as factor

rationally, the activated protein C resistance assay

VIII

identifies latent insensitivity to activated protein C. The

2. Nonspecific inhibitors such as LAs

assay is based on the activated partial thromboplastin time

3. Anticoagulants such as heparins, on a heparin, aprotinin,

(AP) assay with and without reagent activated protein C.

trans, and other direct thrombin inhibitors (discussed later)

The AP in the presence of aPC (CaCl₂/aPC) is given

in this chapter)

by the unactivated (CaCl₂) AP to give a unitless ratio. A

ratio of greater than 2 (a longer clotting time) generally

Acquire inhibitors of clotting proteins, also known as inhibitors of the clotting cascade. A ratio of less than 2 (a circulating anticoagulant, inactivate or inhibit the usual shorter clotting time) indicates a potential factor V (Leiden) anticoagulant activity of coagulation factors. Inhibitors are mutation and resistance to aPC. Factor V—efficient as a reagent y characterize as specific, those directed against a may be a reagent to the test system to correct or any existing coagulation factor, or nonspecific, those directed against a factor deficiencies. The aPC resistance assay may be a reagent co-existence of factors, such as the LA.

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The majority of these inhibitors exhibit biochemical properties, suggesting that they are immunoglobulins. Inhibitors during the postpartum period, most frequently after the may arise following transfusion of blood products or in birth of their first child. Patients with underlying conditions with no previous hemostatic disorders. Acquire no organic disorders such as rheumatoid arthritis, systemic

inhibitors can be a significant cause of hemorrhage.

acute erythematosis, purpura, ulcerative colitis, and

Specific inhibitors against factors II, V, VII, VIII, IX, XII,

bronchial asthma also have an increased tendency to develop

XIII, and vWF have been detected in patients with inherited

factor VIII inhibitors. Many patients have been observed to

have factor deficiencies. However, some inhibitors of factors

develop factor VIII inhibitors with no underlying disease.

II, V, VII, IX, XII, and vWF have been observed in patients

The majority of these patients are of European origin, and

having no deficiencies of coagulation factors. Patients with

both genders are affected.

acquired specific inhibitors may exhibit hemorrhagic di-

Inhibitors against vWF occur in patients with von

Willebrand disease, whereas nonspecific inhibitors are not generally asso-

ciated with bleeding tendencies and underlying diseases such as auto-

immune deficiency.

systemic lupus erythematosus and in previous y

Some inhibitors will demonstrate a specificity inhibitor

healthy persons. A family tendency for the development of

attenuation, with the use of oral anticoagulation. In cases of vWF inhibitors has been noted.

with a specific inhibitor, the activated partial thromboplastin time (APTT) is adequate with correct

Factor IX Inhibitor

to within the reference range, but the incubated APTT—

Inhibitors are found in approximately 2% to 3% of factor IX—

ing studies will be ongoing. Although rare, the presence of

efficient (heterozygous) patients, but the incidence of inhibi—

factor inhibitor, such as factor VIII inhibitor, will increase

tors in severe heterozygous may be as high as 12%. Although

the risk of life-threatening bleeding. The presence of a factor—

these inhibitors are predominantly a result of transfusion of

factor inhibitor can be confirmed by a Bethesda assay or that

bioassays, spontaneous inhibitor formation has been

factor.

reported.

The presence of unusual anticoagulants, antibodies against

protein-homophilic coagulation, increases the risk of

Factor V Inhibitor

thrombosis. The clinical history suggests an LA,
Factor V inhibitors are rare and are not generally associated
further testing includes homozygosity-based screening tests,
with heterozygous Factor V deficiency. Some patients have ha-
mophilic-like enzyme assays, an exclusion of the res—
existence of streptococci but no causal relationship has been
evidence of inhibitors, and in addition, an incubate activate
established.

arterial thrombotic disease (AP), is necessary.

Fibrinogen, Fibrin, and Factor XIII Inhibitors

Etiology

Inhibitors of fibrinogen, fibrin, and factor XIII have been

The incidence of circulating anticoagulants has been bench—

reported. These inhibitors have occurred owing to a

marker at 0.75% of the general population, but certain

transfusions or a rare spontaneous. Some patients have

patients of whom have a higher incidence of inhibitor

a coagulation inhibitor of taking isoniazid, an antitubercu—

event. Inhibitors, found in both serum and as a,

osis rug.

are not inactivated by heating at 56°C for 30 minutes and

remain stable when stored at -20°C. Inhibitors are more

Factor II, VII, IX, and X Inhibitors

stable than clotting factors and are not affected by changes in

Factor II, VII, IX, and X inhibitors are rare. The causes for

Human deficiency. Inhibitors may remain in the circulation even if the patient is
various and include congenital—

tion or months and in some instances have been found in

taste deficiencies, immune disorders, and autoimmunity.

patient's years after the onset.

Factor XI and XII Inhibitors

Specific Inhibitors

Inhibitors of factors XI and XII have been reported in specific inhibitors directly
against specific factors such as

quently in patients with SLE, Warfarin, and other drugs—

Factor VIII or Factor V inhibitors.

emia, and other disorders, as well as with chlorzoxazone

administration.

Factor VIII Inhibitor

Factor VIII inhibitors are the most common specific ac—

Non-specific Inhibitors

une conditions.

Criteria for the Laboratory Diagnosis of

The core clinical manifestation is thrombosis. In women,

Lupus Anticoagulant

it can be associated with recurrent miscarriages. Fetal morbidity and mortality may be due to factors such as placental

thrombosis and placental infarction due to coagulation

- Prolongation of a homologous sensitive clotting assay

thrombosis and placental infarction due to coagulation

- Evidence of an inhibitor demonstrated by a mixing

activation. Secondary risk factors have been suggested. These

study

are age, hypertension, diabetes, obesity, smoking, pregnancy,

- Evidence of a homologous sensitive inhibitor base

surgery, and other genetic hypercoagulable states.

on neutralization of the inhibitor effect with a

Anti-homophilic (aPL) antibodies include

homophilic

- Lack of specific inhibition of any one coagulation factor

- Lupus anticoagulant

- Anticardiolipin antibodies

■ Anti- β 2-glycoprotein 1 antibodies

may have other acquired inhibitors as well. LA occurs in the

In the laboratory, evaluate every antibody and require

presence of disease states other than SLE, such as acquired

to establish a diagnosis. Laboratory diagnosis of antihuman immunodeficiency syndrome (AIDS) and pregnancy, and

idiopathic (aPL) antibodies depends on the detection of an LA,

in procainamide, hydroxychloroquine, or chloroquine therapy.

which prolongs the clotting time in the activated partial thromboplastin

Although LA exhibits an anticoagulant effect, it is rarely

an isolated anticardiolipin antibody.

associated with bleeding.

The relevant antigenic targets in antihuman immunodeficiency (aPL)

LA, an IgM, IgG, or IgA immunoglobulin, interacts with

antibodies are β 2-GP I and thrombin. Coagulation—

prolongs the clotting time in laboratory

variation is suspected because increased coagulation

assays but does not inhibit the activity of any specific coagu—

lators have been found in APS patients who have suffered

thrombotic events. LA is an inhibitor that prolongs the clotting

time in a cerebral ischemic event.

and ent coagulating tests in vitro. LA is the most common cause

Dysregulation and activation may contribute to throm-

botism and activate partial thromboplastin time (APTT).

botism and estimations. Evaluate every sample to determine

In 1995, the Subcommittee on Lupus Anticoagulant

thrombotic and laboratory break down results have been

Standardization Committee published criteria (Box 28.4) or

documented in the urine of APS patients.

the diagnosis of LA. This guideline recommends at least two

Antithrombotic (aPTT) syndrome is clinically and

screening tests based on different assay principles. In addition,

by the presence of one or more antithrombotic antibodies -

mixing study or the verification of the presence of a coagula-

tion (lupus anticoagulant, antithrombotic antibodies and /

tion inhibitor and a confirmation test or the occlusion

or a biologic false-positive test or syphilis associated by

antithrombotic deficiency should also be excluded. A

the simultaneous or subsequent evaluation of any one or

assays should be excluded on citrate anticoagulated speci-

more on a number of false clinical estimations. These

ens that are ate et oor an ree o un er ying e cts.

inc u e venous thro bosis, arteria thro bosis, obstetrica

In co arison, anticar io i in antibo ies (ACAs), IgM,

co ications, thro bocyto enia, b ee ing, neuro ogica

IgG, or IgA i unog obu ins, bin to the hos ho i i s

isease (transient ische ic attacks [IA's] an stroke, ear ycar io i in in the
resence o beta 2-GP 1-car io i in co -

onset e entia, a aurosis ugax an retina venous or arte—

ex. It ay be etecte in hea thy atients an in those with

ria thro bosis, etc.), skin esions, car iac va ve vegetations

a variety o con itions (e.g., SLE).

an itra regurgitation, yocar ia ys unction, ri ary

LA an ACA are risk actors or thro bosis, but the

u onary hy ertension, an a rena insu ciency.

echanis o action is unc ear.

Pri ary anti hos ho i i syn ro e is thro bosis an /

or obstetrica co ications in association with anti hos—

Antip hos p holipid Synd ro m e (APS)

ho i i antibo ies, but without signs o connective tis-

Antiphospholipid syndrome (APS) is e ne by c inica

sue isease. In co arison, secon ary anti hos ho i i

antithrombotic agents that include thrombolysis and/or anticoagulation
synonymous refers to those patients with systemic lupus
pregnancy morbidity in patients with antithrombotic (aPL)
Overall, autoimmune disease is present or subsequent
antithrombotic. Multiple forms of APS exist. Unfortunately, some
identify.

synonyms can be confusing. LA syndrome, or exsanguination, is the kidneys are a major
target organ in antithrombotic

because patients with APS may not necessarily have
syndrome (APS). Neurology in APS is characterized by

SLE and LA is associated with thrombotic rather than hemorrhagic vasculopathy
vasculopathy associated with thrombotic

thrombotic complications. In an attempt to avoid further confusion,

interarterial or interobular arteries, recognizing

APS is currently the reference for the clinical syndrome.

thrombotic in arteries and arterioles, antithrombotic.

Antithrombotic Syndrome (APS) is a thrombotic

The hallmark results from laboratory tests that are

associated with various antithrombotic in patients with a

antithrombotic syndrome (APS) is the presence of

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antithrombotic antibodies (aPL) antibodies or abnormal—

have clinical courses similar to hemorrhage. Patients with

defects in hemostasis - dependent tests of coagulation. Patients

inhibitors. Factor V inhibitors may cause clinical bleeding,

with various autoimmune or rheumatic diseases have a

although the degree of hemorrhage varies considerably.

high incidence of aPL antibodies. These disorders include

Inhibitors of factors XIII, II, VII, IX, and X; fibrin; or fibrinogen—

systemic lupus erythematosus, Sjögren syndrome, rheumatism can result in serious hemorrhagic events.

osteoarthritis, an autoimmune thrombocytopenic purpura.

Some bacteria and viral infections are associated with the

Laboratory Findings

occurrence of aPL. Familial association exists with relatives

Prothrombotic tendency (PT) or activated partial

patients with known APS are more likely to have aPL antithrombotic tendency (APT) are classic laboratory findings.

boles. An association has been found between aCL anti—

Incubation of patient's plasma with normal plasma at 37°C

both an individual who have DRw53 and DR7 HLA genes.

(ixing stu y) an eter ination o AP an P ay

Laboratory n ings in icate ro ngation o c otting
etect the resence o an inhibitor. T e ixing stu y wi be
assays, such as activate artia thro bo astin ti e (AP),
ro onge in the resence o an inhibitor. Inhibitors are ore
kao in c otting ti e, an i ute Russe 's vi er veno ti e
ti e an te erature stab e than their s eci c c otting actors.
(DRVV). T e resence o u us anticoagu ant is con r e
o quantitate the eve s o inhibitors, the Bethes a assay
by a ixing stu y. I a c otting actor is e cient, the a ition
is ost co on y use in the Unite States. One Bethes a
o nor a as a corrects the ro onge c otting ti e. I the
unit is e ne as the a ount o antibo y that wi neutra -
c otting ti e oes not nor a ize uring ixing stu ies, an
ize 50% o the inhibitor activity in a ixture o equa arts
inhibitor is resent; the absence o a s eci c c otting actor
o nor a as a an antibo y-containing as a that has
inhibitor con r s that a u us anticoagu ant is resent.
been incubate or 2 hours at 37°C.

Laboratory tests shou be consi ere in a atient sus—

Detection of antithrombin (aPL) antibody is based on

either having antithrombin synthesis:

or prolongation of thrombin-dependent coagulation assays.

aPL antibody is considered one of the most common causes

■ aCL antibodies (IgG, IgM)

of a prolonged AP. Assays include the Russell's viper

■ Anti-beta-2-glycoprotein 1 antibodies (IgG, IgM)

venotest, kaolin clotting test, aprotinin neutralization ro-

■ Activated partial thromboplastin time (APTT)

cedure, an tissue thromboplastin inhibition test.

Low anticoagulant assays such as DRVV (a threshold

of a ratio of 1.6 or the DRVV ratio) has been rec-

Impaired Fibrinolysis

often occurring in inherited antithrombin synthesis—

Impaired fibrinolysis (Figs. 28.4 and 28.5) has been noted

in both nonantithrombin synthesis.

to be both genetic and acquired in their origin. Inherited

Clinical Presentation

of fibrinolysis may be associated with thrombosis.

Patients with type II hyperfibrinolysis caused by a deficiency

Lupus anticoagulant (LA) is the most commonly acquired

hypercoagulable condition demonstrated by mixing studies.

It has an interesting presentation. In the absence of other

A high incidence of recurrent thrombosis has been noted in

the absence of abnormalities, the LA is rarely associated with

patients with hereditary deficiencies of protein C or antithrom-

bosinogen deficiencies, even with surgical procedures. Bicuspid

aortic. Protein S deficiency also joins the group of others as a

deficiency in these patients are usually the result of thrombotic—

protein deficiencies associated with inherited thrombophilia

cytopenia or another anomaly. Paradoxically, patients with

(Table 28.12). Deficiencies of inhibitors to factors VIII and V

LA are at increased risk of arterial and venous thrombotic

have also been correlated with recurrent thrombosis.

embolism. Venous thrombosis involving the leg veins, with

Characteristic presentations of patients with deficiencies of natu-

ally associated with embolism, is the most frequent complication occurring with anticoagulants are still rare. Deficiencies of 50%

tion. Spontaneous abortion and intrauterine deaths are

of normal protein C, protein S, and antithrombin

also increase in patients with LA.

lead to serious thrombotic events. Frequent retesting confirms the presence of a specific factor inhibitor can be suggestive

conditions include thrombophlebitis, deep venous thrombosis, and

in patients with no history of bleeding episodes who experience only a few. The frequency of protein deficiencies correlates—

with the hemorrhage at various sites or in the orthopedic patients

related with recurrent thrombotic disease is as follows:

not responsive to their usual dosage of blood product infusion.

Bleeding episodes in the orthopedic patients with inhibitors of

■ Protein C: 7%

not appear to be any more frequent or severe than in patients

■ Protein S: 5% to 10%

without inhibitors. When hemorrhagic events do occur, treat-

■ Antithrombin 2% to 4%

of a patient with inhibitor is difficult.

Nonorthopedic patients with acquired inhibitors of

Protein C Deficiencies

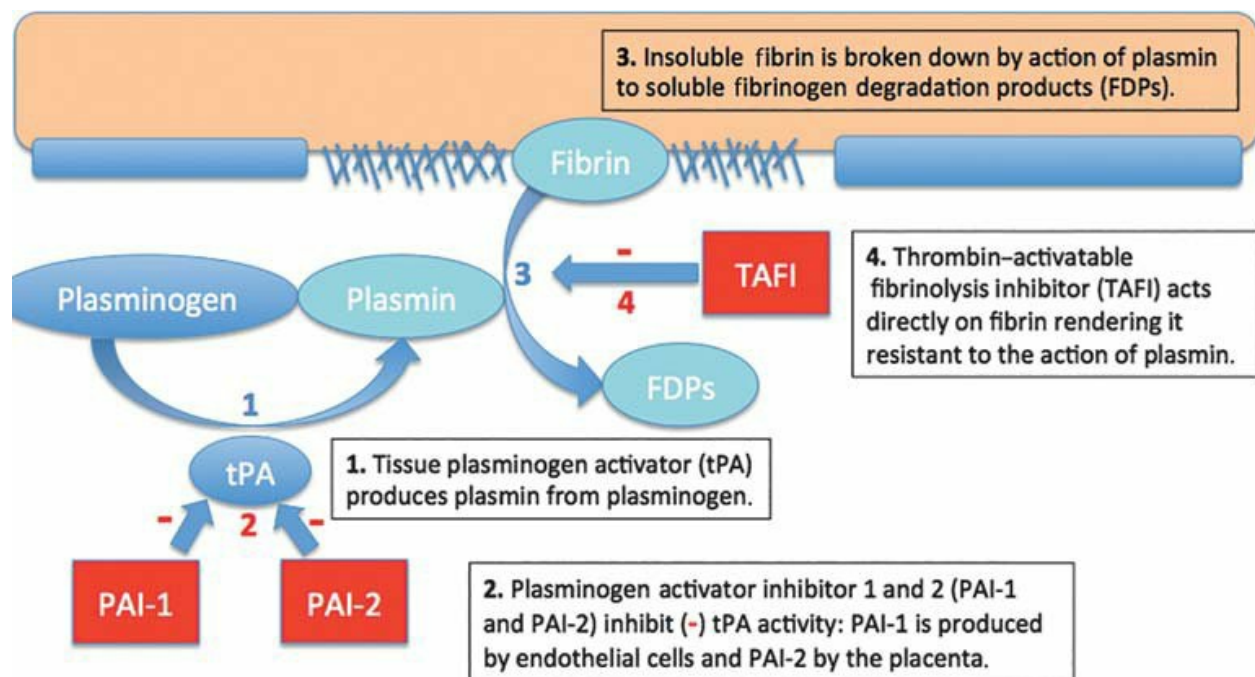
Factor VIII can have a major bleeding requiring transfusion.

Protein C activity has been demonstrated to be related to the

Patients with inhibitors to vWF, Factor XI, and Factor XII

commonly occurring thrombotic episodes in patients with

do not generally exhibit a hemorrhagic tendency. However, an inherited deficiency of protein C and protein S. However, therapy for these patients can be complicated by the presence of the hypercoagulable state in patients with proteinuria is not of the inhibitor. Patients with acquired factor IX inhibitors cause by decrease levels of protein C. Elevated protein C



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PAI-1

Plasminogen

tPA

uPA

α 2AP

α 2M

Plasmin

Endothelial cells

Xa/Va

Fibrin

D-Dimer

FDPs

Prothrombin

Thrombin

Fibrinogen

Thrombomodulin

TAFIa

aPC

Protein C

TAFI

FIGURE 28.4 Fibrinolysis. Thrombin, generated from prothrombin by the action of the Xa/Va prothrombinase complex, activates endothelial cells to release plasminogen activators. These, in turn, cleave plasminogen to plasmin, which degrades fibrin (or, alternatively, by the action of thrombin on fibrinogen) to different fragments and other fibrinogen degradation products. Inhibitors of fibrinolysis (in this case) include PAI-1, which inhibits

the actions of tPA and uPA, and α 2AP, β 2M, and AFI, which inhibit plasmin. Tissue plasminogen activator (tPA), when bound to thrombolytic plasminogen on the surface of endothelial cells, activates AFI to its active form and releases activated protein C, an important vitamin K–dependent protein with anticoagulant and anti-inflammatory properties. α 2AP, a high-molecular-weight plasminogen activator inhibitor; β 2M, a high-molecular-weight plasminogen activator inhibitor; tPA, tissue-type plasminogen activator; uPA, urine-type plasminogen activator; PAI-1, plasminogen activator inhibitor type-1; FDPs, fibrin degradation products; Xa, activated factor X; Va, activated factor V; aPC, activated protein C; AFI, plasminogen activator inhibitor; AFIa, activated AFI. (Source: Shaner DH, Nicholas DG. Rogers' textbook of Pediatric Intensive Care, Philadelphia, PA: Lippincott Williams & Wilkins, 2015.) **FIGURE 28.5** Fibrinolysis. (From Bucklin BA, Baysinger CL, Gabbing D. A Practical Approach to Obstetric Anesthesia, 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins, 2016.) 572

PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

Prevalence of Congenital

Classification of Congenital

TABLE 28.12

TABLE 28.13

Deficiencies

Protein C Deficiency

Patients with

Classification

Functional

Antigenic

Deficient

Recurrent

Protein

All Patients

Thrombosis

Type I

Decreased

Decreased

Type IIa

Decreased*

Normal

Protein C

4%–8%

12%–18%

Type IIb

Normal/abnormal†

Normal

Protein S

2%–8%

15%–18%

*Chromogenic and functional.

†Chromogenic is normal; clotting is abnormal.

even may represent a protective mechanism to the hypercoagulable state in patients with proteinuria because the

anticoagulant activities of antithrombin and protein C are
range indicates heterozygosity. The genetic heterogeneity
probably confers.

Effects in anticoagulation characterized by resistance to aPC

Deficiencies of protein C and protein S can be acquired or

is highly prevalent in patients with venous thrombosis. This

congenital. Acquired deficiencies occur in DIC, severe liver

effects appear to be at least 10 times more common in such

disease, vitamin K deficiency, and oral anticoagulation therapy—

patients than any of the other known inherited deficiencies of

apc. Congenital deficiencies are transmitted in an autosomal

anticoagulant proteins. The anticoagulant cofactor that circulates in plasma. Thrombotic complications usually involve

patients inherit aPC resistance is identical to unactivated factor

the venous system, although more recently, protein S has

V. aPC-resistant plasma contains nor a level of factor V related to—

been associated with arterial thrombosis as well.

coagulant, which suggests that aPC resistance may be caused

Severity of protein C effects have been reported

by a selective defect in an anticoagulant function of factor V.

(Table 28.13). Type I protein C deficiency is characterized by

low antigenic and functional levels of the protein. In those

Protein S Deficiency

with type II deficiency, the antigenic level of protein C is normal—

Family studies indicate that patients with a deficiency of

protein S (PS), but the function of the protein is impaired. Two sub—

types of protein S (PS) have an increased incidence of thrombosis.

Types of the type II defect have been described: classic type IIa,

Early descriptions indicate that PS deficiency is much more

in which both chromogenic and clotting functional assays are

common than either protein C or antithrombin deficiency.

Abnormal, type IIb, in which only the clotting functional

The congenital deficiency of PS is associated with an

ethic abnormality. Protein C deficiencies should, according to

increase risk of recurrent juvenile venous and arterial

thromboses. Type I should be screened by using a protein C functional assay (clot

thrombotic diathesis

base or chromogenic), because this will detect both types I

with acquired PS deficiency is essential.

an II. Once a low level of protein C activity is determined ,

an immunologic assay should be performed to distinguish

Congenital Protein S Deficiency

type I or type II protein C deficiency.

Diagnosis of protein S (PS) deficiency requires significantly

more than a low level of protein S owing

Activated Protein C Resistance

to PS binding with C4b-BP and re-partitioning between free

Activated Protein C (aPC) resistance, a new discovery, has

(functional) and bound (non functional) forms. The clinical

been associated with the risk of thrombotic disease. aPC

Diagnosis of congenital PS is based on the comparison of un-

resistance may be caused by an inherited deficiency of an

functional antigenic (free and total) as well as C4b-BP levels

anticoagulant factor that functions as a cofactor to aPC. aPC

(table 28.14). Currently, three types of congenital deficiencies

resistance appears to be inherited as an autosomal dominant

have been identified : type I, low functional antigenic PS

trait, suggesting that a single gene is involved . It is possible that

levels ; type II, low functional PS levels with a normal antigenic

patients with severe APC resistance are homozygous or the
 rare mutation (heterozygous); and type III, of unknown—
 genetic effect, whereas an APC response closer to the normal
 plasma PS levels corresponds to a decrease in free antigenic

TABLE 28.14 Classification of Congenital Protein S Deficiency

Classification

Functional Clotting

Free PS Antigen

Total PS Antigen

C4b-BP

Type I

Decreased

Decreased

Decreased

—

Type II

Decreased

Normal

Normal

—

Type III

Decreased

Decreased

Normal

Normal

Acute-phase reaction Decreased

Decreased

Normal

Increased

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PS along with a normal C4b-BP. However, a decrease in free/

proteolytic protein synthesis rather than a qualitative

functional PS cause by increase synthesis of C4b-BP can

abnormality. Hereditary factor II deficiency can also be seen -

occur transiently during acute-phase reactions.

constrains in patients with DIC. In these situations, both A

A PS functional assay should be used to screen for a

and hereditary factor II levels are initiated in areas.

types of PS deficiencies. Antigenic levels of both free and

total protein, as well as C4b-BP, will then be deter-

Venous Thromboembolism

ine to differentiate types I, II, and III.

Venous thromboembolism (VTE) has an incidence of

Antithrombin Deficiency

300,000 episodes per year in the United States, and the co-

incidence of pulmonary embolism causes 5% to 10% of a

Hereditary defects of antithrombin (AT) may be caused by

deaths in the hospital. Venous thrombosis can result from

quantitative or qualitative defects. Quantitative deficiency of

hereditary or acquired factors or both (Table 28.15).

AT is transmitted as an autosomal recessive trait.

Patients with venous thromboembolism (VTE) can be

I (quantitative) deficiencies represent the majority of cases.

divided into two groups. The first group includes patients

Family studies reveal that severe thrombotic risk—

with a disease such as cancer, a predisposing factor such as

these usually begin to be manifested at adolescence or

recent surgery, or an acquired abnormality such as the unusual

earthy atherosclerosis. Manifestations of AT deficiency are rare

anticoagulant that is known to increase the risk of thrombo—

in pregnancy. Women with the deficiency have a much higher risk. The pathophysiology is poorly understood.

Incidence of thrombosis because pregnancy, delivery, and oral contraceptives are causative factors.

risk factors that predispose to venous thrombosis. In Defects of a qualitative nature (type II deficiency) are so rare that, for these patients, it is difficult to identify a deficiency or to characterize by decreased heparin cofactor activity.

antithrombin, protein C or protein S and many studies show

The functional estimation of effective A is not associated with a reduction in coagulant concentration. More than

half of patients with type II deficiency have recurrent deep venous thrombosis.

Hypercoagulable States

TABLE 28.15 Associated with Venous

Decreased Antithrombin Levels: Congenital

Thrombosis

The relative incidence of congenital antithrombin (A) deficiency is between 1:2,000 and 1:5,000. A deficiency is inher-

Hypercoagulable State Comments

ite as an autosomal dominant disorder. Homozygotes have

Mutation in factor V gene

Replaces arginine 506 with

not been reported in A deficiency. Patients manifest signs

glutamine, rendering factor

anomaly to start between 10 and 30 years of age, their first

V resistant to inactivation by

thrombotic event. An initial event is spontaneous in a previously

activated protein

state have patients. Women frequently experience an

Mutation in protein C gene

Associated with protein C

gestations during pregnancy or because of oral contraceptive

deficiency

use. Decrease levels of A usually correlate with the severity of venous thrombosis. Arterial thrombosis is a less common

Protein S deficiency

Protein S is a cofactor for

activation in A deficiency.

protein C.

Antithrombin deficiency

Autosomal dominant

Decreased Antithrombin Levels : Acquired inheritance

Acquired antithrombin (AT) deficiency can be caused by Antiphospholipid

Encompasses ACAs and LA;

decreased synthesis, increased consumption, or other disorders—antibodies

associated with venous and

arteries; it can also be triggered in utero. The AT deficiency associated with arterial thrombosis

disorders are decreased synthesis (arteriosclerosis, cardiovascular—

Elevated concentration

Relative risk of venous throm—

vascular disease, chronic hepatitis, cirrhosis, type II diabetes—of factor VIII

thrombosis is 2-fold higher among

hemophiliacs); increased consumption (DIC, hemolytic uremia,

patients with factor V deficiency—

neurotic syndrome, osteoporosis, osteoarthritis, protein-urinations greater than 1,500 IU/L.

ing entero athy, u onary e bo is , stroke, thro bo h ebitis); rug in uce (brino
ysin, he arin, l -as araginase, ora

Frequency in venous

contrace tives); an other isor ers (burns, a ignancies).

thrombosis

Protein C, 2%–4%

Hep a rin Cofa ctor De cien cy

Protein S, 2%–5%

A though e cien cy o antithro bin (A) is the ost co -

Antithrombin, 1%–3%

on, recurrent thro botic co ications have been associate

with a e cien cy o he arin co actor II. T e atter e ect is

Plasminogen, 0.5%–2%

inherit in an autoso a o inant anner. Sy athetic

Source: Goldhaber SZ. Deep vein thrombosis and pulmonary embolism. In:

heterozygous atients exhibit about ha the nor a as a

Intensive Review of Internal Medicine, Boston, MA: Harvard University,

eve s o he arin co actor II activity. T is e cien cy resu ts

1995:75.

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PART 7 ■ Principles and Disorders of Hemostasis and Thrombosis

hereditary effects. Activated protein C resistance occurs in

3. Antithrombotic antibodies—antithrombotic antibodies

about one third of patients. Precipitating factors or throm-

botic anticoagulant.

biosis, such as pregnancy and the use of oral contraceptives,

4. Hyperhomocysteinemia

are identified in 60% of these patients. Activated protein C resistance appears to be 5 to 10 times more common than a

deficiency of antithrombin, protein C or protein S in patients

ANTICOAGULANT THERAPY

with venous thrombosis.

Most clinical laboratories are accustomed to monitoring

Congestive Plasma Monogen Deficiency

patients who are receiving warfarin (Coumadin) or heparin

Congenital plasminogen deficiency is a rare autosomal recessive—

anticoagulant therapy. In addition to these traditional therapies—

specifically characterize clinically by chronic ulcers

ies, new anticoagulants are joining the list of drugs in use.

several venous lesions consisting of subcutaneous bruising

exacerbation and inflammation. Type I plasminogen deficiency

Multi-targeted Anticoagulants : Traditional

is characterized by decrease serum as inogen activity,

Anticoagulants

decrease as inogen antigen levels, an indication of,

whereas type II as inogen deficiency, also known as “hy—

Vitamin K Antagonists

as inogenesis,” is characterized by decrease as inogen

The traditional anticoagulant use osteocalcin

activity with normal slightly reduce antigen levels. Patients

with type II deficiency are usually asymptomatic. Presumably,

risin is low cost.

normal amounts of as inogen antigen with decrease activ—

Warfarin drugs are vitamin K antagonists that interfere

with, as seen in type II, are sufficient or normal wound healing.

with the normal synthesis of factors II, VII, IX, and X as well

The PLG gene directs the creation of the amount of

as Protein C and Protein S. These drugs cause incomplete

function of as inogen. Enzymes called as inogen

coagulation because they lack calcium-binding sites, cannot

activators convert as inogen into as in, which breaks

or enzyme substrate complexes, and are unable to unc—

own brain. Fibrin is the main protein involved in a blood

tion as procoagulants or anticoagulants.

control, thrombus, and is important for wound healing.

Warfarin is a natural product and a inhibitor as a ix—

Congenital as inborn deficiency, caused by muta—

tion of the R and S stereoisomers of the drug. S-warfarin is

mutations in the PLG gene, is a disorder that results in infarct

three to five times more potent an inhibitor of the vitamin

growths on the mucous membranes, such as the eye, is an

Ketoxifen reductase complex, the target of action, than

the intensity of the effect.

R-warfarin. The action of warfarin can be influenced by

drugs and consumption of vegetables containing vitamin K,

Hypothyroidism ocys te inemia

such as spinach and kale and avocado. The osteoporotic

Hypothyroidism ocysteine levels are elevated above-normal concentra—

metabolism via the cytochrome P450 pathway seen

tions of as a/serum homocysteine. Plasma/serum homocysteine or the direct degree of
interaction with

cysteine is the sum of homocysteine and the homocysteiny

an other e ications o a vita in K antagonist. T is ty e
oiety o the isu es ho ocystine an cysteine-ho ocys—
o an un re ictab e res onse requires a syste atic onitor—
teine, whether ree or boun to roteins.

ing o the strength o anticoagu ant an a requent change in
Ho ocysteine ia is a so a eature o severa inherite
osing.

etabo ic isor ers, inc u ing ho ocystinuria, because o
Bio ogica activity is signi cant y ecrease , as revea e
utation in the CBS gene an N-ethy ene tetrahy ro o ate
by the rothro bin (P). T e onset o action o ost war—
re uctase e ciency cause by utation in the M HFR gene.

arin erivatives is between 8 an 12 hours. T e axi u

Hy erho ocysteine ia ay be associate with an
e ect occurs in a roxi ate y 36 hours, an the uration o
increase risk o atherosc erosis an recurrent arteria an
action is a roxi ate y 72 hours.

venous thro bosis usua y in the thir or ourth eca e o

T e rothro bin ti e (P), use to a just the ose o

i e. Ho ocysteine ia/ho ocystinuria an ega ob astic

ora anticoagulants, should be reported according to the
analysis can result in effects in vivo in B12.

INR, not the P ratio or the P expressed in seconds. The

Laboratory Assessment of Hypercoagulable

INR is essentially a corrected P that adjusts for the several

States

often assays used in North America and Europe.

Oral anticoagulant therapy monitoring in patients with

Four major areas of clinical testing are available to evaluate a

whether inhibitors have responded or so the laboratory—

patient or hypercoagulability. These categories are

ries. The thrombin time (PT) can be prolonged in patients

with antithrombin III deficiency or a variety of

1. Natural —Protein C deficiency, Protein S deficiency, factor

reasons:

V (Leiden), antithrombin deficiency, antithrombin co factor

II deficiency

1. Antithrombin use in this syndrome are directed toward

2. Fibrinolysis — plasminogen deficiency, poor tissue plasmin

antithrombin-binding proteins including thrombin.

inogen activator release, excessive as inogen activa—

2. LA or inhibitor interacts with the host cells in the in

tor inhibitor, anys brinogene ia

vitro assays of P and AP .

CHAPTER 28 ■ Disorders of Hemostasis and Thrombosis: Blood Coagulation Factors, Hypercoagulable State, and Anticoagulant Therapy 575

Heparin

Leirudin, a recombinant product, has the same antico—

Heparin anticoagulation is the mainstay of immediate

agulant activity as hirudin, which is produced by elicit—

therapy or conditions such as acute coronary events .

naneech. These drugs act as direct thrombin inhibitors by

Heparin has no anticoagulant activity of its own but acts as

blocking both the active site and the substrate-binding site

an anticoagulant by accelerating the binding of antithrombin

on the thrombin molecule. Leirudin even can be onto target enzymes (e.g., thrombin and factor Xa). Heparin

to be by the AP , ECARIN clotting time, or a chromogenic antithrombin because it helps to prevent new

genetic assay based on the inhibition of thrombin. The AP

thrombus formation and buys time for endogenous brino—

is the most widely used thrombolytic agent. The

mechanism of action is to lyse the clot.

target range for anticoagulation is 1.5 to 2.5 times the base—

Heparin can cause bleeding, thrombocytopenia, and

renal impairment. Among the disadvantages of this indication is

thrombocytopenia. Before initiating heparin, patients should be

tested to monitor patients with a laboratory assay.

screened for clinical evidence of active bleeding. The baseline

Argatroban binds to thrombin directly and acts as an

laboratory evaluation should include complete blood count

anticoagulant by blocking the active site on the thrombin

(CBC), platelets, activated partial thromboplastin time, prothrombin time. This drug is monitored by

thrombin time, stool analysis for occult blood, and urine

thromboplastin time (APTT). The therapeutic level is 1.5

times the control value.

to 3.0 greater than the baseline APTT. Disadvantages of this

Heparin anticoagulation is using intravenous

indication include no known inhibitor to reverse the anti—

transcatheter coronary angioplasty (PTCA) and cardiovascular -

coagulant effect, the need for administration by continuous

onary by ass (CPB) to prevent clot formation.

intravenous infusion, and the need for monitoring by a laboratory to activate clotting time (AC) has been used for

tory assay.

than 25 years to assess the degree of anticoagulation in heparinized patients. Typically, ACs are monitored to establish

Fondaparinux (Arixtra)

a initial target AC to ensure adequate anticoagulation.

Fondaparinux is a synthetic pentasaccharide that accelerates AC was one of the first coagulation tests to be performed

ates the binding of antithrombin (A) to activate factor Xa.

at the point of care, for example, for creating room, cardiac cath—

It has no A activity. Although this drug usually does not

eterization, or the diagnosis, and other interventional procedures—

require monitoring, it is recommended that it be assayed by

ures that require large doses of heparin. Typically, the AC

a system based on the inhibition of factor Xa, monitoring

is easier prior to an immediate reversal of heparinization.

is needed.

Subsequent testing is performed to ensure that adequate anticoagulation continues or a therapeutic heparin is administered.

New Oral Anticoagulants

Low Molecular Weight Heparin

In recent years, the new oral anticoagulants (NOAs) ac—

Low molecular weight heparin (LMWH), a new class of

factor Xa inhibitors apixaban (Eliquis, Pfizer and Bristol-

Myers Squibb), thrombin inhibitor abigatran (Praxa,

heparin, is available for clinical use. These LMWHs (e.g.,

Boehringer Ingelheim), an oral factor Xa inhibitor rivaroxa—

tinzaparin [Innohep], apixaban [Fragmin], an enoxaparin

ban (Xarelto, Bayer Healthcare AG and Janssen Research

[Lovenox]) react with the regulatory protein A to inhibit

& Development LLC, a Johnson & Johnson Company),

activate factor X (factor Xa) but not thrombin (factor IIa).

an oral factor Xa inhibitor edoxaban have revolutionize

Unfractionated heparin, by contrast, is active against both

anticoagulation therapy. Although the NOAs have imita—

rocoagulants. LMWH is less available than standard heparin in terms of patients
who can benefit from them, they

rin to activate resting platelets so that they release platelet

have been shown to be effective for the treatment of thrombo—

factor 4, an antithrombotic essential to platelet factor 4. They only

hy axis o V E an or the revention o stroke in atria
acce te etho o onitoring LMWH is by a chro ogenic
ibri ation.

assay base on the inhibition o actor Xa. T is etho has
T e search to re ace war arin, the anticoagu ant “go
been auto ate in coagu ation instru entation ca ab e o
stan ar ,” was triggere because o the i cu ty o stabi iz—
con ucting chro ogenic assays.

ing atients in the thera eutic internationa nor a ize ratio
LMWH eaks at about 4 hours a er subcutaneous injec-
(INR) range o 2.0 to 3.0. Regu ar onitoring o war arin is
tion. At eak thera eutic eve s, aboratory assay va ues shou
require to avoi the risks o both b ee ing ro excee -
be 0.5 to 1.1 IU/ L or atients who receive the rug twice a
ing the thera eutic range an un ertreat ent that ay not
ay an 1.0 to 2.0 IU/ L or those receiving one ose a ay.
a ow the reso ution o a b oo c otting rob e or ay a ow
c ots to or .

Targe te d Antico ag ulants

In contrast to the uncertainty o war arin, NOAs

may not require a regular laboratory assay to monitor.

Several drugs are direct thrombin inhibitors. These drugs are

Pharmaceutical companies emphasize that it is not neces—

1. Hirudin and eptifibatide

sary to monitor anticoagulant effects. However, in studies

2. Argatroban

with different doses of NOAs, the incidence of clinically

3. Fondaparinux

significant bleeding complications has been directly related

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TABLE 28.16 Comparative Properties of Warfarin, Dabigatran, Rivaroxaban, and Apixaban Warfarin

Dabigatran

Rivaroxaban

Apixaban

Target

Vitamin K

Factor IIa (free and

Factor Xa

Factor Xa

Epoxide reductase

clot-bound thrombin)

Half-life (hours)

40

14–17

5–9

10–14

Peak effect (hours)

72–96

2

2–4

3–4

Antidote

Vitamin K

None

None

None

Source: Modified from Mayo Clinic Proceedings, 88(5):495–511, May 2013.

to the use of NOA. Therapeutic excesses can increase the

inactivate the blood coagulation cascade at two different levels.

risk of bleeding and therapeutic interactions can increase. Rivaroxaban and apixaban inhibit activated factor Xa and thrombotic risk, especially when short-acting NOAs. Dabigatran is a direct thrombin inhibitor. In combination with aspirin are used.

Studies with groups of NOAs active by this route, the incidence of clinically significant bleeding complications was. Advantages and Limitations of Warfarin Versus Directly Related to the Use.

NOAs

Dabigatran etexilate is the pro-drug of dabigatran that. Warfarin has been the only oral anticoagulant for the treatment—reversibly inhibits the thrombin active sites of both free and bound thrombin. The major indications for treatment with warfarin are a narrow therapeutic window, that is, INR, excretion unchanged by the kidney so its administration is and the slow onset of effects and onset of action. Contraindications in patients with renal failure. In contrast, The United States Food and Drug Administration (FDA) has

rivaroxaban is a s a o ecu e with irect inhibitory activa rove a ixaban, abigatran, an rivaroxaban, an these

ity on activate actor X. It is ra i y absorbe an has a

are a inistere as a xe ora ai y ose to a atients an

high bioavai abi ity, is a inistere once ai y, an has

see to share so e a vantages. T e attractiveness o NOAs

a very short ha - i e o 5 to 9 hours in hea thy vo unteers

is a re ictabe e ect without the nee or onitoring, ewer

(ab e 28.16) but is signi cant y higher in the e er y at 9 to

oo an rug interactions, shorter as a ha - i e, an an

13 hours. E i ination by u ti e routes is via the he atic

i rove e cacy-sa ety ratio.

etabo is by cytochro e P450 3A4.

NOAs are ab e to overco e so e o the shortco ings o

war arin, such as s ow onset o action, variab e har aco—

La boratory As s ays

ogic e ects, oo - rug interactions, an the nee or c ose

NOAs o not easure the concentration o the rug

onitoring. NOAs co are avorab y with war arin or

irect y, but ana yze their i act on the coagu ation cas—

stroke revention in atients with atria bri ation. NOAs

ca e. NOAs can be re orte as coagu ation ti e or as the
signi cant y re uce stroke or syste ic e bo ic events by
coagu ation ti e ex resse as a concentration o the cir—
19% co are with war arin ain y riven by a re uction
cu ating NOA concentration. T e re erre re orting o
in he orrhagic stroke. Low-ose NOA regi ens show si i—
resu ts is the concentration o the rug. T e coagu ation
ar overa re uctions in stroke or syste ic e bo ic event to
assays that are use to onitor he arin erivatives or vita—
war arin an a ore avorab e b ee ing ro e but signi -
in K antagonists ay not a ways accurate y ref ect the
cant y ore ische ic strokes.

anticoagu ant activity o the NOAs, an s ecia ize assays
T e i itations o NOAs inc u e high cost; a ack o s e—
ay be nee e (ab e 28.17).

ci c anti otes, i nee e in an e ergency; an the ack o
With rivaroxaban, the P is a ecte in a concentration—
ong-ter sa ety ata. NOA shou be avoi e in regnancy,
e en ent anner. T ro bin generation as easure by the
atients with echanica heart va ves, an atients with

endogenous thrombin potential decreases with rivaroxaban.

severe renal insufficiency. A major advantage of NOAs

For abigatran, the activated partial thromboplastin time

is the absence of an antidote in case of serious bleeding or

(APTT) is chosen, although the dose-response curve is not

when emergency intervention needs immediate correction

linear and results vary by reagent. The EPT time was also

coagulation.

use because it is the parameter of the endogenous thrombin

potential that is influenced most by abigatran. A further

Characteristics of NOAs

require monitoring either of abigatran than the APTT is

In contrast to vitamin K antagonists that reduce the unclotted

the thrombin clotting time (TT), but the assay can be too

tionally even so several coagulation factors, the NOAs

sensitive. Although there is always a linear dose-response

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Coagulation Factors, Hypercoagulable State, and Anticoagulant Therapy 577

**Comparison of Laboratory Assays for Warfarin, Dabigatran, and
Rivaroxaban** TABLE 28.17 **and Apixaban Rivaroxaban and**

Assay

Warfarin

Dabigatran

Apixaban

APTT

Mild increase

Variable, normal or prolonged

Variable, normal or

prolonged

PT/INR

Moderate increase

Variable, normal or prolonged

Variable, normal of

prolonged

TCT

Unaffected

Marked increase

Unaffected

APTT mixing study

Complete correction

Incomplete correction

Incomplete correction

PT mixing study

Incomplete correction

Incomplete correction

Incomplete correction

APTT-dependent clotting factor

Decreased factor IX

Falsely decreased

Falsely decreased factors

assay

factors VIII, IX, and XI

VIII, IX, and XI*

PT-dependent clotting factor

Decreased factors II, VII,

Falsely decreased factors II, V,

Falsely decreased factors II,

assay

and X

VII, and X*

V, VII, and X

Antithrombin activity

Unaffected

Unaffected

Falsely elevated

Factor Xa based

Unaffected

Falsely elevated

Falsely elevated

Factor II based

Protein C activity

Decreased

Falsely elevated

Falsely elevated

Clot based

Decreased

Unaffected

Unaffected

Chromogenic based

Protein S activity

Decreased

Falsely elevated

Falsely elevated

Clot based

*Only at supratherapeutic levels.

APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time; TCT, thrombin clotting time; VKA, vitamin K antagonist.

Source: Modified from Mayo Clinic Proceedings, 88(5):495–511, May 2013.

curve or the direct thrombin inhibitor, once steady-state

NOTE: This is a good time to complete the end of chapter

events are achieved, the assay often becomes increasingly

Review Questions.

prolonged. The prolongation of the heparin-based clotting

time (EC) caused by abigatran is linear curve and does

not exceed easily quantifiable concentrations.

CHAPTER HIGHLIGHTS

When using NOAs, it is important to know exactly when

the NOA was administered relative to the time of bleed

Introduction

said. The time delay between drug intake and bleed

is often uncertain should be carefully recorded when

■ Being an effective blood clot or action are frequently
biological monitoring is error. The AP may provide
relative to a coagulation factor.

a qualitative assessment of the presence of abigatran. In the

■ Disorders of the blood coagulation factors can be grouped
AP is at trough level, that is, 12 to 24 hours after ingestion
into three categories: effective reduction, excessive
of NOA, and it still exceeds the upper limit of normal, this
destruction, and pathological inhibition.

may be associated with a higher risk of being an

Disorders of Defective Production

warrant caution especially in patients with bleeding risk factors. The AP may provide
a qualitative assessment of the

■ Hereditary clotting defects including hemophilia A and
presence of factor Xa inhibitors.

von Willebrand's disease are examples of hereditary disorders—

Quantitative tests for factor Xa inhibitor do exist (in units
that represent functionally inactive factor VIII.

thrombin time and chromogenic assay), but they may not

■ The cloning of factor VIII facilitates the identification of
yet be routinely available in hospitals. There are no data

utations that lead to hemophilia A, an inherited -

on a cut-off these specific tests below in which effective or
ciency of factor VIII coagulant activity that causes severe
urgent surgery is safe. In addition, POC tests to assess the
hemorrhage. von Willebrand's disease may be an acquired
INR should not be used in patients on NOAs.

or inherited disorder.

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- A deficiency of factor IX is known as hemophilia B or

- Acquired inhibitors of clotting proteins, also known as
Christmas disease.

circulating anticoagulants, inactivate or inhibit the usual

- A deficiency of factor XI is referred to as hemophilia C.

procoagulant activity of coagulation factors.

- Fibrinogen deficiency as a genetic disorder may represent

- Specific inhibitors against factors II, V, VII, VIII, IX, XII,
affect prothrombin or physiological processes.

and XIII and vWF have been detected in patients with

- Hereditary deficiencies of the other coagulation factors are

in vivo factor deficiencies.

relative and rare.

- Some inhibitors of factors II, V, VII, IX, and XII and vWF

have been observed in patients having no deficiencies of

Disorders of Destruction and Consumption

coagulation factors.

- Both coagulation factors can be destroyed in vivo by

enzymatic degradation or by pathological activation of

Anticoagulant Therapy

coagulation with excessive utilization of the clotting factors. Enzymatic destruction can result from bites by cer-

- Most clinical laboratories are accustomed to monitoring

patients with snakes whose venom contains an enzyme

patients who are receiving warfarin (Coumadin) or heparin—

that degrades fibrinogen to an effective fibrin monomer.

in anticoagulant therapy

- In vivo activation of coagulation by tissue thromboplastin

- In recent years, new oral anticoagulants (NOAs) acting like vitamin K antagonists can reduce excessive utilization of

factor Xa inhibitor apixaban (Eliquis, Pfizer) and Bristo-

clotting factors. Conditions that can cause this consump-

Myers Squibb), thrombin inhibitor abigatran (Praxa, tioneo coagulation factors include obstetric complications—Boehringer Ingelheim)), an oral Xa inhibitor rivaroxa—tions, trauma, burns, rest and elective surgery, shock, ban (Xarelto, Bayer Healthcare AG and Janssen Research and Development, a Janssen Biotech, Inc. subsidiary, and Janssen-Cilag, a Janssen Pharmaceutica, Inc. subsidiary, and Janssen Pharmaceutica, Inc. subsidiary), heparin.

an oral Xa inhibitor edoxaban have revolutionized

■ Primary and secondary thrombosis are recognized as anticoagulation therapy.

extreme complications of a variety of intravascular and

■ Warfarin drugs are vitamin K antagonists that interfere with the normal synthesis of factors II, VII, IX, and X as consequences.

we as proteins Protein C and Protein S.

■ Primary thrombosis is associated with conditions in

■ Heparin has no anticoagulant activity of its own but acts as which gross activation of the thrombotic mechanism with

an anticoagulant by accelerating the binding of antithrombin to thrombin, thereby inhibiting subsequent fibrinogen and coagulation factor consumption. Antithrombin binds to target enzymes (e.g., thrombin and factor Xa). Heparin is therefore an antithrombin antithrombin.

■ Although the same clinical conditions may also increase fibrin because it helps to prevent new thrombus formation, secondary fibrinolysis or DIC, the distinction between the two is essential in the demonstration of fibrinolysis. The use of the clot.

In secondary fibrinolysis, excessive clotting and fibrinolysis.

■ The search to replace warfarin, the anticoagulant “gold standard,” was triggered because of the difficulty of stabilizing patients in the therapeutic international normalized ratio (INR) range.

standard,” was triggered because of the difficulty of stabilizing patients in the therapeutic international normalized ratio (INR) range.

The Hypercoagulable State (Thrombophilia)

INR range of 2.0 to 3.0. Regular monitoring of warfarin is required to avoid the risks of both bleeding and thrombosis.

■ An increase in the likelihood of blood clot is referred to as hypercoagulability. The therapeutic range for INR is 2.0 to 3.0.

as the hypercoagulable state.

ent that may not allow the resolution of a blood clotting

■ Hypercoagulable states include various inherited

problems or may allow clots to

acquire characteristics characterized by an increase

■ Warfarin has been the only oral anticoagulant for the

risk of thrombosis.

treatment of venous thrombosis or emboli. The

■ The major inherited inhibitor deficiency states include anti-

major deficiencies of treatment with warfarin are as follows—

thrombin deficiency, protein C deficiency, and protein S

therapeutic window, that is, *i.e.* INR, and the slow

deficiency.

onset of effects and onset of action.

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Coagulation Factors, Hypercoagulable State, and Anticoagulant Therapy 579

CASE STUDIES

Case Study 28.1

■ Laboratory Data

On admission, the hemoglobin and hematocrit were 15.0

A 2-year-old boy presented with a back injury. His shoul-

g/L and 44%, respectively. The P was 13 seconds (normal, 10 to 15 seconds), and the AP was 55 seconds (normal, 28 to 35 seconds). The boy's mother took him to the emergency department a few hours after the incident because he was complaining of pain. On physical examination, the physician

Because of the results obtained on the original and repeat note that a large hematoma was in the upper abdomen seen on the AP in conjunction with a vague abdominal pain on the boy's right side. There was no history of surgery (he had a history of being, this patient's surgery was most likely not been circumscribed), injury, or illness. The boy was until a bleeding disorder could be ruled out. receiving no medication.

■ Critical Thinking Group Discussion Questions

Emergency department treatment consisted of irrigating

1. What coagulation deficiencies might be present in this patient? Subsequent to this treatment, the boy began

to be extensive y. He was a little to the hospital .

The following S A laboratory tests were ordered : a

2. What supplementary laboratory assays would be appropriate to obtain an hematocrit, platelet count, and bleeding

time?

time. Because the bleeding continues , a type and cross match

for two units of fresh blood were ordered on a stat basis.

3. How could this be distinguished from other similar conditions in order to determine the other relevant that

orders?

the boy's cousin has a "bleeding problem."

Case Study 28.3

■ Laboratory Data

A 62-year-old white man with a history of abnormal bleeding -

Hemoglobin 8.0 g/L

He was a little to the hospital for a cardiac workup

Hematocrit 26%

before surgery. A brother has died at age 19 of trauma—

Platelet count $200 \times 10^9/L$ (normal , 150 to $450 \times 10^9/L$)

atic bleeding after being injured in a car accident.

Bleeding time 5 minutes (normal , 3 to 8 minutes)

Subsequent coagulation roe tests were or ere be ore

The atient ha his rst b ee ing e iso e at 7 years o age

the trans usion o two units o resh who e b oo . The

owing a y h no e resection. He re orte having sig—

resu ts o these tests were as o ows:

ni cant he orrhaging as a teenager o owing i trau a.

At age 30, the atient ha a tooth extraction o owe by 3

P 12 secon s (nor a , 11 to 15 secon s)

weeks o b ee ing, at which ti e he receive a b oo trans—

AP 60 secon s (nor a , 28 to 35 secon s)

usion. At age 31, he was given a b oo trans usion be ore

T ro bin ti e-re ti ase etho 20 secon s (nor a , 18 to

an a en ecto y an on that occasion ha no b ee ing

22 secon s)

whatsoever. wo years be ore a ission, the atient su -

■ Critical Thinking Group Discussion Questions

ere ro gastrointestina b ee ing o owing surgery or a

1. Do the aboratory ata su ort a iagnosis o a isor er

hiata hernia. At that ti e, he was given two units o bank

o he ostasis?

book and two units of fresh blood. Being subsie and his subsequent recovery was good.

2. What types of disorders can be reliably identified

■ Laboratory Data

by the tests that were performed?

The laboratory findings were as follows:

3. What confirmatory tests must be done in this case?

Hemoglobin 7.0 g/L

Hematocrit 23%

Case Study 28.2

Platelet count $498 \times 10^9/L$

Being tied (Ivy) 2 minutes (normal, less than 8 minutes)

A 21-year-old backpacker in late winter was admitted to the

hospital for a fall from a tree. The patient was 188 minutes (normal,

less than 70 minutes)

His history—

He was concerned that strangulation of the hernia

Platelet aggregation normal

could occur. The patient was in extremely good physical

AP 53 seconds (control, 39 seconds)

condition. He is not remembered for having any unusual

P 13.8 seconds (control, 13.3 seconds)

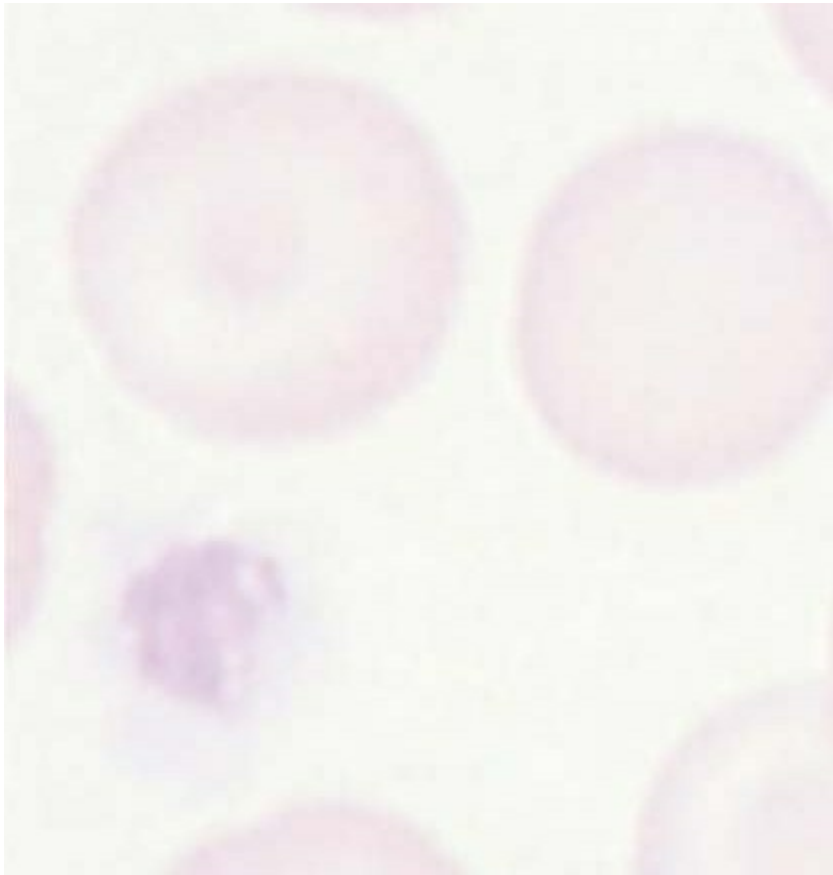
nesses. His family history includes no known

Specific assays for factors VIII and IX were performed. The

results are as follows for his relatives.

Level of activity of factor IX was less than 5%.

(continued)





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C

C A

A S

S E

E S

S T

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d))

■ Critical Thinking Group Discussion Questions

P ate et count $75 \times 10^9/L$

1. What is the iagnosis in this case?

B ee ing ti e 10 inutes

AP 65 seconds (control, 29 seconds)

2. Can this patient safely undergo surgery?

P 19 seconds (control, 11 seconds)

Trobin time 24 seconds (normal, 18 to 22 seconds)

3. What is the role of the laboratory in a surgical case of

Fibrinogen 90 g/L (normal, 200 to 400 g/L)

this type?

FSP screen positive

Prothrombin time test positive

Case Study 28.4

■ Critical Thinking Group Discussion Questions

1. What is the most probable cause of the extensive bleeding?

A woman was admitted in labor to the obstetric unit at 11 pm. Her history and physical examination revealed no significant abnormalities. At the time of admission, she

was having irregular contractions.

2. What is the etiology of this disorder?

In the delivery room, bleeding became extensive. A S A

3. With the transfusion of whole or fresh blood, correct the

hemoglobin, hematocrit, type and cross match or our

being?

units of both, and coagulation profile were ordered.

■ Laboratory Data

Hemoglobin 10.0 g/L

NOTE: This is a good time to write out the answers to the

Hematocrit 27%

Critical Thinking Group Discussion Questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

*4. Unique characteristic associated with a deficiency of

factor XII deficiency is

Disorders of Defective Production

A. frequent nosebleeds

1. Which of the following parameters can be abnormal in

B. no history of bleeding

classic von Willebrand's disease type I?

C. a congenital factor deficiency

A. Bleeding time

D. decrease risk of forming blood clots

B. P

C. Platelet count

Disorders of Destruction and Consumption

D. All of the above

*5. Disseminated intravascular coagulation (DIC) can be initiated by

*2. Platelet aggregation studies in cases of classic von

A. septic shock

Willebrand's disease should reveal

B. severe sepsis

A. normal platelet aggregation when factors such as

C. hemolytic RBC crisis

ristocetin are used for testing

D. all of the above

B. absence of aggregation when factors such as

epinephrine are used for testing

*6. Laboratory results in acute DIC reflect abnormalities in

C. decreased aggregation when factors such as ristocetin—

which of the following coagulation components?

tin are used for testing

A. Platelet function

D. decrease ADP activity when tested

B. Excessive clotting and fibrinolysis

C. Accelerate thrombin formation

*3. The most common form of von Willebrand's

D. Fibrinolysis

disease is

*7. Primary fibrinolysis is characterized by

A. type I

A. gross activation of the fibrinolytic mechanism

B. type II

B. consumption of fibrinogen

C. type III

C. consumption of coagulation factors

D. all have about the same incidence

D. all of the above



CHAPTER 28 ■ Disorders of Hemostasis and Thrombosis: Blood Coagulation Factors, Hypercoagulable State, and Anticoagulant Therapy 581

REVIEW QUESTIONS (continued)

*8. The hallmark of secondary fibrinolysis is the presence of

15. Pregnancy can cause a

A. brin s it ro ucts

A. ri ary hy ercoagu ab e state

B. brin egra ation ro ucts

B. secon ary hy ercoagu ab e state

C. brin ono ers

D. a o the above

*16. A characteristic o circu ating anticoagu ants is which o the o owing?

9. DIC is characterize by

A. T e ost co on s eci c actor inhibitor

A. icrovascu ar thro bosis

B. Acquire inhibitors o c otting roteins

B. brin e osition

C. A so known as anti hos ho i i or anticar io i in

C. active brino ysis

D. None o the above

D. a o the above

*17. A characteristic o u us anticoagu ant is which o the o owing?

T e Hypercoagulable State

A. Tissue-specific factor inhibitor

*10. Which of the following factors can contribute to

B. Acquire inhibitors of clotting proteins

hypercoagulation?

C. A substance known as antithrombin or antithrombin

A. Vascular endothelial damage

D. None of the above

B. Increase blood flow

C. Decrease platelets

*18. A characteristic of factor VIII inhibitor is which of the

D. Decrease titers of clotting factors

following?

A. Tissue-specific factor inhibitor

*11. Antithrombin deficiency can cause a

B. Acquire inhibitors of clotting proteins

A. primary hypercoagulable state

C. A substance known as antithrombin or antithrombin

B. secondary hypercoagulable state

D. None of the above

*12. Oral contraceptives can cause a

Anticoagulant Therapy

A. primary hypercoagulable state

B. secondary hypercoagulable state

19. Warfarin is a vitamin

antagonist.

A. B

*13. Protein C deficiency can cause a

B. C

A. primary hypercoagulable state

C. D

B. secondary hypercoagulable state

D. K

14. Cancer can cause a

A. primary hypercoagulable state

B. secondary hypercoagulable state

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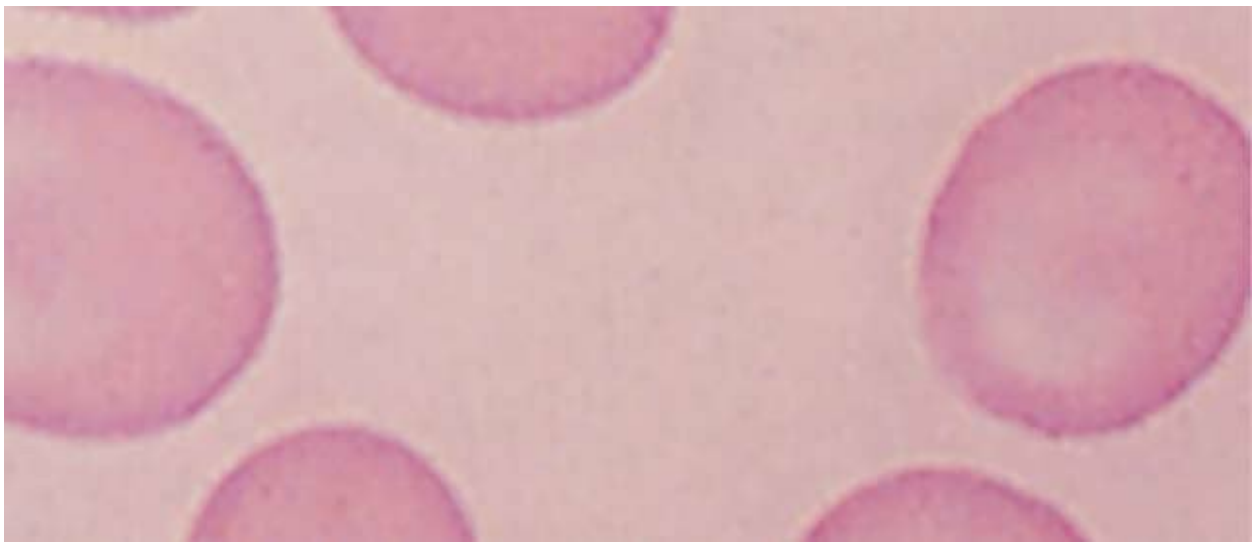
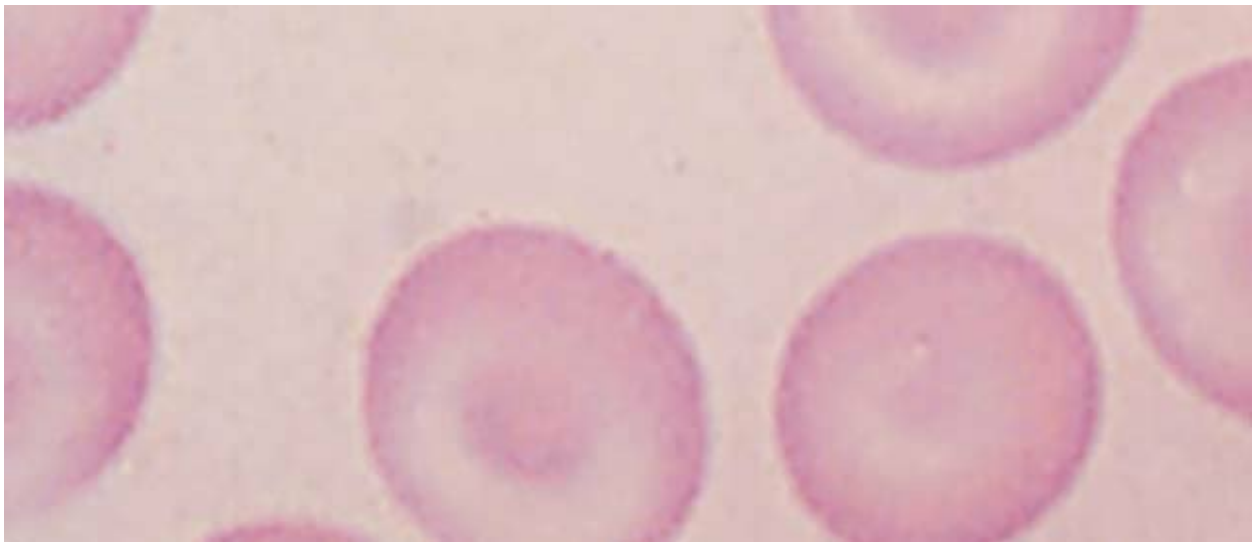
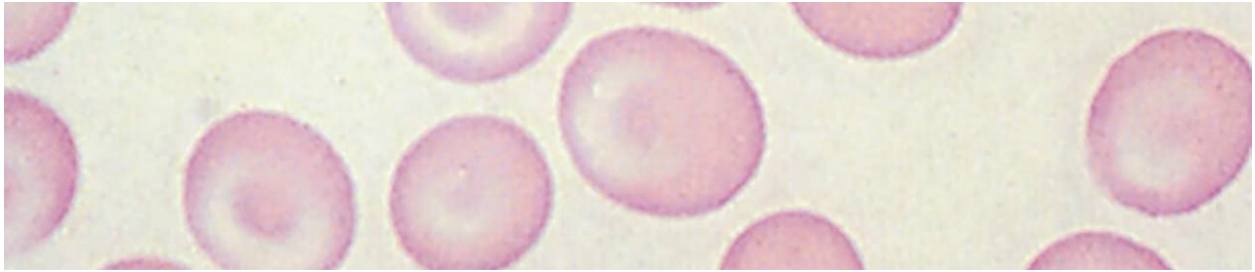
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PART EIGHT

Fundamentals of Hematological Analysis

CHAPTER

Body Fluid Analysis*

29

KEY TERMS

abdominal fluid

cholesterol crystals

pleural effusion

amniotic fluid

effusion

pleural fluid

arthrocentesis

ependyma

semenal fluid

artifacts

exudates

signet ring cells

ascites

lumbar puncture

standard precautions

b a s i c c a l c i u m p h o s p h a t e (BCP)

m a l i g n a n t

s y n o v i a l f l u i d

b l o o d -b a r r i e r

m e s o t h e l i a l c e l l s

t a n y c y t e s

c a l c i u m p y r o p h o s p h a t e d i h y d r a t e

m o n o s o d i u m u r a t e (MSU)

t h o r a c e n t e s i s

(CPPD)

p a r a c e n t e s i s

t r a n s u d a t e s

c a r d i a c t a m p o n a d e

p e r i c a r d i a l e f f u s i o n

x a n t h o c h r o m i a

c e r e b r o s p i n a l f l u i d

p e r i t o n e a l f l u i d

LEARNING OUTCOMES

Intro ductio n

Pleural, pe ritoneal, and pericardial uids

- Associate the various types of body fluids with their respective

- Define the term effusion.

synonyms.

Compare the major characteristics of transudates and exudates.

- Identify the location of the pleura.

Cerebrospinal fluid

- Name at least three disorders associated with the existence of pleu-

- Describe the anatomical structures involved in the circulation of
ral exudate.

cerebrospinal fluid (CSF).

- Associate the various colors of exudate with the typical disorders.

Explain how CSF is produced.

- Name at least two reasons for turbidity in pleural fluid.

Describe the collection procedure for CSF.

- Name the disorder that produces an extremely elevated total leuko-

- Name the appropriate type or types of testing for each of the ali—
cyte count in pleural fluid.

quots of a CSF specimen.

Name the types of common cells that can be encountered in pleural fluid.

Compare the descriptive characteristics of CSF on gross examina-

■ Name the characteristics of malignant cells that can be found in a
tion and the respective associated abnormalities.

pleural effusion.

Compare the characteristics of a recent hemorrhage, an old hemor—

Discuss the cellular abnormalities encountered in pleural and peri—
rhage, and a traumatic tap.

toneal uid.

■ Explain the procedure of manually counting leukocytes in a cere-

■ Identify the location of the peritoneum.

brospinal uid.

■ Name at least three disorders or diseases that can cause a perito—

Describe the common cells seen on microscopic examination and
neal effusion.

the associated disorders.

■ List at least two reasons for a turbid peritoneal effusion.

Name and describe cells unique to the CSF.

Associate a variety of conditions with various colors or appearances

* Describe the appearance of erythrophagocytosis in CSF.

of peritoneal effusion.

*Additional procedures, in CLSI format, are provided on this book's
companion Web site at thepoint.lww.com/Turgeon6e.

- Name several conditions that can produce a high total leukocyte count in peritoneal uid.
- List and describe the types of crystals that may be observed in synovial uid.

List the types of cells that can be seen in peritoneal effusion and

Associate various types of crystals with particular disorders.

associate these cell types with a representative disorder.

Define birefringence and explain its use in the identification of crystals in body fluids.

- Describe the anatomy of the pericardium.

tals in body fluids.

Associate the various types and causes of pericardial effusion.

Describe the normal total cell count and differential in synovial uid.

- Name a cause of an increased total leukocyte count with mostly polymorphonuclear segmented neutrophils (PMNs).
- Compare the laboratory findings in noninflammatory and in inflammatory arthritis.

Synovial uid

Body fluids slide preparation

- Describe the anatomical structures and their respective cellular

- Compare the features of various methods of body fluid sediment and/or chemical components.

preparation.

Describe health, medical, and social behaviors that can negatively

- Differentiate the characteristics of Wright-Giemsa and influence the sperm count or other seminal analysis results.

Papanicolaou's stains.

- Discuss the proper collection and handling of seminal fluid.

Amniotic fluid

- Name the normal number of sperm cells per milliliter or per liter.
- Describe the composition of amniotic fluid and its importance to
- Name the types of microscopic assays and the respective normal values.

- Interpret automated cellular body counts in amniotic fluid.

Synovial fluid

Cases studies

- Define the term arthrocentesis.

Analyze the patient history, clinical signs and symptoms, and labo-

- List at least three disorders that can be diagnosed definitively by

ratory data for the stated case studies, answer the related critical
synovial uid analysis.

thinking questions, and conclude the most likely diagnosis.

- List several sites that may be aspirated.

- Name the tests that should be included in the routine analysis of

NOTE:

synovial uid.

- indicates MLT and MLS core content

- Name the procedures included in the gross examination of synovial

indicates MLT (optional) and MLS advanced content

uid.

INTRODUCTION

TABLE

29.1 Body Fluids

Frequently, the analysis of body fluids is assigned to the hematology laboratory. Gross physical examination, total cell count, microscopic examination, and other special tests

Fluids

Synonyms

are generally within the job responsibility of hematology

Bronchoalveolar lavage

Bronchial washings

technicians and technologists. Because clinical correlations

Cerebrospinal fluid

Spinal fluid

of body fluid analyses are diagnostically important, clinical information is presented in each section of this chapter.

Lumbar puncture fluid

Chemical analyses and microbial and cytological examinations—

Ventricular fluid

nations are generally performed in the chemistry, microbiol—

Meningeal uid

ogy, and cytology departments, respectively. For this reason,

Synovial uid

Joint uid

specific procedures in these disciplines are not included in this chapter.

Peritoneal

Dialysate uid

Sterile body fluid can be found in various body cavities

(abdominal) uid

Paracentesis uid

under normal conditions. In diverse disorders and disease

Ascitic uid

processes, the quantity of these fluids can increase signi -

Pericardial uid

Fluid from around the

cantly. Fluid specimens aspirated from different anatomical

heart

sites (table 29.1) can be analyzed or the total number of

cells, differentiation of cell types, chemical composition, and

Pericardiocentesis fluid

microbial contents. All body fluids should be handled with

Pleural fluid

Chest fluid

caution. Standard precautions must be practiced.

Thoracic fluid

The type of examination performed on the body fluid

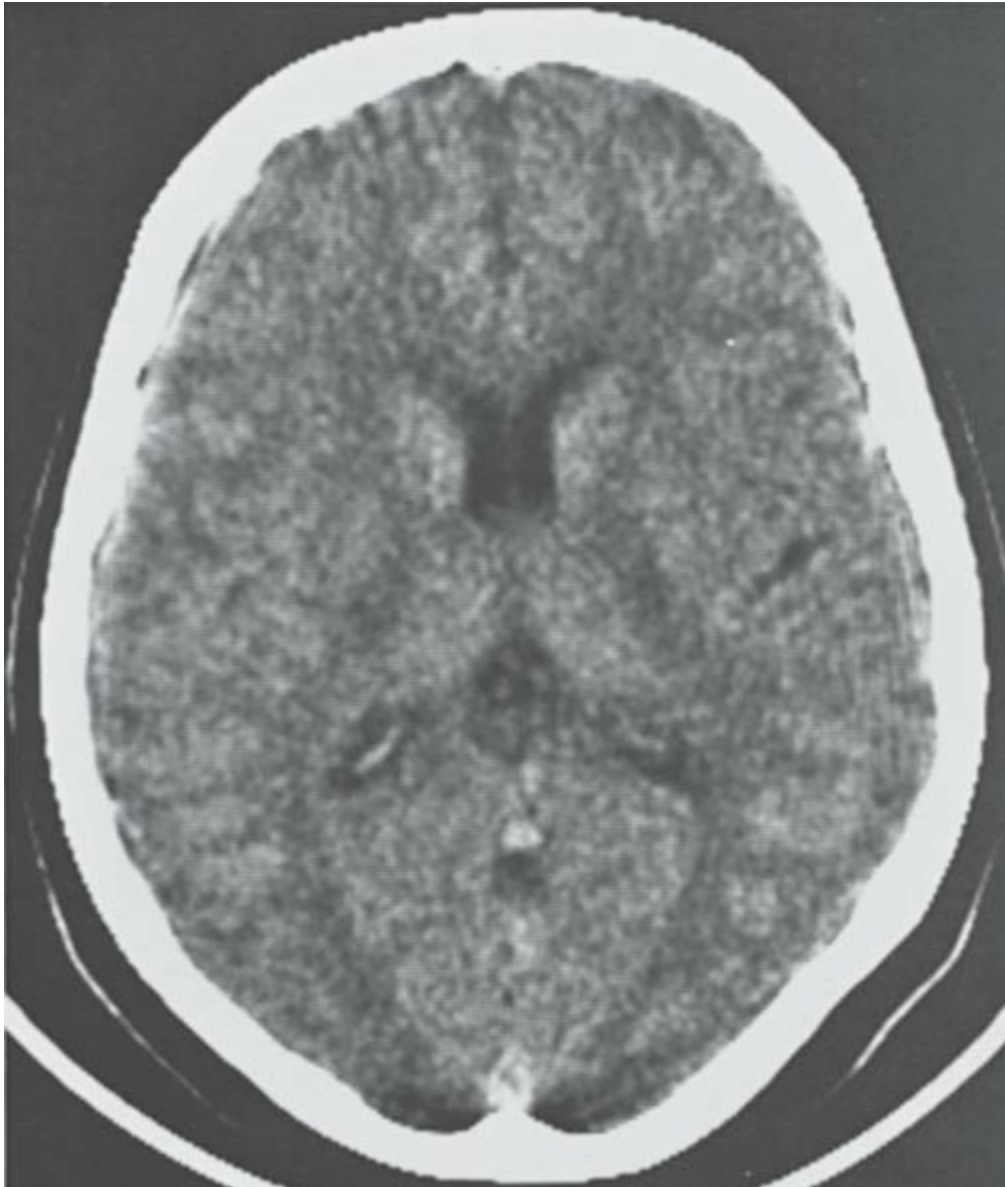
Thoracentesis fluid

depends on the source of the specimen. However, a portion

of the examination of cerebrospinal fluid (CSF); serous fluids

Seminal fluid

Semen



CHAPTER 29 ■ Body Fluid Analysis

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from the pleural, pericardial, and peritoneal cavities; synovial

Three openings in the roof of the fourth ventricle, a pair

fluid; and seminal fluid is frequently performed in the hema—

of lateral apertures (foramina of Luschka) and a median
tology laboratory.

aperture (foramen of Magendie), allow CSF to flow into the
basal cisterns and subarachnoid space of the spinal cord.

From these basal cisterns, CSF migrates over the convexities

NOTE: This is a good time to review the definitions of the Key
toward the cerebral sinuses.

Terms in the Glossary and flash cards on

.

Production of Cerebrospinal Fluid

CEREBROSPINAL FLUID

CSF production is primarily a function of the choroid plexus,

Anatomy and Physiology

with a smaller proportion being derived from the ependymal lining and
perivascular spaces. The plexus is composed

CSF acts as a shock absorber of the brain and spinal cord,

of two layers: the ependyma (the lining epithelium of the

circulates nutrients, lubricates the central nervous sys—

ventricle) and the pia mater. The folded projections of the

tem (CNS), and may also contribute to the nourishment

highly vascularized pia lined with epithelium are referred

of brain tissue. The CSF circulates through the ventricles to as the choroidal epithelium. Choroidal epithelium, blood and subarachnoid space that surrounds both the brain vessels, and interstitial connective tissue form the choroid and the spinal cord. The ventricles (Fig. 29.1) consist of plexus. The plexuses in the lateral ventricles are the largest of our hollow, fluid-filled spaces inside the brain. A lateral and produce most of the CSF. The choroid plexus epithelium—ventricle lies inside each hemisphere of the cerebrum. The epithelium and the endothelium of capillaries in contact with CSF two lateral ventricles communicate with the third ventricle constitute the anatomical structure of the blood-brain barrier. through the foramen of Monro. The third ventricle, a narrow—The ependyma is a single layer of cells with villous projections—row channel between the hemispheres through the area tions and cilia on its surface. Ependymal cells are specialized ependymal cells without cilia, located on the floor of the third located in the pons and medulla, by means of the aqueduct ventricle. The main portion of this cell is directed toward

o Sylvius in the midbrain portion o the brainstem. T is
the ventricle, and the neck and tail portions contact the cap—
ventricle is continuous with the central canal o the spinal
illary wall. T ese cells are not believed to be involved in the
cord.

production o CSF.

Longitudina l ce re bra l

Ca lva ria

fis s ure s

Gre y ma tte r

Ante rior horn of la te ra l ve ntricle

He a d of ca uda te

nucle us

Le nticula r

nucle us

Tha la mus

Qua drige mina l cis te rn

Choroid ple xus

(cis te rn of gre a t ce re bra l ve in)

of la te ra l ve ntricle

Cerebellum

Calcification in

pineal body

Intracranial occipital

protuberance

FIGURE 29.1 Transverse (axial) CT image of the brain. Observe the ventricles, various parts of the brain, and the choroid plexus of the lateral ventricle. (Reprinted from Moore KL, Dalley AF II. Clinical Oriented Anatomy, 4th ed, Baltimore, MD: Lippincott Williams & Wilkins, 1999, with permission.) 586

PART 8 ■ Fundamentals of Hematological Analysis

Tube 3 is for gross examination, cell count, and morphology.

Reasons for Performance of a

Complete Blood Count (CBC) with Differential (CBC with Diff)

TABLE

29.2 Lumbar Puncture and Removal

within 1 hour of specimen collection.

of an Aliquot of CSF

Caution: All CSF specimens should be handled with extreme care. These specimens could potentially harbor

Therapeutic

Relief of increased intracranial pressure

viruses or other infectious organisms.

Diagnostic

Identification of conditions such as subarachnoid hemorrhage, meningeal infection—

Gross Physical Examination

tion (meningitis), multiple sclerosis, and

The spinal fluid is examined visually for turbidity (cloudiness), color, and viscosity. Normal CSF is clear and colorless. Its appearance and viscosity are comparable to those of water.

neoplasms

ness), color, and viscosity. Normal CSF is clear and colorless.

Its appearance and viscosity are comparable to those of water.

Specimen Collection: Lumbar Puncture

Turbidity

CSF is found inside all the ventricles, in the central canal of the

If any turbidity exists, it should be graded using a scale of 0 to 4+.

spinal cord, and in the subarachnoid space around both the

In the absence of a set of known standards for comparison, the

brain and the spinal cord. The subarachnoid space is the area

rating scale is subjective. This scale ranges from 1+, slight cloudiness,

between the arachnoid mater, the middle meningeal membrane,

ness, to 4+, in which newsprint cannot be seen through the tube.

brane covering the brain and spinal cord, and the pia mater,

Cloudiness or turbidity may be caused by pleocytosis the innermost meningeal membrane. The total maximum (increased concentrations of leukocytes, erythrocytes, or volume of CSF in adults is about 150 mL. The maximum volume of microorganisms) or, less commonly, radiographic contrast volume in neonates is approximately 60 mL. The rate of reabsorption media or the presence of fat globules.

Reabsorption in adults is approximately 500 mL/d or 20 mL/h and is constant. Grossly bloody specimens can result from a traumatic tap or from conditions such as a bleeding subarachnoid hemorrhage. In a lumbar puncture, introducing a needle into the subarachnoid or intracerebral hemorrhage. A traumatic tap more commonly occurs in children because of movement during the procedure. The subarachnoid space makes it possible to measure CSF pressure and to obtain fluid for analysis (Table 29.2). This procedure is contraindicated when there is a skin infection at the puncture site or when the patient has septicemia or a general systemic infection.

cal condition. A freshly collected specimen should be examined immediately because of the risk of spreading the infection into the meninges. If the reddish color diminishes between the first and the last tube, the blood in the specimen is due to a traumatic tap. In addition, clots may be observed in trauma with normal skin microbial flora. A stylet needle is introduced into the intervertebral space between the L4 and L5 (lumbar) vertebrae. Up to 20 mL of fluid can be removed from a patient with a subarachnoid block or meningitis. If the patient has a normal opening pressure. The specimen should be placed into sterile tubes. After CSF collection, the Color closing pressure is measured, the stylet replaced, and the needle Any presence of color should be noted. A yellow coloring removed. Specimens must be promptly delivered to the laboratory as a specimen or the supernatant of a centrifuged specimen

tory or analysis. The patient should be given appropriate therapy referred to as xanthochromia. The release of hemoglobin

care because the procedure is not without risk.

from hemolyzed erythrocytes (red blood cells [RBCs]) in

Indications for spinal fluid examination are changing as

the CSF is a potential cause of xanthochromia. The lysis of

other diagnostic methods are improved. Only in a few condi—

RBCs in CSF begins about 2 hours after the occurrence of

tions, such as meningitis, is the lumbar puncture essential and

a subarachnoid hemorrhage (Table 29.3). Other conditions

are of diagnostic value. It may be of differential value in other cases.

Laboratory Analysis

Changes in CSF Following

TABLE 29.3

General Principle

Hemorrhage

A specimen of CSF is examined visually and microscopically.

Gross Examination

The total number of cells can be enumerated, and the types of cells can be morphologically distinguished.

2–12 h

Xanthochromia (pink to orange)

12–24 h Xanthochromia (yellow color, disappears in 2–4 wk)

Specimen

Microscopic Examination

From three to five samples of 2 to 4 mL each are collected in

2–24 h

Erythrocytes, neutrophilic granulocytes (PMNs),

sterile tubes by a physician. The number of tubes and speci—

monocytes, and a few lymphocytes

ed examination related to each tube depends on institutional

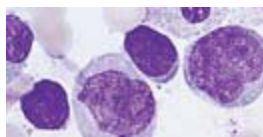
protocol. Typically, tube 1 is for chemical and serological

≥ 48 h

Monocytes and PMNs, erythrophagocytosis,

examination; tube 2 is for microbiological examination; and

siderophages (may persist for 2–8 wk)



CHAPTER 29 ■ Body Fluid Analysis

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(Table 29.4) and a delay in the examination of a specimen—protein or gel formation on standing due to an increased men (which can cause a false-positive result) can produce fibrinogen content.

xanthochromia.

Microscopic Examination: Cellular Enumeration

Viscosity

Electronic cell counters are usually used to count cells in CSF.

Normal CSF has the viscosity of water. Clotting in CSF can

Occasionally, total leukocyte cell counts on body fluids are
be caused by a variety of conditions, including increased
performed manually.

SPINAL FLUID: TOTAL LEUKOCYTE COUNT PROCEDURE

PRINCIPLE

to enumerate the number of WBCs to assist in the development of a differential diagnosis (e.g., bacterial meningitis, viral meningitis, ruptured brain abscess).

W

W

REAGENTS, SUPPLIES, AND EQUIPMENT

1. 10% acetic acid: Prepare by diluting a 100-mL volumetric

R

R

fill flask about half full with distilled water. Using a safety bulb, pipette 10 mL of glacial acetic acid into the flask. Add distilled water to the calibration mark and mix.

R

2. Wright-Giemsa or Wright's stain or 1% methylene blue in methyl alcohol: Prepare by weighing 1 g of methylene blue

R

R

and transferring it to a 100-mL volumetric flask. Dilute to the calibration mark with methyl alcohol. Mix.

3. Small (12- × 75-mm) test tubes, Pasteur pipettes, rubber bulb, and microscope slides

W

W

4. Neubauer's hemacytometer

5. Centrifuge, microscope, and immersion oil

6. Disinfectant solution

7. Disposable gloves and safety goggles

FIGURE 29.2 Neubauer's counting chamber. (R, red cell area; W, white cell area.)

PROCEDURE

the outer lines or the opposite adjacent lines should not

1. Mix the spinal fluid by inversion. With a Pasteur pipette, be counted (see Fig. 29.3).

transfer nine drops of spinal fluid to a small test tube. Add

The number of cells counted in all nine squares should one drop of 10% acetic acid. Mix by gently tapping the not differ by more than five cells. Average the count from tube.

both sides.

2. Allow this mixture to stand for 5 minutes. Mix again.

3. On each side of the chamber of a clean hemacytometer with a coverslip, load a small amount of the diluted spinal fluid. Allow the counting chamber to sit covered, with a moistened filter paper in half of a Petri plate, for a few minutes to allow the cells to settle and the erythrocytes to completely lyse.

4. Place the hemacytometer under the 10× microscopic

objective (low power). Erythrocytes should either be

Counted

absent or appear as ghost cells. The nucleus of poly—

Not counted

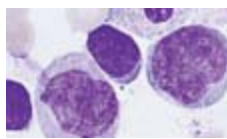
morphonuclear segmented neutrophils (PMNs) will

be bright, whereas the lymphocyte nucleus will be

round.

5. The leukocytes in all nine squares on each side of the chamber should be counted (see Fig. 29.2). Remove the “R” from the inner four squares. If cells touch the inner or middle lines of two adjacent lines, for example, upper and left-hand side, they can be counted. Cells touching

FIGURE 29.3 RBC counting square.



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PART 8 ■ Fundamentals of Hematological Analysis

SPINAL FLUID: TOTAL LEUKOCYTE COUNT PROCEDURE (continued)

6. Soak the hemacytometer in a 10% bleach solution to disinfect—
air bubbles. After use, dispose of the hemacytometer

ect. Discard the capillary pipette and contaminated sup—
in a biohazard container. I a di erent type o dispos—
plies in a biohazard bag.

able hemacytometer is used, ollow the manu acturer's
instructions and use the ormula provided to calculate

CALCULATIONS

the cell count.

2. Clear specimens may be counted undiluted, provided no

10

$9 \times = 11$ leuk ocytes/ μ L

overlapping o cells is seen on microscopic examination.

9

When dilutions are required, calibrated automatic pipettes
are used. Dilutions are made with normal saline, mixed
(T ese calculations may need to be adjusted i the quantity
by inversion, and loaded into the hemacytometer with a
o the specimen varies.)

micropipette. T e appropriate dilution actor must be used
in the calculation.

REFERENCE RANGES

3. Crystal violet stain can be used to facilitate the differentiation—

Normal CSF is crystal clear and colorless. No clots or RBCs

or WBCs from RBCs. Rinse a microhematocrit tube

should be observed. In addition, normal CSF has the vis—

with crystal violet stain to coat the inside. Draw the fluid

viscosity of water.

into the coated microhematocrit tube, mix, and charge the

Normal values: 0 to 5 cells/ μL or 0 to $5 \times 10^6/\text{L}$ (lymphocytes

counting chamber.

and monocytes).

Some use a reference value of 0 to 10/ μL or 0 to $10 \times 10^6/\text{L}$.

CELLULAR ENUMERATION PROCEDURE NOTES

Neonates have a higher normal range, 0 to 30 mononuclear

Sources of Error

cells $\times 10^6/\text{L}$.

If the specimen is not examined promptly after collection,

Values in children are comparable to those in adults.

WBC lysis will give a false impression of the number of

WBCs present. If a delay is anticipated, the specimen should

NOTES :

be refrigerated.

1. A disposable, plastic hemacytometer may be used. The
Clotted specimens result in a falsely low cell count because
C-Chip DHC-N01 has a grid pattern and depth that are
RBCs and WBCs will be trapped in the clot. In unusual cir—
the same as the Neubauer's hemacytometer. It is all-in—
cumstances, manual peripheral blood WBC or platelet counts
one unit does not require a coverslip. Use a micropipette
may be needed. Unopettes or this procedure have been disallowed 10 µL of
sample into the sample injection areas
continued but Bioanalytic Group-H and Biomedical Polymers,
on either end of the chamber. The chamber will fill by
Gardner, MA, www.biomedicalpolymers.com, manufacture
capillary action. Be careful to prevent introduction of
substitutes.

TABLE 29.4

Potential Causes of Xanthochromic CSF

Cause

Example

Clinical Conditions (In Vivo)

Oxyhemoglobin from RBCs lysed "in vivo"

Recent subarachnoid hemorrhage

Bilirubin from RBCs lysed “in vivo”

Older subarachnoid hemorrhage

Increased direct bilirubin with normal blood-brain barrier

Significant jaundice

Premature infants with an underdeveloped blood-CSF barrier

Hemolytic disease of the newborn

and hyperbilirubinemia

Increased CSF protein levels (>150 mg/dL)

Severe meningeal inflammation or infection

Carotenoids in CSF (uncommon)

Meningeal melanoma

Technical Conditions (In Vitro)

“In vitro” RBC lysis

Traumatic tap with detergent in needle, delay in examination

Antiseptic contamination of CSF

Merthiolate or mercurochrome

Delayed examination of CSF specimen

Lysis of intact RBCs

CSF, cerebrospinal fluid.

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Microscopic Examination: Cellular Differentiation

can manifest reactive lymphocytes in their CSF. These con—

Normal CSF contains a few mononuclear cells (lymphocytes

ditions include subacute and chronic meningoencephalitis,

and monocytes) and rare ependymal cells. There is no gen—

tuberculous meningitis, listeriosis, purulent encephalitis,

eral agreement as to the significance of a few neutrophilic

subacute sclerosing panencephalitis, multiple sclerosis, and

leukocytes in a CSF specimen.

bacterial meningitis (recuperative phase).

Cells observed in the CSF resemble comparable cells

In addition, viral inclusions may be seen in patients with

seen in the peripheral blood or bone marrow in terms of

viral meningoencephalitis, but they are rare.

size and nuclear and cytoplasmic features. However, the

Mononuclear Phagocytes

appearance of cells in the CSF that are also seen in peripheral blood may vary in some details. A Wright-Giemsa's

Monocytes

stain is recommended for the microscopic differentiation

The morphological appearance of CSF monocytes is similar to that of cells.

to that of blood monocytes. These cells do, however, degenerate—

Cells that may be encountered in CSF include granulocytes—

ate more rapidly than do lymphocytes in vitro. Young monocytes—

locytes (mature and immature neutrophils, eosinophils,

cytic cells have less cytoplasm than do mature cells, and the

and basophils), mature lymphocytes or reactive lymphocytes—

cytoplasm is more basophilic. The nucleus may be rounder

cytes, mononuclear phagocytes (monocytes, histiocytes,

or more convoluted in younger cells. Activated monocytic

and macrophages), plasma cells, ependymal cells and

cells are larger in overall size, and nucleoli may be observed

choroidal cells, leukemic blasts, and malignant cells (e.g.,

in the nucleus. The cytoplasm may be vacuolated, and cyto—

lymphoma cells or tumor cells). Other types of cells can

plasmic pseudopods may be seen.

include immature, nucleated erythrocytes and intracellular—

Less than 2% of the cells seen in normal CSF should be

lular bacteria. Lupus erythematosus (LE) cells are rarely monocytes. They are more numerous, especially in degenerated and stimulated forms, in infants and small children than in adults. Disease states that can produce an increase in Lymphocytes

monocytes in CSF include tuberculous meningitis, syphilis, The features of CSF lymphocytes are similar to those of small and viral encephalitis. In addition, meningeal irritation and lymphocytes in peripheral blood. Normal CSF has a few

subarachnoid hemorrhage can induce increased numbers of observable lymphocytes. Large lymphocytes and lymphomonocytes. Monocytes also may be seen in leukemic infiltration of the meninges and in infectious states.

normal CSF.

Degenerative changes such as vacuolization, pyknotic

Macrophages

nuclear changes, and variations in the staining pattern

The morphological characteristics of macrophages (histo—

may be present. Actual changes can include overall cell (neutrophils and monocytes) are described in detail in the section describing shrinkage, a shrunken nucleus or dense clumps of very dark pleural fluids. Macrophages can be seen in the CSF from coloration in the nucleus, and an irregular cytoplasmic border—patients with meningitis or meningeal inflammation, under caused by slow drying of the specimen on the slide. In infectious diseases, CNS leukemia, lymphoma, malignant melanoma, or other metastatic tumors that have spread to the meninges of the brain or spinal cord. In addition, macrophages can be seen in patients who have had hemorrhage, encephalitis, aseptic meningitis syndrome (the majority of cases), bacterial meningitis, syphilitic meningoencephalitis, lography, intrathecal chemotherapy, or irradiation therapy and partially treated bacterial meningitis. Noninfectious of the brain.

causes of increased numbers of lymphocytes include condi—

Macrophages with ingested leukocytes can be observed
tions such as multiple sclerosis.

following a surgical procedure that involves the CNS. In

CSF specimens from patients with acute viral meningitis

some pathologic conditions, erythrocytes are randomly

may contain reactive lymphocytes, which must be differenti—

phagocytized. Erythrophagocytosis can be identified when

detected from lymphoblasts associated with leukemia, as well as a

an ingested erythrocyte still contains hemoglobin. If ghost

large number of lymphocytes. In addition, patients who have

spheres are seen within a macrophage, erythrocyte digestion

undergone chemotherapy and irradiation or conditions

can be inferred.

such as leukemia may have reactive lymphocytes in their

CSF subsequent to treatment. Reactive lymphocytes are vari-

Polymorphonuclear Segmented Neutrophils

able in shape and maturation, compared with blasts, which

Very few, if any, polymorphonuclear segmented neutro—

are uniform in shape and degree of maturation. Reactive

phils (PMNs) should be observed in the CSF. PMNs may lymphocytes are also larger, have more cytoplasm, and usually demonstrate rapid disintegration if the specimen is not usually lack the large nucleoli of lymphoblasts.

examined promptly. The cells may appear as shadows or totally

Patients with disorders other than acute viral meningitis or disappear in an aged specimen. In addition, the cytoplasm is patients who have received chemotherapy or radiation therapy usually pale staining, and azurophilic granulation may not be

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PART 8 ■ Fundamentals of Hematological Analysis

evident in a specimen that is a few hours old. Vacuolization of unless accompanied by clinical symptoms, the demonstration of PMNs may be noted in abnormal or old specimens.

The presence of a number of leukemic cells is strongly suggestive of

The overall size of PMNs may be enlarged if the cell is in the involvement of the subarachnoid space in patients with leukemia—process of phagocytosis. The nucleus may be hyperlobulated kemia or lymphoma.

with long and narrow filaments. Older neutrophils can exhibit

pyknosis or karyorrhexis (one or more spherical, densely stain—

Malignant Cells

ing nuclear fragments) and be mistaken for nucleated RBCs.

The presence of even a few cells with malignant features is diag—

The observation of more than an occasional PMN in the

nostic of metastatic involvement of the subarachnoid space.

CSF classically suggests bacterial infection. However, an

These cells may also originate from primary tumors of the

increase in the number of PMNs can be caused by infectious

brain or spinal cord. Approximately 29% of primary tumors of

and noninfectious agents. Infectious disorders with a pre—

the CNS shed identifiable malignant cells into the CSF.

dominance of PMNs include acute, untreated bacterial men—

Malignant cells are recognizable by the dyssynchrony in

ingitis; viral meningoencephalitis during the first few days

maturation between cells. In addition, malignant cells occur

of the infection; early tuberculosis; and mycotic meningitis.

singly or in clusters. Malignant cells are usually accompanied

Aseptic meningitis can exist in cases in which the septic focus

by many histiocytes.

is adjacent to the meninges. Noninfectious causes of increased

Medulloblastoma, a highly malignant tumor, often

PMN numbers include a reaction to CNS hemorrhage (3 to 4

invades the subarachnoid space and sheds cells into the

days afterward), injection of foreign substances such as lido—

CSF. The cells of medulloblastoma are small and hyper—

chrome into the subarachnoid space, and leukemic infiltration.

chromatic. They can occur singly, in rosette formations, or

in clumps. These malignant cells are very similar in appearance—

Other Granulocytic Cells

ance to neuroblastoma, retinoblastoma, and oat cell carcinoma—

Eosinophils and basophils are not normally seen in the CSF.

noma cells.

Their appearance in CSF is similar to that in peripheral blood.

Cells Unique to the Cerebrospinal Fluid

Eosinophils may be increased owing to causes similar

to those of an increase in PMNs (e.g., bacterial infection).

Ependymal Cells

However, unique causes of an increase in eosinophils include

A few ependymal cells, the cuboidal epithelial cells that cover

systemic parasitic or fungal infections, systemic drug reactions, the surface of the cerebral ventricles and the choroid plexus, and idiopathic eosinophilic meningitis.

may be seen in normal CSF. These cells become rounded in appearance after separating from the lining and resemble lymphocytes or monocytoïd cells. Ependymal cells are medium-sized cells with a large nucleus and a thin rim of cytoplasm. Increased basophil numbers can be observed in chronic granulocytic leukemia; purulent meningitis; inflammatory processes; and parasitic infections.

nucleus is round and generally in the center of the cell. The chromatin is dense and may be slightly grainy or pyknotic. In

Plasma Cells

addition, nucleoli may be seen. The nuclear-cytoplasmic ratio is 1:2 to 1:3. Cellular cytoplasm is usually abundant and stains pink in association with viral disorders such as herpes simplex virus infection, meningoencephalitis, syphilitic involvement—

The cytoplasm displays indistinct borders, and ragged indentations of the CNS, and Hodgkin's disease as well as a few projections of cytoplasm or pseudopods may be seen.

subarachnoid hemorrhage.

Although ependymal cells appear similar to choroidal cells on light microscopy, they differ from choroidal epithelial cells because of the absence of intracytoplasmic inclusions.

Erythrocytes

A few erythrocytes (RBCs) may be seen. An increased concentration of RBCs may be seen in traumatic tap specimens and the border of cilia extending into the ventricular cavity.

An increased number of ependymal cells in the CSF is

or in CSF from patients who have conditions such as a bleed—rare. However, they may be observed in specimens from

ing subarachnoid hemorrhage or intracerebral hemorrhage young children and in patients with hydrocephalus, or ol-

(see the discussion of gross examination). The number of

lowing pneumoencephalography. Finding these cells in the

RBCs may also be increased in chronic myelogenous leukemia—

CSF is of limited diagnostic value.

mia or leukoerythroblastic conditions.

Choroidal Cells

Mesothelial Cells

Choroidal cells are medium in size (about the size of a mature Mesothelial cells are not found in normal CSF. Instead, they can lymphocyte) and usually occur in a clump of similar cells.

resemble pia arachnoidal or ependymal cells. Both monocytes

The nucleus is round or cuboidal and eccentrically located. It and mesothelial cells may be transformed into macrophages, has a loose chromatin structure and nucleoli are not visible.

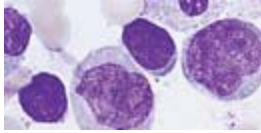
and the morphological distinction is not always obvious.

A generous amount of cytoplasm is evident and is gray or slightly basophilic.

Immature Cells

The nucleus changes from a blue to pink-tinted color in

Immature cells can be seen in patients with leukemias or older samples. In addition, peripheral vacuolization in the malignant lymphomas. Although a single blast is insignificant cytoplasm can be observed in an aging specimen.



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PROCEDURE FOR DIFFERENTIATION OF CELLS IN SPINAL FLUID

PRINCIPLE

increased cellular fragility. In addition, cellular size can be distorted by centrifuge preparation. Cells in the interior of a specimen may be smaller and have a denser nucleus than those at the periphery. A differential count is usually performed.

REAGENTS, SUPPLIES, AND EQUIPMENT

CLINICAL APPLICATIONS

1. Conical centrifuge tubes

Normal CSF is crystal clear and colorless. Gross blood may

2. Microscope slides and Pasteur pipettes

be observed in traumatic tap specimens or in cases of patho—

3. Centrifuge

logical bleeding caused by spontaneous subarachnoid hem—

4. Methylene blue or Wright's stain

orrhage or intracerebral hemorrhage.

Xanthochromia may be indicative of the pathological con-

PROCEDURE

dition subarachnoid hemorrhage, if the erythrocytes have

The sediment to be examined should be prepared using a
been present long enough to hemolyze.

cytocentrifuge, filtration, or sedimentation technique. The

Clotting can be caused by the presence of peripheral

cytocentrifuge is the preferred method of concentrating

blood, increased protein, or gel formation on standing due

CSF specimens. If these methods are not available, an older
to an increased fibrinogen content.

alternative can be used.

turbidity is seen if at least $200 \text{ leukocytes} \times 10^6/\text{L}$, or 400

erythrocytes $\times 10^6/\text{L}$, or microorganisms are present. Increased

ALTERNATIVE METHOD FOR SEDIMENT

segmented neutrophil counts classically suggest bacterial

PREPARATION

in action; however, increased PMNs can be found in infectious

1. Pour 1 to 2 mL of fresh undiluted spinal fluid into a conical centrifuge tube. Balance the centrifuge tube and centrifuge at 2,500 rpm or 10 minutes.

in action), early tuberculosis, and mycotic meningitis. Aseptic

2. Following centrifugation, remove the supernatant fluid. Meningitis can exist in cases in which the septic focus is adjacent to the meninges. Noninfectious causes of increases in cerebrospinal fluid protein include reaction to CNS hemorrhage (3 to 4 days after onset), injection of foreign substances such as lidocaine into the subarachnoid space, and leukemic infiltration.

3. Transfer a small drop of the resuspended sediment onto a glass slide. Increased numbers of lymphocytes are typically associated with viral meningitis.

slide and smear out as a blood smear. Air-dry thoroughly.

ciated with viral infections but may be seen in a variety of

4. Stain with either methylene blue or Wright's stain. The

disorders. These disorders include viral meningoencephalitis—

methylene blue stain is preferred and should be applied to

tis, viral meningitis, syphilitic meningoencephalitis, and

the smear for approximately 12 minutes. Gently wash off

partially treated bacterial meningitis. Noninfectious causes

the stain with distilled water.

of increased lymphocyte numbers include conditions such

5. Allow the smear to air-dry. Examine using the 100× (oil

as multiple sclerosis.

immersion) objective. Count the number of different cells

Other types of cells are rarely encountered. Plasma cells

observed on a total count of 100 leukocytes.

are normally absent. Eosinophils may be increased because

of causes similar to those of an increase in PMNs; increased

REPORTING RESULTS

basophils can be seen in chronic basophilic leukemia, which

Few mononuclear cells (lymphocytes and monocytes) and a

involves the meninges; and monocytes may be seen in leuko—
rare ependymal cells are considered normal findings.
microscopic examination of the meninges and in infectious states.

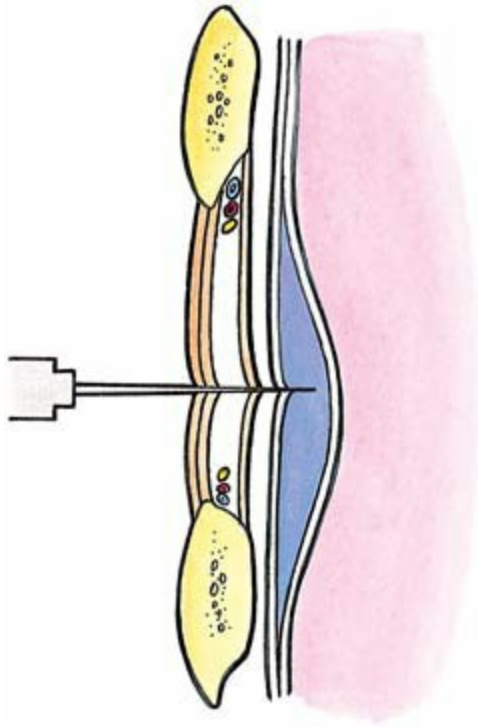
PROCEDURE NOTES

ASSOCIATED FINDINGS

Sources of Error

Glucose and protein values are important to correlate with
actual distortion of cells prepared with a cytocentrifuge
gross and microscopic findings in the CSF. In general, a
can lead to misidentification. Specimens should be prepared
decreased glucose level in the CSF in the presence of a nor—
with a cytocentrifuge, but these preparations may demon—
mal blood glucose level indicates bacterial utilization of glu—
strate actually acts. Portions of fragmented nuclei or cytoplasm
cores. In addition, an elevated total protein concentration is
can be seen. In addition, cells may assume distorted shapes,
also suggestive of an inflammatory reaction or a bacterial
granules may become localized in the cytoplasm, and vacu—
infection. A viral infection will not have a dramatic effect on
ules may appear in the cytoplasm. Abnormal cells are more

CSF glucose levels and may not affect the total protein level
prone to exhibit artifactual disruptions, perhaps because of
significantly.



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PART 8 ■ Fundamentals of Hematological Analysis

Pleural Fluid

NOTE: This is a good time to complete Review Questions
related to preceding content.

Anatomy of the Pleura

The lungs lie in the thoracic (chest) cavity, where they are separated by the
heart in the mediastinum. Each lung is covered by

PLEURAL, PERITONEAL, AND

a serous membrane, the visceral pleura (Fig. 29.4). The interior

PERICARDIAL FLUIDS

of the chest wall, the superior surface of the diaphragm, and the lateral portion of the mediastinum are also lined by a thin mem-

Effusions : Transudates and Exudates

brane, the parietal pleura. The layers of the visceral and parietal

An effusion is an abnormal accumulation of fluid in a par-

pleurae are contiguous, and the potential space between them

ticular cavity of the body. Effusions in the pleural, pericar-

on each side of the thorax forms the pleural cavity. However, the

dial, and peritoneal cavities are divided into transudates

pleural cavity is not a true cavity. It becomes a cavity in an abnor-

mal condition creates an excess accumulation of fluid or air in it.

transudates generally indicate that fluid has

accumulated because of the presence of a systemic disease.

The pleural cavity is lined by a single-cell layer of meso-

In contrast, exudates are usually associated with disorders

thelial cells that form the mesothelium. Mesothelial cells

such as inflammation, infection, and malignant conditions

are supported by layers of connective tissue that contain an

involving the cells that line the surfaces of organs (e.g., lung extensive network of lymphatic vessels and blood capillaries—or abdominal organs).

ies. Although the function of the pleural space is obscure, transudates and exudates frequently differ in character—the stretchable mesothelial cells that line this potential space istics such as color and clarity and in total leukocyte cell provide the lungs and other intrathoracic organs with the count. Classically, transudates have been considered to differ flexibility to expand and retract.

from exudates based on the properties of specific gravity and Pleural fluid is normally produced by the parietal pleura total protein. These characteristics, however, are unreliable and absorbed by the visceral pleura as a continuous process.

in consistently differentiating the two categories of effusions.

Although healthy individuals have 600 to 800 mL of fluid

For example, the mean values of total protein display considerable overlap between transudates and exudates.

estimated at less than 10 mL. This fluid is formed by the filtration

A variety of physical and chemical properties need to be
of blood plasma through the capillary endothelium. The fluid
considered when fluids are categorized as transudates or
is reabsorbed by lymphatic vessels and venules in the pleura.
exudates (Table 29.5).

Transport in and out of the pleural space is dependent on the
balance of hydrostatic pressure in the capillary network of
the parietal and visceral pleurae and capillary permeability,
plasma oncotic pressure, and lymphatic reabsorption.

Comparison of Transudates

TABLE 29.5

Transudates and Exudates*

Characteristics

Transudate

Exudate

Physical Characteristics

pH

7.4–7.5

7.35–7.45

Specific gravity

<1.016

>1.016

Cellular Characteristics

Erythrocytes

Few

Variable

Leukocytes

<1,000

>1,000

Chemical Analyses

Glucose level

Equal to serum

Possibly

decreased

Protein level

<3.0 g/dL

>3.0 g/dL

Pleural uid–serum ratio <0.5

>0.5

FIGURE 29.4 Thoracocentesis. Sometimes it is necessary to insert

of protein

a hypodermic needle through an intercostal space into the pleu—

LDH level

<200

>200 IU/L

ral cavity—the potential space between the parietal pleura lining

Pleural uid–serum ratio

<2:3 (<0.6)

>2:3 (>0.6)

the pulmonary cavity and the visceral pleura covering the lung—to

of LDH†

obtain a sample o pleural f uid or to remove blood or pus. o avoid

damage to the intercostal nerve and vessels, the needle is inserted

*Variations can be observed in examples of various conditions.

superior to the rib, high enough to avoid the collateral branches.

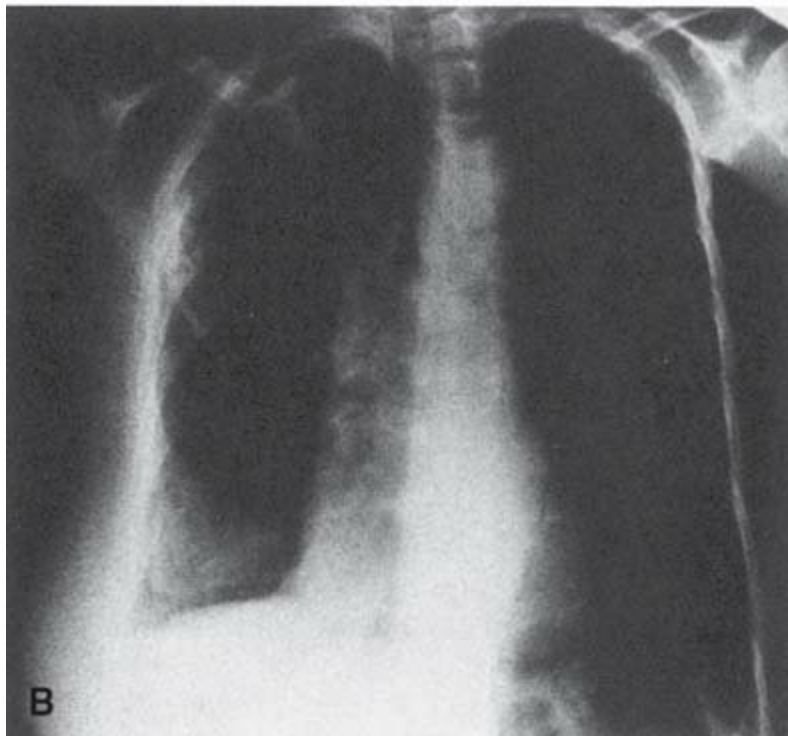
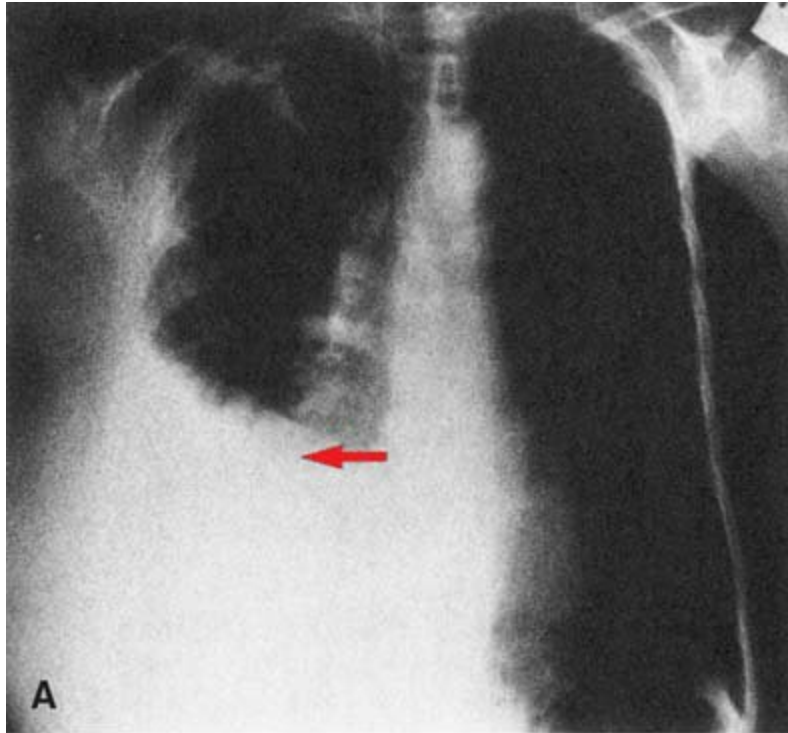
†If nonhemolyzed, nonbloody effusion.

(Reprinted rom Moore KL, Agur A. Essential Clinical Anatomy,

LDH, lactic dehydrogenase.

2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2002,

with permission.)



BOX 29.1

Clinical Correlations (Pleural Fluid)

TRANSUDATES

Congestive heart failure

Cirrhosis with ascites

EXUDATES

Infectious diseases

Empyema

tuberculosis

Malignant neoplasms

Lymphoma

Mesothelioma

Pancreatitis

Rheumatoid arthritis

Laboratory Analysis

Physical Characteristics

Transudates are usually clear, are pale yellow, and do not clot. In comparison, exudates can display a range of colors depending on the associated disorder (Table 29.6). Only

2 mL of circulating blood in 1 L of pleural fluid will produce a blood-tinged appearance. Very viscous fluids, clear

or bloody, are characteristic of mesothelioma. In addition, exudates may be cloudy or purulent and frequently clot on standing because of the presence of fibrinogen.

Specimen turbidity may be caused by lipids or result from

FIGURE 29.5 A. A substantial amount of pleural fluid has accumulated in this patient's right chest cavity (arrow). **B.** After draining and centrifugation indicates the presence of an abundant number of leukocytes, but a white supernatant is caused by chylomicrons. In contrast, chylothorax or pseudochylothorax pleural effusions resemble a chylous effluent. These effusions have a milky or greenish appearance and might have a pearly opalescent sheen. This appearance results from cellular debris and cholesterol crystals. The accumulation of fluid in the pleural space is referred to as a pleural effusion. Excess fluid accumulates if the balance of fluid formation and absorption is in disequilibrium. This

of leukocytes, but a white supernatant is caused by chylomicrons. In contrast, chylothorax or pseudochylothorax pleural effusions

resemble a chylous effluent. These effusions have a milky or greenish

Pleural Effusion

ish appearance and might have a pearly opalescent sheen. This

The accumulation of fluid in the pleural space is referred to as a pleural effusion. Excess fluid accumulates if the balance of fluid formation and absorption is in disequilibrium. This

of fluid formation and absorption is in disequilibrium. This

of fluid formation and absorption is in disequilibrium. This

may be caused by an increased production or a decreased

Representative Exudate

TABLE 29.6

absorption of fluid. Large quantities may need to be drained.

Appearance

Aspiration of pleural fluid is referred to as thoracentesis

Typical Associated

(Fig. 29.5). Failure to remove an increased accumulation of

Appearance

Disorder

leukocytes or blood from the pleural space may lead to the

formation of hemothorax and a subsequent impairment of

Dark red-brown

Amebiasis

pulmonary function.

Greenish to greenish

Classic rheumatoid effusion

The location of a pleural effusion may be suggestive

yellow and turbid

of the type of disorder involved in causing the effusion

(Box 29.1). Typically, left-sided effusions are associated

Yellow and turbid

Infectious process

with conditions such as a ruptured esophagus or acute

Milky

Chylothorax (chylous or

pancreatitis.

pseudochylous)

If a fluid has the general characteristics of an exudate,

Bloody (hemorrhagic)

Traumatic tap, malignancy,

at a minimum, a Gram's stain and culture and cytological

pulmonary infarction, trauma,

studies need to be performed. An open lung biopsy or tissue

pancreatitis, tuberculosis

or examination with histochemical stains and electron

Clearly visible pus (WBCs)

Empyema

microscopy may be required for a diagnosis in suspected

malignant conditions.

Foul odor

Anaerobic bacterial infection

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PART 8 ■ Fundamentals of Hematological Analysis

Cell Count

Lymphocytes

Erythrocyte and leukocyte counts are of limited value in the

Lymphocytes resembling small peripheral blood lympho—

di erential diagnosis of pleural effusions. A massively bloody

cytes are seen in variable numbers in most body fluids.

(hemorrhagic) effusion in the absence of trauma almost always

However, lymphocytes may be variable in size and have an

suggests malignancy or occasionally pulmonary in origin. Pure

immature appearance. The cellular nucleus can be cleaved

blood in the pleural cavity, true hemothorax, results from

and exhibit nucleoli that are often more prominent than

severe chest injuries. In these cases, a microhematocrit deter—

those in peripheral blood lymphocytes.

mination will confirm that the microhematocrit value is simi—

Degenerative changes in aged specimens can include vac—

lar to the patient's peripheral blood packed RBC volume.

uolization, pyknotic nuclear changes, and variations in the

Extremely elevated total leukocyte (WBC) counts o $50.0 \times$

staining pattern. Arti actual changes can include a shrunken

$10^9/L$ or higher are consistent with a diagnosis o empyema.

nucleus or dense clumps o very dark coloration, overall cell

In general, WBC counts less than $1.0 \times 10^9/L$ are associated

shrinkage, and an irregular cytoplasmic border owing to

with transudates, and WBC counts greater than $1,000 \times$

slow drying o the specimen on the slide.

$10^9/L$ are associated with exudates.

E usions rom patients with tuberculosis or malignancies

Use undiluted f uid to per orm the cell count (re er to

requently show a predominance o lymphocytes. E usions

the spinal f uid cell count procedure). Electronic counting

rom patients with non-Hodgkin's lymphoma can mani est

instruments should be used with caution, because debris

malignant lymphocytes that are generally uni orm in compar—

may cause alsely increased counts.

ison to benign conditions in which there is usually a mixture

o different types o lymphocytes (small, medium, and large).

Cell Differentiation Examination

Detection o lymphocyte subsets (T and B lymphocytes)

Smears should be prepared for microscopic examination

in pleural effusion may aid in the differential diagnosis. The

by cytocentrifugation, filter preparation (Millipore), or

subset is considerably higher in fluids from patients with pul—

sedimentation methods. Following preparation o the sedi—

monary tuberculosis than in their blood. The B subset is usually

ment, the smears should be properly stained with Wright's or

significantly lower in pleural fluid than in the circulating blood

Wright-Giemsa stain or differential leukocyte evaluation or

in patients with pulmonary tuberculosis, pulmonary malignant

stained with Papanicolaou's stain or cytological evaluation.

disorders, or nonspecific pleuritis. The presence o a monoclonal

Cell types that can be encountered in the examination o

B-cell population is usually associated with malignant lymphoma.

a Wright-Giemsa-stained specimen include PMNs, eosinophils, basophils, lymphocytes, plasma cells, mononuclear

Mononuclear Cells

phagocytes (monocytes, histiocytes, and macrophages),

Mononuclear phagocytes (monocytes, histiocytes, and macrophages) are seen in variable numbers in both benign and malignant lesions. In addition, in vivo macrophages and histiocytes are used synonymously. Both monocytes and mesothelial cells have also been observed in pleural fluids.

When a cytocentrifuge is used for sediment preparation, artifacts may be transformed into macrophages; the morphology may be encountered. Cells in the interior of a specimen may be smaller in overall size with a denser nucleus than those at the periphery. Abnormal cells in particular are more likely to be affected because of their propensity to be more fragile. In addition, nuclear-induced changes can include distorted shape and segmentation, fragmentation, or holes.

Macrophages vary in size, with a diameter ranging from 15 to 25 μm . The cytoplasm is pale gray and frequently vacuolated. Macrophages may contain phagocytized material such as RBCs. In addition, nuclear-induced changes can include distorted shape and segmentation, fragmentation, or holes.

observable nucleoli. Signet ring cells are a type of macrophage. Cytoplasmic artifacts can include irregular fragmentation, that forms when the small vacuoles of the cell use and form localization of granules, and peripheral vacuolization. one or two large vacuoles that push the nucleus against the side PMNs should be distinguished from mononuclear cells. It of the cell membrane. The nucleus forms the stone component— can be difficult to differentiate lymphocytes from monocytes. nent of the ring. Signet ring macrophages with a normal-size nucleus are commonly seen in sterile inflammatory effusions.

Polymorphonuclear Segmented Neutrophils

The degeneration and death of a macrophage are characterized by an irregular nuclear shape and pyknosis, and cytoplasmic vacuolization and inclusions, with peripheral ruffling. PMNs in pleural fluid may appear morphologically identical to those in the circulating blood or may be difficult to recognize. Immature neutrophils are rarely seen except in chronic granulocytic leukemia or a leukoerythroblastic condition. inflammatory process becomes chronic. Mononuclear cells

In long-standing effusions, signs of cellular degeneration predominate in early inflammatory effusions (e.g., pneumonia, pulmonary infarct, pancreatitis, and subphrenic abscess). After several days, macrophages, lymphocytes, and stained spherical fragments and resemble nucleated erythrocytes (RBCs). Occasionally, the cytoplasm may have a bluish color and resemble the cytoplasm of a lymphocyte.

Eosinophils

An increase in PMNs (Table 29.7) is associated with exudates. An increased number of eosinophils (eosinophilia) in pleural fluid is nonspecific. Eosinophilia in pleural fluid (greater than 100/mm³) is associated with allergic diseases or a bacterial etiology.

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Examples of Cellular Abnormalities Encountered in Pleural and Peritoneal

TABLE 29.7

(Abdominal) Fluids

Condition

Cellular Characteristics

Bacterial Inflammation

Acute

Many neutrophils, histiocytes, and mesothelial cells

May display bacteria

Chronic

Some neutrophils and eosinophils

Many lymphocytes, plasma cells, and histiocytes

Reactive mesothelial cells

May display bacteria

Chronic granulomatous

Elongated or round multinuclear giant cells

inflammation (e.g., tuberculo-Histiocytes, lymphocytes, and plasma cells

sis, sarcoidosis, fungal infection-Some neutrophils

tions, rheumatoid arthritis)

Many reactive mesothelial cells

Amorphous background material from the center of granulomas

May display fungi (special stain), if fungal inflammations

May display tuberculous bacilli (special stains), if tuberculosis

Malignant mesothelioma

Abundant number of cells (single or cluster)

Gland-like peculiar multinucleated cells present

Clusters of cells are made of more than 4–5 cells

Calcified bodies

Occasional psammoma bodies

Metastatic tumors

Malignant cells (single or clusters)

Cytoplasm may display intracellular vacuole, associated with mucin in adenocarcinoma, or squamous cell carcinoma

Intracellular mucin appears as large paranuclear vacuole containing granular blue material Nucleus may be marginated Sarcomas have very large elongated cells with oval to rod-shaped nuclei, small nucleoli and coarse chromatin, and abundant cytoplasm—elongated and finely reticular to granular Poorly differentiated sarcomas have very large tumor cells with large pleomorphic nuclei After chemotherapy or Atypical mesothelial cells

radiation therapy

Increased number of histiocytes

Viral infections

Many lymphocytes, plasma cells, histiocytes, and mesothelial cells

than 10% of total WBCs) may signify that air or blood has

Normally, a small number of cells are sloughed into the

been introduced into the pleural space (e.g., repeated thoracotomy—

serous cavities.

racenteses, pneumothorax, and traumatic hemothorax).

These cells vary in appearance, frequently manifesting

However, it is not diagnostically significant. Eosinophilia

atypical or reactive changes, and usually cause the most difficulty

may also be manifested in parasitic or fungal diseases, pulmonary

cult during the evaluation of cell types. It is extremely difficult

monary in action, and polyarteritis nodosa.

difficult to distinguish between mononuclear phagocytes and

intermediate forms of mesothelial cells. Therefore, they may

Plasma Cells

be mistaken for malignant cells.

The plasma cells resemble those encountered in the

Mesothelial cells may appear as single cells, in clusters, or

bone marrow. An increase in plasma cells accompanies

as sheets. Clustering of cells may be caused by centrifugation

an increase in lymphocytes in patients with multiple

and may closely resemble malignant cells. Clumps of benign

myeloma. Plasma cells may also be seen in effusions from

mesothelial cells can be differentiated from malignant cells by

patients with tuberculosis, rheumatoid arthritis, malignancy, Hodgkin's disease, or other conditions associated with lymphocytosis.

In addition, a uniform, regular arrangement of cells that display fenestrations (openings or windows) between the cyto-

Mesothelial Cells

plasmic membranes of these cells usually indicates that they

Mesothelial cells (middle lining of cells) form the lining are benign.

of the pleural, pericardial, and peritoneal cavities. In vivo,

Mesothelial cells have a large overall size and average

the cells form a single-cell layer or sheet of uniform cells.

from 12 to 30 μm in diameter. Benign mesothelial cells can

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have various appearances; some resemble large plasma cells.

visceral and parietal peritonea. The potential space between

The nucleus or nuclei have a round to oval appearance and

the parietal and visceral layers of the peritoneum is the

occupy about one third to one half of the cell's diameter.

peritoneal cavity. The parietal peritoneum lines the entire

Although one to three nucleoli may be seen, cells may be

abdominal cavity. At the posterior midline, the left and right

multinucleated. Occasionally, a cell may contain 20 or more

sheets of the membrane come together to form a double

nuclei. The nuclear contour is usually smooth and regular,

membrane, the mesentery. Each of the abdominal organs is

with stippled and dark-purple nuclear chromatin.

suspended by this mesentery. As the sheets separate to sur—

The cytoplasm is abundant and varies from light gray to

round an organ, they become the visceral peritoneum of

deep blue. Localized basophilic areas are often seen in the

the organ. In two places within the abdominal cavity, mes—

center of the cell. This perinuclear zone of pallor resembles

enteries extend beyond the organs and form a four-layered

a fried egg in appearance. Cytoplasmic vacuoles of various

thickness, the omenta. Omenta contain phagocytic cells

sizes are often seen. Vacuoles or clear areas at the periphery

that protect the abdominal cavity from infection. However,

of the cytoplasm probably represent glycogen.

peritonitis, an inflammation of these membranes, can result

Degenerative mesothelial cells may show pyknosis and

from infection or chemical irritation.

karyorrhexis. They may also exhibit phagocytosis and trans—

A small amount of fluid, formed by the ultrafiltration of

form into macrophages. Any projections of microvilli may

plasma, lubricates the peritoneum. The presence of this fluid,

be observed extending from the periphery of the cytoplasm;

called peritoneal fluid, reduces friction between the visceral

this is an artifice.

and parietal peritonea as they move against each other.

Mesothelial cells are seen in variable numbers in most

effusions and are increased in sterile inflammations caused

Peritoneal Effusion

by such conditions as pleurisy associated with pulmonary

An abnormal amount of peritoneal fluid (an effusion) can

infection. Few cells, if any, are seen in effusions from patients

accumulate in the peritoneal cavity in the balance between

with tubercular pleurisy or when an increased number of

fluid formation and reabsorption is altered by a disease process. If pyogenic organisms are present in the effusion. The number of cells. The collection of fluid in the peritoneal cavity, ascites, number of large mesothelial cells, differing from macrophages, is results from increased hydrostatic pressure in the systemic more than 5%, tuberculosis is ruled out.

circulation, increased peritoneal capillary permeability, decreased plasma oncotic pressure, or decreased fluid reabsorption. Cytological Examination

sorption by the lymphatic system. The procedure for removing fluid from the peritoneal cavity is paracentesis. Most malignant effusions are caused by metastatic adenocarcinoma because of its peripheral location and high incidence.

Analysis of body fluids, secretions, and tissue biopsy specimens can be valuable in the diagnosis of carcinoma. Another

Causes of Peritoneal Effusions

causes of peritoneal effusions range from disorders and source of the diagnosis of pleural malignancy is sputum.

The causes of peritoneal effusions range from disorders and diseases that directly represent involvement of the peritoneum. The presence of a massive bloody (hemorrhagic) effusion

neum, such as bacterial peritonitis, to abdominal condition in the absence of trauma is highly suggestive of malignancy. The number of malignant cells varies. On microscopic examination, tumor cells frequently aggregate in clumps and sometimes show gland-like formation. Characteristics of malignant cells include the following:

miscellaneous disorders such as myxedema, ovarian diseases, pancreatic disease, and chylous ascites.

1. Variation in cell sizes and shapes (pleomorphic) or similar in appearance (monomorphic)

Conditions that may conform with the definition of exudates can be associated with congestive heart failure, hepatic cirrhosis, and hypoproteinemia.

2. Multiple, round aggregates of cells

3. High nuclear-cytoplasmic (N:C) ratio

Conditions that may conform with the definition of exudates can be associated with congestive heart failure, hepatic cirrhosis, and hypoproteinemia.

4. Irregularity in nuclear size and shape

Conditions that may conform with the definition of exudates can be associated with congestive heart failure, hepatic cirrhosis, and hypoproteinemia.

5. High nuclear-cytoplasmic (N:C) ratio

dates can be associated with primary or secondary peritonitis—

5. Coarseness and clumping of chromatin

tis, malignant disorders, trauma, and pancreatitis.

6. Large, prominent, irregular nucleoli

7. Possible giant vacuoles

Laboratory Analysis

8. Basophilic or vacuolated (mucin-containing) cytoplasm

The laboratory criteria for distinguishing transudates from

9. Irregular and abnormal mitosis

exudates are less clearly defined for peritoneal (ascitic) fluid

10. Engulfment of malignant cells by other malignant cells

than for pleural fluid. Transudates are usually clear and pale

Peritoneal Fluid

yellow. Exudates are cloudy or turbid because of an increased

concentration of leukocytes, elevated protein levels, and

Analysis of the Peritoneum

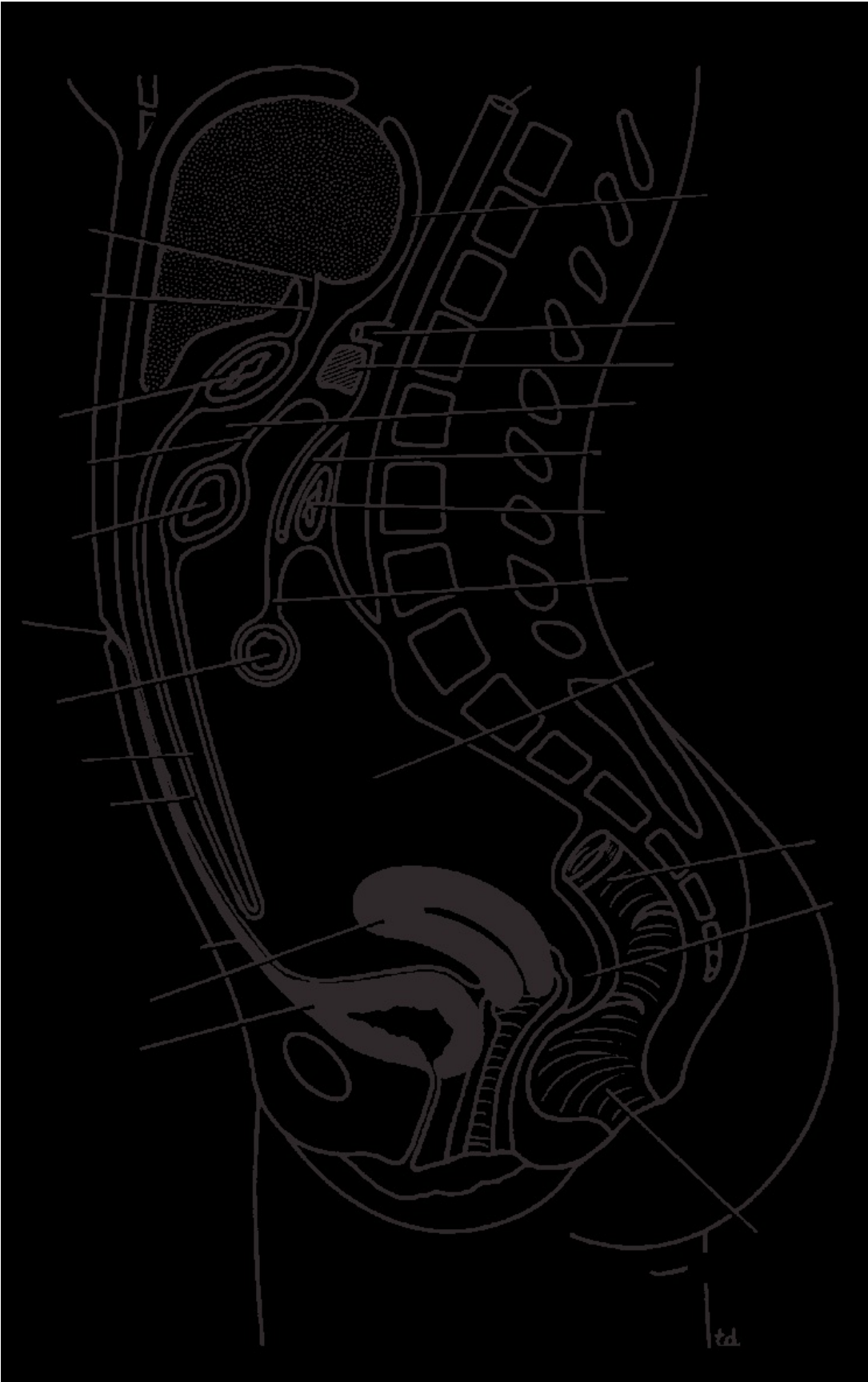
occasionally microorganisms. Exudates may be seen in peritonitis. The peritoneum is a smooth membrane that covers the

peritonitis, cases of perforated or infected intestine, and pancre—

abdominal walls and viscera of the abdomen and pelvis

atitis. An evaluation of ascitic fluid includes gross inspection,

(Fig. 29.6). The continuous sheet of single-cell layers of total cell count, microscopic examination of sediment or mesothelial cells supported by connective tissue forms the cell differentiation, cytological studies, chemical analysis or



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Diaphragm

Aorta

Superior recess

Portohepatis

of lesser sac

Lesser omentum

Celiac artery

Pancreas

Stomach

Lesser sac

Transverse mesocolon

Superior mesenteric artery

Third part of duodenum

Transverse colon

Mesentery

Umbilicus

Greater sac

J e junum

Infe rior re ce s s of le s s e r

s a c

Gre a te r ome ntum

Re ctum

Re ctoute rine pouch

Me dia n umbilica l liga me nt

Ute rus

Bla dde r

Ana l ca na l

FIGURE 29.6 Sagittal section of the female abdomen showing the arrangement of the peritoneum. (Reprinted from Snell RS. Clinical Anatomy By Regions, 8th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2008, with permission.) constituents such as total protein and lactic dehydrogenase, resulting from trauma, lymphoma, tuberculosis, hepatic cir—

and microbial culture.

rhosis, or carcinoma. Malignant lymphoma and carcinoma

are the two most common causes of chylous peritoneal fluid.

Physical Characteristics

In contrast, pseudochylous fluid has a milky or greenish

Pale yellow abdominal fluid can be differentiated from urine

appearance because of the presence of cellular debris and

because urine can be tested for pH, glucose, and protein. A cholesterol crystals. This abnormality may be associated with a variety of clinical conditions (Table 29.8) can produce a deviation from the anticipated yellow or straw-colored fluid. Grossly bloody (hemorrhagic) peritoneal fluid may be seen in trauma. Chronic effusions produced by a wide variety of causes.

tion from the anticipated yellow or straw-colored fluid. Grossly bloody (hemorrhagic) peritoneal fluid may be seen in trauma.

Total Cell Count

patients with a ruptured spleen or liver, intestinal infection, total erythrocyte (RBC) and leukocyte (WBC) counts are performed on ascitic fluid. Use undiluted fluid to perform the cell count (refer to the spinal fluid cell count procedure). Use electronic counting instruments with care because those with duodenal ulcers. Green-colored effusion results usually performed on ascitic fluid. Use undiluted fluid to perform the cell count (refer to the spinal fluid cell count procedure). Use electronic counting instruments with care because those with duodenal ulcers. Greenish fluid, however, may also be present in patients with cholecystitis (inflammation of the gallbladder) or acute pancreatitis. The presence of bile can be

lter preparation (Millipore), or sedimentation methods.

confirmed with a spot test or bilirubin.

Cell counts improve the accuracy and specificity of diag—

Chylous (milky-appearing) peritoneal fluid is rare.

nosis by peritoneal lavage (flushing of space with Ringer

Chylous ascites is caused by a leakage of lymphatic vessels

lactate solution). However, the total cell count is of less

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laparotomy. If the test results are equivocal, another lavage

Variations in Peritoneal

TABLE 29.8

may be indicated in 1 to 2 hours.

(Abdominal) Fluid Appearance

total WBC counts are of limited value in differential diagnosis, but a total WBC count higher than $0.3 \times 10^9/L$ is consid-

Examples of Conditions

ered to be abnormal. More than half of patients with infected

Color

ascites have a total WBC count higher than $0.3 \times 10^9/L$, with

Pale yellow

Normal

more than 25% PMNs on the leukocyte differential smear.

Straw colored

Normal

Leukocyte counts greater than $0.5 \times 10^9/L$ are considered to

Congestive heart failure

be useful presumptive evidence in distinguishing between

Cirrhosis

bacterial peritonitis and cirrhosis. In bacterial peritonitis,

Neoplasm

the total WBC count is higher than $0.5 \times 10^9/L$, with more than 50% PMNs.

Reddish brown

Neoplasm

A wide variation in the peritoneal WBC count is seen in

or bloody

Pancreatitis

patients with chronic liver disease because of extracellular

Pulmonary infarct

shis in fluid associated with ascites formation or resolution.

Trauma

During diuresis, the total leukocyte concentration may

Traumatic thoracentesis

increase dramatically, but the concentration of PMNs usually

Tuberculous peritonitis

remains low. Therefore, the variance of the total WBC

Appearance

count usually does not lead to confusion between cirrhosis

Clear

Normal

and bacterial peritonitis.

Tuberculous peritonitis

total WBC counts may occasionally be elevated in peritonitis.

Turbid (cloudy)

Bacterial peritonitis

peritoneal fluid independently of the RBC count. This is particularly

Pancreatitis

usually true in patients with penetrating abdominal trauma

Conditions with increased cellular

with visceral injury. If lavage is performed immediately after
components

the injury occurs, the WBC count may not yet be elevated
(Table 29.10).

Mucinous

Neoplasm

Chylous*

Obstruction of lymphatic duct (e.g.,

Cellular Differential Examination

(milky)

lymphoma)

Tuberculous peritonitis

Following preparation of the sediment, the smears should be

Trauma

properly stained with Wright's or Wright-Giemsa stain or

Pancreatitis

differential leukocyte evaluation or with Papanicolaou's stain

or cytological evaluation.

Purulent

Bacterial peritonitis

A differential cell count should be performed on the

*Supernatant is white because of chylomicrons.

Wright-Giemsa–stained smear. If a cytocentrifuge is used or sediment preparation, artifacts may be encountered (see “Pleural Fluid” or a discussion of the artifact induced by accuracy in the diagnosis of penetrating trauma (gunshot cytocentrifuge preparation).

and stab wounds) of the abdomen than in other conditions

Although the quantities of some cells in peritoneal

(Table 29.9). A positive result by lavage is indicative of

fluid compared with pleural fluid may vary in some

Criteria for Diagnosing Blunt and Penetrating Trauma by Analysis of Peritoneal TABLE 29.9

Lavage Fluid

Diagnosis

Gross Findings

Laboratory Analysis

Positive

Blood in aspirate or lavage

RBC count $>0.1 \times 10^{12}/L$; $>0.05 \times 10^{12}/L$ in cases of

Lavage fluid retrieved via Foley catheter or chest tube

penetrating trauma

Evidence of food, foreign particle, or bile

WBC count $>0.5 \times 10^9/L$

Amylase level $>2 \times$ serum amylase level

Indeterminate

Small amount of bloody fluid noted in dialysis catheter

RBC count $0.05\text{--}0.1 \times 10^{12}/L$; $0.01\text{--}0.05 \times 10^{12}/L$ in
on insertion

cases of penetrating trauma

WBC count $0.001\text{--}0.005 \times 10^9/L$

Amylase levels slightly higher than serum amylase levels

Negative

RBC count $<0.025 \times 10^{12}/L$

WBC count $<0.001 \times 10^9/L$

Amylase level lower than serum amylase level

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Mesothelial Cells

TABLE 29.10 Examples of Cell Count Variations

In contrast to pleural effusions, tuberculous peritoneal effusions may contain many mesothelial cells. Ascitic fluid

Erythrocytes (RBCs)

associated with cirrhosis may contain many highly atypical mesothelial cells.

High

Neoplasm, tuberculous peritonitis

Variable

Pancreatitis

Malignant Cells

Low

Cirrhosis, bacterial peritonitis, congestive

It is possible to observe malignant tumor cells in peritoneal effusions in patients with congestive heart failure

peritoneal effusions. Cytological examination should be performed if a malignancy is suspected. It is important to distinguish

Leukocytes (WBCs)

between malignant and mesothelial cells because the cells

High

Bacterial peritonitis (PMNs)

most difficult to differentiate from malignant cells are mesothelial cells.

Congestive heart failure (mesothelial)

Neoplasm (>50% lymphocytes)

Dia gnosis of Ascites

Tuberculous peritonitis (>70%

Ascites is a condition in which fluid accumulates within

lymphocytes)

the peritoneal space (cavity). This constitutes a peritoneal

effusion. More than several hundred milliliters of peritoneal fluid must usually be present before the effusion can be

detected by physical examination. Small amounts of effusion

disorders, the cell types that can be encountered are the

may be asymptomatic. Increasing amounts, however, cause

same as those that can be seen in pleural fluids. These

abdominal distention and discomfort, anorexia, nausea,

cells include PMNs, eosinophils, basophils, lymphocytes,

early satiety, heartburn, flank pain, and respiratory distress

plasma cells, mononuclear phagocytes (monocytes, his—

in patients.

tiocytes, and macrophages), mesothelial cells (normal, Radiographic studies such as ultrasonography and computed tomography (CT) scans are very sensitive and allow (malignant) cells. In addition, in vivo LE cells have also been the radiologist to observe the presence of an effusion and to observed.

distinguish it from a cystic mass. Rarely is a laparoscopy or exploratory laparotomy required.

Polymorphonuclear Segmented Neutrophils

Diagnostic abdominal paracentesis with the removal of 50 to 100 mL of fluid is essential for the establishment of an abnormal. A high proportion of PMNs is suggestive of bacterial infection. Aspiration may be combined with lavage.

alcoholic cirrhosis demonstrate a ratio of PMNs in excess of 50%. Patients with abdominal pain who have chronic ascites—

o 30%.

tes or ascites o unknown origin, sudden onset o ascites

In addition, an absolute neutrophil count may also be

(intraperitoneal hemorrhage, in arct, or pancreatic ascites),

help ul. A count greater than $0.25 \times 10^9/L$ is a airly sen—

suspected per oration o a peptic ulcer or bowel per oration,

sitive indicator o spontaneous or secondary bacterial

or blunt trauma to the abdomen need to have a paracen—

peritonitis.

tesis per ormed. wo o the most common indications or

paracentesis are complications o cirrhosis (e.g., spontane-

Eosinophils

ous bacterial peritonitis) and suspected intra-abdominal

Eosinophilia o the peritoneal f uid is less common than

malignancy.

that o the pleural f uid. Eosinophilic ascites is rare, but

T e e usion specimen needs to be analyzed promptly.

when present, more than 50% o the cells in the perito—

Laboratory assessment includes gross examination or char—

neal f uid are eosinophils. Eosinophilic ascites mani ests in

acteristics such as color and clarity; total erythrocyte and patients with eosinophilic gastroenteritis, ruptured hydatid leukocyte cell counts; differential leukocyte examination; cysts, lymphoma, or vasculitis. In addition, patients with chemical assays such as total protein, amylase, and lactate dehydrogenase; and microbial studies including Gram's ascites.

stain, routine cultures, anaerobic cultures, tuberculosis cultures, and cytological examination.

Lymphocytes

A predominance of lymphocytes is seen in transudates

Pericardial Fluid

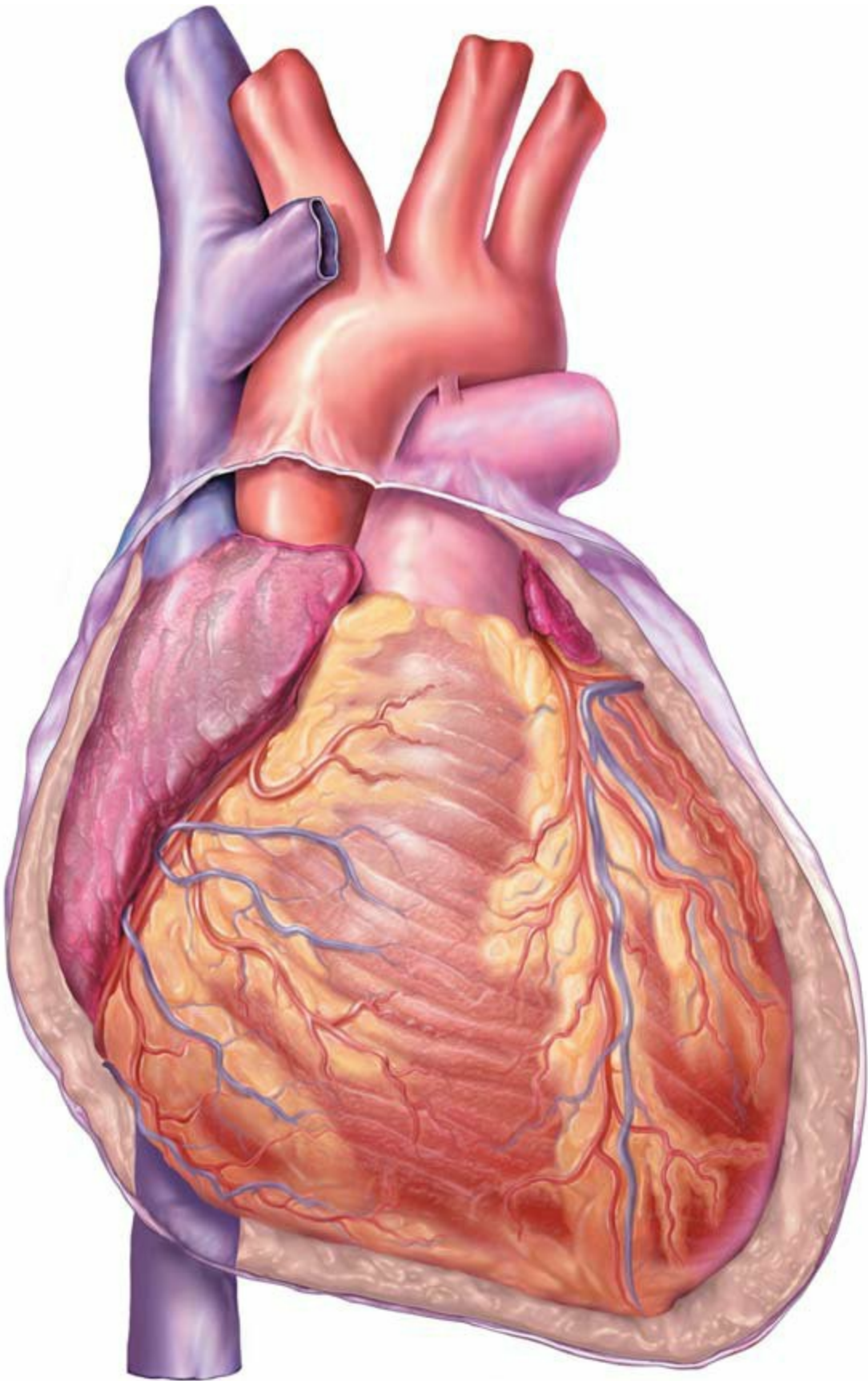
from patients with congestive heart failure, cirrhosis, or nephrotic syndrome. On differential examination, lympho—

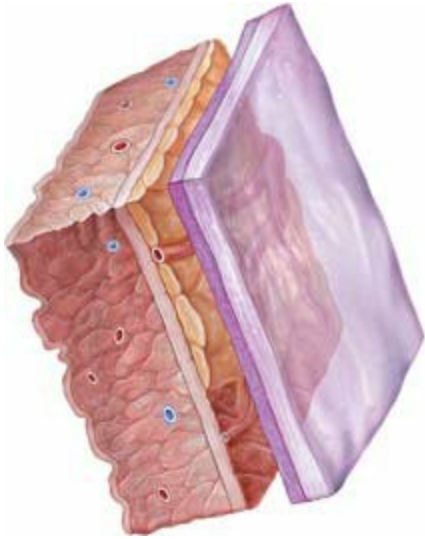
Analysis of the Pericardium

cytes may represent the majority of leukocytes in chylous

The pericardium (Fig. 29.7) is a fibrous sac, composed of the parietal and visceral layers, that encloses the heart. In patients with tuberculous peritonitis or malignancies.

the heart and roots of the great blood vessels. The inner





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serous portion of the pericardium consists of the parietal and visceral layers. The outer parietal layer is in contact with the restraining effect of the pericardium is essentially reflected by the mean central venous pressure. The term cardiac as the epicardium, is in contact with the heart and roots of tamponade is often used to indicate a critical state of cardiovascular compromise, usually with hypotension, caused by fluid accumulation between the parietal and visceral layers, which is filled with a small amount of

by pericardial fluid under increased pressure. It is widely
fluid to reduce friction between the layers, is the pericardial
accepted that any elevation of central venous pressure that is
cavity.

caused by pericardial effusion constitutes cardiac tamponade. Therapeutic
removal of pericardial fluid, pericardiocentesis—

Pericardial Effusion

Pericardiocentesis, is usually indicated if the central venous pressure rises

An abnormal accumulation of fluid in the cavity, a pericardial
to approximately 10 mm Hg.

effusion, is most frequently caused by damage to the lining of

Pericardial effusion is usually accurately assessed by echo—

the cavity and increased capillary permeability. In addition,

cardiography, but there are pitfalls in the interpretation of

in acute pericarditis, interference with pericardial venous

such studies. For example, tamponade can be produced by

and lymphatic drainage predisposes the patient to effusion

localized pockets of pericardial effusion that may not be

development.

evident by echocardiography, particularly if the pocket is

The physiological function of the normal pericardium

located adjacent to the right atrium laterally. C scans and is considered to be pericardial restraint, which tends to magnetic resonance imaging (MRI) are also accurate means

Section of the heart wall

Fibrous pericardium

Parietal pericardium

Pericardial space

Epicardium

Myocardium

Endocardium

Fluid in pericardial space

FIGURE 29.7 Pericardial effusion. (Asset provided by Anatomical Chart Co.)

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o demonstrating pericardial effusion and are less subject to (microhematocrit), however, can be valuable in distinguishing the limitations of echocardiography in localized effusions. distinguishing a hemorrhagic effusion from aspirated blood in a Pericardial disease causes effusions that are loculated or specimen. The quantity of erythrocytes is usually lower in a

bilateral; they are rarely exclusively right sided. Patients with hemorrhagic effusion than in a simultaneously assayed circulating blood specimen. In contrast, aspirated blood, if bilateral effusions.

cient in quantity, will exhibit an erythrocyte volume that is comparable to that in the circulating blood.

Cause of Pericardial Effusion

Pericardial fluid is relatively acellular. An increase (more than $1 \times 10^9/L$) is suggestive of microbial infection or cardiac effusion (Table 29.11). Neoplastic disease produces malignancy.

duces a significant volume of fluid in the pericardium and is one of the most common causes of pericardial effusion.

Evaluation of Samples

Primary tumors of the pericardium (mesothelioma) are rare. However, metastatic tumors of the pericardium and as previously described in the section "Pleural Fluid." The

heart are common in patients with advanced malignant disease—sediment should be stained and examined for leukocytosis. Cells from primary sites (such as the lung and breast) and in cells and malignant mesothelial cells.

patients with leukemia or lymphoma. These types of metastases are the most common causes of malignant effusions.

Leukocyte Differential

Therefore, one of the most important parts of the laboratory value of a differential leukocyte count in establishing a

tory examination of pericardial fluid is cytological studies

differential diagnosis is debatable. However, an elevated total or malignant cells.

leukocyte count in conjunction with mostly PMNs can be observed in bacterial pericarditis. In contrast, pericardial fluid

Laboratory Analysis

may demonstrate increased lymphocytes in viral pericarditis.

Gross Examination

Mononuclear phagocytes (monocytes, histiocytes, and

Normal fluid is transparent and pale yellow. Hemorrhagic macrophages) can be seen in variable numbers in pericar-

(bloody) effusions may result from a variety of abnormal

dial effusions. In addition, in vivo LE cell formation has been observed in pericardial fluids.

specimen. On visible examination, a hemorrhagic effusion should not form clots in a plain (nonanticoagulant) tube, but

Cytological Examination

aspirated blood usually exhibits clotting. A milky-appearing Smears should be closely examined for the presence of malignancy. An effusion may be a true or pseudochyloous fluid (see “Pleural Effusions” or a discussion of chylous fluids). The appearance of these cells was previously described in “Pleural Fluids.”

Fluid” or a discussion of milky effusions).

The value of the measurement of pH is not well established. However, specimens with a pH less than 7.0 may be

NOTE: This is a good time to complete Review Questions associated with infectious or rheumatoid disease. In addition, related to the preceding content.

tion, hemorrhagic specimens typically demonstrate a pH that is lower than the pH in circulating blood.

Serous Fluid

Cell Counts

The main function of seminal fluid (semen) is to transport

Erythrocyte and leukocyte cell counts are of limited value

sperm to female cervical mucus. After deposition in the

in the differential diagnosis of a pericardial effusion.

female reproductive tract, sperm remain in the seminal

Erythrocyte counts or a determination of packed cell volume

plasma or a short time while attempting to enter the mucus.

TABLE 29.11 Causes of Pericardial Effusion

Type

Cause

Infectious agents

Viruses, especially coxsackie group viruses, bacteria (e.g., tubercular, fungal)

Cardiovascular disease

Myocardial infarction, Dressler's (postinfarction) syndrome, cardiac rupture, congestive heart failure, acute aortic dissection Collagen vascular disease

Rheumatic disease

Hemorrhagic

Trauma, anticoagulant therapy, leakage of aortic aneurysm

Renal disease

Kidney failure and uremia (common), long-term dialysis

Neoplastic disease

Mesothelioma, metastatic carcinoma, leukemia, lymphoma

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The analysis of sperm is associated with fertility studies.

prostate surgeries, and large abdominal surgeries performed. In an evaluation of a man's fertility, each aspect of the semen

formed or testicular and rectal cancers can impact fertility.

analysis is considered. Abnormal results are considered to

Environmental factors can also contribute to infertility.

decrease the chances of fertilization. Various factors can

Overexposure to environmental conditions such as toxins,

affect a sperm count or other semen analysis values, including—

chemicals, or heat can reduce sperm production or sperm

lifestyle behaviors such as the use of alcohol, tobacco,

function. Specific causes include

cocaine, some prescription drugs and herbal medicines such

as St. John's wort. Anabolic steroids, cocaine, and marijuana

■ Industrial chemicals

can impact sperm production. Emotional stress, obesity, and

■ Heavy metal exposure

occupation are influencing factors. Certain occupations can

■ Radiation

increase the risk of infertility, including those associated with extended use of computers or video display monitors, Anatomic and Physiology shift work, and work-related stress. Sports activities such as Each of the male reproductive structures (Fig. 29.8) contribute to the formation of semen. Prolonged bicycling can increase heating of the testicles and contributes specific components to seminal fluid. In addition, bicycle seat pressure on the perineum can cause numbness in the testicles. In addition to spermatozoa, this fluid has a highly varied composition and contains hormones that regulate the function of the penis and erectile dysfunction. (Table 29.12).

Health issues and medical factors can also impact male fertility. On ejaculation, sperm, which constitute only a small part of the total volume of seminal fluid, are released from the epididymal stores and combine with fluids from accessory glands to form seminal fluid. Anatomical problems such as undescended testicles or medical conditions such as celiac disease can decrease fertility. Other factors include the following: Initially, secretions are added

■ **Varicocele.** A varicocele is a swelling of the veins that drain from the prostate gland and then from the seminal vesicles. the testicle. This is the most common reversible cause of Prostatic fluid has an acidic pH and provides components male infertility. A varicocele may prevent normal cooling (e.g., fibrinolysis or liquefaction of the clot that forms at of the testicle, leading to reduced sperm count and fewer ejaculation) to the semen. The seminal vesicle, which has an moving sperm.

alkaline pH, contributes 70% of the seminal fluid volume and

■ **Infection.** Some infections can interfere with sperm production or can cause blockage of the passage of sperm.

The first part of the ejaculated seminal fluid contains

Infections include some sexually transmitted infections sperm and prostatic secretions. The second part of the semi— such as Chlamydia and gonorrhea.

nal fluid is composed primarily of seminal vesicle secretions.

■ **Ejaculation issues.** Retrograde ejaculation occurs when semen enters the bladder during orgasm instead of emerging—

Analysis of Seminal Fluid

coming out the tip of the penis.

Principle

- Antisperm antibodies. Antisperm antibodies mistakenly

Seminal fluid (semen) is examined macroscopically and

identify sperm as nonsemen.

microscopically. These procedures are performed to deter-

- Tumors. Malignant and nonmalignant tumors can affect

mine the physical and chemical properties of the fluids such

the male reproductive organs directly or can affect the

as liquefaction, to quantitate the number of sperm cells, and

glands that release hormones related to reproduction.

to examine cellular motility and morphology.

- Hormone imbalances. Infertility can result from hormonal

Semen analysis is the primary test for the evaluation of male

systems including the hypothalamus, pituitary, thyroid,

infertility. Although no specific measures are diagnostic of

and adrenal glands.

infertility, sperm concentration, motility, and morphology can

- Sperm duct defects. Some men experience blockage in the

be used to classify men as sub fertile, or indeterminate fertility, epididymis or a blockage of one or both of the tubes that are fertile (Table 29.13). Semen also can be analyzed for a variety of reasons, including artificial insemination protocols, postvasectomy assessment, and evaluation of probable sexual assault. Men with cystic fibrosis and other inherited conditions may be born without sperm ducts.

■ **Genetic defects.** Inherited disorders such as Klinefelter's syndrome (XXY)—in which a male is born with two X

Specimen Collection

chromosomes—cause abnormal development of the male

A fresh specimen is needed. The specimen may be collected from the reproductive organs.

in a clean, sterile, glass or plastic container. Ideally, seminal

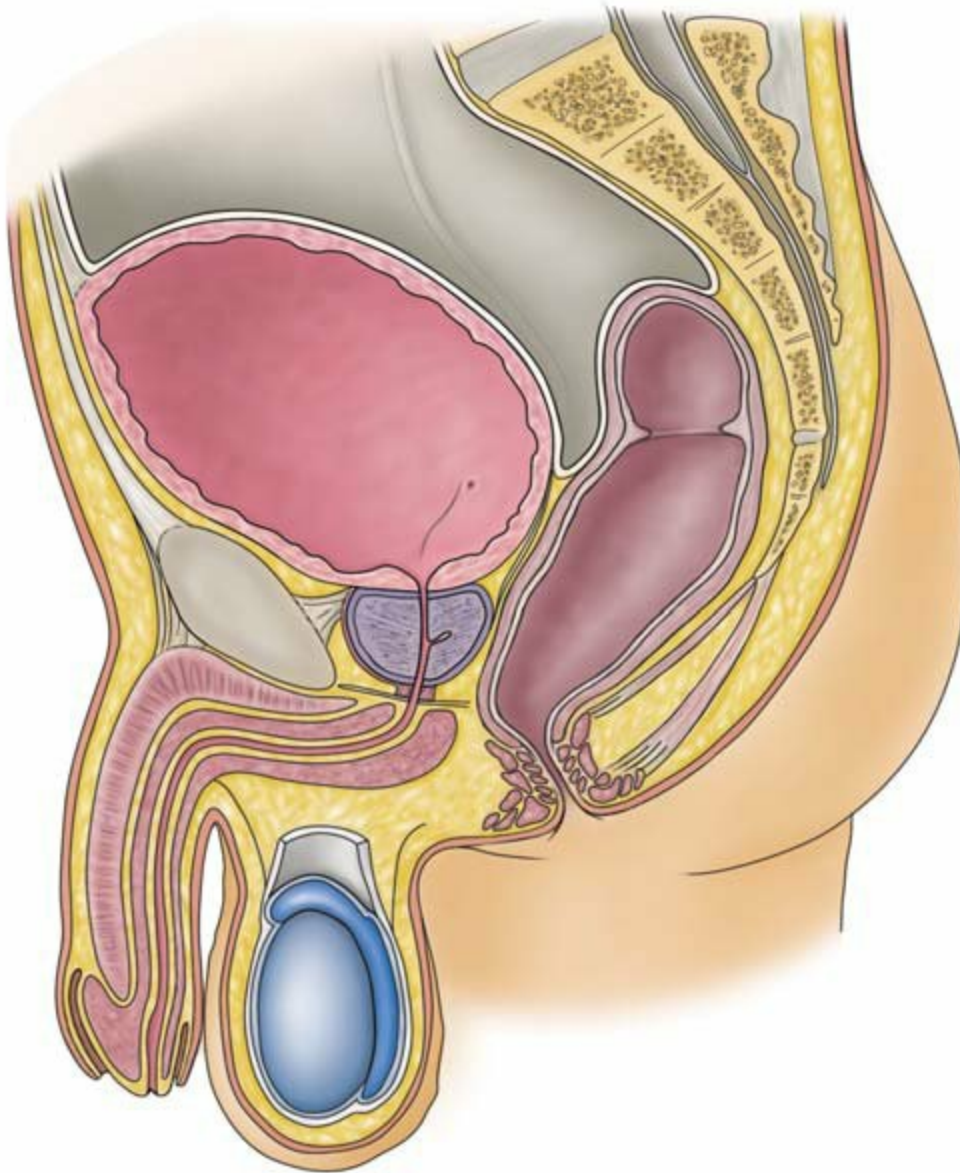
■ **Difficulties with sexual intercourse.** These can include erectile dysfunction, premature ejaculation, painful intercourse, or mandatory that the specimen be kept at 37°C and examined for anatomical abnormalities, or psychological problems.

within 1 hour of collection. After 60 minutes of storage in

■ Medications. testosterone replacement therapy, long-term use of a plastic container, sperm motility is significantly reduced. anabolic steroid use, chemotherapy, certain antifungal medications. Most laboratories examine two specimens collected a few days apart. Collection, proper transport, and prompt examination—impair sperm production.

These factors are critical factors in the analysis of seminal fluid.

■ Surgical history. Certain surgeries including vasectomy, Standard precautions should be adhered to when handling inguinal hernia repairs, scrotal or testicular surgeries, semen, blood, and other body fluids.



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Pelvic cavity

Pelvic um

Superior surface (roof)

Urinary bladder

of bladder

Urethral orifice

Superficial fossa

Rectovesical pouch

Median umbilical ligament

Transverse rectal fold

Apex of bladder

Internal urethral orifice

Linea alba

Ampulla of rectum

Rectovesical septum

Pubic symphysis

Prostate

Retropubic space / fat pad

Prostatic utricle

Intramural part of urethra

Prostatic and

Fundiform ligament of penis

intermediate urethra

Pubopros ta tic liga me nt

Pe rine a l me mbra ne

Sus pe ns ory liga me nt of pe nis

Exte rna l ana l s phincte r

Exte rna l ure thra l s phincte r

Spongy ure thra

Ana l ca na l

Inte rna l a na l s phincte r

Gla ns pe nis

Navicula r fos s a

P re puce

He a d of e pididymis

Exte rna l ure thra l ope ning

Te stis

FIGURE 29.8 Sagittal section o the male pelvis. T e peritoneum drapes over the relatively simple topology o the bladder and rectum. T e prostate gland, which is subject to hyperplasia with advancing age, can be palpated via the rectum. (Reprinted rom Moore KL, Dalley AF. Clinically Oriented Anatomy, 5th ed, Baltimore, MD: Lippincott Williams & Wilkins, 2006, Fig. 3.17A, p. 397, with permission.) It is recommended that a 3-to 5-day period o sexual

it is collected at home. In addition, they must be aware that it

abstinence be observed be ore specimen collection. wo days

must be delivered promptly to the laboratory.

may be sufficient; the period should not exceed 5 days. Condoms

treated with spermicide or lubricants with spermicidal

Macroscopic Examination

properties must be avoided during specimen collection. In addition—

A fresh specimen should be examined for coagulum formation, patients need to be advised to keep the specimen warm, i

tion (liquefaction), color, pH, volume, and viscosity. This procedure, in CLSI format, is provided on this book's companion

Web site at thepoint.lww.com/urgeson6e. The results may be

used not only in the assessment of fertility but also in the

TABLE 29.12 Composition of Seminal Fluid

detection of other disorders.

Structure

Component

Testicle

Sperm, steroids: testosterone

and dihydrotestosterone

TABLE 29.13 Threshold Values of Semen

Testicle or epididymis

Androgen-binding protein

Testicle (most probable

Proteins (enzymes), lipids,

Classification*

Values

source)

electrolytes

Subfertile

$<13.5 \times 10^6/\text{mL}$ sperm concentration

Epididymis

Carnitine, acetyl carnitine,

$<32\%$ of sperm with motility

glyceryl phosphorylcholine

$<9\%$ of sperm with normal

Seminal vesicles

Flavin, fructose, prostaglandins

morphologic features

Seminal vesicles and

Magnesium

Fertile

$>48.0 \times 10^6/\text{mL}$ sperm concentration

prostate

$>63\%$ of sperm with motility

Prostate

Citric acid, enzymes, zinc, p30

glycoprotein

$>12\%$ of sperm with normal morphologic features

Cowper's glands,

Unknown

glands of Litter

* Values between subfertile and fertile are classified as indeterminate.

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Microscopic Examination

determine whether the sperm cells are able to penetrate the

Several microscopic procedures may be valuable in the assessment—
cervical mucus.

ment of seminal fluid. These procedures, in CLSI format, are
In medicolegal cases, identification and security are para—
provided on this book's companion Web site at thepoint.lww.com/surgeon6e. Enumeration of the number of sperm and
jurisdiction. In cases of alleged rape or suspected sexual
examination of the morphological characteristics of the cells
assault, vaginal smears may be submitted for evaluation of
are routinely performed procedures. Other microscopic pro—
the presence of sperm. Sperm can be detected in the vagina
cedures include motility, viability, and agglutination studies.
or 24 to 72 hours after intercourse. However, the absence of
Agglutination may indicate sperm-agglutinating antibodies
sperm does not mean that intercourse has not taken place.
or prostatitis. A significant increase in abnormal movements
Procedures for the identification of semen stains on cloth—
of sperm, notably immobilizing-type motion, is highly sugges—
ing may also be requested. These procedures can include
tive of the presence of sperm-immobilizing antibodies in the
screening for A, B, or H blood group substances, the labile

fluid. Viability and mobility studies should also be correlated. enzyme marker peptidase A, and phosphoglucomutase Morphological characteristics, the commonly encountered in combination with ABO typing. Other procedures can variant forms, are presented in table 29.14. Increases in the include examination or fluorescence under ultraviolet light, number of tapered spermatozoa and immature forms are re— acid phosphatase test, and enzyme-linked immunosorbent quently characteristic of patients with a varicocele and those assay or p30 male-specific semen glycoprotein of prostatic who have been under extreme stress. Increases in both of these origin or an immunological precipitin test to identify semen variants are referred to as a nonspecific stress pattern. Other of human origin on clothing.

variants have no direct correlation with specific disorders.

Sperm viability requires a very simple two-step staining

Other Microscopic Features

procedure using eosin Y as the stain and nigrosin as a coun—

When sperm are being examined or morphological charac—

terstain. Using these stains, sperm that do not take up the

teristics, the presence of other cellular elements (e.g., erythrocytes, leukocytes, or bacteria) in the specimen should be observed. Debris (e.g., precipitated stain) should not be observed. Additionally, a peroxidase stain, for example, Leucostain, can be used to identify peroxidase-positive leukocytes. Mistakenly, bacteria. All specimens should be observed for peroxidase-positive leukocytes.

Trichomonas parasites, particularly donor semen.

Semen pH should be between 7.2 and 7.8, fructose at 150 mg/dL. Technical Notes: If bacteria are observed, a sterile portion of the specimen should be cultured. However, the probability of a positive finding is low. Semen or artificial insemination in action, whereas a pH of 8.0 or higher may indicate an infection, whereas a pH less than 7.0 suggests contamination should be tested for infectious diseases (e.g., Neisseria gonorrhoeae). If a man is being evaluated for infertility, the specimen should be tested for agglutination of sperm occurs when sperm stick together.

men should be cultured for Mycoplasma.

in a specific and consistent manner (head to head, tail to tail, etc.) suggesting an immunologic cause to infertility.

Synovial Fluid

Clumping of sperm in a nonspecific manner may be due to

Synovial (joint) fluid is a transparent, viscous fluid secreted by the synovial membrane. This fluid is found in joint cavities, bursae, and tendon sheaths (Fig. 29.9). Its function is to lubricate the joint space and transport nutrients to the articular cartilage. Impaired function of synovial fluid with age or disease may play a role in the development of degenerative joint disease (osteoarthritis). A variety of disorders produce changes in the number and types of cells and the chemical composition of the fluid. Analysis of synovial fluid plays a

Additional Laboratory Procedures

Other techniques for the examination of semen may be requested in various situations. In cases of infertility, cervical mucus and sperm compatibility tests may be warranted to

Other techniques for the examination of semen may be

Other techniques for the examination of semen may be

Other techniques for the examination of semen may be

Other techniques for the examination of semen may be

Other techniques for the examination of semen may be

Other techniques for the examination of semen may be

Other techniques for the examination of semen may be

Sperm Morphology (Variant

TABLE 29.14

major role in the diagnosis of joint diseases.

Forms)

Type

% Normal Limits

Anatomy and Physiology of Joints

Immature sperm cells (spermatids)

<15

Diarthrodial joints are lined at their margins by a synovial membrane (synovium), with synovial cells lining this

Tapered heads

<15

space. The lining cells synthesize protein and are phago—

Poorly formed heads

<15

cytic. Mechanical, chemical, immunological, or bacterio—

Double heads

<5

logical damage may alter the permeability of the membrane

Large heads

<5

and capillaries to produce varying degrees of inflammatory response. In addition, inflammatory joint fluids contain lytic

Small heads

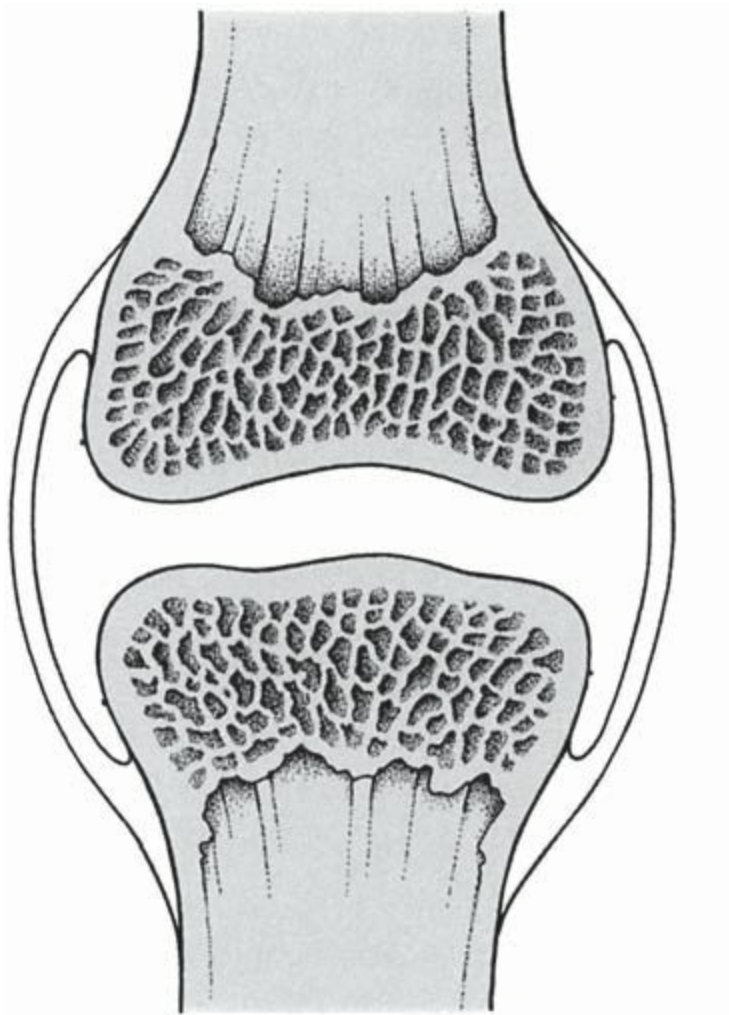
<5

enzymes that produce depolymerization of hyaluronic acid,

Double or broken tails

<5

which greatly impairs the lubricating ability of the fluid.



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Although aspiration was once performed in surgery, it is

TYPICAL

SYNOVIAL JOINT

now considered to be a bedside procedure. As with other procedures involving potentially infectious fluids, gloves should

be worn when performing an aspiration or handling the fluid.

Infiltration of the site with lidocaine to decrease pain into the

Bone

deeper, pain-sensitive structures of the capsule or periosteum

increases the risk of injecting anesthetic into the joint space

and can interfere with the results of some assays.

Synovial membrane

Laboratory Assays

Capsular ligament

Routine analysis of synovial fluid (see procedures, in

Synovial fluid

CLSI format, provided on this book's companion Web site at

Articular cartilage

thepoint.lww.com/ surgeon6e) should include microscopic examination of a wet preparation, crystals, Gram's stain, and microbiological culture. If the fluid is very turbid, or if septic arthritis is considered for other reasons, the specimen should be sent for Gram's stain and culture. A Gram's stain is needed if a high likelihood of infection exists.

Gross Examination

Other observations or procedures can include volume and

FIGURE 29.9 Synovial joint. (Reprinted from Werner R, Benjamin BE. *A Massage Therapist's Guide to Pathology*, 2nd ed, Baltimore, appearance, viscosity, mucin clot test, and chemical analysis MD: Lippincott Williams & Wilkins, with permission.) or protein and glucose.

Purpose of Arthrocentesis

Appearance

Synovial fluid is a plasma dialysate; however, certain molecules—Arthrocentesis constitutes a liquid biopsy of the joint. It is molecules are preferentially excluded from the joint. Normal, a fundamental part of the clinical database, together with noninflamed joints have small quantities of clear or trans—

the medical history, physical examination, and plain radio—
parent fluid. The fluid is viscous and slightly alkaline and, i
graphic films. Analysis of aspirated synovial fluid is essential
normal, does not clot.

in the evaluation of any patient with joint disease because
Inflammatory fluid can be translucent or opaque. In general,
it provides a better reflection of the events in the articular
the more inflammatory the fluid is, the more opaque or puru—
cavity compared with blood tests. For example, abnormal
lent its appearance is, but there is no discrete gross appearance
test results such as antinuclear antibody (ANA), increased
that separates in ected from nonin ected fluid. Cloudiness is
erythrocyte sedimentation rate (ESR), elevated uric acid
not always the result of leukocytes. Fluids can also be opaque
concentration, and rheumatoid factor can be seen in healthy
because of crystals or other materials (Box 29.2).

individuals or in those with unrelated joint diseases.

The color of synovial fluid ranges from pale yellow to straw
Disorders such as gout, calcium pyrophosphate dihydrate
colored depending on the amount of albumin, bilirubin,

(CPPD) deposition disease, and septic arthritis can be diagnosed cells, and other debris present. Edema produces relatively definitively by synovial fluid analysis and may allow or consider—colorless fluid because of its low protein content. The preservation or exclusion of rheumatoid arthritis and systemic lupus erythematosus (SLE). Synovial fluid analysis can also support a number of disorders (Box 29.3).

diagnosis of diseases as disparate as amyloidosis, hypothyroidism, ochronosis, hemochromatosis, and even simple edema.

Viscosity

In addition, arthrocentesis may alleviate elevated intra-articular pressure. The removal of fluid will relieve symptoms. The description of viscosity is a time-honored test. In fact, this property gave synovial fluid its name. If synovial fluid is allowed to drip from the aspirating needle, a long string implies high viscosity and potentially decrease joint damage. Removal of the products of inflammation is an important component in the treatment of infectious arthritis and may be beneficial in implied justification or estimating viscosity is to differentiate

other forms of arthritis.

between noninflammatory (high-viscosity) and inflammatory (low-viscosity) fluids. Unusually, viscous fluids are obtained

Aspiration

from ganglia, hypothyroid lesions, and patients with SLE.

Arthrocentesis is the process performed by a physician to obtain synovial fluid. Synovial fluid is readily obtained by

Mucin Clot Test

aspiration from most joints. Frequent sites of aspiration

The best use of the mucin clot test is to distinguish the ana—

include the knee, shoulder, elbow, wrist, interphalangeal

tomical origin of bloody or other fluids. The presence of a

joints, hip, and ankle.

mucin clot implies that it is synovial fluid.

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Microscopic Examination

BOX 29.2

Wet Preparation Examination

Fresh synovial fluid should be examined under a clean coverslip by routine microscopy for cells and particulate material.

Particulate Matter Found in Synovial Fluid

Cytoplasmic inclusions within granulocytes sparkle and can

Adipose tissue fragments

appear to be light or dark. These inclusions are believed to

Amyloid bodies

represent distended phagosomes or droplets containing lipid.

Bacteria and fungi

It is important to note whether crystalline material is intra—

Cartilage fragments

cellular or extracellular. In acute attacks of gout and pseudo—

Cells

gout, crystals are engulfed by leukocytes. During intercritical

Collagen bodies

periods, crystals may lie free in the fluid. Immunofluorescent

Crystals

studies have demonstrated that some inclusions contain

Fibrin strands and clumps

immunoglobulins and complement.

Immune complexes

Fracture or trauma to a joint can produce free lipid droplets—

Lipid

lets in the synovial fluid, which can also be seen in aseptic

Metal and plastic fragments

necrosis and fat embolism. These droplets are rarely seen in

Parasites

inflammatory effusions. Irregular strands of fibrin or fibrillar

Rice bodies

fragments of cartilage may be observed in specimens from

Synovial fragments

patients with degenerative arthritis.

Unrecognizable junk

Cells and other particulate matter should not be confused

with crystalline materials. One basis of differentiation is that

crystals have straight, parallel edges. In addition to routine light

the mucin clot procedure estimates the density and refractive

microscopy, examination with a polarized light microscope is

ability of the precipitate that forms when synovial fluid

recommended for the identification of crystals (Table 29.15).

is placed in acetic acid. The addition of several drops of

normal synovial fluid to a dilute (2% to 5%) acetic acid

solution results in the formation of a tight, sticky, rope-

Basic Calcium Phosphate

like mass (polymerization of synovial fluid hyaluronate)

Basic calcium phosphate (BCP) crystals include hydroxyapatite

that remains intact when shaken. A good or excellent clot

(HA), octacalcium phosphate, and tricalcium phosphate. The

implies high molecular weight hyaluronic acid and not—

size of BCP crystals is below the limits of resolution of optical

mal hyaluronate-protein interactions. A fair or poor clot

microscopy. If aggregated, they are visible by light microsc—

implies in inflammatory arthritis. There are, however, no

copy and appear as shiny, laminated, printed coins. With a

standard criteria of performance of the test, and the end

polarized microscope, BCP crystals are nonbirefringent.

point is subjective.

BCP crystals are associated with subcutaneous calcification and calcific
periarthritis and tendinitis. In addition, BCP

crystals can be found in both acute and chronic synovitis.

BOX 29.3

Monosodium Urate

Monosodium urate (MSU) crystals are 8 to 10 μm in length

Exam ple s of Co nditions Associated w ith

and are needle or rod shaped. T e crystals may be pointed

Hem arthros is

and intracellular or extracellular. With a polarization microscope, MSU crystals appear as strongly bire ringent rods or

Anticoagulant therapy

needles that are bright against a dark, ully polarized back—

Hereditary de ciency o clotting actors (e.g., hemophilia

ground. With a red compensator, they appear yellow in color

or von Willebrand's disease)

when the longitudinal crystal axis is parallel (negative bire—

In ection

ringence) to the slow component o the compensator. T e

Metallic joint prostheses

crystals appear to be blue when perpendicular to the axis o

Osteoarthritis

the compensator.

Postsurgery or prosthesis

MSU crystals are pathognomonic or gouty arthritis.

Preexisting arthritis

Pseudogout

Calcium Pyrophosphate Dihydrate

Rheumatoid arthritis

Calcium pyrophosphate dihydrate (CPPD) crystals are more eas—

Sickle cell disease (crisis)

ily seen with a good light microscope. CPPD crystals assume

Synovial hemangioma

multiple, three-dimensional forms: rods, rhomboids, and

T rombocytopenia

parallelepipeds occur simultaneously.

T rombocytosis

With a polarizing microscope, CPPD crystals are less bire—

rauma with or without racture

ringent than MSU crystals and are more di cult to identi y.





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TABLE 29.15 Characteristics of Synovial Fluid Crystals

Crystal

Microscopic Appearance

With Polarization

Associated Disorders

Monosodium urate (MSU)

Acicular

Strongly negatively birefringent

Acute gouty arthritis

Red compensator in a polarizing—

Tophaceous gout

ing microscope—yellow color

Calcium pyrophosphate

Polymorphic

Weakly positively birefringent

CPPD deposition disease

dihydrate (CPPD)

Red compensator in a polarizing microscope—blue color

Calcium hydroxyapatite

Too small to identify; with

Not birefringent

Calcific tendinitis

electron microscopy, as small

Apatite-associated destruc—

needles or rods

tive arthritis

Soft tissue calci cations

in the connective tissue

diseases

Calcium oxalate

Polymorphic, classically

Variable; positively birefrin—

Chronic renal disease,

bipyramidal

gent or strongly to weakly

oxalosis

birefringent

Lipid liquid

“Maltese cross”

Strongly positively

Acute and chronic arthritis

birefringent

Cholesterol

Large, plate-like, with

Strongly variably birefringent

Chronic rheumatoid effusions

punched-out corners

Corticosteroids

Polymorphic clumps, rods,

Strongly variably birefringent

Iatrogenic

or rhomboids

Talc

Varying size

Strongly positively

Contaminant

birefringent

MSU, monosodium urate; CPPD, calcium pyrophosphate dihydrate.

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With a red compensator, CPPD crystals appear blue when the

longitudinal axis is parallel to the slow component of the com—

TABLE 29.17 Classification of Synovial Fluid

compensator. They exhibit positive birefringence. CPPD crystals are yellow when perpendicular to the axis of the compensator.

Group

Description

CPPD crystals are associated with CPPD deposition disease.

However, they may be identified in effusions from a number of

I

Noninflammatory

inflammatory joint diseases, particularly rheumatoid arthritis.

II

Inflammatory

Cholesterol Crystals

III

Infectious

Cholesterol crystals are usually easy to distinguish because of

IV

Crystal induced

their large size and flat, plate-like shape. Characteristically,

V

Hemorrhagic

these rectangular plates have notched corners. They may, however, appear as long, birefringent needles or as rhomboids, resembling MSU or CPPD crystals.

the traditional push method. This method has the advantage

The presence of cholesterol crystals is considered to be

requiring no special equipment, but the recovery of cells

nonspecific. However, they are usually found in chronic effusions variable and a considerable amount of cellular damage is

sions from patients with rheumatoid arthritis.

produced.

More effective methods of concentrating cells include

Artifacts

sedimentation, cytocentrifugation, and filtration.

Artifacts can be mistaken for crystals, although crystals have sharp, clearly defined edges and straight sides. Particulate

Staining of Body Fluid Sediment

matters that can be confused with crystals include plastic joint prostheses, nail polish, dust particles, immersion oil

Morphological descriptions of cells encountered in body droplets, and refractile collagen fibrils.

fluids reflect their microscopic appearance with Wright

CPPD and MSU crystals can be confused with other birefringent materials including crystalline anticoagulants, such as calcium oxalate, ethylenediaminetetraacetic acid (EDTA), and lithium heparin; certain corticosteroid preparations; and talcum powder. However, the Papanicolaou's stain (Figure 29.18) is somewhat different. This procedure, in CLSI format, is provided on this book's companion Web site at thepoint.lww.com/nurse6e.

The Wright-Giemsa stain is basically a cytoplasmic stain

Clinical Applications

with moderate nuclear staining ability. In contrast, the

The distinction between various types of arthritis is not always

Papanicolaou's stain is predominantly a nuclear stain with a

easy to make based on clinical observations. Traditionally,

modest ability for cytoplasmic differentiation. The Wright—

synovial fluids have been classified into several categories

Giemsa staining method is simpler than the Papanicolaou

(Figures 29.16 and 29.17) based on gross appearance, total

method because it requires no immediate fixation of the leukocyte and differential cell counts, and physical and slide and therefore fewer steps in the staining procedure. chemical examinations.

However, a difference in cell size is evident between the two staining protocols. Cells appear larger when prepared by the

Body Fluid Slide Preparation

air-dried, Wright-Giemsa procedure. It is most helpful, if possible, to prepare and stain specimens by both methods to. A differential cell count on a body fluid should be performed to gain as much information as possible. The criteria for diagnosis on stained smears prepared from a concentrated preparation are exactly the same for normal or abnormal cells by fixation—not in a hemacytometer. Some of the techniques of either method.

Sediment preparation and staining are different for body fluids than for blood. The procedure, in CLSI format, is provided on this book's companion Web site at thepoint.lww.com/nurse6e.

Amniotic Fluid

Ordinary centrifugation can be used to concentrate cells—

Amniotic fluid is the nourishing and protecting liquid containing elements in the sediment, and slides can be prepared with fluid obtained by the amniocentesis of a pregnant woman. It consists of mostly water but also contains proteins, carbohydrates, lipids and phospholipids, urea, and electrolytes, all of which aid in

Comparison of Inflammatory and

the growth of the fetus. In the late stages of gestation, most of

TABLE 29.16 Noninflammatory Synovial Fluids

the amniotic fluid consists of fetal urine.

The volume of amniotic fluid increases until about

Total WBC % Polymorphonuclear

34 weeks of gestation, at which time the amount of amniotic

Category

Count

Segmented Neutrophils

fluid is about 800 mL and is reduced to about 600 mL at the time of birth (about 40 weeks).

Inflammatory

$>2 \times 10^9/L$

$>75\%$

Amniotic fluid is continually being swallowed and

Noninflammatory $<2 \times 10^9/L$

$<75\%$

“inhaled” and replaced through being “exhaled.” It is essential

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TABLE 29.18 Papanicolaou Stained Morphology

Cell Type

Nucleus

Cytoplasm

Neutrophils (mature)

Multilobulated; hyperchromatic

Green or pink; granules not evident

Neutrophils (immature)

Green or pink; primary granules not visible; secondary granules present

Lymphocytes

Round with finely granular chromatin; no nucleolus

Green; absent or scanty

visible

Lymphocytes (reactive)

Oval to cleaved; finely granular with evenly

Green; moderate amount

distributed chromatin; small chromocenter

Monocyte (usually macro—

Lacy

Some color; moderate amount; slightly

phages in pleural fluid)

vacuolated

Macrophage

Lacy chromatin pattern; irregular shape

Green; degenerating cell may be pink.

Mesothelial cell

Size variable (may occupy up to 50% of the cell);

Deep pink or green; homogeneous distribution—

round to oval; usually central; well-defined membrane—

may be more densely stained in

periphery; evenly distributed granular chromatin; small

center of the cell and around the nucleus;

nucleoli may be present; multinucleated

pale cytoplasmic vacuoles may be seen.

Ependymal cells

Round; central dense chromatin; may be grainy or

Green or pink; may have “brush” border—

possible nucleoli

abundant; generous amount

Choroidal cells

Round; central, smooth, dense chromatin

Pale green; moderate amount

Plasma cells

Round to oval; eccentrically located; clumped

Dense and green; abundant; paranuclear

chromatin

perinuclear area present; may contain small vacuoles (e.g., Russell’s bodies, “grape cells”)

Basophils

Granules do not stain

that the amniotic fluid be breathed into the lungs by the fetus

negative FN can reduce unnecessary hospitalizations and

in order for the lungs to develop normally.

drug therapies. High levels can be due to causes other than
The analysis of amniotic fluid, tapped from the mother's
risk of preterm delivery. The American College of Obstetrics
abdomen, is called amniocentesis. The fluid contains fetal
and Gynecology currently does not recommend routine FNC
cells that can be examined for genetic defects, and chemi—
screening of pregnant women, as its use has not been shown
cal analysis, for example, bronectin, and other assays can
to be clinically effective in predicting preterm labor in low—
determine fetal lung maturity. Fetoplacental function can be
risk, asymptomatic pregnancies.

assessed by analyzing the lecithin/sphingomyelin ratio.

Lamellar body counts (LBCs) in amniotic fluid are

Fetal bronectin (FN) is a protein produced during preg—
another assessment of fetal lung maturity and the associ—
nancy and functions as a biological glue, attaching the fetal
ated risk of developing respiratory stress syndrome in a
sac to the uterine lining. FN is performed if a woman is 26 to
premature in ant. Respiratory distress syndrome is caused
34 weeks pregnant and having symptoms of premature labor.

by insufficient surfactant in a newborn's lungs. The number

The goal then is to intervene to prevent the potentially serious lamellar bodies present in the amniotic fluid is propor—

ous health complications of a preterm baby.

tional to amount of available surfactant. Lamellar bodies

A cervical or vaginal fluid sample is collected and analyzed

have concentrated layers of phospholipid secreted by type

or FN. During the first trimester and for about half of the sec—

II alveolar cells and act as storage packets of surfactants in

second trimester (up to 22 weeks of gestation), FN is normally

amniotic fluid. Lamellar bodies can be counted using

present in the cervicovaginal secretions of pregnant women.

platelet channels in automated instrumentation (see

In most pregnancies, after 22 weeks, this protein is no lon—

Chapter 30). Fetal lung maturity cutoff values can be estab—

lished by three methods according to CLSI document

be before labor). The presence of FN during weeks 24 to 34 of a

C58-A (2011).

high-risk pregnancy, along with symptoms of labor, suggests

that the “glue” may be disintegrating ahead of schedule and

alerts doctors to a possibility of preterm delivery.

A negative FN result is highly predictive that preterm

NOTE: This is a good time to complete end of chapter
delivery will not occur within the next 7 to 14 days. A

Review Questions.

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PART 8 ■ Fundamentals of Hematological Analysis

CHAPTER HIGHLIGHTS

Seminal Fluid

- Seminal fluid is examined physically, chemically, and

Cerebrospinal Fluid

microscopically.

- CSF acts as a shock absorber for the brain and spinal cord,

- Procedures are performed to determine the physical and chemical properties, to quantitate the number of sperm cells, and to examine cellular motility and morphology.
- circulates nutrients, lubricates the CNS, and may also contribute to the nourishment of brain tissue.

- Clinically, the examination of spinal fluid is useful in

- Fresh specimens should be examined for color, pH, volume, and viscosity.

hemorrhage, meningeal infection (meningitis), multiple

- Seminal fluid can be analyzed for a variety of reasons, including in fertility studies, artificial insemination proto-
- sclerosis, and neoplasms.

- Normal CSF is crystal clear and colorless. A yellow color—

cols, postvasectomy assessment, and evaluation of probing of a specimen is referred to as xanthochromia.

able sexual assault.

- Normal CSF has the viscosity of water.

Synovial Fluid

- Total WBC counts are useful in developing a differential diagnosis. Very few leukocytes should be seen in normal

- Synovial fluid is a transparent, viscous fluid secreted by the CSF. Elevated WBC counts can be observed in acute, synovial membrane.

untreated, bacterial meningitis. Very high WBC counts

- Turbid fluid is found in joint cavities, bursae, and tendon are unusual and suggest intraventricular rupture of a brain sheaths.

abscess.

- The function of the fluid is to lubricate the joint space and

- Normal CSF contains a few mononuclear cells and rare transport nutrients to the articular cartilage.

ependymal cells.

- Analysis of synovial fluid plays a major role in the diagno-

■ Cells that may be encountered in CSF include granulocytes and cells associated with joint diseases.

cytic leukocytes, mature lymphocytes or reactive lymphocytes.

■ Synovial fluid analysis can also support a diagnosis of diseases. Cells, mononuclear phagocytes, plasma cells, ependymal cells and choroidal cells, leukemic blasts, and malignant cells as disparate as amyloidosis, hypothyroidism, ochronosis, hemochromatosis, and even simple edema. In addition, cells. Other types of cells can include immature, nucleated cells. Arthrocentesis may alleviate elevated intra-articular pressure. erythrocytes, or intracellular bacteria.

■ A decreased glucose level in the CSF in the presence of a

Body Fluid Slide Preparation

normal blood glucose level indicates bacterial utilization of glucose.

■ A differential cell count on a body fluid should be performed.

■ An elevated total protein concentration is suggestive of an inflammation. Formed on stained smears prepared from a concentrated preparation—not in a hemacytometer.

- A viral infection will not have a dramatic effect on CSF
 - Ordinary centrifugation can be used to concentrate cellular elements in the sediment, and slides can be prepared significantly.
- with the traditional push method.

- The Wright-Giemsa stain is basically a cytoplasmic stain with moderate nuclear staining ability. In contrast, the

Pleural, Peritoneal, and Pericardial Fluids

Papanicolaou's stain is predominantly a nuclear stain with a modest ability for cytoplasmic differentiation.

- An effusion is an abnormal accumulation of fluid in a particular space of the body. Effusions in the pleural, pericar-

Amniotic Fluid

dial, and peritoneal cavities are divided into transudates or exudates.

- Amniotic fluid is the nourishing and protecting liquid
- Transudates generally indicate that fluid has accumulated consisting of mostly water but also contains proteins, carbohydrates, lipids and phospholipids, urea, and electro-

■ Exudates are usually associated with disorders such as
lytes, all of which aid in the growth of the fetus.

inflammation, infection, and malignant conditions involving

■ In the late stages of gestation, most of the amniotic fluid
lining the cells that line the surfaces of organs (e.g., lung or
consists of fetal urine.

abdominal organs).

■ It is essential that the amniotic fluid be breathed into the

■ Transudates and exudates frequently differ in characteristics—
lungs by the fetus in order for the lungs to develop normally.
tics such as color and clarity and total leukocyte cell count.

■ The analysis of amniotic fluid, tapped from the mother's

■ A hemorrhagic effusion in the absence of trauma almost
abdomen, is called amniocentesis.

always suggests malignancy or occasionally pulmonary

■ The fluid contains fetal cells that can be examined or
in vitro.

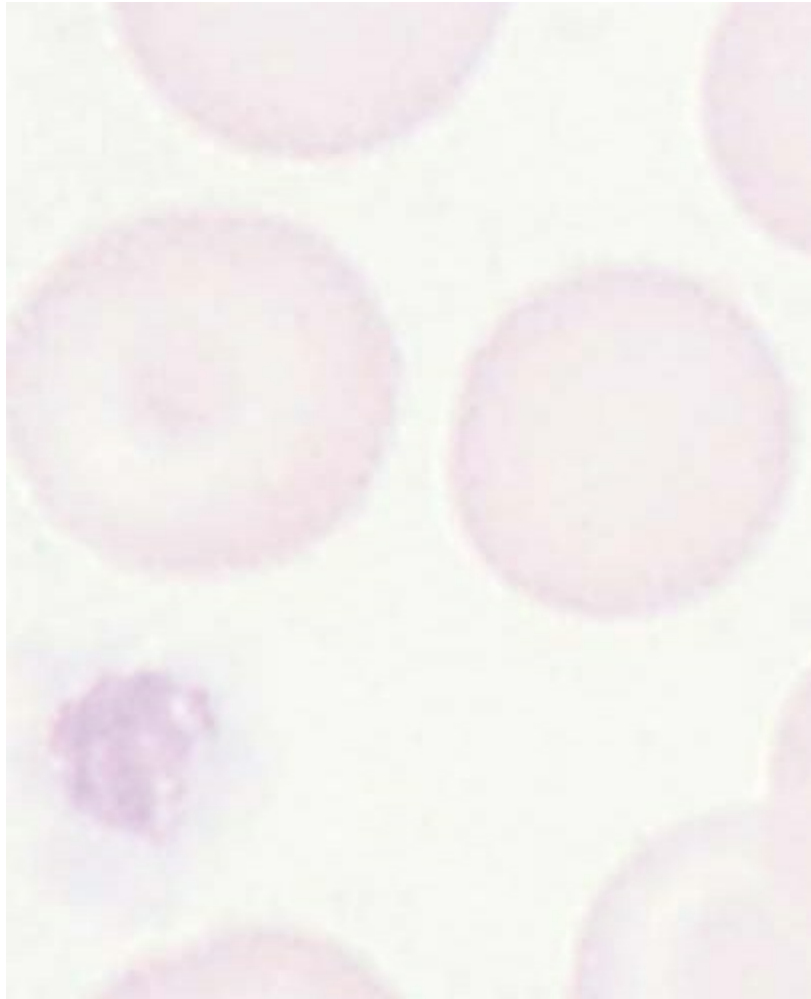
genetic defects, and chemical analysis, for example, bro-

■ Extremely elevated total WBC counts in pleural fluids are
nectin, and other assays can determine fetal lung maturity.

consistent with a diagnosis of empyema.

- LBCs are associated with the risk of developing respiratory
- Pericardial fluid is relatively acellular.

stress syndrome in a premature infant.





CHAPTER 29 ■ Body Fluid Analysis

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CASE STUDIES

Case Study 29.1

Case Study 29.2

An 18-year-old college student with fever, chills, and
T is married couple was seen by an obstetrical-gynecologic
severe headache is seen in an urgent care clinic. She elt
ertility specialist. An examination o the wi e had revealed
nauseated and vomited when she visited to the clinic. At
no apparent abnormalities related to the ability to conceive.
the clinic, her temperature is 103.5°F; she has neck rigidity
and complains o back pain. Some small petechial spots are
On this o ce visit, the husband was examined. He had no
noted in the mouth. Blood is drawn or a complete blood
history o infectious diseases and was in good health. A
count (CBC) and blood glucose, and lumbar puncture is
semen analysis was ordered.
per ormed. Cerebrospinal f uid is collected sequentially in
three sterile tubes and examined.

■ Laboratory Results

otal volume: 1.8 mL (reference range 1.5 to 5.5 mL/ejaculation)

Sperm count: $115 \times 10^6/\text{mL}$ (reference range $\geq 20 \times 10^6/\text{mL}$)

■ Blood Results

Sperm cell morphology: 35% = normal (reference range

White cell count: $25 \times 10^9/L$

greater than 12%)

Differential: 80% neutrophils, 10% lymphocytes, 10% mono—

Sperm cell morphology: No defects in the head or tail
cytes

Note: Greater than 50% of those cells examined must be

Glucose: 95 mg/dL

normal in size, shape, and length.

■ Cerebrospinal Fluid Results

Sperm motility specimen stored @ 37°F or 45 minutes $\geq 70\%$

CSF pressure: Increased

Note: At least 50% should be motile 1 hour after ejaculation,

Gross appearance: All tubes equally cloudy, not bloody
moving forward in a straight line with good speed.

Glucose: 15 mg/dL

Sperm viability test: Within normal reference range

CSF white cell count: $12.0 \times 10^9/L$; 90% neutrophils

Culture for Mycoplasma: Negative

Gram's stain: Many gram-negative cocci in pairs, some

Additional observation—No bacteria or white blood cells

intracellular

were seen in the specimen.

■ Critical Thinking Group Discussion Questions

■ Critical Thinking Group Discussion Questions

1. Based on the Gram's stain, what is the likely diagnosis or

1. Do any of the laboratory results suggest a subertile condition—
this patient? Explain the reason for your answer.

dition?

2. How can this patient's diagnosis be differentiated from

2. How would sperm viability be determined?

other similar clinical presentations?

Note: This is a good time to write out the answers to the
review questions.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

*3. CSF is collected from an intervertebral space between
the

and

vertebrae.

Cerebrospinal Fluid (CSF)

A. 4, 5

1. The anatomical structures associated with the circula—

B. L2, L3

tion of CSF are

C. L3, L4

A. ventricles and subarachnoid spaces

D. L4, L5

B. subarachnoid space and pia mater

C. ependyma and pia mater

4. Tube 1 is commonly used for

D. arachnoid mater and pia mater

A. gross examination, cell counting, and morphology

examination

*2. CSF production is associated with the

B. microbial examination

A. arachnoid mater and pia mater

C. chemical and serological examination

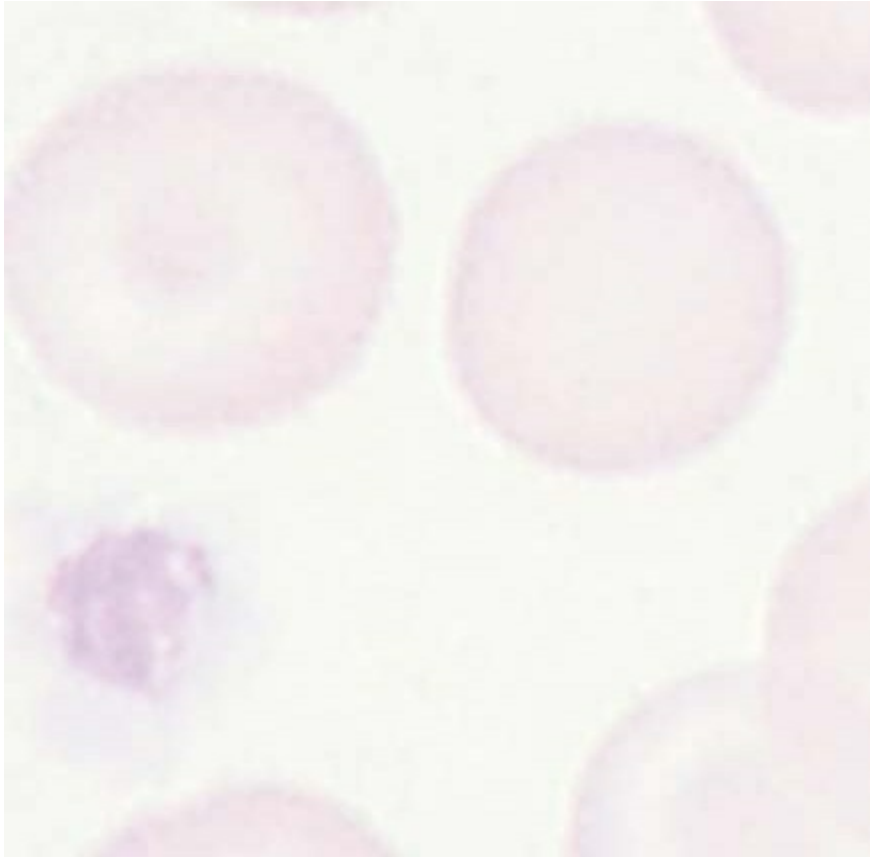
B. choroid plexus and ependymal lining

D. a discard tube

C. arachnoid mater and subarachnoid space

D. subarachnoid space and pia mater

(continued)



PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS (continued)

5. Tube 2 is commonly used for

*12. Viral infection is associated with

A. gross examination, cell counting, and morphology

A. lymphocytosis

examination

B. increased polymorphonuclear segmented neutro—

B. microbial examination

phils (PMNs)

C. chemical and serological examination

C. macrophages

D. a discard tube

D. extremely elevated leukocyte count in CSF

E. normal leukocyte reference range in CSF

6. Tube 3 or the nail tube is commonly used for

A. gross examination, cell counting, and morphology

13. A leukocyte count of 0 to $5 \times 10^6/L$ is associated with

examination

A. lymphocytosis

B. microbial examination

B. increased polymorphonuclear segmented neutro—

C. chemical and serological examination

phils (PMNs)

D. a discard tube

C. macrophages

D. normal leukocyte reference range or CSF

7. A cloudy and turbid specimen is most commonly

*14. Bacterial infection is associated with

caused by

A. lymphocytosis

A. increased fibrinogen

B. increased polymorphonuclear segmented neutro—

B. subarachnoid hemorrhage

phils (PMNs) in CSF

C. subarachnoid hemorrhage (more than 12 hours

C. macrophages

after the bleed)

D. normal leukocyte reference range or CSF

D. increased numbers of leukocytes

*15. CNS leukemia or lymphoma is associated with

*8. A grossly bloody specimen is most commonly caused

A. lymphocytosis

by

B. increased polymorphonuclear segmented neutro—

A. increased fibrinogen

phils (PMNs)

B. subarachnoid hemorrhage

C. macrophages

C. subarachnoid hemorrhage (more than 12 hours

D. extremely elevated leukocyte count in CSF

after the bleed)

D. pleocytosis

16. Normal CSF contains

A. lymphocytes and ependymal cells

9. A xanthochromic (yellow color) specimen is most

B. ependymal and choroidal cells

commonly caused by

C. mesothelial and ependymal cells

A. increased fibrinogen

D. erythrocytes and leukocytes

B. subarachnoid hemorrhage

C. subarachnoid hemorrhage (more than 12 hours

17. The cell count on a CSF specimen should be performed
after the bleed)

within

of collection.

D. pleocytosis

A. 30 minutes

B. 1 hour

10. Gel formation in a specimen is most commonly caused

C. 2 hours

by

D. 12 hours

A. increased fibrinogen

E. 24 hours

B. subarachnoid hemorrhage

C. subarachnoid hemorrhage (more than 12 hours

*18. Clotting in CSF may be caused by

after the bleed)

A. increased protein concentration

D. pleocytosis

B. increased electrolyte concentration

C. increased glucose concentration

*11. Intraventricular rupture of brain abscess is associated with

D. the presence of bacteria

A. lymphocytosis

*19. An increased total leukocyte count in a CSF specimen

B. increased polymorphonuclear segmented neutro—

can be caused by

phils (PMNs)

A. bacterial meningitis

C. macrophages

B. viral meningoencephalitis

D. extremely elevated leukocyte count in CSF

C. intravascular rupture of a brain abscess

D. both A and C

CHAPTER 29 ■ Body Fluid Analysis

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REVIEW QUESTIONS (continued)

*20. An increase in the number of lymphocytes in a CSF

*28. Conditions not associated with pleural effusion include
specimen can be caused by

A. tuberculosis

A. multiple sclerosis

B. infectious diseases

B. viral meningoencephalitis

C. mesothelioma

C. fungal meningitis

D. viral pneumonia

D. all of the above

*29. Yellow and turbid is a representative exudate appearance

*21. Based on the information in case 29.1, the observation
appearance typically associated with
of cloudy CSF samples suggests

A. empyema

A. normal condition of a CSF specimen

B. infectious process

B. viral infection of the meninges

C. anaerobic bacterial infection

C. parasitic infection of the brain

D. chylothorax

D. bacterial infection present

*22. Based on the information in case 29.1, the differential

*30. Milky is a representative exudate appearance typically
diagnosis of the patient could be

associated with

A. multiple sclerosis

A. empyema

B. acute brain hemorrhage

B. infectious process

C. pulmonary embolism

C. anaerobic bacterial infection

D. bacterial meningitis

D. chylothorax

Pleural, Peritoneal, and Pericardial Fluids

*31. Bloody is a representative exudate appearance typically

associated with

23. Which of the following is (are) characteristic of an effusion?

- A. empyema
- B. infectious process
- A. Abnormal accumulation of fluid
- C. anaerobic bacterial infection
- B. Can be a transudate
- D. malignancy in the absence of trauma
- C. Can be an exudate
- D. All of the above

*32. Clearly visible pus is a representative exudate appearance.

*24. A transudate can be described as

appearance typically associated with

A. specific gravity greater than 1.016, low to moderate

A. empyema

number of leukocytes, and lactic dehydrogenase less

B. infectious process

than 200 IU/L

C. anaerobic bacterial infection

B. specific gravity less than 1.016, pH 7.4 to 7.5, and

D. malignancy in the absence of trauma

lactic dehydrogenase less than 200 IU/L

C. pH 7.35 to 7.45 and protein concentration greater

*33. Foul odor is a representative exudate appearance type—

than 3.0 g/dL

cally associated with

D. lactic dehydrogenase less than 200 IU/L and protein

A. empyema

concentration greater than 3.0 g/dL

B. infectious process

C. anaerobic bacterial infection

*25. Pleura

D. chylothorax

A. covers abdominal walls and viscera of the abdomen

B. covers the lungs

*34. Pleural fluid can have a white supernatant fluid after

C. is a fibrous sac around the heart

centrifugation owing to

D. is lining of the spinal cord

A. increased concentration of leukocytes

*26. Peritoneum

B. presence of lipids

A. covers abdominal walls and viscera of the abdomen

C. presence of chylomicrons

B. covers the lungs

D. both A and B

C. is a fibrous sac around the heart

*35. An extremely elevated leukocyte concentration in pleural fluid is typically associated with

D. is lining of the spinal cord

ral fluid is typically associated with

*27. Pericardium

A. hemothorax

A. covers abdominal walls and viscera of the abdomen

B. malignancy

B. covers the lungs

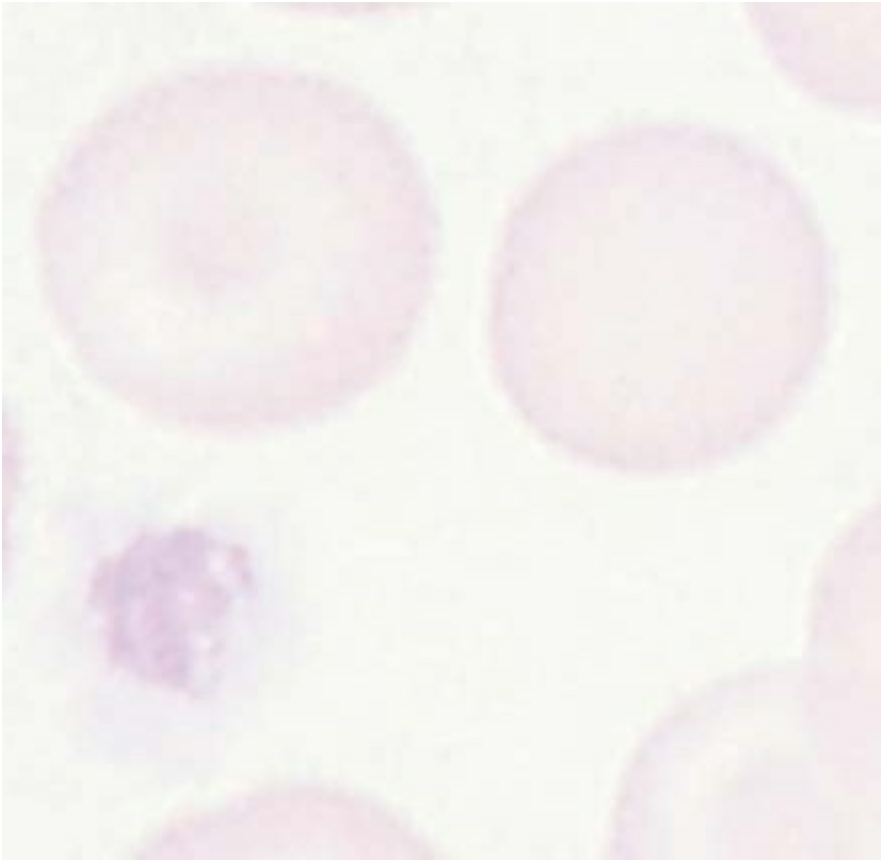
C. empyema

C. is a fibrous sac around the heart

D. classic rheumatoid effusion

D. is lining of the spinal cord

(continued)



PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS (continued)

*36. Which of the following cells can be seen in pleural fluid?

44. The causes of peritoneal effusion include all of the following except

lowing except

B. Mononuclear phagocytes

A. bacterial peritonitis

C. Mesothelial cells

B. hepatic cirrhosis

D. All of the above

C. congestive heart failure

D. tuberculosis

*37. All of the following describe the characteristics of

malignant cells except

*45. An abnormal-appearing peritoneal effusion can be

A. multiple round aggregates of cells

caused by all of the following except

B. high N:C ratio

A. bacterial peritonitis

C. large, irregular nucleoli

B. pancreatitis

D. smooth chromatin

C. neoplasm

*38. Many neutrophils, histiocytes, and mesothelial cells are

D. tuberculous peritonitis

associated with (a)

A. viral infection

*46. The peritoneal effusion color of pale yellow is associated with

B. acute bacterial inflammation

ated with

C. metastatic adenocarcinoma

A. normal

D. malignant mesothelioma

B. pulmonary infection

C. congestive heart failure

*39. Abundant, multinuclear cells and clusters of cells are

D. sepsis

associated with a(n)

A. viral infection

47. The peritoneal effusion color of straw colored is associated—

B. acute bacterial inflammation

ated with

C. metastatic adenocarcinoma

A. normal

D. chronic granulomatous inflammation

B. pulmonary infarct

C. congestive heart failure

*40. Many malignant cells (in clusters) are associated with

D. sepsis

a(n)

A. viral infection

*48. The peritoneal effusion color of bloody is associated

B. acute bacterial inflammation

with

C. metastatic adenocarcinoma

A. normal

D. malignant mesothelioma

B. pulmonary infarct

*41. Many lymphocytes, mesothelial cells, histiocytes, and

C. congestive heart failure

plasma cells are associated with a(n)

D. sepsis

A. viral infection

B. acute bacterial inflammation

*49. An extremely increased leukocyte concentration in

C. malignant mesothelioma

peritoneal fluid can be caused by

D. chronic granulomatous inflammation

A. bacterial peritonitis

B. pancreatitis

*42 and 43. In a pleural effusion, the percentage of

is

C. cirrhosis

extremely high in pneumonia and the percentage of

D. none of the above

is extremely high in viral peritonitis.

*50. Eosinophils are associated with

42.

A. chronic peritoneal dialysis

A. polymorphonuclear segmented neutrophils

B. congestive heart ailure, cirrhosis, and nephrotic

B. eosinophils

syndrome

C. basophils

C. tuberculous peritonitis

D. monocytes

*51. Lymphocytes are associated with

43.

A. chronic peritoneal dialysis

A. polymorphonuclear segmented neutrophils

B. congestive heart ailure, cirrhosis, and nephrotic

B. eosinophils

syndrome

C. basophils

C. tuberculous peritonitis

D. lymphocytes

CHAPTER 29 ■ Body Fluid Analysis

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REVIEW QUESTIONS (continued)

*52. Mesothelial cells are associated with

60. Cowper glands are associated with

A. chronic peritoneal dialysis

A. ructose and prostaglandins

B. congestive heart ailure, cirrhosis, and nephrotic

B. unknown

syndrome

C. sperm

C. tuberculous peritonitis

D. p30 glycoprotein

D. coxsackie group viruses

61. Sperm motility can become decreased i the specimen is

*53. In ectious agents are associated with

A. stored at room temperature

A. rheumatic disease

B. stored in a plastic container or more than 1 hour

B. mesothelioma

C. examined after 2 hours of storage

C. Dressler's postinfection syndrome

D. all of the above

D. coxsackie group viruses

62. The normal value of sperm cells is

$\times 10^9/L$.

A. 15 to 30

*54. Collagen vascular disease is associated with

B. 30 to 45

A. rheumatic disease

C. 30 to 60

B. mesothelioma

D. 60 to 150

C. Dressler's postinfection syndrome

D. coxsackie group viruses

63. The reference value for sperm motility (fresh specimen) is

*55. Neoplastic disease is associated with

A. 40% to 90% (mature and oval headed)

A. rheumatic disease

B. test or infectious disease

B. mesothelioma

C. prostatitis or sperm-agglutinating antibodies

C. Dressler's postinfection syndrome

D. greater than 60%

D. coxsackie group viruses

64. The reference value for sperm morphology is

56. A cause of an increased concentration of cells in pericardial fluid is

B. 40% to 90% (mature and oval headed)

A. microbial infection

C. test or infectious disease

B. malignancy

D. greater than 60%

C. congestive heart failure

65. The reference value for sperm agglutination is

D. both A and B

A. at least 50%

B. 40% to 90% (mature and oval headed)

Seminal Fluid

C. test or infectious disease

57. testicles are associated with

D. greater than 60%

A. fructose and prostaglandins

B. unknown

66. A consideration value of specimens used or artificial

C. sperm

insemination

D. p30 glycoprotein

A. at least 50%

B. 40% to 90% (mature and oval headed)

58. Seminal vesicles are associated with

C. test or infectious disease

A. fructose and prostaglandins

D. prostatitis or sperm-agglutinating antibodies

B. unknown

*67. Based on the information presented in case 29.2, the

C. sperm

patient would be considered

D. p30 glycoprotein

A. in ertile

B. sub ertile

59. Prostate gland is associated with

C. ertile

A. ructose and prostaglandins

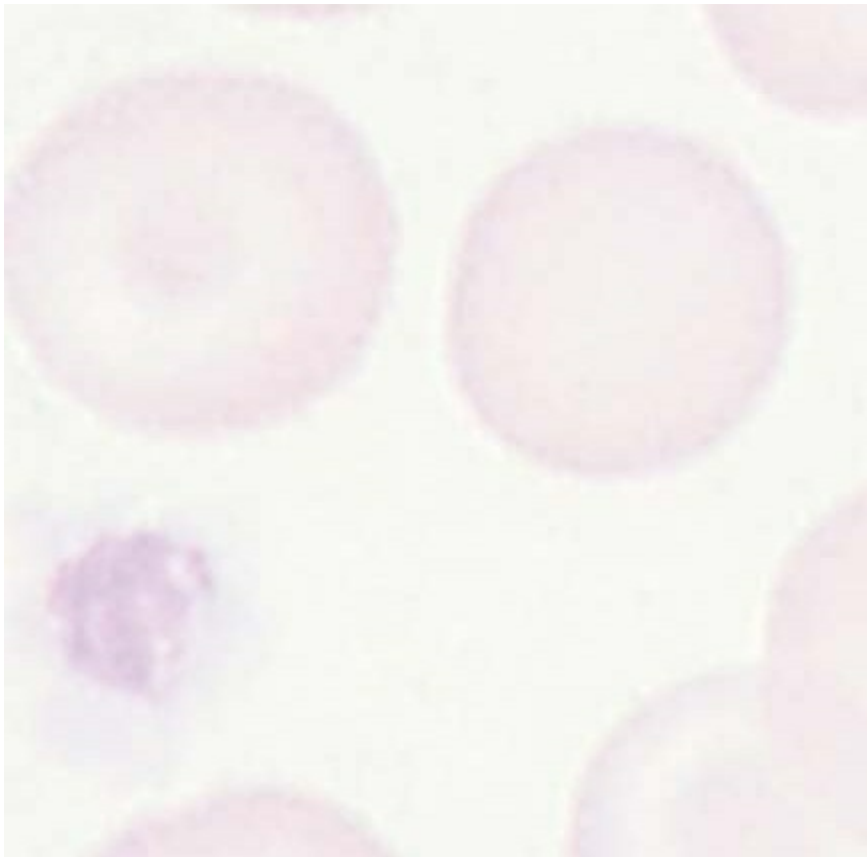
D. super ertile

B. unknown

C. sperm

D. p30 glycoprotein

(continued)



PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS (continued)

*68. Based on the information presented in case 29.2, when

*75. MSU is associated with

a seminal sample was stained with eosin Y as the stain

A. chronic renal disease

and nigrosin as a counterstain, viable sperm cells

B. chronic rheumatoid effusions

would be

C. acute and chronic arthritis

A. unstained

D. acute gouty arthritis

B. pink

C. orange

*76. Calcium oxalate is associated with

D. blue

A. chronic renal disease

B. chronic rheumatoid effusions

Synovial Fluid

C. acute and chronic arthritis

69. Arthrocentesis is

D. acute gouty arthritis

A. a bone biopsy

B. a liquid biopsy

*77. Cholesterol is associated with

C. not as accurate as blood testing

A. chronic renal disease

D. a good test to monitor the effects of chemotherapy

B. chronic rheumatoid effusions

C. acute and chronic arthritis

70. Disorders that can be diagnosed definitively by synovial fluid analysis are

D. acute gouty arthritis

A. gout, CPPD deposition disease, and rheumatoid

*78. Lipid-laden "maltese cross" is associated with

arthritis

A. chronic renal disease

B. CPPD deposit disease, rheumatoid arthritis, and

B. chronic rheumatoid effusions

SLE

C. acute and chronic arthritis

C. rheumatoid arthritis, SLE, and septic arthritis

D. acute gouty arthritis

D. gout, CPPD deposition disease, and septic arthritis

Amniotic Fluid

71. Which of the following would not be an aspiration site

79. Amniotic fluid consists of

or synovial fluid?

A. water

A. Knee

B. proteins

B. Elbow

C. carbohydrates

C. Posterior iliac crest

D. all of the above

D. Ankle

80. Fetal fibronectin (FN) is

*72. If a synovial fluid aspirate is very turbid and septic

A. a protein produced during pregnancy

arthritis is suspected, a

should definitely be

B. a biological glue, attaching the fetal sac to the uterus—
performed.

ine lining

A. total cell count and differential count

C. associated with fetal lung maturity

B. crystal examination

D. all of the above

C. Gram's stain and culture

D. all of the above

*81. Lamellae bodies are

A. associated with the risk of developing respiratory

*73. Crystals that are in multiple three-dimensional forms

stress syndrome in a premature infant

are

B. composed of concentrated layers of phospholipid

A. CPPD crystals

secreted by type II alveolar cells

B. BCP crystals

C. act as storage packets or surfactants in amniotic

C. MSU crystals

fluid

D. cholesterol

D. all of the above

*74. An increased percentage of polymorphonuclear segmented neutrophils (PMNs) is characteristic of

A. chronic urticaria

B. septic arthritis

C. rheumatoid arthritis

D. rheumatic fever

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Here you will find additional learning tools to increase

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Principles of Blood Collection and

CHAPTER

Instrumentation in Hematology*

30 Processing

KEY TERMS

bimodal cellular distribution

Gaussian distribution

photon

Delta checks

histogram

red cell distribution width (RDW)

digital cell morphology

LASER

sample

electrical impedance

monoclonal antibodies

statistic

ow-cell

mean peroxidase index

Stoke's Shift

o w ce ll cyto m e try

m e a n p la te le t volu m e (MPV)

VCS (volu m e , con du ctivity, s ca tte r)

u o ro ch ro m e

MPV n o m o g ra m

viscosity

u o ro p h o re

p a ra m e te r

LEARNING OUTCOMES

Ins trum ental principles

■ Describe the relationship of the RDW to the mean corpuscular vol-

■ Describe the basic theory of the electrical impedance principle of
ume (MCV).

cel counting and sizing.

■ Name the six classi cations of erythrocytes based on the RDW and

■ Describe the basic theory of the optical detection principle of cell
MCV.

counting and sizing.

■ Explain how the red cel mean index (RCMI) is calculated and give

■ Explain the fundamental concepts of laser technology.

the normal value.

- Describe the principles of flow-cell cytometry and two basic uses of
- Describe the appearance of a leukocyte histogram generated by the this technology in hematology.

electrical impedance method.

- Describe the appearance of a leukocyte histogram generated by the

Whole blood cell analysis

optical detection method.

- Define the terms parameter and sample.
 - Describe the construction of a platelet histogram.
 - List the parameters measured by basic benchtop hematology
 - Explain how the mean platelet volume (MPV) is calculated.
- analyzers.
- Compare the relationship between MPV and the platelet count.
 - Describe the methods used to measure the parameters named in
 - Name at least four disorders in which the MPV is abnormal.
- the preceding objective.
- Explain the purpose of the platelet distribution width (PDW) and its
 - Name the parameters measured by total cell counting systems.
- normal value.

- Define the abbreviation RDW.

Laser technology

- Describe the process and output of total cell and histogram electrical impedance systems.

- Describe the generation, by laser technology, of a histogram for red

- Describe the process and output of a laser scatter technology

blood cells (RBCs).

system.

- Explain how a platelet histogram is generated.

Compare the process and output of the continuous flow system to

- Describe the analysis and interpretation of the peroxidase analysis.

the other two types of total cell and differential cell counters.

- Explain the output of the basophil/lobularity channel.

- Describe the general characteristics of histograms.

- Describe the process of lymphocyte subtyping.

Analysis of instrumental data output

Applications of flow cytometry

- Describe the appearance of microcytic and macrocytic erythrocytes

- Describe the general functions that flow cytometry analysis can

on a histogram.

provide.

- Name two conditions that would contribute to a bimodal cellular
- Name the three factors that have contributed to the rapid advance distribution on an erythrocyte histogram.
- of the technology of ow cytometry.
- Explain how the red cel distribution width (RDW) is calculated and
- Name and discuss three hematological applications of ow cytometry.

*

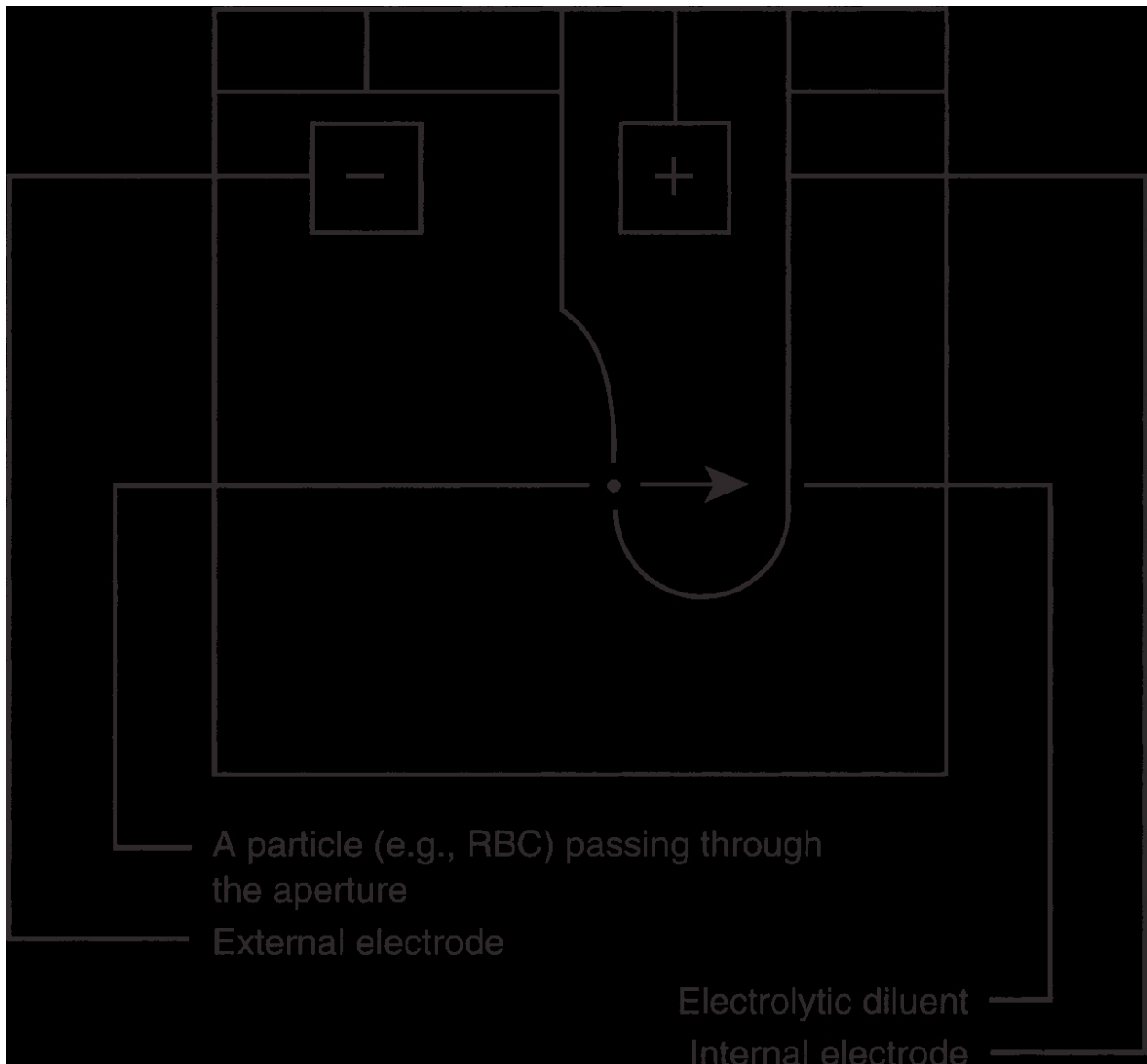
give the normal range.

- Name and discuss three other cel ular applications of ow cytometry.

*Additional procedures, in CLSI format, are provided on this book's companion Web site at thepoint.lww.com/Turgeon6e.

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Digital microscopy

Case studies

Describe the function of artificial neural networks.

Analyze and discuss the significance of the erythrocyte and leuko—

Explain the benefits and advantages of digital microscopy.

cyte histograms and the nomogram presented in the six case studies, answer the critical thinking group discussion questions, and

Instruments in coagulation studies

conclude a diagnosis.

- Describe the two most common types of instruments used in the clinical laboratory for the detection of fibrin clots.

NOTE:

- Explain the principles of electromechanical and optical detection
- indicates MLT and MLS core content

systems.

indicates MLT (optional) and MLS advanced content

Describe the methodological principle of platelet aggregation.

The use of automation and robotics is being used in clinical laboratories around the globe. Since the introduction of the automated hematology instrumentation in the 1950s, laboratory automation has continued to evolve and expand in available pulse produced indicates the cell's volume (Fig. 30.2). The output histogram is a display of the distribution of cell volume and

assays that can be automated.

frequency. Each pulse on the x-axis represents size in femto—

oliters (fL); the y-axis represents the relative number of cells.

ogy and hemostasis laboratories is impacted by automation.

Although larger laboratories have automation such as speci-

The Optical Detection Principle

men preparation and identification, preanalytical automation, and digital morphology not found in small laboratories,

In the optical or hydrodynamic focusing method of cell

laboratory professionals need to understand the basic prin—

counting and cell sizing, laser light is used. A diluted blood

principles of various levels of hematology automation.

specimen passes in a steady stream through which a beam of

laser light is focused. As each cell passes through the sensing

zone of the flow cell, it scatters the focused light. Scattered

INSTRUMENTAL PRINCIPLES

light is detected by a photodetector and converted into an

electrical pulse. The number of pulses generated is directly

Instrumentation and the automation of procedures continue

to increase in the clinical hematology laboratory. Since the first

Coulter Cell Counter Model A was introduced in the 1950s, the types of automated equipment and instrumental capabilities of instrumentation have become more diverse and sophisticated.

Cell counting and automated differential analysis are now routinely found in most laboratories. Microprocessor applications

have increased instrument programming capabilities and data output in ways that were unimagined a decade ago.

The counting of the cellular elements of the blood (erythrocytes, leukocytes, and platelets) can be based on one of two

classic methods:

1. Electrical impedance
2. Optical detection

The Electrical Impedance Principle

Electrical impedance is a method of cell counting was originally developed by Coulter Electronics and is referred to as

the Coulter principle. Cell counting and sizing are based on the detection and measurement of changes in electrical impedance (resistance) produced by a particle as it passes

FIGURE 30.1 Coulter aperture: electronic impedance principle.

through a small aperture. Particles such as blood cells are

When the aperture of an electronic particle counter is immersed

in a dilution of whole blood in an electrolyte solution, changes nonconductive but are suspended in an electrically conductive— in electrical resistance can be measured. The passage of each cell into a diluent. As a dilute suspension of cells is drawn through the aperture, the resistance of the electrical path between two electrodes that are located on each side of the aperture. (Adapted from Pierre R. Signicant Advances in Hematology, Hialeah, FL: Coulter Electronics, 1985:6, with permission.)

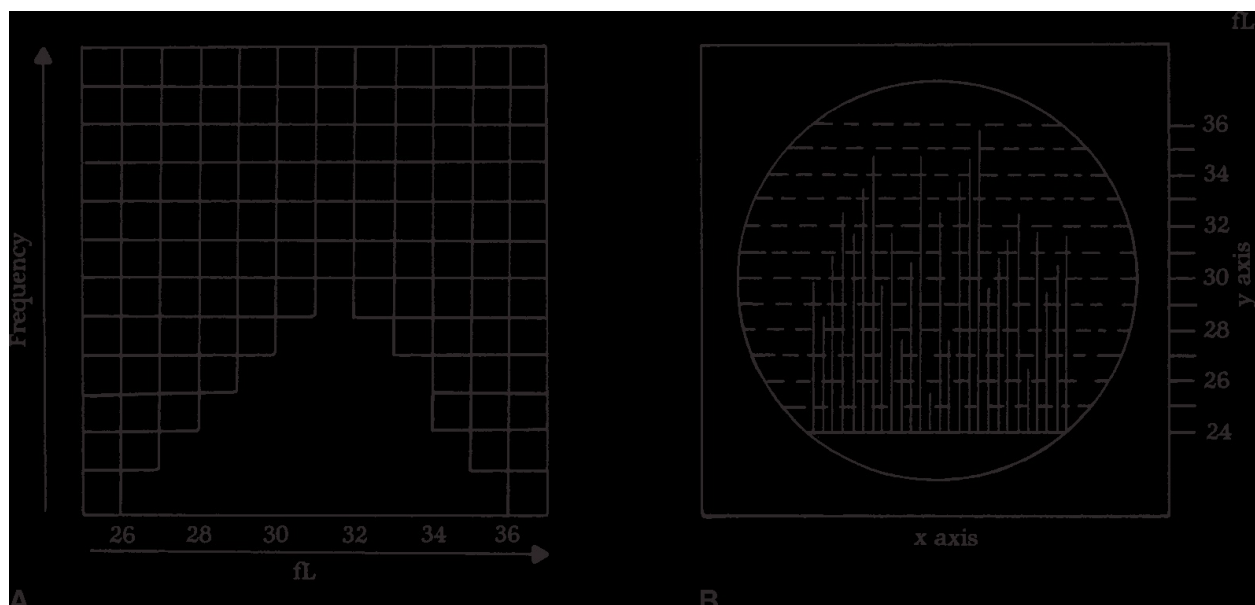


FIGURE 30.2 Cell counting: impedance principles. The number of pulses on the oscilloscope screen indicates the number of particles passing through the aperture. The height (amplitude) of each pulse reflects the volume of each cell. **A.** Histogram distribution of normal erythrocytes. **B.** Oscilloscope appearance as erythrocytes pass through the cell counting aperture and produce an electrical resistance. (Adapted from Pierre R. Seminars and Case Studies: The Automated Differential, Hialeah, FL: Coulter Electronics, 1985:4, with permission.) proportional to the number of cells passing through the

4. Orthogonal light scatter 90 degrees. The result of this sensing zone in a specific period.

application of light scatter is the production of data based

The application of light scatter means that as a single cell

on reflection and refraction of internal components,

passes across a laser light beam, the light will be reflected

which correlates with internal complexity.

and scattered. The patterns of scatter are measured at various angles (forward scatter 180 degrees and right angle 90

Radio frequency

degrees). Scattered light provides information about cell structure, shape, and reflectivity. These characteristics can be used

In this newer application, high-voltage electromagnetic cur—

to differentiate the various types of white blood cells (WBCs)

rent is used to detect cell size, based on the cellular density.

and to produce scatter plots with a ve-part differential.

The radio frequency (RF) pulse is directly proportional to the nuclear size and density of a cell. RF or conductivity is related

Characteristics of Light Scatter

to the nuclear-cytoplasmic ratio, nuclear density, and cytoplasmic granulation.

Optical Light Scatter

In this category, light amplification is generated by stimu-

Fundamentals of Laser Technology

lated emission of radiation. Three independent processes are

In 1917, Albert Einstein speculated that under certain conditions, atoms or molecules could absorb light or other radiation. These are as follows:

1. Diffraction and the bending of light around corners with

tion and then be stimulated to shed this gained energy. In the use of small angles

1950s, physicists theorized how this borrowed energy could

2. Reflection and the bending of light because of a change in be multiplied and emitted in high quantities. A decade later, speed with the use of intermediate angles

new lasers were developed and used in medical and industrial

3. Reflection and light rays turned back by the surface or an

trial applications.

obstruction with the use of large angles

The electromagnetic spectrum ranges from long radio waves to short, powerful gamma rays (Fig. 30.3). Within this

Angles of Light Scatter

spectrum is a narrow band of visible or white light, which

Various angles of light scatter can aid in cellular analysis.

is composed of red, orange, yellow, green, blue, and violet

These are as follows:

light. Light amplified by stimulated emission of radiation

(

1. Forward light scatter 0 degrees. This is directed light,

LASER) light ranges from the ultraviolet and infrared spectrum through all the colors of the rainbow.

which relates to the volume of the cell.

In contrast to other forms of radiation, laser

2. Forward low-angle light scatter 2 to 3 degrees. This character—

light is:

characteristic can relate to size or volume.

3. Forward high angle 5 to 15 degrees. This type of measure-

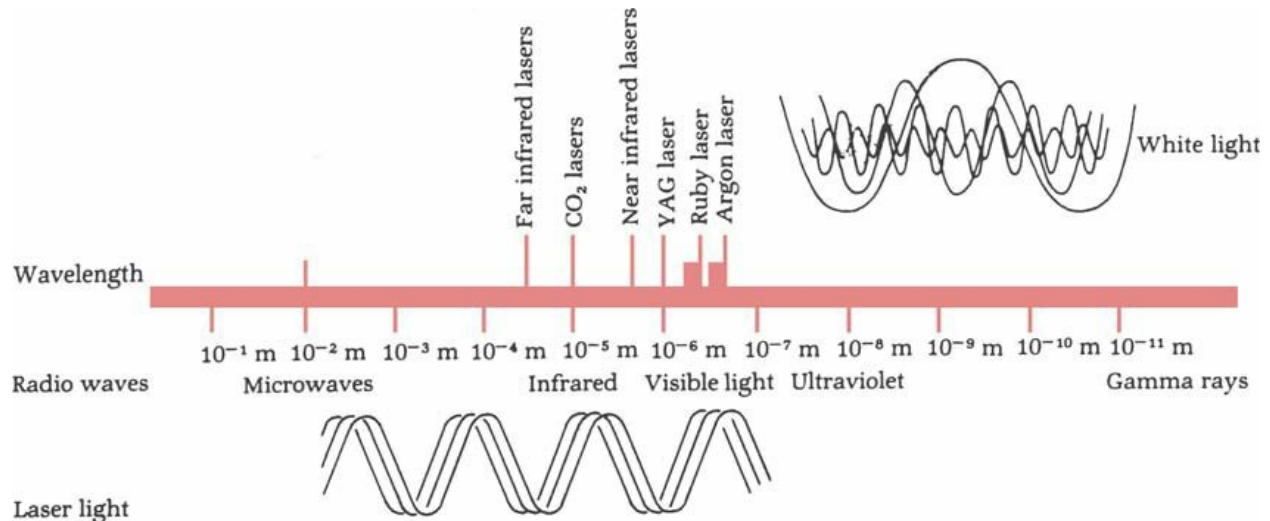
■ concentrated,

ment allows or description o the re ractive index o cel-

- almost exclusively o one wavelength or color,

lular components.

- the parallel waves travel in one direction.



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FIGURE 30.3 The electromagnetic spectrum ranges from long radio waves (10^{-1} m) to short gamma rays (10^{-11} m). The narrow band of the electromagnetic spectrum that constitutes white or visible light is composed of red, orange, yellow, green, blue, and violet light. A comparison of white light and laser light demonstrates that visible or white light and all radiation waves are disordered and jumbled. Laser light by comparison is organized and concentrated. (YAG, yttrium, aluminum, garnet.) Lasers sort the energy in atoms and molecules, concentr—

radiation. When a photon reaches an atom of the medium,

trates it, and release it in powerful waves. Through the use of

the energy exchange stimulates the emission of another photon—

fluorescence, laser light can occur in numerous wavelengths.

emission in the same wavelength and direction. This process continues until a cascade of growing energy sweeps through the neon lasers; the yttrium, aluminum, garnet (YAG) type, medium.

medium of gas, liquid, or crystal is energized by high-intensity light, an electrical discharge, or even nuclear radiation.

When an atom extends beyond the orbits of its electrons or when a molecule vibrates or changes its shape, they

to as Brewster windows, are slanted at a precise angle, which

instantly snap back and shed energy. A fluorescent chemical

polarizes the laser's light. The photons, which are reflected

back and forth, finally gain so much energy that they exit as

laser light.

When an atom extends beyond the orbits of its electrons or when a molecule vibrates or changes its shape, they

to as Brewster windows, are slanted at a precise angle, which

instantly snap back and shed energy. A fluorescent chemical

polarizes the laser's light. The photons, which are reflected

back and forth, finally gain so much energy that they exit as

laser light.

When an atom extends beyond the orbits of its electrons or when a molecule vibrates or changes its shape, they

to as Brewster windows, are slanted at a precise angle, which

instantly snap back and shed energy. A fluorescent chemical

polarizes the laser's light. The photons, which are reflected

back and forth, finally gain so much energy that they exit as

a er exposure to a light particle. T e shorter the wavelength
a power ul beam. T e power o lasers to pass on energy and
o an electromagnetic disturbance, the more energy each
in ormation is measured in watts.

photon contains.

Flu oro pho re s

NOTE: This is a good time to review the de nitions of the Key

When discussing f uorophores, the terms, excitation and

Terms found in the Glossary and ash cards on

. It is

emission wavelengths, are used. T e shorter wavelength light

also a good time to complete review questions related to the

is the light that is used as the excitation light or f uorophores.

preceding content.

T e shorter wavelength light is absorbed by an electron o the

f uorophore and as a result, this higher energy photon excites

the f uorophore. Excitation doesn't last long because the natu-

Principles o f Flow Cytom etry

ral state o the f uorophore is the ground state. In returning to

this ground state, the f uorophore emits a photon at a longer

Flow cell cytometry combines fluid dynamics, optics, laser wavelength (lower energy) and returns once more to a relaxed light, high-speed computers, and fluorochrome-conjugated state. In the fluorophores used in the clinical laboratory, the monoclonal antibodies (MAbs) that rapidly classify groups cycle of excitation and emission typically happens in about 0.5 of cells in heterogeneous mixtures. Laser light is the most to 20 ns. Recurrent cycles will continue, if there is continued common light source used in flow cytometers because of exposure to the excitation light, until photobleaching occurs. the properties of intensity, stability, and monochromatism. The unit of wavelength is the nanometre (nm). The Stokes Flow cytometry is defined as the simultaneous measurement Shift is the difference, in nanometres, between the peak excitation and the peak emission wavelengths. Each fluorophore flows in suspension through a measuring device. has a distinct and individual Stokes Shift. Virtually, all flow cytometric assays use fluorescent stains. Fluorescent dyes, called fluorochromes, are dyes that stain a

Photons

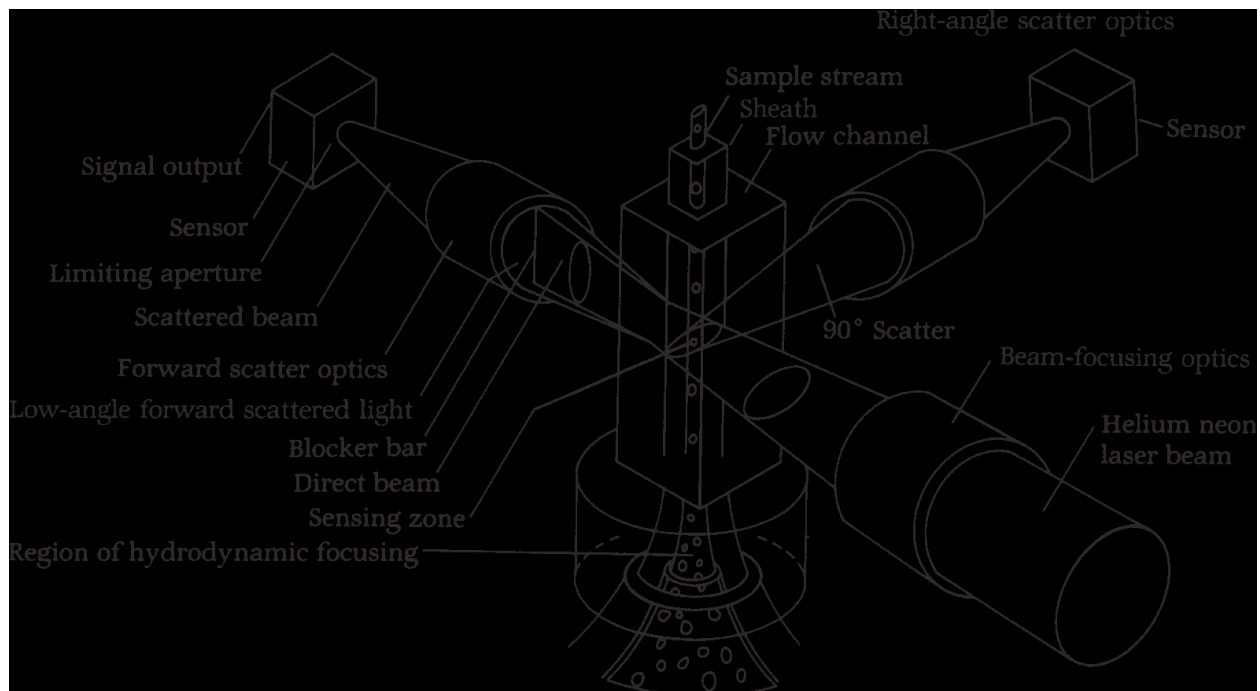
specific component of a cell or some other cellular marker

The term, **photon**, describes the energy packets of an emit—

with specific protein expression and nucleic acid content.

ted visible-light particle. A photon is the basic unit of all

Fluorescent dyes used in flow cytometry must bind or



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PART 8 ■ Fundamentals of Hematological Analysis

react specifically with the cellular component being studied—

The stained cells next pass through the laser beam. The

dyes such as reticulocytes, peroxidase enzyme, or DNA content—

laser activates the dye and the cell fluoresces. The interaction

event. Fluorescent dyes include acridine orange, thioflavin ,

between each cell and the laser beam provides the following

pyronin Y, fluorescein isothiocyanate (FITC), and phycoerythrin—

two types of information:

thrin (PE). FITC and PE are used when dual color analysis

1. The amount of light scattered by each cell hit by the laser

is desired.

beam

Many flow cytometric assays use direct immunofluorescence—

2. The intensity of the fluorescence emitted by labeled antigen staining with fluorochrome-conjugated monoclonal

bodies bound to antigens on the different types of cells—

antibodies to identify cellular characteristics. Fluorochromes

stained cells

are molecules that absorb light of one wavelength and emit

light of a higher wavelength. Fluorochromes are covalently

Although the fluorescence is emitted throughout a

bonded to monoclonal antibody molecules. This provides

360-degree circle, it is usually collected via optical sensors

a mechanism that allows for the determination by the flow

located at 90 degrees relative to the laser beam. The fluorescence—

cytometer if a labeled antibody has bound to the cell surface.

Fluorescence information is then transmitted to a computer. Flow

Each fluorochrome has a maximal excitation wavelength

cytometry performs fluorescence analysis on single cells at

at or near the wavelength of the laser and has a characteristic—

rates up to 50,000 cells/minute. The computer is the heart of

tic emission spectrum. The fluorochrome, excited by the laser light, will fluoresce at a longer wavelength. Fluorescein emits a green fluorescence, PE emits orange, and peridinin chlorophyll protein or PE coupled to cyanin 5 emits a red fluorescence. The basis of cellular identification

cence. An argon laser, which produces blue light, is the most commonly used laser. Some instruments add a red helium—

The Basis of Cellular Identification

One of the major advantages of flow cytometry is that more than one measurement can be made on every cell during its passage through the laser beam. Each cell can be optically measured or the intensity of scattered light. Some flow cytometers have a second laser that can excite other fluorochromes. Like the side-scattered blue light, all of these fluorescent signals pass through the objective set at 90 degrees to the incident laser light. The number of colors

The cellular light scatter patterns can be used to identify

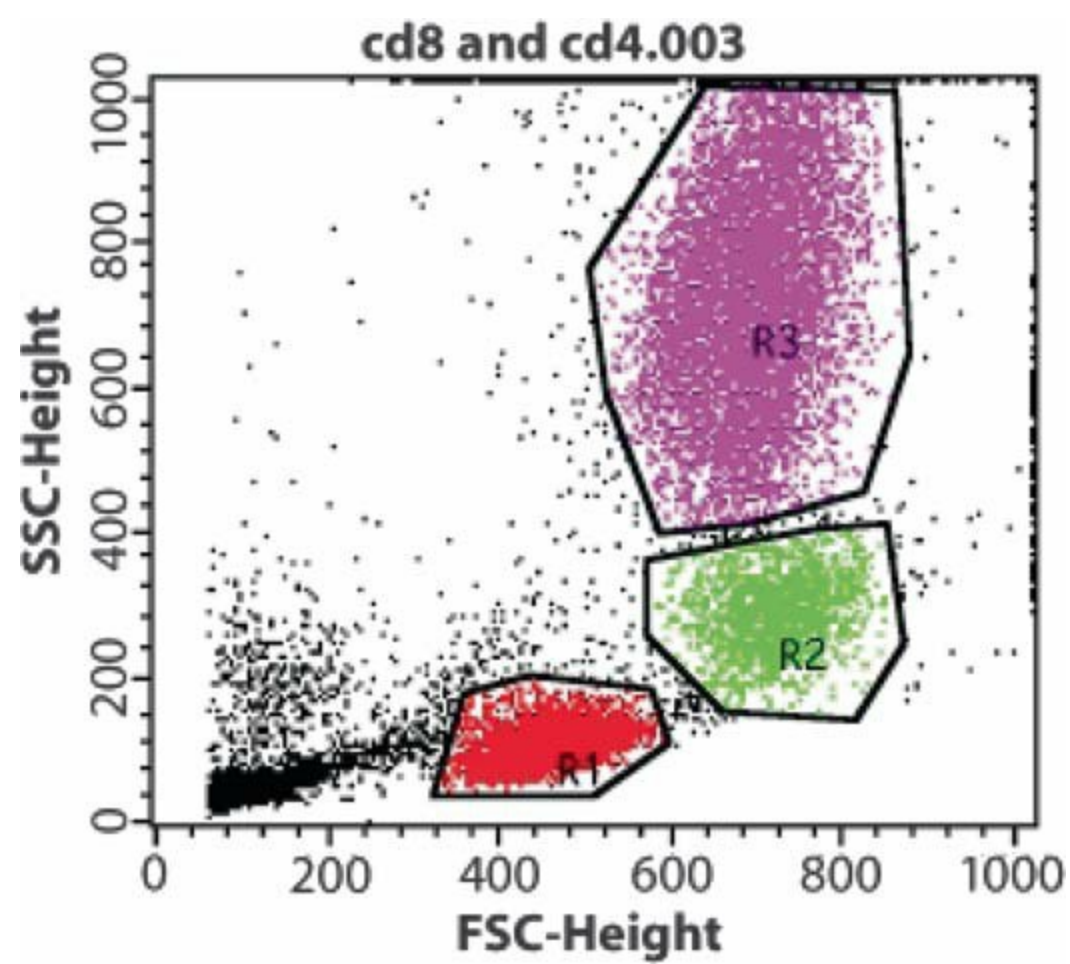
in flow cytometry output refers to the number of individual
tissue cells. Both intrinsic and extrinsic properties of cells
fluorescence-labeled antibodies used simultaneously in a
can be analyzed by flow cytometry. Intrinsic properties
given reaction tube. For example, mixing a cell suspension
include forward- and right-angle light scatter, which cor—
with a combination of antibodies labeled with two fluoro—
relate with size and granularity of a cell, respectively. This
chromes is referred to two-color flow cytometry.

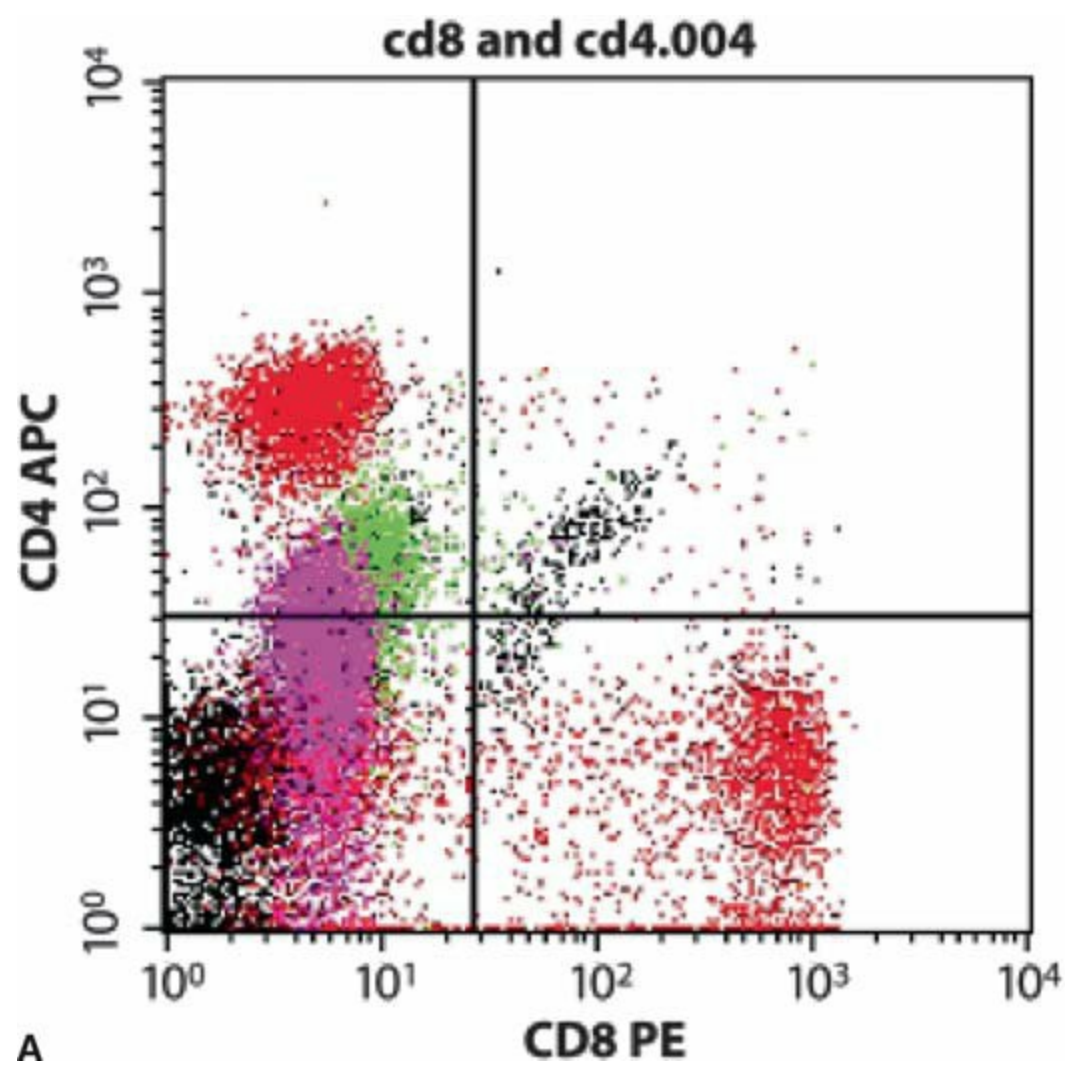
data output does not require addition of dyes or stains or
A suspension of stained cells is pressurized using gas and
detection. In contrast, extrinsic properties rely on the bind—
transported through plastic tubing to a quartz flow cham—
ing of various probes to the cells. The scattered light passes
ber (Fig. 30.4) within the instrument. In the flow chamber,
through a variety of filters and lenses and is then measured
the specimen is injected through a needle into a stream of
by photomultiplier tubes, which convert the light signals
physiological saline solution called the sheath. The sheath
into electronic signals for computer analysis. Light scat—

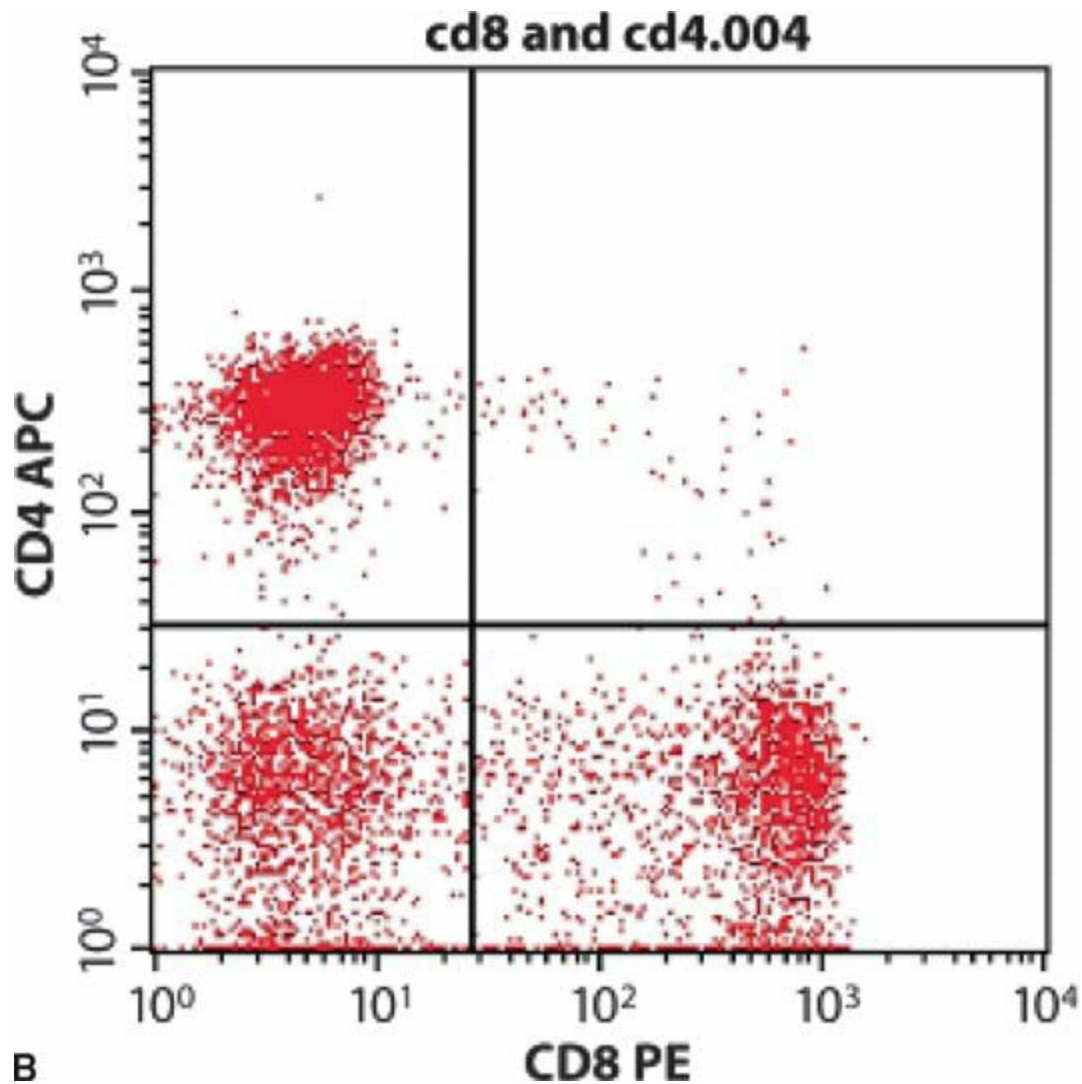
and specimen both exit the flow chamber through a 75- μ m
tered along the axis of the laser beam is “forward scatter,”
ori ce. This laminar flow design confines the cells to the very
and light scattered perpendicular to the axis is “side scat—
center of the saline sheath with the cells moving in single file.
ter” or “orthogonal scatter.” Forward scatter is roughly

FIGURE 30.4 Laser flow cytometry.

The optical detection of forward- and
right-angle light scatter using a laser
light source is accomplished by using
a sensor as the cells pass through the
beam under conditions of laminar flow.
(Courtesy of Ortho Diagnostic Systems,
Westwood, MA, 1985.)







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Data Analysis

Data are plotted on a histogram. Populations of similar cells form discrete and characteristic two-dimensional “clusters” of scatter when the forward and side scatters are plotted

against each other.

Most hematological samples contain multiple cell populations. It is necessary to first identify the population of interest

for further analysis. Whole blood is commonly used for platelet and erythrocyte assays. Data collected by the flow cytometer

can be displayed as a 1-parameter histogram or as 2-parameter

plots. A 1-parameter histogram is described as either the percentage of cells within a set of markers or as the mean fluorescent intensity of a population. A 2-parameter plot is usually

divided into four quadrants, each containing a percentage of

the total population. This is used to distinguish between fluorescent and nonfluorescent cells. It also defines the expression

and nonexpression of a cell molecule marked by a fluorescent

antibody or other fluorochrome. It is often necessary to analyze a single population within several populations and debris.

FIGURE 30.5 Forward light scatter (FSC) versus side light scatter

Electronic gating allows isolation of a specific cluster of cells

(SSC) of normal peripheral blood. Electronic regions (gates) have

been set to identify lymphocytes (R1), monocytes (R2), and granulocytes (R3). (Reprinted from McCoy JP. Flow cytometry, Am Assoc

graphic boundary (Fig. 30.5) around a population of cells.

been set to identify lymphocytes (R1), monocytes (R2), and granulocytes (R3). (Reprinted from McCoy JP. Flow cytometry, Am Assoc

graphic boundary (Fig. 30.5) around a population of cells.

graphic boundary (Fig. 30.5) around a population of cells.

Clin Chem Clin Lab News, 29(9):8–10, 2003, with permission.)

Quadrant markers divide two-parameter plots into four sections called quadrants. The quadrants are used to distinguish negative, single-positive, and double-positive populations from one another (Fig. 30.6). A negative population is located in the lower left (LL) quadrant.

FIGURE 30.6 A. The peripheral blood in Figure 30.8A was stained with phycoerythrin (PE)-conjugated CD8

antibodies (x-axis) and allophycocyanin (APC)-conjugated CD4 antibodies (y-axis) in a two-color immunophenotyping procedure. This figure is ungated and illustrates the difficulty in analyzing ungated data from heterogeneous specimens. **B.** This histogram illustrates the CD4 and CD8 staining on only the gated lymphocytes in the peripheral blood from Figure 30.7. By eliminating monocytes and granulocytes from the analysis, interpretation of staining is made much easier. (Reprinted from McCoy JP. Flow cytometry, Am Assoc Clin Chem Clin Lab News, 29(9):8–10, 2003, with permission.)



WHOLE BLOOD CELL ANALYSIS

TABLE

30.1 Calculate d Output

Clinical laboratory automation has been evolving rapidly during the past 40 years. A significant innovation is auto-

Type

He m atocrit RDW

MCV

mated front-end (preanalytical [preexamination]) instrumentation/robotics and total work cells linked by a track or

Beckman $RBC \times MCV$

CV% of RBC Mean of RBC

conveyor. Because specimen processing is perhaps the most

Coulter

histogram

size distribution

labor-intensive portion of the testing process, automating

histogram

this preanalytical (preexamination) process is one option or

Abbott

$RBC \times MCV$

Relative

Mean of RBC

streamlining procedures to reach maximum efficiency and to
value to CV

size distribution

improve customer service. The critical workload volume to
histogram

reach to invest in front-end automation is between 1,500 and

Sysmex

Mean pulse

RDW-SD (fL) Hct/RBC

2,000 specimens per day.

Bayer

$\text{RBC} \times \text{MCV}$

CV%

Mean of RBC

Automated analyzers now form the backbone of clinical

volume histogram

laboratories both large and small. Smaller analyzers are commonly used in S A labs, reestanding clinics, physicians'

RDW, red cel distribution width; MCV, mean corpuscular volume; RBC, red

o ces, and small hospital laboratories. Larger and more

blood cel ; CV, coef cient of variation; RDW-SD, red cel distribution width

based on standard deviation; Hct, hematocrit.

complex systems are used in larger clinical and research

laboratories.

T e degree o instrumental sophistication is requently

capabilities continue to be developed. Some o the innova—

described by the number o parameters that the instrument

tions include the ollowing:

generates. T e term parameter is a statistical term that re ers

to any numerical value that describes an entire population.

■ Quantitation o nucleated erythrocyte (NRBC) counts

Parameter should be clearly distinguished rom the term

■ A channel or enumeration o immature granulocytes

sample, which is a subset o a population. Any numerical

(IGs)

value describing a sample is called a statistic.

- Random access CD4 lymphocyte counting

The smaller hematology instruments measure erythro-

- Analysis of CD34, CD38, and CD61 cell markers

cytes (RBCs), leukocytes (WBCs), and platelets. Entry-level

- Measurement of reticulocyte hemoglobin

hematology instruments generate eight measured or calcu-

- Enumeration of hematopoietic progenitor cells (HPCs)

lated parameters (WBC, RBC, hemoglobin [Hgb], hema-

- Counting of IGs

tocrit [Hct], MCV, mean corpuscular hemoglobin [MCH],

mean corpuscular hemoglobin concentration [MCHC], and

Types of Automated Cell Counting

platelets). Computerized systems generally flag high or low

Instruments

patient results. These systems are automated from sample

aspiration through result printout (Fig. 30.7). Additional

Major types of automation are representative of the ways

basic parameters include erythrocyte morphology in or—

that blood cells can be counted, leukocytes differentiated,

mation expressed as red cell distribution width (RDW), Mean and other components (e.g., MCH and MCHC) calculated. Platelet Volume (MPV), or leukocyte histogram differential. Hemoglobin is measured by the traditional cyanmethemoglobin flow-cell method at 525 and 546 nm, depending on the manufacturer (Table 30.1).

the instrument manufacturer. Models and features of instrumentation continue to deliver new automation capabilities in hematology. For example, automated reticulocyte counting was a leading edge technology a few years ago but the respective manufacturers' Web sites or any updates.

counting was a leading edge technology a few years ago but

Examples of Automated Instruments

is a routinely measured parameter in many clinical laboratories today. Some of the latest instruments prepare and stain

CELL-DYN Series (<http://www.abbot.com>)

peripheral blood smears and automatically correct or leukocyte (WBC) interference. Newly developed instrumental (MAPSS) flow cytometry with hydrodynamic focusing of the

FIGURE 30.7 Sysmex lavender top hematology and diabetes testing solution. (Courtesy of Sysmex America, Inc.)

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cell stream universal to Abbott ve-part differential analyz—
cell size and hemoglobin concentration. The light absorp—
ers. The CELL-DYN Sapphire is the most advanced of the
tion measurement is proportional to ribonucleic acid (RNA)
CELL-DYN analyzers. These analyzers feature dual leukocyte
content because stained reticulocytes absorb more light than
counting methods. The leukocyte differential without the use
mature erythrocytes. The output of these three parameters is
of a stain is accomplished with light scatter with 0, 90, 10,
plotted on a reticulocyte volume histogram, on a hemoglo—
and 90 degrees (depolarized) as well as nuclear optical count
bin concentration histogram, and on the reticulocyte hemo—
by light scatter at 0 and 10 degrees. In addition, the CELL—
globin content histogram. The reticulocyte hemoglobin
DYN Sapphire uses three-color fluorescence measurements
content (CHr) demonstrates the functional state of eryth—
made on cells that are stained with fluorochromes dyes: FL1

reticulocytosis. CHr is an important indicator of asymptomatic iron deficiency anemia, which is particularly important in children under the age of 2 years and pregnant women. The reference range for fluorescence analysis. This mode uses a more economical, low flow rate of 0 to 200 L.

red (632.8 nm) wavelength laser or MAPSS analysis.

Two separate methods are used by the ADVIA 120/2120.

Erythrocytes and platelets are counted by light scatter at 90 and 10 degrees. The total WBC count is measured by impedance. Red cell indices (MCV, MCH, MCHC) are derived from two reaction chambers: the peroxidase chamber and the spherule/nuclear density chamber. In the peroxidase reaction (IRF) are measured and reported.

In the spherule chamber, WBCs are lysed and peroxidase reagent is used.

A unique feature is cyanide-free hemoglobinometry.

This chamber is heated to a high temperature to lyse RBCs.

Rapid cell lysis followed by the formation of an imidazole—

and platelets and to x the WBCs. T e WBC size is mea—
hemoglobin complex is measured. T e HB Flow Cell is illu—
sured by orward-angle laser light scatter. Peroxidase activity
minated by a light-emitting diode (LED) and a photodetector
is measured by tungsten light optics. Myeloperoxidase is a
measures transmitted light at 540 nm.

granulocyte enzyme marker. Data are displated on a PEROX

In addition, the CELL-DYN 4000 incorporates the tech—
cytogram with light absorption depicted on the x-axis and
nologies used in the basic model and eatures three inde—
orward scatter on the y-axis. In the lobularity/nuclear den—
pendent measurements and ocused f ow impedance. A
sity reaction chamber, an aliquot o whole blood is intro—
hydrodynamically ocused impedance method is employed
duced into an acid bu er that selectively lyses the cytoplasm
or primary erythrocyte counts, secondary platelet counts,
o all cells, except basophils. Samples f ow through a laser
and erythrocyte and platelet size distribution analysis.

light path, where low-angle scatter and high-angle scatter are

Multidimensional light scatter and f uorescent detection

measured. Basophils are not lysed and appear larger, scatter—
are used as well. DNA and RNA fluorescence are used to
ter more light, and appear higher on the vertical axis of a
distinguish NRBCs from viable and nonviable leukocytes
scattergram compared to the bare nuclei of other WBCs. A
(WBCs). RNA fluorescence can measure reticulocytes and
primary WBC count and basophil count are generated from
the IRF.

this channel.

Data generated by the ADVIA system indicate relative
Horiba ABX Diagnostics, Inc. (<http://www.abx.com>)
percentages and absolute values of granulocytes (neutro—
The Pentra 60 C+ is a small benchtop instrument. It reports
phils, eosinophils, basophils), lymphocytes, and monocytes.
20 parameters and RBC, platelets (PL), and basophil

In addition, interpretative data to signal the presence of
(BASO) histograms. Two patented methodologies are used.

abnormalities in the sample are generated as well as the percentage of large
unstained cells (LUCs). An increased num—

Siemens HealthCare Diagnostics (<http://www.siemens-healthcare-diagnostics.com>)

ber of LUCs suggests the presence of variant lymphocytes or

medica.l.s iem en s .com)

blast cells.

The ADVIA series uses two separate and independent flow cytometers to count and identify cells. This technology uses light scatter to analyze RBCs and WBCs. Cells are counted and differentiated by light scatter, a dark-field optical method. Once a specimen is differentiated on three optical measurements: low-angle light scatter, high-angle scatter, and absorbance. These data points are used to identify cell populations. The specimen is divided into separate aliquots for analysis in various reaction chambers. Dual leukocyte methods of peroxidase staining and basophil lobularity are used. Erythrocytes and platelets are counted in the RBC reaction chamber by flow cytometry. Hemoglobin has dual readings and colorimetric analysis. The latest generation of Beckman Coulter (the LH Series) is a hematology analyzer that can analyze hemoglobin and corpuscular hemoglobin con—

ully automated complete blood count (CBC) and differential
centration mean.

analyzer. The use of the Beckman Coulter AccuCount tech—
Reticulocyte enumeration using oxazine 750 stain is
nologies, the Coulter Principle, and volume, conductivity and
determined by low-angle light scatter, high-angle light scat—
scatter (VCS) (see Box 30.1) technologies delivers expanded
ter, and absorption measurements as the aliquot of speci—
productivity and advances in cellular flow analysis of indi—
men travels through the reticulocyte reaction chamber. The
vidual cells. These applications allow for nucleated red blood
low-angle scatter and high-angle scatter are proportional to
cell (NRBC) counts with a corrected total leukocyte (WBC)
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PART 8 ■ Fundamentals of Hematological Analysis

to aspirate a specimen of anticoagulated whole blood with—

BOX 30.1

out removing the rubber stopper of an evacuated tube. The
Manual closed mode is essentially the same as the Sampler
mode, but mixing and continuous analysis cannot be per-

VCS Technology

formed automatically. The Capillary mode is used to analyze a very small sample of whole blood that has been diluted 1:5.

Volume (V): Using direct current impedance, the volume

The XS series of Sysmex instruments are automated so each cell is measured.

hematology systems consisting of two units: the Main Unit

Conductivity (C): Radio frequency penetrates the cell, that aspirates, dilutes, mixes, and analyzes anticoagulated which generates the data points of cell size and cell whole blood specimens and the IPU that processes data internal structure.

from the Main Unit and provides an operator interface. Flags

Scatter (S): Midangle scatter detected by a beam of laser and error messages alert laboratory personnel to specimen light that generates data about cellular granularity and abnormalities.

cell surface structure.

Red cell distribution width, an expression of anisocytosis—

VCS: Single channel that analyzes approximately 8,000

sis, is reported as RDW-SD and RDW-CV. The RDW-SD is cells in a near-native condition.

an actual measurement of the width of the RBC histogram

The RDW-CV, by comparison, is a mathematically derived parameter. The RDW-CV is dependent on the average size

count, correction for WBC interference, and the ability to

of the RBCs or the MCV. Some models produce nucleated

analyze body fluids, for example, CSF. In the analyzer and

RBC and reticulocyte counts. Reticulocytes are enumerated

work cells are combined, many of the preevaluation and

by fluorescent flow cytometry using laser light and a nucleic postevaluation steps are automated.

acid fluorescent dye. Results are reported as RBC-O (opti—

A second Beckman Coulter technology, AccuGate, uses a

cal) and reticulocyte number (RE #) and reticulocytes per—

gating method to separate WBCs or RBCs and reticulocytes

cent (RE %). Reticulocyte maturation can be assessed. The

by using contour gates around cell populations. Leukocyte

RE-Hb or reticulocyte hemoglobin equivalent is used to

analysis is performed in three dimensions and is displayed

monitor the availability of iron in RBCs.

as a 3D cube. Individual cells are represented as points on

Hemoglobin is measured using sodium lauryl sulfate

a scatterplot reflecting cell volume, conductivity, and laser

(SLS-hemoglobin method), a noncyanide compound. In the

light scatter characteristics. Reticulocytes is conducted by

Sysmex series, erythrocytes and platelets are analyzed by

combining traditional supravital staining with new methy—

Hydrodynamic focusing

lene blue stain and flow cytometry using VCS technology.



Direct current (DC)

NRBC is performed using the proprietary VCS technology.



Automatic discrimination

A third technology, AccuFlex, allows end users to optimize



the levels of individual flags or improved data point per or the leukocyte count is analyzed by the DC detection

mance. Contour discriminators examine areas between di -

method and automatic discrimination. A ve-part di er—

erent cell populations. Flagging messages identify the type of cell population (e.g., WBC), the suspected variation (e.g., channel (analyzed by RF and DC). A differential scattergram variant lymphocytes), and cell data related condition (e.g., and an immature myeloid in formation (IMI) scattergram are abnormal WBC population). A definitive condition, or produced by fluorescent flow cytometry. Each cell is measured by forward-scatter laser light, lateral-scatter laser light, and lateral fluorescent light.

Sysmex (<http://www.sysmex.com>)

Three additional parameters can be performed on the

In the Sysmex X series, there are four modes of sample

XE-2100 analyzer. These are IG, immature platelet fraction introduction:

(IPF), and hematopoietic progenitor cell (HPC).

RBC and WBC cell counts can be performed on body fluids—

1. Sampler mode

ids, including CSF, serous fluid, and synovial fluid.

2. Manual mode

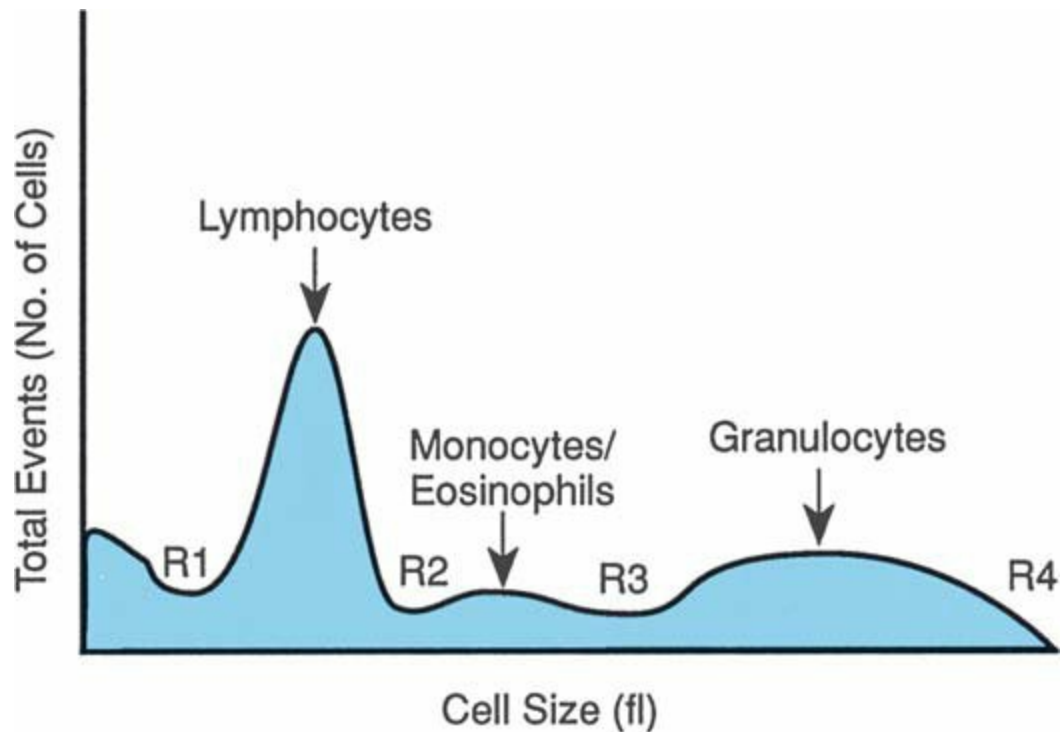
3. Manual closed mode

In strum e nt Da ta Out put

4. Capillary mode

Flags or messages are generated i abnormal results are genT e Sampler mode is the primary mode o operation. T is

erated. Di erent cell types are distinguished electronically
mode automatically mixes, aspirates, and analyzes samples
by impedance by the pulses they generate. T e pulses that
with removing the rubber stopper o an evacuated tube lled
are generated are sorted according to size. T ese individual
with anticoagulated blood. In Manual mode, the most com—
pulses appear on the oscilloscope and are categorized by
monly used method or S A assay, the rubber stopper o an
the computer. From the WBC histogram, the percent and
evacuated tube lled with anticoagulated blood, is manually
absolute number o lymphocytes, mononuclear cells, and
removed, and the individual blood sample is aspirated with a
granulocytes are determined. Each channel on the x-axis
pipette. Using the Manual closed mode, the sampler is used
represents size increasing by 1 L ($1\text{ L} = 1\text{ }\mu\text{m}^3$) rom le



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General Histogram Characteristics

Histograms are graphic representations of cell frequencies versus sizes. In a homogeneous cell population, the curve assumes a symmetrical bell-shaped or Gaussian distribution.

A wide or more flattened curve is seen when the standard deviation (SD) from the mean is increased. Histograms not only provide information about erythrocyte, leukocyte, and platelet frequency and their distribution about the mean but

also depict the presence of subpopulations.

Histograms provide a means of comparing the sizes of a patient's cells with those of normal population's. Shifts in one direction or the other can be of diagnostic importance. The position of the curve on the x-axis reflects the cell size. In the Coulter system, the size (volume in femtoliters) is represented on the x-axis.

FIGURE 30.8 Blood cell histograms (Coulter S KS). Example of a WBC count differential histogram based on volumetric studies. The various components of the WBC are shown on the graph. R1 to R4

ANALYSIS OF INSTRUMENTAL DATA

correspond to flags generated by the instrument representing distri-

OUTPUT

bution abnormalities warranting manual observation. R1, nucleated RBCs, platelet clumps, large platelets, cryoglobulin, small lympho-

The Erythrocyte Histogram

cytes, unlysed RBCs; R2, reactive lymphocytes, lymphoblasts, basophils, clotted sample; R3, eosinophilia, monocytosis, blasts, clotted

The erythrocyte histogram reflects the native size of erythro-

cytes or any other particles in the erythrocyte size range. The

Clinical Laboratory Medicine, 2nd ed, Philadelphia, PA: Lippincott

erythrocyte histogram in the Coulter system displays cells as

Williams & Wilkins, 2002, with permission.)

small as 24 L, but only those greater than 36 L are counted

as erythrocytes. The extension of the lower end of the scale

to right. Each division on the y-axis represents one in that

from 36 to 24 L allows for the detection of erythrocyte frag—

channel, providing the relative number of cells (Fig. 30.8).

ments, leukocyte fragments, and large platelets.

Although normal quantities of leukocytes are present in

the erythrocyte bath and are included in the erythrocyte

NOTE: This is a good time to complete Review Questions

count, they are not significant in the histogram. The system

related to the preceding content.

can be calibrated to compensate for $7.5 \times 10^9/L$ leukocytes.

If the leukocyte count is significantly elevated, the histogram

will be affected.

Quality Assurance of Output Data

If the cells are larger than normal, the histogram curve

will be more to the right, as in the megaloblastic anemias. If

The Joint Commission, College of American Pathologists
the cells are smaller than normal, the curve will be more to
(ISO 15189 CAP), and the Clinical Laboratory Improvement
the le (Fig. 30.9), as in untreated iron deficiency anemia.

Amendments require a quality assurance/quality control

After appropriate treatment of the underlying cause of an
system. A variety of quality control methods are available
anemia, the curve should move toward the normal range.

via computerized programming. These include instrument

In the normal unimodal distribution is altered, the early
checks that ensure that the background is acceptably low

stages of an underlying disorder may be revealed. A histo—

and confirm the calibration stability of the electronic system.

gram distribution that is a bimodal cellular distribution can be

Control specimen data can be monitored with the generation

seen in various situations, including cold agglutinin disease,

on a Levey-Jennings graph for each parameter.

after the transition of normal erythrocytes into a person

Patient results can be monitored with continuous XB

with abnormally sized erythrocytes, in the presence of eryth—

analysis (weighted moving averages), which uses the erythrocyte fragments, or with agglutination.

patient's own data to monitor population values and instrument performance. Batches of 20 samples are used to track

Quantitative Descriptors of Erythrocytes

MCV, MCH, and MCHC values. This method can be used to detect changes in sample handling, reagents, or instrument

An expression of erythrocyte size is the RDW in the Coulter performance.

series. This term refers to variation in erythrocyte size.

Delta checks are another quality control method or

Correlations between the RDW and the MCV exist or comparing a patient's own leukocyte, hemoglobin, MCV, various types of anemias. A classification of erythrocyte and platelet values with previous results. If the difference populations has been proposed based on the similarities between the two is greater than laboratory-set limits, the or dissimilarities in the erythrocyte population and in the current result is immediately flagged for review.

RDW and MCV.

PART 8 ■ Fundamentals of Hematological Analysis

o the right side o the curve that is excluded represents grouped or clumped erythrocytes.

T e RDW is calculated by dividing the SD by the mean o the red cell size distribution.

Microcytic

Ma crocytic

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SD

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RDW =

×100

re d blood

re d blood

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Mean size

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cells

cells

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The RDW is expressed numerically as the coefficient of

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variation percentage. The normal range is 11.5% to 14.5%.

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Abnormalities can be observed on the high side but no

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abnormalities have been noted on the low side. The RDW

e

v

is increased above the normal limits in iron deficiency,

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a

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vitamin B12 deficiency, and folic acid deficiency. In the

To

hemoglobinopathies, the RDW is increased in proportion

to the degree of anemia that accompanies the hemoglobin

disorder.

Relationship of RDW and MCV

Quantitative descriptors of erythrocyte size include both the

60

120

MCV

cell size (fl)

RDW and the conventional erythrocyte index, the MCV. The

RDW is independent of high, low, or normal MCV and is

FIGURE 30.9 Histogram illustrating normal RBC size distribution

with smaller than normal RBCs to the left; larger than normal RBCs

an earlier sign of nutritional deficiency than the MCV. The

to the right.

relationship of the RDW and MCV can characterize various

erythrocytic abnormalities (Table 30.2).

The MCV of a specimen is calculated using the entire area

Red Cell Distribution Width

under the erythrocyte curve. Because the RDW is a mathematical

A new parameter, the RDW, expresses the coefficient of

variation of the erythrocyte volume distribution. It is calculated

heterogeneous distribution curve and a normal

culated directly from the histogram. A portion of the curve

RDW. Patients with a low MCV may have a distribution

(Fig. 30.10) at the extreme ends is excluded from the computation

curve with a normal (homogeneous) width, which produces

tation to exclude clumps of platelets, large platelets, or electrical

a high RDW. A particularly valuable distinction based on the

trical interference on the left side of the curve. The portion
 RDW is one between iron deficiency anemia (high RDW and
 either low or normal MCV) and anemia of chronic disease
 (normal RDW and normal or low MCV).

Examples of the Relationship of

TABLE

30.2 Mean Corpuscular Volume and

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Red Cell Distribution Width

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MCV

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RDW

High

Normal

Low

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Re

High

Megaloblastic Normocytic

Iron deficiency

anemias

anemias

anemia

Normal Aplastic

Reticulocytosis* Heterozygous

anemia in
thalassemias.

FL

adults

Anemias

FIGURE 30.10 Red cell distribution width (RDW) calculation. The
of chronic

RDW is an expression of the coefficient of variation of the red cell
in anemia

volume distribution. Both the MCV and RDW are calculated from
or disorders

the erythrocyte (RBC) histogram. The MCV is calculated from the

* The MCV and RDW are normal because the reticulocytes are only slightly
entire area under the curve, but the RDW is calculated only on the
larger than the cells into which they will mature in compensated hemolytic
basis of the trimmed histogram (middle area). (Adapted from Pierre
anemia.

R. Seminars and Case Studies: The Automated Differential, Hialeah,
MCV, mean corpuscular volume; RDW, red cell distribution width.

FL: Coulter Electronics, 1985:39, with permission.)

CHAPTER 30 ■ Instrumentation in Hematology

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Classification of Anemias Based

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TABLE

30.3 on Red Cell Distribution Width

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and Mean Corpuscular Volume

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MCV

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Re

RDW

High

Normal

Low

fL

High

Macrocytic

Normocytic

Microcytic

FIGURE 30.11 Electrical impedance leukocyte histogram. Three

different cell types can be identified using the impedance principle—

Normal

Macrocytic

Normocytic

Microcytic

ciple. Because the lytic agent acts on the cellular membrane and RDW, red cell distribution width; MCV, mean corpuscular volume. cytoplasm and produces cellular shrinkage, these populations can be distinguished from one another. The cellular population on the extreme left represents lymphocytes, the middle population represents mononuclear cells, and the population of cells on the Classification of Erythrocytes Based on MCV and extreme right side represents granulocytes. (Adapted from Pierre RDW

R. Seminars and Case Studies: The Automated Differential, Hialeah, As long as the red cell volume distribution histogram is uni— FL: Coulter Electronics, 1985:39, with permission.) modal, erythrocyte size is described efficiently by the mean (MCV) and coefficient of variation (RDW). An increased RDW may occur with a low MCV even when the width of On the x-axis of the histogram, four regions are noted the curve is normal. at approximately 35, 90, 160, and 450 fL. There are certain Erythrocytes with a normal RDW are homogeneous in

expected characteristics of the curves at these locations. A character and exhibit very little anisocytosis on a peripheral blood smear. Erythrocytes with an increased RDW are referred to as heterogeneous and exhibit a high degree of anisocytosis on a peripheral blood smear. A classification of erythrocytes that includes the homogeneity or heterogeneity of the three populations; however, each differential analysis is individualized to determine the position of the populations has been proposed (Table 30.3).

The computer program uses these locations to determine erythrocytes that includes the homogeneity or heterogeneity of the three populations; however, each differential analysis is individualized to determine the position of the populations has been proposed (Table 30.3).

in each specimen. Leukocytes normally occur at 35 L or above; the region below 35 L should be clear. Particles such

The Leukocyte Histogram

as clumped or giant platelets, NRBCs, and nonlysed erythrocytes might produce interference at or below 35 L.

Size-referenced leukocyte histograms display the classification of leukocytes that the instrument detects abnormal patterns. The types of

classification of leukocytes according to size following lysis.

Alert signals include the following:

It does not display the native cell size. The lytic reagent causes a cytochemical reaction. As a result of the reaction,

1. Cells below 35 fL.

the cytoplasm collapses around the nucleus, producing

2. Cells between the lymphocyte and mononuclear cell

differential shrinkage. Therefore, the histogram of leu—

region. Lymphocytes that are larger than normal, such as

leukocyte subpopulations reflects the sorting of these cells

variant lymphocytes, certain blast forms, plasma cells, or,

by their relative size, which is primarily related to their

in some cases, eosinophilia and basophilia, can trigger an

nuclear size.

Alert.

The Coulter model system classifies approximately 20,000

3. Cells between the mononuclear and granulocyte popula—

particles when the leukocyte count is at the 10.0×10^9

tions; an increase in IGs or other abnormal cell popula—

3 cells/ μ L

level. As the leukocytes pass through the aperture in the

tions, such as certain types of blasts and eosinophils.

electrical impedance system, they displace their volume in

4. Cells to the far right region of the curve, usually a high

a conductive fluid, which causes a change in electrical resis—

absolute granulocyte count.

tance as each cell passes through the aperture. This change is

5. An abnormality detected at exactly the 35-L threshold.

proportional to the cell volume. The histogram generated by

6. A significant increase in the mononuclear population.

the Coulter principle provides size information.

7. A multiple alert, when more than one of these regions is

Although the Coulter leukocyte histogram displays

affected.

all cells as small as 30 L, only those greater than 35 L are

In ADV differential technology, there is a correlation of

counted as leukocytes. The histogram differentiates lymph—

signals to Di Plot populations (Figs. 30.12 and 30.13).

phocytes, mononuclear cells, and granulocytes (Fig. 30.11).

Lymphocytes are typically small with a regular shape. They

Mononuclear cells include blasts or other immature cells, are smaller in volume and lower in absorbance than the other such as promyelocytes and myelocytes, as well as monocytes; cells and are positioned in the lower part of the Di Plot.

however, in a normal specimen, monocytes represent the Neutrophils will absorb light depending on the presence of mononuclear cells.

cytoplasmic granules and segmented nuclei.

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PART 8 ■ Fundamentals of Hematological Analysis

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FIGURE 30.14 Platelet histogram. Platelet counting and sizing

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in both electrical impedance and optical systems reflect the native

Ne

cell size. (Adapted from Pierre R. Seminars and Case Studies: The Automated Differential, Hialeah, FL: Coulter Electronics, 1985, with permission.)

s

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yt

c

the RBC aperture. In the optical system, forward light scatter

o

h

p

pattern discrimination between erythrocytes and platelets

ym

L

in the flow cell determines the platelet count and frequency

distribution.

Debris

In the electrical impedance system, the analyzer's computer classifies particles that are greater than 2 L or less than

Absorbance

20 L as platelets. In optical systems, the cell pulse area is

FIGURE 30.12 Correlations of signals to Di Plot populations

determined. The raw data from either the RBC aperture or in Coulter Ac . (Reproduced with kind permission of Beckman forward light scatter are sorted. These raw data histograms Coulter, Inc.)

are then smoothed and tested against mathematical criteria that eliminate nonplatelet particles and are finally fitted to a

Platelet Histograms

lognormal distribution curve in the impedance method. This distribution curve has a range of 0 to 70 L. The normal platelet Platelet counting and sizing in both the electrical impedance count is derived from the integrated area under this best-fit and optical systems reflect the native cell size. In the electrical lognormal curve (Fig. 30.14).

trical impedance method, counting and sizing take place in

The expected cell coincidence error (more than one cell passing through the aperture at the same time) is corrected

Blasts,

Myelocytes,

based on mathematical probability. In the Coulter models, a

Promyelocytos

Metamyelocytes,

e

minimum of 400 particles per aperture must be detected and

Immature neutrophils

m

u

l

evaluated. If an insufficient number of particles are present in

Vo

the 2-to 20-L range, a no-count condition is reported. The data

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or the size distribution histogram are taken from three sensing

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channels in this system. T is method additionally creates three

e

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Im

curves and compares the counts. All three must agree statisti—

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cally. I any inconsistency exists, an alert results. An alert is also

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Atypical

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generated i the results are not within the range o 3 to 15 L.

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lymphs

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Particles within the platelet size range can inter ere with

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the platelet count and histogram. Small particles, such as

Ne

bubbles or dust, can overlap at the low end of the histogram.

Microcytic erythrocytes can interfere at the upper end.

s

e

However, the curve-fitting process attempts to eliminate

yt

c

interference at the upper and lower ends to obtain a correct

o

h

p

platelet count. If the histogram does not return to the base—

ym

L

line at both the right and the left of the peak, either there is

Abnormal

severe thrombocytopenia or nonplatelets are being counted.

lymphocytes

Either erythrocyte or leukocyte ragments may be responsi—

De bris, NRBCs

ble. In such cases, the platelet count and derived parameters

o MPV and PDW are not reliable.

Abs o rbanc e

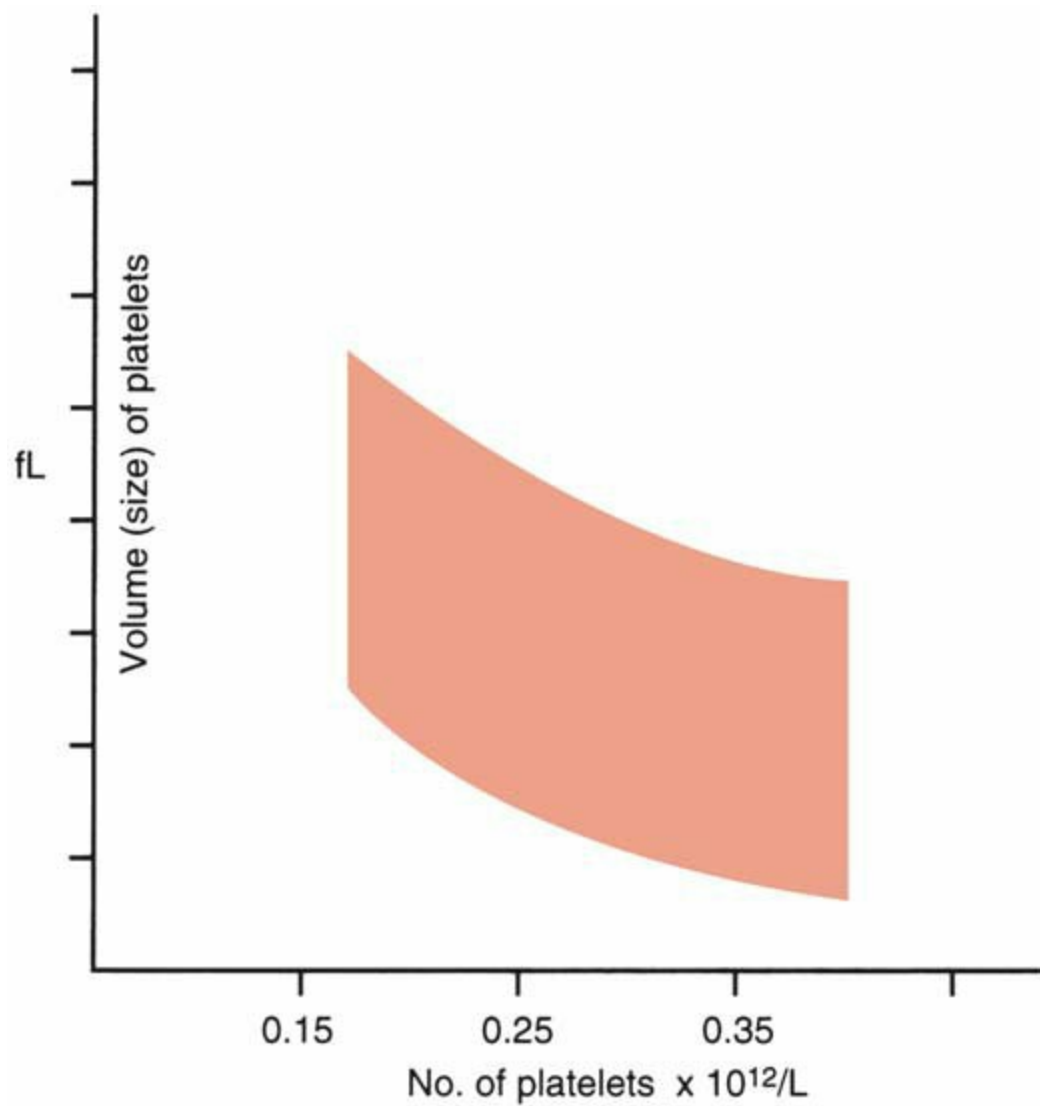
FIGURE 30.13 Di Plot normal and abnormal cell populations

NOTE: This is a good time to complete Review Questions

with Coulter Ac . (Reproduced with kind permission o Beckman

related to preceding content.

Coulter, Inc.)



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Derived Platelet Parameters

Mean Platelet Volume in

TABLE

30.4

Platelet size has been measured for more than a decade by

Selected Disorders

either micrometry or flow cytometry methods. However, sizing information from data obtained from whole blood speci-

Decreased MPV

Increased MPV

mens and the application of computer technology now make it

Aplastic anemia

Idiopathic thrombocytopenic

possible or additional parameters to be generated instrumen—

purpura

tally. The Coulter model systems yield the additional paramete—

Megaloblastic anemia

After splenectomy

ters of MPV and PDW. These parameters are derived from the

platelet histogram and allow for a size comparison between a

Wiskott-Aldrich syndrome

Sickle cell anemia

patient's specimen and the normal population's. Size compari—

After chemotherapy

sons are useful as an indicator of certain disorders.

MPV, mean platelet volume.

Mean Platelet Volume Calculation

The MPV is a measure of the average volume of platelets in

MPV is normal. The distribution of platelet size is generally

a sample. The MPV is analogous to the erythrocytic MCV. It

a right-skewed, single peak.

is derived from the same data as the platelet count. In eth—

No single normal range exists. Patients with a lower plate—

elylenediaminetetraacetic acid (EDTA)-anticoagulated blood,

let count normally have a higher MPV, and patients with a

platelets undergo a change in shape. This alteration (swell—

higher platelet count have a lower MPV. Analysis of a nomo—

ing) causes the MPV to increase approximately 20% during

gram demonstrates that an MPV between 9.0 and 9.8 L is

the first hour. After this time, the size is stable or at least

in the normal range if the platelet count is normal. MPVs

12 hours; however, MPV values should be based on speci—

from 7.8 to 8.9 L or from 9.9 to 12.0 L may be in the normal

means that are between 1 and 4 hours old.

range, depending on the platelet count.

In healthy patients, there is an inverse relationship

between platelet count and size (Fig. 30.15). The volume

Disorders of Mean Platelet Volume

increases as the platelet count decreases. Because of this

Various disorders are associated with altered MPV values

inverse relationship, the MPV and the platelet count must

(Table 30.4). The MPV is often decreased in aplastic anemia,

be considered together. This relationship between the

in megaloblastic anemia, or as the result of chemotherapy.

platelet count and MPV is illustrated as a graph, the **MPV**

Hypersplenism is associated with an MPV that is inappropri-

nomogram, and is used to determine whether a patient's

ately low or the platelet count. In septic thrombocytopenia,

the nomogram varies as thrombocytopenia develops, with

the MPV rising as the platelet count falls. Platelet destruction

associated with disseminated intravascular coagulation causes

an increase in the MPV proportional to the severity of thrombocytopenia. The

MPV is often increased in patients with

myeloproliferative disorders or heterozygous thalassemia.

Platelet Distribution Width

The PDW is a measure of the uniformity of platelet size in a blood specimen. This parameter serves as a validity check and monitors assay results. A normal PDW is less than 20%.

The PDW can be increased in aplastic and megaloblastic anemias, in chronic myelogenous leukemia, and as the

result of antileukemic chemotherapy. The causes of increased

PDW are not known but are probably related to dysfunctional megakaryocytic development. Falsely elevated results

can be caused by extraneous particles, such as erythrocyte

fragments, which broaden the platelet volume distribution

beyond that of actual platelets.

LASER TECHNOLOGY

Some systems use the principle of flow cytometry based on

FIGURE 30.15 Mean platelet volume (MPV) nomogram. An differential light scattering and cytochemistry. Three distinct inverse relationship between platelet size and platelet count exists steps are involved in its function:

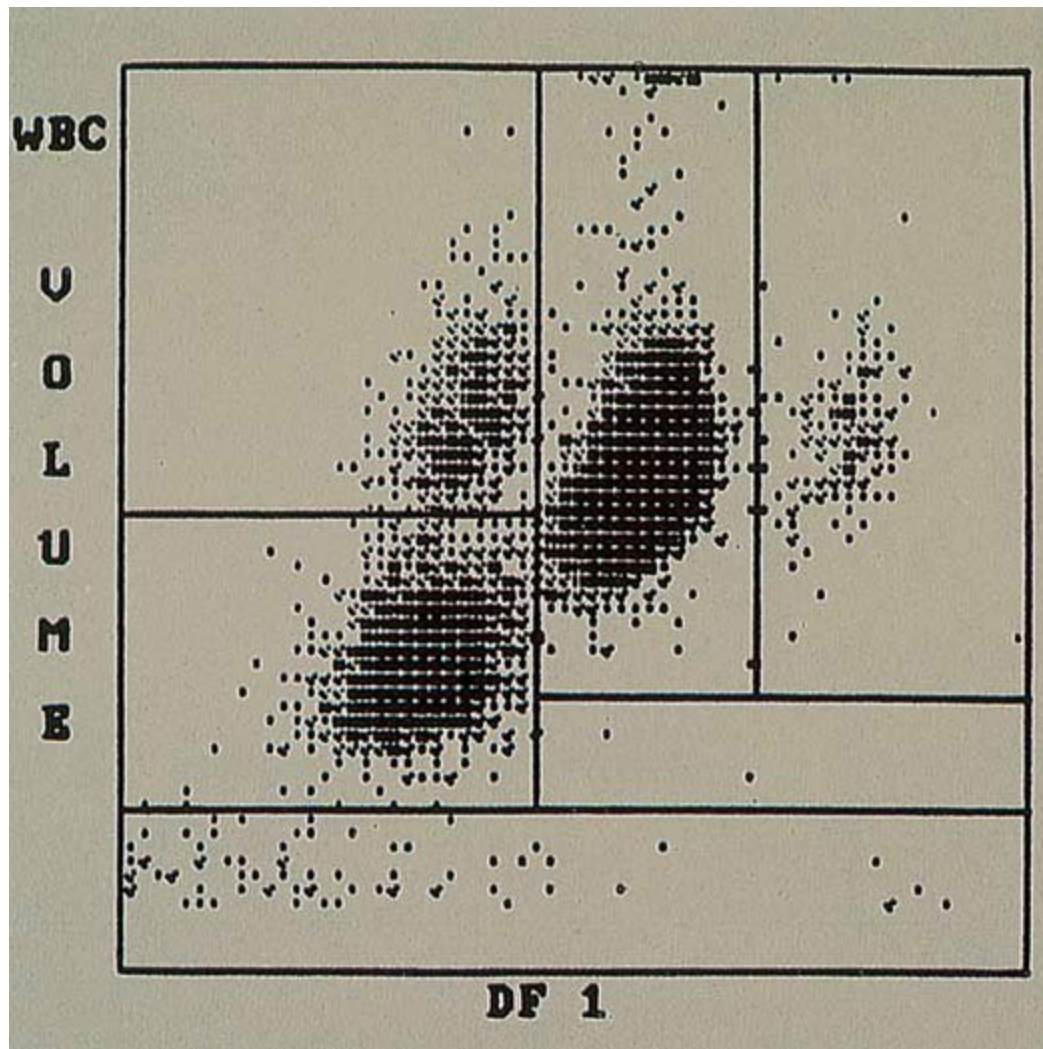
and is expressed as the MPV nomogram. (Adapted from Pierre R.

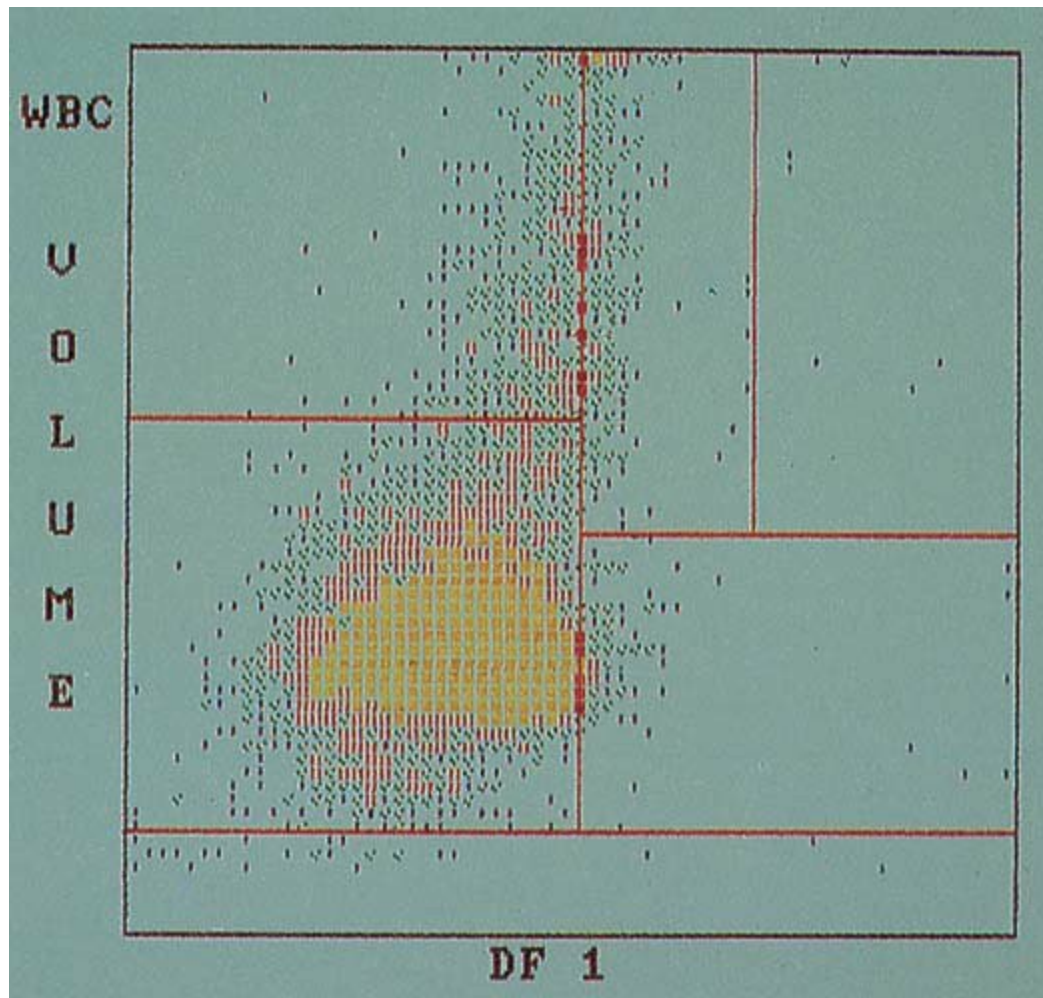
Seminars and Case Studies: The Automated Differential, Hialeah, FL:

1. Cytochemical reactions prepare the blood cells for analysis.

Coulter Electronics, 1985, with permission.)

2. A cytometer measures specific cell properties.





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PART 8 ■ Fundamentals of Hematological Analysis

3. Algorithms convert these measurements into familiar results or cell classification, cell count, cell size, and

hemoglobinization.

The instrument's sampling mechanism divides blood

samples into aliquots that are treated in our separate reaction chambers:

1. Hemoglobin
2. Red cell/platelet

3. Peroxidase

4. Basophil/lobularity or nuclear channel

Red Blood Cells / Platelets

The RBC/platelet channel uses a laser-based optical assembly

that is shared with the basophil/lobularity channel. A bu -

ffered reagent isovolumetrically spheres and fixes RBCs and

platelets. The light scattered at low and high angles simultaneously measures RBC volume (size) and optical density

(hemoglobin concentration) of each cell. The signal pairs are

transformed by a computer into a cytogram and two histograms (Fig. 30.16).

FIGURE 30.17 Histogram—chronic lymphocytic leukemia.

Additional parameters (see Chapter 10 or a full discus-

(Reprinted from McClatchey KD. Clinical Laboratory Medicine,

sion of RBC parameters) obtained from the histograms are

2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2002,

with permission.)

MCV and the RDW (reference range, 10.2% to 11.8%). Based

on the hemoglobin concentration of each cell, the cellular

hemoglobin concentration mean (CHCM) is determined.

The platelet histogram (Fig. 30.17) is derived from mea—

The hemoglobin distribution width (HDW) is determined. Measurements made with the high-angle detector. The MPV is The HDW is the SD of the hemoglobin concentration his—the mode of the measured platelet volumes.

togram. Hematocrit, MCH, and MCHC are calculated from the measured hemoglobin, RBC count, and MCV. The red

Peroxidase

cell cytogram enables simultaneous observation of cell volume—In this tungsten light-based optics channel, RBCs are lysed volume and hemoglobin concentration.

and WBCs are fixed and then stained. A dark precipitate forms in the primary granules of leukocytes containing peroxidase when a chromogen is added with hydrogen peroxide as the substrate. Eosinophils and neutrophils are strongly positive and monocytes are weakly positive.

Peroxidase is not present in basophils, lymphocytes, blasts, or LUCs.

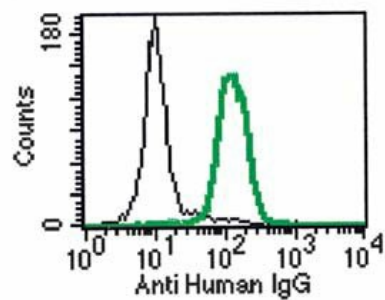
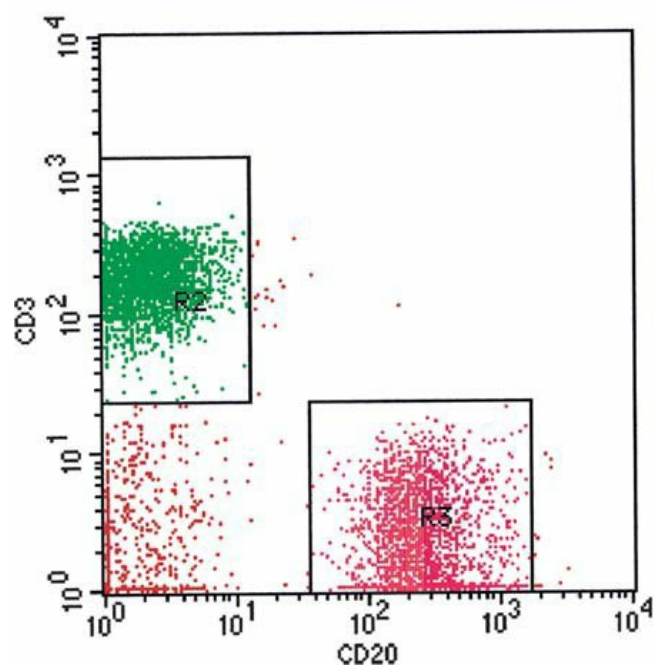
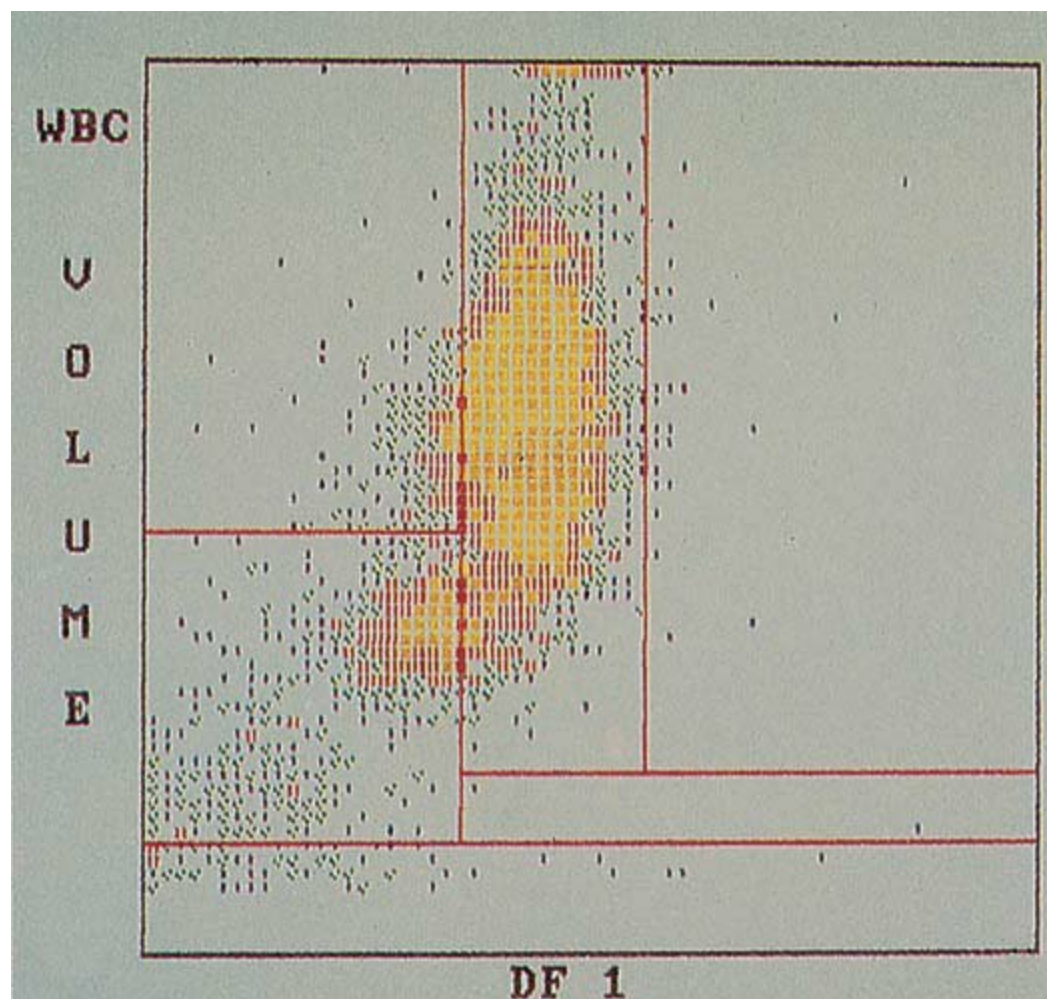
Thousands of cells are characterized by a combination of their size (scatter) and peroxidase activity (absorbance) (Fig. 30.18). Scatter is plotted on the y-axis and absorption

on the x-axis. Each cell is represented by a dot. The position of the dot is dependent on the combination of the light scattered and absorbed by each cell.

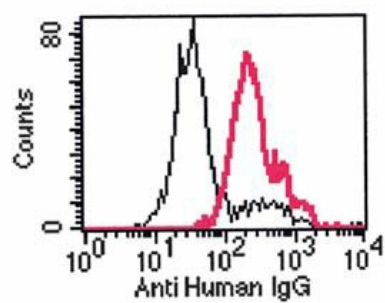
The clusters of dots that are generated are defined and analyzed, the number of cells in each is counted, and the cells are classified based on information stored in the computer. This information is used to generate the total WBC count and differential count, except for basophils. The relative percentages and absolute values of leukocytes are included. The parameter mean peroxidase index (MPXI), the index of the mean peroxidase activity of neutrophils as measured by their stain intensity, is generated. Increased myeloperoxidase activity may be associated with megal-

FIGURE 30.16 Normal differential. (Reprinted from McClatchey
blastic anemia, hyperproliferative granulopoiesis, or reactive states. Increased numbers of LUCs may indicate the presence of blasts or abnormal lymphocytes. The reference

KD. Clinical Laboratory Medicine, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2002, with permission.)



T Cells



B Cells

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a low pH, the membranes and cytoplasm of specific leukocytes, neutrophils, eosinophils, lymphocytes, and monocytes

disintegrate and only the bare nuclei remain.

The nuclear channel cytometer distinguishes leukocytes

by differences in nuclear shape and counts basophils. This

laser-based cytometer measures light scattering at two di-

fferent angles, low (0 to 5 degrees) and high (5 to 15 degrees).

Low-angle scatter measures size, and the low-angle scatter of

intact basophils is much greater than the bare nuclei of other

leukocytes. A fixed horizontal threshold separates basophils

from the nuclei of other leukocytes (Fig. 30.18). High-angle

scatter is responsive to the lobularity of nuclei. The more lobulated the nuclei, the larger the high-angle signal.

On the cytogram, polymorphonuclear neutrophil (PMN)

appears on the right and mononuclear nuclei (MN) appear

on the left with a valley between them. A vertical threshold

separates the two clusters. The ratio of PMN:MN, the lobularity index (LI), is an index of the degree of PMN nuclear

segmentation; a low value suggests a leshi. Blast cells appear to the le o the normal mononuclear cells on the x-axis and are counted or f agging purposes. (A system o

FIGURE 30.18 Histogram—acute myelogenous leukemia.

f ags or abnormal morphology alerts the instrument opera- (Reprinted rom McClatchey KD. Clinical Laboratory Medicine, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2002, tor that additional work, such as microscopic examination with permission.)

o the blood, may be required.) Nucleated RBCs, i present, appear within the PMN cluster.

ranges o 0% to 3.7% or LUCs and 0% to 5.4% or HPX have been established.

Lym pho cyte S ubtyping

Bas ophil/ Lo bularity (Nuclear) Channe l

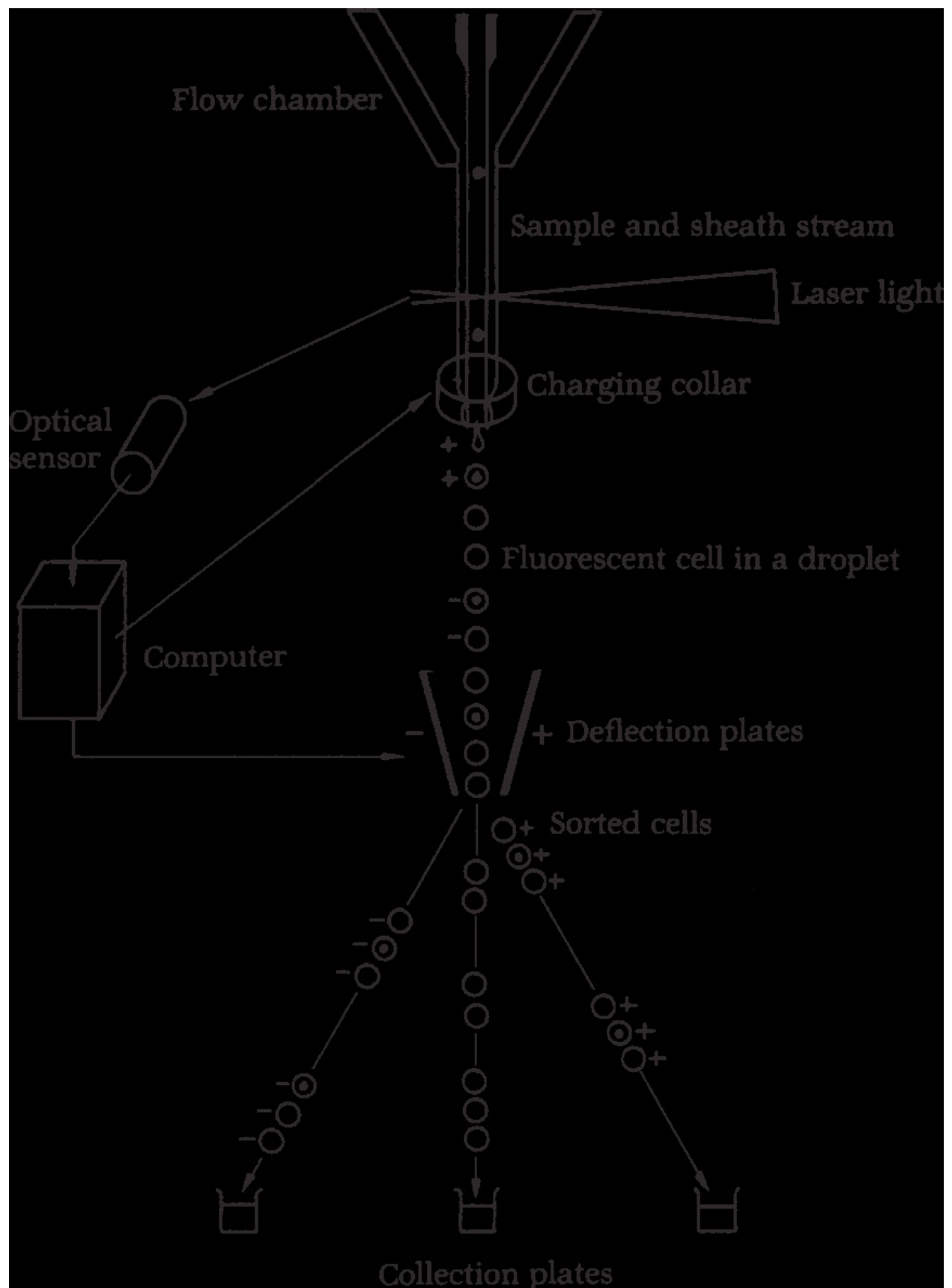
An immunoperoxidase reaction is used or lymphocyte subtyping (Fig. 30.19). A speci c monoclonal antibody is rst

T e nuclear channel is used to measure the con ormation o reacted with whole blood. A second biotinylated antibody, the nucleus o WBCs. T e principle o the reaction in this which binds only to the monoclonal antibody, is added, ol—

channel is that when WBCs are exposed to a surfactant at lowed by an avidin-peroxidase reagent. Peroxidase is used

FIGURE 30.19 Three-color flow cytometric crossmatch. **Left.** Dot plot displaying CD-20 PE staining of B cells (x-axis) versus CD3-PerCP staining of cells (y-axis). **Right.** Single parameter histogram of and B cells.

Staining fluorescence with the normal human serum control is shown in black; staining fluorescence observed with a positive serum sample is shown in green and red color for and B cells, respectively.



PART 8 ■ Fundamentals of Hematological Analysis

as a stain, using a similar method to that of the peroxidase

General Properties of Flow Cytometry

channel. Lymphocytes, which have been labeled by the immunoperoxidase reaction, appear between the unlabeled

Most flow-cell instruments can simultaneously analyze multiple labeled lymphocyte population and the monocytes. Cells with multiple parameters at the rate of 5,000 to 10,000 cells/second.

endogenous peroxidase such as neutrophils stain intensely

The cellular analysis yields quantitative data about the chemical and appear as to the right.

ical and physical properties of individual cells, and after analysis, cells can be physically separated into subpopulations

for further study at the rate of 5,000 cells/second. The major

APPLICATIONS OF FLOW CYTOMETRY

advances in this technology are owing to several factors:

The introduction of the flow cytometer into the clinical laboratory

1. The ability to produce monoclonal antibodies resulted in laboratory is a major technological advance. Flow cytometry is a the subsequent development of specific surface markers

eld that has evolved rapidly during the past three decades.

or various subpopulations o cells.

Instruments based on the f ow cytometry principle were ini—

2. T e development o new f uorescent probes or DNA,

tially designed to count and size cells. Later modi cations

RNA, and other cellular components increased the variety

were designed to per orm di erential leukocyte counts by

o possible applications at the molecular and cellular level.

identi ying speci c cytochemical reactions in the cells. T e

3. T e expansion o computer applications has improved the

current types o f ow cytometry instruments can analyze

instrumentation technology, making it easier to operate

cells or many constituents and sort cells into subpopulations

and more practical or use in clinical as well as research

(Fig. 30.20).

laboratories.

He mato log ical Applicatio ns

Flow cytometry can be applied practically to several techniques in the clinical hematology laboratory. T ese applications include automated leukocyte di erentiation and

reticulocyte enumeration.

Automated Differentials

Automated differentials can be based on a variety of principles. These include determination of cell volume by electrical

impedance or forward light scatter, cytochemistry or peroxidase staining, and VCS technology. Evaluation of internal

cellular organelles and nuclear characteristics can be by:

- 90-Degree laser scatter

- Polarizing laser light

- RF

Separate measurements can be made of individual measurements of volume, conductivity, and light scatter. An

additional method is to integrate the three in VCS technology into a three-dimensional (3D) leukocyte analysis. The

volume aspect is by volumetric sizing by impedance and RF

opacity or internal composition. In addition, helium-neon

laser light scatter is applied so that laser light can produce

scattering characteristics of each cell at different angles or

granularity and nuclear structure.

In addition, different reagents can be used to lyse certain

cells. Different types of technologies are used by instrument

manufacturers to produce an automated leukocyte differential. These include the following:

FIGURE 30.20 Laser and cell-sorting schematic. In a flow cytometer—

Beckman Coulter: VCS

metry system, stained cells flow through a sample tube. As the cells

Abbott: MAPPS—0, 90, 10, and 90 degrees depolarized

and a stream of saline solution leave the flow chamber, they move

Roche (Sysmex): RF and DC

like a string of beads in the center of the sheath. The fluorescence of

Siemens Healthcare Diagnostics (formerly Bayer and

the cells is detected by a sensor. Cells can be appropriately charged

as they move through a charging collar or deflection plates. Sorting

Technicon): peroxidase staining; optical scatter and

of the cells is accomplished by deflecting charged cells depending

absorption; basophils: differential lysis laser scatter

on the charge (either positive or negative).

high and low

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Clinical Applications of Flow Cytometry

Other platelet assays include platelet surface receptor

quantitation and distribution or the diagnosis of congeni—

Because single-cell suspensions of peripheral blood and bone marrow are easy to obtain, most clinical applications of flow cytometry are in the specialties of hematology and immunology. A number of instruments are currently manufactured for use in the study of platelet function disorders, platelet-associated immunology, and platelet crossmatching or transfusion. Other assays include fibrinogen receptor occupancy and various uses.

studies or monitoring the clinical efficacy of platelet-directed

Counting Reticulocytes and Platelets

anticoagulation in thrombosis. Detection of activated platelet surface markers, cytoplasmic calcium ion measurements,

Reticulocytes

and platelet microparticles or the assessment of hypercoagulability

Manual counting of reticulocytes has been conducted

and reliable states can be performed.

since the 1940s. It is tedious and time consuming and analyzes fewer erythrocytes than do flow cytometry systems.

Other Cellular Applications

Enumeration of reticulocytes by flow cytometry is more

accurate, precise, and cost-effective than manual counting.

Flow cytometry applications are extended to various areas of

Flow cytometry also provides additional reticulocyte parameters—specialized study.

parameters of the IRF, or reticulocyte maturity index (RMI), and

Immunophenotyping

the measurement of reticulocyte maturity.

Reticulocytes can be counted by using a stain or residue—

Monoclonal antibodies, identified by a cluster designation

of RNA in erythrocytes (e.g., new methylene blue, thiazole

(CD), are used in most flow cytometry immunophenotyping

azole orange, and oxazine 750); proprietary fluorescent dye

(Fig. 30.21; Table 30.6). Cell surface molecules recognized by

CD45 is used by one manufacturer. The Coulter system

monoclonal antibodies are called antigens because antibodies—

uses new methylene blue and sulfuric acid as reagents.

Antibodies can be produced against them or markers because they

In addition, fully automated flow cytometers specifically

identify and discriminate between (“mark”) different cell

designed for reticulocyte enumeration by optical light scatter

populations. Markers can be grouped into several categories.

have been incorporated into existing hematology analyzers

Some are specific for cells of a particular lineage (e.g., CD4+

(see table 30.5).

lymphocytes) or maturational pathway (e.g., CD34+ progenitor stem cells), and the expression of others varies according

Platelets

to the state of activation or differentiation of the same cells.

Measurement of platelets provides an estimate of young,

Measuring T Cells for Acquired Immunity

reticulated platelets by counting platelets that stain with

Syndrome Analysis

an RNA dye (e.g., thiazole orange or coriphosphine-O).

Platelets in whole blood are also labeled with PE-conjugated

The quantitation of T and B cells using monoclonal surface

CD41 antibody to distinguish them from other small par-

markers can be performed using flow cytometry. With the

techniques. CD-41-positive platelets are evaluated for RNA content. The finding of elevated reticulated platelets indicates

CD34

“stress” platelets from increased bone marrow production

and is consistent with a diagnosis of immune thrombocytopenic purpura.

Stem Cell

Examples of Hematology

CD45

CD45

CD45

CD45

CD45

TABLE

30.5 Analyzers with a Reticulocyte

Enumeration Feature

Manufacturer

Instrument

CD15

CD14

CD3

CD19

CD161

Abbott Diagnostics

CELL-DYN 3700, CELL-DYN Ruby,

Gra nulocyte

Monocyte T-Lymphocyte B-Lymphocyte Thrombocyte

CELL-DYN Sapphire

Beckman Coulter Inc.

HmX, LH 500, LH 700/750, LH 785,

CD45

CD45

Horiba

LH 1500, LH 7801

ABX Diagnostics

Pentra DX 120

CD4

CD8

Siemens Healthcare

Advia 120, Advia 2120, Advia 2120i

Diagnostics

CD3

CD3

Sysmex

XE 2100, XE 5000, XE-alpha N,

T-Lymphocyte

T-Lymphocyte

XT-2000i

FIGURE 30.21 CD membrane markers.

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and may be used as a guide or timing the administration

Exam ple s of Co mm o nly Us ed

o antiretroviral therapy as well as monitoring the level o

TABLE

30.6 Monoclonal Antibodie s in Flow

immune reconstitution ollowing initiation o therapy.

Cyto me try

Ba s ic Lym ph ocyte S cre en ing Pa ne l

CD De s igation

Targe t Ce ll

A basic immune screening panel typically consists o detection

CD3

T lymphocytes

and quantitation o CD3, CD4, CD8, CD19, and CD16/56.

CD4

T lymphocytes (helper cells),

Anti-CD45/CD14 is included to assist in distinguishing lymphocytes from monocytes (dimly expressed)

lymphocytes from monocytes. This panel reveals the frequency of T cells (CD3+), B cells (CD19+), and natural killer cells

CD8

T lymphocytes (cytotoxic),

(CD3+, CD16+, CD56+). It also provides the frequency of macrophages

Th-inducer cells (CD3+, CD4+) and Th-suppressor/cytotoxic

CD19

B lymphocytes

cells (CD3+, CD8+).

CD34

Progenitor (hematopoietic stem cells)

Typical ranges of lymphocyte subset percentages in adult

donors are CD3, 56% to 86%; CD4, 33% to 58%; CD8, 13% to 39%; CD16+ CD56, 5% to 26%; and CD19, 5% to 22%.

Flow cytometer, 10,000 cells can be assayed into subsets in

It does not provide information on cell activation or signaling—
1 minute with multiparameter analysis. Through the use of
signaling pathway receptors, frequency of subsets (e.g., T1 or
monoclonal antibodies, T- and B-cell populations can be
T2), stem or blast cells, B lymphocytes (e.g., immunoblasts
divided into subpopulations with specific functions. For
or plasma cells), or nonlymphoid elements.

For example, cells are divided into two functional subpopulations, T-helper (TH) and
T-suppressor (TS) cells. Normal

Hematological Malignancy

Individuals have a TH/TS ratio of 2 to 3:1. This ratio is

Flow cytometry has become an important tool in the diagnosis—
involved in certain disorders and diseases. These conditions
diagnosis and classification of hematologic neoplasia by immu—
include the acute phase of cytomegalovirus mononucleosis,
immunophenotyping. Numerous, well-characterized antibodies
subsequent to bone marrow transplantation, and acquired
and their various combinations used in flow cytometry allow
immunodeficiency syndrome (AIDS).

For rapid, reliable identification and characterization of these

The CD4 (helper subset) T-lymphocyte cell count is one

neoplasms.

o the standard measures for diagnosing AIDS and the man—

Intracellular staining is most often used to aid in the

assessment of disease progress in patients with human immu—

diagnosis of acute leukemias and lymphomas as an adjunct

to HIV disease. The analysis of the T-cell

to surface antigen detection. For these assays, multiple cell

and B-cell ratio is clinically useful in evaluating the immune

surface and intracellular antigens may be studied simulta—

neously. The status of patients who may be at an increased risk of

opportunistic infections. In addition, the absolute number

each one is conjugated to a unique fluorochrome to char—

acterize the cells in each tube. This technique is referred

to as three-color or four-color immunophenotyping.

Examples of commonly used antibodies in hematopathol—

well as monitoring the level of immune reconstitution (table 30.7) are

CD3, CD13, CD22, myeloperoxidase

guide or timing the institution of antiretroviral therapy as

Examples of commonly used antibodies in hematopathol—

well as monitoring the level of immune reconstitution (table 30.7) are
CD3, CD13, CD22, myeloperoxidase

lowing initiation o therapy.

(MPO), terminal deoxynucleotidyl trans erase (d), and

In these cases, two cell sur ace antigens—CD3, which is present on mature lymphocytes, and CD4, which is only

Re lationship Am ong

present on the helper subset o lymphocytes—are used.

Re prese ntative Mem brane

T e percentage o CD4 lymphocytes is determined by using

TABLE

30.7 Antigens, Hematopoietic Cells,

a f uorochrome-conjugated CD3 antibody (e.g., FI C-CD3)

and Malig nancies

together with a CD4 antibody conjugated to a second f uorochrome (e.g., PE-CD4). T e absolute CD4 count can

Ce llular

He m atologic

be determined by a single-plat orm method, which uses a

IC Antige n

Dis tribution

Malignancy

sample spiked with a predetermined number o beads per

unit volume to index the CD4 count comparatively. A sec—

CD3

T lymphocytes

T acute lymphoblastic

ond approach is a dual-platform method. The absolute count

leukemia

o CD4-bearing lymphocytes is calculated by multiplying

CD 13

Granulocytes

Acute myelogenous

the percentage o CD4-bearing lymphocytes by the absolute

leukemia

lymphocyte count (calculated independently from the total

CD22

B lymphocytes

B acute lymphoblastic

leukocyte count and percent o lymphocytes in a peripheral

leukemia

blood smear differential).

The absolute number o CD4 lymphocytes is reflective o

TdT

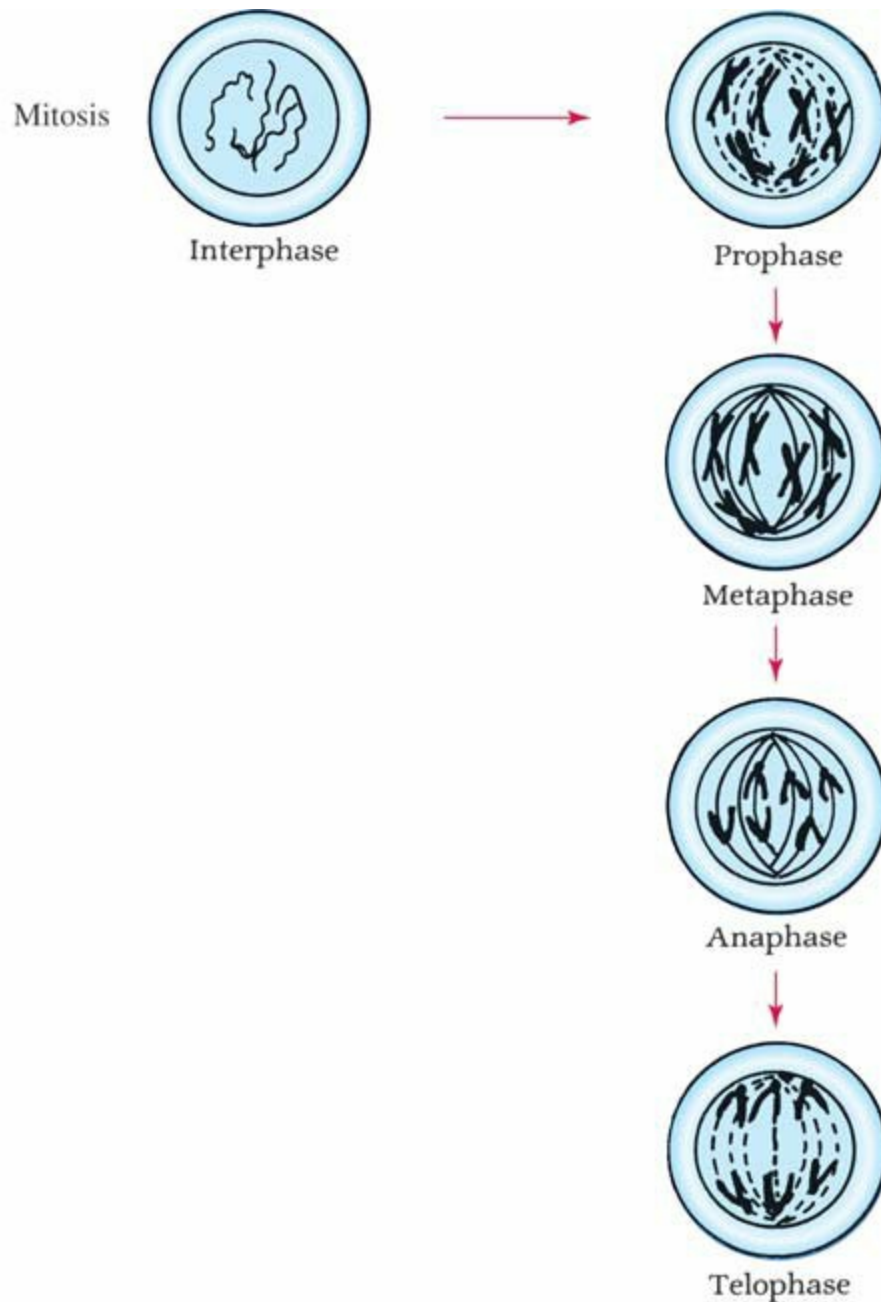
Usually immature

Acute lymphoblastic

the degree of immune deficiency in HIV-infected patients

lymphocytes

leukemia



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cytoplasmic immunoglobulins (kappa and lambda light chains, M heavy chain).

Research applications of immunocytochemistry (IC)

immunophenotyping include IC cytokine expression to examine functional subtypes of lymphocytes in acquired and primary immunodeficiencies and to measure engraftment success after a transplant procedure. Detection of cancer—related markers in tumors as prognostic indicators (e.g., estrogen and progesterone receptors, oncoproteins, p53) is an additional application.

DNA Ploidy and Cell Cycling

One of the earliest clinical applications of flow cytometry was the detection of aneuploidy and cell cycling status of solid tumors, particularly selected breast tumors. Since 1996,

G

the use of DNA analysis has significantly decreased. It is now

1

o

most often performed in patients with node-negative breast

8 or more hours 6-8 h

S

u

r

s

cancer and other tumors in which the clinical correlation

prognostic significance is strongest. Recent technological

e

m

i

innovations may lead to a revival o interest in clinical DNA

T

e

t

i

2

n

G

G2

analysis.

0

i

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f

5

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d

h

Because approximately a 2-week lag exists between bone

o

I

n

u

M

r

s

marrow activity and its resultant expression in the peripheral

I T O S I S

blood, it is important to assess the current status of the bone

T

marrow cells under certain conditions (i.e., cell cycle kinetics—

A M P

ics). Flow cytometry allows for analysis of the bone marrow

cell cycle parameters with no time lag.

FIGURE 30.22 Cell cycle. G

Flow cytometry techniques with bone marrow cells

0, nondividing cell; G1, cell growth; S,

DNA replication; G2, protein synthesis; M, mitosis, which lasts for

are applicable to DNA cell cycle analysis, which quanti—

1 to 3 hours and is followed by cytokinesis or cell division (telo—

tates the number of cells in various phases of the cell cycle

phase [], anaphase [A], mitosis [M], prophase [P]). (Adapted

(Fig. 30.22). The cell cycle stage is important in drug therapy.

from Porth CM. Pathophysiology Concepts of Altered Health States,

Antineoplastic drugs exhibit specificity of different phases of

7th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2005, with

the cell cycle. Inhibitors of microtubule function affect cells

permission.)

in M phase; glucocorticoids inhibit cells in G1; antimetabo—

lites and folate pathway inhibitors inhibit cells in S phase;

nucleic acid stains are also detected. In bone marrow trans—

antitumor antibiotics inhibit cells in G2; topoisomerase

plantation, flow cytometry applications can include pretrans—

inhibitors inhibit cells in S phase and G2. Alkylating agents

plantation determinations of the efficacy of ex vivo -cell

and platinum complexes affect cell function in all phases and

graft depletion, posttransplantation evaluation of immune

are therefore cell cycle nonspecific. Different cell cycle speci—

recovery, graft rejection, graft versus host disease, and the
techniques allow various drug classes to be used in combination
graft versus leukemia effect.

to target different populations of cells. Specific drugs can be
administered to target actively replicating neoplastic cells,

Monitoring Monoclonal Antibody Therapy

and nonspecific agents can be used to target nonreplicating

In conjunction with IC and molecular techniques, flow
neoplastic cells.

cytometry has been essential for measuring the expression of cell surface and
intracellular markers of multiple

Solid Organ Transplantation

drug resistance (MDR) in cancer patients, assessing the

Flow cytometric crossmatching uses fluorochrome-conjugated

intracellular accumulation and effects of chemotherapeutic

labeled antihuman IgG to detect the binding of alloantibodies

drugs, and studying the other mechanisms leading to MDR.

to donor lymphocytes in allogeneic organ transplantation.

Ligand, antigen, or molecule-targeted biologic therapy using

CD3 and CD19 coupled with anti-IgG in a three-color

monoclonal antigens (e.g., Mylotarg [CD33] and rituximab

assay can distinguish T-lymphocyte and B-lymphocyte mismatches. Patient serum can be screened against known HLA antigens or the detection of corresponding antibodies.

directly disrupting cell proliferation and antiapoptosis by blocking the cell membrane receptors and circulating ligands

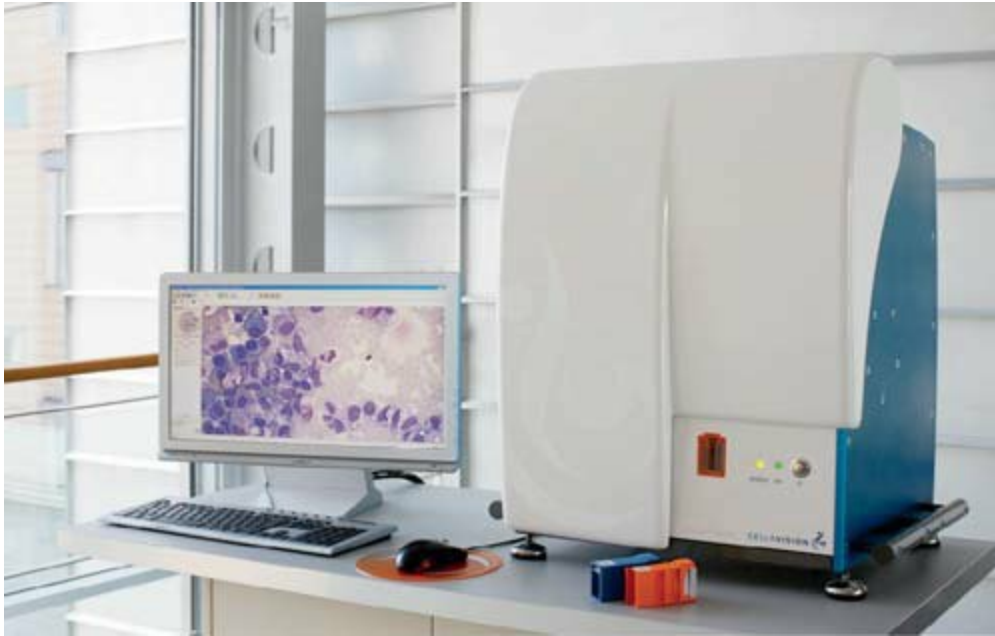
Stem Cell Transplantation

associated with signal transduction. Others serve as the target

Flow cytometry is widely used to enumerate the CD34+

positive implanted stem cells. In some cases, CD45 and

new class of pharmaceutical agents was anti-CD3.



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More recently, monoclonal antibodies directed against fluorescence measurements. The cell type of interest can be CD20, CD25, CD33, CD45, and CD52 have been developed. separated from a complex mixture of cell types even though Before treatment, flow-cell analysis is critical for confirming it may be an extremely rare or minor subpopulation.

that the antigen is expressed by the remaining cells. During and after treatment, flow cytometry is used to verify bind-

DIGITAL MICROSCOPY

ing of the antibody and to monitor the efficacy of tumor cell

eradication.

Recent advances in artificial neural networks (ANNs), image analysis, and slide handling have combined to produce

Paroxysmal Nocturnal Hemoglobinemia Testing instruments that automate manual differentials in new ways.

The detection of paroxysmal nocturnal hemoglobinemia

This new technology, referred to as automated digital cell morphology (PNH) by the traditional methods of Ham's (acid hemolysis) morphology (Fig. 30.23), provides an unprecedented level of

and the sucrose lysis test has been replaced in many clinical efficiency and consistency. In its simplest form, automated

laboratories by flow cytometry analysis. The glycosylphosphatidylinositol (GPI)-linked proteins, CD55 and CD59, are

automatically located and preclassified into categories of blood cells. Images of these cells are retained or confirmed—

surface markers exists. A deficiency or absence of these cell blood cells. Images of these cells are retained or confirmed—

surface markers exists. A deficiency or absence of CD55

tion by a technologist and can be shared electronically and

and CD59 is established, the condition is diagnostic of PNH.

stored as digital images. This adaptability allows for future review and comparisons by laboratory professionals and fetal hemoglobin physicians.

Detection of fetal hemoglobin and F cells by flow cytometry is becoming common. The assay uses monoclonal antibodies

Artificial Neural Networks

to hemoglobin F. This analysis allows for the detection of a variety of diseases including sickle cell disease and fetal—

An ANN is an information-processing model that simulates maternal hemorrhage. In addition, this methodology allows the way the human brain processes information. ANN emulates or quantitation of fetal hemoglobin.

lates the neural structure of the brain, which is composed of a large number of highly interconnected processing elements

Blood Parasites

(neurons) working together to solve specific problems.

Malarial parasites can be screened by flow cytometry method—

ANNs have been around since the 1940s, but it was not until 1980s. Erythrocytes are stained with acridine orange, the

the mid-1980s that algorithms became sophisticated enough
mature erythrocytes containing no DNA do not fluoresce
and computers powerful enough for general applications to
with this stain. However, malarial erythrocytes contain DNA
develop.

and thus will fluoresce.

Digital Cell Morphology

Cell Functioning Analysis

New hardware and the development of databases have aided

Every event that occurs during the process of lymphocyte

in developing image analysis systems that can really meet

activation can be measured by flow cytometry. The measure—

the demands of the hematology laboratory. The most dra—

ments with the greatest clinical significance include tyrosine

phosphorylation, calcium flux, oxidative metabolism, neo—

the ability to digitize image specimens and transmit these

antigen expression, and cellular proliferation.

images electronically or remote analysis. This capability is

Flow cytometry measurement of the oxidative burst in

now called virtual microscopy.

neutrophils has been used as a screening test for chronic granulomatous disease (CGD).

Chromosomal Analysis

Flow cytometry can be used for karyotyping analysis. A

chromosomal histogram consists of seven peaks that represent the different groups of chromosomes. By evaluating the

peaks, various disorders can be diagnosed.

Cell Sorting

Some flow cytometers have additional hardware that allows

them to act as cell sorters. After quickly making the appropriate measurements, the computer makes the decision to sort

or isolate a single cell by applying a charge to that cell just as

it leaves the flow cell. The cell is electrostatically deflected

into a test tube. Any cell type can be sterilely sorted and

FIGURE 30.23 CellaVision DM-1200. (Courtesy of Cellavision, Inc.)

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In 2000, CellaVision (Lund, Sweden) launched the

Di Master Octavia. The system consists of an automated

BOX 30.3

microscope with a 100× objective, a stepper motor and light control unit, and a progressive three-chip CCD color camera connected to a computer with software for localization,

Fibrinolytic Hemostasis Panel Assays

segmentation, and classification of white and RBCs. The sys-

α -2-Antiplasmin

tem processes eight slides per batch, utilizing a slide holder.

Plasminogen

It allows for remote review of a smear and storage of up to

Plasminogen activator inhibitor

20,000 slides with images in a database.

issue plasminogen activator

In comparison with earlier attempts by other manufacturers, the Di Master Octavia handles wedged smears stained

Solution

Container No.

Time

according to the Wright, Wright-Giemsa, or May-Grünwald—

Giemsa staining protocols and uses ANNs trained on a large

Fixative

2

30 s

database of cells. It was the first image analysis system to

Wright stain

3

3 min

locate and preclassify cells into 15 different categories and

Stain buffer

4

6 min

automatically precharacterize six RBC morphologic charac—

Deionized water

5

1.5 min

teristics. The platelet estimates and erythrocyte precharacterization are performed in an overview image corresponding

Drying stage

6

3 min

to eight high-power fields (100×). Review and release of

results can be done remotely.

Electromechanical Methods

NOTE: This is a good time to complete Review Questions

The earliest instruments to detect blood clotting were developed between 1920 and 1940. These instruments were based primarily on detection of the formation of a fibrin clot and replaced visual observation of the formation of a fibrin clot

in a test tube. By the mid-1960s, electromechanical instruments were in widespread use. In the 1970s, photo-optical

INSTRUMENTS IN COAGULATION

STUDIES

Many different manufacturers produced laboratory-based methods replaced electromechanical devices in most laboratories, except student laboratories or as a backup method in coagulation analyzers, many of which are capable of performing clotting clottable, immunoassay, and chromogenic assays. The difficulty with some instruments is that many of them are unable

The principle of electromechanical methodology is the

to transmit an industry-standard test identifier (LOINC code)

measurement of conduction or impedance of an electrical

to the host laboratory information system (LIS).

cal current by the formation of fibrin. An example of such

Various models are available in a wide range of prices

a semiautomated instrument is the brometer. This system

designed for different size laboratories. Each instrument

consists of a 37°C heat block, an automatic pipette, and a

offers unique advantages (e.g., high throughput, reduced

mechanical mixer and timer block.

reagent volume, integral bar-code reader, cap piercing, or

After the appropriate containers are filled and plasma

automatic sample predilution). Many instruments offer user—

samples and thromboplastin substrate are incubated, plasma

programmable methods and preprogrammed methods.

is added to the substrate to initiate the timing mechanism.

Suggested screening panels include thrombotic hemoT is timing mechanism
triggers a digital readout time and

stasis panel (Box 30.2) and a fibrinolytic hemostasis panel

the probe unit. The probe arm holds two electrodes. When

(Box 30.3).

in operation, it drops down and allows the electrodes to all into place within the reaction well containing the plasma-thromboplastin mixture. The stationary probe does not move when the instrument is in operation but functions in con—

BOX 30.2

junction with the moving electrode. The stationary electrode is responsible for creating an electrical potential between it and the moving electrode. The moving electrode is located in

Thrombotic Hemostasis Panel Assays

front of the stationary electrode in the probe arm. When a test

Antithrombin

is being performed, this electrode cycles through the plasma—

Factor VIII:C

thromboplastin mixture every half second until a clot forms.

Heparin

A detection circuit is activated when a fibrin strand is formed

Lupus anticoagulant

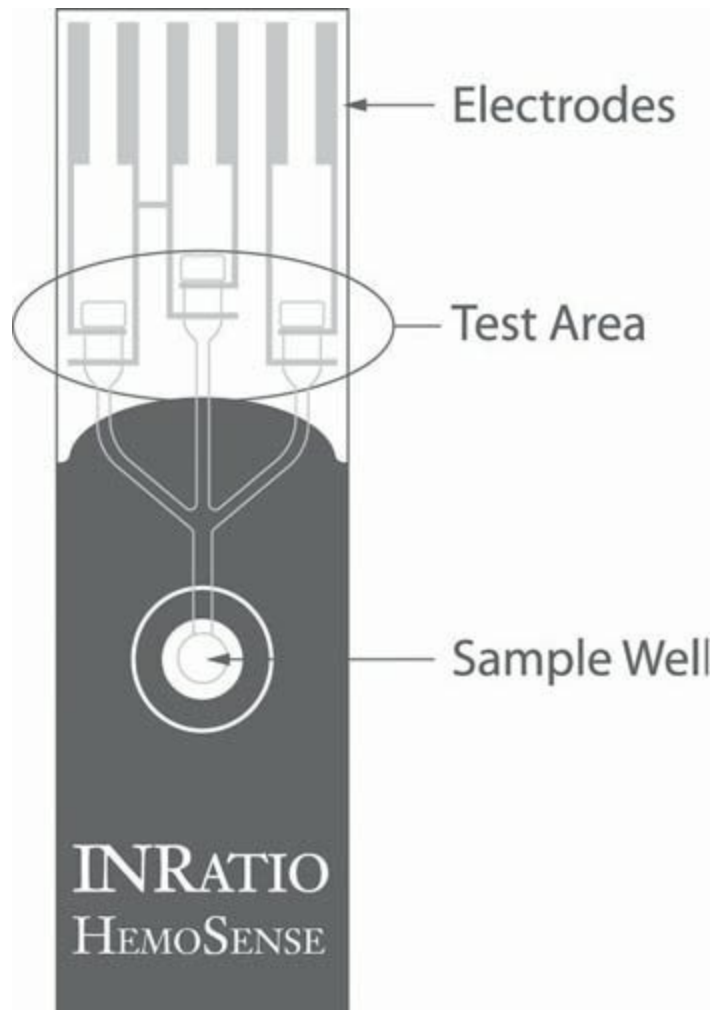
between the two electrodes, thus completing the circuit. Circuit

Protein C

activation stops the timer and prevents further movement of

Protein S and free protein S

the moving electrode. Electromechanical methods, such as the brometer, can be used for various coagulation assays.



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PART 8 ■ Fundamentals of Hematological Analysis

These include activated partial thromboplastin time (APT), signal. An amplified signal is converted to a digital value or prothrombin time (PT), and factor assays.

Further processing. The computer-processed results are submitted to a new generation of

P point of care testing is the

sequently sent to the visual display monitor and printer.

HEMOSENSE INRatio2 IN/P (Milpitas, CA). This hand—

A system is ready for operation when the temperature

held instrument provides the P and corresponding inter—

indicator reads $37 \pm 1^{\circ}\text{C}$. Preprogrammed modes select the

national normalized ratio (INR) value by measuring the

test parameters for each test method, which determines the

electrical impedance using fresh capillary whole blood. The

proper volumes of specimen and reagents. The appropriate

INR system performs a modified version of the one-stage P

amounts of reagents are placed in the specific reagent stor—

test. The clot formed in the reaction is detected as a change

age wells. Pressing the start button initiates the test cycle.

in the electrical impedance of the blood sample that occurs

The optical density (absorbance) of the reaction mixture is

when fibrinogen is converted into fibrin. The test strip itself

then monitored until the rate of change exceeds a predetermined—

consists of layers of transparent plastic, one of which is an

mined level or a defined period, indicating the presence of a

electrode layer (Fig. 30.24).

brin clot end point. The time (in seconds) of the end point is stored and may be printed or displayed on demand at the

Photo-Optical Methods

end of each series of determinations.

Quality control in these systems includes automatic sel -

The principle of photo-optical measurement is that a change checking of the optical system and the storage of standard in light transmission measured as optical density (absorbance) versus time can be used to quantitatively determine to check precision.

the activity of various coagulation stages or factors. Photo—

In addition to routine clot testing, newer instruments

optical clot detection systems can be used for the determina—

tion of chromogenic channel models to automate the growing

tion of a wide variety of assays (e.g., AP, P, brinogen

range of specialized diagnostic tests in coagulation.

levels, and thrombin time). Quantitative factor assays based

on the AP (factors VIII, IX, XI, and XII) and quantitative factor assays based on the P (factors V, VII, and X) are

Viscosity-Based Detection System

examples of available assays.

Viscosity is defined as the resistance that a material has to a change in its form. This principle is used as a mechanism for rate detector cells with their own red LED light source, which for clot detection, the natural thickening (viscosity) is monitored is driven by a constant current regulator to give each a noise—by the motion (amplitude of an oscillating steel ball in a special light beam. The light beam passes through a specially designed cuvette) as a change in form takes place. The where it is altered by fibrin clot formation. The light beam final result is accurate and is insensitive to colored plasma, then passes through a diaphragm and falls on the sensor, which for lipemic plasmas, bilirubin, or turbid reagents and reliably instantly converts the transmitted light into an electrical measurement for the hemostasis laboratory. Diagnostica Stago manufactures an instrument based on this principle. The steps in the viscosity-based detection system (VDS) are as follows:

1. Movement of the steel ball is triggered by two activating coils, working alternately to induce and maintain a natural oscillation.
2. When the start reagent is added, the detection starts immediately.
3. When the ball starts oscillating left and right, a chronometer (clock) begins to time the clotting of the sample.
4. As the ball oscillates left and right, the amplitude of motion is also measured. A peak is formed when the ball is detected in the center of the cuvette.
5. Amplitude is monitored during the entire clotting process.
6. As the clot appears, the viscosity increases, and the amplitude decreases.
7. Based on different algorithms, the chronometer is stopped even if the clot is peak, and/or the ball is still in motion.

Platelet Agglutination

The ristocetin cofactor assay measures the ability of a patient's

FIGURE 30.24 HEMOSENSE INRatio2 IN/P . (Courtesy of plasma to agglutinate normal platelets in the presence HEMOSENSE Milpitas, CA.)

of ristocetin. The rate of ristocetin-induced agglutination is

related to the concentration of von Willebrand factor, and the number of pulses generated is directly proportional to the percent normal activity can be obtained from the standard curve. Patient values are determined by comparison to a standard curve. Patient values are determined by comparison to a specific period.

a standard curve, allowing quantitation of percent ristocetin

■ Based on the original ideas of Einstein and physical theories in the 1950s, laser light was applied to medical and scientific instrumentation. Lasers are able to sort the

Platelet Aggregation

energy in atoms and molecules, concentrate it, and release it in powerful waves.

Most platelet aggregation procedures are based on some

Flow-cell cytometry is another method that is applied

variation of Born method. Agents such as adenosine diphos-



in the study of cells. The principle of flow cytometry is

phate (ADP), collagen, epinephrine, snake venom, thrombosed on the act that cells can be stained specifically with

bin, and ristocetin can also be used to aggregate platelets.

a fluorescent dye to identify exact cell types. Laser light is

The principle of the test is that platelet-rich plasma is

combined with this method in state-of-the-art instrument—

treated with a known aggregating agent. It aggregated, cloud—

tation or cell identification and sorting.

iness or turbidity patterns are determined by photometry—

The values that an instrument generates are referred to as

usually comparing the light transmitted through a suspension



parameters. The simplest units count erythrocytes, leuko—

of aggregated platelets with that of a suspension of nonag—

cytes, and platelets. The most sophisticated instruments

aggregated platelets using an aggregometer. The curve that is

generate many additional parameters.

obtained can be used to assess platelet function.

■ Some instruments are based on a variety of principles

Primary Response

including the electrical impedance principle and the optical principle of laser

scatter technology.

Primary response is the reversible aggregation of platelets by

In addition to numerical outputs, the larger instruments

the aggregating agent. The appearance of a biphasic reaction,



are capable of generating graphic displays of the frequency

showing both primary and secondary response, can occur

distributions of erythrocytes, leukocytes, platelets, and

or some agonists at low concentrations.

histograms.

Secondary Response

■ Quality control systems, such as Levey-Jennings charts,

are also generated by the larger instruments.

Secondary response is the result of enhancement of the initial aggregation process caused by the release of endogenous

ADP and the formation of thromboxane A

Whole Blood Cell Analysis

2. The secondary

response is irreversible.

■ A significant innovation is automated front-end (preanalytical [preexamination]) instrumentation/robotics and

New er Autom atio n

total work cells linked by a track or conveyor.

T e PFA-100 (Siemens Healthcare Diagnostics) is an auto-

■ Automated analyzers now orm the backbone o clinical
mated system that incorporates a high shear f ow system
laboratories both large and small. Smaller analyzers are
to simulate the in vivo hemodynamic conditions o plate—
commonly used in S A labs, reestanding clinics, phy—
let adhesion and aggregation as encountered at a vascular
sicians' o ces, and small hospital laboratories. Larger
lesion. T e system evaluates the ability o platelets to occlude
and more complex systems are used in larger clinical and
an aperture in a biochemically active membrane. Results are
research laboratories.

reported as closure time (C). T is instrument o ers several

■ T e degree o instrumental sophistication is reaquently
advantages over traditional aggregometry because it assesses
described by the number o parameters that the instru—
multiple acets o primary hemostasis—adherence, activa—
ment generates. T e term parameter is a statistical term

tion, and aggregation.

that refers to any numerical value that describes an entire population. Parameter should be clearly distinguished from the term sample, which is a subset of a popula-

CHAPTER HIGHLIGHTS

tion. Any numerical value describing a sample is called

Instrumental Principles

a statistic.

- Smaller hematology instruments measure erythrocytes
- Various principles of cell counting are used in instrumentation—electrical impedance, and optical.

hematology instruments generate eight measured or cal-

- The impedance principle is based on the detection and calculated parameters (WBC, RBC, Hgb, Hct, MCV, mean measurement of changes in electrical resistance produced by a particle as it passes through a small aperture.

hemoglobin concentration [MCHC], and platelets).

- In the optical principle, the degree of scatter and the
- Computerized systems generally flag high or low patient amount of light reaching the sensor depend on the volume results. Additional basic parameters include erythrocyte

of the cell. The volume of each cell is proportional to the morphology information expressed as RDW, MPV, or leukocyte forward scatter of light. In both systems, leukocyte histogram differential.

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PART 8 ■ Fundamentals of Hematological Analysis

- Technology continues to deliver new automation capabilities.
- Other cellular applications of flow cytometry include analyses in hematology. For example, automated reticulocyte analysis of the ratio of cells to B cells in immunodeficiency counting was a leading edge technology a few years ago states such as AIDS, the study of DNA in cell cycle kinetics but is a routinely measured parameter in many clinical labs, and the investigation of chromosomes.

laboratories today.

- Some of the newer instruments prepare and stain peripheral

Digital Microscopy

eral blood smears and automatically correct for leukocyte (WBC) interference.

- Advances in artificial neural networks (ANNs), image

analysis, and slide handling have combined to produce

- Newly developed instrumental capabilities continue to be developed. Some of the innovations include quantitation of instruments that automate manual differentials in new nucleated erythrocyte; a channel or enumeration of immature granulocytes; random access CD4 lymphocyte counting; technology, referred to as automated digital cell morphology, provides an unprecedented level of efficiency and consistency.

poietic progenitor cells; and counting immature granulocytes.

- In its simplest form, automated digital cell morphology is a process where blood cells are automatically located and preclassified into categories of blood cells.

Quality Assurance of Instrumental Data

Output

- Images of these cells are retained for confirmation by a technologist and can be shared electronically and stored

■ Erythrocyte histograms are valuable in determining the as digital images. This adaptability allows for future similarity of the population of RBCs being tested.

review and comparisons by laboratory professionals and

■ Quantitative parameters that express variation in the physicians.

erythrocyte population are either the RDW or the RCMV.

The RDW and MCV can be correlated and classified in

Instruments in Coagulation Studies

various disease categories.

■ Manual methods have been replaced in the clinical

■ The WBC histogram classifies WBCs into three categories—hematology laboratory by electromechanical and optical systems: lymphocytes, mononuclear cells or monocytes, and systems.

granulocytes. Computer programming allows for the di-

In the older electromechanical system, two electrodes

differentiation of leukocytes graphically, in terms of percent-

■

work in conjunction with one another. When a fibrin

age and absolute values.

strand forms in the plasma-thromboplastin mix-

■ Platelet histograms, the MPV, and the DPW can be gener—
ture between these two electrodes, a complete circuit
ated by computer-assisted instruments in addition to the
is formed. Completion of the circuit automatically
platelet count. The MPV is an expression of the measure
stops the timer and the length of the reaction time is
of the average volume of the platelets in the sample. The
displayed.

PDW is a measure of the uniformity of platelet size.

■ In the optical system, a change in light transmission

Laser Technology

through the reaction mixture of plasma-thromboplastin
is measured as optical density versus time. Formation of a

■ Some systems are based on the principle of differential
brin clot alters the light path, and after the data are pro—
light-scattering cytochemistry.

cessed by the onboard microprocessor, the time in seconds

■ Cytochemical reactions prepare the blood cells for analy—

that the reaction took is displayed or printed.

sis, a cytometer measures specific cell properties, and algo-

- Both methods can measure AP, P, factor levels, and rhythms convert these measurements into cell classification, various other parameters.

cell count, cell size, and hemoglobinization.

- Platelet aggregation procedures are used to test the qualitative response of platelets to various aggregating

Applications of Flow Cytometry

agents.

- Instruments based on the flow-cell cytometry principle

Case Studies

were initially designed to count and size cells; later modifications included leukocyte differential analysis.

Because the relationship between histogram and nomogram

- Today, the applications of the technology are highly information is important to understanding the data output diverse and include both cellular component identification—capabilities of modern instrumentation, specific examples tion and cell-sorting capabilities.

become important in establishing a diagnosis and monitor-

■ Monoclonal antibodies and fluorescent probes have had a
ing treatment of a patient. A knowledge and understanding
major effect on advances in flow cytometry applications.

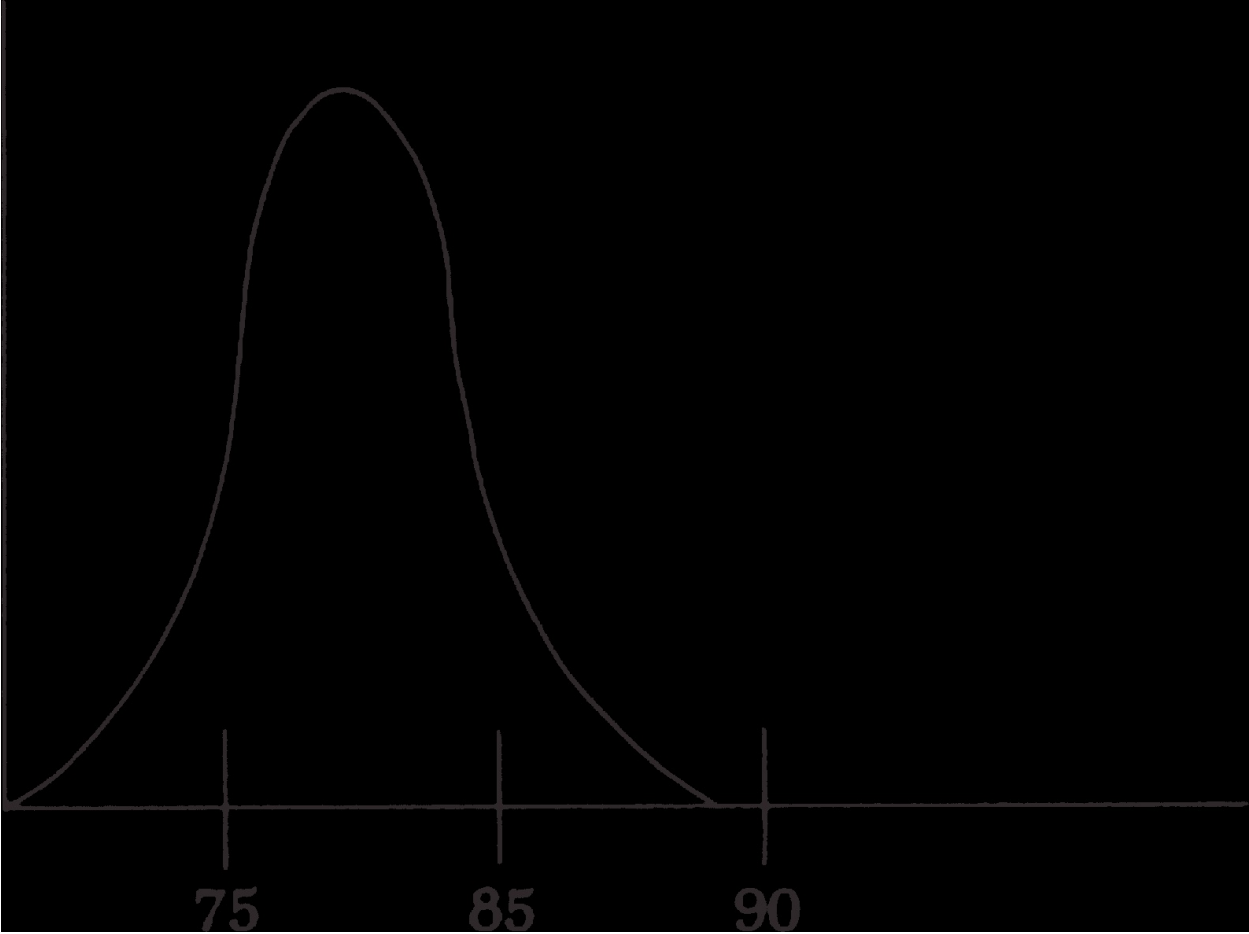
of these newer sources of patient information is important to

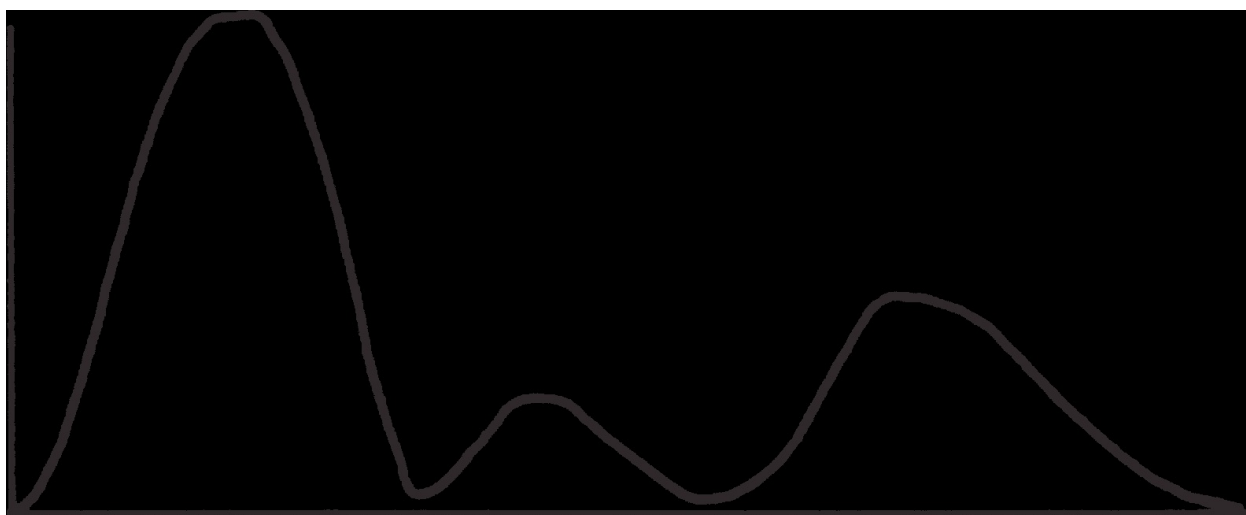
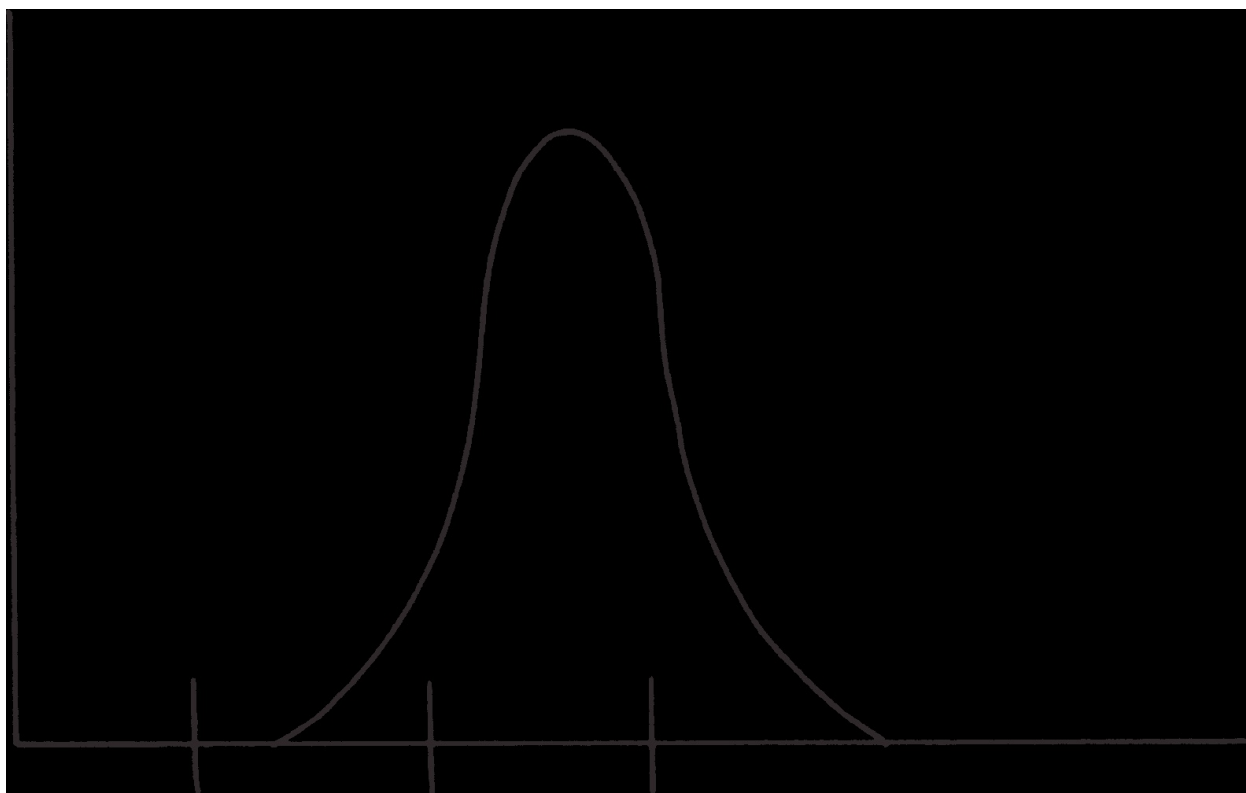
■ In the hematology laboratory, in addition to leukocyte di -
the clinical laboratory scientist. Each of the cases presented

differentiation, applications can include reticulocyte count in this chapter
represents a fairly typical example of a specific

ing and screening for malarial parasites.

type of disorder.





CHAPTER 30 ■ Instrumentation in Hematology

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CASE STUDIES

Case Study 30.1

■ Laboratory Data

A 28-year-old white woman had the following erythrocyte results:

RBC count $3.2 \times 10^{12}/L$

Hemoglobin 8.7 g/dL

Hematocrit 26%

MCV 81 L

MCH 19 pg

MCHC 27.2 g/dL

■ Critical Thinking Group Discussion Questions

RCMI 13.2

1. Do the histogram or other erythrocyte results demonstrate any abnormalities?

Her RBC histogram appears below. All other parameters were within normal ranges.

2. What type of disorder, if any, is suggested by the RBC results and histogram?

3. What further laboratory testing should be considered?

Case Study 30.3

■ Laboratory Data

A 57-year-old white man with a total leukocyte count of $15 \times 10^9/L$ had a three-part leukocyte differential:

Lymphocytes 68%

Mononuclear cells 4%

■ Critical Thinking Group Discussion Questions

Granulocytes 28%

1. Do the histogram or other erythrocyte results demon—

Absolute lymphocyte value $10.2 \times 10^9/L$

strate any abnormalities?

Absolute granulocyte value $4.2 \times 10^9/L$

2. What type of disorder, if any, is suggested by the RBC

The WBC histogram appears below. All other parameters

results and histogram?

were within normal ranges.

3. What further laboratory testing should be considered?

Case Study 30.2

■ Laboratory Data

■ Critical Thinking Group Discussion Questions

A 48-year-old black woman had the following RBC results:

1. Do the histogram or other leukocyte results demonstrate

RBC $2.36 \times 10^{12}/L$

any abnormalities?

Hemoglobin 8.6 g/dL

Hematocrit 27%

2. What type of disorder, if any, is suggested by the WBC

MCV 114 L

results and histogram?

MCH 36.4 pg

3. What further laboratory testing should be considered?

MCHC 32 g/dL

RDW 28%

Case Study 30.4

Her RBC histogram appears below. All additional parameters were within normal ranges.

■ Laboratory Data

A 14-year-old white girl with a total leukocyte count of

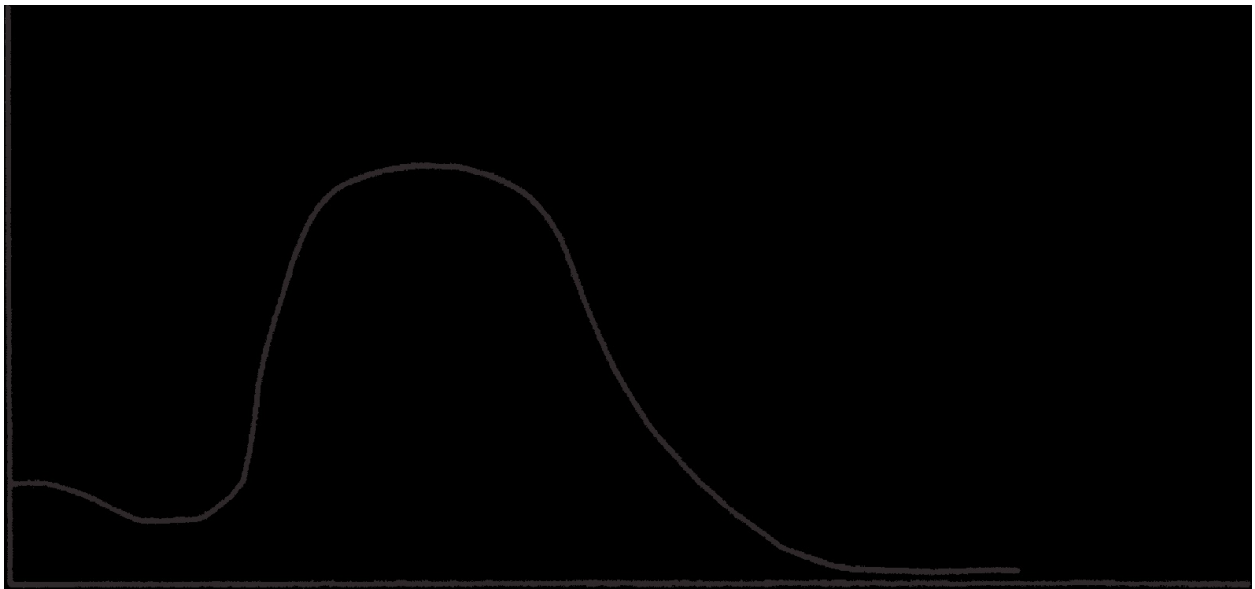
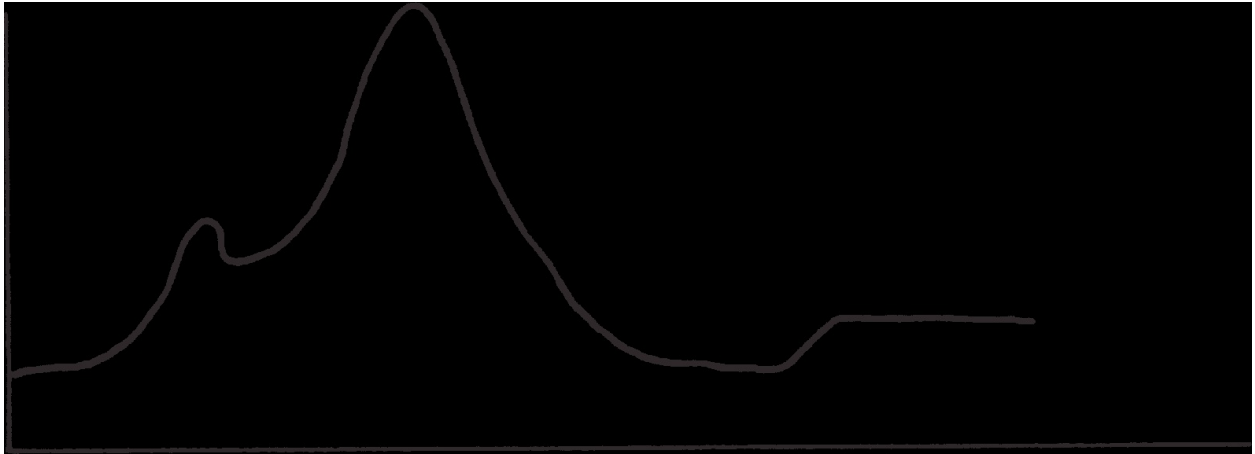
$29 \times 10^9/L$ had the following histogram results:

Lymphocytes 15%

Mononuclear cells 48%

Granulocytes 37%

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

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Her WBC histogram appears below. T e platelet count was

23

decreased. All erythrocytic parameters were within normal ranges; however, the values tended to be in the low ends o the ranges.

21

25

19

fL

29

17

■ Questions

15

27 1

1. Do the histogram or other leukocyte results demon—

9

13

11

7

strate any abnormalities?

2. What type o disorder, i any, is suggested by the WBC results and histogram?

3. What urther laboratory testing should be considered?

0.2

0.3

0.4

P la tele t count ($\times 10^{12}/L$)

Cas e Study 30.5

■ Critical Thinking Group Discussion Questions

■ Laboratory Data

1. Did the patient have an initial abnormality?

A 12-year-old black boy had a total leukocyte count of $55.0 \times 10^9/L$. His histogram appears below.

2. Did the patient develop any abnormalities following treatment? His platelet count was low, as were his erythrocyte

parameters. Additionally, his platelet histogram was abnormal.

3. Did the patient's values return to normal?

Case Study 30.7

■ History and Physical

M.W., a 50-year-old male clinical laboratory scientist, saw his primary care provider for an examination prior to a 6-month international volunteer assignment. He felt well and had no symptoms of any abnormalities.

A urinalysis and CBC were ordered.

■ Critical Thinking Group Discussion Questions

■ Laboratory Data

1. Do the histogram or other leukocyte results demon—

Urinalysis: All results within re erence ranges.

strate any abnormalities?

■ Hematology Laboratory

2. What type o disorder, i any, is suggested by the WBC

Me as ure m e nt

Patie nt Data

Re fe re nce Range*

results and histogram?

RBC

$5.03 \times 10^6/\mu\text{L}$

4.00–6.20

3. What urther laboratory testing should be considered?

Hgb

15.2 g/dL

11.0–18.8

Hct

45%

35.0–55.0

Cas e Study 30.6

WBC

$5.1 \times 10^3/\mu\text{L}$

6.0–11.0

■ Laboratory Data

PLT

$175 \times 10^3/\mu\text{L}$

150.0–400.0

A 28-year-old white woman underwent a chemothera—

MCV

89 fL

80.0–100.0

peutic regimen or the treatment o leukemia. Her initial platelet count and MPV as well as successive platelet

MCH

30 pg

26.0–34.0

counts and MPVs, beginning on the 7th day a er the ter—

MCHC

34 g/dL

31.0–35.0

mination o treatment, were assayed every other day and

RDW

18%

10.0–20.0

charted by the laboratory.

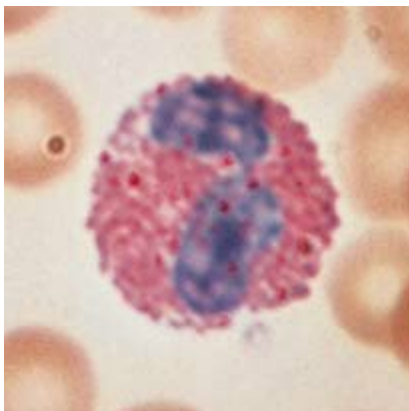
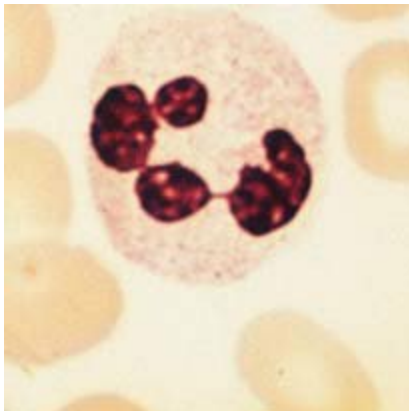
MPV

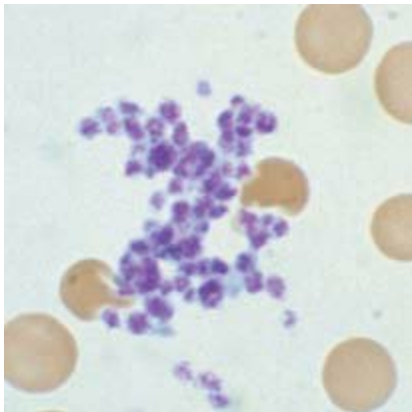
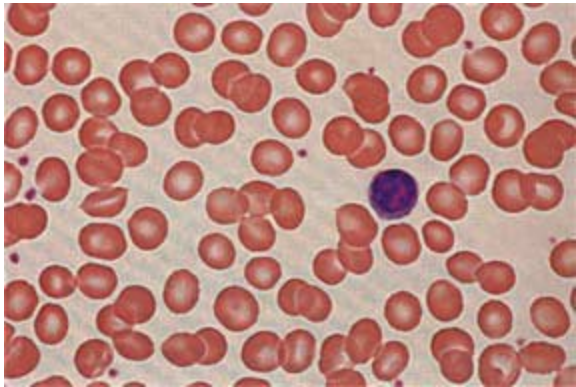
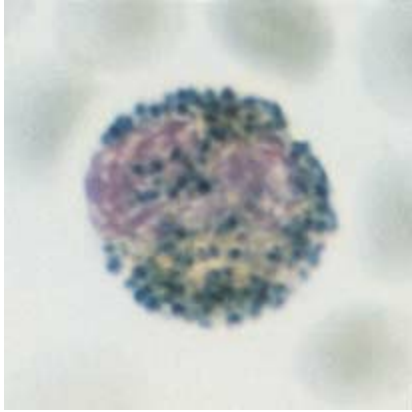
9.1 fL

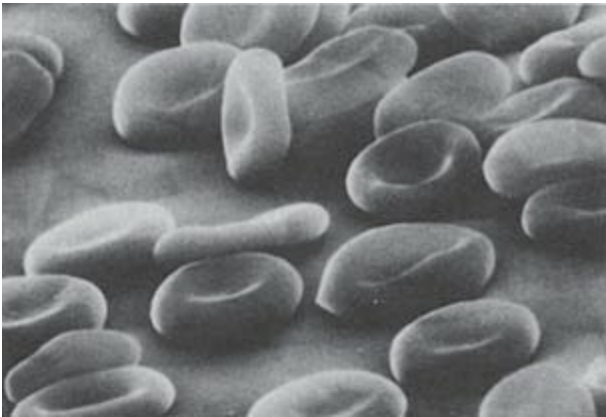
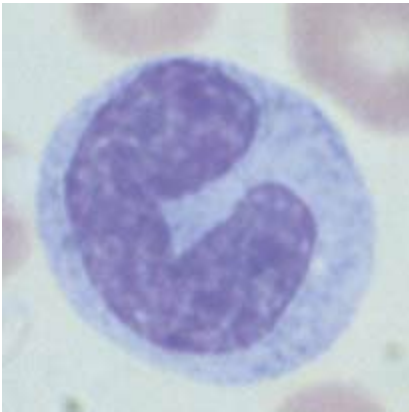
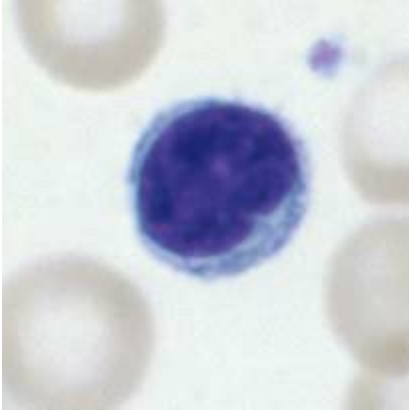
6.0–10.0

fL, femtoliters; pg, pictogram.

* Published for Beckman Coulter AC TTM.







CHAPTER 30 ■ Instrumentation in Hematology

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■ Leukocyte Differential Examination

■ Absolute Cell Counts

Ce ll Type

%

Patie nt

Re fe re nce

Units

Value

Range

Neutrophils (band neutrophils + polymorphonuclear

58

segmented neutrophils [PMNs])

Neutrophils

103/ μ L

2.96

2.0–8.0

Lymphocytes

35

Lymphocytes

103/ μ L

1.79

1.0–5.0

Monocytes

4

■ Critical Thinking Group Discussion Questions

Eosinophils

2

1. Are any of the laboratory values abnormal?

Basophils

1

2. Why are the absolute cell counts important data?

Total

100

3. Should additional laboratory assays be ordered?

VOL

RBC

PLI

WBC/

BASO

ABS

Neutrophil

Eosinophil

Basophil

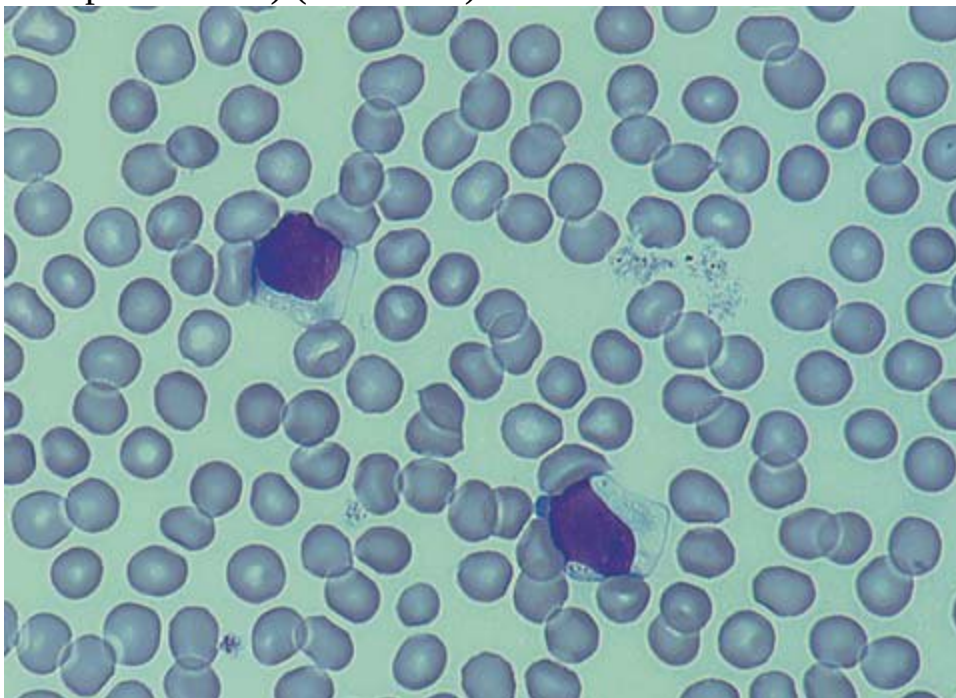
Blood smear

Red blood cells and platelets

Lymphocyte

Monocyte

(Reprinted from Cohen BJ, Wood DL. Memmler's Textbook of the Human Body in Health and Disease, 9th ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2000, with permission.) (continued)



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PART 8 ■ Fundamentals of Hematological Analysis

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Cas e Study 30.8

Le ukocyte Diffe re ntial

%

■ History and Physical

S.S., a 10-year-old white emale, presented to the pediatrician

Band neutrophils

0

complaining of a cold and cough with pain in the neck and

Polymorphonuclear segmented neutrophils (PMNs)

30

chest. She had an elevated temperature and a slight cough.

Lymphocytes

62

Physical examination revealed enlarged lymph nodes,

Monocytes

5

wheezing, and an elevated temperature.

Eosinophils

2

VOL

Basophils

1

Total

100

ABS

RBC

P LI

WBC/

BAS O

(Reprinted from McClatchey KD. Clinical Laboratory
Medicine, 2nd ed, Philadelphia, PA: Lippincott Williams
& Wilkins, 2002, with permission.)

A urinalysis, CBC, and throat culture were ordered.

■ Absolute Cell Counts

■ Laboratory Data

Patient

Reference

Urinalysis: all results within reference ranges.

Units

Value

Range

■ Complete Blood Count

Neutrophils

103/ μ L

1.11

2.0–8.0

Measurement

Units

Reference Range*

Lymphocytes

$103/\mu\text{L}$

22

1.0–5.0

RBC

$5.14 \times 10^6/\mu\text{L}$

4.00–6.20

■ Critical Thinking Group Discussion Questions

Hgb

13.8 g/dL

11.0–18.8

1. Are any of the laboratory values abnormal?

Hct

41%

35.0–55.0

2. Why are the absolute cell counts important data?

WBC

$35 \times 10^3/\mu\text{L}$

6.0–11.0

PLT

$200 \times 10^3/\mu\text{L}$

150.0–400.0

3. Should additional laboratory assays be ordered?

MCV

81 fL

80.0–100.0

MCH

27 pg

26.0–34.0

MCHC

32 g/dL

31.0–35.0

RDW

15%

10.0–20.0

MPV

9.0 fL

6.0–10.0

fL, femtoliters; pg, pictogram.

*Published for Beckman Coulter ACTTM.

CHAPTER 30 ■ Instrumentation in Hematology

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Cas e Study 30.9

■ Absolute Cell Counts

■ History and Physical

Patie nt

Re fe re nce

K.C., a 70-year-old nurse, su ered rom degenerative arthri-

Units

Value

Range

tis. She visited her rheumatologist because o increasing pain

Neutrophils

103/ μ L

13

2.0–8.0

in her knees.

A CBC and erythrocyte sedimentation rate (ESR) were

Lymphocytes

103/ μ L

1.44

1.0–5.0

ordered. Synovial fluid was removed from one knee or

Monocytes

103/ μ L

3.6

0.1–1.0

examination.

■ Critical Thinking Group Discussion Questions

■ Laboratory Data

1. Are any of the laboratory values abnormal?

Measurement

Units

Reference Range*

2. Why are the absolute cell counts important data?

RBC

$3.41 \times 10^6/\mu\text{L}$

4.00–6.20

Hgb

11.0 g/dL

11.0–18.8

3. Should additional laboratory assays be ordered?

Hct

31%

35.0–55.0

Case Study 30.10

WBC

$18 \times 10^3/\mu\text{L}$

6.0–11.0

PLT

210×10^3

History and Physical

$3/\mu\text{L}$

150.0–400.0



M, a 45-year-old white female, began to experience abdom—

MCV

92 fL

80.0–100.0

inal pain over the last several days. She went to a primary

MCH

32 pg

26.0–34.0

care clinic or help.

MCHC

35 g/dL

31.0–35.0

A CBC was ordered.

RDW

12%

10.0–20.0

Me as ure me nt

Units

Re fe re nce Range*

MPV

9 fL

6.0–10.0

fL, femtoliters; pg, pictogram.

RBC

5.1 × 10⁶/μL

4.00–6.20

* Published for Beckman Coulter AC TTM.

Hgb

13.0 g/dL

11.0–18.8

Hct

37%

35.0–55.0

WBC

23.0 × 10³/μL

6.0–11.0

Leukocyte Differential

%

PLT

450 × 10³/μL

150.0–400.0

MCV

72 fL

80.0–100.0

Band neutrophils

5

MCH

25 pg

26.0–34.0

Polymorphonuclear segmented neutrophils (PMNs)

65

MCHC

35 g/dL

31.0–35.0

Lymphocytes

8

RDW

17%

10.0–20.0

Monocytes

20

MPV

8.0 fL

6.0–10.0

Eosinophils

1

Basophils

1

fL, femtoliters; pg, pictogram.

* Published for Beckman Coulter AC TTM.

Total

100

VOL

RBC

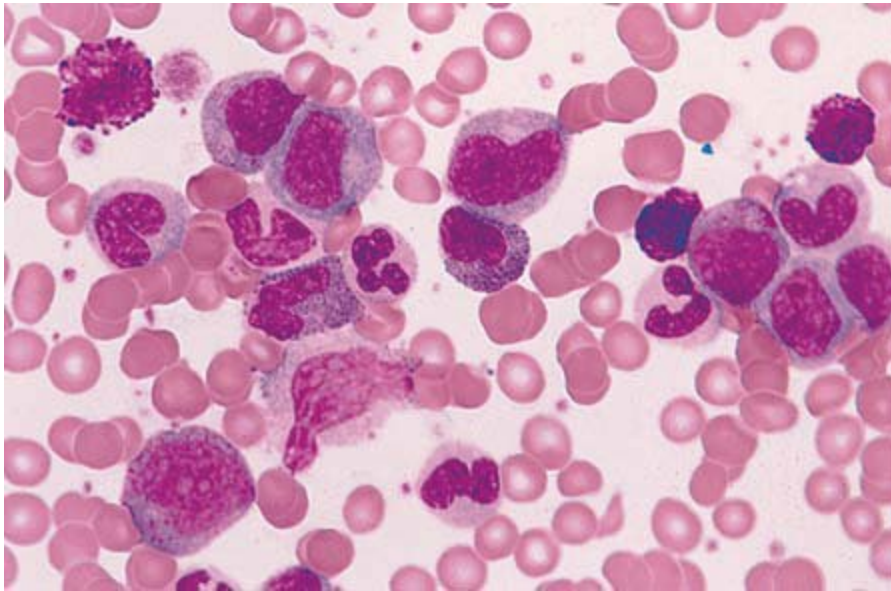
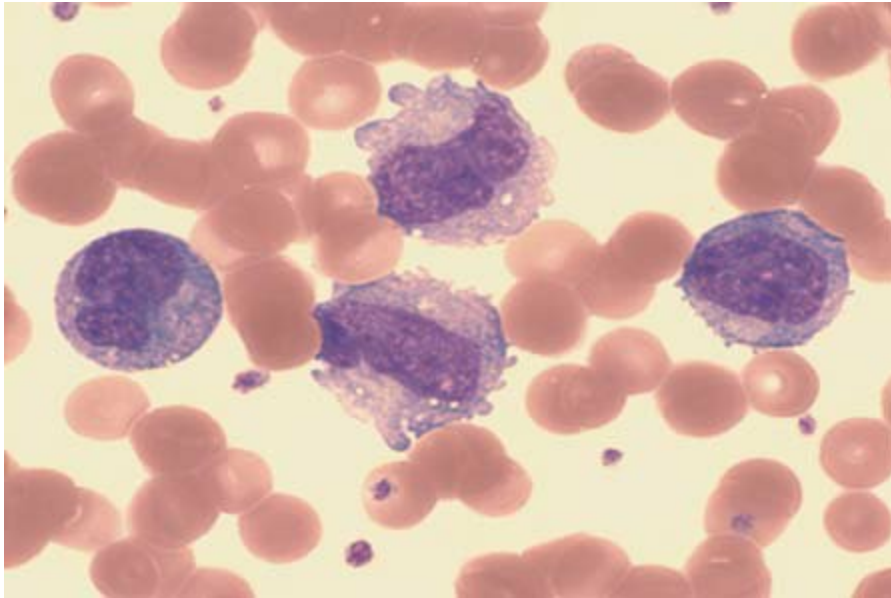
P LI

WBC/

BAS O

ABS

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

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■ Laboratory Data

VOL

Le ukocyte Diffe re ntial

%

Band neutrophils

10

Polymorphonuclear segmented neutrophils (PMNs)

80

Lymphocytes

7

Monocytes

1

Eosinophils

1

Basophils

1

ABS

Total

100

RBC

P LI

RBC morphology: 3+ microcytosis, 3+ hypochromia, 1+ anisocytosis.

■ Absolute Cell Counts

Neutrophils

103/ μ L

20.7

2.0–8.0

WBC/

Lymphocytes

10³/μL

1.6

1.0–5.0

BAS O

■ Critical Thinking Group Discussion Questions

1. Are any of the laboratory values abnormal?
2. Why are the absolute cell counts important data?
3. Should additional laboratory assays be ordered?

(Reprinted from McClatchey KD. Clinical Laboratory Medicine, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2002, with permission.)

CBC was ordered.

(Reprinted from Anderson SC. Anderson's Atlas

Measurement

Units

Reference Range*

of Hematology, Philadelphia, PA: Wolters Kluwer

Health/Lippincott Williams & Wilkins, 2003, with

RBC

$4.0 \times 10^6/\mu\text{L}$

4.00–6.20

permission.)

Hgb

11 g/dL

11.0–18.8

Hct

34%

35.0–55.0

Case Study 30.11

WBC

$9.0 \times 10^3/\mu\text{L}$

6.0–11.0

PLT

$20.0 \times 10^3/\mu\text{L}$

150.0–400.0

■ History and Physical

MCV

85 fL

80.0–100.0

A.C. is a 27-year-old white male. On New Year's day, he discovered swollen lymph nodes in his arm pits. He had been

MCH

28 pg

26.0–34.0

becoming progressively tired and weak over the last several

MCHC

33g/dL

31.0–35.0

weeks but thought that he had the flu.

RDW

15%

10.0–20.0

He went to the emergency department where a stat CBC

MPV

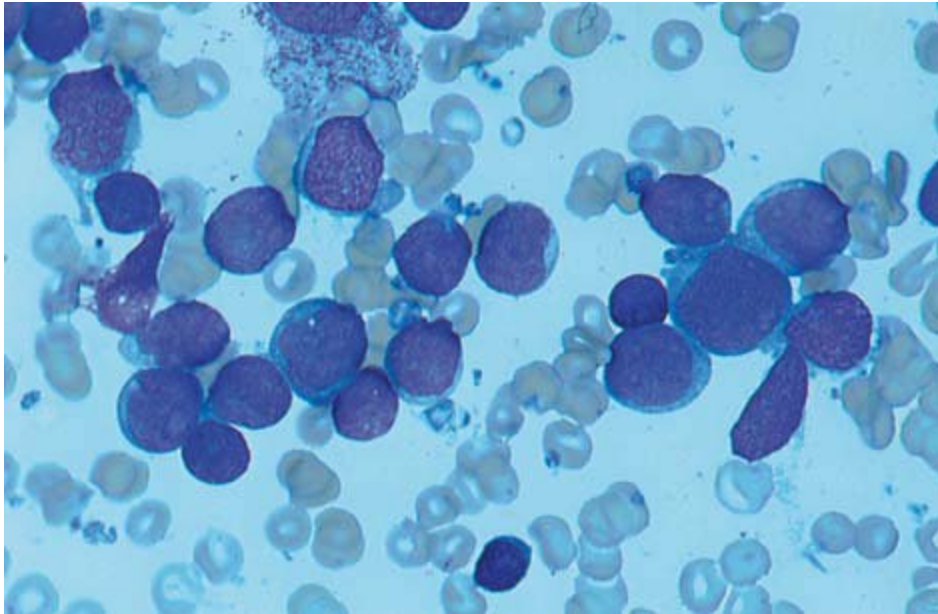
9.0 fL

6.0–10.0

was ordered.

fL, femtoliters; pg, pictogram.

*Published for Beckman Coulter ACTTM.



CHAPTER 30 ■ Instrumentation in Hematology

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CA

CA S

S E

E S

S T

T U

U D

D IIE

E S

S (c

(c o

o n

n ttiin

n u

u e

e d

d))

■ Laboratory Data

■ Absolute Cell Counts

Le ukocyte Diffe re ntial

%

Neutrophils

103/ μ L

23.4

2.0–8.0

Blasts

7

Lymphocytes

103/ μ L

4.5

1.0–5.0

Metamyelocytes

2

■ Critical Thinking Group Discussion Questions

Band neutrophils

10

1. Are any of the laboratory values abnormal?

Polymorphonuclear segmented neutrophils (PMNs)

14

2. Is the histogram abnormal?

Lymphocytes

50

Monocytes

14

3. Should additional laboratory assays be ordered?

Eosinophils

0

Basophils

3

NOTE: This is a good time to write out the answers to the

Total

100

Critical Thinking Group Discussion Questions.

VOL

RBC

P LI

WBC/

BAS O

ABS

(Reprinted from McClatchey KD. Clinical Laboratory

Medicine, 2nd ed, Philadelphia, PA: Lippincott

Williams & Wilkins, 2002, with permission.)

PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

9. Which parameters are calculated rather than directly

1. Which of the following is not a benefit of laboratory
measured?

instrumentation to the hematology laboratory?

A. Hematocrit and erythrocyte distribution width

A. Produces faster results from specimens

B. Erythrocyte count and leukocyte count

B. Reduced cost on rarely performed procedures

C. Leukocyte count and hematocrit

C. Less variation in technique from technologist to

D. Platelet count and platelet volume

technologist

D. Increased accuracy because data are collected on

10. The delta check method of quality control

more cells counted or analyzed

A. uses the patient's own data to monitor population

values

2.

Electrical impedance principle

B. uses batches of 20 samples to track MCV, MCH, and

A. The volume of each cell is proportional to the degree

MCHC values

of light scatter.

C. compares the patient's leukocyte and platelet counts

B. Each cell momentarily increases resistance.

with his or her previous results

D. monitors the patient's values within two SDs of the

3.

Optical detection principle

mean

A. The volume of each cell is proportional to the degree

of light scatter.

11. Applying the optical principle of laser scatter technology

B. Each cell momentarily increases resistance.

to cell counting and analysis, discrimination between

erythrocytes and platelets depends on the

4. The abbreviation LASER stands for

A. cellular volume

A. light-associated simulated emission of radiation

B. cellular refractive index

B. largely amplified by simulated emission of radiation

C. time of flight through the sensing zone

C. light amplified by stimulated emission of radiation

D. all of the above

D. liquid amplified by stimulated emission of radiation

12. In an erythrocyte histogram, the erythrocytes that are

5. A photon is

larger than normal will be to the

of the normal

A. a distribution of energy

distribution curve.

B. a piece of equipment in a laser assembly

A. right

C. the basic unit of all radiation

B. less

D. equivalent to an atom

C. in the middle

6. The major application of flow-cell cytometry is

13. A bimodal histogram distribution is suggestive of

A. determining cell size and granularity

A. cold agglutinin disease

B. sorting of cells and cellular identification using

B. posttransfusion of normal red cells to a person with monoclonal antibodies

abnormally sized red cells

C. treating cancer cells and identifying specific virus

C. the presence of RBC fragments

types

D. all of the above

D. counting leukocytes and platelets

14. The formula for the RDW is

7. The term parameter means

Patient RBC variation – average normal RBC variation

A. a subset of a population

A.

SD of average normal RBC variation

B. the mean value of a sample

C. two SDs on either side of the mean value

SD

D. any numerical value that describes an entire

B.

$\times 100$

Mean

population

15. The formula for the red cell measurement is

8. Data output from three-part differential counters

Patient RBC variation – average normal RBC variation

includes

A.

SD of average normal RBC variation

A. an erythrocyte histogram

B. a leukocyte histogram

SD

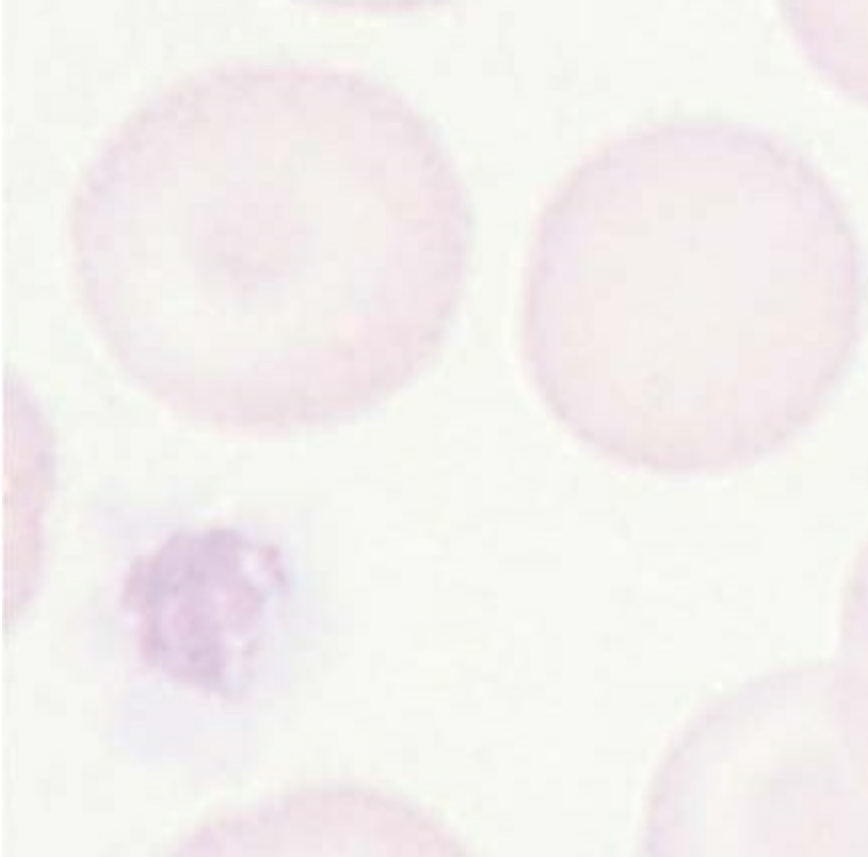
C. a platelet histogram

B.

$\times 100$

Mean

D. all o the above



CHAPTER 30 ■ Instrumentation in Hematology

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REVIEW QUESTIONS (continued)

16. The RDW and MCV are both quantitative descriptors of erythrocyte size. If both are increased, the most

24. The MPV is often decreased

probable erythrocytic abnormality would be

A. in sickle cell anemia

B. in megaloblastic anemia

A. iron deficiency anemia

C. in idiopathic thrombocytopenic purpura

B. acquired aplastic anemia

D. after splenectomy

C. megaloblastic anemia

D. hemoglobinopathy

25. A normal PDW is

A. less than 5%

*17. If the RBC distribution on a histogram demonstrates

B. less than 10%

a homogeneous pattern and a small SD, the peripheral

C. less than 15%

blood smear would probably exhibit

D. less than 20%

A. extreme anisocytosis

B. very little anisocytosis

26. Which of the following can be an application of flow—

C. a single population of spherocytes

cell cytometry?

D. a single population of macrocytes

A. Screening erythrocytes for malaria

B. Counting of reticulocytes

18. The

can be determined from a WBC histogram.

C. Quantitation of and B cells

A. percent of lymphocytes

D. All of the above

B. absolute number of lymphocytes

C. frequency distribution of granulocytes

27. Major systems in a flow cytometer include all of the

D. all of the above

following except

A. fluidics

19 and 20. The sorting of leukocyte subpopulations in the

WBC histogram determined by electrical impedance reflects

B. optics

the (19)

, which is primarily related to their (20)

.

C. computerized electronics

D. gating

19.

*28. The restriction of data analysis to one cell population

A. overall size

is accomplished by

B. relative size

A. amplification

C. nuclear size

B. gating

D. chromatin pattern

C. compensatory monitoring

20.

D. data limitation

A. cytoplasmic size

B. nuclear size

29. Which cell surface membrane marker is used for enumeration of HPC?

C. concentration of granules

enumeration of HPC enumeration?

D. cytoplasmic color

A. CD4

B. CD8

21. The mononuclear cells in a WBC histogram can include

C. CD34

A. blast cells

D. CD45

B. promyelocytes

C. monocytes

30. Reticulocytes can be detected by using

stain.

D. all of the above

A. new methylene blue

B. thiazole orange

*22. A combined scatter histogram measures

C. propidium iodide

A. overall size versus nuclear size

D. both A and B

B. cytoplasm-to-nucleus ratio

C. cell size and granularity

31. The newer clinical instruments for measuring blood

D. cell shape and cytoplasmic color

clotting are based on

A. clot elasticity

23. The MPV is

B. fibrin adhesion

A. analogous to the MCHC

C. conduction of impedance of an electrical current by

B. a direct measure of the platelet count

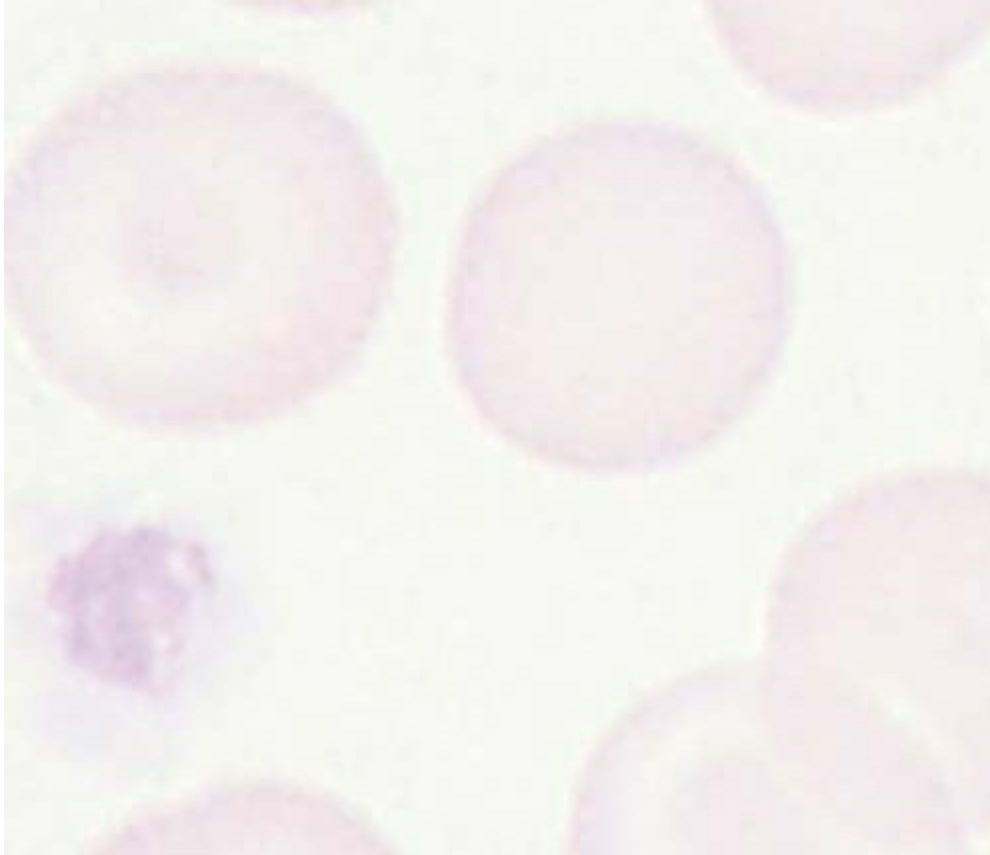
fibrin

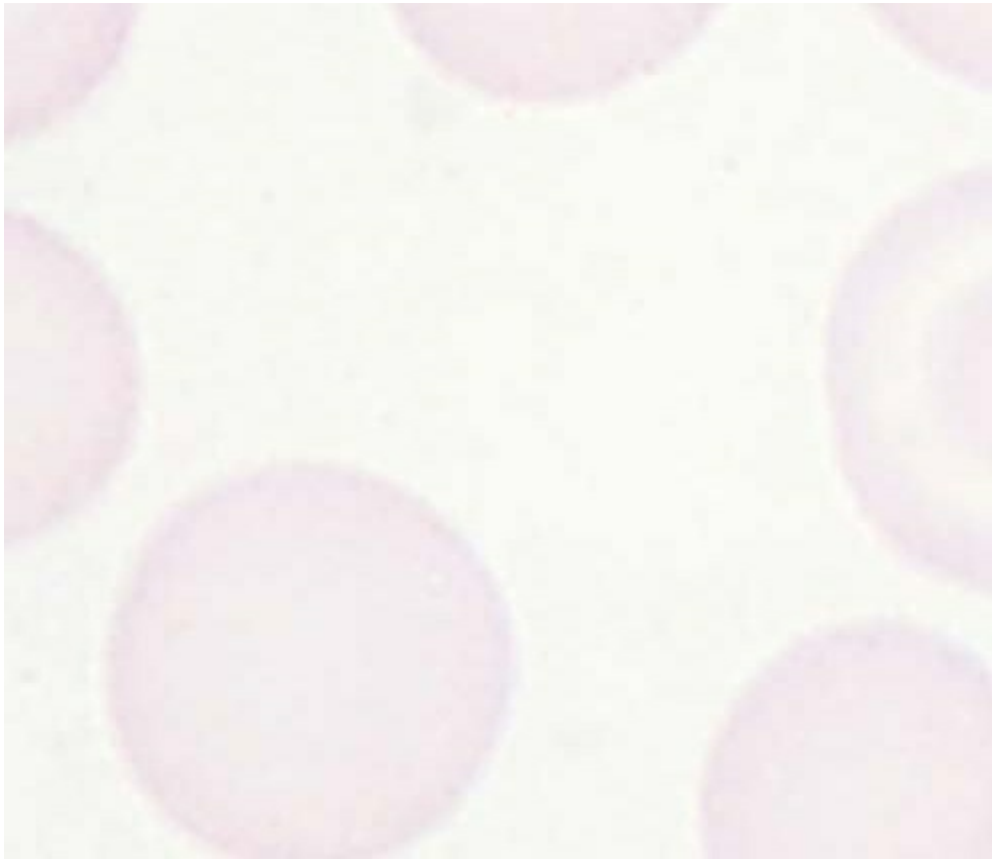
C. a measurement of the average volume of platelets

D. changes in optical density

D. a comparison o the patient's value to the normal value

(continued)





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PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS (continued)

32. The brometer relies on the principle of

35. With a particle-counting instrument, a high back—

A. clot elasticity

ground count can be due to

B. brin adhesion

A. a partial obstruction o the aperture

C. conduction or impedance o an electrical current by

B. an electrical line inter erence

brin

C. contaminated diluent

D. changes in optical density

D. bubbles in the diluent

33. In the photo-optical method, the change in light trans-

*36. A source o error when using the brometer in coagu—

mission versus the

is used to determine the

lation studies can be

activity o coagulation actors or stages.

A. improper reaction temperature

A. amount o patient's plasma

B. overincubation o the substrate reagent

B. amount of test reagent

C. overincubation of the test plasmas

C. time

D. all of the above

D. temperature

*34. In measuring platelet aggregation, platelet-rich plasma

can be treated with

to aggregate platelets.

A. saline

B. collagen

C. epinephrine

D. both B and C

Gonder J, Mell LD. Everything you wanted to know about automated

COMPANION RESOURCES

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14(21):17–20, 2002.

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Jamieson B. The continued climb of hematology automation, Adv

Each student is encouraged to access and use the Web—

Admin Lab, 12(4):56–60, 2003.

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Here, you will find additional learning tools to increase

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Molecular Diagnostic Techniques

CHAPTER

31 and Applications

KEY TERMS

clonality

genome

next-generation sequencing (NGS)

deoxyribonucleotide

hematology

nucleotide

deoxyribonucleic acid (DNA)

loop-mediated isothermal amplification

polymerase chain reaction (PCR)

diode method (Sanger method)

tion (LAMP)

primers

exome

minimal residual disease

LEARNING OUTCOMES

- Discuss the goal and findings of the Human Genome Project.

Compare DNA sequencing by the Sanger and related analysis by melt-

- Explain an overview of molecular techniques in hematology.

ing curve analysis, pyrosequencing, and capillary electrophoresis.

- List and give examples of the benefits and applications of molecular

- Discuss the Southern blot technique including clinical applications.
techniques in hematopathology.

- Describe the principle, advantages, and disadvantages of FISH.

Summarize the importance and examples of gene rearrangement

- Describe the advantages and outline the generalized steps in next—
studies.

generation sequencing (NGS).

Discuss the importance of molecular techniques to detection of

Explain how microarrays are applied to immunologic testing.

minimal residual disease.

- Correctly answer end of chapter review questions.

- Describe characteristics of single nucleotide polymorphisms (SNPs).

- Describe the principle of the polymerase chain reaction (PCR) ampli—

NOTE:

cation technique, including strengths and weaknesses.

- indicates MLT and MLS core content

- Compare various PCR adaptations.

indicates MLT (optional) and MLS advanced content

- Identify and briefly describe other amplification techniques.

THE HUMAN GENOME PROJECT

microscopic recognition of genome regions smaller than 2 to

3 million bp, chromosome stretches sufficient to accommo—

The goal of the Human Genome Project was to sequence the

date about 50 to 100 genes. In contrast, gene probing proce—

exact order of the base pairs in a segment of deoxyribonucleic

acids are capable of discerning differences as small as 10 to

acid (DNA) in order to establish our genetic database. Genetic

50 bp in fragments of individual cloned genes. The strength of

variations associated with specific disease or increased risk

cytogenetics is not in characterizing gene structure but in its

of specific diseases are the target of genome investigations.

utility in locating major rearrangements, which can then be

The International Human Genome Sequencing Consortium

characterized at the gene level by methods of DNA analysis.

published the first draft of the human genome in the jour—

It is estimated that about 19,500 genes are present in

nal, Nature, in February 2001. Today, the study of the human genome is as complete as it can be. Small gaps that are uncovered by scientists working on the Human Genome Project in 2003 were not coverable with any current sequencing method remain, first-generation DNA sequencing, Sanger sequencing. Today, second-generation sequencing or next-generation sequencing amounts to about 1% of the gene-containing portion of the genome, or euchromatin. The Human Genome Project (NGS) analyzes millions of fragments of DNA in sequenced regions of the genome, or euchromatin. The goal was to sequence only nuclear euchromatin regions of the genome, which makes up about 90% of the genome. The commonly used technology to confirm nucleotide changes in other regions of the cell, called heterochromatin found in centromeres and telomeres, were not sequenced under the project. Sanger sequencing developed in 1975 is still the “gold standard” in sequencing because the error rate is still fairly high.

The creation of NGS platforms

Gene sizes range from a few thousand to several hundred thousand base pairs. This resolution limit precludes expanded the clinical use of nucleic acid sequencing.

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PART 8 ■ Fundamentals of Hematological Analysis

Hematopathology

the diagnosis of B-cell malignancies. The Ig and CR gene rearrangements during normal B and T-lymphocyte development, respectively, generate unique junctions of variable, joining include

diversity, and joining (VDJ) segments, interspersed by random

- Faster turnaround time

dom nucleotide (N) insertion and/or deletion. These B and

- Smaller required sample volumes

-clonal recombinations generate patient-specific DNA

- Increased specificity and sensitivity

length and sequences, which represent ideal molecular

markers or detection and quantification of leukemic cells. CML was the first human malignancy to be consistently associated with a chromosome abnormality, the Philadelphia (Ph) chromosome. Although it is sensitive, the technology is susceptible to false negatives. Today, molecular methods due to clonal evolution during natural history of the disease; are used to identify changes ranging from a single chromosome—thus, some patients may relapse with a clone different to that of the original disorder to alterations involving the interchange of chromosomes observed at presentation.

Abnormalities of erythrocytes (sickle cell disease, and α - and β -thalassemias), leukocytes

Minimal Residual Disease

(acute myelogenous leukemia [AML], acute lymphoblastic leukemia [ALL], chronic myelogenous leukemia [CML], Molecular techniques, for example, PCR, real-time quantitative PCR (RQ-PCR), flow cytometry, and cytogenetic and lymphoma), and coagulation factors (hemophilia A, hemophilia B, and factor V Leiden defect) can be detected

marker studies, are more sensitive to a low number of cells by molecular methods.

than morphologic appearance in the peripheral blood. PCR

The study of hematological malignancies, hematopathology—is able to detect one malignant cell in a population of 1 million cells, was the first form of human cancer to be studied in

depth at the molecular level. Investigation of the Philadelphia

Molecular techniques permit early detection of leukemia

chromosome at the molecular level revealed a translocation—

relapse at subclinical levels, allow for early clinical interven—

induced gene rearrangement involving the Bcr and Abl

tion, perhaps before early progenitor cells, including CD34+

genes that results in activation of the Abl cellular oncogene.

cells, acquire genetic lesions that increase the aggressiveness

Fluorescence In Situ Hybridization (FISH) analysis is common to the clone.

commonly performed in the search for translocation (9;22)

In the past, molecular detection and monitoring of

(BCR/ABL), which is diagnostic of CML.

patients with chronic myeloid leukemia patients have been

Cytogenetic, FISH, and other molecular genetic tech—
successful.

techniques can aid in establishing a diagnosis of a malignancy,

Now, the current state of the art and development of

for example, ALL, detecting blast transformation emerging

molecular techniques in other leukemias, for example, child—

from CML, or determining a patient's prognosis. In addition,

childhood ALL, is of growing interest. tumor load, type of leu—

molecular techniques provide a diagnostic tool for clinicians

leukemia, whether disease specific marker is identifiable, and

in order to

technological limits will determine the optimum methodology for monitoring MRD.

- Detect minimal residual disease (MRD) in hematological malignancies

- Purge malignant cells (e.g., bcr-positive cells) from autolo-

MOLECULAR TECHNIQUES

gous bone marrow before infusion

IN HEMATOLOGY

- Monitor patients following bone marrow transplantation

- Discover an early relapse in patients treated for a hemato—

Since the inception of research on the Human Genome

logical malignancy

Project, molecular biology has a high profile in the field of

medicine. Molecular techniques have enabled more accurate

Gene Rearrangement Studies

classification of various disorders and advanced progress on

treatment of these disorders.

Gene rearrangement studies are important in diagnostic

Characteristics of nucleic acids are discussed in Chapter 3.

hematopathology as indicators of clonality and as aids in

Molecular genetic testing focuses on examination of nucleic

determining the cellular lineage of a particular malignant

acids (DNA or RNA) by special techniques to determine if

proliferation. Immunophenotyping categorizations are aided

if a specific nucleotide base sequence is present. Applications

by the use of cluster designation (CD) or specific lineages of

of nucleic acid testing have expanded, despite higher costs

cells. CDs indicate a known cluster of monoclonal antibodies

associated with testing, in various areas of the clinical laboratory—

binding to a known antigen on the cell surface of hematopoietic

tory, including hematology (hematopathology). The distinct
etic cells.

advantages of molecular testing include greater accuracy in

Molecular diagnostic assays to detect heavy chain or

diagnosis, faster turnaround time, smaller required sample

kappa chain rearrangements are useful for establishing the

volumes, and increased specificity and sensitivity in the

diagnosis of B-cell neoplasms. T-cell receptor (TCR) beta,

detection of minimal residual disease after treatment of

gamma, and delta rearrangements are useful in establishing

cancer.

CHAPTER 31 ■ Molecular Diagnostic Techniques and Applications

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MOLECULAR GENETICS IN HEMATOLOGY

In laboratories in which PCR is performed frequently, any

false-positives are generally caused by amplicon contamination—

techniques in molecular genetics are beginning to be used

cautiously. A broken capillary tube or a PCR plate left carelessly at

extensively in hematology. A wide range of abnormalities

the edge of a table can aerosolize those amplicons, which can

can be detected with these techniques. PCR is an in vitro

then adhere to lab coats and objects in the room.

method that amplifies low levels of specific DNA sequences

A simple and effective way to combat amplicon contamination—

in a sample to higher quantities suitable for further analysis.

contamination is to wipe down everything—equipment, worksta—

PCR analysis can lead to the detection of gene mutations that

contaminations, and pipettes—with bleach. Generously spray with 10%

signifying the early development of cancer.

bleach and then let it sit for 15 to 30 minutes.

Microarrays (DNA chips) are basically the product of

Polymersase Chain Reaction

bonding or direct synthesis of numerous specific DNA probes

on a stationary, often silicon-based, support. Molecular biology—

PCR is an in vitro method that amplifies low levels of specific

biology provides new ways to establish a diagnosis, determine

DNA sequences in a sample to higher quantities suitable for

patient prognosis, and monitor.

further analysis. The three important applications of PCR are

1. Amplification of DNA

Single Nucleotide Polymorphisms

2. Identification of a target sequence

Single nucleotide polymorphisms (SNPs) comprise the most

3. Synthesis of a labeled antisense probe

abundant source of genetic variation in the human genome.

PCR is unrivaled as a means of direct cloning and gene

Since the decoding of the human genome and the result—

sequence analysis. The first diagnostic application of PCR tech—

ing greater than 3 million SNPs, laboratory techniques have

nology was in prenatal diagnosis of sickle cell anemia through

been able to associate disease states and pharmacological

amplification of beta globin sequences. PCR has become

responses with individual SNPs. SNPs have various charac—

increasingly popular for detecting chromosomal breakpoints,

teristics (Box 31.1).

fusion genes, and MRD after chemotherapy for leukemia and

lymphoma. However, PCR does have limitations (Box 31.2).

Polymerase Chain Reaction

To use this technology, the target sequence to be amplified

Amplicon and Amplicon Control Measures

must be known. typically, a target sequence ranges from

An amplicon is a piece of genetic material, such as DNA, that can be formed as the product of a natural event or artificial amplification technique, such as a polymerase chain reaction

BOX 31.2

(PCR). A molecular diagnostic laboratory that performs in vitro amplification reactions needs to practice techniques to

Limitations and Potential Problems with PCR

control contamination. This is especially true if a high number of thermal cycles is used for the PCR.

Weaknesses of PCR technique include the following:

PCR is highly sensitive, but a disadvantage to the use of this assay is that it is prone to producing false-positive results.

- Contamination

- Large deletions in sequence result in no place for a primer to bind

- Must know that PCR can amplify across from a large

BOX 31.1

deletion from an adjacent site

- Should not use PCR for mutation search unless it is

Single Nucleotide Polymorphisms

known that the mutation does not involve large deletions

Potential problems with PCR include the following:

- Are not completely synonymous with point mutation
 - Often cause a premature stop codon or a missense
 - Assumes 100% efficiency of replication with each cycle
- codon

- Amplification in the second phase may not be truly

- Are the most common clinically significant DNA
- exponential

polymorphism

- A variety of variables cannot be fully controlled
- Can be studied by allele-specific PCR, melt curve analysis, and microarrays
- The number of cycles is difficult to determine because

of the rapidity of exponential amplification

- Occur at specific regions in the genome
 - Very low amounts of starting material may fail to
 - Can vary between populations or ethnic groups
- amplify

From Heriot K. Welcome to the beginning: molecular pathology or the

From Heriot K. Welcome to the beginning: molecular pathology or
community hospital pathologist and medical technologist, ASCP Annual
the community hospital pathologist and medical technologist, ASCP
Meeting, ampa, FL, 2014.

Annual Meeting, ampa, FL, 2014.

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PART 8 ■ Fundamentals of Hematological Analysis

100 to 1,000 base pairs in length. wo short DNA “prim—

Region of DNA to be a mplifie d

ers” that are typically 16 to 20 base pairs in length are used.

S tra nd 1 3'

Namely, the oligonucleotides (small portions o a single

S tra nd 2 5'

DNA strand) act as a template or the new DNA. T ese

Hea t to s e pa ra te

s tra nds

primer sequences are complementary to the 3' ends o the

Cycle 1

Cool a nd a dd

sequence to be ampli ed. T is enzymatic process is carried

primers

out in cycles (Figs. 31.1 and 31.2).

Strand 1 3'

Each cycle theoretically doubles the amount of specific

DNA sequence present and results in an exponential accumulation

of the target sequence

of the DNA fragment being amplified (amplicons)

DNA polymerase

(Table 31.1). In general, this process is repeated approximately

Strand 1 3'

30 times. At the end of 30 cycles, the reaction mixture

5'

should contain approximately 230 molecules of the desired

5'

Strand 2 5'

product. After cycling is completed, the amplification products

are then cooled

and can be examined in various ways. Typically, the contents

Cycle 2

(with primers and

o the reaction vessel are subjected to gel electrophoresis.

DNA polymerase

present)

This allows visualization of the amplified gene segments

Strand 1 3'

Target DNA

5'

5'

Strand 2 5'

Heat

5'

Strand 1 3'

5'

Primers

5'

Strand 2 5'

5'

Repeat the a ting

Cycle 3

Extension of new DNA using Taq polymerase

and cooling cycle

Strand 1 3'

Repeat process multiple times

Final amplified products

Strand 2 5'

Cycles 4

Multiple heating

to 20

and cooling cycles

Detection probe

Amplified DNA present in about 10

FIGURE 31.1 The PCR is depicted. The target DNA is first melted

6 copies

using heat (generally around 94°C) to separate the strands of DNA.

FIGURE 31.2 Polymerase chain reaction (PCR). (Reprinted from

Primers that recognize specific sequences within the target DNA are

Wilcox BR. High-Yield Biochemistry, Baltimore, MD: Lippincott

allowed to bind as the reaction cools. Using a unique, thermostable

Williams & Wilkins, 1999, with permission.)

DNA polymerase called Taq and an abundance of deoxynucleoside

triphosphates, new DNA strands are amplified from the point of the (e.g., PCR products, bands) and a determination of their primer attachment. The process is repeated many times (called cycles) specificity. Additional product analysis by probe hybridization—until millions of copies of DNA are produced, all of which have the tion or direct DNA sequencing is often performed to further same length defined by the distance (in base pairs) between the primer verify the authenticity of the amplicon.

binding sites. These copies are then detected by electrophoresis and staining or through the use of labeled DNA probes that, similar to

Nested Primers

the primers, recognize a specific sequence located within the amplified

Adaptations of the PCR technique have been developed. One

ed section of DNA. (Reprinted from Porlts CM. Pathophysiology:

Concepts of Altered Health States, 7th ed, Philadelphia, PA: Lippincott

adaptation uses nested primers. This adaptation uses a two—

Williams & Wilkins, 2005, with permission.)

step amplification process. In the first step, a broad region

CHAPTER 31 ■ Molecular Diagnostic Techniques and Applications

which anneals between forward and reverse primers.

TABLE

PCR Amplification*

31.1

Hydrolysis is one of many methods now available for detection and quantification of target sequences.

Number of Cycles

Number of DNA

A sensitivity of 1×10^{-5} is achievable by Q-PCR, but contamination is a major concern, and hence, strict working

1

2

practices must be adhered to, for example, RNA extraction,

2

4

cDNA synthesis, and post PCR analysis must be geographically separated. Equally, false negatives due to a lack of

3

8

mRNA or suboptimum integrity of mRNA and/or cDNA

4

16

must be controlled or. This is achieved by concomitantly

5

32

measuring one of the ubiquitously expressed housekeeping

10

1,024

genes, for example, ABL1, BCR.

20

>1,000,000

Consensus Primer PCR and Allele-Specific

*The number of copies of a specific DNA sequence doubles with each

Oligonucleotide PCR

amplification. PCR usually consists of a series of 20 to 40 repeated

temperature changes called cycles; each cycle typically consists of two

Consensus primer PCR and allele-specific oligonucleotide

to three discrete temperature steps. Most commonly, PCR is carried out

PCR (ASO-PCR) are the two Ig PCR strategies or MRD

with cycles that have three temperature steps. The temperatures used

studies. ASO-PCR utilizes primers designed to anneal to a

and the length of time they are applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the unique patient-specific Ig sequence and subsequently is used to monitor sequential samples in follow-up studies. The concentration of divalent ions and dNTPs in the reaction, and the melting temperature (T_m) of the primers. PCR may reach a plateau with no more qualitative method has a sensitivity of 1×10^{-2} to 1×10^{-4} .

copies produced because of reagent limitations, inhibitors, *etc.*

ASO-PCR significantly improves the sensitivity of MRD studies, but ASO-PCR is time consuming and expensive.

Combination of ASO primers and consensus oligonucleotide DNA surrounding the sequence of interest is amplified—

oligonucleotide probes make it accessible to Q-PCR, permitting pre—

ed. This is followed by a second round of amplification to

precise quantification of MRD with a sensitivity of 1×10^{-4} to

amplify the specific gene sequence to be studied. Another

1×10^{-5} .

A recent modification of the PCR technique has been used successfully to differentiate alleles of the same gene.

Other Amplification Methods

Real-Time PCR

PCR is not the only method of amplification. Transcription—mediated amplification of ribosomal RNA and strand displacement

Another method based on PCR is real-time PCR (RT-PCR).

placement that uses primers to bind on the same side as the

However, this method is not used as much now as in the

DNA target can be used.

past. RT-PCR detects RNA from viable cells and thus is a method that is making its way from the research laboratory

gets genes expressed that are likely to have a functional role,

likely to becoming a mainstream technique is loop-mediated

directly or indirectly, in cellular proliferation. The amplification

isothermal amplification (LAMP). This technique differs from

amplification of this technique is in the quantitation of specific

traditional PCR because it starts with two primers, not the

DNA sequences of interest and for identification of point

mutations used by PCR. Two of the two primers each have a second

mutations.

tion, which is complementary to one end of a target DNA

This PCR variation uses fluorescence resonance energy

transfer (FRET) sequence but each also has an intentionally “mirrored” second

transfer (FRET). This PCR variation is particularly appealing

tion of the target. This allows each primer to have the capability because the procedure is less susceptible to amplicon contamination or forming a hairpin structure. The hairpin primers delineate the region to be detected through its amplification, copy number.

if it is present in the specimen. The two other primers act as primer initiating points which cause flipping of the intact Quantitative PCR (Q-PCR)

double-stranded ends to hairpins needed to continue the Q-PCR assumes 100% efficiency, but there is variation in the replications.

efficiency of amplification. In very small specimens, there Selection of primers is more challenging with LAMP than may be no amplification.

traditional PCR. Currently, this method is being to be or

Quantification of specific sequences of DNA has been infectious disease detection, but further applications could greatly simplified by real-time quantitative polymerase emerge.

chain reaction (RQ-PCR or Q-PCR). In Q-PCR, the rate of accumulation of amplicons is proportional to the number of

Analysis of Amplification Products

target transcripts in the starting material during the exponential phase of the PCR. This technique also offers increased

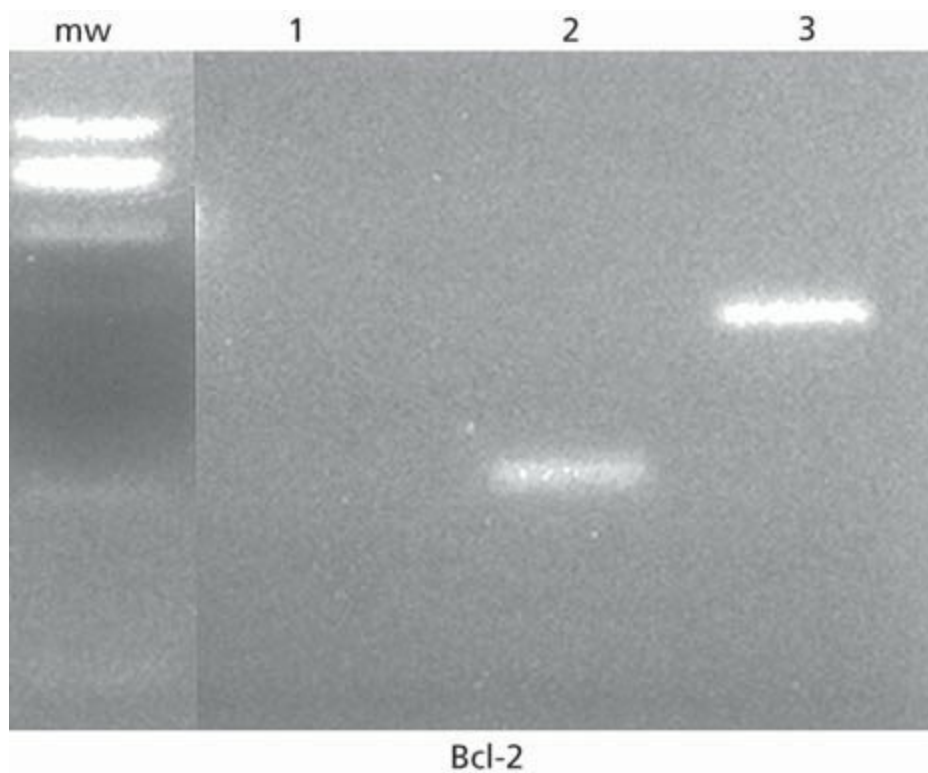
Detection of DNA products by PCR assay can be conventional—

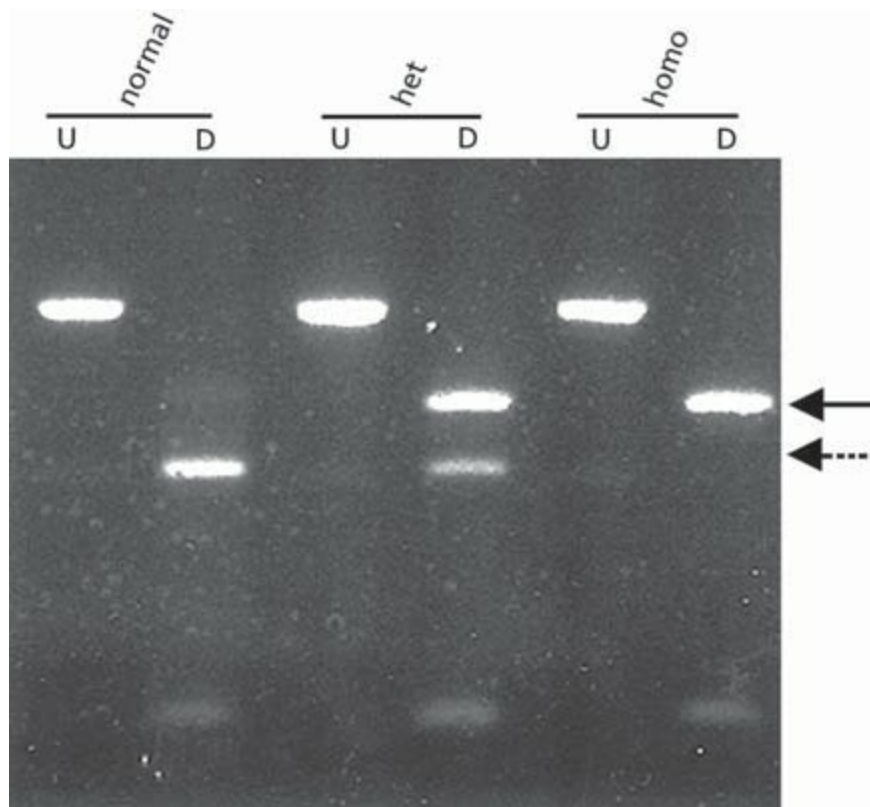
specificity with the inclusion of the third reporter labeled

ally analyzed using agarose gel electrophoresis and ethidium

oligonucleotide probe using hydrolysis-based technology,

bromide staining. This technique is simply an extra step and a





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PCR assay has been run. DNA and other biomolecules can be separated based on charge, size, and shape (see Figs. 31.3 and 31.4). DNA has a net negative charge and will migrate toward the anode (positive pole). PCR products are loaded into an agarose gel and electrophoresed. Ethidium bromide is a dye that intercalates into nucleic acids and will fluoresce with an orange color under ultraviolet (UV) irradiation. An image analyzer uses UV light to capture computer images of the PCR products. Melting curve analysis (MCA) is a method of assessing

the dissociation characteristics of double-stranded (DS)

DNA using a fluorophore during heating. When the temperature is raised, the DS begins to dissociate, which leads

to a rise in the absorbance intensity, hyperchromicity. Factor

V Leiden coagulopathies are a good example, in a particular mutation is known.

The principle of MCA is that slightly mismatched DNA

will denature at a lower temperature than perfectly matched

FIGURE 31.3 PCR. Molecular gene structure of chromosomes 14

DNA. The temperature is gradually increased, and the change

and 18. Example of bcl-2 translocation detection by PCR. Lane 1, a

in fluorescence is measured, such as Factor V Leiden.

reactive lymphoid tissue showing no evidence of bcl-2 translocation

while in lanes 2 and 3 show 2 patients with follicular lymphoma

DNA Sequencing

having bcl-2 rearrangement. mw, molecular size markers. (From

McClatchey KD. Clinical Laboratory Medicine, 2nd ed, Philadelphia,

In the past decade, DNA sequencing has enabled the system—

PA: Lippincott Williams & Wilkins, 2002.)

atic sequencing of more than 10,000 cancer exomes and 2,500

whole cancer genomes. This has revolutionized the under-

- Faster turnaround time

standing of the genetics of cancer and has led to previously

- Smaller required sample volumes

unrecognized cancer genes and new mutation signatures.

- Increased specificity and sensitivity

Mutations arise from replication errors or from DNA

damage that is either repaired incorrectly or left unrepaired.

Capillary electrophoresis (CE) (Figs. 31.5 and 31.6) is a

Leukemias, such as AML, typically have the lowest numbers of

relatively new, powerful separation technique that is ideally

mutations compared to tumors, such as lung cancer. Different

mutational processes lead to idiosyncratic patterns of mutations, termed
“mutational signatures.” These patterns allow

identification of known and novel mutations. Features that can

characterize the action of a given mutation process include the

type of mutations observed, local sequence context, distribution across the
genome, and evidence of repair. Since the

discovery of the Philadelphia chromosome, mutation reoccurrence has proven
to be a powerful tool for the identification of

new cancer genes. Estimates of the mutation rate in human

and B lymphocytes are thought to be on the order of 2×10^{-8} to 10^{-7}

mutations per diploid genome per cell division. Sequencing studies in normal blood has revealed insights into patterns of clonal expansion associated with driver mutations. In the blood, mutations reflect about the relationship between aging, particularly individuals older than 65 years of age, and the typical epidemiological pattern seen in leukemias.

Many of the revolutionary changes that have occurred in research in the biological sciences, particularly the Human

FIGURE 31.4 PCR detection of factor V Leiden. Exon 10 of Factor

Genome Project, can be directly attributed to the ability to

V gene is PCR amplified and cut with (D) restriction enzyme MnlI.

manipulate DNA in defined ways. Molecular genetic testing

DNA from normal (N) individuals contains MnlI, recognition site

focuses on the examination of nucleic acids (DNA or RNA)

(GAGG), which is lost in individuals carrying mutation. Undigested

by special techniques to determine whether a specific nucle—

DNA in normal (N) individuals shows 267 bp (upper bands) DNA

otide base sequence is present.

fragment. After restriction enzyme treatment, normal individuals show

163 bp fragment (dashed arrow). In homozygous (Hom) patients,

The applications of nucleic acid testing have expanded, digestion shows 200 bp fragment (solid arrow). In heterozygous (Het) despite higher costs associated with testing, in various areas patients, both 163 bp and 200 bp fragments are seen. Internal control of the clinical laboratory. These include genetic testing or of 67 bp in digested samples is seen in the bottom. U 5, undigested; diagnosis and monitoring. Molecular testing has the following— D 5, digested. (From McClatchey KD. Clinical Laboratory Medicine, ing advantages: 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2002.)

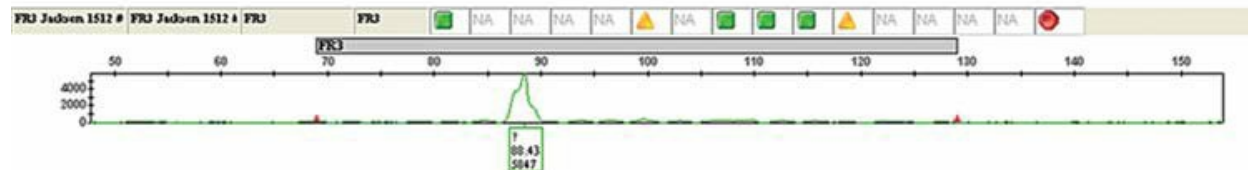
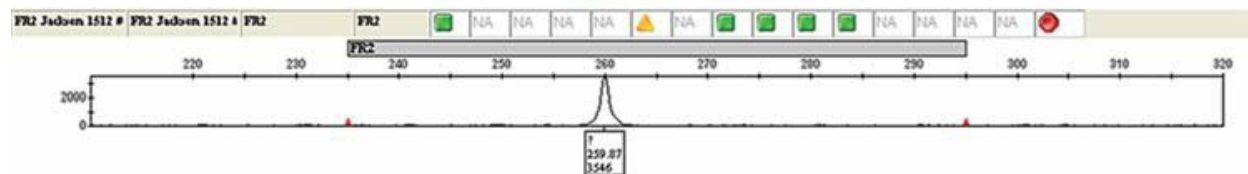
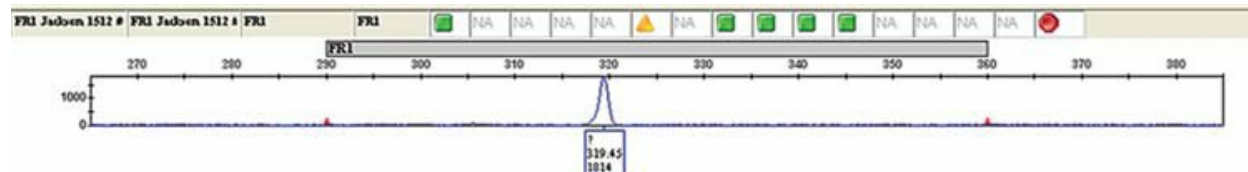
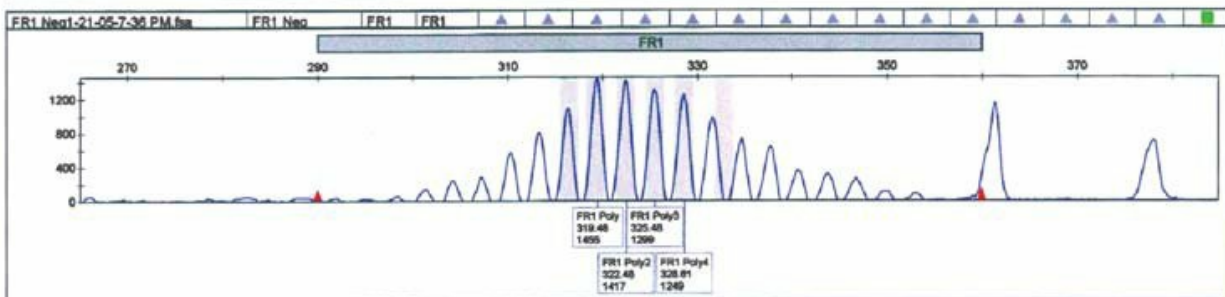
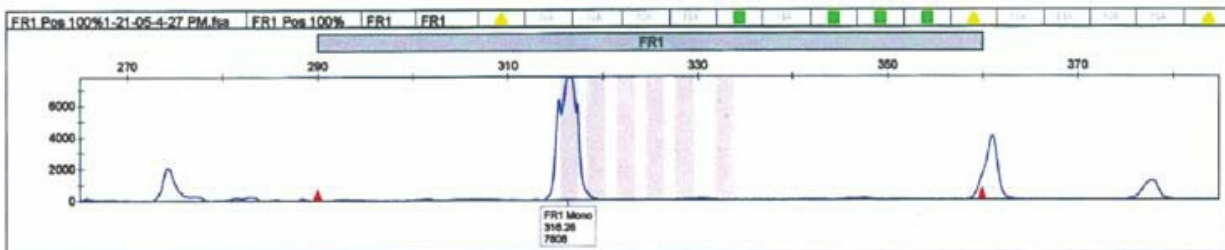
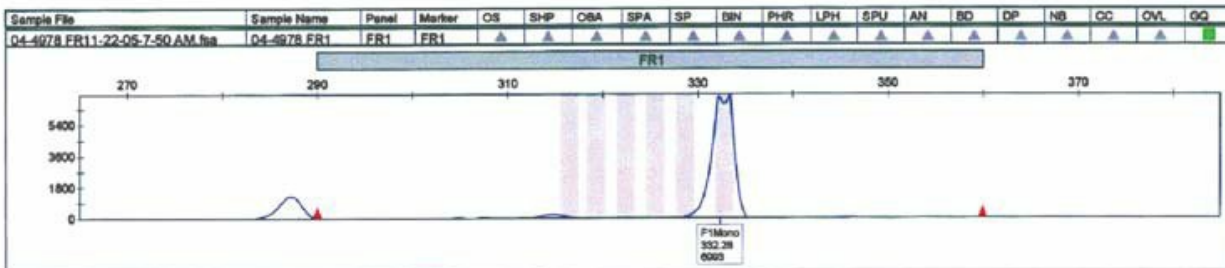


FIGURE 31.5 PCR with capillary electrophoresis technique using three pairs

of primers or the detection of immunoglobulin heavy-chain gene rearrangements. Frameworks I and II show

a monoclonal peak, representing gene

rearrangement. Framework III shows a

polyclonal pattern. (From Sun . Flow

Cytometry, Immunohistochemistry, and

Molecular Genetics or Hematologic

Neoplasms, 2nd ed, Philadelphia, PA:

Lippincott Williams & Wilkins, 2012.)

suited for handling small amounts of DNA. The first pub—

method is considered to be the “gold standard” method to

published papers on DNA analysis by CE only appeared in 1988.

which other molecular methods are compared. However,

Various CE methods and their applications to nucleic acid

the Sanger method does have limitations (Box 31.3). DNA

analysis, specifically those dealing with nucleosides, nucleo—

sequencing displays the exact nucleotide or base sequence of

nucleosides, oligonucleotides, and dsDNA (PCR) fragments, have

a fragment of DNA that is targeted.

been developed.

The Sanger method uses a series of enzymatic reactions

In contrast to conventional gel electrophoresis, CE takes

to produce segments of DNA complementary to the DNA

advantage of two types of driving forces: (1) the force causing

being sequenced. This method is also called the chain-terminating

the electrophoretic migration and (2) the force exerted by

termination method because of the synthetic nucleotides that

electroosmotic flow (EOF) through the capillary. CE offers

lack the $-OH$ at 3' carbon atom are added to the growing

several similarities to high-performance liquid chromatography—

DNA strand, there is no 3'-OH on the next nucleotide to be

raphy (HPLC), that is, ease of use, high resolution, speed,

attached to, and the DNA chain stops elongating.

on-line detection, and full automation capability. CE, however—

Automated sequencing techniques use primers with fluorescent

ing taken essential components from both HPLC and electrophoresis—

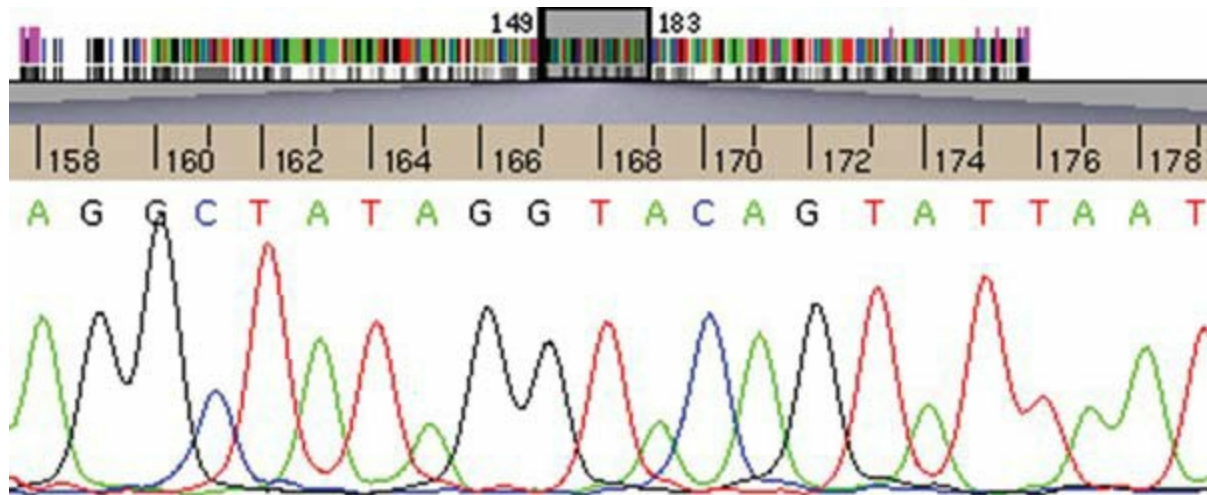
different fluorescent labels.

trophoresis, can be viewed as an instrumental approach to

electrophoresis.

1. The first step in sequencing a target is usually to amplify it. DNA sequencing (Figs. 31.7 and 31.8) is the determination of the precise sequence of nucleotides in a sample of amplified DNA isolated from the clinical specimen (the target DNA). The most popular method for doing this is called the **dideoxy method** or Sanger method. This DNA sequencing method involves the separation of double-stranded DNA (dsDNA) into single strands (ssDNA).

FIGURE 31.6 Splenic B-cell marginal zone lymphoma. Polymerase chain reaction with capillary electrophoresis technique reveals a monoclonal peak in each of the three frameworks, representing immunoglobulin heavy-chain gene rearrangement. (From Sun . Atlas of Hematologic Neoplasms, New York, NY: Springer, 2009.)



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There are two different pyrosequencing strategies that

BOX 31.3

are currently available: solid-phase pyrosequencing and liquid-phase pyrosequencing. Solid-phase pyrosequencing utilizes immobilized DNA in a three-enzyme system. In this

Limitations of DNA Sequencing

system, a washing step is performed to remove the excess substrate after each nucleotide addition. Using this method,

- Large amounts of normal tissue required

the DNA to be sequenced is broken up into fragments of

- Sensitivity—tumor mixed with normal cells

~100 base pairs and denatured to form single-stranded DNA

■ Heterozygosity—most mutation, halo DNA is (ssDNA). Then single ssDNA amplicons are immobilized normal onto microscopic beads and placed into separate wells. In liquid— From Heriot K. Welcome to the beginning: molecular pathology or the mid-phase pyrosequencing, a nucleotide-degrading enzyme community hospital pathologist and medical technologist, ASCP Annual is introduced to make a four-enzyme system. Addition of Meeting, Tampa, FL, 2014.

this enzyme has eliminated the need for solid support and intermediate washing and enables the pyrosequencing reaction to be performed in a single tube.

2. The second step involves adding primers to the ssDNA.

Primers are short synthetic segments of ssDNA that con-

Southern Blot Technique

tain a nucleotide sequence complementary to a short

The Southern and Northern blot techniques are historic

strands of target DNA. The patient's DNA serves as a

techniques used to detect DNA and RNA, respectively. The

template to copy. DNA polymerase catalyzes the addition

Southern blot procedure (Figs. 31.9 and 31.10) can be used

o the appropriate nucleotides to the preexisting primer.

in clinical laboratories, but the Northern blot technique is
DNA synthesis is terminated when the deoxynucleotide is
used in research setting.

incorporated into a growing DNA chain.

Specimen DNA is denatured and treated with restriction enzymes to create
DNA fragments; then, the ssDNA

Pyrosequencing

fragments are separated by electrophoresis (Fig. 14.7). The

In attempt to find a faster and less expensive way of molecu—

electrophoretically separated fragments are then blotted to

lar sequencing, pyrosequencing has emerged as a method.

a nitrocellulose membrane, retaining their electrophoretic

Pyrosequencing is a DNA sequencing technique that is based

position and hybridized with radiolabeled single-stranded

on the detection of released pyrophosphate (PPi) during

DNA fragments with sequences complementary to those

DNA synthesis. In a cascade of enzymatic reactions, visible

being sought. The resulting dsDNA bearing the radiolabel, i

light is generated that is proportional to the number of incor—

present, is then detected by radiography.

porated nucleotides.

The Southern blot procedure has clinical diagnostic appli—

The cascade starts with a nucleic acid polymerization

cations or disorders associated with significant changes in

reaction in which inorganic PPi is released as a result of

DNA, a deletion or insertion of at least 50 to 100 bp (e.g.,

nucleotide incorporation by polymerase. The released PPi is

fragile X syndrome), and determination of clonality in

subsequently converted to AMP by AMP sulurylase, which

lymphomas of or B cell origin. In a single-base mutation

provides the energy to luciferase to oxidize luciferin and

changes an enzyme restriction site on the DNA, resulting in

generate light. Because the added nucleotide is known, the

an altered band or fragment size, the Southern blot proce—

sequence of the template can be determined.

cedure can detect these changes in DNA sequences.

FIGURE 31.7 Sequence from HIV. A DNA sequence from the HIV following R-PCR is demonstrated here and was obtained by using sequencing by

termination (i.e., traditional Sanger sequencing). The most commonly used

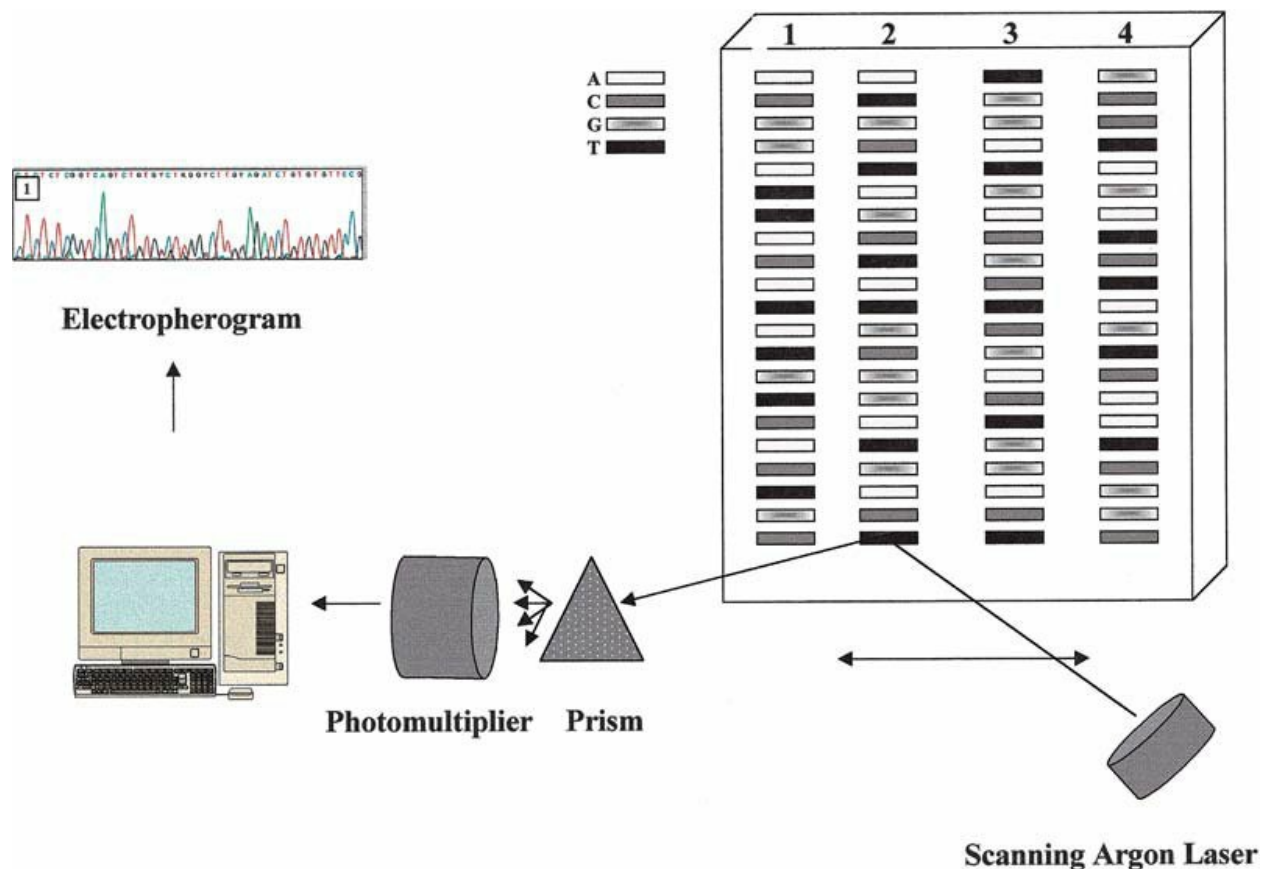
methods of sequencing by termination utilize capillary electrophoresis rather

than gel-based methods, 2016. (From Procop GW, Koneman EW.

Koneman's

Color Atlas and textbook of Diagnostic Microbiology, 7th ed, Philadelphia, PA:

Lippincott Williams & Wilkins, 2016.).



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FIGURE 31.8 DNA sequencing. Automatic DNA sequencing. Single-lane automatic DNA sequence analysis. The 377 ABI Prism automatic DNA sequencer uses fluorescently labeled primers or fluorescently labeled dideoxynucleotides to tag the newly synthesized DNA fragments. The four reactions are combined into a single tube and are separated by electrophoresis

in a single lane. When the tagged DNA reaches the area where the laser is located, it emits a light that passes through a prism.

The prism divides the light emitted from the dyes and sends it to a photomultiplier and subsequently to a computer where the sequence is determined. The order of the colors as they pass through the laser reflects the DNA sequence. (From McClatchey KD. Clinical Laboratory Medicine, 2nd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2002.) Fluorescent In Situ Hybridization (FISH)

and IgG immunoassay has been developed mostly as a research

FISH is a tissue-based molecular diagnostic assay (Fig. 31.9).

tool. This immunoassay uses highly active gold nanoparticles

The rapid expansion in the availability of polyclonal and

as the label and can be confirmed by clinical testing. The

monoclonal antibodies has fostered a dramatic increase in

method has many desirable features, including rapid detec—

light microscopic immunohistochemistry (IHC) and in situ

tion, selectivity, and minimal instrumentation. The protocol

hybridization.

has potentially broad applications for clinical immunoassays

FISH analysis is used in the diagnosis of hematological

and DNA hybridization analysis (Figs. 31.11).

malignancies including CML, AML, Burkitt lymphoma,

and other lymphomas (e.g., follicular lymphoma, mantle

Next-Generation Sequencing

cell lymphoma, MAL lymphoma, and anaplastic large cell lymphoma). FISH analysis (Box 31.4) is generally better or Although sequencing of the entire human genome is possible, detection of deletions and inversions than PCR.

physicians are typically interested in only the protein-coding

FISH is locus specific for a particular sequence of DNA.

regions of the genome, the exome. The exome comprises

The basic principles of FISH analysis are as follows:

slightly more than 1% of the genome. Exome sequencing

is helpful in the identification of disease-causing mutation,

- A probe is a specifically designed sequence of nucleic acid—when the genetic cause is unknown.

usually DNA that is labeled with a fluorescent compound.

Molecular characterization of tumors typically include

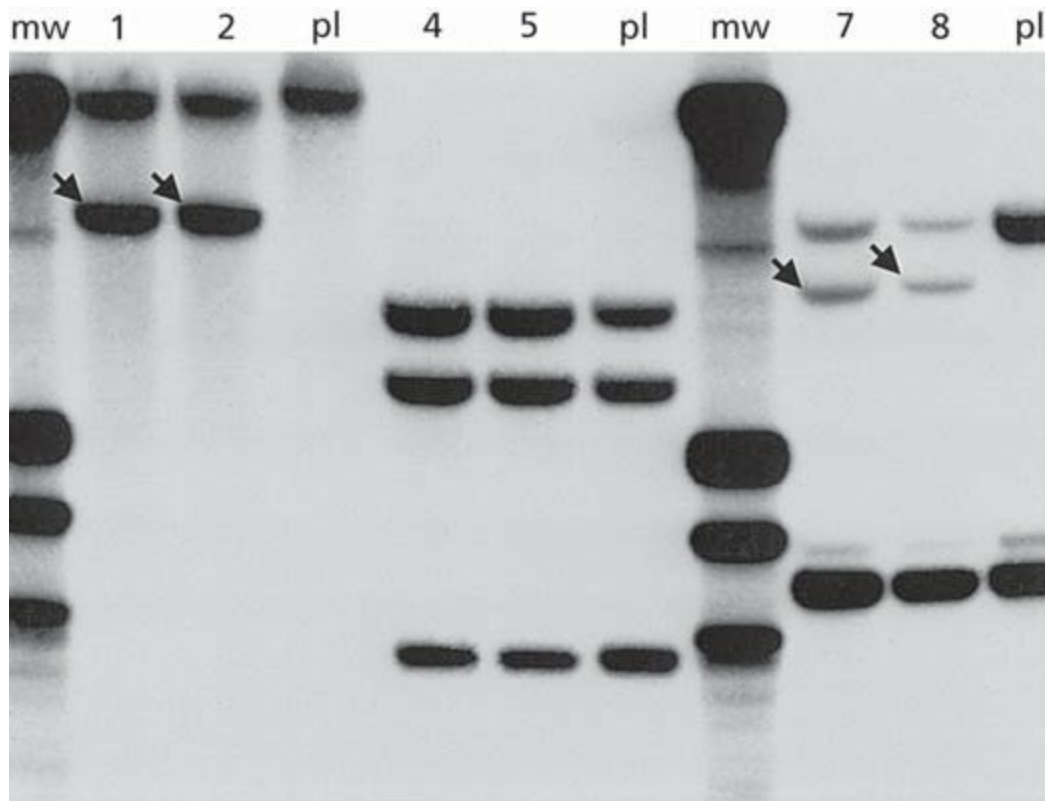
The target is DNA or RNA from the patient being tested.

Sanger sequencing (described previously) of a limited

- After exposure of the patient sample to the probe and a number of genes known to harbor mutations with well—washing step, the presence of the fluorochrome in the

described clinical appearances. If several genes need to be sampled, it indicates that the target sequence is present. In a laboratory setting, Sanger sequencing can be costly and time consuming. Because fluorescence microscopy is required, the target must be labeled. However, the Sanger method continues to be the default method. It may have a weak fluorescent counterstain to permit it to be seen, but the primary fluorescent material is the probe. Sanger sequencing is considered the “gold standard” when the newer next-generation sequencing methods are compared. Sanger sequencing approach does not yield clear cut results.

As a follow-up, a simple sensitive method or in-situ amplification is required. Next-generation sequencing (NGS) is not a method, it is a technology. It uses chemiluminescent detection of sequence-specific DNA. Sanger sequencing is an approach. In the last decade, NGS technologies have been



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A gene A (normal)

C C T G A G G

BOX 31.4

Ms t II site

gene S (sickle)

C C T G T G G

S tre ng ths and Lim itations of FIS H

(no Ms t II site)

STRENGTHS OF FISH

B

Restriction site

- Is better than PCR for deletions and inversions

β -globin

absent in sickle-cell

gene

- Is the next generation of cytogenetic techniques

β -globin

banding

Mst II

Mst II Mst II

- Can be correlated with morphology
- Can be used to assess DNA or mRNA
- Can be performed on metaphase spreads but can also

gene A

1.1kb

be done on paraffin block sections

gene S

1.3kb

LIMITATIONS OF FIS H

- Problems in tissue that has been decalcified or not fixed

C

in formalin

Southern blot

- Requires a fluorescent microscope

of DNA cut with

Probes must be specifically designed

Mst II and

■

β S(1.3kb)

hybridized

- Can only be used to detect the presence or absence of

with β -globin

β A(1.1kb)

previously identified chromosomal aberrations

probe

- Not good as PCR or very small mutations

- Labor intensive

■ issue that is xed cannot be cultured or metaphase

S ickle -ce ll control

spreads

Norma l control

Ca rrie r

Modi ed rom Heriot K. Welcome to the beginning: molecular pathol—

Affe cte d individua l

ogy or the community hospital pathologist and medical technologist,

FIGURE 31.9 Restriction ragment length polymorphism (RFLP)

ASCP Annual Meeting, ampa, FL, 2014.

caused by loss o a restriction site. I a mutation occurs in a cleavage site or a restriction enzyme, the pattern o restriction ragments

di ers rom normal. **A.** T e mutation that causes sickle cell anemia

results in the loss o an MstII site in the β -globin gene. **B.** Samples

o DNA rom individuals are treated with restriction endonucleases

and then subjected to electrophoresis on gels. With the Southern blot

technique, the restriction ragments on the gel are hybridized with a

radioactive cDNA probe or the β -globin gene. T e sickle cell allele

produces a ragment o 1.3 kilobases (kb) when treated with MstII.

A normal allele produces a ragment o 1.1 kb (plus a ragment o 0.2

kb that is not seen on the gel). For a person with sickle cell disease,

both alleles produce 1.3-kb restriction fragments. In a normal person, both alleles produce 1.1-kb fragments. For a carrier, both the

1.3- and 1.1-kb fragments are observed. (From Lieberman M, Ricer

R (eds.). BRS Biochemistry, Molecular Biology, and Genetics, 6th ed,

Philadelphia, PA: Lippincott Williams & Wilkins, 2013.)

developed (Figs. 31.12 and 31.13). This approach overcomes

the limitations of traditional Sanger sequencing by providing

highly parallel sequencing with a separate sequence result for

every sequence of interest. This has positioned NGS as the

FIGURE 31.10 Southern blot detection of B-cell receptor (BCR)

method of choice for targeted resequencing of regions of the

gene rearrangement in a patient with peripheral B-cell lymphoma.

human genome. The approach of NGS technologies has the

two separate lesions were investigated for evidence of a monoclonal

potential to be more cost-effective and be able to simultaneously

B-cell population. Restriction digestion with BamHI (1, 2) and

usually sequence complete genomes of patients to deliver per—

EcoRI (7, 8) show extra bands (arrow), which are not detected in

personalized medicine. NGS can produce thousands to millions

the lane with DNA isolated from placenta (pl). Although HindIII

digest (4, 5) shows no extra band, two rearranged bands detected by

o genome sequences at one compared to the 96 sequences

BamHI and EcoRI are sufficient to establish clonality. Because both processed by the traditional Sanger method.

lesions show similar banding pattern, these lesions are considered

NGS technologies permit analysis of mutation, rearrange—

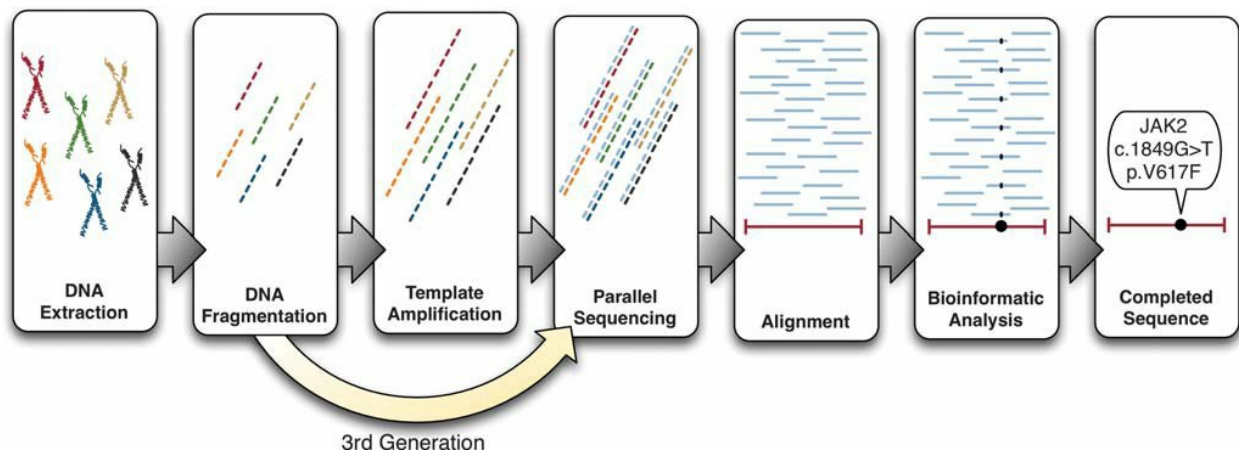
to be clonally identical. (Reprinted from McClatchey KD. Clinical

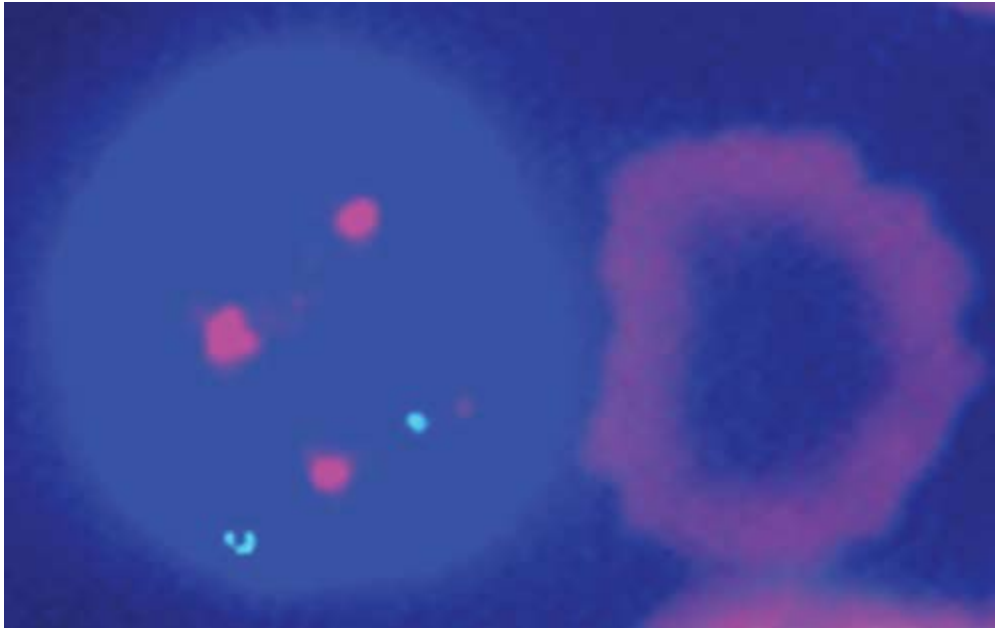
ment, amplifications and deletions (DNA sequencing), or

Laboratory Medicine, 2nd ed, Philadelphia, PA: Lippincott Williams

coding and noncoding RNA (RNA sequencing). Most clinical

& Wilkins, 2002, with permission.)





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1. emplate preparation
2. Sequencing and imaging
3. Data analysis

emplate Preparation. This step consists of building a DNA or complementary DNA (cDNA) library and amplification of that library. A sequencing library is constructed by fragmenting the DNA or cDNA specimen and attaching adapter sequences, synthetic oligonucleotides of a known sequence, to the ends of the DNA fragments. A constructed

library is clonally amplified in preparation for sequencing.

Amplification of single library fragment on microbeads is

unique to the One Touch system; bridge amplification is

used to form template clusters on a flow cell by the Illumina

FIGURE 31.11 FISH technique demonstrates trisomy 12 in a system.

case of CLL with prolymphocytoid transformation. (From Sun .

Sequencing and Imaging. For the next step, the two com—

Flow Cytometry, Immunohistochemistry, and Molecular Genetics

or Hematologic Neoplasms, 2nd ed, Philadelphia, PA: Lippincott

mercial systems rely on sequencing by synthesis. The library

Williams & Wilkins, 2012.)

fragments act as a template from which a new DNA fragment is synthesized. Sequencing occurs through a cycle of

washing and flooding the fragments with the known nucle—

cally used assay is based on DNA sequencing. Commercial

otides in a sequential order. As nucleotides incorporate

panels available through Ion Torrent PGM One Touch

into a growing DNA strand, they are digitally recorded as

system and Illumina are popular or design own panels based

sequence. One system, PGM, does semiconductor sequenc—

on genes of interest. NGS permits analysis of all the exomes
ing that relies on detection of pH changes induced by the
or even entire genes. This has led to expansion of the number
release of a hydrogen ion upon the incorporation of a nucleotide genes that can be
analyzed at one time and identification
otide into a growing strand of DNA. The Illumina MiSeq
of patients at risk.

relies on detection of fluorescence generated by the incorporation—

Steps in NGS

incorporation of fluorescently labeled nucleotides into the growing

Each NGS platform is unique in how sequencing is
strand of DNA.

accomplished, but the generalized sequencing protocol for

Data Analysis. The final step after sequencing is complete

the two commercially available NGS platforms (Ion Torrent

is that raw sequence data must undergo several analysis

PGM (Ion Torrent system and Illumina) includes

steps. Preprocessing of data removes adapter sequences and

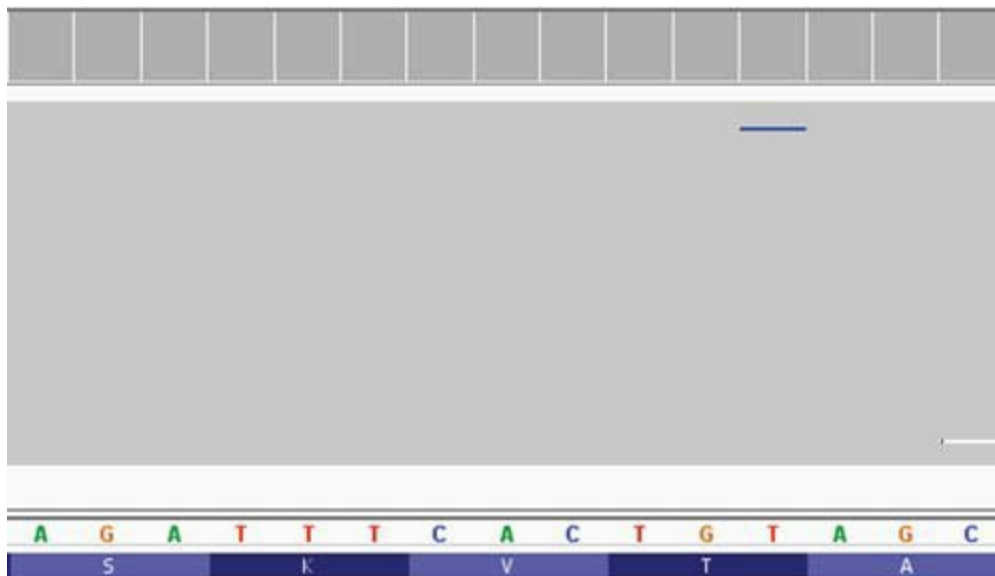
FIGURE 31.12 Generalized overview of next-generation sequencing.
Genomic DNA is isolated and then fragmented.

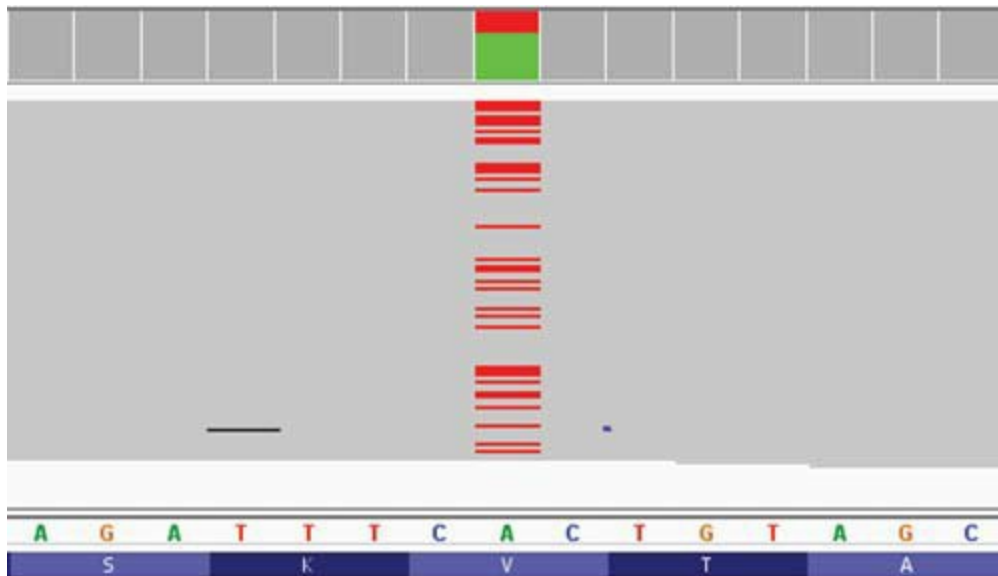
Although not depicted, the fragmented DNA template is typically

immobilized on a solid support (e.g., slide or bead) as a means to spatially separate the templates during the massive parallel sequencing step. Most current methods (i.e., “second-generation” NGS technology) utilize a DNA template amplification step. In contrast, emerging “third-generation” NGS

technologies are not reliant on template amplification, thus reducing a potential source of error and bias (51). The next step involves a multitude of short sequencing reactions that are carried out in parallel across the entire template. These reactions result in a number of overlapping sequencing reads such that each DNA base position in the template is independently evaluated multiple times. Once the massive parallel sequencing reactions are completed, the individual sequencing reads (light blue lines) are aligned to a reference sequence (dark red line) using software tools. Using bioinformatic tools, the final sequence is assembled, interpreted, and annotated for sequence variants. (From Orazi A, Foucar K, Knowles D, *et al.*

Knowles Neoplastic Hematopathology, 3rd ed, Philadelphia, PA: Lippincott Williams & Wilkins, 2013.)





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Targeted Sequencing

Whole genome and whole-exome sequencing are available.

In many instances, sequencing of specific genes or genomic regions is preferred. Targeted sequencing is less expensive and yields much higher coverage of genomic regions of interest.

Sequence panels that target hundreds of genomic “hotspots” or disease-causing mutations are being developed. Targeted

sequencing of either individual genes or whole panels of

genomic regions aids in rapid diagnosis of many genetic disorders. This can aid in therapy decision making.

A

G

A

T

T

T

C

A

C

T

G

T

A

G

C

S

K

V

T

A

Microarray Gene Chips

A

BRAF V600 mutation

Microarray gene chip technology represents the merger

of three fields: the Human Genome Project, fabrication of

integrated circuits mounted on a substrate, and sophisticated

computer power. The microarray chip technology is becoming

a routine tool for the high-throughput analysis of gene expression in a wide range of biological systems, including hematopathology applications such as diffuse large B-cell lymphoma.

Microarrays basically represent the product of bonding or

direct synthesis of numerous specific DNA probes on a stationary, often silicon-based, chip. The chip may be tailored to

particular disease processes. It is easily performed and readily automated (Fig. 31.14).

A

G

A

T

T

T

C

A

C

T

G

T

A

G

C

Microarrays are miniature gene fragments attached to glass

S

K

V

T

A

chips. The microarrays have material immobilized on a sub-

B

BRAF wild type

strate that is of a known sequence and is frequently called the

probe, a known sequence. The unknown sequence of mate-

FIGURE 31.13 Next-generation sequencing (NGS). Hundreds to

rial that is applied to the array, for example, patient's serum,

thousands of sequence reads are mapped and horizontally aligned to specific targeted regions in the reference genome (sequence is called the target. In microarrays, the known sequence is shown on bottom of each panel). A software-assisted analysis assists immobilized, and the unknown sample is in solution.

in the detection of mutations, displayed as colored bars in each read

These chips are used to examine gene activity of thousands above the mutation site. A wild-type sequence within each read is or tens of thousands of gene fragments and to identify genetic displayed in gray. Mutation frequency correlates to the number of mutations, using a hybridization reaction between the sequences times the mutant sequence is detected compared to the total number on the microarray and a fluorescent sample. Following hybridization, the chips are scanned with high-speed fluorescent results from BRAF V600E mutation positive (**A**) and negative (**B**) detectors, and the intensity of each spot is quantitated. The identity and amount of each sequence are revealed by the location mutation is displayed in red. Patients with metastatic melanoma

and intensity of fluorescence displayed by each spot.

that harbors the BRAF V600E mutation are candidates for targeted

Computers are used to analyze the data. Software is avail—

therapy. (From DeVita V Jr, Lawrence S, Rosenberg SA. DeVita,

Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology,

able to provide an estimate of background fluorescence, using

Philadelphia, PA: Lippincott Williams & Wilkins, 2012.)

fluorescence from negative controls, and to estimate signal to

noise ratio. Software is also available to identify signals that

low-quality read, mapping of data to a reference genome or

are suspicious because of their faintness; because the shape of

de novo alignment of the sequence reads, and analysis of the

the fluorescent area suggest a probe that is improperly made,

compiled sequence. Analysis can include identification of

shaped, or placed; or because the signal appears to have been

somatic and germ-line mutations.

modified by dust particles. All data obtained from microarrays

With NGS technology, the process begins with template

must be corrected for artifacts in a process called

preparation by shearing DNA (or cDNA) to create fragments

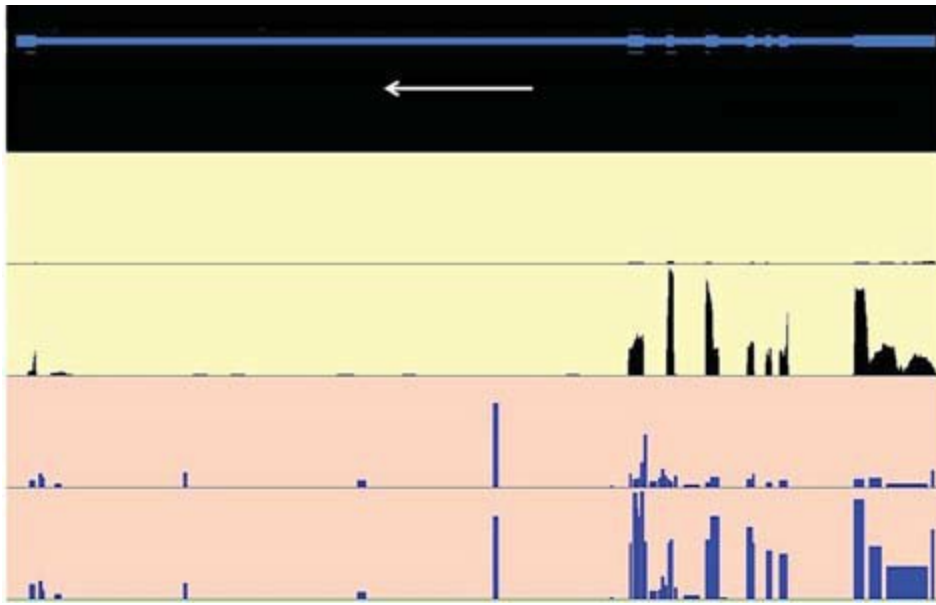
normalization. Simultaneous statistical analyses are necessary libraries. Adaptor sequences are added to these fragments to analyze the signals emanating from the thousands of probes and serve as primers for amplification usually by emulsion on the substrate.

PCR or bridge PCR methods. The resulting amplified signal A comparison of RNA gene analysis by microarray versus beads or clusters are analyzed using a variety of platform—NGS is presented in Fig. 31.15).

specific chemical analyses, but all are based on the addition

NOTE: This is a good time to complete end of chapter of labeled nucleotides. Digital images are captured and analyzed to determine the sequence of the target DNA.

Review Questions related to the preceding content.



Microscope slide

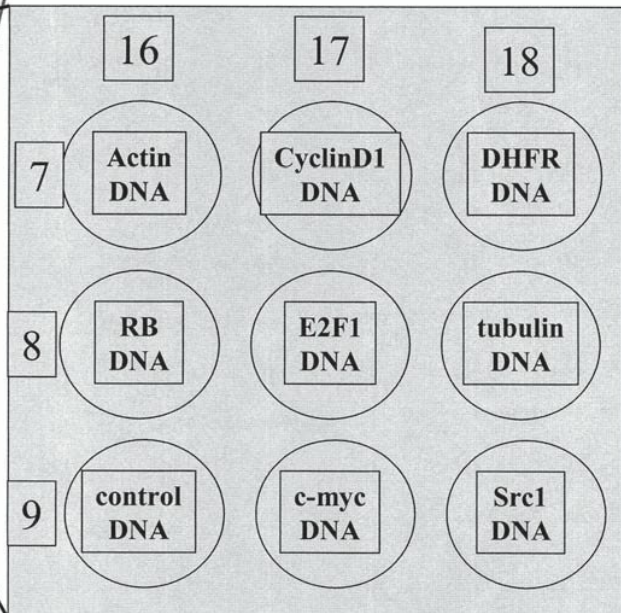
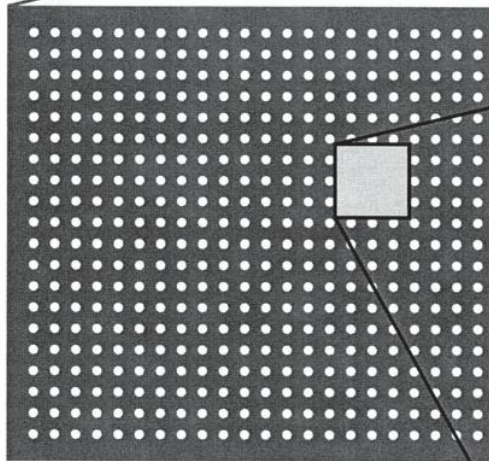
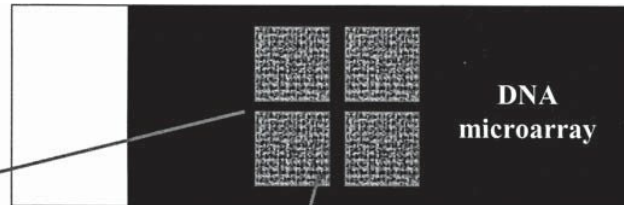


FIGURE 31.14 Microarray gene expression profiling using microarray analysis. A solid surface (in this example, a glass microscope slide) contains thousands of spots. Each spot contains a large number of DNA fragments. For each spot, the DNA fragments are derived from one specific gene. (From Courtesy of Ron Kerkhoven, Netherlands Cancer Institute, Amsterdam, The Netherlands.) **Gene Exons : 8**

7 6 5 4 3 2

1

RNA Seq:

Primary

Metastasis

Mic ro array:

Primary

Metastasis

FIGURE 31.15 RNA expression analysis, next-generation sequencing versus microarray. Here, a single gene is portrayed (**upper black panel**), with exons indicated by thicker portion of the locus, introns by the thinner portions. Note that both methods detect exon level expression. The **upper yellow panels** are paired with the lower mauve pair; the former represent “pile up” quantitation of reads at a given locus, first in the primary tumor (**upper panel**), then the metastatic tumor (**lower panel**). The pattern is repeated in the lower pair of boxes, but for microarrays. It is apparent in both that the gene is expressed more highly in the metastasis than the primary. However, the magnitude of the difference is far greater by NGS, a reflection of the limited dynamic range of microarrays. In addition, occasional exons appear to be detected by microarrays that are not found by NGS. In general, there is close agreement

between the two. (From Pizzo PA, Poplack DG.

Principles and Practice of Pediatric Oncology, Philadelphia, PA: Lippincott Williams & Wilkins, 2015.)

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PART 8 ■ Fundamentals of Hematological Analysis

CHAPTER SUMMARY

history of the disease; thus, some patients may relapse with a clone different to that observed at presentation.

The Human Genome Project

Minimal Residual Disease

- The goal of the Human Genome Project was to sequence the exact order of the base pairs in a segment of DNA in
 - Molecular techniques, for example, PCR, real-time quantitative PCR (RQ-PCR), flow cytometry, and cytogenetic
 - Genetic variations associated with specific diseases or marker studies, are more sensitive to a low number of cells increased the risk of specific diseases are the target of than morphologic appearance in the peripheral blood. genome investigations.
 - PCR is able to detect one malignant cell in a population
 - It is estimated that about 19,500 genes are present in 1 million cells.
- human beings.

- tumor load, type of leukemia, whether disease specific
- The initial method of analysis used by scientists working on marker is identifiable, and technological limits will determine the optimum methodology for monitoring MRD.

DNA sequencing, Sanger sequencing. Today, second-generation sequencing or next-generation sequencing (NGS)

Molecular Genetics in Hematology

analyzes millions of fragments of DNA in sequenced uni-

- Techniques in molecular genetics are beginning to be used soon from a single patient specimen.
- extensively in hematology.

- A wide range of abnormalities can be detected with these

Molecular Techniques in Hematology

techniques. PCR is an in vitro method that amplifies low

- Molecular genetic testing focuses on examination of levels of specific DNA sequences in a sample to higher nucleic acids (DNA or RNA) by special techniques to quantities suitable for further analysis. PCR analysis can determine if a specific nucleotide base sequence is present.
- lead to the detection of gene mutations that signify the

■ The distinct advantages of molecular testing include early development of cancer.

greater accuracy in diagnosis, faster turnaround time, smaller required sample volumes, and increased specificity

Single Nucleotide Polymorphisms

ity and sensitivity in the detection of minimal residual disease—

Single nucleotide polymorphisms (SNPs) comprise the most abundant source of genetic variation in the human genome. ease a better treatment for cancer.



most abundant source of genetic variation in the human genome.

Hematopathology

■ Since the decoding of the human genome and the resulting

■ The benefits of molecular techniques in hematopathology

including greater than 3 million SNPs, laboratory techniques

diagnosis and monitoring include faster turnaround time,

have been able to associate disease states and pharmacogenomics—

smaller required sample volumes, and increased specificity—

logical responses with individual SNPs.

ity and sensitivity.

■ Today, molecular methods are used to identify changes

Polymerase Chain Reaction

ranging from a single chromosome disorder to alterations involving the interchange of DNA between chromosomes.

■ An amplicon is a piece of genetic material, such as DNA, that can be formed as the product of a natural event or

■ Hematological malignancies were the first form of human cancer to be studied in depth at the molecular level.

artificial amplification technique, such as a polymerase

Investigation of the Philadelphia chromosome at the chain reaction (PCR).

molecular level revealed a translocation-induced gene

■ In laboratories in which PCR is performed frequently, rearrangement involving the Bcr and Abl genes that results

any false positives are generally caused by amplicon

in activation of the Abl cellular oncogene.

contamination.

■ PCR is an in vitro method that amplifies low levels of spe-

Gene Rearrangement Studies

cific DNA sequences in a sample to higher quantities suitable for further analysis.

- Gene rearrangement studies are important in diagnostic

- The three important applications of PCR are amplification of DNA, identification of a target sequence, and synthesis of a labeled antisense probe.

proliferation.

- PCR has become increasingly popular for detecting chro-

- Immunophenotyping categorizations are aided by the use of chromosomal breakpoints, fusion genes, and MRD analysis—cluster designation (CD) or specific lineages of cells. CDs in chemotherapy for leukemia and lymphoma.

indicate a known cluster of monoclonal antibodies binding

- One adaptation of PCR uses nested primers.

to a known antigen on the cell surface of hematopoietic cells.

- Real-time PCR is another method based on PCR.

- Although it is sensitive, the technology is susceptible to

- Q-PCR assumes 100% efficiency, variation in efficiency of amplification, and no amplification of some specimens.



CHAPTER 31 ■ Molecular Diagnostic Techniques and Applications

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■ Other techniques are used to enhance the sensitivity and size, the Southern blot procedure can detect these changes specificity of amplification techniques. The selection of in DNA sequences.

one technique over another is often based on factors such as sensitivity and specificity profiles, cost, turnaround

Fluorescent In Situ Hybridization (FISH)

time, and local experience.

■ This is a tissue-based molecular diagnostic assay. The rapid

DNA Sequencing

expansion in the availability of polyclonal and monoclonal antibodies has fostered a dramatic increase in light

■ Molecular genetic testing focuses on the examination of microscopic immunohistochemistry (IHC) and in situ nucleic acids (DNA or RNA) by special techniques to hybridization.

determine whether a specific nucleotide base sequence is

■ FISH analysis is used in the diagnosis of hematological present.

malignancies including CML, AML, Burkitt lymphoma,

■ The applications of nucleic acid testing have expanded, and other lymphomas (e.g., follicular lymphoma, mantle despite higher costs associated with testing, in various cell lymphoma, MALT lymphoma, and anaplastic large areas of the clinical laboratory.

cell lymphoma).

■ Molecular testing has the following advantages: faster

■ FISH analysis is generally better for detection of deletions turnaround time, smaller required sample volumes, and and inversions than PCR.

increased specificity and sensitivity.

■ DNA sequencing is the determination of the precise

Next-Generation Sequencing

sequence of nucleotides in a sample of DNA. The most popular method of doing this is called the dideoxy

- NGS is not a method, it is an approach.

method or Sanger method.

- This approach overcomes the limitations of traditional

- Melting curve analysis (MCA) is a method of assessing the Sanger sequencing by providing highly parallel sequencing—dissociation characteristics of double-stranded (DS) DNA using a separate sequence result for every sequence of using a fluorophore during heating.

interest.

- Capillary electrophoresis (CE) is a relatively new, power-

- This has positioned NGS as the method of choice or target—ul separation technique that is ideally suited for handling targeted resequencing of regions of the human genome.

small amounts of DNA.

- NGS has the potential to be more cost-effective and be

- Pyrosequencing is an attempt to find a faster and less able to simultaneously sequence complete genomes of

expensive way of molecular sequencing.

patients to deliver personalized medicine.

- NGS technologies permit analysis of mutation, rearrange-

Southern Blot Technique

ment, amplifications and deletions (DNA sequencing), or

coding and noncoding RNA (RNA sequencing).

- The Southern blot procedure is used less commonly than in the past.

Microarray Gene Chips

- Specimen DNA is denatured and treated with restriction enzymes to create DNA fragments; then, the ssDNA frag-

- Microarray gene chip technology represents the merger of two technologies: DNA microarrays and gene chips. DNA fragments are separated by electrophoresis.

of three fields: the Human Genome Project, fabrication of

- The Southern blot procedure has clinical diagnostic appli—

integrated circuits mounted on a substrate, and sophisticated computers or disorders associated with significant changes

cated computer power.

in DNA, a deletion or insertion of at least 50 to 100 bp

- The microarray chip technology is becoming a routine method for the detection and determination of clonality in lymphomas of B

tool or the high-throughput analysis of gene expression cell origin.

in a wide range of biological systems, including hema-

■ In a single-base mutation changes an enzyme restriction topathology applications such as di use large B-cell site on the DNA, resulting in an altered band or fragment lymphoma.

REVIEW QUESTIONS

*Indicates ML (optional) and MLS advanced content.

2. The first inherited hematologic disorder to be diagnosed

1. Molecular techniques are being used to detect abnormalities—using molecular biologic assay was

A. hemophilia A

A. erythrocytes

B. factor V Leiden

B. leukocytes

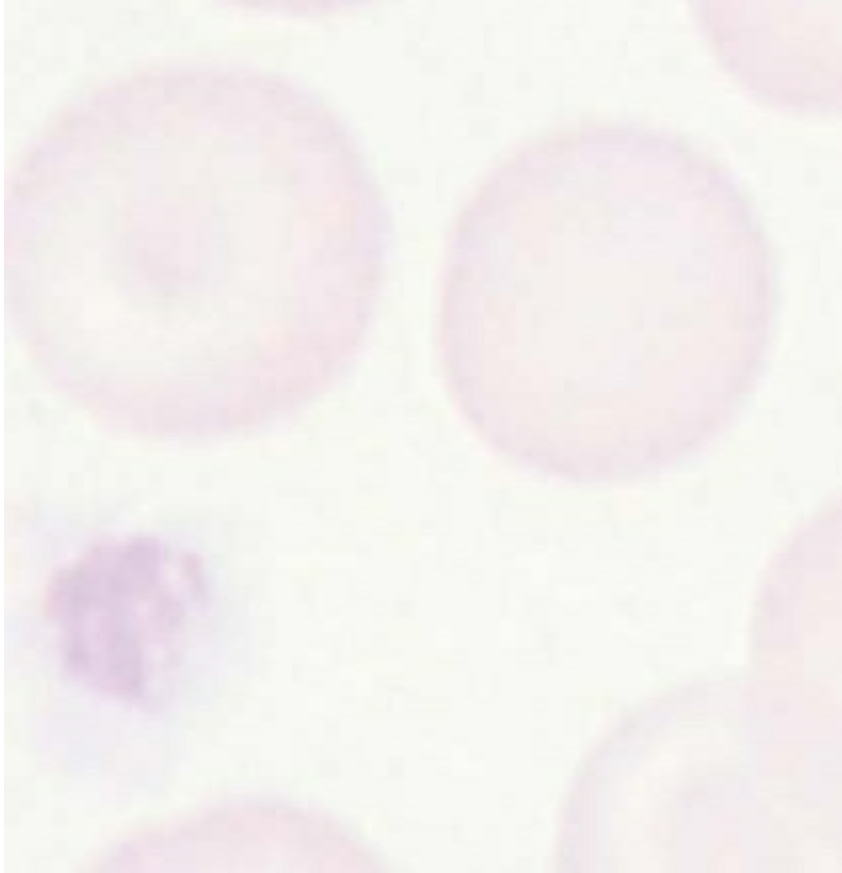
C. sickle cell anemia

C. some coagulation factors

D. CML

D. All o the above

(continued)





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PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS (continued)

3. PCR testing is useful in

9. All of the following are true of FISH except

A. forensic testing

A. the acronym stands for fluorescent in situ

B. genetic testing

hybridization.

C. disease diagnosis

B. It is a tissue-based molecular diagnostic assay.

D. All of the above

C. It is a prenatal diagnosis of a genetic disorder.

D. It is useful in the diagnosis of various anemias.

4. The traditional PCR technique

A. extends the length of the genomic DNA

*10. Microarrays are

B. alters the original DNA nucleotide sequence

A. DNA probes bonded on glass chips

C. amplifies low levels of specific DNA sequences

B. tissue-based probes

D. amplifies the target region of RNA

C. used to identify single-base mutations

D. used to determine clonality in lymphomas

5. PCR protocol

A. doubles the specific amount of DNA with each cycle

*11. Molecular techniques provide a diagnostic tool to

B. typically has three temperature steps

A. detect MRD in hematological malignancies

C. repeats the number of cycles about 30

B. monitor patients following bone marrow

D. all of the above

transplantation

C. detect an early relapse in a patient treated or a

6. Variations of PCR include

hematological malignancy

A. nested primers

D. all of the above

B. real-time PCR

C. microarray analysis

*12. Which of the following best describes the molecular

D. both A and B

alteration of the Philadelphia chromosome to chronic

myelogenous leukemia (CML)?

7. The method that continues to be considered the “gold

A. The mutation produces an overly active tyrosine standard" or DNA sequencing kinase that increases apoptosis.

A. Sanger method

B. The mutation reduces serine enzyme activity in

B. Southern blot method

affected cells.

C. Northern blot method

C. The mutation involves translocation of a gene that is

D. Dot blot method

critical to myelocytic maturation.

D. Hematopoietic cells gain a proliferation advantage

8. The Southern blot procedure has diagnostic applications because of tyrosine kinase activity.

or diseases or disorders associated with

A. significant changes in DNA (e.g., deletion)

B. determination of clonality in lymphomas of B-cell origin

C. detection of restriction fragment length polymorphisms

D. all of the above

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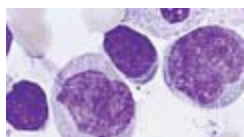
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CHAPTER

32 Pr

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ology

LEARNING OUTCOMES

■ Describe the general principles of basic and selected special-

■ Perform the stated procedure.

ized procedures in hematology, special stains, and coagulation

■ Perform any calculations needed for reporting the results in the
procedures.

procedure.

■ Describe the proper type of specimen collection and handling for

■ State the reference range values for the parameters measured by
the stated procedure.

the procedure.

- Prepare the necessary reagents for the stated procedure.
 - Describe the sources of error and clinical applications of the
 - Describe the quality control steps needed for the stated procedure.
- procedure.

PROCEDURAL FORMAT

9. Reporting results (normal values)

10. Procedure notes including sources of error, clinical

The procedures in this chapter are presented in a format that is applications, and limitations of the procedure

consistent with the guidelines set forth by the CLSI. This format is:

11. References

1. Procedure title and specific method

All specimens should be treated with caution. All blood,

2. Test principle including type of reaction and the clinical

tissues, and blood derivatives should be considered potentially reasons for the test

tially infectious. Specimen handling notes that are particularly

3. Specimen collection and preparation

important to coagulation studies are presented on the page

4. Reagents, supplies, and equipment

immediately preceding the Coagulation Procedures section.

5. Calibration of a standard curve

Many of the procedures in this chapter are classic methods that

6. Quality control

are frequently performed in the working clinical laboratory.

7. Procedure

These procedures are included or used in special circumstances

8. Calculations

such as in the student laboratory or small clinical laboratories.

HEMATOLOGY PROCEDURES

TOTAL LEUKOCYTE COUNT PROCEDURE

Reagents, Supplies, and Equipment

Principle

1. Disposable Leuko-IC capillary pipette and Leuko-IC

A capillary blood specimen or an EDTA-anticoagulated whole blood specimen is collected in a 20- μ L capillary pipette mixture vial

blood specimen is collected in a 20- μ L capillary pipette mixture

2. Handheld cell counter (optional)

with a solution of acetate buffer and gentian violet stain. The

3. Conventional microscope and lens paper

erythrocytes will lyse, and the stain will impart a light violet—

4. traditional hemocytometer and coverslip or disposable

blue color to leukocytes to facilitate enumeration. Although total hemocytometer.*

WBC counts are most frequently performed with automated

Note: The improved Neubauer hemocytometer consists

equipment, the manual enumeration of WBCs is important in

of two raised counting chambers. A specially weighed

cases where the counts are very low.

Specimen

*

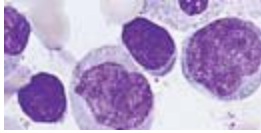
Anticoagulate whole blood or capillary blood can be used.

A disposable, glass hemocytometer (www.bioanalytic.com) has a grid pattern and depth that are the same as the traditional Neubauer hemocytometer.

EDTA is the preferred anticoagulant. A hemolyzed specimen

cytometer. This all-in-one unit does not require a coverslip. Follow the manufacturer's instructions for use.

manufacturer's instructions for use.



CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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HEMATOLOGY PROCEDURES (continued)

coverslip or the hemocytometer is placed over the chambers

5. Begin counting in the upper left-hand square and count
for cell counting. The four large outer squares are used for
the leukocytes, which appear as round particles.

leukocyte counting.

Continue to count in the remaining three squares.

The variation in the number of cells counted in the four

Quality Control

squares should not differ by more than 5 to 10 cells.

A normal control specimen should be counted.

6. Count the leukocytes on the other side of the hemocytometer; this total should
be within 10 cells of the other side.

Procedure

Average the count from both sides.

1. Follow the manufacturer's directions for specimen preparation—

7. The total leukocytes counted on each side are a representation of the counting chamber.

together and divide by 2 to obtain the average. It is

2. On each side of the chamber of a clean hemocytometer

important to note that the distribution of cells should be

with a coverslip, load a small amount of the dilute blood.

roughly equivalent throughout the count and that no clumps

Allow the counting chamber to sit covered, with a moist—

of cells should be seen. If clumping is noted, another dilution

using filter paper in half of a Petri plate, or a few minutes

incubation must be made.

to allow the cells to settle and the erythrocytes to completely

8. Soak the hemocytometer in a 10% bleach solution to is—
completely lyse.

infect. Discard the capillary pipette and contaminate

3. Place the hemocytometer under the 10× microscopic
supplies in a biohazard bag.

objective (low power) to focus using either a phase-contrast optics or bright field (lower concentration) at 10×

CALCULATIONS

magnification or the total count. Erythrocytes should be

Shortcut Formulas

lyse. The nucleus of polymorphonuclear segmented neutrophils will be bright, but the lymphocyte nucleus will be

total leukocyte count from 4 large corner squares $\times 0.05 =$

round.

WBCs $\times 10^9/L$

4. The leukocytes in all large four corner squares that are

or

further rule into 16 smaller squares in each large square,

total leukocyte count from 4 large corner squares $\times 50 =$

of each side of the chamber, should be counted (see

WBCs/ μL

Fig. 32.1). If cells touch the inner or middle lines of two

adjacent lines, for example, upper and lower sides, they

Classic Formula

can be counted. Cells touching the outer lines or the opposite—

Dilutional Factor = 20. This represents the reciprocal of the

side of adjacent lines should not be counted.

1:20 dilution of blood.

Number of squares counted = 4

Area of each square = 1 mm^2

Depth of solution = 0.1 mm

total number of cells counted \times Reciprocal

of the dilution

W

W

= cell m

/ m³

Number of squares counted \times Area of each

square \times Depth of the solution

R

R

Example: If the average total of leukocytes counted was 180,

the total leukocyte count would be:

R

$180 \times 20 = 3600 \times 10^3 / \mu\text{L}$ or $3600 \times 10^9 \text{ L}/(\text{SI unit})$ s

4×1

2

mm \times 0.1

. mm

R

R

Reporting Results

Reference value: $4.5 \text{ to } 11.0 \times 10^9/\text{L}$

W

W

Procedure Notes

Sources of Error

Erroneous results can be due to contamination of diluting fluid, incorrect dilution or loading of the hemocytometer, or an uneven

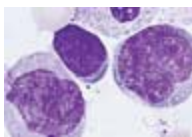
FIGURE 32.1 Neubauer counting chamber. (R, red cell area;

distribution of leukocytes in the counting chamber. Prompt

W, white cell area.)

counting of cells is important to the accuracy of the count.

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

HEMATOLOGY PROCEDURES (continued)

Clinical Applications

In most cases, leukocyte counts are only performed manually when there are extremely low total leukocyte counts.

The total leukocyte count in whole blood specimens can be decreased or increased due to a variety of disorders.

Select quantitative leukocyte disorders

Decrease leukocytes (leukopenia)

FIGURE 32.2 The method of slide examination in the leukocyte

Viral disorders

differential count.

Radiation- or chemotherapy-induced leukopenia

Aplastic anemia

Reagents, Supplies, and Equipment

Megaloblastic anemia

1. A manual cell counter designed for differential counts

Increase leukocytes (leukocytosis)

2. Microscope, immersion oil, and lens paper

Bacterial infections

Quality Control

Inflammation

Leukemias

gaining an experience in examining immature and abnormal cell morphology are essential. A set of reference slides

CORRECTION OF TOTAL LEUKOCYTE COUNT

with established parameters should be established to assess

FOR NUCLEATED RED BLOOD CELLS

the competence of an individual to perform differential and

If more than 10 nucleated erythrocytes are seen on a differential

morphological identification of leukocytes and erythrocytes.

differential blood smear, the total leukocyte count should be

Participation in a quality assurance program continues to

corrected.

document the expertise of the hematologist in microscopy.

Questionable or abnormal smears should be referred to a

Average total WBC count $\times 100$

Corrected WBC =

supervisor or verification.

$100 + \text{number of nucleated RBCs}$

Procedure

100 WBCs in differential count

1. Begin the slide examination with a correctly prepared and stained smear (see Chapter 2 or specimen preparation).

REFERENCES

2. Focus the microscope on the 10× objective (low power).

Scan the smear to check for cell distribution, clumping, and

Henry JB (ed.). Clinical Diagnosis and Management by Laboratory

abnormal cells. A drop of immersion oil and switch

Methods, Philadelphia, PA: Saunders, 1984:1444.

to the 100× (oil immersion) objective. Begin the count by

urgeon ML, Benner J. Hematology and Coagulation Laboratory

determining a suitable area (Fig. 32.2). Extend the exami—

Manual, Corning, NY: Corning Community College Press, 1985.

nation from the area where approximately half of the

LEUKOCYTE DIFFERENTIAL COUNT

erythrocytes are barely overlapping to an area where the

Principle

erythrocytes touch each other. It is important to examine

cellular morphology and to count leukocytes in areas that

A stained smear is examined to determine the percentage

are neither too thick nor too thin. In areas that are too thick,

of each type of leukocyte present and assess the erythrocyte

cellular details such as nuclear chromatin patterns are i -

an platelet morphology. Increases in any of the normal leukocytes are difficult to examine. In areas that are too thin, distortion of leukocyte types and the presence of immature leukocytes or cells makes it risky to identify a cell type.

Erythrocytes in peripheral blood are important diagnostically

3. Count the leukocytes using a tracking pattern. Each cell in a wide variety of inflammatory disorders and leukemia.

Identify should be immediately tallied as a neutrophil

Erythrocyte abnormalities are clinically important in various

(band), neutrophil (segmented), or polymorphonuclear

anemias. Platelet size irregularities are suggestive of particular

neutrophil (PMN); lymphocyte; monocyte; eosinophil;

large thrombocyte disorders.

or basophil. A brief leukocyte morphology reference is

Specimen

include (Table 32.1); however, refer to specific chapters in

Peripheral blood, bone marrow, or body fluids,

the text or a complete discussion of leukocyte and eryth-

such as spinal fluid, are appropriate specimens. Whole blood

erythrocyte cellular morphology.

smears may be made from EDTA-anticoagulated blood or

4. Abnormalities of leukocytes, erythrocytes, and platelets

prepare from free-flowing capillary blood. Smears should

be noted. Normally, 8 to 20 platelets are present in

fields made within 1 hour of blood collection from EDTA. A special oil immersion field in a properly prepared smear (where

cells are spread at room temperature to avoid distortion of cells

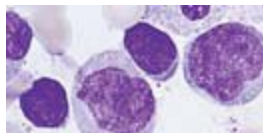
the RBCs barely touch each other). After examining at least

100 fields, unstained smears can be stored indefinitely

in a dry place. In different fields, the average number of platelets can be

counted, but stained smears gradually fade.

Counted platelets are multiplied by a factor of 20,000 to arrive at an approximate



CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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HEMATOLOGY PROCEDURES (continued)

TABLE

32.1 A Comparison of Normal Leukocytes in Peripheral Blood

Segmented

Band

Neutrophil

Neutrophil Lymphocyte Monocyte

Eosinophil

Basophil

Nuclear shape

Lobulated

Curved

Round

Indented or twisted Lobulated

Lobulated

Chromatin

Very clumped

Moderately Smooth

Lacy

Very clumped Very clumped

clumped

Cytoplasmic color

Pink

Blue, pink

Light blue

Gray-blue

Granulated

Granulated

Granules

Many

Many

Few or absent Many

Many

Many

Color of granules

Pink, a few blue Pink

Red

Dusty blue

Orange

Dark blue

Average percentage

56%

3%

34%

4%

2.7%

0.3%

total circulating platelet concentration. Nucleate eryth—

bu y coat. I this layer o concentrate cells is remove by

rocytes are not inclu e in the total count but are note

pipetting, push-we ge-type smears can subsequently be preper 100 white
bloo cells (WBCs). A total o at least 100

pare an staine or microscopic examination. T is tech—

leukocytes shoul be counte . Express the results as a per—

nique is use ul in the per ormance o leukocyte i erential

centage o total leukocytes counte .

counts on patients with extremely low total leukocyte counts

or in special testing proce ures.

Reporting Results

Re erence values, particularly the ban neutrophil percent—

Specimen

age, may vary. Values or chil ren i er rom a ult re erence

A reshly rawn specimen o ED A-anticoagulate whole

values. See insi e back cover or a ull iscussion o re erence

bloo is nee e .

values.

Procedure

Procedure Notes

1. Centrifuge the specimen of whole anticoagulated blood

A well-made and well-stained smear is essential to the accuracy of the differential count. The knowledge and ability of the cell morphologist are critical to high-quality results.

or at least 5 minutes at 2,000 to 2,500 rpm.

2. With a Pasteur pipette, remove most of the top plasma layer and discard.

A minimum of 300 leukocytes must be within the acceptable working area, when the total leukocyte count is no less

3. The interface layer along with a small amount of plasma and a small volume of erythrocytes can then be removed using a Pasteur pipette.

than $4 \times 10^9/L$. The neutrophils, monocytes, and lymphocytes should appear evenly distributed in the usable fields of the

4. A drop of this suspension can be placed on a microscope slide and a push-weather smear prepared. Air-dry and stain.

nonidentifiable forms except in certain forms associated with

Alternative Technique

pathological states. If a disrupted cell is clearly identifiable,

include it in the differential count. Classify nonidentifiable

A refinement of the classic buoy coat technique has been

disrupted cells (smudges or baskets) as “other,” and note them

envelope or use with automated blood smear equipment.

on the report if more than a few are observed.

In this technique, saline solution and 22% albumin are

The blood smear preparation techniques describe in

add to the interface layer. This enhancement produces better—

Chapter 2 are commonly used in the laboratory for the preparation—

cell separation on the peripheral smear and minimizes

distortion of blood smears. In certain circumstances, the preparation—

the spreading artifact during centrifugation. One of this

distortion of a buoy coat peripheral blood smear increases the

enhancement to the basic technique:

accuracy of the leukocyte differential count.

1. Proceed from step 3 above by transferring the interface

layer to a disposable Wintrobe (ESR) tube. This tube is

Preparation of Buffy Coat Smears

place into a 16 × 100-mm test tube and centrifuge for a

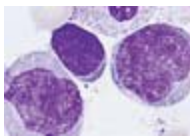
Principle

minimum of 5 minutes at 2,000 rpm.

An anticoagulated specimen of whole blood is centrifuged to

2. After centrifugation, the top plasma layer is removed with a Pasteur pipette and discarded. Remove approximately 0.03 mL of the interface layer and a small volume of erythrocytes and platelets, and erythrocytes. The interface layer between the plasma and erythrocytes is referred to as the buffy coat. Transfer to a 20-mL test tube or plastic vial.

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

HEMATOLOGY PROCEDURES (continued)

A known volume of isotonic (0.85%) saline solution is added to the test material. The mixture is then centrifuged at a concentration to calculate the mean corpuscular hemoglobin

tube or vial to prepare a 1% to 2% suspension of cells.

concentration (MCHC).

3. Add 22% albumin to the suspension at the rate of three

Specimen

drops of albumin for each 10 mL of resulting cell suspension. If the amount of albumin is too great, the cells will

Venous blood anticoagulate with EDTA or capillary blood

appear too dark and may have pseudopossibilities.

Collect directly into heparinized capillary tubes can be

4. This preparation can then be transferred to the sample

use. Specimens should be centrifuged within 6 hours of col—

lection and treated according to the instrument manufacturer's

instructions. Hemolyzed samples cannot be used for testing.

turer's instructions.

Reagents, Supplies, and Equipment

Clinical Applications

1. Capillary tubes (75 mm long with an ID of 1.155 mm).

Select disorders associated with increases in normal leuko—

Blue-banded tubes contain no anticoagulant and are used

leukocyte types are

with EDTA-anticoagulated blood. Red-banded tubes are

heparinize or use with capillary blood.

Neutrophils

2. Clay-type tube sealant.

1. Bacterial infections

3. Microhematocrit centrifuge and reading device.

2. Inflammation

Calibration

3. Stress

The calibration of the centrifuge should be checked regularly—

4. Chronic leukemia

for timer accuracy, speed, and maximal packing of cells.

Lymphocytes

Use a stopwatch for accuracy, a tachometer for speed, and

1. Viral infections

a time versus constant volume method to check packing of

2. Whooping cough

erythrocytes. Check the capillary tube reading device against

3. Chronic leukemia

another reader periodically.

Quality Control

Monocytes

Commercially available whole blood can be used to check the

1. tuberculosis

accuracy of normal and abnormal levels.

2. Rheumatoid arthritis

3. Fever of unknown origin

Procedure

1. Well-mixed anticoagulant blood should be drawn into

Eosinophils

two microhematocrit tubes by capillary action. The tubes

1. Active allergies

should be filled to about three-fourths of their length.

2. Invasive parasites

Wipe the outside of the tubes with gauze or wipes.

Basophils

Free-flowing capillary samples should be collected in the

1. Ulcerative colitis

same manner.

2. Hyperlipidemia

2. Seal one end of each tube with a small amount of clay-like

material. Place the syringe into the sealant, holding the index finger over the opposite end to prevent

BIBLIOGRAPHY

blood from leaking out of the tube onto the sealant.

3. Place the filled and sealed capillary tubes into the centri—

Provision on this book's companion Web site at <http://thepoint.lww.com/surgeon6e>.

usage. The sealed ends should point toward the outside of

thepoint.lww.com/surgeon6e.

the centri usage. The duplicate samples should be placed

opposite each other to balance the centri usage. Record the

PACKED CELL VOLUME OF WHOLE BLOOD:

position number of each specimen.

MICROHEMATOCRIT METHOD

4. Securely fasten the latch on top of the capillary tubes.

Principle

Close the centri usage top and secure the latch. Set the timer

The packed cell volume (PCV) is a measurement of the ratio

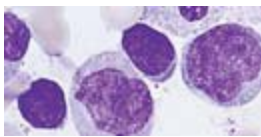
of 5 minutes. The centrifuge speed of centrifugation should be

of the volume occupied by the RBCs to the volume of whole

10,000 to 15,000 rpm.

blood in a sample of capillary or venous blood. Following

5. After the centrifuge has stopped, open the top and remove centrifugation, this ratio is measured and expressed as a percentage of the cover plate. Promptly read the PCV on an appropriate percentage or decimal fraction. Clinically, the PCV is used to piece of equipment or specially designed car. Measure detect anemia, polycythemia, hemodilution, or hemoconcentration— the PCV by adjusting the top of the clay sealant to the zero position. In conjunction with an erythrocyte count, the PCV mark and reading the top of the red cell column. A reader is used to calculate the mean corpuscular volume (MCV). with an ocular that has cross-markings produces the most The PCV is also used in conjunction with the hemoglobin accurate reading.



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HEMATOLOGY PROCEDURES (continued)

Note: When taking readings, be sure that the bottom of the

2. Conventional microscope and lens paper

packed cell column is lined up correctly to the zero mark. Do

3. Rational hemocytometer and coverslip or disposable

not include the buoy coat in reading the packed erythrocyte

hemocytometer*

column. Do not allow the tubes to remain in the centrifuge

Note: The improved Neubauer hemocytometer consists of two

or more than 10 minutes because the interface between the

raised counting chambers. A specifically weighed coverslip or

plasma and the cells will become slanted and an inaccurate

the hemocytometer is placed over the chambers or cell count—

reading will result.

ing. The five millile squares are used for RBC counting.

Reporting Results

Quality Control

The PCV is preferentially expressed as a decimal fraction,

A normal control specimen should be counted.

such as 0.45 L/L, rather than as 45%. In current practice, the

percentage expression is commonly used. Reference values:

Procedure

males, 41.5% to 50.5%; females, 36.0% to 45.0%.

1. Prepare the dilution (1:200) and fill the counting chamber

Procedure Notes

following the manufacturer's instructions.

Sources of Error

2. Allow the hemocytometer to sit for at least 3 minutes (it may be covered with a Petri dish cover). During this time,

Erroneous results can be caused by inclusion of the buoy. The erythrocytes will settle in the chamber.

coat in reaching the packed column, hemolysis of the specimen.

3. Place the hemocytometer on the microscope stage and examine, and inadequate mixing. If the centrifugation time is too short or the speed is too low, an increase in trapped plasma will occur in the central area (Fig. 32.1). This central area is (1% to 3%) will occur in normal blood. Increase amounts of trapped plasma can produce errors in cases in which an entire square is used for the erythrocyte count (Fig. 32.3).

erythrocyte abnormality exists, such as sickle cell anemia.

4. Switch to the 43 to 44× objective (high power) and begin

Other sources of error include prolonged tourniquet stasis

counting the appropriate cells in the five squares—

an excess EDTA, which causes cells to shrink and pack more

tightly than they should.

The number of cells counted in each of the

five small squares should not vary by more than 10 cells.

Clinical Applications

It is important to note that the distribution of cells should

The PCV is used for detecting anemia, polycythemia, hemo—

be roughly equivalent, and no clumps of cells should be

seen. If clumping is seen, another dilution must be made.

Count the erythrocytes on the other side of the hemocytometer. This total should be within 20 to 30 cells of

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the other side. The total erythrocytes counted on each

side are added together and divided by 2 to obtain the

Provide on this book's companion Web site at <http://thepoint>.

average.

lww.com/urgeson6e.

5. Soak the hemocytometer in a bleach solution to disinfect.

RED BLOOD CELL TOTAL COUNT

Erythrocyte Count

Principle

A specimen containing some cellular elements, such as erythrocytes and leukocytes, is diluted in specific volumes.

The isotonic diluting fluid will not lyse erythrocytes, which

facilitates enumeration. Manual determinations of erythrocytes may be performed if an automated cell counter is not

available or in cases of extremely low erythrocyte counts.

Counted

Not counted

Specimen

Anticoagulate whole blood or capillary blood can be used.

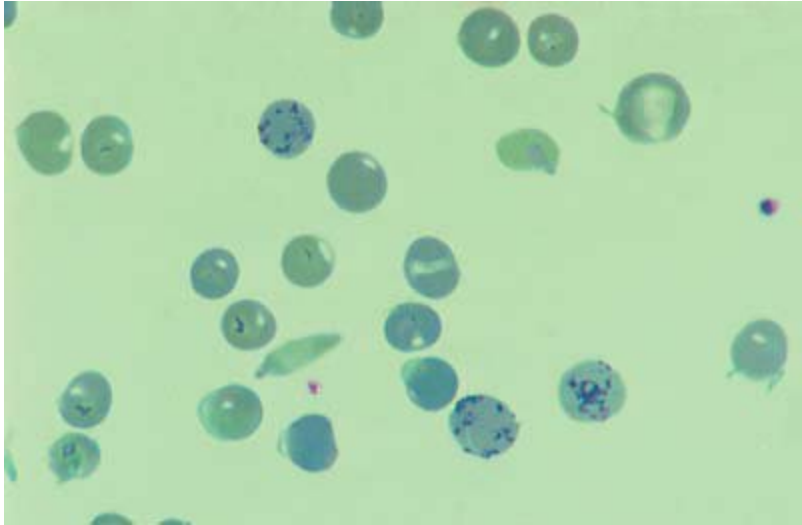
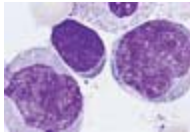
EDTA is the preferred anticoagulant. A hemolyzed specimen is inappropriate for an accurate erythrocyte count.

Reagents, Supplies, and Equipment

1. Disposable Ery-IC capillary pipette and Ery-IC diluent vial

FIGURE 32.3 RBC counting square.

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

HEMATOLOGY PROCEDURES (continued)

Calculation

No.o erythrocytes = average

ra itional Formula: totalo RBCscounte in 5squares

× ilution correction actor

×volume correction factor

1. Five of the 25 squares in the large 1-mm square are counted.
2. The specimen dilution factor is 200.
3. The volume correction factor is 50. This number represents the total volume of the five squares reported in

terms of 1.00 μL . This is calculated by dividing the volume

FIGURE 32.4 Peripheral blood smear with reticulocytes; staining

residue (1.00 μL) by the volume used (0.02 μL).

is with new methylene blue dye. The blue granules represent pre—

Example: If the average number of erythrocytes counted is

400, the total erythrocyte count would be:

Laboratory Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams

& Wilkins, 2002, with permission.)

400 200 50 40

.

106/ μL or 40

.

1012

$$\times \times = \times$$

×

/ L (.SI).

the few remaining mitochondria and erritin masses in living

Shortcut formula: A simplification of this formula is to use a

young erythrocytes to form microscopically visible dark-blue

factor of 10,000, which represents 200×50 . The total average of

clusters and filaments (reticulum). An erythrocyte still possess—

$400 \text{ erythrocytes} \times \text{the factor of } 10,000 = 4,000,000 \text{ or } 4 \times 10^{12}/\text{L}$.

ing RNA is referred to as a reticulocyte (see Fig. 32.4). The enumeration of reticulocytes is important in assessing the status of

Reporting Results

erythrocyte production in the bone marrow (erythropoiesis).

Reference values: Males: $4.5 \text{ to } 6.0 \times 10^{12}/\text{L}$; females: 4.0 to

$5.5 \times 10^{12}/\text{L}$

Specimen

Whole blood that is anticoagulated with either EDTA or heparin—

Sources of Error

air is suitable. Capillary blood drawn into heparinized tubes

Increase or erratic results may be seen if contaminated

or immediately mixed with stain may also be used. The test

utilizing clean, wet or dirty pipettes, a dirty hemocytometer,

should be performed promptly after blood collection. Staining or drying of the dilution in the hemocytometer occurs. smears retain their color for a prolonged period.

Clinical Applications

Reagents, Supplies, and Equipment

A manual count may be appropriate in specimens with low

Reagent

erythrocyte counts.

New methylene blue solution: this solution is prepared as follows:

REFERENCE

1. Weigh out 0.5 g of new methylene blue N, 1.4 g of potassium oxalate, and 0.8 g of sodium chloride. Place these

Henry JB (ed.). Clinical Diagnosis and Management by Laboratory

Methods, Philadelphia, PA: Saunders, 1984:1444.

chemicals in a 100-mL volumetric flask, dilute to the calibration mark with distilled water.

RED BLOOD CELL INDICES

2. Mix well. Place in a clean brown bottle that is properly

The erythrocyte indices (see Chapter 10) are used to math—

label with the name of the reagent, date of preparation,

ematically of the cell size and the concentration of hemoglobin—

and name of the individual who prepared the solution.

bin within the cell. They are as follows:

3. Filter solution daily or immediately before use to remove any precipitate.

1. MCV

2. Mean corpuscular hemoglobin (MCH)

Supplies and Equipment

3. MCHC

1. Capillary tubes

2. Glass slides

RETICULOCYTE COUNT: NEW METHYLENE

3. Wright or Wright-Giemsa stain

BLUE METHOD

4. Microscope, lens paper, and immersion oil

Principle

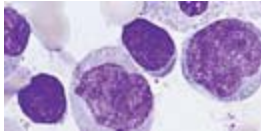
5. Miller ocular disc (optional)

Supravital stains, such as new methylene blue N or brilliant

An alternative specimen collection and processing method

cresyl blue, bin, neutralize, and cross-link RNA. These stains

is Ret-tic (bioanalytic GmbH.com). See package insert or the cause the ribosomal and residual RNA to coprecipitate with procedure.



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HEMATOLOGY PROCEDURES (continued)

Procedure

1. One third of a capillary tube should be filled with well-mixed blood.
2. An equal amount of fuchsine stain is then drawn into the tube. The tube is rotated back and forth by hand.
3. An alternative method is to mix two drops of blood and two drops of fuchsine stain.

A

4. Allow this mixture to stand for at least 10 minutes.
5. Gently remix and expel small drops of the stain and blood mixture onto several microscope slides and prepare

B

smears.

6. Air-ry.

7. wo or three rie sli es may be counterstaine

with Wright stain (see proce ure or staining bloo
smears).

FIGURE 32.5 Miller ocular isc. Square A is nine times the area

8. Using the 10× microscope objective, ocus the smear. A

o square B. Reticulocytes are counte in square A; erythrocytes are

a rop o oil to the sli e, an move to the oil immersion

counte in square B in successive microscopic f el s until at least

(100×) objective. T e appropriate counting area is the por—

300 RBCs are counte .

tion o the smear where the erythrocytes are evenly istribute an not
overlapping. Be ore beginning the count,

or anemia is a itionally help ul or clinical interpretation o

scan the sli e to check that reticulocytes can be locate

the reticulocyte count (see Chapter 5).

on that sli e.

9. o count the reticulocytes, a minimum o 1,000 (both

Miller Ocular Disc

reticulin-containing and nonreticulate) erythrocytes

Principle

must be counted. Normally, 500 erythrocytes will be counted on each of two slides. The number of reticu—

A Miller ocular is inserted into the eyepiece of the micro—

scopelococytes on these two slides do not agree within 20%,

the microscope permits a rapid survey of erythrocytes. This is (see

Figure 32.5) imposes two squares (one nine times the area of the

sure to count all cells that contain a blue-staining fla—

other) onto the field of view.

ment, fragment, or granule of reticulum in the erythrocyte. The counting field

can be reduced by using paper

Procedure

hole reinforcements or small pieces of paper cut to fit the

Reticulocytes are counted in the large square and erythro—

oculars with a small hole cut out in the middle of each.

cytes in the small square in successive microscopic fields

this makes counting easier than viewing the entire

until at least 300 RBCs are counted. This allows for an esti—

field.

mate o reticulocytes among a minimum population o 200 erythrocytes. T e absolute reticulocyte count can be eter—

Note: A Miller ocular isc can be use to acilitate counting mine by multiplying the reticulocyte percentage by the the number o reticulocytes an total RBCs.

RBC count.

Calculations

Calculations

I 47 reticulocytes are oun when 1,000 erythrocytes are examine (47 reticulocytes an 953 mature erythrocytes),

No. o reticulocytes in large squares

the reticulocyte count is calculate as ollows:

Reticulocytes =

× 100

(expresse in percentage)

No. o RBCs in sm

all squares × 9

47

× 100

= 4 7

. %reticulocytes (uncorrected)

1,000

Example: Given that there are 50 reticulocytes in the large squares and 300 red blood cells in the small squares,

Reporting Results

50

Reticulocytes =

×

100 = 1.886

Reference values: 0.5% to 1.5%; neonates, 2.5% to 6.5%. Some (expressed in percentage)

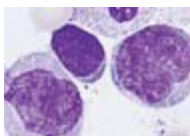
300×9

laboratories express the reticulocyte count in absolute rather than proportional terms. Reporting in absolute terms is

Note: Reticulocytes may also be counted by automated

becoming the preferred method of reporting. The correction methods.

(continued)



PART 8 ■ Fundamentals of Hematological Analysis

HEMATOLOGY PROCEDURES (continued)

se imente in a special tube that is place perpen icular in

De cre as e d

a rack or 1 hour. T e clinical value o this proce ure is in

Re ticulocyte s**Incre as e d Re ticulocyte s**

the iagnosis an monitoring o in ammatory or in ectious

Aplastic anemia

Blood loss

states.

Aplastic crises of

Crisis associated with

Specimen

hemolytic anemia

hemolytic anemia

Fresh anticoagulate bloo collecte in either so ium citrate

Chemotherapeutic

Subsequent to treatment of

or EDTA may be used. Sodium citrate is the preferred anti—

or radiation-induced

pernicious anemia, folic acid

coagulant, and the specimen must fill the entire tube—in an

hypoproliferation

deficiency, or iron deficiency

evacuate tube is used—to achieve the correct ratio of blood

Pernicious anemia

to anticoagulant. The ratio is 4 vol of blood to 1 vol of sodium

citrate. If EDTA anticoagulant is used, it must be diluted to the

Decreased erythropoiesis

ratio of 4 vol of blood to 1 vol of 0.9% sodium chloride.

Sources of Error

Blood should be at room temperature for testing and should

be no more than 2 hours old. If anticoagulated blood is refrigerated—

A refractile appearance of erythrocytes should not be con—

ferate, the test must be set up within 6 hours. Hemolyze

use with reticulocytes. Refractile bodies are due to poor

specimens cannot be used.

rying owing to moisture in the air. Filtration of the stain is

essential because precipitate can resemble a reticulocyte.

Reagents, Supplies, and Equipment

Erythrocyte inclusions should not be mistaken for reticulo—

1. Westergren pipettes.

locytes. Howell-Jolly bodies appear as one or sometimes two,

2. Vertical rack: this special rack is equipped with a leveling

deep-purple, dense structures. Heinz bodies stain a light blue—

bubble device to ensure that the tubes are held in a verti—

green and are usually present at the edge of the erythrocyte.

cal position within 1 hour. The fittings on the rack should

Pappenheimer bodies are more often confused with reticulo—

be clean and uncracked to prevent leakage of the dilute

cytes and are the most difficult to distinguish. These purple—

blood.

staining iron deposits generally appear as several granules in

a small cluster. If Pappenheimer bodies are suspected, stain

Procedure

with Wright-Giemsa to verify their presence.

1. Mix the blood citrate or blood-EDTA-saline mixture

Falsely decrease reticulocyte counts can result from

thoroughly.

unstaining the blood with new methylene blue. High glucose—

2. Aspirate a bubble-free specimen into a clean analytical
cose levels can also cause reticulocytes to stain poorly.

Westergren pipette. Fill to the zero mark. Do not pipette
by mouth.

Clinical Applications

3. Place the pipette into the vertical rack at 20°C to 25°C in
Selecte Disorders Associate with Abnormal Results
an area free from vibrations, drafts, and direct sunlight.

4. After 60 minutes, read the distance in millimeters from

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the bottom of the plasma meniscus to the top of the sedimented erythrocytes.

Provide on this book's companion Web site at <http://thepoint.lww.com/surgeon6e>.

5. Record the value as millimeters in 1 hour.

<http://thepoint.lww.com/surgeon6e>.

Reporting Results

The reference value of this test varies depending on age. In per-

SEDIMENTATION RATE OF ERYTHROCYTES:

persons younger than 50 years of age, the average reference values

WESTERGREN METHOD

are up to 10 mm/h in males and 13 mm/h in females. For the Westergren method has been selected as the method of

persons older than 50 years of age, average reference values are up to 13 mm/h in males and up to 20 mm/h in females. choice by the CLSI.

to 13 mm/h in males and up to 20 mm/h in females.

Principle

Procedure Notes

The erythrocyte sedimentation rate (ESR), also called the sedimentation rate,

Sources of Error

rate, measures the rate of settling of erythrocytes in dilute

Numerous sources of error have been cited for the ESR procedure—

human plasma. This phenomenon depends on an interrelationship—

ure. The age of the specimen is important, the test should be

relationship of variables, such as the plasma protein composition,

performed at 20°C to 25°C, and the blood should be at room

the concentration of erythrocytes, and the shape of the erythrocyte—

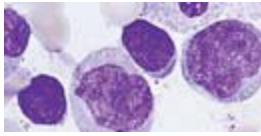
temperature. Other sources of error include incorrect ratios of

erythrocytes. The ESR value is determined by measuring the distance—

blood and anticoagulant, bubbles in the Westergren tube, and

distance from the surface meniscus to the top of the erythrocyte

tilting of the ESR tube. Tilting of the tube accelerates the fall of



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HEMATOLOGY PROCEDURES (continued)

erythrocytes, at an angle of even 3 degrees from the vertical

3. Place the tip of the pipette (filled with blood) into the

can accelerate sedimentation by as much as 30%.

Wintrobe tube until the tip touches the bottom of the tube.

4. Gently begin to press the pipette bulb and slowly move

Clinical Applications

the pipette tip up from the bottom of the tube. Continuous

the ESR is directly proportional to the weight of the

pressure must be kept on the pipette bulb while the pipette

cell aggregate and inversely proportional to the surface

tip is moved up from the bottom of the tube. The pipette

area. Microcytes sediment more slowly than macrocytes.

tip must be in continuous motion to avoid introducing air

Erythrocytes with abnormal or irregular shapes, such as bubbles into the column of blood.

sickle cells or spherocytes, hinder rouleaux formation and

5. The Westergren tube must be filled to the zero mark.

lower the ESR. The removal of fibrinogen by defibrination

6. Place the tube into a Westergren tube holder that has been also produces a decrease ESR.

adjusted to a perfectly level position.

An increase ESR value can be seen owing to various

7. Allow the tube to stand for 1 hour at room temperature in abnormal blood conditions: rouleaux, increase of fibrinogen at room temperature.

levels, a relative increase of plasma globulins caused by the

8. Read the tube from the bottom of the plasma meniscus to

loss of plasma albumin, and an absolute increase of plasma

the top of the sedimented erythrocytes. Each line on the

globulins. Clinical conditions associated with increase

tube represents 1 mm.

ESR values include anemia, infections, inflammation, tissue necrosis (such as myocardial infarction), pregnancy, and

Reporting Results

some types of hemolytic anemia.

The patient's value is reported in millimeters per hour. The reference value is 0 to 20 mm/h for men and 0 to 9 mm/h

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for men.

Procedure Notes

Provide on this book's companion Web site at <http://thepoint.lww.com/surgeon6e>.

One of the major drawbacks of this procedure is that the 100-mm tube length and the narrow bore of the tube limit readings in excess of 60 mm/h. Care must be taken to avoid introducing

air bubbles into the column and to fill the tube to the zero mark.

SEDIMENTATION RATE OF ERYTHROCYTES:

air bubbles into the column and to fill the tube to the zero mark.

WINTROBE METHOD

Principle

Sources of Error

See Principle at the Westergren Method (above) for details.

Falsely increased results can be produced by

1. Positioning the tube at an incline rather than in a vertical

Specimen

position

Fresh blood collected in EDTA anticoagulant may be used. A

2. Allowing the tube to stand for longer than 1 hour

minimum of 2 mL of whole blood is needed. The specimen

3. A room temperature above normal

must be well mixed, and the procedure must be performed

4. Falsely decreased results can be produced by

within 2 hours of blood collection.

5. An improper concentration of anticoagulant-whole blood

Reagents, Supplies, and Equipment

ratio

6. Anticoagulate blood that is more than 2 hours old

1. Wintrobe sedimentation tubes: these tubes are available in either

7. Allowing the tube to stand for less than 1 hour

reusable glass or disposable form. Depending on the type

8. Refrigerate blood or decrease room temperature

of Wintrobe rack use, the choice of tube includes graduated or plain.

Clinical Applications

2. Wintrobe sedimentation rack (graduated or plain).

Refer to the Westergren Method above.

3. Pasteur pipette (long-tippe) an rubber pipette bulb.

Procedure

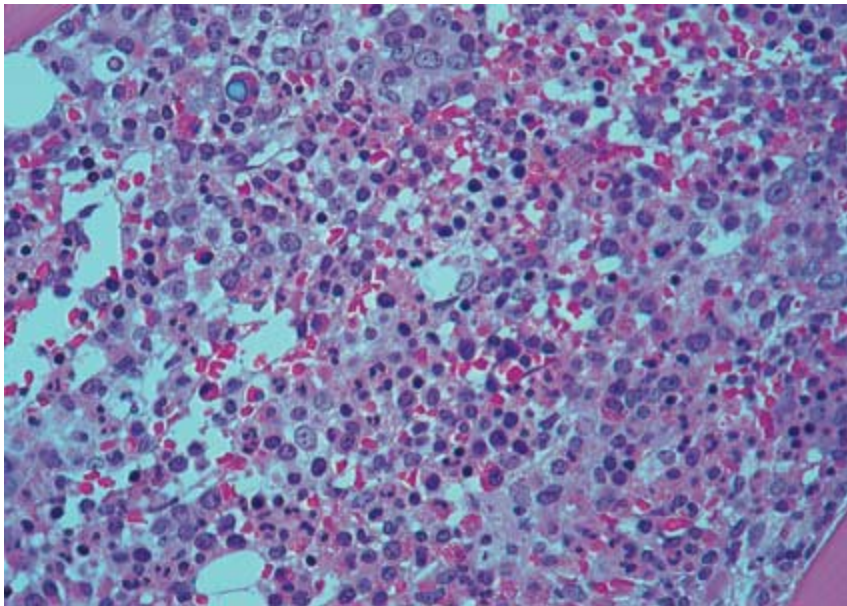
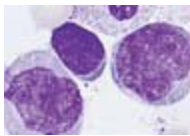
BIBLIOGRAPHY

1. Gently an thoroughly mix the anticoagulate bloo .
2. Draw as much bloo as possible into the Pasteur pipette

Provi e on this book's companion Web site at <http://thepoint.lww.com/surgeon6e>.

with the attache pipette bulb.

lww.com/ surgeon6e.



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PART 8 ■ Fundamentals of Hematological Analysis

SPECIAL HEMATOLOGY PROCEDURES

ACIDIFIED SERUM LYSIS TEST: HAM METHOD

Principle

Erythrocytes are incubated with fresh and heated serum to test

for hemolysis. Weak acid is used in specific serum cell mixtures to maximize hemolytic activity. The presence of hemolysis, depending on the test conditions, may be observed in

cases of antibody-sensitized coated erythrocytes, spherocytes,

or paroxysmal nocturnal hemoglobinuria (PNH).

This procedure, in CLSI format, is provided on this book's

companion Web site at <http://thepoint.lww.com/nurse6e>.

BONE MARROW EXAMINATION

Principle

FIGURE 32.6 Bone marrow biopsy sections demonstrate normal

A bone marrow aspiration is performed by a physician to

cellularity. Virtually 100% cellular marrow from a newborn boy.

examine the cellular activities of the marrow. Properly prepare

(Reprinted from McClatchey KD. Clinical Laboratory Medicine,

specimens are usually stained with a Wright-Giemsa stain and

2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins, 2002,

special stains, such as Prussian blue, and cytochemical stains

with permission.)

or various enzymes. A specimen of the marrow is also examined histologically using a hematoxylin-eosin (H&E) stain.

osteoclasts, and mast cells. Reticulum cells are present

Bone marrow examination is valuable in the diagnosis of

macrophages that represent the skeletal and structural

disorders specifically involving the marrow, such as multiple

components of the marrow sinuses. Osteoblasts and osteo—

myeloma, and in the study of leukemias and some types of

clasts are uncommon in marrow aspirates because they are

anemia. In most cases, the bone marrow presents the early

not involved in hematopoiesis. The function of osteoblasts

developmental events that produce the blood picture seen in

and osteoclasts is formation and remodeling of bone. Mast

peripheral blood or evidence of an underlying systemic disease.

cells are connective tissue cells with no definite ancestral

relationship to the blood basophil or its precursors. Mast

Specimen

cells along with plasma cells are characteristic of marrow

Refer to Chapter 2 for details on specimen collection and

amplification or depletion. Clusters of metastatic neoplastic

Wright-Giemsa staining. For details on special stains, refer

cells may also be observed in bone marrow smears.

to the specific staining procedure in this section. A peripheral

3. Using duplicate bone marrow smears, any special stains

blood smear should also be collected on the same day as the

(e.g., iron) should be promptly performed and examined.

bone marrow aspiration.

4. The peripheral blood smear should be simultaneously

examined.

Procedure

Examination of Bone Marrow Slides

Reporting Results

1. Using the 10× objective, the smear is scanned for any

1. The technologist usually refers the slides and slide examined—

apparent overall cellular abnormalities. An estimation of

cellularity report to a pathologist or comparison with the

cellularity can also be appraised. Semiquantitative assessment—

H&E preparation. The cellularity of the specimen is usually—

measurements of cellularity in aspirates can be classified into hypoplastic, normoplastic, or hyperplastic levels. Cellularity varies

myeloid-erythroid (M/E) ratio is determined from the bone

with a patient's age and the site of the bone marrow aspiration—
marrow aspiration slides. The pathologist will then assign
a rating. Marrow cellularity is expressed as the ratio of the
diagnosis to the case and suggest supplementary tests, if
volume of hematopoietic cells to the total volume of the
necessary.

marrow space (cells plus fat as well as other stromal elements) (see Fig. 32.6).

Normal Distribution of Bone Marrow Cells in an Adult

2. Using the 100× (oil) immersion objective, a differential

Cell Type

Mean %

count of at least 200 cells is performed. Any abnormalities in distribution will be
apparent by this examination.

Rubricytic series

21.5

Erythrocyte maturational and morphological abnormalities—

Rubriblasts

0.6

ties and megakaryocyte morphology should be examined using cell differentiation.
Nonhematopoietic cells

Prorubricytes

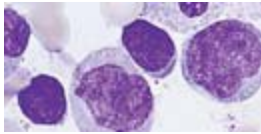
2.0

o normal bone marrow may also be seen. These cells are

Rubricytes

12.4

reticulum cells (marrow macrophages), osteoblasts and



CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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SPECIAL HEMATOLOGY PROCEDURES (continued)

nucleotides, when activated with long-wave (340 to 370 nm)

Cell Type

Mean %

ultraviolet (UV) light, is used for visual examination of G6PD

Metarubricytes

6.5

activity. The observed rate of the appearance of bright fluorescence is proportional to the blood G6PD activity. G6PD

Neutrophil series

56.0

efficiency is one of the most prevalent hereditary erythrocyte

Blasts

1.0

enzyme deficiencies. A deficiency of this enzyme can produce

Promyelocytes

3.4

drug-or stress-induced hemolytic anemia in alcoholic persons.

Myelocytes

11.9

This procedure, in CLSI format, is provided on this book's companion Web site at <http://thepoint.lww.com/nurseon6e>.

Metamyelocytes

18.0

Bands

11.0

HEMOGLOBIN ELECTROPHORESIS : CELLULOSE ACETATE METHOD

PMNs

10.7

Principle

Eosinophil series

3.2

Electrophoresis may be defined as the movement of charge

Basophils

<0.1

particles on various media under the influence of an electric

Lymphocytes

15.8

current. Particles move at different speeds because of their

Monocytes

1.8

weight and electric charge.

Megakaryocytes

<0.1

In hemoglobin electrophoresis, a hemolysate prepared from

intact erythrocytes is placed on a medium such as cellulose

Reticulum cells

0.3

acetate. The strips of cellulose acetate are placed in an alkali—

Plasma cells

1.8

line buffer (pH 8.0 to 8.6), an electrical charge is applied. The

M/E ratio

2.5:1

strips are stained to see the hemoglobin reactions. A comparison of the unknown hemolysate with hemolysates from known

hemoglobin types is made. Hemoglobin electrophoresis by cel-

BIBLIOGRAPHY

lulose acetate is useful in identifying and quantifying hemoglobin variants and abnormal quantities of hemoglobin reactions.

Provided on this book's companion Web site at <http://thepoint.lww.com/surgeon6e>.

This procedure, in CLSI format, is provided on this book's

<http://thepoint.lww.com/surgeon6e>.

companion Web site at <http://thepoint.lww.com/surgeon6e>.

DONATH-LANDSTEINER'S SCREENING TEST

Analysis of Hemoglobin

Principle

Since the first hemoglobinopathy was described in 1910, sev—

The Donath-Landsteiner antibody test is used to demonstrate

eral techniques have been developed to study hemoglobin.

the presence of this extremely potent hemolysin. This antibody

They include hemoglobin electrophoresis with various media, requires col incubation to exhibit hemolysis in the patient's such as paper, cellulose acetate, or agar; solubility testing; an serum. A positive result is diagnostic of paroxysmal col hemoglo— enaturation of hemoglobin through the use of acid or alkali— binuria (PCH), the rarest form of autoimmune hemolytic anemia. line solutions (see Chapter 17). A method for the preliminary This procedure, in CLSI format, is provided on this book's identification of abnormal hemoglobin is electrophoresis. companion Web site at <http://thepoint.lww.com/nurse6e>.

Alkaline Electrophoresis

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

This method of separating hemoglobin fractions is based on

ACTIVITY IN ERYTHROCYTES : VISUAL

the principle that hemoglobin molecules in an alkaline solu-

FLUORESCENT SCREENING TEST

tion have a net negative charge and move toward the anode in

Principle

an electrophoretic system. This screening procedure can separate enzyme glucose-6-phosphate dehydrogenase (G6PD)

variant hemoglobins A, F, S, and C and other variant hemoglo—

catalyzes the following reaction:

bins (Fig. 32.7). Those with a greater electrophoretic mobility than hemoglobin A at pH 8.6 are classified as the fast hemo—

Glucose-6-phosphate + NADP → G6PD

6

→

globins. Examples of fast hemoglobins are Bart hemoglobin (not fluorescent)

and the two fastest variants, hemoglobin H and hemoglobin -ph

6

6-phosphogluconate + NADPH

I. Hemoglobin C is the slowest common hemoglobin.

(not fluorescent)

Various media, such as paper, cellulose acetate, or starch blocks,

The reaction mixture containing glucose-6-phosphate,

and different buffers may be used for electrophoresis. These alter—

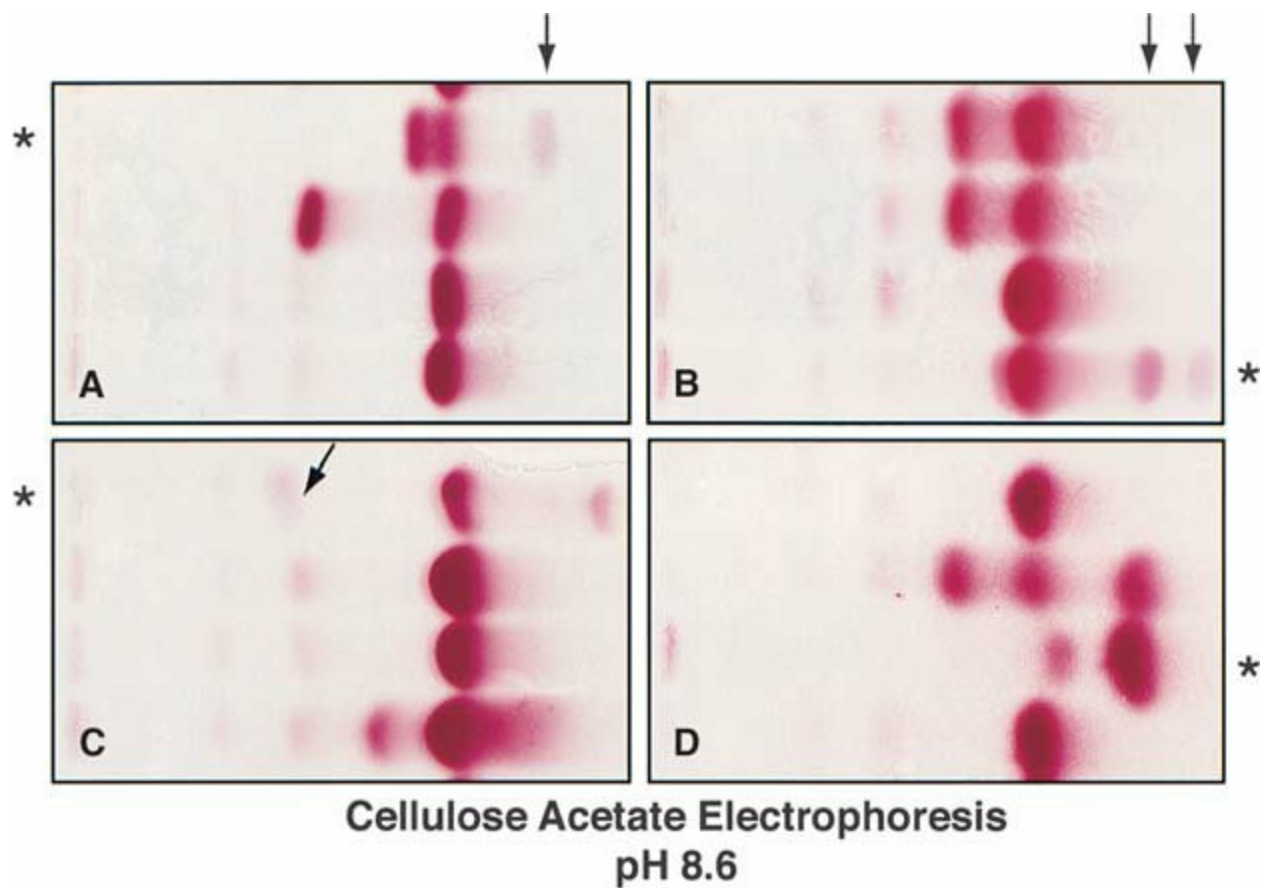
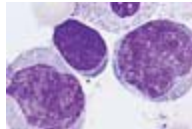
nicotinamide adenine dinucleotide phosphate (NADP),

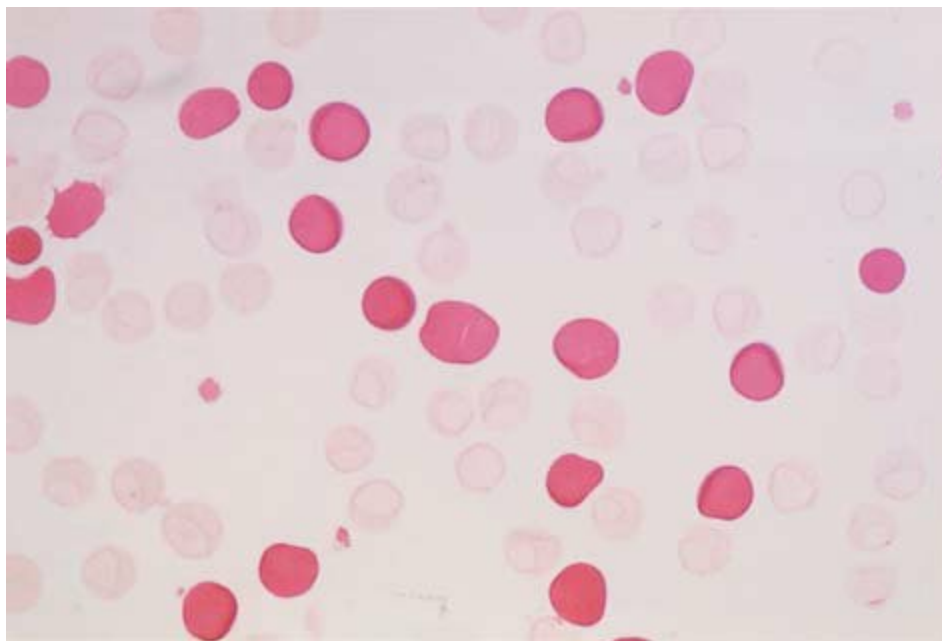
native methods vary in their efficiency of separation. For example,

and blood is incubated, and at time intervals, drops of the

cellulose acetate at alkaline pH is rapid and reproducible. The same mixture is applied to filter paper. The fluorescence of the mixture separates the hemoglobin fractions S, F, A, C, and A₂.

(continued)





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PART 8 ■ Fundamentals of Hematological Analysis

SPECIAL HEMATOLOGY PROCEDURES (continued)

FIGURE 32.7 Electrophoresis. **A.** Specimen from a

neonate. Hb-Barts is present (arrow) in indicating a thalassemia. **B.** Specimen from a 3-month-old. In addition to

Hb-Barts, there is also a small amount of Hb H (double

arrow). **C.** Hb H-Hb-Constant Spring. There is a dullness in the Hb A₂ area (arrow) in indicating the presence

of Hb-Constant Spring. **D.** Hb-Barts hydrops fetalis.

There is no Hb A present. The majority of the hemoglobin is Hb-Barts, with a small amount of Hb-Portland.

(Reprinted from McClatchey KD. Clinical Laboratory

Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams

& Wilkins, 2002, with permission.)

Citrate Agar Electrophoresis

This test involves acid denaturation of hemoglobin. Fetal

This process takes place at an acid pH. In this method, hemoglobin—

hemoglobin resists denaturation, whereas adult hemoglobin

bands are separated on the basis of a complex interaction between

does not. Occasionally, intermediate (partially denatured)

hemoglobin, agar, and citrate buffer ions. Citrate agar separates

cells may be seen that are almost surely fetal hemoglobin—

hemoglobin fractions that migrate together on cellulose acetate.

containing cells. Elevated levels of hemoglobin F can be seen

These fractions are hemoglobins S, D, G, C, E, and O. All hemo—

in beta-thalassemia, a form of anemia, and paroxysmal nocturnal

hemoglobin specimens that show an abnormal electrophoretic pat—

ternal hemoglobinuria (PNH).

tern in alkaline media should undergo electrophoresis on acid

Chromatography

citrate agar. The combination allows for a complete

identification of many variant hemoglobins.

Quantitation of hemoglobin A1 can be accomplished by cation

exchange minicolumn chromatography. However, the results

Denaturation Procedures

o this technique can be affected by several types of hemoglobin—

A procedure commonly used to determine the amount

of fetal hemoglobin in addition to hemoglobin A1. Cellulose acetate and citrate

buffered gel that has mixed with maternal blood follow—

agar electrophoresis should be used in conjunction with staining. Delivery is the Kleihauer-Betke (Fig. 32.8) procedure.

ion exchange chromatography to eliminate the possibility of

interference by hemoglobin variants. Other assay methods or

glycosylated hemoglobin include high-pressure liquid chromatography (HPLC) and colorimetric methods.

Analysis of Hemoglobin

■ Several techniques have been developed to study hemoglobin including hemoglobin electrophoresis, solubility

testing, and denaturation of hemoglobin through the use

of acidic or alkaline solutions.

■ Alkaline electrophoresis is a method of separating hemoglobin fractions based on the principle that hemoglobin

molecules in an alkaline solution have a net negative

charge and move toward the anode in an electrophoretic

system.

■ Various media and different buffers may be used for elec-

FIGURE 32.8 Kleihauer-Betke stain. Kleihauer-Betke acid elu—

trophoresis. For example, cellulose acetate at alkaline pH

tion of fetal hemoglobin (Hb). Red blood cells containing HbF

is rapidly reproducible.

are deeply stained red; red cells containing HbA appear as pale

■

pink ghosts. (Reprinted from Greer JP (ed). Wintrobe's Clinical

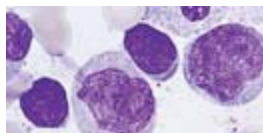
A procedure used to determine the amount of fetal blood

Hematology, 11th ed, Philadelphia, PA: Lippincott Williams &

that has mixed with maternal blood following delivery is

Wilkins, 2004, with permission.)

the Kleihauer-Betke procedure.



CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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SPECIAL HEMATOLOGY PROCEDURES (continued)

■ Chromatography can be used to quantitate hemoglobin

o solution A an 73.3 mL o solution B. Bu er (6.8 pH).

A1. Assay metho s or glycosylate hemoglobin inclu e

Mix 49.6 mL o solution A an 50.4 mL o solution B.

high-pressure liqui chromatography (HPLC) an colo—

Refrigerate all solutions to store.

rimetric metho s.

7. Giemsa stock stain: Prepare by a ing 5 g o pow ere

Giemsa stain to 330 mL o reagent-gra e glycerol. Mix.

HEMOGLOBIN S S CREENING TEST: QUALITATIVE

Place in a 60°C oven or 2 hours. Allow to cool. Mix. With

DIFFERENTIAL S OLUBILITY TEST

constant stirring, slowly a 330 mL o methanol. rans er

Principle

to a stoppere brown bottle an shake or a ew minutes.

T is is a biphasic system consisting o an upper organic phase

Label. Filter before use.

o toluene an a lower, aqueous phase containing phosphate

Working Giemsa Stain: Prepare by a ing 10 mL o f ltere

bu er, saponin, an re ucing agents. Erythrocytes are lyse

Giemsa stock stain to 90 mL o bu er solution. Mix.

by toluene and saponin, with the released hemoglobin being reduced by sodium hydrosulfite. The resulting colors of the

1. Coplin or other type of staining jar and slide holder aqueous phase and the interface phase allow for the identification of hemoglobin types AA, AS, and SS.
2. Microscope, immersion oil, and lens paper

Quality Control

Detection of the abnormal Hb S is diagnostic of sickle cell disease. Hb S, if inherited in the homozygous state (SS), results in sickle cell anemia. Inheritance of the heterozygous state (AS) produces a benign and asymptomatic condition, except in individuals who are involved in strenuous physical sports, such as long-distance runners. Participation in a quality assurance program, such as the College of American Pathologists program, is important in maintaining expertise in this area, particularly in laboratories that frequently encounter positive results.

istance runners, or in individuals whose occupations have

Procedure

the potential to reduce oxygen levels, such as test pilots.

This procedure, in CLSI format, is provided on this book's

1. Following blood smear preparation, allow the smears to
companion Web site at <http://thepoint.lww.com/nurseon6e>.

air-dry for approximately 30 minutes. Fix the thin smears
in methanol for a few seconds. Do not fix the thick smears.

MALARIAL SMEARS

2. Place the smears in a Coplin jar containing Giemsa stain—

Principle

ing solution for 30 minutes. Rinse the smears in running
tap water and allow to air-dry.

Thick and thin blood smears are prepared, stained, and exam—

3. Examine the smears using the oil immersion objective.

ine microscopically for the presence of one of our malaria

The erythrocytes on the thick, unfixed smears will be

types. Detection and correct identification of the species of

destroyed, making examination easier. The thick smear is

malaria (*Plasmodium malariae*, *P. vivax*, *P. falciparum*, or

use as a screening test to establish the presence of the

P. ovale) are important to ensure proper treatment.

parasite. The thin smear, which allows for careful exami—

Specimen

nation of cellular morphology, permits identification of

Smears of capillary or EDTA-anticoagulated blood are pre—

the species of malaria.

prepare as describe in the section on specimen preparation

Reporting Results

in Chapter 2.

The diagnosis of malaria is based on the demonstration of

Reagents, Supplies, and Equipment

the *Plasmodium* species in the blood. Refer to Figure 32.9

1. American Chemical Society (ACS)-grade methanol

or illustrations of the typical appearance of various species.

2. ACS-grade glycerol

A brief morphological description is given in Table 32.2. For

3. Buffer (pH 6.4 or 6.8)

a complete discussion of each of the *Plasmodium* species, as

4. Solution A: Prepare by placing 9.47 g of anhydrous sodium

well as other actors relate to malaria, see Chapter 7.

sodium phosphate (Na_2HPO_4) or 11.87 g of hydrate sodium

phosphate (Na

Procedure Notes

$2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) into a 1-L volumetric flask.

Dilute to the calibration mark with deionized water. Mix.

Sources of Error

5. Solution B: Weigh and transfer 9.080 g of primary potassium—

Malarial parasites can be confused with platelets. It is

sodium phosphate (KH_2PO_4) into a 1-L volumetric flask.

important to distinguish between malarial parasites in the

Dilute to the calibration mark with deionized water. Mix.

erythrocyte and platelets that are superimposed on the eryth—

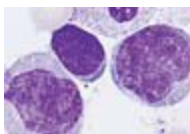
6. Solutions A and B can be mixed in various proportions

erythrocyte. Malarial parasites are never seen in the spaces between

to achieve a different pH. Buffer (6.4 pH). Mix 26.7 mL

erythrocytes.

(continued)



PART 8 ■ Fundamentals of Hematological Analysis

SPECIAL HEMATOLOGY PROCEDURES (continued)

Early "ring" forms

Trophozoite

Schizont

Gametocytes

m

Not usually

Not usually

u

r

a

seen in

seen in

p

i

peripheral

peripheral

c

l

a

blood

blood

f

P.

e

l

a

v

o

P.

&

x

a

v

i

v

P.

e

a

i

r

a

l

a

m

P.

FIGURE 32.9 Malarial parasites in blood cells. This schematic drawing illustrates the most prominent morphological features that distinguish human malarial species in blood smears. The nuclear chromatin bodies of all of the malarial parasites are shaded in dark blue-gray in this diagram but actually appear red in Giemsa-stained preparations. *Plasmodium falciparum* usually appears as small, fine, ring forms, sometimes more than one per red blood cell.

More-mature forms of the species are not usually seen in the peripheral blood. The gametocyte is characteristically banana shaped. *P. vivax* and *P. ovale* are distinguished by details not illustrated here. In infected cells, ring forms are larger than those of *P. falciparum*. At the trophozoite stage, red schüner dots are seen. The schizont contains more than a dozen merozoites before it ruptures. *P. malariae* infects smaller, senescent cells. Schüner dots are not present.

At the schizont stage, 8 to 12 merozoites are arranged peripherally around the central malarial pigment. (Reprinted from Engleberg NC, Dermody J, DiRita V. Schacter's Mechanisms of Microbial Disease, 4th ed., Baltimore, MD: Lippincott Williams & Wilkins, 2007, with permission.) TABLE

32.2 Morphological Comparison of Plasmodium sp.

RBC Appearance

Parasitic Appearance

Species

Size

Inclusions

Cytoplasm

Merozoites

P. vivax

Enlarged

Schuffner dots

Blue discs with red nucleus

12–24

Acute forms

Signet ring forms

P. falciparum

Normal

Maurer dots

Minute rings

6–32

Two chromatic dots

Accole forms

Gametes: crescent shaped

P. malariae

Normal

Ziemann dots

One ring with one dot

6–12

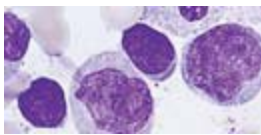
P. ovale

Enlarged

Schuffner dots

One ring form

6–14



CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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SPECIAL HEMATOLOGY PROCEDURES (continued)

Clinical Applications

type. The basic principle of the absorption steps in this process—

It is important to recognize and distinguish between the vari—

ure is comparable to that originally described by Davidsohn

ous types of malaria to properly treat the patient. Treatment is

in his sheep agglutinin test. Serum or plasma is absorbed with

important because plasmodia infect and destroy erythrocytes.

both guinea pig kidney and bee erythrocyte stroma. Guinea

pig kidney contains only the Forssman antigen, and bee eryth—

Limitations

rocytes contain only the antigen associated with infectious

The process is tedious, and it is frequently very difficult to

mononucleosis. Guinea pig kidney will absorb only heterophile

located in infected erythrocytes.

antibodies of the Forssman type, and bee erythrocytes will

absorb only the heterophile antibody of infectious mononucleo-

BIBLIOGRAPHY

sis. Agglutination of horse RBCs by the absorbed patient specimen is indicative of a positive reaction or heterophile antibody.

Provide on this book's companion Web site at <http://thepoint.lww.com/surgon6e>.

This procedure, in CLSI format, is provided on this book's

<http://thepoint.lww.com/surgon6e>.

companion Web site at <http://thepoint.lww.com/urgeson6e>.

OSMOTIC FRAGILITY OF ERYTHROCYTES :

MONOS POT TEST (ORTHO DIAGNOSTICS,

DACIE METHOD

RARITAN, NJ)

Principle

Principle

In the osmotic fragility test, whole blood is exposed to varying

This procedure is based on agglutination of horse erythrocytes

concentrations of sodium chloride solution and allowed to

by heterophile antibody present in infectious mononucleosis.

incubate at room temperature. The amount of hemolysis is

Because horse RBCs exhibit antigens directly against both

then determine by examining the supernatant fluid either

Forssman and infectious mononucleosis antibodies, a difference—

visually or with a spectrophotometer.

tial absorption of the patient's serum is necessary to distinguish

Erythrocytes are placed in an isotonic solution (0.85%)

the specific heterophile antibody from those of the Forssman

sodium chloride, water molecules will pass in and out of

Incubated Osmotic Fragility

100

Positive osmotic

90

fragility

80

70

Normal

60

%

s

Control

i

50

ys

l

o

m

e

40

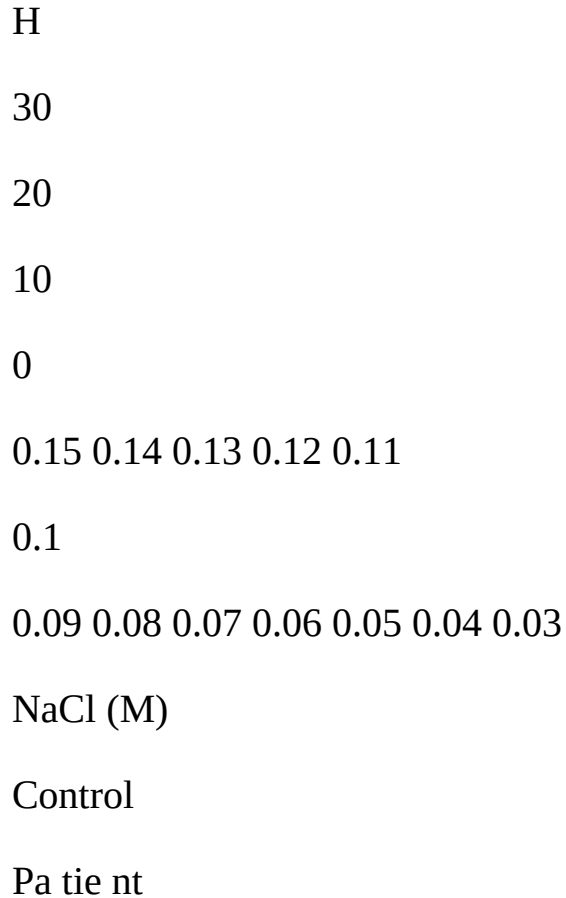
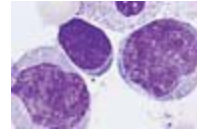
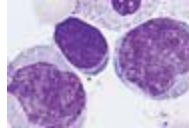


FIGURE 32.10 Osmotic fragility plot. The solid line depicts a leishman curve characteristic of hereditary spherocytosis.

However, other conditions such as immune hemolytic anemias characterized by increase numbers of spherocytes may also demonstrate a positive osmotic fragility result. The osmotic fragility test measures the in vitro lysis of red cells suspended in solutions of decreasing osmolarity. The red cell is freely water permeable, and thus, when red cells are placed into a hypotonic solution, water is osmotically drawn into the red cell. As the cell swells, it becomes spherical. After the critical volume of the cell is reached, the membrane begins to leak large molecules such as hemoglobin. This release of hemoglobin into the supernatant is measured spectrophotometrically. As a result of membrane loss, spherocytes have less capacity for additional swelling and thus are more fragile than normal red cells when placed into hypotonic solution. (Pereira I, George I, Arber DA. Atlas of Peripheral Blood. Philadelphia, PA: Lippincott Williams

& Wilkins, 2011.) (continued)



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PART 8 ■ Fundamentals of Hematological Analysis

SPECIAL HEMATOLOGY PROCEDURES (continued)

Normal

enter into the cell than leave. This net influx of water molecules

Fresh

Incubated

Range

cells eventually ruptures the cell membrane.

100

Hereditary

s

Spherocytosis

i

The main factor in this process is the shape of the erythrocytes

ys

l

o

rocyte, which is dependent on the volume, surface area, and

75

m

e

unctional state of the erythrocytic membrane. A spherocytic

h

t

n

50

erythrocyte ruptures much more quickly than normal erythrocyte

c

r

rocytes or erythrocytes that have a large surface area per volume

P

25

ume, such as target or sickle cells. The fragility of erythrocytes

is increased when the rate of hemolysis is increased. The rate

0

0.7 0.6 0.5 0.4 0.3 0.2

0.7 0.6 0.5 0.4 0.3 0.2

o hemolysis is decrease, the erythrocytic fragility is consi-

NaCl (g/dL)

ere to be decrease. The clinical value of the procedure is in

differentiating various types of anemias (Figs. 32.10 and 32.11).

FIGURE 32.11 Osmotic fragility (as manifested by percent hemolysis) of normal and hereditary spherocytosis (HS) erythrocytes

This procedure, in CLSI format, is provided on this book's

after incubation in salt solutions of varying tonicity. In fresh HS

companion Web site at <http://thepoint.lww.com/nurse6e>.

erythrocytes, note the “tail” of cells with increased sensitivity as a

result of splenic conditioning (**left**). In the incubated blood cell

SUCROSE HEMOLYSIS TEST

(RBC), note that the entire HS population of RBCs is more osmoti—

Principle

cally sensitive (**right**). (From Glauber BE, Naumovski L. Hereditary

erythrocytosis. In: Rimoin DL, Connor JM, Pyeritz RE,

Erythrocytes in PNH lyse when exposed to serum solutions of

et al., eds. Principles and Practice of Medical Genetics. New York:

low ionic strength containing complement. This test demon—

Churchill Livingstone, 1996, with permission.)

strates the sensitivity of erythrocytes to the protein, complement.

Normal erythrocytes under similar circumstances do not lyse.

the membrane in equal amounts. In hypotonic solutions,

This procedure, in CLSI format, is provided on this book's

erythrocytes will hemolyze because more water molecules

companion Web site at <http://thepoint.lww.com/nurse6e>.

SPECIAL STAINS

ACID PHOSPHATASE IN LEUKOCYTES :

than other leukocytes. Most of the acid phosphatase isoen-

CYTOCHEMICAL STAINING METHOD WITH AND

zyme is inhibited by L-tartaric acid. The cells of hairy cell

WITHOUT TARTRATE

leukemia, Sézary syndrome, and some T-cell acute lympho—

Principle

blastic leukemias are tartrate resistant.

Peripheral blood or bone marrow smears are fixed and incu—

In the absence of tartaric acid, all leukocytes demonstrate

activity in a solution of naphthol AS-BI phosphoric acid and

granular sites of enzyme activity. When incubated with tartrate, an occasional granule may be observed in lymphocytes and some specialized macrophages (e.g., Gaucher cells and hairy cells). In blood smears, a positive reaction is noted by the presence of more than two cells with intense acid phosphatase activity.

an intense activity (i.e., more than 40 granules).

Naphthol AS-BI phosphate → acid phosphatase → naphthol

Clinical Applications

AS-BI

This procedure distinguishes lymphocytes from other types of mononuclear cells. Because the cells of hairy cell leukemia are among the few abnormal cell types that are tartrate resistant, it is considered to be a significant marker in this disease.

Duplicate blood or bone marrow smears are incubated in a solution that also contains L-(+)-tartrate-containing substrate. This procedure in CLSI format is provided on this book's companion Web site at thepoint.lww.com/nurseon6e.

Cells containing tartrate-sensitive acid phosphatase do

ALKALINE PHOSPHATASE IN LEUKOCYTES :

not exhibit any deposits, whereas those mononuclear

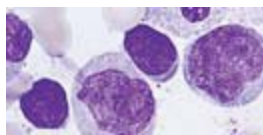
CYTOCHEMICAL STAINING METHOD

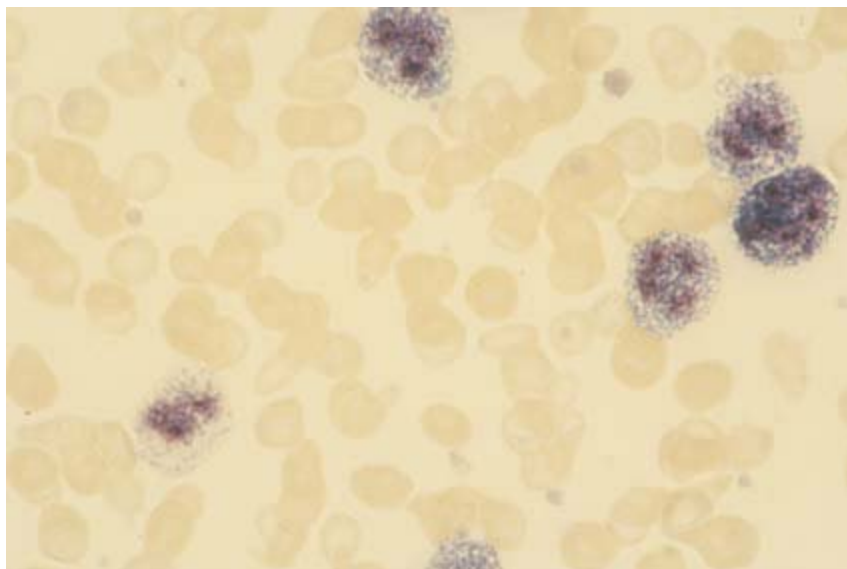
cells containing tartrate-resistant acid phosphatase are not affected by such treatment.

Principle

Most leukocytes exhibit a positive acid phosphatase reaction—

Leukocyte alkaline phosphatase (LAP) activity can be positive to varying degrees. Lymphocytes display less activity than granulocytes. LAP activity can be increased, normal, or decreased in a variety of conditions.





CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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SPECIAL STAINS (continued)

Leukocyte Alkaline Phosphatase

TABLE

32.3 (LAP) Chemical Stain

Scoring Characteristics

3+

Cell

Amount Size of

Rating (%)*

Granule

Stain Intensity

2+

0

None

—

None

1+

50

Small

Faint to moderate

2+

50–80

Small

Moderate to strong

3+

80–100

Medium to large Strong

FIGURE 32.12 Leukocyte alkaline phosphatase (LAP) stain. Cell

4+

100

Medium to large Brilliant

type: Granulocytes; distinguishes leukemoid reaction from chronic myelogenous leukemia. Description: LAP is an enzyme associated

*Percent volume of cytoplasm occupied by dye precipitate.

with the specific granules; presence of activity indicates intracellular metabolic activity; positivity is indicated by either a ruby red color or

Example:

a blue-purple color; positivity is quantitated; 100 consecutive bands
50 cells with a 4 rating 200

or segmented neutrophils are scored using the following criteria: 0,
colorless; 1, diffuse positivity, occasional granules; 2, diffuse posi—
30 cells with a 3 rating 90

tivity, moderate numbers of granules; 3, strong positivity, numerous—
20 cells with a 2 rating 40

ous granules; 4, very strong positivity, dark, confluent granules. The
total 100 cells 330 LAP score

scores of the 100 cells are summed. Clinical conditions: Increase
leukemoid reactions in polycythemia vera, pregnancy, infections,

Reporting Results

CML blast crisis, myelofibrosis. Decrease leukemoid reactions in

The normal range depends on the type of azo dye used. In fast
chronic myelogenous leukemia, paroxysmal nocturnal hemoglobinuria,
blue RR is used, the normal range is 32 to 182. In fast violet B
binuria, some myelodysplastic syndromes, idiopathic thrombocy—
topenic purpura. (Reprinted from Anderson SC. Anderson's Atlas
of Hematology, Philadelphia, PA: Wolters Kluwer Health/Lippincott
Clinical Applications
Williams & Wilkins, 2003, with permission.)

This test is clinically most useful in differentiating chronic
myelogenous leukemia from leukemoid reactions. Leukemoid
reactions may result from infections, toxic conditions, and neo—
plasms as well as miscellaneous conditions such as the treatment
leukemia.

Peripheral blood or bone marrow smears are fixed and

Alkaline phosphatase activity can be associated with vari—
incubate in an alkaline dye solution of naphthol AS-MX

Alkaline phosphatase activity can be associated with vari—
incubate in an alkaline dye solution of naphthol AS-MX

Alkaline phosphatase activity can be associated with vari—
incubate in an alkaline dye solution of naphthol AS-MX

Alkaline phosphatase activity can be associated with vari—
incubate in an alkaline dye solution of naphthol AS-MX

Alkaline phosphatase activity can be associated with vari—
incubate in an alkaline dye solution of naphthol AS-MX

Alkaline phosphatase activity can be associated with vari—
incubate in an alkaline dye solution of naphthol AS-MX

ous conditions and disorders. Postsurgical patients experience phosphate and fast blue RR salt or fast violet B salt. As the a rise in activity with a peak 2 to 3 days postoperatively and a result of phosphatase activity, naphthol AS-MX is liberated gradual return to normal values within 1 week. Persisting elevation of the LAP score is strong evidence of an active inflammatory process. LAP scores are useful in diagnosing ectopic (Fig. 32.12). The following reactions occur at cellular sites of pregnancy and an anovulatory menstrual cycle. The LAP score alkaline phosphatase activity:

is also useful in differentiating choriocarcinoma from hydatidiform mole because the test score is normal in choriocarcinoma and high in cases of hydatidiform mole.

This procedure in CLSI format is provided on this book's companion Web site at thepoint.lww.com/surgeon6e.

or

Naphthol AS-MX + fast violet B salt → violet pigment

ESTERASE (ALPHA-NAPHTHYL ACETATE ESTERASE) IN LEUKOCYTES: CYTOCHEMICAL

Obtaining Results

STAINING METHOD

To obtain the LAP activity score, the number of cells counted

Principle

In each category (0 to 4+) is multiplied by the value of that

Esterases are ubiquitous in nature and encompass a variety of

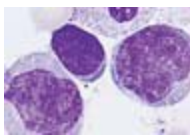
categories. These scores are summed or the cumulative total

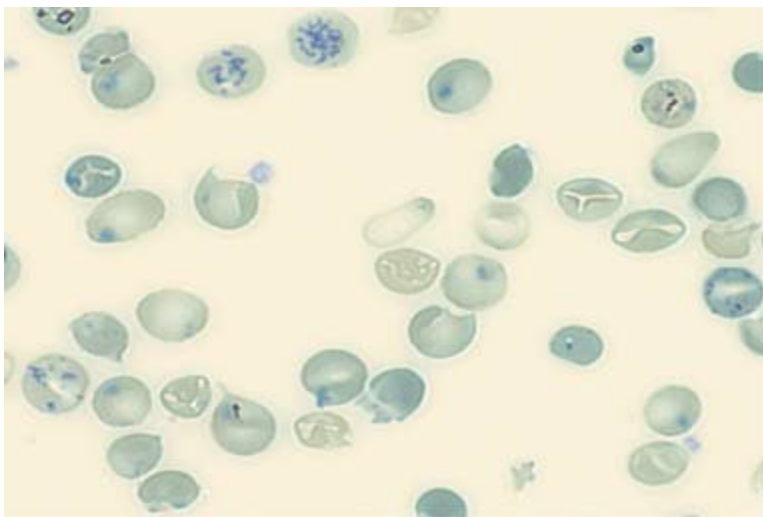
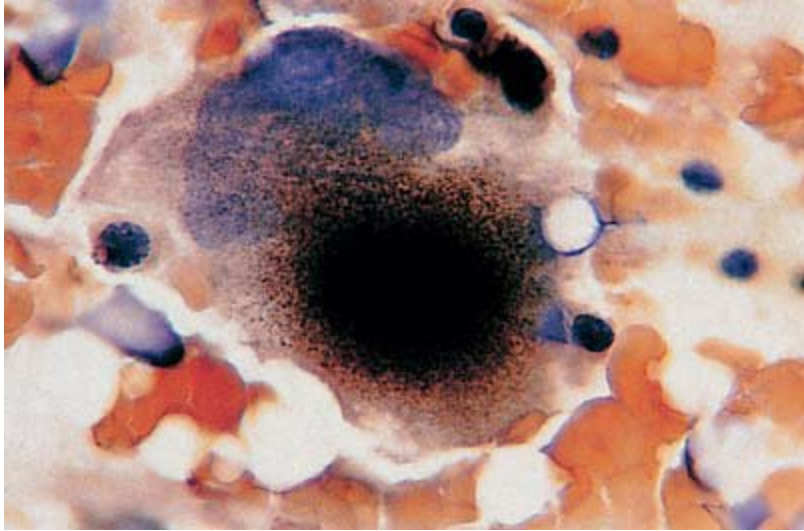
of different enzymes acting on selective substrates. In this process

(see table 32.3)

ure, blood or bone marrow smears or touch preparations are

(continued)





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PART 8 ■ Fundamentals of Hematological Analysis

SPECIAL STAINS (continued)

This procedure in CLSI format is provided on this book's companion Web site at thepoint.lww.com/nurseon6e.

ESTERASE (NAPHTHOL AS-D CHLOROACETATE ESTERASE) IN LEUKOCYTES: CYTOCHEMICAL

STAINING METHOD

Principle

See Alpha-Naphthyl Acetate Esterase.

In this procedure, blood or bone marrow smears are incubated with naphthol AS-D chloroacetate in the presence of a

stable diazonium salt. Naphthol compounds are coupled with

FIGURE 32.13 Strong α -naphthyl acetate esterase activity in

diazonium salt, forming highly colored deposits at the sites

of a normal megakaryocyte. (Reprinted from Mills SE. Histology

of enzyme activity.

for Pathologists, 3rd ed., Philadelphia, PA: Lippincott Williams &

Naphthol AS-D chloroacetate esterase enzyme is usually

Wilkins, 2007, with permission.)

considered specific for cells of granulocytic lineage. They

should show no granulation. Activity is weak or absent in

incubated with alpha-naphthyl acetate in the presence of a stable

monocytes and lymphocytes.

diazonium salt. Enzymatic hydrolysis of ester linkages liberates

This procedure in CLSI format is provided on this book's

free naphthol compounds. These naphthol compounds then

companion Web site at thepoint.lww.com/nurseon6e.

couple with diazonium salt, forming highly colored deposits at the sites of enzyme activity (Fig. 32.13). Under effective conditions,

HEINZ BODIES

this method provides a means to distinguish cells of the

Principle

granulocytic series from cells of the monocytic series. This is particularly useful in the differentiation of leukemias.

Whole blood is mixed with crystal violet stain and allowed to incubate. Moist preparations of the blood and stain mixture

Clinical Applications

are examined for the presence of Heinz bodies in the erythrocytes.

Alpha-naphthyl acetate esterase enzyme is detected primarily in monocytes. Heinz bodies represent unstable types of hemoglobin, which are denatured by dyes, such as crystal violet or brilliant cresyl blue, and appear as intraerythrocytic stained bodies. Monocytes should demonstrate black granulation.

Lymphocytes may occasionally exhibit some activity.

Heinz bodies appear as blue, refractile, intracytoplasmic

This procedure in CLSI format is provided on this book's

inclusions. They are irregularly shaped bodies of varying

companion Web site at thepoint.lww.com/nurseon6e.

sizes, up to 2 μm in diameter, and are found close to the cellular membrane. There may be more than one Heinz body

Alpha-Naphthyl Acetate Esterase with Fluoride Inhibition

present in an erythrocyte. The test detects in vivo precipitation

to differentiate positive reacting cells conclusively from

Heinz bodies (Fig. 32.14).

monocytes, so sodium fluoride is incorporated with the incubation system. The monocyte enzyme is inactivated in the

presence of this compound.

All cells of monocytic origin will be negative for enzyme

activity, with the exception of differentiated histiocytes or

specialized macrophages in tissue, which may also be resistant to sodium fluoride.

Clinical Applications

Esterase reactions may be used in the classification of acute

nonlymphoblastic leukemia. The staining characteristics are:

M0 and M1 → negative

FIGURE 32.14 Heinz bodies. Cell type: Young and mature red blood

M2 and M3 → naphthol AS-D chloroacetate esterase (NCAE)-

cells. Description: Round, refractile inclusions on the periphery

positive, alpha-naphthyl acetate esterase (NAE)-negative

of the cell (arrow) when stained with a supravital dye; consists of denatured globin produced by the destruction of hemoglobin; they may

M4 → variable reactions with NCAE and NAE

occur in multiple numbers. Clinical conditions: Drug-induced anemias

M5a → NCAE-negative, NAE-variable

Thalassemia G-6-PD deficiency and other red blood cell enzymopathies—

M5b → NCAE-negative, NAE-positive

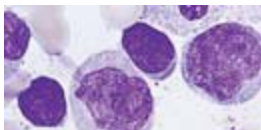
thias. Unstable hemoglobinopathies. (Reprinted from Anderson SC.

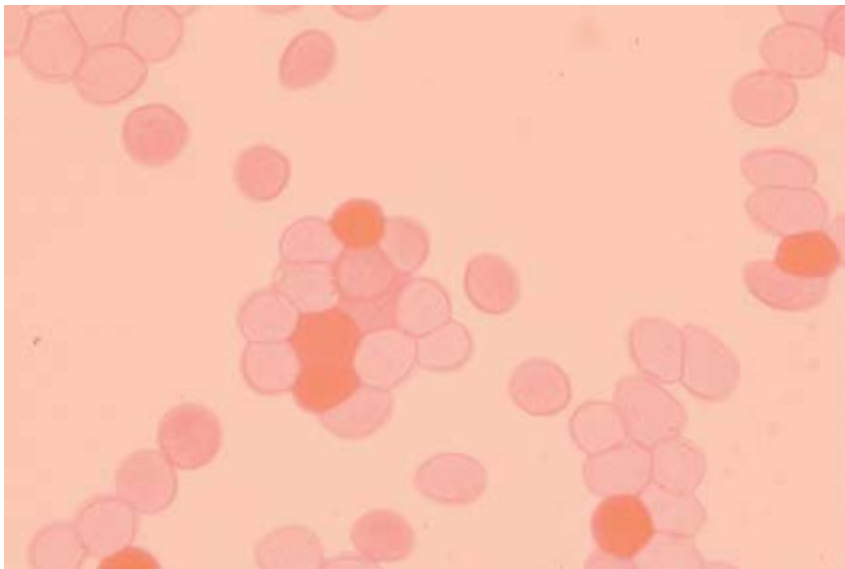
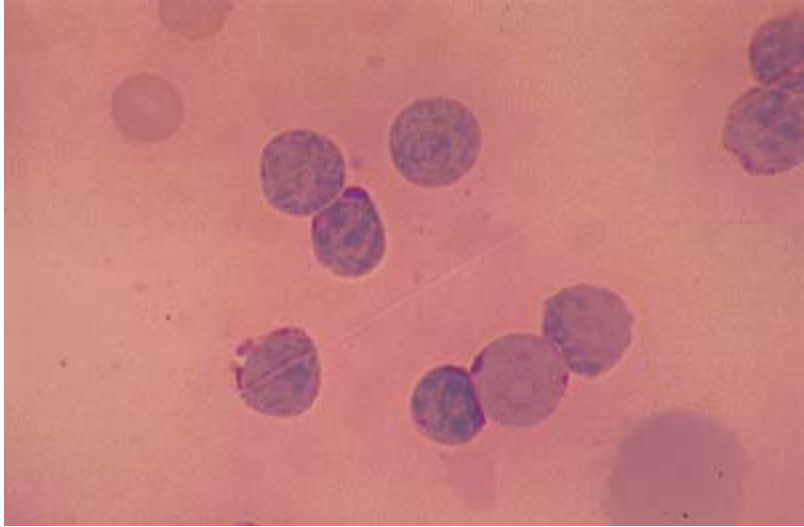
M6 → NCAE-negative, NAE-variable

Anderson's Atlas of Hematology, Philadelphia, PA: Wolters Kluwer

M7 → NCAE-negative, NAE-variable

Health/Lippincott Williams & Wilkins, 2003, with permission.)





CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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SPECIAL STAINS (continued)

Clinical Applications

bloo . T ese cells shoul appear rarely. T e results are

Heinz bodies are formed when the glycolytic enzymes in the erythrocytes are unable to prevent the oxidation of hemoglobin (Hb F)-containing cells. In adults, values are higher, with newborn infants having 70% to 90% Hb F-containing cells. This leads to the formation of a precipitate to form Heinz bodies. Erythrocytic enzyme

Clinical Applications

Enzyme levels decrease as the cell ages; therefore, occasional Heinz bodies will be observed in normal blood.

Increased amounts of Hb F are found in various hemoglobinopathies such as hereditary persistence of fetal hemoglobin, forms of hemoglobin that are present in a number of hemoglobinopathies, such as sickle cell anemia, and the thalassemias.

lytic disorders. Heinz bodies occur in disorders such as G6PD

This procedure in CLSI format is provided on this book's companion Web site at thepoint.lww.com/nurse6e.

oxidant drugs, and in the presence of unstable hemoglobins

PERIODIC ACID-SCHIFF (PAS) IN LEUKOCYTES:

such as Hb Zurich and Hb H.

CYTOCHEMICAL OR HISTOCHEMICAL STAINING

This procedure in CLSI format is provided on this book's

METHOD

companion Web site at thepoint.lww.com/nurseon6e.

Principle

HEMOGLOBIN F DETERMINATION BY ACID

When treated with periodic acid, glycols are oxidized to

ELUTION: KLEIHAUER AND BETKE METHOD

aldehydes. After reaction with Schiff's reagent (a mixture of

MODIFIED BY SHEPARD, WEATHERALL, AND

pararosaniline and sodium metabisulfite), a pararosaniline

CONLEY

product is released that stains the glyco-containing cellular

Principle

elements (Fig. 32.16). Clinically, the PAS stain is helpful in

After blood smears are fixed with ethyl alcohol, a citric

recognizing some cases of erythroleukemia and acute lym—

acid phosphate buffer solution removes (elutes) hemoglobin

phoblastic leukemia.

other than Hb F from erythrocytes. The Hb F (fetal hemo—

Clinical Applications

globin)—containing erythrocytes are visibly identifiable

In normal bone marrow, the earliest myeloid precursors of

on microscopic examination when appropriately stained

do not stain, but as granular staining increases as a

(Fig. 32.15). Shortly after birth, the amount of Hb F in

in function of maturation along myeloid pathways. Erythrocytic

in humans decreases to low levels. Increased amounts of Hb F

precursors do not stain, but megakaryocytes and platelets

are found in various hemoglobinopathies such as hereditary

stain intensely. Monocytes stain faintly and may display

persistence of fetal hemoglobin, sickle cell anemia, and the

granules.

thalassemias.

In acute lymphoblastic leukemia, PAS activity is highly

An adult specimen should have approximately the same

variable. In most cases, some precursor cells show coarse

number of dense Hb F-containing cells as the normal adult

granules or block-like positivity. In acute granulocytic leukemia, myeloblasts are usually negative, although a faint

positive reaction product may occasionally be observed.

In erythroleukemia, intense cytoplasmic granular PAS staining may be observed in early erythroid precursors.

Adult

Fetal

cell

cell

FIGURE 32.15 Acid elution or Kleihauer-Betke stain. Cell type:

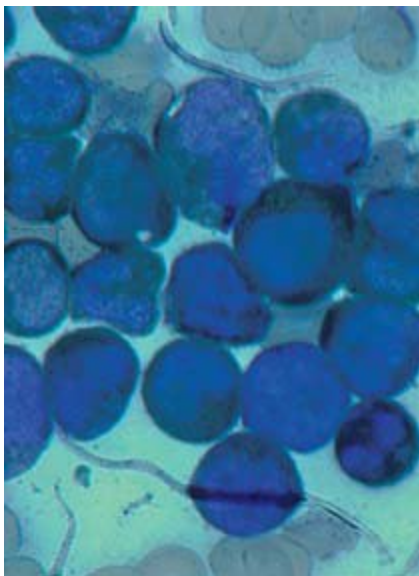
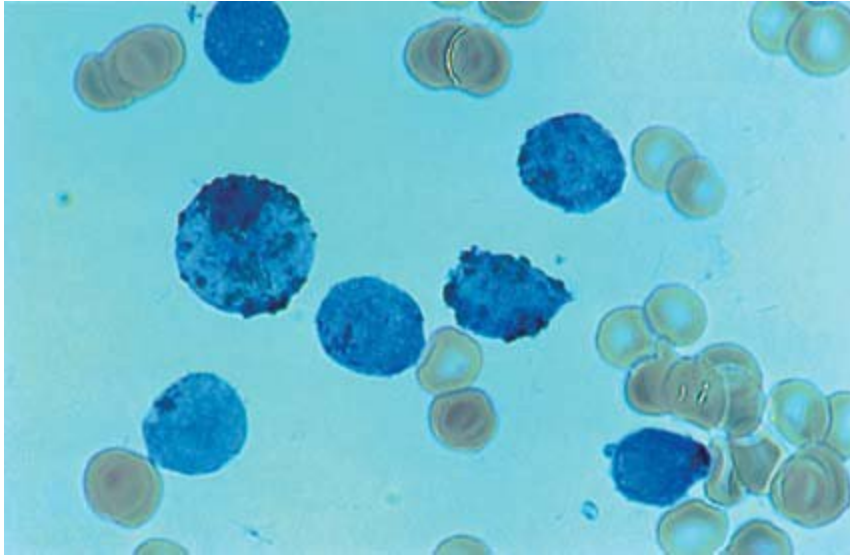
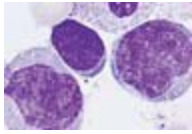
Red blood cell. Description: Cells containing hemoglobin F will appear pink to red; cells containing no hemoglobin F will only have their outer membrane visible (ghost cells). Clinical conditions: Hereditary persistence of fetal hemoglobin MDS some leukemias.

FIGURE 32.16 Periodic acid-Schiff stain of acute lymphoblastic

(Reprinted from Anderson SC. Anderson's Atlas of Hematology, leukemia L1. (Reprinted from McClatchey KD. Clinical Laboratory Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins, Wilkins, 2003, with permission.)

2002, with permission.)

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

SPECIAL STAINS (continued)

Di use staining may be present in more mature nucleate

SIDEROCYTE STAIN: PRUSSIAN BLUE STAINING

erythrocytes.

METHOD

This procedure in CLSI format is provided on this book's

Principle

companion Web site at thepoint.lww.com/nurseon6e.

The Prussian blue reaction precipitates free iron into small blue

PEROXIDASE (MYELOPEROXIDASE) IN

or blue-green granules in erythrocytes. Free iron is not identified

LEUKOCYTES : CYTOCHEMICAL STAINING

identified on Wright-or Wright-Giemsa-stained blood smears. An

METHOD

immature or mature erythrocyte containing free iron is referred

Principle

to as a sideroblast or siderocyte, respectively. Increased numbers

of siderocytes are seen in disorders such as thalassemia major or

Myeloperoxidase (MP) is detected by means of the enzyme's

in patients after a splenectomy. The iron granules encircle the

interaction with diaminobenzidine (DAB), a benzidine substi—

nucleus of the erythrocyte, it is referred to as a ring sideroblast.

The brown reaction product is first intensified with copper

Although alcoholism is the most common cause of ring sidero—

salts followed by Gill's modified Papanicolaou stain, which results

blasts, they may also be seen in cases of lead poisoning or anemia.

in intense gray-black granules at sites of neutrophil and monocyte MP activity (Fig. 32.17). The reaction can be illustrated as:

Clinical Applications

$\text{DAB} + \text{H}_2\text{O}_2 \text{ MP} \rightarrow \text{oxidized DAB}$

An increase in sideroblasts is associated with thalassemia major

2

2

(light brown pigment)

or minor and with the sideroblastic anemias. The sideroblastic

anemias are a miscellaneous group of diseases caused by drugs

Oxidized DAB + Cu (NO

) \rightarrow gray-black pigment

3

or chemicals, as well as various disorders, and are idiopathic

2

or idiopathic origin. Siderocytes are uncommon in peripheral

This procedure differentiates cells of lymphoid origin from blood but may be seen after a splenectomy.

granulocytes and their precursors and monocytes.

This procedure in CLSI format is provided on this book's Clinical Applications

companion Web site at thepoint.lww.com/nurseon6e.

The most probable results to be expected with peroxidase-

SUDAN BLACK B STAIN: CYTOCHEMICAL

staining procedures in cases of acute nonlymphocytic leukemia-

STAINING METHOD

mia (ANLL) are:

Principle

M1 → 5% to 15% of blasts may be positive

Following fixation, blood or bone marrow films are immersed

M2 and M3 → positive

in a buccal Sudan black B solution. After rinsing, slides are

M4 → positive (usually a mixed population of cells)

counterstained with Mayer's hematoxylin. Cells are examined

M5a → may be positive

microscopically for the presence of blue-black discrete granules—

M5b → a fine granular deposit may be observed in more maturation (Fig. 32.18). Cells committed to the lymphoid pathway display negative staining reactions, whereas myeloid and mature cells

display negative staining reactions, whereas myeloid and M6 and M7 → myelocytic cells are positive. Monocytic forms display characteristic positive reactions.

ALL → negative (L1, L2, and L3)

The Sudan black B staining pattern usually parallels the MP stain and is useful in the identification of myelogenous and

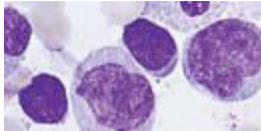
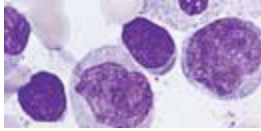
This procedure in CLSI format is provided on this book's myelomonocytic leukemias.

companion Web site at thepoint.lww.com/nurse6e.

FIGURE 32.17 Myeloperoxidase stain of acute myelogenous leu-

FIGURE 32.18 Sudan black B (left) stain of acute myelogenous leukemia M2. (Reprinted from McClatchey KD. Clinical Laboratory leukemia M4. (Reprinted from McClatchey KD. Clinical Laboratory Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams & Medicine, 2nd ed., Philadelphia, PA: Lippincott Williams & Wilkins, Wilkins, 2002, with permission.)

2002, with permission.)



CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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SPECIAL STAINS (continued)

Clinical Applications

irreversible addition of oxynucleotides to the 3'-hydroxy

This cytochemical stain in conjunction with other testing is

groups on the end of DNA. The primary methods of detection—

used in the identification of myelogenous and myelomonocytic

leukemias are immunofluorescence and immunoperoxidase using

cytic leukemias.

a monoclonal antibody.

This procedure in CLSI format is provided on this book's

is a cell marker found on immature and neoplastic

companion Web site at thepoint.lww.com/nurseon6e.

cells frequently seen in leukemic states.

Clinical Applications

TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE TEST

This procedure is useful in identifying lymphoblastic cells on peripheral blood and/or bone marrow smears.

Principle

This procedure in CLSI format is provided on this book's

terminal deoxynucleotidyl transferase () is a non-

companion Web site at thepoint.lww.com/nurseon6e.

template-directed DNA polymerase that catalyzes the

COAGULATION PROCEDURES

In coagulation testing, replicate analysis is frequently per-

1. It has a closer osmolality to plasma and produces more
normal. However, in a study of replicate testing, it was con-
sidered accurate activate partial thromboplastin time (APTT)
conclude that repeat testing does not enhance the precision of
results when patients are being treated with heparin.

the accuracy of coagulation tests. Accuracy and precision are

2. This citrate concentration is used as the mean of the nor-
malized by quality assurance procedures, such as frequent

mal range in the calculation of the international normal—
calibration checks and multilevel commercial controls, as
ize ratio. Other anticoagulants (e.g., heparin, EDTA, or
well as the practices describe below.

oxalate) should not be used.

Specimen Quality

The introduction of plastic tubes for safety reasons

All coagulation testing critically depends on the quality of
requires that new reference ranges for all coagulation assays
the specimen. Minimum tissue trauma and the avoidance
be established. At least 10 female and 10 male patients who
of hemolysis are essential. Proper phlebotomy techniques
are in the laboratory's patient population and who are not
describe in Chapter 2 must be strictly followed.

receiving anticoagulation therapy should be tested to establish a reference range.

Special Collection Techniques

Because of the importance of the ratio of blood to anti—
to reduce the possibility of introducing tissue thromboplastin—
coagulant, the proper vacuum in an evacuated tube must be
tied into a whole blood sample and the subsequent utilization—

maintained. The expiration date on the tube container necessitates attention to certain factors with clot formation, certain techniques must be monitored. If tubes are stored in sealed metal containers, they should be allowed to warm. Specimens collected for coagulation studies, precautions should be taken to monitor the premature clotting. Studies are not normally drawn initially, in multiple samples to avoid loss of the tubes' vacuum. Tubes are stored best in open containers when they are collected. If a single sample is collected using an evacuated container in an upright position.

In a heparin tube, a small amount of blood should be allowed to clot. The ratio of 9:1 in a specimen anticoagulated with sodium citrate. Enter the plastic needle holder before collecting blood in the tube. Citrate is achieved with a properly collected specimen in a tube. Nonwetable evacuated tubes should be used to prevent patient's PCV is between 0.20 and 0.60 L/L. However, in polycythemic or grossly anemic patients, a correction in the amount of blood in the container.

The amount of anticoagulant or the amount of blood drawn must be made.

If a sample is collected using the syringe technique, two

to determine the amount of anticoagulant in extreme
 syringes are used. Approximately 1 mL of venous blood is
 cases, the following correction formula is used:
 collected in the first tube, and this syringe is disconnected
 from the needle hub. Immediately, a second syringe is con—

00

$.0185 \times \text{volume of blood (mL)}$

$\text{L} \times 100 - \text{patient's}$

connected to the hub of the needle, and the specimen is collected

packed cell volume

in this syringe.

to determine the amount of whole blood needed,

Anticoagulants

Sodium citrate (3.2%) is the anticoagulant of choice (see

60

$\times 45$

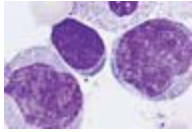
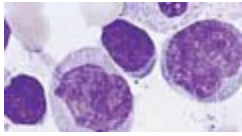
.

Chapter 2). The 3.2% citrate concentration has several

$100 - \text{patient's packed cell volume}$

advantages:

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

COAGULATION PROCEDURES (continued)

Specimen Handling

Barium sulfate adsorption: to 1 mL of plasma, add 50

Once a sample is in vitro, changes begin to occur, and labile

mg of barium sulfate. Incubate at 37°C for 15 minutes with

actants, such as actin VIII, begin to deteriorate quickly.

requent mixing. Centrifuge at 2,000 rpm to obtain a clear

It is strongly recommended that testing for prothrombin

supernatant will. Use immediately after preparation.

time (P) should be complete within 24 hours, and AP

Aluminum hydroxide adsorption: an aluminum hydroxide

should be complete within 4 hours. Samples should only be

suspension is prepared by adding 1 g of aluminum hydroxide

held for a maximum of 2 hours at 4°C, if testing cannot be completed within this time, the suspension should be added to 4 mL of distilled water. A 1 volume of the suspension to 9 volumes of water is recommended for immediate use.

vol o citrate plasma. Mix well an incubate or 5 minutes at

I testing is not con ucte , plasma shoul be separate

37°C. Centrifuge at 2,500 rpm to obtain a clear supernatant.

from the cells by centrifugation to produce a platelet-poor

Aged serum: a specimen of nonanticoagulated whole blood

plasma (platelet count less than $10 \times 10^9/L$). T is is prepare

should be allowed to clot. The specimen should be incubated

by centrifuging the whole blood specimen or a minimum of

at 37°C or 3 hours. A 0.1 M sodium citrate in the ratio

20 minutes at 2,500 rpm.

o 1 vol o 0.1 M so ium citrate to 9 vol o whole bloo to

Specimens can be store at -20°C or up to 2 months or

the specimen tube. Allow the tube to incubate at 37°C or an

-70°C or up to 6 months. Frost-free freezers should not be

additional 2 hours. Centrifuge for 10 minutes and remove the

use or storage of specimens. Storage in a frost-free freezer

serum. Serum should be used immediately or stored at -20°C .

or at temperatures higher than -20°C can compromise spec—

Before use, dilute the age serum with 0.85% sodium chloride

improves quality (e.g., prolongs clotting time of a lupus antico—

using a ratio of 1 vol of serum to 4 vol of sodium chloride.

agulant screening assay).

General Sources of Error

For use of a frozen specimen, it should be rapidly thawed at

Glassware must be clean. Disposable glassware is preferred

37°C while being gently inverted to prevent denaturation

of reusable equipment because detergent residues can

interfere with fibrinogen testing should be conducted immediately. If

scratched glassware can produce erroneous results. All previously thawed sample
cannot be tested immediately, the sample

centrifugation directions must be strictly followed because varia—

may be held for a maximum of 2 hours at 4°C until tested.

variations in pH, reagent concentration, and temperature are

For other assays (e.g., lupus anticoagulant), the capillary

major sources of error.

specimens should be centrifuged at $1,500g$ or no less than

15 minutes at room temperature, and the plasma removed

Quality Control

and then centrifuge again.

Water baths and heat blocks must be monitored continually for accurate temperatures. Refrigerators and freezers should

Specimen Preparation

also be monitored to ensure the stability of specimens and test

Specialized testing or coagulation factors may require

reagents. Reagents must not be beyond their state expiration

adsorbed plasma or aged serum. Plasma can be a sorbent

ate. Procedural technique must be consistent and appropriate.

using either barium sulfate or aluminum hydroxide. After

adsorption, the resulting adsorbed plasma contains factors I,

BIBLIOGRAPHY

V, VIII, XI, and XII. Age serum contains factors VII, IX, XI,

and XII. Refer to the factor substitution studies procedure in

Provide on this book's companion Web site at <http://thepoint.lww.com>.

this section or the technique.

<http://thepoint.lww.com/surgeon6e>.

COAGULATION PROCEDURES

BRECKER-CRONKITE MANUAL METHOD:

Specimen

PLATELET COUNT

ED A-anticoagulate whole blood is preferred. Capillary

Principle

blood may be used if venous blood is not available. The assay

Whole blood is diluted with an oxalate buffer, which com—

should be performed within 5 to 6 hours of the time that the

platelets hemolyze the erythrocytes. Platelets can then be

blood specimen is collected or within 24 hours, if the speci—

counts, preferably using a phase hemocytometer and phase

microscopy is recommended.

microscope. Enumeration of platelets is performed to detect

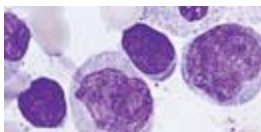
Reagents, Supplies, and Equipment

either decrease states (thrombocytopenia) or increase

1. Prepare a 1:100 dilution of blood with Thrombo-IC cap—

states (thrombocytosis).

capillary pipettes and a diluent vial.



COAGULATION PROCEDURES (continued)

2. Conventional microscope or phase-contrast hemocytom—

Calculations

eter an coverslip. T is type o hemocytometer has a at

T e number o platelets per microliter is:

bottom rather than the concave type use or counting

other cellular elements.

otal

3. Petri ish with a piece o moistene f lter paper inserte

average

ilution

volume

in the bottom.

no. o

4. Phase contrast microscope and lens paper.

Platelets/ μ L = platelets

\times correction \times correction

5. Microscope slides and Wright-Giemsa stain. This is an

in 5 squares

factor

factor

optional but recommend a quality control procedure.

The dilution and volume correction factors are derived in

Quality Control

the same manner as for the erythrocyte count.

Quantitative results should be checked against a well-made,

Example: If the average total of the platelets counted in five

stained peripheral smear.

squares is 20, the platelet count per microliter is:

Procedure

20×200

$\times 50$

$= 200,000/\mu\text{L}$ or

1. Thoroughly mix the blood sample and fill the hemocytometer according to the manufacturer's directions.

$200 \times 10^9/\text{L}$ (SI unit)

s

cytometer according to the manufacturer's directions.

Note: to load or charge the counting chamber, the first

Reporting Results

new drops of the dilution are left in the bore of the pipette

The reference value is 150,000 to 400,000/ μL or 150 to

should first be expelled. Fill one side of the counting

$400 \times 10^9/\text{L}$ (SI units).

chamber with the platelet dilution, being careful not to

Sources of Error

let any of the solution run over the edges into the surrounding grooves. Repeat this procedure, using the second

A variety of technical errors can produce incorrect results.

on Trombo-IC dilution vial, and fill the opposite side

These include the age of the specimen, clumping of platelets,

of the counting chamber.

adhesion to glass, and

2. Place the hemocytometer into a Petri dish with moisture incorrectly diluting the specimen.

filter paper on the bottom and allow to stand for 15 to 30

An even distribution of platelets through the counting minutes. Patient identification should be included on the area is critical. Clumping of the platelets results from inadequate mixing or poor technique. If clumps are seen, the number and loss of moisture will be prevented by covering the sample must be rediluted and recounted.

hemocytometer.

In cases of thrombocytopenia, the dilution may have to be

3. Carefully wipe off any moisture that may have accumulated. The new dilution factor must be included in the calculation formula. In cases of thrombocytosis, fewer squares are counted. Using the 10× objective can be counted. In either thrombocytopenia or thrombocytosis (low power), focus the microscope on the large middle

tosis, refer to the manufacturer's package insert or additional

square of the counter chamber. The background should

instructions on changing and calculating platelet counts.

appear pink, whereas the platelets, leukocytes, and

Clinical Applications

nebris, and markings of the hemocytometer appear

The enumeration of platelets has now become a routine com—

illuminate .

ponent of the automated complete blood cell count (CBC).

4. Switch to the 43 to 44× objective (high power) and refoIn cases of severe thrombocytopenia or whenever cellular

cus, if necessary, with only the fine adjustment. Reduce the

abnormalities may be spuriously affecting the automated

amount of light reaching the microscope stage.

count, a manual count should be performed .

5. The platelets should appear round or oval in shape and

Selected Quantitative Disorders of Platelets

give a faintly purple-ochre appearance. Any extraneous

nebris will appear to be refractile when the epth of focus

is altered using the fine adjustment.

Increased Platelets

Decreased Platelets

6. Five squares (the same as an erythrocyte count) are

(Thrombocytosis)

(Thrombocytopenia)

counted on each side of the hemocytometer. Both sides

of the chamber must be counted. The total number of

Postsplenectomy

Aplastic anemia

platelets counted on each side should be within 10 of

Polycythemia vera

Idiopathic thrombocytopenia

each other. At the total number of platelets counted

Chronic myelogenous

Acute leukemias

on each side together and divide by 2. This number represents

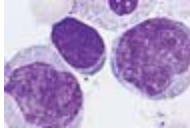
leukemia

represents the average number of platelets counted in five

squares.

Notes

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

COAGULATION PROCEDURES (continued)

REFERENCES

Procedure

1. Place an aliquot of 0.025 M calcium chloride in a test tube

Brecker G, Cronkite EP. Morphology and enumeration of human blood

and incubate at 37°C for a minimum of 5 minutes and a

platelets. J Appl Physiol, 3:365, 1950.

maximum of 60 minutes.

Henry JB (ed.). Clinical Diagnosis and Management by Laboratory

2. Pipette 0.1 mL of the partial thromboplastin substrate into

Methods, Philadelphia, PA: Saunders, 1984:1444.

a test tube and incubate for 2 minutes.

Seivar CE. Hematology for Medical Technologists, 4th ed., Philadelphia,

3. Pipette 0.1 mL of patient or control plasma into the sub—

strate. Shake briskly to mix. Begin timing or exactly

strate. Shake briskly to mix. Begin timing or exactly

COAGULATION PROCEDURES: ACTIVATED

5 minutes.

PARTIAL THROMBOPLASTIN TIME

4. After 5 minutes of activation, transfer 0.1 mL of pre—

Principle

Warm calcium chloride to the mixture. Immediately begin to time with a stopwatch. Insert nichrome loop and sweep it across the bottom of the tube at the rate of two complete thrombin and fibrin polymers via the intrinsic pathway. The AP procedure measures the time required to generate a visible fibrin web. The test is performed by sweeping the nichrome loop across the bottom of the tube at the rate of two complete thrombin and fibrin polymers via the intrinsic pathway. times per second.

Although a partial thromboplastin time test can be performed—

5. At the first appearance of fibrin, stop the stopwatch.

Normally, contact factors can be activated more thoroughly

Record the number of seconds. Repeat this procedure

by the addition of substances such as kaolin in the activated

(steps 2 through 5) for each assay.

Form of this assay.

In the AP, calcium ions and phospholipids that substitute—

Note: Performance of this test by automated methods is

tute or platelet phospholipids are added to blood plasma. These are described in Chapter 30.

generation of fibrin is the endpoint. Clinically, the APTT is

Reporting Results

used to identify any quantitative deficiencies in the intrinsic clotting system and to control anticoagulant therapy.

Reference values are dependent on the activator and phospholipid reagents used; however, 20 to 35 seconds is typically

Specimen

normal. In some laboratories, ranges may be from 28 to 42

seconds. Fresh plasma from citrate whole blood is needed. Centrifuge for 10 minutes, with 42 to 46 seconds being marginal.

Unopened whole blood specimens at 2,500 rpm or 20 min—

Procedure Notes

minutes. Promptly transfer the plasma to a labeled plastic tube.

Sources of Error

Place in an ice bath until tested. Specimens should be tested within 2 hours of collection.

Various sources of error include poor specimen collection or storage, improper reconstitution and storage of reagents,

Reagents, Supplies, and Equipment

reaction temperature, timing, and clot detection.

1. Partial thromboplastin substrate containing an activator

Clinical Applications

(such as Platelin Plus Activator or automated AP from

The AP is widely advocated as the test of choice for the

Organon Teknica Corp. or equivalent)

control of heparin therapy. It is also important in the screen—

2. 0.025 M calcium chloride

in profile of prekallikrein; high-molecular-weight kininogen—

3. Ice bath

gen; factors XII, XI, IX, VIII, X, V, II, and I; and inhibitors

4. (If manually performed) Stopwatch, 12 × 75-mm test

against these factors.

tubes, nichrome loop, 0.1-mL pipettes, and a 37°C water

bath or heat block

BIBLIOGRAPHY

Quality Control

The routine testing of control materials is essential. Both normal

Provision on this book's companion Web site at <http://thepoint>.

Normal and abnormal controls should be tested simultaneously

lwww.com/ urgeon6e.

with patient specimens. Results within an outside the normal range are equally important to monitor.

ANTITHROMBIN III: CLOTTING ASSAY METHOD

Commercial normal and abnormal control plasmas

Principle

should be used or a comprehensive quality control program.

In the presence of heparin, thrombin is neutralized at a rate

to prepare these controls, reconstitute normal and abnormal

that is proportional to the antithrombin (A III) concentration—

plasma with the exact amount of reagent-grade water specified—

tion. Following defibrination, plasma is assayed in a two-stage

defined on the label. Allow to stand for 30 minutes at room temperature—

procedure that utilizes standardized amounts of heparin,

temperature. Swirl gently to ensure complete rehydration. Mix

defibrinogen, and thrombin. The resulting clotting time is intermediately before use.

Store rehydrated vials at 2°C to 8°C and use

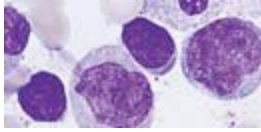
prepared using a calibration curve. Clinically, the A III assay is

within 24 hours. Unopened vials should be stored at 2°C to

use until prior to and subsequent to treatment with heparin in

8°C and use before the expiration date on the label.

cases of disseminated intravascular coagulation (DIC).



CHAPTER 32 ■ Laboratory Manual: Manual Procedures in Hematology

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COAGULATION PROCEDURES (continued)

This procedure, in CLSI format, is provided on this book's

Quality Control

companion Web site at <http://thepoint.lww.com/nurseon6e>.

A normal patient plasma should be tested at the same time as that of the unknown patient.

BLEEDING TIME: STANDARDIZED IVY METHOD

Principle

Procedure

The bleeding time test is an in vivo measurement of platelet

1. Using fresh plasma, prepare the following dilutions:

Let a lesion and aggregation on locally injured vascular

2. Incubate the control and patient specimens and mix—

subendothelium. This test provides an estimate of the integ—

tures at 37°C and perform an AP or P assay on each
rity of the platelet plug and thereby measures the interac—
plasma, control, and plasma-control mixture after 10, 30,
tion between the capillaries and platelets. The bleeding time
and 60 minutes.

reflects this aspect of platelet function by measuring the
length of time two standardized punctures of the ventral

Reporting Results

forearm take to stop bleeding. Clinically, the bleeding time
If the abnormality is that of a deficiency, a normal plasma
is prolonged in thrombocytopenia, qualitative platelet disorder—
sample will correct the assay results to a reference value. If the
errors such as von Willebrand's disease, aspirin ingestion, or
abnormality is caused by a circulating anticoagulant (inhibi—
the presence of vascular problems.

tor), a greater correction is demonstrated as the ratio of nor—

This procedure, in CLSI format, is provided on this book's
mal plasma increases in the mixture.

companion Web site at <http://thepoint.lww.com/surgeon6e>.

Patient

Normal

Deficiency

Inhibitor

CIRCULATING ANTICOAGULANTS

Principle

9 parts

1 part

Significant

No significant

Some coagulation deficiencies are caused by inhibitors to
correction

correction

specific factors rather than the lack of a factor. These inhibitors—

5 parts

5 parts

Significant

Some

factors are sometimes referred to as circulating anticoagulants.

correction

correction

to detect a circulating anticoagulant, the APTT and the PT

1 part

9 parts

Significant

More

that were originally abnormal are repeated using various

corrections

corrections

mixtures of patient plasma and normal plasma. The illustration—

Note: It is important to incubate the test specimens for 60 minutes because

mixtures are incubated at 37°C and tested after 10, 30, 60, and

some inhibitors act progressively, and it may take time for the APTT and/

120 minutes of incubation. If the abnormality is a deficiency,

or PT results of the patient plasma and patient plasma-normal control mix—

10% normal plasma will correct the test result to be close to

normal values to show the effects of the inhibitor (a prolonged clotting time). To interpret

the normal range, as will the addition of 50% normal plasma.

To interpret the results, the end point of the normal control has to be compared to

If the abnormality is caused by a circulating anticoagulant,

the patient-normal plasma mixtures. As the specimens incubate, a slight

increase in the end points will be observed because of the loss of labile clot—more correction will usually be shown as the ratio of normal—ting factors. The degree of prolongation in the patient and patient-control mal plasma increases in the mixture. The detection of an mixtures must be greater than the normal control plasma.

inhibitor may show up immediately or may require incubation of the normal plasma in the presence of the inhibitor.

Differentiation between a coagulation factor deficiency and

Factor

Disorder

a circulating anticoagulant is important in the correct treatment of a patient.

II

Myeloma, systemic lupus erythematosus (SLE)

V

Streptomycin administration, idiopathic

Patient Plasma

Normal Plasma

VIII

SLE, rheumatoid arthritis, drug reaction,

asthma, inflammatory bowel disease,

9 parts

1 part

postpartum

5 parts

5 parts

VIII, IX

Following replacement therapy for hereditary

1 part

9 parts

deficiency

IX

SLE—rare

Specimen

X

Amyloidosis

Refer to the AP procedure or treatment of the original specimen.

X, V

SLE—common

Reagents, Supplies, and Equipment

XI

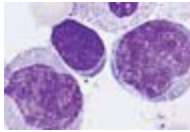
SLE—very rare

Refer to the AP procedure.

XIII

Isoniazid administration, idiopathic

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

COAGULATION PROCEDURES (continued)

Clinical Applications

Second-generation

TinaQuant (Roche), Liatest

Various types of anticoagulants may interfere with coagulation—

latex agglutination

(Stago), MDA D-dimer

tion at different stages, especially factor VIII, heparin-like

(immunoturbidimetric)

(bioMérieux)

activity, an antithromboplastin. Most acquire antico—

Source: Adapted from Righini M, Perrier A, De Moerloose P, *et al.*

agglutnants are autoantibodies (usually IgG, sometimes IgM)

D-Dimer

for venous thromboembolism diagnosis: 20 years later, *J Thromb Haemost*,

directed against specific coagulation factors. Below is a list of

6:1059–1071, 2008.

circulating anticoagulants associated with clinical disorders.

Approximately 5% to 10% of patients with SLE, many patients

on phenothiazine therapy, patients taking a variety of medications. The classic microplate ELISA was considered the gold stan—

dition, and patients with lymphoproliferative disorders may interfere with measuring d-dimer. Today, more than 30 different

assays for inhibitors known as lupus-like anticoagulants.

d-dimer assays are commercially available. These assays represent—

In addition to testing for circulating anticoagulants to spe—

represent a wide range of techniques.

Specific coagulation factors, the platelet neutralization procedure

EUGLOBULIN LYSIS TIME

(PNP) and the tissue thromboplastin inhibition test (TT)

may be valuable. The PNP test separates lupus-like inhibitors

Principle

rom factor VIII, X, and V inhibitors but does not inhibit—

The euglobulin fraction of plasma contains plasminogen, fibrin—

gen, and activators with the potential for transforming plasminogen to plasmin. This reaction is precipitated with 1% acetic

acid and resuspended in a borate solution. The euglobulins are

lupus-like anticoagulant with inhibitory activity against tissue

thromboplastin. Details of the PNP and I procedures

then clot by the addition of thrombin. The clot is incubated ,

can be found in works of Lenahan and Smith.

and the time of lysis is reported . Clinically, the euglobulin lysis

test is a screening procedure for fibrinolytic activity.

This procedure, in CLSI format, is provided on this book's

BIBLIOGRAPHY

companion Web site at <http://thepoint.lww.com/surgeon6e>.

Provided on this book's companion Web site at <http://thepoint.lww.com/surgeon6e>.

MIXING STUDIES AND FACTOR ASSAYS

Mixing Studies (Circulating Anticoagulant Screen,

D-DIMER ASSAY

Screening for Circulating Inhibitor)

Principle

Principle: P mixing study is indicated when the P is

The fundamental principle of the d-dimer assay has remained unchanged in the absence of heparin therapy. The assay is largely unchanged: recognition of the unique neo-epitope in the presence of a thrombin time to detect therapeutic heparin. D-dimer is detected by specific antisera. D-dimer contains a neo-epitope that is formed following the cross-linking of adjacent D-dimers by the action of thrombin. A mixing study is performed by measuring the APTT in the patient's plasma, then mixing the patient's plasma with normal plasma. It is this epitope that is recognized by specific antisera used in clinical assays.

plasma (NPP) and repeating the APTT tests immediately and after 37°C incubation.

Commercially Available D-Dimer Assays

If the results of the mixing study show correction or both the immediate and incubated APTT tests, the patient

Method

Vendor

most likely has a coagulation factor deficiency (or multiple factor deficiencies). If the results of the mixing study show

Microplate ELISA

Asserachrom D-di (Stago),

no correction in either the immediate or incubate PT/aPTT,

Enzygnost (Dade-Behring)

the patient may have a coagulation inhibitor, such as lupus

ELISA and uorescence Vidas DD (bioMérieux), Stratus

anticoagulant. If the mixture's PT result does not correct,

(ELFA)

D-dimer (Dade-Behring)

the patient may have an inhibitor. There are specific inhibi—

ELISA and

Pathfast (Mitsubishi), Immulite

tors such as anti-factor VIII, associated with bleeding, and

chemiluminescence

(Siemens)

lupus anticoagulants, associated with thrombosis. If the

Immuno filtration and

NycoCard (Nycomed), Cardiac

results of the mixing study show correction or the immediate—

sandwich-type

D-dimer (Roche)

ate P aP results, but no correction in the incubate P

aP, the patient may have a slow-acting inhibitor such as

Semiquantitative latex

Dimertest latex (IL), Fibrinosticon

anti-factor VIII (table 32.4).

agglutination

(bioMérieux)

Note: Correction of the AP in the mixing study suggests

Manual, whole-blood

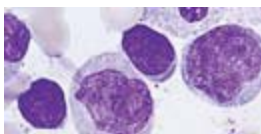
SimpliRED (Agen), Clearview

a coagulation factor deficiency in either the intrinsic path—

agglutination

Simplify D-dimer (Agen)

way (factors VIII, IX, XI, and XII; high-molecular-weight



COAGULATION PROCEDURES (continued)

with anti-fibrin split products. If fibrin split products are

TABLE

32.4 Mixing Studies

present, agglutination will occur. If these products are present

in increase amounts with normal hepatic and renal function, it is assumed that a recent fibrinolytic event has taken

PT or APTT

place or is taking place. Clinically, elevated results demon-

After Mixing

strate that the activation of plasmin has occurred or is occur-

PT or

and Waiting

ring, such as in primary fibrinolysis and DIC with secondary

APTT After

for a 2-Hour

fibrinolysis.

Inhibitor or

Immediate

Incubation at

This procedure, in CLSI format, is provided on this book's

Deficiency

Mixing

37°C

companion Web site at <http://thepoint.lww.com/surgeon6e>.

Factor deficiency

Corrected

Corrected

Factor VIII inhibitor

Corrected

Not corrected

FIBRINOGEN ASSAY: METHOD CLOTTING ASSAY

Factor V (Leiden)

Not corrected

Not corrected

Principle

inhibitor

Fibrinogen

thrombin actor II → fibrin

Lupus anticoagulant

Not corrected

Not corrected

When plasma is diluted and clotting time with excess thrombin, kininogen [HMWK]; or prekallikrein [PK]) or in the the fibrinogen concentration is inversely proportional to the common pathway (also prolonged PT) such as factors II, V, clotting time, yielding a linear relationship when plotted on a log-log paper. Deficiency of factors VIII, IX, and XI will present with bleeding. Deficiency of factor XII, or prekallikrein, will not increase bleeding risk but may increase thrombotic risk. Fibrinogen assays are useful in detecting deficiencies of fibrinogen and in detecting an alteration in the conversion of fibrinogen to fibrin.

This procedure, in CLSI format, is provided on this book's

Substitution Studies

companion Web site at <http://thepoint.lww.com/surgeon6e>.

Substitution studies and factor assays can be used to identify

Platelet Aggregation

specific coagulation factor deficiencies.

See Chapter 25.

Principle

PROTAMINE SULFATE ASSAY

Substitution studies may be performed using a sorbe

Principle

plasma and age serum with the AP to identify deficiencies of blood coagulation. Substitution studies may also be

Protamine is used to neutralize the effects of heparin. It prot—

performed using a sorbe plasma with the P to identify a

amine is administered in excess therapeutically, it is capable

factor VII deficiency.

of interfering with factor IX activity and thromboplastin generation. A positive result indicates the inappropriate presence

Specific Factor Assays

of intravascular fibrin monomers. (See Chapter 25 or more

Principle

information on platelet aggregation.)

Factor assays are based on the ability of the plasma in question. This procedure, in CLSI format, is provided in this book's

tion to correct a factor-deficient substrate, such as factor VIII.

companion Web site at <http://thepoint.lww.com/surgeon6e>.

The actual assay is the same as the AP with the exception of the factor-deficient substrate. Identification of specific

PROTHROMBIN TIME

Factor deficiencies is valuable in both the diagnosis and the

Principle

treatment of patients.

This basic procedure involves adding plasma to an excess of

This procedure, in CLSI format, is provided on this book's

extrinsic thromboplastin-calcium substrate. Thromboplastin

companion Web site at <http://thepoint.lww.com/surgeon6e>.

is derived from tissues that supply phospholipoprotein, such

as animal brain. The length of time required to form a fibrin

FIBRIN SPLIT PRODUCTS : THROMBO-

clot is measured in seconds.

WELLCOTEST METHOD

Clinically, this procedure is used to monitor oral anticoagulant therapy, as a screening test in the diagnosis of coag—

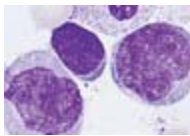
Principle

ulation deficiencies, and as a component of a liver profile

Whole blood is added to thrombin (to ensure complete

assessment. Prolonged results can indicate a deficiency of clotting) and soya bean enzyme inhibitors (to prevent any one or more factors in the extrinsic pathway: factors VII, X, break down of fibrin). After incubation, the patient's serum is added to a mixture of latex particles that have been coated with factor II or I. The presence of an inhibitor will also produce prolonged values.

(continued)



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PART 8 ■ Fundamentals of Hematological Analysis

COAGULATION PROCEDURES (continued)

TABLE

32.5 Probable Coagulation Deficiencies Based on APTT and PT Test Results Deficient Factor

Test

V

VII

VIII

IX

X

XI o r XII

PT

Abnormal

Abnormal

Normal

Normal

Abnormal

Normal

APTT

Abnormal

Normal

Abnormal

Abnormal

Abnormal

Abnormal

Adsorbed plasma

Corrects

No change

Corrects

No change

No change

Corrects

Aged serum

No change

Corrects

No change

Corrects

Corrects

Corrects

PT, prothrombin time; APTT, activated partial thromboplastin time.

Specimen

Reporting Results

Fresh plasma from citrate whole blood is preferred,

Reference values range from 10 to 15 seconds. Report both

although oxalate plasma may be used. The sample should

be the patient and control specimens in seconds. An order alter—

be centrifuge promptly after collection, with the plasma

native method of reporting is to express the percentage of

removed from the erythrocytes. Plasma may be stored or

patient activity. T is is calculate as
several hours at 2°C to 6°C before testing.

Control time s

(seconds)

Reagents, Supplies, and Equipment

×

100 = %activity of patient

Patient's time (seconds)

1. Thromboplastin
2. 12 × 75-mm test tubes

Clinical Applications

3. Pipettes: 0.1 mL (100 µL)

This test depends on the activity of factors VII, V, X, II, and

4. 37°C water bath or heat block
5. Stopwatch

I. A deficiency of any of these may produce a 3-to 4-second
prolongation in the test (Table 32.5).

6. Nichrome loop

Quality Control

BIBLIOGRAPHY

Normal and abnormal citrate or oxalate test plasma should be run with each patient assay or test batch.

Provide on this book's companion Web site at <http://thepoint.lww.com/surgeon6e>.

Procedure

This procedure is commonly performed using automated

THROMBIN TIME

equipment. However, in some cases, a manual procedure may

Principle

be used.

The thrombin time test determines the rate of thrombin—

1. Prewarm plasma at 37°C for a minimum of 2 minutes and in use cleavage of fibrinogen to fibrin monomers and the a maximum of 10 minutes.

subsequent polymerization of hydrogen-bonded fibrin poly—

2. Prewarm thromboplastin at 37°C for a minimum of 2 minutes. Clinically, extremely low fibrinogen levels, abnormal minutes and a maximum of 60 minutes.

fibrinogen, thrombin inhibitors, and high concentrations

3. Add 0.1 mL of plasma to 0.2 mL of thromboplastin. If per immunoglobulin (e.g., myeloma proteins) will produce

forming this procedure manually, pipette quickly. Start a stopwatch simultaneously.

trations of fibrin-fibrinogen degradation products will also

4. Using the nichrome loop technique, the loop is swept through the mixture at 2 sweeps per second until the first other parameters, such as the AP and P, are prolonged. A strand of fibrin appears. The tube may also be tilted using this procedure, in CLSI format, is provided in this book's a magnifier to observe clot formation.

companion Web site at <http://thepoint.lww.com/surgeon6e>.

5. Repeat this procedure in duplicate or all specimens, Refer to the reference values on the inside back cover as including controls. The duplicate results should be within well as additional procedures at <http://thepoint.lww.com/surgeon6e>. 1 second or one another.

<http://thepoint.lww.com/surgeon6e>.

REVIEW QUESTIONS

*Indicates MLT (optional) and MLS advanced content.

7. The correct reference value for hemoglobin assay (adult male) is

Hematology Procedures

A. 0.15 to $0.3 \times 10^9/L$

1. What is the appropriate reagent for the reticulocyte

B. 12.0 to 16.0 g/L

count?

C. 4.5 to $5.9 \times 10^{12}/L$

A. New methylene blue

D. 22% to 40%

B. Phloxine B

C. Solution lyses erythrocytes and releases the cells to

8. The correct reference value for absolute lymphocyte

be counted

(adult) is

D. 10% HCl solution

A. $1.2 \text{ to } 3.4 \times 10^9/\text{L}$

B. 12.0 to 16.0 g/ L

2. What is the appropriate procedure and characteristic of

C. $4.5 \text{ to } 5.9 \times 10^{12}/\text{L}$

the Westergren method?

D. 22% to 40%

A. The diluting solution lyses erythrocytes with propylene glycol and contains sodium carbonate and water.

9. The reference value for total leukocyte count is

B. The procedure measures the rate of erythrocyte

A. up to 13 mm/h

settling.

B. 2.5% to 6.0%

C. Ferrous ions are oxidized to the ferric state.

C. $150 \text{ to } 450 \times 10^9/\text{L}$

D. The diluting solution is either 1% hydrochloric acid

D. $4.5 \text{ to } 11.0 \times 10^9/\text{L}$

or 2% acetic acid.

10. The reference value for PCV (adult, female) is

3. What source of error will have greatest effect on PCV

A. up to 13 mm/h

(hematocrit)?

B. 2.5% to 6.0%

A. Incorrect dilution of blood and diluent

C. 150 to $450 \times 10^9/L$

B. Hemolysis of whole blood specimen

D. 36% to 45%

C. Excessive anticoagulant will produce shrinkage of cells

11. The reference value for direct platelet count is

D. Specimen stored in EDTA or 1 week

A. up to 13 mm/h

B. 2.5% to 6.0%

4. In a platelet count, what error will have the greatest

C. 150 to $400 \times 10^9/L$

effect on the test result?

D. 4.4 to $11.3 \times 10^9/L$

A. Reactive bodies can produce a false-positive observation.

12. The reference value for the reticulocyte count (newborn

B. Specimens stored at room temperature or more

in ant) is

than 5 hours will produce inaccurate results.

A. up to 13 mm/h

C. An oil stain

B. 2.5% to 6.5%

D. Incorrect pH of stain

C. 150 to 450 $\times 10^9/L$

D. 36% to 45%

5. In a reticulocyte count, what error will have the greatest effect on the test result?

13. The reference value for the Westergren ESR method

A. Reactive bodies can produce a false-positive

(adult male over 50 years) is

observation.

A. up to 15 mm/h

B. Specimens stored at room temperature or more

B. 2.5% to 6.0%

than 5 hours will produce inaccurate results.

C. $150 \text{ to } 450 \times 10^9/\text{L}$

C. An oil stain

D. 36% to 45%

D. Incorrect pH of stain

14. What clinical or specimen condition will produce an

6. The correct reference value for an erythrocyte count

increase total leukocyte count?

(adult male) is

A. Active allergies

A. $0.15 \text{ to } 0.3 \times 10^9/\text{L}$

B. Immediate hypersensitivity reactions

B. 12.0 to 16.0 g/L

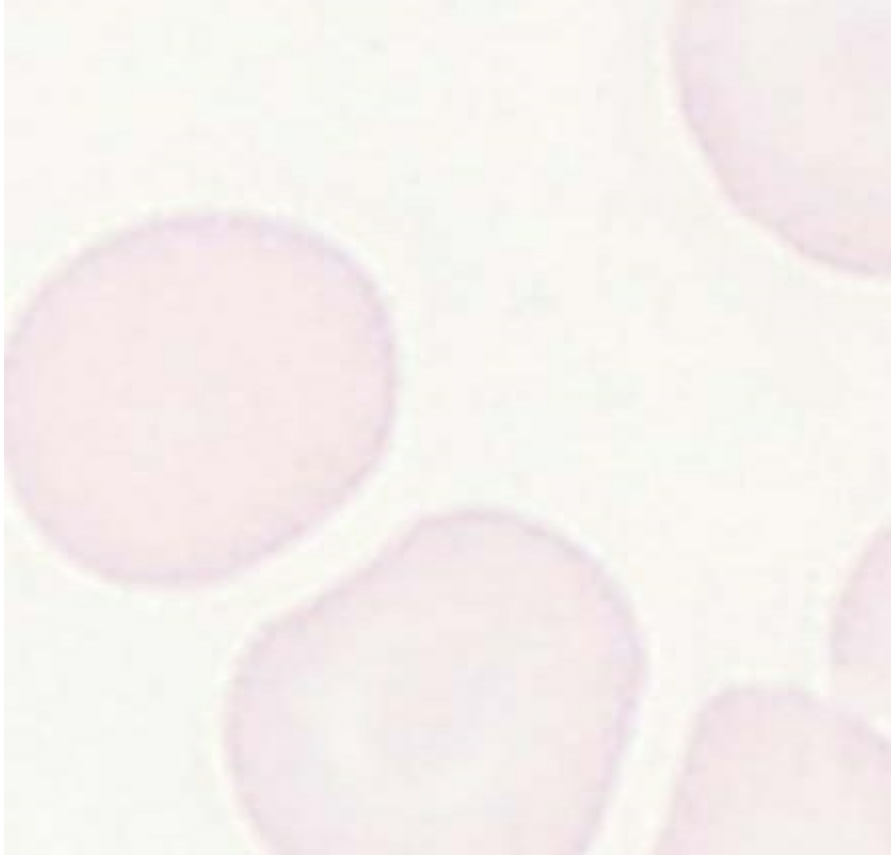
C. Inflammation

C. $4.5 \text{ to } 5.9 \times 10^{12}/\text{L}$

D. A lipemic blood specimen

D. 22% to 40%

(continued)



PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS (continued)

15. Which clinical or specimen condition will produce an

23. What clinical condition will produce a decrease

increase PCV test result?

Westergren ESR value?

A. Splenectomy

A. Polycythemia vera

B. Rouleaux formation

B. Acute leukemias

C. Polycythemia

C. Megaloblastic anemia

D. Hemolytic anemia crisis

D. Viral infection

16. Which clinical or specimen condition will produce an

24. A normal blood smear should have no more than

increase reticulocyte count test result?

approximately

(maximum) number of platelets

A. Splenectomy

per oil immersion field in an area where the erythroB. Rouleaux formation
cytes are just touching each other.

C. Polycythemia

A. 10

D. Hemolytic anemia crisis

B. 15

C. 20

17. Which clinical or specimen condition will produce an
increase Westergren ESR method test result?

D. 25

A. Splenectomy

25. The PCV procedure can be affected by the

B. Rouleaux formation

A. speed of the centrifuge

C. Polycythemia

B. length of time of centrifugation

D. Hemolytic anemia crisis

C. ratio of anticoagulant to whole blood

18. What clinical condition will produce an increase value

D. all of the above

o neutrophils?

A. Invasive parasites

26. Which of the following erythrocytic inclusions contain

B. Bacterial infections

RNA and can be observed by staining with new methy—

C. Viral infections

lene blue?

D. tuberculosis

A. Howell-Jolly bodies

B. Heinz bodies

19. What clinical condition will produce an increase value

C. Pappenheimer bodies

of lymphocytes?

D. Reticulocytes

A. Invasive parasites

B. Bacterial infections

27. The sedimentation rate of erythrocytes can be affected

C. Viral infections

by the

D. tuberculosis

A. ratio of anticoagulant to whole blood

B. position of the tube

20. What clinical condition will produce an increase value

C. temperature of the specimen or laboratory

of monocytes?

D. all of the above

A. Invasive parasites

Special Hematology Procedures

B. Bacterial infections

C. Viral infections

*28. Cellulose acetate at pH 8.6 separates the hemoglobin

D. tuberculosis

fractions

A. S

21. What clinical condition will produce an increase value

B. H

of eosinophils?

C. A

A. Invasive parasites

D. both A and C

B. Bacterial infections

C. Viral infections

*29. In an alkaline (pH 8.6) electrophoresis is performed,

D. tuberculosis

hemoglobin E has the same mobility as hemoglobin

A. S

22. What clinical condition will produce a decrease in reticu—

B. F

leucocyte count?

C. A

A. Polycythemia vera

D. C

B. Acute leukemias

C. Megaloblastic anemia

D. Viral infection

REVIEW QUESTIONS (continued)

Special Stains

Coagulation Procedures

*30. Heinz bodies are

37. Which of the following is the appropriate principle

A. precipitation of free iron into blue or blue-green

or description of the activated partial thromboplastin
granules

time (APTT)?

B. enhanced by crystal violet stain

A. In the presence of heparin, thrombin is neutralized.

C. lymphocytes that do not stain

B. Measures the time required to generate thrombin

D. intense cytoplasmic granular staining particles in
an fibrin polymers via the intrinsic pathway

erythroleukemia

C. Measures inhibitors of specific factors

D. An in vivo measurement of platelet adhesion

*31. Prussian Blue stain produces

an aggregation on locally injured vascular

A. precipitation of free iron into blue or blue-green

subendothelium

granules

B. enlargement of blood cells

38. Which of the following is the appropriate principle for

C. non-staining in lymphocytes

description of the antithrombin assay?

D. intense cytoplasmic granular staining particles in

A. In the presence of heparin, thrombin is neutralized.

erythroleukemia

B. Measures the time required to generate thrombin

*32. Periodic acid-Schiff produces

an fibrin polymers via the intrinsic pathway

A. precipitation of free iron into blue or blue-green

C. Measures inhibitors of specific factors

granules

D. An in vivo measurement of platelet adhesion

B. enature re bloo cells

an aggregation on locally injure vascular

C. non-staining in lymphocytes

suben othelium

D. intense cytoplasmic granular staining particles in

erythroleukemia

39. Which o the ollowing is the appropriate principle or
escription o the circulating anticoagulant assay?

*33. Peroxi ase stain pro uces

A. In the presence o heparin, thrombin is neutralize .

A. precipitation o ree iron into blue or blue-green

B. Measures the time require to generate thrombin
granules

an f brin polymers via the intrinsic pathway

B. enature re bloo cells

C. Measures inhibitors o specif c actors

C. non-staining in lymphocytes

D. An in vivo measurement o platelet ahesion

D. intense cytoplasmic granular staining particles in
an aggregation on locally injure vascular

erythroleukemia

subendothelium

*34. The result of an Leukocyte alkaline phosphatase

40. The appropriate reference value or diagnostic charac-

(LAP) stain is:

teristic of the activated partial thromboplastin time

A. normal, if the result is 32 to 182 with ast blue RR

(aP) is

ye

A. positive result: increase ratio of normal plasma to

B. positive, if 10% to 50% hemolysis is present

patient plasma.

C. normal, if < 10% of cells exhibit hemoglobin F in

B. Normal: 2 to 8 minutes

analysis

C. Normal: 20 to 35 seconds (28 to 42 seconds), the

D. normal in newborns, if the result is 70% to 90%

range depending on the activator and phospholipid

*35. Leukocytes that demonstrate a positive reaction in tar—

reagents

taric aci -resistant phosphatase cytochemical staining

D. Normal: 80% to 100% (range, 107% \pm 19%)

are the lymphocytes seen in

A. in ectious mononucleosis

41. T e appropriate re erence value or iagnostic charac—

B. malignant lymphoma

teristic o the antithrombin level is:

C. ALL (non-type)

A. positive result: increase ratio o normal plasma to

D. Hairy Cell leukemia

patient plasma.

B. Normal: 2 to 8 minutes

*36. LAP bloo smears shoul be staine with _____ o

C. Normal: 20 to 35 secon s (28 to 42 secon s), the
specimen collection.

range open ing on the activator an phospholipi

A. 8 hours

reagents

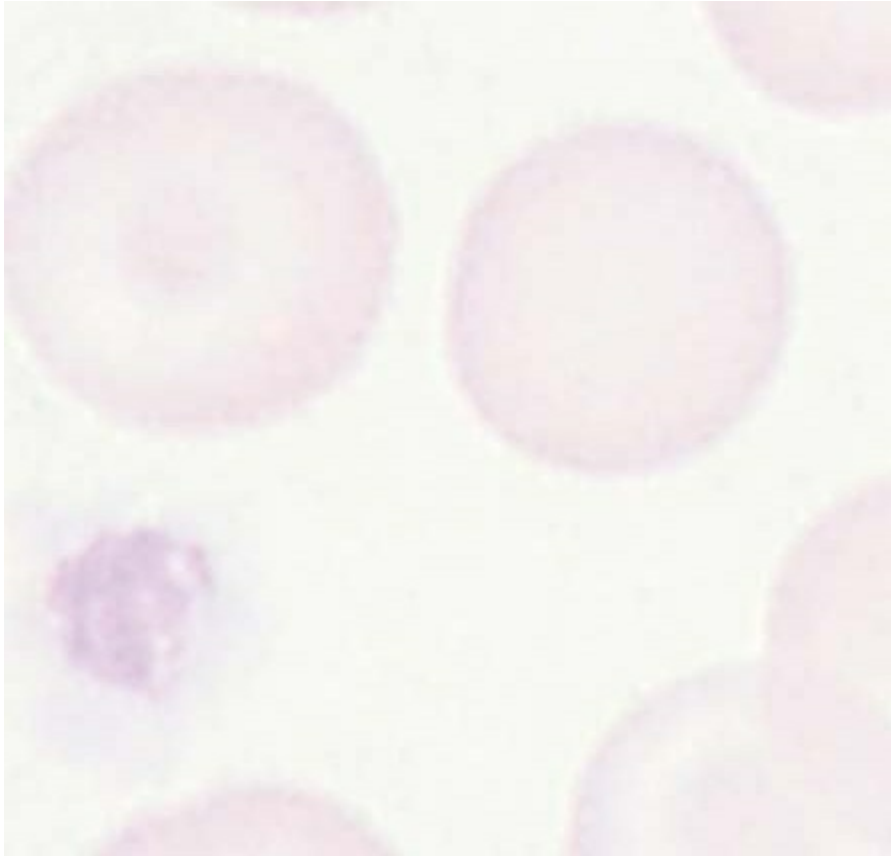
B. 48 hours

D. Normal: 80% to 100% (range, 107% \pm 19%)

C. 72 hours

D. 5 ays

(continued)



PART 8 ■ Fundamentals of Hematological Analysis

REVIEW QUESTIONS (continued)

42. The appropriate reference value or diagnostic character—

45. Which of the following is the appropriate reference

value of circulating anticoagulants is:

value of fibrin split products?

A. positive result: increase ratio of normal plasma to

A. Normal: no dissolution of the clot at 24 hours

patient plasma.

B. Normal: less than 8 to 10 $\mu\text{g/mL}$

B. Normal: 2 to 8 minutes

C. Normal: 50% to 150%

C. Normal: 20 to 35 seconds (28 to 42 seconds), the

D. Normal: 200 to 400 mg/L or a titer of 1:123 to 1:256

range depending on the activator and phospholipid

reagents

46. Which of the following is the appropriate reference

D. Normal: 80% to 100% (range, $107\% \pm 19\%$)

value of fibrin-stabilizing factor?

A. Normal: no dissolution of the clot at 24 hours

43. The appropriate reference value or diagnostic character—

B. Normal: less than 8 to 10 µg/mL

istic of the prothrombin time is:

C. Normal: 50% to 150%

A. Normal 10 to 14 seconds

D. Normal: 200 to 400 mg/L or a titer of 1:123 to 1:256

B. Normal: 2 to 8 minutes

C. Normal: 20 to 35 seconds (28 to 42 seconds), the

47. Which of the following is the appropriate reference

range depending on the activator and phospholipid

value of fibrinogen assay?

reagents

A. Normal: no dissolution of the clot at 24 hours

D. Normal: 80% to 100% (range, 107% ± 19%)

B. Normal: less than 8 to 10 µg/mL

C. Normal: 50% to 150%

44. Which of the following is the appropriate reference

D. Normal: 200 to 400 mg/L or a titer of 1:123 to 1:256

value of factor VIII assay?

- A. Normal: no dissolution of the clot at 24 hours
- B. Normal: less than 8 to 10 $\mu\text{g/mL}$
- C. Normal: 50% to 150%
- D. Normal: 200 to 400 mg/L or a titer of 1:123 to 1:256

COMPANION RESOURCES

<http://thepoint.lww.com/nurse6e>

Each student is encouraged to access and use the web—

base companion resources envelope or this chapter.

Here, you will find additional learning tools to increase

your understanding of the concepts and clinical applications of the chapter.

APPENDIX

Answers to Review Questions

A

CHAPTER 1

17. D

31. D

25. D

18. D

32. A

26. D

1. A

19. D

33. D

27. C

2. A

20. D

34. A

28. B

3. B

21. D

35. B

29. A

4. A

22. D

36. B

5. D

23. B

37. A

6. B

24. D

38. A

CHAPTER 5

7. B

25. D

39. D

8. B

26. C

40. D

1. B

9. D

27. A

41. C

2. D

10. A

28. B

42. A

3. A

11. C

29. A

43. D

4. A

12. D

30. C

44. D

5. D

13. B

45. C

6. D

14. A

46. D

7. C

15. B

CHAPTER 3

47. D

8. A

16. A

48. A

9. C

17. D

1. B

49. D

10. D

18. B

2. B

50. A

11. D

19. D

3. B

12. D

20. D

4. D

13. C

21. B

5. D

CHAPTER 4

14. D

22. C

6. D

15. B

23. A

7. A

1. D

16. B

24. D

8. C

2. C

17. A

25. D

9. B

3. C

18. C

26. B

10. D

4. D

19. D

11. C

5. B

20. D

12. A

6. B

21. D

CHAPTER 2

13. B

7. B

22. B

14. C

8. D

23. B

1. B

15. D

9. D

24. B

2. B

16. A

10. D

25. D

3. D

17. B

11. B

26. A

4. B

18. D

12. D

27. D

5. D

19. D

13. B

28. A

6. C

20. B

14. C

29. A

7. A

21. D

15. C

30. A

8. E

22. A

16. C

31. C

9. B

23. C

17. C

32. B

10. A

24. B

18. D

11. D

25. D

19. D

12. C

26. A

20. B

CHAPTER 6

13. A

27. D

21. B

14. C

28. B

22. A

1. C

15. B

29. C

23. A

2. D

16. D

30. B

24. B

3. D

703

704

4. A

17. D

28. A

42. C

5. B

18. D

29. A

43. B

6. A

19. B

30. C

44. D

7. C

20. B

31. A

8. B

21. D

32. C

9. A

22. A

33. A

CHAPTER 10

10. A

23. B

34. B

11. D

24. C

35. A

1. D

12. D

25. D

36. C

2. C

13. B

26. B

37. A

3. A

14. B

27. D

38. B

4. C

15. B

28. C

39. A

5. B

16. D

29. A

6. C

17. B

30. D

7. B

18. D

31. B

CHAPTER 9

8. C

19. B

32. D

9. C

20. C

33. A

1. B

10. C

21. B

34. B

2. D

11. D

22. A

35. C

3. B

12. A

23. C

36. D

4. A

13. C

24. A

37. B

5. A

14. A

25. D

38. D

6. B

15. B

26. B

39. A

7. D

16. D

27. C

40. C

8. A

17. D

28. D

41. C

9. C

18. C

29. C

42. A

10. D

19. B

30. A

11. B

20. C

31. A

12. B

21. A

32. C

CHAPTER 8

13. C

22. B

33. B

14. B

23. D

34. C

1. A

15. B

24. A

35. A

2. A

16. A

25. A

36. D

3. A

17. B

26. A

37. D

4. C

18. B

27. D

38. D

5. A

19. A

28. A

39. D

6. B

20. B

29. C

40. B

7. B

21. A

30. A

8. C

22. B

31. C

9. B

23. A

32. C

CHAPTER 7

10. B

24. C

33. D

11. D

25. B

34. B

1. C

12. D

26. B

35. C

2. B

13. D

27. D

36. C

3. C

14. D

28. D

37. C

4. A

15. A

29. A

38. C

5. C

16. D

30. A

39. C

6. A

17. A

31. B

40. D

7. C

18. D

32. D

41. D

8. A

19. D

33. B

42. B

9. D

20. D

34. A

10. D

21. B

35. C

11. A

22. A

36. A

CHAPTER 11

12. C

23. B

37. D

13. B

24. D

38. D

1. D

14. A

25. D

39. D

2. D

15. C

26. B

40. C

3. D

16. B

27. C

41. D

4. C

APPENDIX A ■ Answers to Review Questions

705

5. A

21. D

8. B

7. A

6. D

22. C

9. C

8. A

7. A

23. D

10. A

9. C

8. C

24. D

11. D

10. C

9. B

25. D

12. D

11. B

10. A

26. A

13. B

12. B

27. A

14. A

13. A

28. D

15. B

14. D

CHAPTER 12

29. D

16. A

15. D

30. C

17. B

16. A

1. B

31. A

18. A

17. D

2. A

32. B

19. C

18. B

3. A

33. C

20. D

19. C

4. B

34. D

21. B

20. B

5. B

35. B

22. B

21. A

6. C

36. D

23. A

22. B

7. A

37. B

24. C

23. A

38. C

25. C

24. B

39. B

26. D

25. B

CHAPTER 13

27. C

26. B

28. C

27. A

1. D

CHAPTER 15

29. C

28. A

2. B

30. A

29. A

3. D

1. C

30. B

4. D

2. A

31. B

5. D

3. D

CHAPTER 17

32. C

6. A

4. D

33. D

7. C

5. A

1. C

34. B

8. B

6. A

2. D

35. A

9. A

7. C

3. A

36. C

10. A

8. D

4. A

37. B

11. C

9. A

5. D

38. B

12. A

10. D

6. B

39. A

11. D

7. B

12. C

8. B

CHAPTER 14

13. A

9. B

CHAPTER 19

14. A

10. C

1. C

15. B

11. A

1. D

2. D

16. C

12. C

2. B

3. D

17. C

13. D

3. B

4. D

18. D

14. C

4. D

5. A

19. C

15. A

5. A

6. B

20. C

16. A

6. A

7. D

21. C

17. A

7. A

8. C

22. B

18. D

8. B

9. C

23. C

19. A

9. B

10. D

20. D

10. A

11. A

11. A

12. C

CHAPTER 16

12. A

13. B

CHAPTER 18

13. A

14. D

1. A

14. B

15. B

2. B

1. D

15. C

16. A

3. A

2. D

16. A

17. C

4. B

3. A

17. C

18. C

5. B

4. D

18. D

19. B

6. A

5. D

19. B

20. B

7. C

6. D

20. A

706

APPENDIX A ■ Answers to Review Questions

21. B

39. B

CHAPTER 24

8. A

22. B

40. D

9. A

23. C

41. B

1. B

2. D

3. C

CHAPTER 27

CHAPTER 20

CHAPTER 22

4. C

5. D

1. B

1. D

1. B

6. D

2. A

2. B

2. C

7. D

3. C

3. B

3. B

8. B

4. D

4. B

4. C

9. B

5. D

5. D

5. D

10. A

6. C

6. C

6. D

11. C

7. A

7. D

7. B

12. B

8. A

8. A

8. C

9. D

9. D

10. A

10. B

CHAPTER 25

11. C

CHAPTER 21

11. D

12. B

12. A

1. B

13. C

1. D

13. C

2. E

14. B

2. B

14. C

3. B

15. D

3. C

4. A

16. A

4. A

5. D

17. B

5. D

CHAPTER 23

6. C

18. C

6. C

7. B

19. A

7. D

1. C

8. A

20. B

8. C

2. A

9. B

21. B

9. D

3. B

10. A

22. B

10. B

4. A

11. D

23. D

11. A

5. C

12. D

24. C

12. D

6. B

13. D

25. D

13. C

7. B

14. D

26. A

14. D

8. D

15. A

27. C

15. A

9. C

16. B

28. D

16. D

10. C

17. D

29. B

17. A

11. D

18. D

30. D

18. D

12. D

19. D

31. A

19. A

13. C

20. B

32. C

20. D

14. D

21. A

33. D

21. C

15. B

22. B

34. B

22. A

16. D

23. D

35. D

23. A

17. D

24. C

36. D

24. C

18. D

25. D

37. A

25. D

19. A

26. A

38. D

26. B

20. D

27. B

27. C

21. A

28. B

28. A

22. D

CHAPTER 28

29. A

23. B

30. D

24. D

CHAPTER 26

1. A

31. B

25. C

2. C

32. D

26. C

1. D

3. A

33. A

27. C

2. B

4. B

34. B

28. C

3. C

5. D

35. D

29. D

4. A

6. B

36. A

30. D

5. C

7. D

37. B

31. A

6. A

8. D

38. C

32. C

7. B

9. D

APPENDIX A ■ Answers to Review Questions

707

10. A

37. D

3. A

CHAPTER 32

11. A

38. B

4. C

12. B

39. C

5. C

1. A

13. A

40. D

6. B

2. B

14. B

41. A

7. D

3. C

15. B

42. A

8. D

4. B

16. B

43. D

9. A

5. A

17. C

44. D

10. A

6. C

18. A

45. D

11. D

7. B

19. D

46. A

12. A

8. A

47. C

13. D

9. D

48. B

14. B

10. D

CHAPTER 29

49. A

15. A

11. C

50. A

16. C

12. B

1. A

51. B

17. B

13. A

2. B

52. C

18. D

14. C

3. D

53. D

19. B

15. C

4. C

54. A

20. B

16. D

5. B

55. B

21. D

17. B

6. A

56. D

22. C

18. B

7. D

57. C

23. C

19. C

8. B

58. A

24. B

20. D

9. C

59. D

25. D

21. A

10. A

60. B

26. D

22. C

11. D

61. D

27. D

23. A

12. A

62. D

28. B

24. C

13. D

63. D

29. C

25. D

14. B

64. B

30. D

26. D

15. C

65. A

31. D

27. D

16. A

66. C

32. C

28. D

17. B

67. C

33. C

29. C

18. A

68. B

34. D

30. B

19. D

69. B

35. C

31. A

20. D

70. D

36. D

32. D

21. D

71. C

33. C

22. D

72. C

34. A

23. D

73. A

CHAPTER 31

35. D

24. B

74. B

36. A

25. B

75. D

1. D

37. B

26. A

76. A

2. C

38. A

27. C

77. B

3. D

39. C

28. D

78. C

4. C

40. C

29. B

79. D

5. D

41. D

30. D

80. D

6. D

42. A

31. D

81. D

7. A

43. A

32. A

8. D

44. C

33. C

CHAPTER 30

9. D

45. B

34. C

10. A

46. A

35. C

1. B

11. D

47. D

36. D

2. B

12. C

APPENDIX

Pr

Fre i

qn

u ceip

ntle

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B

ADH

anti iuretic hormone

HMWK

high molecular weight kininogen

AG

antiglobulin test

IA

indirect antiglobulin test

AHG

antihuman globulin

IF

intrinsic factor

AIDS

acquired immune deficiency syndrome

Ig

immunoglobulin

ANA

antinuclear antibody

IL

interleukin

AP

activated partial thromboplastin time

IM

infectious mononucleosis

ASCLS

American Society of Clinical Laboratory

IU

international unit

Science

IV

intravenous

ASCP

American Society of Clinical Pathologists

L

liter

ASO

antistreptolysin O

LAP

leukocyte alkaline phosphatase

CAP

College of American Pathologists

M

meter

CBC

complete blood count

MCH

mean cell hemoglobin

CDC

Centers for Disease Control and Prevention

MCHC

mean cell hemoglobin concentration

CFU

colony-forming unit

MCV

mean cell volume

CLIA'88

Clinical Laboratory Improvement Amendments

MPV

mean platelet volume

o 1988

MSDS

material safety data sheets

CL

clinical laboratory technician

M

medical technologist

COLA

Commission on Office Laboratory Accreditation

NAD

nicotinamide adenine dinucleotide, oxidized

CSF

colony-stimulating factor; cerebrospinal fluid

form

DA

direct antihuman globulin test

NADH

nicotinamide adenine dinucleotide, reduced form

DIC

disseminated intravascular coagulation

OSHA

Occupational Safety and Health Administration

DNA

eoxyribonucleic aci

PCV

packe cell volume

EA

early antigen

PEP

postexposure prophylaxis

EBV

Epstein-Barr virus

PKK

plasma prekallikrein

ED A

ethylene iaminetetraacetic aci

PMN

polymorphonuclear neutrophil

EIA

enzyme immunoassay

P

prothrombin time

ELISA

enzyme-linked immunosorbent assay; enzyme—

P

partial thromboplastin time

labeled immunosorbent assay

QA

quality assurance

ESR

erythrocyte sedimentation rate

QC

quality control

FIA

fluorescence immunoassay

RBC

red blood cell

FISH

fluorescent in situ hybridization

RDW

red cell distribution width

HBV

hepatitis B virus

SI

International System of Units

Hct (or Ht) hematocrit

SLE

systemic lupus erythematosus

HCV

hepatitis C virus; previously called non-A,

SPIA

solid-phase immunosorbent assay

non-B hepatitis virus

JC

The Joint Commission

HDN

hemolytic disease of newborn

LC

thin-layer chromatography

Hgb

hemoglobin

thrombin time

HHS

Department of Health and Human Services

vWD

von Willebrand's disease

HIV

human immunodeficiency virus

vWF

von Willebrand's factor

HLA

human leukocyte antigen

WBC

white blood cell

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APPENDIX

Safety Data Sheets

C

The Hazard Communication Standard (HCS) (29 CFR information of the supplier. The requirement in 1910.1200(g)), revised in 2012, requires that the manufacturers, distributors, or importers provide Safety

Product identifier use on the label and any other com—

Data Sheets (SDSs) (formerly MSDSs or Material Safety Data



mon names or synonyms by which the substance is known
Sheets) or each hazardous chemical to downstream users to
Name; address; phone number of the manufacturer,
communicate information on these hazards. The information



importer, or other responsible party; an emergency phone
number contained in the SDS is largely the same as the MSDS,
number

except that now the SDSs are required to be presented in a
Recommended use of the chemical (e.g., a brief description—
consistent user-friendly, 16-section format. This brief provides



information on what it actually does, such as flame retardant) and
guidance to help workers who handle hazardous chemicals to
any restrictions on use (including recommendations given
become familiar with the format and understand the contents
by the supplier)¹
of the SDSs.

The SDS includes information such as the properties

o each chemical; the physical, health, an environmen—

SECTION 2: HAZARD(S) IDENTIFICATION

tal health hazar s; protective measures; an sa ety precautions or han ling, storing, an transporting the chemical.

T is section i entif es the hazar s o the chemical presente

T e in or mation containe in the SDS must be in English

on the SDS an the appropriate warning in or mation associ-

(although it may be in other languages as well). In a iate with those hazar s. T e require in or mation consists

tion, OSHA requires that SDS preparers provi e specif c

o the ollowing:

minimum in or mation as etaile in Appen ix D o 29 CFR

■ T e hazar classif cation o the chemical (e.g., ammable 1910.1200. T e SDS preparers may also inclu e a itional liqui , category1).

in or mation in various section(s).

■ Signal wor .

Sections 1 through 8 contain general in or mation about

■ Hazar statement(s).

the chemical, i entif cation, hazar s, composition, sa e

■ Pictograms (the pictograms or hazar symbols may be

handling practices, an emergency control measures (e.g., presented as graphical reproductions of the symbols in the pictogram). This information should be helpful to those who are blind or have low vision or be a description of the name of the hazard that needs to get the information quickly. Sections 9 through 11 and 16 contain other technical and scientific information.

■ **Precautionary statement(s).**

Information, such as physical and chemical properties, stability and

■ **Description of any hazards not otherwise classified.**

Reactivity information, toxicological information, exposure

■ **For a mixture that contains ingredient(s) with unknown**

control information, an other information including the

toxicity, a statement describing how much (percentage) of

the mixture consists of ingredient(s) with unknown acute

that no applicable information was known when the pre-

toxicity. Please note that this is a total percentage of the

preparation or last revision. The SDS must also state

the mixture consists of ingredient(s) with unknown acute

that no applicable information was known when the pre-

toxicity. Please note that this is a total percentage of the

preparation or last revision. The SDS must also state

the mixture consists of ingredient(s) with unknown acute

element.

The SDS must also contain Sections 12 through 15, to be consistent with the UN Globally Harmonized System of

SECTION 3: COMPOSITION/INFORMATION ON

Classification and Labeling of Chemicals (GHS), but OSHA

INGREDIENTS

will not enforce the content of these sections because they concern matters handled by other agencies.

This section identifies the ingredient(s) contained in the

Description of all 16 sections of the SDS, along with

product information on the SDS, including impurities and sta—

their contents, is presented below:

balizing activities. This section includes information on substances, mixtures, and all chemicals where a trade secret is

claimed. The required information consists of the following:

SECTION 1: IDENTIFICATION

Substances

This section identifies the chemical on the SDS as well as

■ Chemical name

the recommended uses. It also provides the essential contact

■ Common name and synonyms

709

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APPENDIX C ■ Safety Data Sheets

■ Chemical Abstracts Service (CAS) number and other

and cleanup practices to prevent or minimize exposure to

unique identifiers

people, properties, or the environment. It may also include

■ Impurities and stabilizing additives, which are themselves

recommendations distinguishing between responses or

classification and which contribute to the classification of the

large and small spills where the spill volume has a significant

chemical

impact on the hazard. The required information may consist

of recommendations or

Mixtures

■ Use of personal precautions (such as removal of ignition

■ Same information required for substances

sources or providing sufficient ventilation) and protective

■ The chemical name and concentration (i.e., exact percent—

equipment to prevent the contamination of skin, eyes, and

age) of all ingredients which are classified as health hazards—
clothing

are as are

- Emergency procedures, including instructions or evacu-

- Present above their cut-off /concentration limits or

ations, consulting experts when needed, and appropriate

- Present a health risk below the cut-off /concentration

protective clothing

limits

- Methods and materials use or containment (e.g., cover-

- The concentration (exact percentages) of each ingredient

ing the rains and capping procedures)

must be specified except that concentration ranges may be

Cleanup procedures (e.g., appropriate techniques or

use in the following situations:

-

neutralization, decontamination, cleaning, or vacuum-

- A false secret claim is made,

ing; a sorbent material; and/or equipment required or

- There is batch-to-batch variation, or

containment/cleanup)

- The SDS is used for a group of substantially similar mixtures.

Chemicals where a trade secret is claimed

SECTION 7: HANDLING AND STORAGE

- A statement that the specific chemical is an entity and/or

This section provides guidance on the safe handling practices. If the exact percentage (concentration) of composition has been claimed as a trade secret, the conditions or safe storage of chemicals. The requirements for a trade secret are as follows:

Information consists of the following:

SECTION 4: FIRST-AID MEASURES

- Precautions for safe handling, including recommendations for handling incompatible chemicals, minimizing

This section describes the initial care that should be given by the release of the chemical into the environment, and procedures for untrained responders to an individual who has been exposed. It also includes advice on general hygiene practices (e.g., eating, drinking, and smoking in work areas is prohibited).

- Recommendations on the conditions or safe storage,

- Necessary first-aid instructions by relevant routes of exposure—including any incompatibilities. Provide advice on specific exposure (inhalation, skin and eye contact, and ingestion) storage requirements (e.g., ventilation requirements).

- Description of the most important symptoms or effects, and any symptoms that are acute or delayed

- Recommendations for immediate medical care and special

SECTION 8: EXPOSURE CONTROLS/PERSONAL

treatment needed, when necessary

PROTECTION

SECTION 5: FIREFIGHTING MEASURES

This section indicates the exposure limits, engineering controls, and personal protective measures that can be used to

This section provides recommendations for fighting a fire to minimize worker exposure. The required information concerning cause by the chemical. The required information consists of

- Recommendations of suitable extinguishing equipment,

- OSHA permissible exposure limits (PELs), American and information about extinguishing equipment that is

Conference of Governmental Industrial Hygienists

not appropriate or a particular situation

(ACGIH) threshold limit values (LVs), and any other

- Advice on specific hazards that develop from the chemical exposure limit use or recommended by the chemical during the fire, such as any hazardous combustion products from the manufacturer, importer, or employer preparing the safety data sheet, where available

- Recommendations on special protective equipment or

- Appropriate engineering controls (e.g., use local exhaust precautions or fume hoods, ventilation, or use only in an enclosed system)

- Recommendations on personal protective measures to

SECTION 6: ACCIDENTAL RELEASE MEASURES

prevent illness or injury from exposure to chemicals, such as personal protective equipment (PPE) (e.g., appropriate

This section provides recommendations on the appropriate types of eye, face, skin or respiratory protection needed

response to spills, leaks, or releases, including containment (based on hazards and potential exposure)

APPENDIX C ■ Safety Data Sheets

711

- Any special requirements or PPE, protective clothing, or

- Indication of any safety issues that may arise should the

respirators (e.g., type of glove material, such as PVC or

product change in physical appearance

nitrile rubber gloves; an breakthrough time of the glove

Other

material)

- Indication of the possibility of hazardous reactions, inclu -

ing a statement whether the chemical will react or polym—

SECTION 9: PHYSICAL AND CHEMICAL

erize, which could release excess pressure or heat, or create

PROPERTIES

other hazardous conditions. Also, a description of the conditions under which hazardous reactions may occur.

This section identifies physical and chemical properties asso-

- List of all conditions that should be avoided (e.g., static

charge with the substance or mixture. The minimum require

discharge, shock, vibrations, or environmental conditions

information consists of

that may lead to hazardous conditions).

- Appearance (physical state, color, etc.)

- List of all classes of incompatible materials (e.g., classes of

- Upper/lower flammability or explosive limits

chemicals or specific substances) with which the chemical

- Oxidizing or

could react to produce a hazardous situation.

- Vapor pressure

- List of any known or anticipated hazardous decomposition

- Oxidizing or threshold

products that could be produced because of use, storage, or

- Vapor density

heating. (Hazardous combustion products should also be

- pH

included in Section 5 [Fire-Fighting Measures] of the SDS.)

- Relative density

- Melting point/freezing point

- Solubility(ies)

SECTION 11: TOXICOLOGICAL INFORMATION

- Initial boiling point and boiling range

- Flash point

This section identifies toxicological and health effects in or-

- Evaporation rate

mation or indicates that such data are not available. The

- Flammability (solid, gas)

required information consists of the following:

- Partition coefficient: n-octanol/water

- Information on the likely routes of exposure (inhalation,

- Auto-ignition temperature

ingestion, skin and eye contact). The SDS should indicate

- Decomposition temperature

if the information is unknown.

- Viscosity

- Description of the delayed, immediate, or chronic effects

The SDS may not contain every item on the above list

from short- and long-term exposure.

because information may not be relevant or is not available.

- The numerical measures of toxicity (e.g., acute toxicity

When this occurs, a notation to that effect must be made or

estimates such as the LD₅₀ [median lethal dose]—the

that chemical property. Manufacturers may also determine the estimated amount (of a substance) expected to kill 50% of relevant properties, such as the dust concentration in air (Kst) or test animals in a single dose.

combustible dust, use to evaluate a dust's explosive potential.

- Description of the symptoms. This description includes the symptoms associated with exposure to the chemical including symptoms from the lowest to the most severe exposure.

SECTION 10: STABILITY AND REACTIVITY

- Indication of whether the chemical is listed in the National Toxicology Program (NTP) Report on Carcinogens (latest

This section describes the reactivity hazards of the chemical (edition) or has been found to be a potential carcinogen in

and the chemical stability information. This section is based on the International Agency for Research on Cancer (IARC)

divided into three parts: reactivity, chemical stability, and other.

Monographs (latest editions) or found to be a potential

The required information consists of the following:

carcinogen by OSHA.

Reactivity

- Description of the specific test data for the chemical(s).

These data can be for a class or family of the chemical in

SECTION 12: ECOLOGICAL INFORMATION

such data adequately represent the anticipated hazard of

(NONMANDATORY)

the chemical(s), where available.

This section provides information to evaluate the environ-

Chemical stability

mental impact of the chemical(s) if it were released to the

environment. The information may include

- Indication of whether the chemical is stable or unstable

under normal ambient temperature and conditions while

- Data from toxicity tests performed on aquatic and/or terrestrial storage and being handled

terrestrial organisms, where available (e.g., acute or chronic

- Description of any stabilizers that may be needed to maintain—

aquatic toxicity data on fish, algae, crustaceans, and other

to maintain chemical stability

plants; toxicity data on birds, bees, plants)

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APPENDIX C ■ Safety Data Sheets

- Whether there is a potential for the chemical to persist and

Construction and Equipment of Ships Carrying Dangerous
Materials in Bulk) (International Bulk Chemical Code
Chemicals in Bulk) (International Bulk Chemical Code
tion or other processes, such as oxidation or hydrolysis
[IBC Code])

- Results of tests of bioaccumulation potential, making reference to the octanol–water partition coefficient (K_{ow})
- Any special precautions that an employee should be aware of or needs to comply with, in connection with transportation and conveyance either within or outside their premises
- The potential for a substance to move from the soil to the ground water (inicate results from a sorption studies or leaching studies)
- Other adverse effects (e.g., environmental fate, ozone layer depletion potential, photochemical ozone creation potential, endocrine disrupting potential, and/or global warming—

SECTION 15: REGULATORY INFORMATION

(NONMANDATORY)

tial, endocrine disrupting potential, and/or global warming—

ing potential)

This section identifies the safety, health, and environmental regulations specific to the product that is not indicated anywhere else on the SDS. The information may include

SECTION 13: DISPOSAL CONSIDERATIONS

where else on the SDS. The information may include

(NONMANDATORY)

- Any national and/or regional regulatory information on the chemical or mixtures (including any OSHA, Department

This section provides guidance on proper disposal practices,

transportation, Environmental Protection Agency, or

recycling or reclamation of the chemical(s) or its container,

Consumer Product Safety Commission Regulations)

and safe handling practices. To minimize exposure, this section

should also refer the reader to Section 8 (Exposure Controls/

Personal Protection) of the SDS. The information may include

SECTION 16: OTHER INFORMATION

- Description of appropriate disposal containers to use

This section indicates when the SDS was prepared or when

- Recommendations of appropriate disposal methods to

employ

the last known revision was made. The SDS may also state where the changes have been made to the previous version.

- Description of the physical and chemical properties that may affect disposal activities

You may wish to contact the supplier for an explanation of the changes. Other useful information also may be included

- Language discouraging sewage disposal here.

- Any special precautions or handling or incineration activities

Employer Responsibilities

SECTION 14: TRANSPORT INFORMATION

Employers must ensure that the SDSs are readily accessible to employees for all hazardous chemicals in their workplace.

(NONMANDATORY)

This may be done in many ways. For example, employers

This section provides guidance on classification information

may keep the SDSs in a binder or on computers as long as the

for shipping and transporting of hazardous chemical(s) by

employees have immediate access to the information without

road, air, rail, or sea. The information may include

leaving their work area when needed and a backup is available or rapid access to the SDS in the case of a power out-

- UN number (i.e., four-figure identification number of the hazard or other emergency. Furthermore, employers may want substance)¹

to designate a person or persons responsible for obtaining

- UN proper shipping name¹

and maintaining the SDSs. If the employer does not have an

- transport hazard class(es)¹

SDS, the employer or designated person(s) should contact

- Packing group number, if applicable, based on the degree of hazard

the manufacturer to obtain one.

of hazard ²

- Environmental hazards (e.g., identify if it is a marine pollutant)

Reference

Accordant to the International Maritime Dangerous

Goods Code [IMDG Code]

OSHA, 29 CFR 1910.1200(g) and Appendix D. United

- Guidance on transport in bulk (accordant to Annex II

Nations Globally Harmonized System of Classification

o MARPOL 73/783 and the International Code of the
on Labelling of Chemicals (GHS), third revision,
United Nations, 2009. These references and other information—
1 Chemical, as defined in the HCS, is any substance, or mixture of
information relate to the revised Hazard Communication Standard
substances.

can be found on OSHA's Hazard Communication Safety and
2 Found in the most recent revision of the United Nations
Health topics page, located at: [http://www.osha.gov/sg/](http://www.osha.gov/sg/Recommendations%20on%20the%20Transport%20of%20Dangerous%20Goods)
Recommendations on the Transport of Dangerous Goods.

3
[hazcom/in-ex.html](http://www.osha-hazcom/in-ex.html).

MARPOL 73/78 means the International Convention on the
Prevention of Pollution from Ships, 1973, as modified by the Protocol

Disclaimer: This brief provides a general overview
of 1978 relating thereto, as amended

of the safety data sheet requirements in the Hazard

APPENDIX C ■ Safety Data Sheets

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Communication Standard (see 29 CFR 1910.1200(g))

Review Commission and the courts or additional guidance (see Appendix D of 29 CFR 1910.1200). It does not alter the requirements on OSHA compliance requirements. Please note that for states with OSHA-approved state plans may have additional requirements or chemical safety data sheets, outside of interpretations and enforcement policy may change over those outline above. For more information on those standards, the reader should consult current OSHA interpretations, please visit: <http://www.osha.gov/csp/osp/state-standards.html>.





APPENDIX

D Tube Guide BD

BD Vacutainer® Venous Blood Collection

Tube Guide

For the full array of BD Vacutainer® Blood Collection Tubes, visit www.bd.com/vacutainer.

Many are available in a variety of sizes and draw volumes (for pediatric

applications). Refer to our website for full descriptions.

BD Vacutainer® Tubes

BD Vacutainer® Tubes

Inversions

with

with

at Blood

Your Lab's

BD Hemogard™ Closure

Conventional Stopper

Additive

Collection*

Laboratory Use

Draw Volume/Remarks

5

d chemistry

separation

rou dono

Re d/

scree t serum

Gold

Gray

disease.** Tu inversion

ensure blood.

c minutes.

8

chemistry.

Tu ensure anticoagulant

Light

Gre e n/

Gre e n

Gray

separation

pr clotting.

(glass)

0

chemistry.

activator Silicone

5

ro

scre

Re d

Re d

(plastic)

disease.** Tu

ensure

minutes.

Vacutainer®

5 to 6

chemistry

Tube

Tu ensure

Orange

Thr

minutes.

activator

Thro

8

chemistry

activator

Tu i ensure activato

Orang e

minutes.

8

toxicology

serum)

8

pr

Royal

K2EDT (plastic)

Blue

Tu ensure

(EDT blood.

heparin

8

chemistry

heparin

8

Tu ensure

Gre e n

Gre e n

pr clotting.

oxalate/

8

d and

fluoride

EDTA

Gray

Gray

fluoride/Na₂ EDTA

8

fluorid

8

T

tube)

ensur pr blood.

K₂EDT (plastic)

8

Tan

Tu pr clotting.

Sodium

8

cultur

(SPS)

microbiology.

dextrose

(

Solution A -

8

patern testing.

citrate

Ye llow

a g/

Tu ensure

dextrose

pr clotting.

Solution B -

8

citrate

a g/

dextrose

714



APPENDIX D ■ Tube Guide BD

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BD Vacutainer® Tubes

BD Vacutainer® Tubes

Inversions

with

with

at Blood

Your Lab's

BD Hemogard™ Closure

Conventional Stopper

Additive

Collection*

Laboratory Use

Draw Volume/Remarks

Liquid K3EDTA (glass)

8

K2EDTA and K3EDTA for whole blood

Spray-coated K

8

hematology determinations. K

2EDTA

2EDTA may be

used for routine immunohematology testing,

Lave nde r

Lave nde r

(plastic)

and blood donor screening.***

Tube inversions ensure mixing of anticoagulant

(EDTA) with blood to prevent clotting.

BD Vacutainer®

8

For use in molecular diagnostic test methods

PPT™ Tube

(such as, but not limited to, polymerase chain

reaction [PCR] and/or branched DNA [bDNA]

White

K2EDTA with gel

amplification techniques.) Tube inversions

ensure mixing of anticoagulant (EDTA) with blood to prevent clotting.

Spray-coated K₂EDTA

8

For whole blood hematology determinations.

(plastic)

May be used for routine immunohematology testing and blood donor screening.***

Pink

Pink

Designed with special crossmatch label for patient information required by the AABB.

Tube inversions prevent clotting.

Buffered sodium citrate

3-4

For coagulation determinations. CTAD for

0.105 M (≈3.2%) glass

selected platelet function assays and routine

Lig ht

Lig ht

0.109 M (3.2%) plastic

coagulation determination. Tube inversions

Blue

Blue

Citrate, theophylline,

3-4

ensure mixing of anticoagulant (citrate) to

adenosine, dipyridamole

prevent clotting.

(CTAD)

Cle ar

None (plastic)

0

For use as a discard tube or secondary

Ne w

specimen tube.

Cle ar

Re d/

Light Gray

Note: BD Vacutainer® Tubes for pediatric and partial draw applications can be found on our website.

BD Diagnostic

* Invert gently, do not shake

BD Global Technical Services: 1.800.631.0174

Preanalytical Systems

** The performance characteristics of these tubes have not been established for infectious disease testing in general; therefore, users must **BD Customer Service** : 1.888.237.2762

validate the use of these tubes for their specific assay-instrument/reagent system combinations and specimen storage conditions.

1 Becton Drive

www.bd.com/vacutainer

*** The performance characteristics of these tubes have not been established for immunohematology testing in general; therefore, users must Franklin Lakes, NJ 07417 USA

validate the use of these tubes for their specific assay-instrument/reagent system combinations and specimen storage conditions.

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VS5229-11

VACUETTE® Tube Guide

Venous Blood Collection Tubes



Cap Color	Additive	Number of Inversions	Testing Disciplines	Comments
	No Additive	5-10	Discard tube Transport/Storage Immunohematology Viral Markers	
	Sodium Citrate 3.2% (0.109 M) 3.8% (0.129 M)	4	Coagulation	If a winged blood collection set is used AND the coagulation specimen is drawn first, a discard tube is recommended to be drawn prior to this tube to ensure the proper anticoagulant-to-blood ratio.
	Clot Activator	5-10	Chemistry Immunochemistry Immunohematology Viral Markers	For complete clotting, 30 minutes minimum clotting time is required. Incomplete or delayed mixing may result in delayed clotting.
	Clot Activator w/Gel	5-10	Chemistry Immunochemistry TDMs	For complete clotting, 30 minutes minimum clotting time is required. Incomplete or delayed mixing may result in delayed clotting.
	Lithium Heparin Lithium Heparin w/Gel Sodium Heparin	5-10	Chemistry Immunochemistry	
	K ₃ EDTA K ₂ EDTA	8-10	Hematology Immunohematology Molecular Diagnostics Viral Markers	
	K ₂ EDTA Gel	8-10	Molecular Diagnostics	
	Potassium Oxalate/ Sodium Fluoride	5-10	Glycolytic Inhibitor Glucose and Lactate	
	Sodium Heparin	5-10	Trace Elements	

Ring Indicator



yellow - Gel Separation



black - Standard Draw



green - Sodium Heparin



white - Pediatric Draw

see reverse for pediatric cap information

Greiner Bio-One
888.286.3883 Phone | 800.726.0052 Fax
office@us.gbo.com | www.gbo.com

VACUETTE®
one step ahead ▶

APPENDIX

ube Guide Greiner

E

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VACUETTE® Tube Guide

Pediatric or Small Volume Draw Tubes (2 ml or Less)



Cap Color	Additive	Number of Inversions	Testing Disciplines	Comments
	Clot Activator	5-10	Chemistry Immunochemistry Immunohematology Viral Markers	For complete clotting, 30 minutes minimum clotting time is required. Incomplete or delayed mixing may result in delayed clotting.
	Lithium Heparin Sodium Heparin	5-10	Chemistry Immunochemistry	
	Sodium Citrate 3.2% (0.109M) 3.8% (0.129M)	4	Coagulation	If a winged blood collection set is used AND the coagulation specimen is drawn first, a discard tube is recommended to be drawn prior to this tube to ensure the proper anticoagulant-to-blood ratio.
	Potassium Oxalate/ Sodium Fluoride	5-10	Glycolytic Inhibitor Glucose and Lactate	
	K ₃ EDTA K ₂ EDTA	8-10	Hematology Immunohematology Molecular Diagnostics Viral Markers	

Ring Indicator



white - Pediatric Draw

For further assistance: 888.286.3883 Phone | 800.726.0052 Fax | office@us.gbo.com | www.gbo.com

0731003R1

VACUETTE®
one step ahead ▶

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APPENDIX

Interleukins

F

Summary of Interleukins (ILs)

Name

Source

Target Blood Cells

Function

IL-1

B cells, monocytes, dendritic T helper cells

Costimulation

cells.

B cells

Maturation and proliferation

Appears to influence dif—

NK cells

Activation

ferent progenitor cells indi—

Macrophages

In ammation

rectly in hematopoiesis. It

may act in synergy with IL-3,

M-CSF, G-CSF, and GM-CSF

to stimulate cel s.

IL-2

Th1 cells

Activated T cel s and B cells, In uences the proliferation and regulation of

NK cel s, macrophages

T cells, B cel s, natural killer (NK) cells, and

monocytes. It acts on activated B cel s as a

growth and differentiation factor.

IL-3

Activated Th3 cells, mast

Hematopoietic stem cells

Promotes the growth of early hematopoietic cel

cel s, NK cel s, endothelium, Mast cel s

lines (e.g., proliferation of CFU-GEMM, CFU-M,

eosinophils

CFU-Meg, CFU-Eo, and CFU-Bs colonies from

bone marrow). IL-3 acts with M-CSF to stimulate proliferation of monocytes and macrophages. It also stimulates granulocyte, monocyte, eosinophil, and mast cell production.

Growth and histamine release

IL-4

Th2 cells, just activated

Activated B cells

Proliferation and differentiation, IgG1 and IgE

naive CD4⁺ cells, memory

T cells

synthesis

CD4⁺ cells, mast cells,

Proliferation

macrophages

Interacts with G-CSF to proliferate myeloid progenitor cells.

IL-5

Th2 cells, mast cells, eosinophils

Stimulates eosinophil colony production and
phils

B cells

interacts with GM-CSF and IL-3 in eosinophil
induction.

Differentiation, IgA production

IL-6

Macrophages, Th2 cells, B

Activated B cells

Differentiation into plasma cells

cells, astrocytes, endothelial

Plasma cells

Antibody secretion

lumen

HSCs

Differentiation

T cells, others

Induces acute phase reaction, hematopoiesis,
differentiation, inflammation

IL-7

Bone marrow stromal cells

Pre/pro-B cell, pre/pro-T

Differentiation and proliferation of lymphoid

and thymus stromal cells

cell, NK cells

progenitor cells, involved in B, T, and NK cell

survival, development, and homeostasis,

↑ proinflammatory cytokines

718

APPENDIX F ■ Interleukins

719

Summary of Interleukins (ILs) (continued)

Name

Source

Target Blood Cells

Function

IL-8

Macrophages, lymphocytes, Neutrophils, basophils,

An inflammatory cytokine that is chemotactic

epithelial cells, endothelial

lymphocytes

for both neutrophils and T cells. It is a potent

cells

stimulator of neutrophils, and it activates the

respiratory burst and the release of both specific and azurophilic granular contents.

IL-9

Th2 cells, specifically by

T cells, B cells

Acts as a potent CD4⁺ T lymphocyte growth

CD4⁺ helper cells

factor. In addition, it has been demonstrated

to support growth of BFU-E.

IL-10

Monocytes, Th2 cells, CD8⁺

Macrophages

Cytokine production

T cells, mast cells, macro—

B cells

Activation inhibits Th1 cytokine production

phages, B cell subset

Mast cells

(IFN- γ , TNF- β , IL-2)

Th1 cells

Stimulation

Th2 cells

IL-11

Bone marrow stroma

Bone marrow stroma

Multifunctional regulator of hematopoiesis

IL-12

Dendritic cells, B cells,

Activated T cells

Differentiation into cytotoxic T cells with IL-2,

T cells, macrophages

NK cells

↑ IFN- γ , TNF- α , ↓ IL-10

↑ IFN- γ , TNF- α

IL-13

Activated Th2 cells, mast

Th2 cells, B cells,

Stimulates growth and differentiation of

cells, NK cells

macrophages

B cell s (IgE), inhibits Th1 cells and the production of macrophage inflammatory cytokines

(e.g., IL-1, IL-6), ↓ IL-8, IL-10, IL-12.

IL-14

T cell s and certain malignant Activated B cell s

Induces growth and proliferation of B cell s,

B cells

inhibits Ig secretion

IL-15

Mononuclear phagocytes

T cells, activated B cell s

Induces production of natural killer cells

(and some other cell s),

especially macrophages following infection by virus(es)

IL-16

Lymphocytes, epithelial

CD4⁺ T cell s (Th cells)

CD4⁺ chemoattractant, increases the mobility

cells, eosinophils, CD8⁺

of CD4⁺ T cell s

T cells

IL-17

T helper 17 cells (Th17)

Epithelium, endothelium,

↑ In inflammatory cytokines

other

IL-18

Macrophages

Th1 cells, NK cells

Induces production of IFN- γ , ↑ NK cell activity

Acts as a synergist with

IL-12 in some of its effects

IL-19

—

Regulates the functions of macrophages;

suppresses the activities of Th1 and Th2

IL-20

Biological activities similar

Regulates proliferation and differentiation of
to IL-10

keratinocytes

IL-21

Activated T helper cells,

All lymphocytes, dendritic

Costimulates activation and proliferation of

NKT cells

cells

CD8+ T cells; augments NK cytotoxicity; augments CD40-driven B cell
proliferation, differentiation, and isotype switching; promotes

differentiation of Th17 cells

IL-22

Similar to IL-10

Activates STAT1 and STAT3 and increases

—

production of acute phase proteins such as

serum amyloid A, Alpha 1-antichymotrypsin,

and haptoglobin in hepatoma cell lines

720

APPENDIX F ■ Interleukins

S um m ary of Interleukins (ILs) (continued)

Nam e

S ource

Targe t Bloo d Ce lls

Function

IL-23

—

Increases angiogenesis but reduces CD8

T cel in ltration

Acts as a stimulant on particular populations

of memory T cells

IL-24

—

Plays important roles in tumor suppression,

wound healing, and psoriasis by in uencing

cell survival

IL-25

Supports proliferation of cells in the lymphoid lineage

Induces the production of IL-4, IL-5, and IL-13, which stimulate eosinophil expansion

IL-26

Enhances secretion of IL-10 and IL-8 and cell surface expression of CD54 on epithelial cells

IL-27

Regulates the activity of B lymphocytes and T lymphocytes

IL-28

Plays a role in immune defense against

viruses

IL-29

—

Plays a role in host defenses against microbes

IL-30

—

Forms one chain of IL-27

IL-31

—

May play a role in inflammation of the skin

IL-32

—

Induces monocytes and macrophages to
secrete TNF- α , IL-8, and CXCL2

IL-33

—

Induces helper T cells to produce type 2

cytokine

IL-35

Regulatory T cells

Suppression of T helper cell activation

M-CSF, macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; CFU-GEMM, colony-forming unit-granulocyte, erythrocyte, monocyte, and megakaryocyte; CFU-M, colony-forming unit-macrophage; CFU-Meg, colony-forming unit-megakaryocyte; CFU-Eo, colony-forming unit-eosinophil; CFU-Bs, colony-forming unit-basophil; BFU-E, burst-forming unit-erythroid.

APPENDIX F ■ Interleukins

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Characteristics of Human Hematopoietic Growth Factors

Growth Factor

Cellular Source

Progenitor Cell Target

Mature Cell Target

Erythropoietin

Peritubular cells of the kidney,

CFU-E, late BFU-E, CFU-Meg

None

Kupffer's cells

IL-3

Activated T lymphocytes

CFU-blast, CFU-GEMM, CFU-GM,

Eosinophils, monocytes

CFU-G, CFU-M, CFU-Eo, CFU-Meg,

CFU-Baso, BFU-E

G-CSF

Monocytes, broblasts,

CFU-G

Granulocytes

endothelial cells

M-CSF

Monocytes, broblasts,

CFU-M

Monocytes

endothelial cells

GM-CSF

T lymphocytes, monocytes, eo—

CFU-blast, CFU-GEMM, CFU-GM,

Granulocytes

sinophils, monocytes, broblasts, CFU-G, CFU-M, CFU-Eo, CFU-Meg,

endothelial cells

BFU-E

G-CSF, granulocyte colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; CFU-blast, colony-forming unit-blast; CFU-GEMM, colony-forming unit granulocyte, erythrocyte, monocyte, and megakaryocyte; CFU-GM, colony-forming unit-granulocyte and macrophage; CFU-Eo, colony-forming unit-eosinophil; CFU-Meg, colony-forming unit-megakaryocyte; BFU-E, burst-forming unit-erythroid; CFU-G, colony-forming unit-granulocyte; CFU-M, colony-forming unit-macrophage; CFU-E, colony-forming unit-erythroid; CFU-Baso, colony-forming unit-basophil.

APPENDIX

T e Microscope

G

EQUIPMENT AND SUPPLIES

USE OF THE MICROSCOPE

1. Microscope

A microscope is a elicate instrument. Follow the proce ural

2. Lens paper

step in exact or er.

3. Kimwipes

4. Immersion oil

Step

Procedural Steps

1

Remove the microscope dust cover and clean the lens of the objectives (see below).

2

Turn on light source. Rotate the low power, for example, 10×, objective lens slowly until an audible click is heard.

Note: Always begin initial y focusing with the low power objective.

3

Look at the microscope from the side to increase the working distance as far as possible with the coarse adjustment.

4

Put on gloves as directed. Select a specimen slide and place on the microscope stage. Secure with clamp on the stage.

5

Center the slide on the stage by rotating the stage knobs located under the stage.

6

On the ocular, turn the diopter ring to the middle of the adjustment range. While looking through the right eyepiece, slowly turn the coarse adjustment

to bring the specimen into focus. Once in focus, use the fine adjustment knob to make a sharper image adjustment.

7

While looking through the left eyepiece, turn the diopter adjustment ring to focus the specimen.

8

Adjust the interpupillary distance of the eyepieces until both left and right fields of vision coincide completely. The image should appear as one single field with a sharply focused image. The image should remain in sharp focus if either eye is closed.

Note: Lift out one eyepiece and adjust aperture diaphragm to about 3/4 full. Specific adjustments are 0.25 for 10×, 0.65 for 40×, and 1.3 for 100×. Replace eyepiece.

9

After the specimen slide is in focus, open the iris diaphragm in the substage condenser to its widest adjustment. Set the light source intensity control to about 2/3 maximum output. A blue filter may be needed to reduce yellow emissions from the light source.

10

If the condenser has a swing out lens, position this lens in the light path.

11

Lower the condenser and close the field iris diaphragm. Raise the condenser until the image of the circle of light is in sharp focus.

12

Center the field iris diaphragm by manipulating the two centering screws on the compensating lens.

Note: To check, open the eld iris diaphragm until its image touches the perimeter of the eld of view. If the image is not precisely in the eld of view, center again.

13

Open the eld iris diaphragm until the edge of the leaves disappear from your eld of vision.

14

Select and position the objective lens of choice. Adjust the iris diaphragm until the appropriate amount of light is available. The amount of light is also controlled by the light source.

Note: Less light is needed for 10× and the most amount of light is needed when using the oil immersion (100×) objective lens. The amount of light needs to be adjusted each time a new objective lens is selected.

15

Use the ne adjustment knob to refocus to the sharpest image. With parfocal objectives, once an image is in focus with 10×, it will remain in focus when higher power objectives, for example, 40× or 100×, are selected.

If 100× objective lens is selected, move the slide slightly sideways and place a drop of immersion oil direct on the slide over the area to be observed. Return the slide to the appropriate viewing area.

Caution: Always begin focusing with the low power objective, for example, 10×.

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APPENDIX G ■ The Microscope

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Step

Procedural Steps

16

Following examination of a specimen slide, remove the slide and clean the microscope by wiping off the objective lens with lens paper. Be careful to clean the oil immersion lens last.

17

Return the low power (10×) objective to the viewing position and completely decrease the working distance by lowering the objective to the lowest possible position.

18

Cover the microscope and return to storage, if it is not stored on the laboratory benchtop.

TIPS FOR CLEANING THE MICROSCOPE

a circular motion, beginning at the center and moving outward. Repeat with new ampere lens paper as necessary.

Cleaning the Exterior

Finally, blot dry with clean lens paper. Do not rub; this may scratch the surface of the lens.

The surface of most microscopes may be kept clean by wash—

Lenses should never be touched with the fingers.

ing it with a neutral soap and water. To clean the metal and

Objectives must not be taken apart because even a slight

enamel, a gauze or so cloth should be moistened with

alteration of the lens setting may ruin the objective. Merely the cleaning agent and rub over the surface with a circular motion. The surface should be immediately cleaned. A dirty objective may be unscrewed from the nosepiece with a Kimwipe or clean, dry piece of gauze. Kimwipes or then held upside down and checked for cleanliness by using gauze should never be used to clean the optical parts of the microscope. The ocular (remove from the body tube) as a magnifying

glass. Dust or lint can also be removed from the rear lens of the objective by blowing it away with an air syringe. Such

Cleaning Optical Lenses : General

removal of the objective from the nosepiece is not a routine

Comments

cleaning procedure. The final step after using the microscope The glass surfaces of the ocular, the objectives, and the condenser should always be wiped with clean lens paper. These lenses must be

kept meticulously clean. Optical glass is softer than ordinary glass and should never be cleaned with paper tissue or gauze;

Cleaning the Ocular

these materials will scratch the lens. To clean the lenses of the

The ocular (eyepiece) is susceptible to dirt because of its location on the microscope and contact with the observer's eye. To clean the ocular, use lens paper.

When polishing with lens paper, take care that nothing

Mascara presents a constant cleaning problem. Dust can be

is present that will scratch the optical glass in the polishing

remove from the lens of the ocular with an air syringe or

process. Such potentially abrasive dirt, dust, or lint can easily

camel's hair brush; air is probably easier to use and more

be blown away before polishing.

efficient. The lens should then be polished with lens paper.

The ocular can be checked for additional dirt by holding it

Cleaning the Objectives

up to a light and looking through it. When looking into the

Oil must be removed from the oil-immersion (100×) objective—

microscope, dirt on any part of the ocular will rotate with the

tive immediately after use by wiping with clean lens paper.

ocular when it is turned. The ocular should not be removed

If not removed, oil may seep inside the lens or dry on the

for more than a few minutes; dust can collect in the body tube

outside the surface of the objective. The high-mag (40×) objective

can settle on the rear lens of an objective.

should never be used with oil, but if this or any other objective or microscope part comes into contact with oil, it should

Cleaning the Condenser

be cleaned immediately. If a lens is especially dirty, it may

The light source and condenser should also be removed,

be cleaned with a small amount of commercial lens cleaner,

lint, and dirt. First, blow away the dust with an air syringe

methanol, or manufacturer-recommended solution applied

or camel's hair brush, then polish the light source and condenser lens paper then wipe across the surface.

condenser with lens paper. It may be necessary to clean these

to clean the oil-immersion lens properly, first lower the

parts further with lens paper moistened with a commercial

stage, then rotate the objective to the front and wipe gently

lens cleaner or methanol before polishing them with lens

with clean lens paper. Clean off the immersion oil with lens paper.

paper ampene with special lens cleaner.

Alternatively, the cleaning agent may be applied to a

Cleaning the Stage and Adjustment Knobs

wooden applicator stick wrapped with cotton or lens paper

and moistened with the cleaning agent. Do not use a plastic

The stage of the microscope should be cleaned after each use

applicator stick; it will be dissolved by the solvent, ruining

by wiping with gauze or a tissue. After it has been cleaned

the objective. Apply the cleaning agent by blotting and using

thoroughly, the stage should be wiped dry.

APPENDIX

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QUICK CALCULATION REFERENCES

Low Blood Platelet Counts

Absolute Leukocyte Cell Count

Number of platelets = average total of platelets in five squares ×

dilution factor × volume correction factor = Platelets × 10⁹/L

Absolute cell value = total leukocyte count

×%o WBC type on differential smear

Reticulocytes

evaluation

Corrected reticulocyte count (%) =

Absolute reticulocyte count = %reticulocytes \times RBC count

Hematocrit (PCV)

Reticulocyte count \times

= % (corrected)

Normal PCV based on

Total Leukocyte Count Corrected for

age and gender

Nucleated Erythrocytes

Average total leukocyte

Reticulocyte Production Index (RPI)

count $\times 100$

Corrected total WBC =

Corrected reticulocyte count (%)

100 + number of nucleated RBCs /

RPI (%) =

100 WBC in the differential count

Maturation time in days

Maturation Time Correction Factor

Erythrocyte Measurements

Hematocrit (L/L)

Maturation time

Mean Corpuscular

Hematocrit (PC V) L/ L

0.45

1.0

Volume (MCV)

=

L

RBC count (1

× 012 / L) = a

0.35

1.5

0.25

2.0

0.15

2.5

Mean Corpuscular

Hemoglobin (1

× 0 g / L)

=

= pgb

Miller Ocular Disc Calculation for

Hemoglobin (MCH)

RBC count (1

× 10¹² / L)

Reticulocytes

Mean Corpuscular

Number of reticulocytes

Hemoglobin (g / L)

Hemoglobin

(large square)

=

= g / L

Reticulocyte =

× 10¹⁰ = % uncorrected

Concentration (MCHC) Hematocrit (PCV) L/ L

Number of erythrocytes

(small square) × 9

microliters

b picograms

International Normalized Ratio (INR)

$\text{INR} = (\text{patient P} / \text{control})^{\text{ISI}}$

Red Cell Distribution Width (RDW)

Derive from RBC histogram

$\text{CV of RBC distribution} = (\text{SD}$

$\times 100)$

mean

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APPENDIX H ■ Quick References

725

BODY FLUIDS

Neonates

Segmente polymorphonuclear neutrophils

0%–5%

Cerebrospinal Fluid

Lymphocytes

2%–38%

Mononuclear cells

50%–94%

Manual Cell Counts

Ependymal cells

rare

Body Fluids

Histiocytes

1%–6%

Number of leukocytes (WBCs) = average total of WBCs in

Pleural Fluid

four large squares \times dilution factor \times

volume correction factor = WBCs \times 10⁹/L

Total Cell Count

≤ 1000 WBCs/ μ L, 0 RBCs/ μ L

Total Cell Count

Differential PMN $\leq 25\%$, Mononuclear 0%–75%

Adults 0–5 mononuclear cells/ μ L, 0 RBCs/ μ L

Seruminal Fluid

Neonates 0–30 mononuclear cells/ μ L, 0 RBCs/ μ L

total volume: 1.5–5.0 mL

Differential Cell Count

total cell count 20–160 million/mL

Adults

Synovial Fluid

Segmented polymorphonuclear neutrophils

0%–5%

Lymphocytes

28%–96%

Total cell count

Mononuclear cells

16%–56%

200–600 WBCs/cells/μL, 0 RBCs/μL

Ependymal cells

rare

Histiocytes

rare

Differential PMN 0%–25%, mononuclear cells 0%–75%

QUICK GUIDE TO REFERENCE (NORMAL) VALUES*

Erythrocytes¹

Adult Values

Male

Female

Hematocrit

Packed cel volume

41.5%–50.4%

36%–45%

Erythrocyte count

$4.5\text{--}5.9 \times 10^{12}/\text{L}$

$4.5\text{--}5.1 \times 10^{12}/\text{L}$

Hemoglobin concentration

13.5–17.5 g/dL

12.0–16.0 g/dL

Mean corpuscular volume (MCV)

80–96 fL

80–96 fL

Mean corpuscular hemoglobin (MCH)

27.5–33.2 pg

27.5–33.2 pg

Mean corpuscular hemoglobin concentration (MCHC)

33%–36%

33%–36%

Pediatric Values

At birth (cord blood)

Packed cel volume

51%

Erythrocyte count

$4.7 \times 10^{12}/L$

Hemoglobin concentration

16.5 g/dL

MCV

108 fL

MCH

34 pg

At 6–12 years of age

Packed cel volume

40%

Erythrocyte count

$4.6 \times 10^{12}/L$

Hemoglobin concentration

13.5 g/dL

MCV

86 fL

MCH

29 pg

*Values vary by testing methods and patient populations.

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APPENDIX H ■ Quick References

Other Erythrocyte-Related Measurements

Representative Hemostasis and

Conventional Units

Coagulation Assays

Red Cell Distribution Width

11.5%–14.5%

Platelets (Aver. Adult)

150–400 × 10⁹/L

Reticulocyte Count

Mean platelet volume (MPV)

7.4–10.4 L

Newborn

2.5%–6.5%

Activate partial thromboplastin time (aP) 32–48 secon s

A ult

0.5%–1.5%

d-Dimer

0.0–0.4 µg/mL

Fibrinogen

200–400 mg/ L

Hemoglobin

Prothrombin time (P)

12–15.5 secon s

Electrophoresis

T rombin time

15–20 secon s

A1 hemoglobin

95%–97%

A2 hemoglobin

2%–3%

Erythrocyte Reference:

Fetal newborn

60%–90%

1. Greer JP, *et al.* (e s.). Wintrobe's Clinical Hematology, 11th e .,

>1 year

1%–2%

2004:2697–2719.

Erythrocyte Sedimentation Rate (ESR)

Male 0–15 mm/h

Leukocyte References:

(Adults <50 Years of Age)

Female 0–20

2. Greer JP, *et al.* Wintrob's Clinical Hematology, Volume Two, 12th

mm/h (Westergren

e ., Philadelphia, PA: Lippincott Williams & Wilkins, 2009:2584–

Metho)

2585. ables B.2 an B.4.

3. McPherson RA, Pincus MR, *et al.* Henry's Clinical Diagnosis and

Leukocyte s 2

Management by Laboratory Methods, 22n e ., St. Louis, MO: El-

otal2

sevier, 2011:1502. ables A.5–A.9.

Birth

9.0–30.0 × 10⁹/L

6 months

6.0–17.5 × 10⁹/L

10 years

4.5–13.5 × 10⁹/L

21 years an ol er

4.5–11.0 × 10⁹/L³

A ult

Average Relative Percentage³

Neutrophils (Segs, Polys)

56%

Immature (ban s) neutrophils 3%

Eosinophils

2.7%

Basophils

0.3%

Monocytes

4%

Lymphocytes

34%

Examples of Absolute

Leukocyte Cell Counts

Segmented Neutrophils

$1.4\text{--}6.5 \times 10^9/\text{L}$

Lymphocytes

$1.2\text{--}3.4 \times 10^9/\text{L}$

Comparative Average Relative

Percentage²

Birth

Neutrophils 61%,

Lymphocytes 31%

1 year of age

Neutrophils 31%,

Lymphocytes 61%

10 years of age

Neutrophils 54%,

Lymphocytes

GLOSSARY

A

aleukemic leukemia – a form of leukemia in which little change

abdominal fluid – abnormal fluid from the large cavity of the body

is seen in the total leukocyte count or cellular maturity in the

with major organs below the diaphragm

peripheral blood. An increased number of immature cells can

abetalipoproteinemia – rare hereditary disorder where abnormally

be found in the bone marrow

shaped red blood cells, acanthocytes, are seen

alkaline – a basic solution (pH 7.1 to 14.0) with the ability to neu—

absolute iron deficiency anemia – a decrease in total body iron

metabolize acids

caused by conditions such as blood loss due to chronic bleeding

ALL – acute lymphoblastic leukemia

or trauma, decreased intake of iron, or increased utilization of iron

allele(s) – one or two or more genes that occur at the same locus on

absolute lymphocytosis – an increase in the total number of lymphocytes

homologous chromosomes

lymphocytes in the circulating blood. Seen in viral infections such as

allogeneic stem cell transplantation – a transfer of primitive cells

infectious mononucleosis and rubella (German measles)

from a donor to a recipient

absolute number – a number such as an absolute cell count where

alpha granule – a type of storage granule found in the mature platelet

the concentration of a particular type of cell is calculated using

ambient temperature – temperature of surrounding environment,

the differential percentage of a specific cell times the total number

for example, air temperature

number of cells counted

amniotic fluid – the watery fluid surrounding a fetus in the womb

absolute polycythemia – see secondary polycythemia

amplitude – height or magnitude

absolute reticulocyte count – see corrected reticulocyte count

amyloidosis – the abnormal deposition of amyloid, a protein, in

absorbance – optical density

various tissues

acanthocytes – an abnormally shaped red blood cells seen in con—

anaphase – a stage in cellular division (mitosis)

ditions such as abetalipoproteinemia, cirrhosis of the liver with

anaphylaxis – a severe and often life-threatening reaction to a or—

hemolytic anemia or neonatal hepatitis

foreign protein

accuracy – describes how close a test result is to the true value

anaplasia – highly pleomorphic and bizarre cytologic features asso—

acidosis – a condition of venous blood pH being less than 7.42

ciated with malignant tumors that are poorly differentiated

acquired hemolytic anemia – a condition of accelerated destruc—

anemia – a condition of decreased or dysfunctional erythrocytes

tion of red blood cells caused by external factors

anemia of chronic diseases or disorders (ACD), anemia of in am—

acquired immunodeficiency syndrome (also acquired immunodeficiency (AID) – a term used to describe anemia associated with

immunodeficiency disorder) – an immune disorder caused by a virus in

inflammation, chronic infection, malignancy, or various sys—

the case of AIDS

temic diseases. Also known as anemia of chronic diseases (ACD)

activate clotting time – a type of laboratory measurement of the

aneuploidy – a deviation from the diploid number of chromosomes

time required for blood to form a clot

characteristic of Hodgkin disease

activate partial thromboplastin time (APTT or aPTT) – a labora—

angina – any condition characterized by spasmodic feelings of

tory assay to assay the first stage of blood coagulation

suffocation

active transport – the movement of molecules across a cellular

angiogenesis – the growth of new blood vessels

membrane that requires energy

anisochromia – variation of the color of erythrocytes caused by

actomyosin (thrombosthenin) – a contractile protein found in

unequal hemoglobin concentration

platelets

anisocytosis – a general term used to denote an increased variation

acute – severe and of short duration

in cell size

acute blood loss – sudden loss of circulating blood

ANLL – acute nonlymphoblastic leukemia

adenopathy – swelling of the lymph nodes

anomaly – a significant deviation from normal

adhesiveness – in coagulation, the process of platelets sticking to

anorexia – loss of appetite

the blood vessel wall

anoxia – without oxygen. The reduction of oxygen in the tissues

ADP – adenosine diphosphate

below physiological levels

agammaglobulinemia – the absence or severe decrease of the

antibody – an immunoglobulin produced in response to an antigen

gamma globulin protein fraction in the blood

anticoagulants – substances that prevent or delay the clotting of blood

agglutination – clumping of cells

antigen – a chemical substance that can elicit an antibody response

agglutinin – an antibody produced in response to a specific antigen

or can be used to identify cells

(foreign substance)

antihemophilic factor – factor VIII

aggregate – a clump of cells or particles

anti-human globulin test (AHG) – previously referred to as the
aggregation – in blood coagulation, the process in which platelets
Coombs' test. May be either a direct or an indirect test to detect
stick or clump together

the presence of antibodies on erythrocytes (direct test) or the pres—
agranulocytosis – a severe lack of the normal concentration of
ence of antibodies capable of coating erythrocytes (indirect test)
granulated peripheral blood cells

antiphospholipid syndrome (APS) – an immune disorder character—
Alder-Reilly inclusions – abnormal purple-red particles represent—
ized by elevated levels of two primary classes of antiphospholipid
ing precipitated mucopolysaccharides that are seen primarily
(aPL) antibodies-anticardiolipin antibodies and lupus anticoagu—
in neutrophilic, eosinophilic, and basophilic leukocytes seen in
lant. Associated with both arterial and venous thrombosis

Alder-Reilly anomaly

anuclear – a cell lacking a nucleus

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Glossary

antithrombin – an alpha-2 globulin that circulates in the plasma

basophilic granules – blue-staining granules

aplastic anemia – a congenital or genetic predisposition to bone

BCP – see basic calcium phosphate

marrow ailure

BCR-ABL1 usion gene – the abnormal recombination o two

apo erritin – a protein that combines with iron to orm erritin

dif erent segments o genes seen in disorders such as chronic

apoptosis – programmed cell death

myelogenous leukemia

appen icular skeleton – the bones o the limbs o the body

Bence Jones protein – the abnormal protein requently ound in

APTT – activated partial thromboplastin time

the urine o patients with multiple myeloma. It precipitates at

argon – an inert gas used in lasers

50°C, disappears at 100°C, and reappears on cooling to room

arteries – distributing blood vessels that leave the heart

temperature

arterioles – microscopic continuations o arteries that give of

benign – nonmalignant or noncancerous

branches called metarterioles, which in turn join the capillaries

Bernard-Soulier syndrome – a disorder characterized by the largest

arthritide – an eruption of the skin caused by gout

platelets seen in a platelet disorder

arthrocentesis – entry into a joint cavity to aspirate fluid

beta-thalassemia – a form of anemia in which beta-chain synthesis

arthrography – radiographic (x-ray) study of a joint

is impaired

artifacts – any artificial particles seen in stained preparations, diluted—

bilirubin – a breakdown product of heme from hemoglobin

in fluids, *etc.*

biliverdin – a breakdown product arising from the oxidation of

ascites – an abnormal accumulation of fluid in the abdominal (peri—

bilirubin

itoneal) cavity

bimodal cellular distribution – two populations of cells when mea—

aspirate – the process of physically removing, usually with a syringe,

sorted by size

fluid from a body cavity or space

biohazard – a substance capable of causing harm

assay – the determination of the purity of a substance or the amount

bite cells – an abnormal type of red blood cell

of a particular substance in a mixture or compound

bit map – a polygonal figure with as many as 16 sides drawn around

ATP – adenosine triphosphate

the cells to be analyzed or sorted in flow cell cytometry

atrophy – a decrease in the number or size of cells that produces a

blasts – the most immature form of cells

reduction in the size of a normal organ or tissue

blast crisis – the dominance of immature blood cells in the blood

atypical antibody – an antibody not usually found in the blood

or bone marrow of patients with a treated leukemia previously

plasma. Also referred to as an alloantibody

in remission

Auer rods or Auer bodies – these cellular inclusions are aggregates

blister cells – an abnormal red blood cell that appear to have a blis—

of cytoplasmic granules that appear as red, elongated structures.

ter-like, clear space

They may occur alone or in groups in myeloblasts and occasionally—

blood-brain barrier – walls of blood vessels of the central nervous

ally monoblasts

system that prevent or delay the entry o certain blood sub—

autoantibody – antibodies capable o reacting with one's own cells.

stances into the brain tissue

In autoimmune hemolytic anemia, patients develop antibodies

blotting – trans fer or xation o nucleic acids onto a solid matrix,

that produce hemolysis o the patient's own cells

such as nitrocellulose, so that they may be hybridized with a probe

autoimmune hemolytic anemia – the destruction and reduction

bone marrow – the material in the cavities o bones. Red marrow is

o red blood cells because o the rupturing o the cellular mem—

the site o hematopoiesis

brane due to self-directed antibodies

buffer solution – a solution that will resist sudden changes in acid—

autosomal dominant – a genetic trait that expresses itself, i pres—

ity or alkalinity

ent, and is carried on one o the chromosome pairs 1 through 22

buffy coat – the interface layer in a tube o anticoagulated blood

axial skeleton – the bones o the head and trunk o the body

between the plasma and erythrocytes. This layer contains leuko—

azurophilic granules – granules that stain red due to azure dyes

cytes and thrombocytes

burr cells – an abnormal type of red blood cell

B

bursa – a small fluid-filled sac

B-cell disease – disorders associated with B-type lymphocytes such

burst-forming unit-erythroid – the most primitive identifiable

as CLL

unipotent erythroid stem cell in primitive fetal cells

B cells or B lymphocytes – the primary source of cells responsible

for antibody responses

C

B-cell prolymphocytic leukemias – a type of leukemia

Cabot rings – ring-shaped, figure-eight, or loop-shaped inclusions

babesiosis – a tick-borne, parasitic infection of red blood cells

seen in stained erythrocytes

bacteremia – a bacterial infection of the blood

calcium pyrophosphate dihydrate – an abnormal crystal found in

base pair – a nucleotide (adenine, guanine, cytosine, thymine, or

uracil) in DNA or RNA

uracil) and its complementary base on the opposite strand

calibration – the comparison of an instrument measurement or

basic calcium phosphate (BCP) – a type of crystal that can be seen

reading to a known physical constant

in joint (synovial) fluid

CAP – College of American Pathologists

basilic vein – an acceptable vein in the arm for venipuncture

capillaries – a unit of the microcirculation that functions as the link

basophilia – an abnormal increase in the number of erythrocytes

between the arterial and venous blood circulation

with a blue appearance. The presence of one, evenly distributed—

capillary blood – blood obtained from the capillaries of sites such

as the fingertip, toe, or heel

as the fingertip, toe, or heel

Wright-stained blood smears

carboxyhemoglobin – a variant form of hemoglobin that displaces

basophilic stippling – abnormal, dark-staining granules in red

blood cells

oxygen and creates hypoxia in carbon monoxide poisoning

cardiac tamponade – a critical condition of cardiovascular compromise

basophils – white blood cells (leukocyte) seen in normal peripheral
promote, usually hypotension, caused by pericardial fluid under
blood or bone marrow

increased pressure in the enclosed sac around the heart

Glossary

729

carrier – a term used in genetics to denote a parent who has a dis—

chronic lymphocytic leukemia – a malignant form of increased
ease causing gene

peripheral blood cells involving lymphocytes and of long duration

catecholamines – biologically active amines such as epinephrine

chronic myelogenous leukemia (CML) – a malignant form of
that are derived from the amino acid tyrosine

increased peripheral blood cells involving granulocytes and of

CDC – Centers for Disease Control and Prevention

long duration

cDNA – complementary DNA, produced from mRNA using reverse

chronic myelomonocytic leukemia – a malignant form of increased
transcriptase

peripheral blood cells involving granulocytes and monocytes

celiac disease – an uncommon malabsorption syndrome (also
and of long duration

known as nontropical sprue) characterized by an inability to
circulating anticoagulants – abnormal substances that can produce
digest and utilize fats, starches, and sugars

bleeding

cell-based coagulation (physiologic, in vivo) – a model of blood

circulating pool – form blood cells such as platelets that are present
coagulation that recognizes some of the aspects of hemostasis

in the circulating blood

that the classic cascade, protein-centric model does not

CLL – chronic lymphocytic leukemia

cell coincidence error – more than one cell passing through the aperture—

clonal – referring to a group of cells with a common cell of origin

ture of an impedance cell-counting instrument at the same time

(see clone)

cellular proteases – derived from lysosomes of granulocytes that

clonality – referring to a clone

may be trapped within a thrombus

clone – daughter cells descended from the same single cell, all have—

centrioles – a pair of central spots inside the centrosome

ing identical phenotypes and growth characteristics as the origi—

centrosome – the area of the cell where the cytoplasm is homogenous precursor cell

neous, where there are no mitochondria, and where there are

cluster of differentiation (CD) – a grouping of cell surface antigen

two tiny spots at the center

markers

cephalic vein – an appropriate vein in the arm for venipuncture

coagulation inhibitor – a substance such as an anticoagulant that

cerebrospinal fluid – a fluid formed continuously in the choroid plexus

prevents the clotting of blood

of the cerebral ventricles. It is found in the subarachnoid space, surrounding

coefficient of variation (CV) – a statistical term denoting the precision—

ventricles of the brain, and the central canal of the spinal cord

sion of results

CFU-E – colony-forming units-erythroid

coincidence – in automated impedance cell counting, if more than

CFU-GEMM – colony-forming-unit-granulocytes-erythrocyte—

one cell is within the boundaries of the aperture at the same

macrophage-megakaryocyte

time, only a single pulse is counted

CFU-GM – colony-forming-unit-granulocyte-macrophage

col agglutinins – antibodies in the plasma that react best at 0°C

CH – constant region of the immunoglobulin heavy chain gene locus
to 20°C

channel analyzer – a device in which individual pulses are categorized

collagen – a protein found in the skin, tendons, bone, and cartilage

colony-stimulating growth factor – a soluble substance that promotes

collagen disease – diseases of the skin, tendons, bone, and cartilage,

on the x-axis and frequency on the y-axis

such as systemic lupus erythematosus and rheumatoid arthritis

Charcot-Leyden crystals – colorless, hexagonal, needle-like crystals

colony-stimulating growth factor – a soluble substance that promotes

cell growth

cell growth

bronchial secretions, and feces

combined scatter histogram – a type of histogram that includes

Chéiak-Higashi anomaly (also Chéiak-Higashi syndrome) –

both forward- and right-angle scatter information

a rare inherited autosomal recessive trait that is characterized

complement – a group of proteins associated with inflammation
by the presence of large granules and inclusion bodies in the
that enhance phagocytic activity and disrupt cellular membranes
cytoplasm of leukocytes. The leukocytic neutrophils display
when activated

impaired chemotaxis and delayed killing of ingested bacteria

complement system – a series of proteins that when activated cul—

chemoimmunotherapy – treatment of a malignant condition with
minate in the membrane attack unit (MAC) that punctures the
an targeted monoclonal antibody

cellular membrane and lyses the cell

chemokinesis – chemically induced movement of white blood cells

complete blood count – traditionally consisting of a red blood cell

chemotaxis (related to chemotactic factors) – the release of sub—

count, a microhematocrit, hemoglobin, total white blood cell

stances that attract phagocytic cells as the result of traumatic or

count, and white blood cell differential count

microbial damage

congenital – a condition present at birth

chloroma – a malignant tumor arising from myeloid tissue

conjugate bilirubin – a water-soluble, albumin-bound form of

cholesterol crystals – precipitation solid form of a lipid

bilirubin

Christmas disease – factor IX deficiency

constitutional aplastic anemia – a type of bone marrow failure

chromatids – halves of a chromosome pair bound together in duplicate

resulting in impaired hematopoietic blood cell growth due primarily

during cell division

mainly to a congenital disorder

chromatin – the network of small fibers in the nucleus of a cell

contact group – blood coagulation factors XI and XII, prekallikrein

chromoprotein – a conjugated protein having respiratory functions

(Fletcher factor), and high molecular weight kininogen (Fitzgerald

(e.g., hemoglobin)

factor)

chromosomes – structures consisting of DNA wrapped around a

core (nucleosome) or control specimen – a specimen of which the

protein core that are visible in the nucleus of a cell during cell

division is known that is used for comparison with the unknown

division

specimen

chronic – gradual or of long duration

control (v.) – to keep within limits

chronic blood loss – the loss of blood over an extended period of time

Cooley's anemia – thalassemia major is usually equivalent to beta—

chronic granulomatous disease – a sex-linked autosomal recessive

thalassemia in a homozygous form and is sometimes called

genetic disorder that produces defective phagocytosis because

Cooley's anemia

the cells are unable to destroy previously engulfed bacteria

Coombs' test – see anti-human globulin test

730

Glossary

coproporphyrin – a porphyrin formed in the intestine from bili—

dense granules – a type of granule found in platelets, megakaryocytes

rubin. Abnormal amounts may be found in the urine in some

deoxygenated hemoglobin – reduced hemoglobin

forms of anemia

deoxyribonucleotide – components of DNA (phosphate, sugar and an

cord blood – blood obtained from the umbilical vessels at birth

organic base such as A P, C P, G P, P) that are required by
corrected reticulocyte count – a mathematical adjustment of the
the DNA enzyme, DNA polymerase, or DNA synthesis
reticulocyte count to account for variations caused by erythrocyte
deoxyribonucleic acid – DNA

cyte quantity

DH – diversity region of the immunoglobulin heavy chain gene locus

Coulter principle – a method of cell counting and volumetric sizing

diabetes mellitus – a disorder of carbohydrate metabolism caused

based on the detection and measurement of changes in electrical

by an insufficiency of insulin

cal resistance produced by a particle, suspended in a conductive

diagnosis – determination of the nature of a disorder or disease

liquid, traversing a small aperture

dialysate – the soluble materials and fluids (e.g., water) that pass

counterstain – a stain used to enhance a previously applied primary

through a semipermeable membrane

stain

dialysis – the passive process of movement of soluble substances

CPPD – see calcium pyrophosphate dihydrate

and water molecules through a semipermeable membrane

cryoglobulin – a serum globulin that precipitates, gels, or crystal—

Diamond-Blackfan anemia – an inherited (congenital) form of

leukemia that spontaneously at low temperatures. May be found in mul—

pure red blood cell aplasia

multiple myeloma and collagen disease

amoeboid movement of cells

crystalline inclusions – rod-shaped deposits of IgG

Diels-Alder reaction (Sanger's method) – a classic molecular assay

CSF – (A) refers to colony-stimulating factor, a specific glycoprotein

method

macromolecule that stimulates the growth of granulocytes and

chemotaxis – the movement of a substance from an area of high con—

macrophage cells; (B) an abbreviation for cerebrospinal fluid

concentration to an area of low concentration

curve-fitting – in computerized automated instruments, the instru—

digital cell morphology – an digital electronic form of cell recognition

computer's computer process of fitting a lognormal curve to the

infection – a method to remove or reduce infectious organisms

platelet raw data

from inanimate objects such as table tops

cytochemical stains – staining reactions that produce a colored pre—

ispersion – scattered

precipitate from a specific insoluble compound in a cell

disseminate intravascular coagulation (DIC) – this is a serious

cytochemistry – the identification of specific types of molecules in a cell

coagulation disorder that consumes platelets and blood coagula—

cytogenetic analysis – the examination of the chromosomes of a

cell. It is an example of a major breakdown of the hemo—

nucleated cell

static mechanism that occurs when the procoagulant factors

cytogenetics – the branch of genetics concerned with the cellular

outweigh the anticoagulant system

elements of heredity

diverticulitis – inflammation of the small blind pouches that form

cytokines – soluble factors secreted by T-lymphocyte populations

in the lining or wall of the colon

cytokinesis – cytoplasmic division during cellular division (mitosis)

DNA – deoxyribonucleic acid

cytological – refers to cells

Döhle bodies (Amato bodies) – abnormal inclusion bodies that

cytology – the study of cells

appear as light-blue-staining vacuoles predominantly in neutro—

cytomegalovirus infection – a herpes family virus that can cause

phils in viral diseases and other toxic conditions

congenital infections in the newborn and a clinical syndrome

Down's syndrome – a chromosomal abnormality. Previously

resembling infectious mononucleosis

referred to as mongolism

cytoreduction – decreasing the number of cells

Downey cells – an early classification system of certain forms of

variant lymphocytes. Downey I types have many vacuoles in the

D

cytoplasm; Downey II types resemble plasma cells; Downey III

d-dimer – a molecular form encountered in excess in disseminated

types are an immature form of lymphocyte

intravascular coagulation (DIC)

DPG – diphosphoglycerate (2,3-DPG) combines with the beta

definitive hematopoiesis – specifically committed blood cell lines

chains of deoxyhemoglobin and diminishes the molecule's affinity—

egmacytes – an abnormal type of red blood cell

ity or oxygen

egranulation – the loss of granules such as in the basophil when

repanocytes – an abnormal red blood cell form

an antigen binds to two adjacent IgE antibody molecules located

rif – a statistical observation of movement in a specific direction

on the surface of mast cells

away from a measured point on a quality control chart

elation – a chromosomal aberration in which a segment of a chromosome

rumsticks – an appendage of nuclear material attached to the nucleus

mosome is lost

of a segmented neutrophil. May be seen in some cells in women

elta check(s) – a quality assurance/quality control initiative that

DsDNA – double-stranded DNA

monitors a patient's results or significant differences between

yscrasia – an abnormal or pathological condition of the blood

repeat testing results

yserythropoiesis – defective red blood cell maturation

elta granule – a type of storage granule found in the mature platelet

yserythropoietic anemia – a condition of red cell development

e novo – a newly presented, primary case of a disorder or disease

because of abnormal red blood cell development

enaturation – the process of treating a protein with agents such as

ysgranulopoiesis – defective white blood cell maturation

heat or acid and causing it to lose its native properties because of

ysmegakaryocytopoiesis – a condition of platelet development

disruption of secondary and tertiary bonding such as hydrogen

because of abnormal megakaryocytic development

bonds

ysmegapoiesis karyocyte – defective platelet maturation

enature DNA – double-stranded helix separates into two sin—

ysplasia – (adj. dysplastic) abnormal development (e.g., defective

gle strands, breaking hydrogen bonds; caused by changes in

cellular development). Abnormal cytological features and tissue

temperature, pH, or nonphysiological concentrations of salt,

organization, often is a premalignant change

detergents, or organic solvents

yspnea – difficulty in breathing

Glossary

yspoiesis – an abnormality in the development o blood cells

Epstein-Barr virus – the virus associated with the development

yspoietic syn rome – a combination o de ective and disrupted

o in ectious mononucleosis in Western countries and Burkitt's

cell line development

lymphoma in A rica

Ehrlichia – a parasitic disease that in ectis white blood cells

E

elliptocytes – abnormally shaped red blood cells in an elliptical shape

EAC – erythrocyte-antibody-complement rosette test

en othelins – a amily o peptides in tissues that modulate vasomo—

ecchymoses – large patches o bleeding into the tissues

tor tone, cell proli eration, and hormone production associated

echinocytes – an abnormal red blood cell shape

with vasculature physiology

eclampsia – a toxic condition o pregnancy

epigenetics – stable changes in gene unction that are transmitted

e ema – an abnormal accumulation o uid in the body's intercel—

rom one cell to its progeny

lular spaces

erythroblastic islands – see blood islands

EDTA (K3 EDTA) – tripotassium ethylenediaminetetraacetate.

erythroblastopenia – a deficiency of the red blood cell maturation

A commonly used anticoagulant in blood collection

stage, erythroblast

effusion – an abnormal accumulation of fluid in a particular cavity

erythroblasts – the earliest differentiated stage of red blood cell

of the body such as the chest

maturation

electrical impedance – also called electrical resistance

erythrocyte sedimentation rate – a laboratory method of detecting

electrical impedance principle – a method of cell counting and

analysis of inflammation or infection

sizing based on the detection and measurement of changes in

erythrocytosis – an increase in the red blood cell mass in circulation

electrical resistance

in blood

elution – Removal of antibodies from the erythrocytes that they are

erythroleukemia – a form of leukemia that is usually acute and rapidly

coating or bound to

resents the overproliferation of both immature granulocytic and

Embryonic-Meyerhof glycolytic pathway – the major, anaerobic,

erythrocytic cell types

energy-yielding pathway associated with the breakdown of glu—

erythropoiesis – the process of red blood cell (erythrocyte)

production in erythrocytes (glycolysis)

production

embryonic hemoglobin – primitive hemoglobins such as Gower I,

erythropoietin – a glycoprotein hormone (mol wt 46,000) that

Gower II, and Portland that are formed in the yolk sac

stimulates erythropoiesis. It is produced mainly by the kidneys

in ocularitis – an inflammation of the lining membrane of the

in response to tissue hypoxia

heart

ESR – erythrocyte sedimentation rate. Also referred to as sedimentation rate

phagocytosis – the process in which specialized cells engulf particles

essential thrombocythemia – a hereditary disorder of platelets

and molecules, with the subsequent formation of membrane—

etiology – the study of the cause(s) of disease

bound vacuoles within the cytoplasm

euchromatin – chromatin that is rich in nucleic acid, is genetically
endoplasmic reticulum (ER) – an extensive, lace-like network com—
active, and stains lightly. It is considered to be partially or fully
composed of pairs of membranes enclosing interconnecting cavities
uncoiled
or cisternae

exons – protein-coding regions of the genome
endoreplication (endomitosis) – the process that occurs in the
exons – a coding sequence of nucleotides in a gene that is tran—
megakaryocyte during early maturation. In this process, chro—
scribed into mRNA

chromosomal materials (DNA) and the other events of mitosis occur
extramedullary hematopoiesis – the formation and development
without subsequent division of the cytoplasmic membrane into
of blood cells outside the bone marrow in sites such as the liver
identical daughter cells
and spleen

endothelial cells or endothelium – simple squamous epithelium
extravasation – the movement of neutrophils
that lines blood and lymphatic vessels and the heart

extravascular hemolysis – rupturing of red blood cells outside of
endothelial dysfunction – nonadaptive changes in endothelial
the blood vessels

structure and function provoked by pathophysiological stimuli

extravascular destruction (also extravascular catabolism) – the

enzyme-linked immunosorbent assay (ELISA) – technique in

destruction of an erythrocyte through phagocytosis and digestion—

in which an enzyme is complexed to an antigen or antibody and

uptaken by macrophages of the mononuclear phagocyte system

a substrate added that generates a color proportional to the

extrinsic hemolytic anemias – a factor outside of the red blood cell

amount of binding

causes rupture of the cell

enzymopathy – a pathological enzyme deficiency

extrinsic pathway – the initiation of blood clotting begins with

eosin – an acidic stain that stains some cytoplasmic structures of

either the extrinsic or the intrinsic pathway. The extrinsic path—

the cell an orange-red color. The red-staining structures are aci—

way is activated by the entry into the blood of phospholipopro—

dophilic or eosinophilic substances

teins and organelle membranes from disrupted tissue cells

eosinophilia – an increase in the number of eosinophilic leukocytes

extrinsic system – a system or the third phase of initiation of
in the peripheral blood

coagulation

eosinophilic granules – orange-staining granules found in a spe—

exudates – an abnormal accumulation of fluid (effusion) in a par—

ci c leukocyte type

ticular body cavity

ependyma – the membrane lining the cerebral ventricles and the
central canal of the spinal cord

F

epidemiology – the study of infectious diseases or conditions in many

FAB – French-American-British classification

individuals in the same geographical location at the same time

factor V (Leiden) – a coagulation factor mutation

epinephrine – a hormone produced by the adrenal medulla that

factor V R506Q (Leiden) – also called factor V (Leiden)

acts as a vasoconstrictor

familial polycythemia – an unusual genetic disorder that produces

EPO – erythropoietin

a defect in the regulation of erythropoietin production

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Glossary

Fanconi's anemia – a congenital form of aplastic anemia

glycosylate hemoglobin – a substitution of normal hemoglobin

ferritin – a storage form of iron

that is formed during the maturation of the erythrocyte

fibrin – a meshy protein clot formed by the action of thrombin on

Golgi apparatus – a horseshoe-shaped or hook-shaped cellular

fibrinogen

organelle with an associated stock of vesicles or sacs

fibrinogen – blood coagulation factor I

gout – a form of arthritis characterized by excessive quantities of

fibrinogen group – factors I, V, VIII, and XIII; clotting factors

uric acid in the blood, with possible deposition in the joints and

fibrinolysis – the dissolution of a fibrin clot

other tissues

fibrin split products – components of fibrinogen breakdown

granulocytes – segmented neutrophils, band neutrophils, metamy—

brin-stabilizing actor – actor XIII

elocytes, basophils, and eosinophils

lamin – a contractile protein found in platelets

granulocytic kinetics – the collective term for the development,

nal common pathway – the pathway combining activated coagu—

distribution, and destruction of neutrophils, eosinophils, and

lution actors initiated via different mechanisms leading to blood

basophils

clotting

granules – solid particles in cellular cytoplasm that can contain

Fitzgerald actor – high molecular weight kininogen

substances such as enzymes or iron

xe macrophages – macrophages that line the endothelium of

grape cell (Mott's cell) – a plasma cell whose cytoplasm contains

capillaries, the bone marrow, and the sinuses of the spleen and

inclusions that are transparent blue sacs or crystal-like in nature

lymph nodes

ame cells – red-staining plasma cells

H

Fletcher actor – prekallikrein

Hageman factor – factor XII

flow-cell – a type of laboratory automation such as flow cell cytometry

haptoglobin – a plasma globulin that binds with the alpha-beta

flow cell cytometry – a method of identifying various cell types

dimers of hemoglobin

or inclusions

hairy cell leukemia – a form of leukemia that exhibits lymphocytes

fluid mosaic model – the description of a cellular membrane

with ragged cytoplasmic edges

urochrome – a visually detectable molecule used in flow cytometry—

hazard communication standards – a set of U.S. governmental

entry studies

safety standards

folate – one of the vitamins of the B complex

Hb (Hgb) – hemoglobin

folate antagonists – substances that inhibit the synthesis of folate

Hct – hematocrit or packed cell volume (PCV)

folate deficiency – an absence of folate from the diet

Heinz bodies – an accumulation in the erythrocyte of oxidized

French-American-British classification – FAB classification

glutathione that forms an insoluble complex with hemoglobin

frequency distribution – the grouping of data in classes and determi—

because of the absence of NADPH

number of the number of observations that fall in each of the classes

Helmet cells – an abnormally shaped red blood cell, also called

functional iron deficiency – an inadequate utilization of stored iron

schizocytes

functional iron deficiency anemia – a lack of normal red cell func—

hemochromatosis – a condition acquired or hereditary, of iron over—

tion due to inadequate utilization of stored iron

load and excessive accumulation in various tissues and organs

hemagglutination inhibition – the prevention of erythrocyte

G

clumping

G phase – the gap period in cellular division referred to as a part of

hematocrit – a packed red blood cell measurement included in a

interphase (e.g., G

complete blood count

1, G2, G0)

gametes – a term for ova and sperm

hematology – the study of blood

gastric mucosa – the lining membrane of the stomach

hematoma – accumulation of blood in the tissues or space in the body

Gaucher's disease – a monocytic disorder that represents a deficiency

hematopathology – the study of disorders of the blood and bone

marrow

marrow

glucose from its parent sphingolipid, glucosylceramide

hematopoiesis – (adj. hematopoietic). The formation and development

Gaussian distribution – a symmetrical bell-shaped curve

of blood cells, chiefly in the bone marrow

gene – the functional unit of the chromosome that is usually

hematopoietic dysplasia – see myelodysplastic syndrome

responsible for the structure of a single protein or polypeptide

hematopoietic progenitor cells – early cell forms in the maturation

genetics – the study of the transmission of inherited characteristics

of blood cells

genome – the complete set of genetic information (genes) or genetic

hematuria – blood in the urine

cellular material in an organism or cell

heme – an iron-bearing compound that is the nonprotein pigment

genotype – the total genetic composition of an individual

portion of the hemoglobin molecule. It is responsible for oxygen

Giemsa stain – a Romanowsky-type blood stain

and carbon dioxide transport

gingival hyperplasia – excessive proliferation of gum tissue, often

hemochromatosis – a disorder of iron metabolism characterized by

producing a white appearance

the deposition of excessive iron in the tissues

Glanzmann's thrombasthenia – a blood coagulation disorder char—

hemoglobin – a chemical substance composed of iron and proteins

acterized by a failure of platelets to aggregate in response to all

that is responsible for transporting oxygen to tissues and car—

aggregating agents

born dioxide away from tissues

glial cells – certain cells of the brain

hemoglobin A – the major form of normal adult hemoglobin

glucose-6-phosphate dehydrogenase (G6PD) deficiency – an

hemoglobin electrophoresis – a separation method of hemoglobin

inherited defect of an essential red blood cell enzyme

reactions based on the principle that hemoglobin molecules in
glycocalyx – a fuzzy outer coat that surrounds the platelet's cellular
an alkaline solution have a net negative charge and move toward
membrane

the anode in an electrophoretic system

glycogen – a long-chain polysaccharide composed of repeating
hemoglobin F – fetal hemoglobin. The predominant hemoglobin
units of glucose

variety in the fetus and neonate

Glossary

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hemoglobin S – sickle-type hemoglobin found in sickle cell anemia

human immunodeficiency virus (HIV) – an RNA retrovirus that
and/or sickle cell trait

causes acquired immune deficiency syndrome (AIDS)

hemoglobinemia – the presence of free hemoglobin (not mem—

human stem cells – undifferentiated cells

brane-enclosed) in the blood plasma

hybridization – interaction between two single-stranded nucleic

hemoglobinopathies – inherited (genetic) defects related to hemo—

acid molecules to form a double-stranded molecule

globin. These defects may result in an abnormal structure of the

hydrophilic – water-attracting

hemoglobin molecule or a deficiency in the synthesis of normal

hydrophobic – water-repelling

adult hemoglobin

hypercellular – accelerated blood cell production in the bone marrow

hemoglobinuria – free hemoglobin in the urine

hypercoagulable state – an increase in the likelihood of blood to

hemolysis – a substance that liberates hemoglobin from

clot in vivo

erythrocytes

hyperplasia – excessive tissue growth or cellular multiplication

hemolytic anemias – a deficiency of red blood cells in the circulation—

hypersegmentation – an abnormal condition in which more than

normal blood caused by a disruption of the red blood cell membrane

where nuclear segments are observed in segmented neutrophils

hemolytic disease of the newborn (HDN) – a disorder seen in

hypertension – increased blood pressure

unborn and newborn infants in infants maternal antibodies that cross—

hypertrophy – increase in the size of cells that produces an enlargement of tissue mass or organ size

hemophilia A – classic hemophilia. A hereditary disorder that produces factor VIII deficiency

hyperviscosity – decreased fluidity of blood plasma because of

increased blood proteins

hemophilia B – Christmas disease. A hereditary disorder that produces factor IX deficiency

hypervolemia – an increased total blood volume

hypochromia (also hypochromic) – when the central pallor of

erythrocytes exceeds one third of the cell's diameter

hemocytin – granular, iron-rich, brown pigment found in body

hypoglobulia – a condition of neutrophils in which normal segmentation fails to occur

hemocytinuria – the presence of granular, iron-rich, brown pigment in the urine

hypoproliferative disorders – a term that may be substituted or the

term in the urine

reduced growth or production of cells, particularly erythrocytes

hemostasis – the stoppage of bleeding from a blood vessel

such as hypoproliferative anemia

heparin – an anticoagulant that acts as an antithrombin

hypossegmentation – failure of cellular nucleus to segregate into an

hepatomegaly – excessive enlargement of the liver

expected number of pieces (segments)

hepatosplenomegaly – an enlarged liver and spleen

hypothyroidism – decreased thyroid activity

hepcidin – a peptide hormone produced by the liver. It is an important

hypoxia – a decrease of oxygen in the body tissues

important constituent in the regulation of iron metabolism

hereditary – inherited

I

Hermansky-Pudlak syndrome – a blood coagulation disorder

iatrogenic – a condition induced or relating to an illness or disorder

characterized by storage granule abnormalities of the platelets

that was caused by medical examination or treatment

(thrombocytes)

idiopathic – a disorder or disease without an identifiable external

heterochromatin – a type of chromatin that is tightly coiled,

etiology, or self-originated

assumes a dark stain, and is genetically inactive

idiopathic cytopenia of undetermined significance (ICUS) – a

heterogeneous – dissimilar

myelodysplastic syndrome

heterozygous – in genetics, possessing the alternate characteristics

immune deficiency disease – a defect in the ability to detect and/or a pair of homologous chromosomes

gens and/or to produce antibodies against foreign antigens

hexose monophosphate shunt – this ancillary energy-yielding

immune thrombocytopenia (ITP) – a decrease in platelets caused

system is also referred to as the oxidative pathway. The system

by an immune mechanism such as an antibody

couple oxidative metabolism with pyridine nucleotide and glu—

immune thrombocytopenic purpura – large patches of blood

tathione reduction

under the skin due to a decrease of platelets caused by an

high molecular weight kininogen – Fitzgerald factor

immune mechanism

histogram – a pictorial display of frequency and class limits of a

immunity – the process of being protected from or resistant to or—

sample

foreign antigens

histones – positively charged protein. Proteins associated with

immunocompetent – the ability to recognize and respond to a or—

nucleic acids

foreign antigen

Hodgkin disease – a major form of malignant lymphoma

immunodeficiency – a dysfunction in the body defense mechanism

holo – the area of the cell cytoplasm encircled by the concavity of

that detects foreign antigens and produces antibodies against them

the nucleus

immunoglobulin – a protein belonging to the gamma globulin

homeostatic proliferation – balanced cell multiplication

reaction. Immunoglobulins are divided into five classes, with

homeostasis – the tendency of a biological system to maintain equilibrium—

IgG being the most abundant

equilibrium or balance

immunological dysfunction – refers to immune deficiency disease

homogeneous – uniform or same

immunophenotype – the appearance of antigens associated with

homozygous – in genetics, when the genes for a trait on homologous

various types of cells

chromosomes are the same

incidence – the frequency of an occurrence, for example, a disease

Howell-Jolly bodies – very coarse, round, solid-staining dark-blue

infective erythropoiesis – underperformance in red blood cell

to purple DNA remnants seen in abnormal erythrocytes

production

HTLV (human T-cell leukemia virus) – this virus family is associated

with – an area of necrosis in a tissue due to obstruction of the

circulation associated with T-cell leukemia, hairy cell leukemia, and acquired

blood circulation

immune deficiency syndrome (AIDS)

infectious mononucleosis – a benign lymphoproliferative disorder

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Glossary

infectious waste – the remains of materials that harbor disease

krypton – an inert gas used in lasers

causing microorganisms

Kupfer's cells – cells in the liver that have the ability to engulf or
inflammation – tissue reaction to injury caused by physical or
phagocytize foreign particles as part of the mononuclear phago—
chemical agents, including microorganisms. Symptoms include
cytic system

redness, tenderness, pain, and swelling

kwashiorkor – a severe protein deficiency seen in infants and children

interleukin 1 (IL-1) – a soluble cell mediator released by macrophages and
activates helper cells

L

interleukin-2 (IL-2) – also called cell growth factor

labile – unstable

interleukins – soluble protein molecules that work with hematopoietic

growth factors to stimulate proliferation and differentiation

LAP – leukocyte alkaline phosphatase cytochemical stain

of specific blood cell lines

laparotomy – incision in the abdomen

international normalized ratio (INR) – the standardized expression

LASER – light amplification by stimulated emission of radiation

of a measurement of the effect of the anticoagulant warfarin

lazy leukocyte syndrome – a genetic disorder that produces a susceptibility to infections in part because of poor white blood cell

internuclear bridging (INB) – a morphological feature seen in

myelodysplastic syndromes

mobility to an entry site of infection

intramedullary hemolysis (catabolism) – an alternate pathway for

leptocytes – an abnormal red blood cell shape

erythrocyte breakdown (hemolysis) that normally accounts for

leukemia – a neoplastic proliferative disease characterized by an

less than 10% of red cell destruction

overproduction of immature or mature cells of various leukocyte

intravascular catabolism – a term used to refer to the breakdown

types in the bone marrow or peripheral blood

of red blood cells within blood vessels

leukemoid reaction – an assay used to differentiate chronic myelogenous

intravascular hemolysis – erythrocyte breakdown within the blood

leukemia from a severe infection or inflammation that

vessels

resembles leukemia

intrinsic factor (IF) – substance secreted by the parietal cells of the

leukocyte – white blood cell

mucosa in the undus region o the stomach

leukocyte ahesion deficiency (LAD) – a defect that causes abnor—

intrinsic hemolytic anemia – accelerated breakdown o red blood

mal binding (adherence) o phagocytic cells

cells due to caused within the cell

leukocyte alkaline phosphatase (LAP) – a cytochemical stain used

intrinsic pathway (intrinsic system) – the initiation o blood clot—

to differentiate a leukemoid reaction rom other disorders

ting begins with either the intrinsic or the extrinsic pathway. In

leukocytopenia – less than the normal reference range o total

the intrinsic pathway, coagulation begins with the activation o

white blood cells (leukocytes)

actor XII to XIIa

leukocytosis – a significant increase in the total white cell count

introns – intervening noncoding sequences o nucleotides that

leukoerythroblastosis – an abnormal condition o increased imma—

determines gene unction

ture orms o red and white blood cells

in vitro – in the test tube or outside the body

leukopenia – a severe decrease in the total white cell count

in vivo – within the living organism

leukostasis – a pathological finding of slightly dilated, thin-walled

iron deficiency anemia – lack of oxygen-carrying capacity because
vessels filled with leukemic cells

of inadequate hemoglobin in red blood cells

leukotrienes – a newly identified class of compounds that mediate

iso – equal. Isotonic saline solution has a concentration of 0.85%,
the inflammatory functions of leukocytes

which is equal to the concentration of sodium chloride in cells—

Levey-Jennings chart – a quality control chart used to graphically
lular cytoplasm

display the assay values of controls versus time

isoimmune – possessing antibodies to antigens of the same system

LIF – leukocytosis-inducing factor. A regulator that influences the

isoimmune hemolytic anemia – disruption of red blood cells

release of neutrophils from the bone marrow into the circulation—

caused by antibodies that results in a lack of red blood cells and
tory system

associated functions

lipids – one of the three major biochemical classes. This class

isolation technique – precautions used to prevent the transmission

includes the fatty acids and steroids

of disease either to or from a patient or patient specimen

lipophilic yes – stains with an affinity for fatty substances

liquefaction – the process of conversion into liquid form

J

loop-mediated isothermal amplification (LAMP) – a molecular

jaundice – a yellow appearance of the skin, sclerae, and body excretions

method

JH – joining region of the immunoglobulin heavy chain gene locus

Luebering-Rapoport pathway – an important oxygen-carrying pathway of erythrocytes that permits the accumulation of 2,3-DPG

K

lumbar puncture – a spinal fluid tap into the vertebral column

kallikrein – activated Fletcher factor

lupus anticoagulant – an antiphospholipid antibody

karyokinesis – the division of the nuclear membrane during cell—

lymphadenopathy – disease of the lymph nodes

lular division

lymphoblastic leukemia – a major form of leukemia characterized

karyorrhexis – a stage of cellular degeneration when chromatin is

by the presence of increased numbers of immature lymphocytes

distributed irregularly throughout the cytoplasm

in the peripheral blood, bone marrow, and lymph nodes

karyotype – the full complement of chromosomes in an organism

lymphocyte recirculation – the ree movement of lymphocytes

kb – kilobase pairs, 1,000 bases

between the blood and lymphoid tissue

keratocytes – an abnormally shaped

lymphocytes – a type of leukocyte

kinins – small biologically active peptides

lymphocytopenia – a severe decrease in the total number of lym—

kinin system – a system of small biologically active peptides

phocytes in the peripheral blood

Kleihauer-Betke test – a semiquantitative test for fetal hemoglobin

lymphocytosis – a significant increase in the total number of lym—

knizocytes – abnormal red blood cell shape

phocytes in the peripheral blood

Glossary

lymphoma – solid, malignant tumors of the lymph nodes and associated

megakaryocyte – the largest cell found in bone marrow that produces

platelets

lymphoproliferative disorders – a group of diseases characterized

by the proliferation of lymphoid tissues and/or lymphocytes

cytoplasm during erythrocyte maturation

lymphosarcoma – malignant neoplastic disorders of the lymphoid

tissues, excluding Hodgkin's disease

results in large cells without coordinated maturation of the

lyse – to break apart or dissolve

nucleus and cytoplasm

lysosomal storage diseases – a large group of genetically inherited

disorders that produces defect in any enzymes involved in

cytoplasm

degradation processes in the cell leading to an accumulation of

megablastic – uncoordinated maturation of the nucleus and

cytoplasm

degradation processes in the cell leading to an accumulation of

megablastic anemia – a deficiency of red blood cells and function

products that cause cellular dysfunction

due to a deficiency of vitamin B12 or folic acid

lysosomes – cytoplasmic organelles that contain lytic enzymes

megalocyte – an extremely large erythrocyte with a diameter exceeding 12 μm . This cell is larger than a macrocyte

M

meiosis – the process in which ova or sperm with half the normal

M phase – the phase of cellular division in which the cell actually divides
number of chromosomes ($1n$) are produced

macrocytic – a larger than normal cell

memory cells – antigen-sensitized lymphocytes

macrocytosis – a condition of having larger than normal cells

mesenteric thrombosis – a condition of clotting in the membranous

macroglobulin – a high molecular weight protein of the globulin type

tissues attaching the small intestine to the posterior abdominal wall

macrophage – a large mononuclear phagocytic cell of the tissues

mesothelial cells – a single-cell layer of epithelial cells that line the

that exists either as a fixed type that lines the capillaries and

pleural cavity and form the mesothelium

sinuses of organs such as the bone marrow, spleen, and lymph

metamyelocyte – an immature granulocytic leukocyte

nodes or as a wandering type

metaphase – a period in cellular division (mitosis)

malabsorption syndrome – impaired absorption of nutrients in the

metaplasia – change from one adult cell type to another (e.g., glandular
intestine

intestine to squamous epithelium metaplasia)

malaise – a general feeling of tiredness or discomfort

metarubricyte – normoblasts (acidophilic) or nucleated red blood cells

malaria – a parasitic infection of the red blood cells

metastatic carcinoma – a malignancy that has spread from its origin

malignant – cancerous

nasal local point

manifestation – the display of symptoms of a disease or disorder

metastatic disease – see metastatic carcinoma

marginating pool – the granulocytes that adhere to the vascular

methemoglobin – a hemoglobin variant

endothelium

methemoglobinemia – the presence of an increased amount of the

marrow reserve – the segmented neutrophils in the maturation—

hemoglobin variant, methemoglobin

storage compartment

methemoglobin reductase deficiency – a deficiency of a red blood

mast cells – tissue basophils

cell enzyme

maturation-storage compartment – the stage following the proli-

methemoglobin reductase pathway – an erythrocytic metabolic

erative stage. The site where metamyelocytes, band neutrophils,

pathway that functions to prevent the oxidation of heme iron

and a portion of segmented neutrophils are stored

methylene blue – a basic stain that stains the nucleus and some

mature erythrocyte – a red blood cell lacking a nucleus or remnant

cytoplasmic structures of a cell a blue color. The blue-stained

RNA

structures are basophilic substances

Maurer's dots – red dots seen in stained erythrocytes infected with

microcytic – a small cell

the malaria parasite *Plasmodium falciparum*

microcytosis – a condition of increased small cells

May-Hegglin anomaly – an abnormal genetic condition character—

micro filaments – cellular ultrastructures consisting of the protein actin

indicated by the presence of Döhle body–like inclusions in neutro—

micro filaments – small, hollow cellular ultrastructures composed

of filaments, eosinophils, and monocytes. Abnormally large platelets

of polymerized, macromolecular protein subunits, tubulin

and thrombocytopenia frequently coexist in this condition

microhematocrit – packed cell volume

MCH – mean corpuscular hemoglobin of an erythrocyte

microRNA – 21 to 25 nucleotide RNAs

MCHC – mean corpuscular hemoglobin concentration of an

migration inhibition factor (MIF) – affects macrophage migration

erythrocyte

associated with delayed hypersensitivity reactions

MCV – mean corpuscular volume of an erythrocyte

minimal residual disease – a minimum number of cells in a malign—

MDS – see myelodysplastic syndrome

metastatic mass after treatment

mean – the arithmetic average

mixing study – a coagulation study to identify groups of missing

mean corpuscular hemoglobin (MCH) – average weight (content)

coagulation factors

o hemoglobin per red blood cell

mitochondria – cellular ultrastructures composed of an outer,

mean corpuscular hemoglobin concentration (MCHC) – average

smooth membrane and an inner, folded membrane, the cristae.

concentration of hemoglobin per unit volume of red blood cells

These organelles are associated with cellular energy-yielding

mean corpuscular volume (MCV) – Average red blood size or volume

activities

mean peroxidase index – the average peroxidase activity of neutro—

mitogen – a substance that stimulates cell division (mitosis)

phils as measured by staining intensity using automated equipment

mitosis – the process of body cellular division

mean platelet volume (MPV) – the average size of a platelet

mode – the number of values that occur with the greatest frequency

median – the middle value of a set of numbers arranged according

monoclonal antibodies – immune globulins directed against anti—

to size

clones derived from a single cell line

median cubital vein – a vein in the arm suitable for venipuncture

monocyte – a large mononuclear type of leukocyte

megakaryoblastic leukemia – a form of acute myeloid leukemia

mononuclear cells – cells with a single large nucleus such as monocytes, promyelocytes, myelocytes, and blasts

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Glossary

mononuclear phagocyte system – the body defense system that

neutrophilia – a significant increase in the number of neutrophils
consists of a variety of types of cells that have the ability to engulf
granulocytes in the peripheral blood

or phagocytize substances such as foreign particles

neutrophilic reaction – when both the basic and the acidic stains

monosodium urate – an abnormal crystal that may be observed in
stain the cytoplasmic structures, a pink or lilac color develops
synovial fluid

new oral anticoagulants – a group of newly synthesized blood

morphology – the visual appearance, form, and shape of a cell
anticoagulants

motility – movement

next generation sequencing (NGS) – a molecular assay approach

Mott cells – see Grape cells

Niemann-Pick disease – a monocytic disorder that represents the

MPD – myeloproliferative disorders

deficiency of an enzyme that normally cleaves phosphocholine—

MPV – mean platelet volume. A measure of the average volume of

platelets from its sphingolipid, sphingomyelin

the platelet population contained within the platelet curve

nonhistones – less positively charged proteins associated with

MPV nomogram – a picture of the volume or size of platelets

nucleic acids

mRNA – messenger RNA; a processed transcript of the structural

non-Hodgkin lymphoma – a type of malignant lymphoma

gene, present in the cytoplasm, from which a protein is produced

normochromic – normal color

MSU – monosodium urate

normocytic – normal size cell

mtDNA – mitochondrial DNA

Northern blot – hybridization technique similar to the Southern

mucopolysaccharidosis – a genetic disorder characterized by

blot, using RNA instead of DNA as a target

Alder-Reilly inclusions in granulocytes

nosocomial infections – hospital or medically acquired infections

mucosa-associated lymphoid tissue (MALT) – components of

NRBC/100 WBC – the number of nucleated erythrocytes counted

reticuloendothelial system

during a 100-cell leukocyte differential count

multiple myeloma – a malignant disorder of plasma cells that is

NRF – neutrophil-releasing factor. A regulator that influences the

also known as plasma cell myeloma

release of neutrophils from the bone marrow into the circulation—

multipotential hematopoietic stem cell – the progenitor of all

hematopoietic system

blood cells. Also called hematopoietic progenitor cells (HPCs)

nuclear-cytoplasmic ratio – the amount of space occupied by the

myeloblastic – a major form of leukemia characterized by large

nucleus in the relationship to the space occupied by the cytoplasm

numbers of immature or mature granulocytes or related cells

nucleated red blood cells – NRBCs

such as monocytes in the peripheral blood and/or bone marrow

nucleoli – the region of the nucleus rich in RNA

myelocyte – an immature granulocytic leukocyte

nucleoside – RNA is composed of its phosphate group, deoxyribose sugar

myelodysplastic syndrome (MDS) – a group of disorders associated

with abnormalities of erythrocytes, platelets, granulocytes,

nucleobases: adenine (A), uracil (U), guanine (G), and cytosine

and monocytes

(C). A pairs with U; C pairs with G

myelogenous – refers to the myeloid or granulocytic type cell line

nucleosome – a structural unit of a eukaryotic chromosome composed

of a section of DNA coiled around a core of histones

culating blood

nucleotide – basic building block of nucleic acids, consisting of a

myelomas – malignant growth of plasma cells

nitrogenous base, a pentose sugar, and phosphoric acid. Each

myeloperoxidase (MPO) deficiency – an inadequate amount of

phosphorylated deoxyribose sugar is attached to a purine (adenine)

enzyme to destroy microorganisms when phagocytized

nine [A] or guanine [G]) or pyrimidine (cytosine [C] or thymine
myeloproliferative neoplasms (MPNs) – a malignant overproduc-

[]) base. A pairs with ; G pairs with C

tion of myeloid blood cells

null cells – a type of lymphocyte without either T- or B-cell surface

myocardial infarction – necrosis of the muscular tissue of the heart
markers

myosin – a contractile protein

O

N

ochronosis – a peculiar discoloration of body tissue

naïve B cells – lymphocytes never exposed to an antigen

oncogenes – transforming genes of cellular origin that are con—

natural anticoagulant systems – systems in the body that prevent
tained in retroviruses and associated with acute leukemias.

inappropriate coagulation of the blood

Altered version of normal genes

natural killer (NK) lymphocytes – a subtype of lymphocytes that

operational iron – iron used for oxygen binding and biochemical
are able to directly kill a microorganism

reactions

necrosis – cell death

opportunistic infections – microbial diseases that infect a debili—

Neisseria gonorrhoeae – a gram-negative bacteria that causes
tated host

gonorrhea

opsonins – chemical that enhances phagocytosis

neonatal autoimmune thrombocytopenia – a deficiency of blood

opsonization – the process of coating a particle with immunoglob—

platelets in a newborn infant due to self antibodies

ulin and/or complement that enhances phagocytosis

neoplasm (a) neoplastic – a new growth

organelles – small cellular ultrastructures that are the functional

nephropathy – a disease of the kidneys

units of a cell

neutropenia – a severe decrease in the number of neutrophils

osmosis – the movement of water molecules through a semiperme—

granulocytes in the peripheral blood

able membrane

neutrophil – the proportionally largest type of mature granulocytic

osmotic fragility – the ability or exhibility of the cellular membrane

leukocyte

to withstand pressure

neutrophil extracellular traps (NETs) – structures formed by the

osteoarthritis – degenerative joint disease characterized by degen—

release of the nuclear contents of the neutrophil into the extracellular space of the articular cartilage

cellular space

osteopathology – any disease of the joints and bones

Glossary

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oval macrocytes – an oval-shaped red blood cell

plasma cell leukemia – a malignant proliferation of plasma cells

oxidant stress – a decrease in the level of oxygen available to the

plasma thromboplastin antecedent – factor XI

tissues caused by agents such as drugs

plasma thromboplastin component – factor IX

oxidative pathway – see hexose monophosphate shunt

plasmids – small, circular, self-replicating molecules of DNA in bac—

oxyhemoglobin – oxygenated hemoglobin

transformation; foreign genetic material can be introduced into the plasmid and amplified

as the plasmid replicates; a cloning vector

P

plasmin – a proteolytic enzyme with the ability to dissolve ormed

packe cell volume – hematocrit or microhematocrit

brin clots

pallor – paleness o the skin and mucous membranes

plasminogen – the inactive precursor o plasmin that is converted

pancytopenia – a severe decrease in all o the blood cells

to plasmin by the action o substances such as urokinase

Papanicolaou stain – a cytological stain used most commonly to

plasminogen activators – the kinase enzymes

detect uterine and cervical cancer

platelet – also called thrombocyte, megakaryocyte

Pappenheimer bo ies (si erotic granules) – abnormal basophilic

platelet a hesion – the ability o platelets to stick together

iron-containing granules seen in erythrocytes

platelet aggregation – the gathering together o platelets

paracentesis – collection o uid rom the peritoneal cavity

platelet plug – the meshing together o platelets into a solid mass

parameter – any numerical value that describes an entire population

pleocytosis – the presence of a greater than normal number of

paroxysmal cold hemoglobinuria – a hemolytic anemia caused by
cells

cold-reacting antibodies

pleural effusion – an abnormal, excessive amount of fluid from the

paroxysmal nocturnal hemoglobinuria – a hemolytic anemia that
chest cavity

expresses itself during night hours

pleural fluid – watery liquid in the chest cavity

PAS stain – the periodic acid–Schiff stain reaction for cellular

PLT – platelet count

carbohydrates

PNH – paroxysmal nocturnal hemoglobinuria. A rare, acquired

pathogen – a disease-causing organism

chronic hemolytic anemia

pathogenesis – the origin of disease

poikilocytosis – alterations or variations in the shape of erythrocytes

pathophysiology – the mechanism of a pathological disorder

point mutation – a change that affects a single base in DNA

Patient Care Partnership – A Joint Commission initiative

polychromasia – see polychromatophilia

PDW – platelet distribution width

polychromatic normoblast – a red blood cell with a blue-tinged

Pelger-Huët anomaly – an autosomal dominant genetic disorder
cytoplasm

that produces hyposegmentation of neutrophils

polychromatophilia – red, evenly distributed basophilic (blue)

pericardial effusion – an accumulation of fluid in the cardiac sac
granules that impart a blue color to Wright-stained erythrocytes

pericardial fluid – watery liquid in the sac surrounding the heart

polycythemia – an increase in erythrocytes in the circulatory

peripheral blood – blood in the extremities (e.g., capillary blood)
blood

peritoneal fluid – watery liquid in the abdominal cavity

polycythemia vera – a blood dyscrasia in which the erythrocytes,

pernicious anemia – an erythrocytic disorder associated with

leukocytes, and thrombocytes are all increased above normal

defective vitamin B12 uptake

polymerase chain reaction (PCR) – method for synthetically

petechiae – small purple hemorrhagic spots on the skin or mucous

amplifying known DNA sequences in vitro using many cycles of
membranes

denaturation and polymerization with synthetic oligonucleotide

pH – a numerical value expressing acid, neutral, or alkaline (basic)

primer extension employing aqueous polymerase

conditions of a solution. A pH of 7.0 is neutral. Values from 0 to

polymorphism – a condition of multiple appearance

6.9 are acidic, and values from 7.1 to 14.0 are alkaline

polysaccharide – a carbohydrate containing 10 or more

phagocyte – any cell that is capable of engulfing and destroying

monosaccharides

foreign particles such as bacteria

porphyrin – any of a group of iron- or magnesium-free cyclic tetra-

phagocytosis – a form of endocytosis. This important body defense

heme derivatives that form the basis of the respiratory pigment—

mechanism is the process by which specialized cells engulf and

destroy foreign particles

destroy foreign particles

iron form hemes

phagosome – an isolated vacuole formed in phagocytosis

precision – the closeness of test results when repeated analyses of

pharyngitis – an inflammation of the throat

the same material are performed

phenotype – the outward or physical expression of an inherited

prefix – the beginning portion of a medical term

characteristic

prekallikrein – Fletcher factor

Philadelphia chromosome – the Philadelphia chromosome (Ph1) is

preleukemia – an older term for a condition preceding acute

a translocation involving chromosomes 22 and 9. It is translocated—

leukemia

It is present in the precursors and megakaryocytes of patients

primary lymphoid tissues – the bone marrow and thymus gland are

with chronic myelogenous leukemia

classified as primary or central lymphoid tissue

phlebotomy – the collection of venous blood or venipuncture

primary myelofibrosis – a myeloproliferative neoplasm

photon – a basic unit of radiation

primitive hematopoiesis – begins in the embryonic yolk sac in

pia mater – the innermost of the three meninges covering the brain

structures called blood islands and generates erythrocytes, macrophages, and platelets but does not generate granulocytes or lymphocytes

pinocytosis – a form of endocytosis. It is the process in which specialized cells engulf fluids

primer – short nucleic acid sequence that pairs with ssDNA and

plasma – the straw-colored fluid component of blood

provides a free 3'-OH end to “prime,” or begin polymerase synthesis

plasma cell (plasmacyte) – a mature plasma cell that is not normally found in the circulating blood

prothrombin – factor V

prothrombin – factor V

prothrombin – factor V

prothrombin – factor V

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Glossary

probe – a known, labeled sequence of DNA or RNA used to

RCMI – red cell morphology index. Derived from a comparison of the

detect complementary sequences in target polynucleotides by

patient's measured red cell volume distribution with a distribution

hybridization

representing the average patient population served by the labora—

procoagulant – a nonactivated coagulation actor

tory. T e calculation o RCMI relates to a statistical unction called

proconvertin – actor VII

z, which measures the dif erence between a random variable and

pro ciency testing – a method o assessing per ormance quality in

the mean under the curve. I the RCMI is outside the -2.0 to $+2.0$

testing specimens

range, this indicates a signi cant number o abnormal red cells

progenitor bloo cells – early orms o blood cells

RDW – red cell distribution width. An index o the variation in red

progenitor cells – see progenitor blood cells

cell size. It is computed rom the red cell histogram by dividing

prognosis – a orecast o the probable outcome o a condition, dis—

the standard deviation by the mean and multiplying by 100

order, or disease

reactive eosinophilia – an increase in eosinophils caused by in am—

proli erative compartment – the site in the bone marrow where

mation or allergic reaction

blood cells multiply

reactive lymphocytosis – a condition of lymphocyte response to

pronormoblast – an early red blood cell maturation stage

an event

promyelocyte – an immature granulocytic leukocyte

reactive neutrophilia – a increased concentration of neutrophilic

prophase – the first stage in cellular division (mitosis)

granulocytes in response to an event

prorubricyte – basophilic normoblast

red cell distribution width (RDW) – a measurement of the variation

prostaglandins – naturally occurring fatty acids that stimulate the

contraction in red blood cell size

contraction of uterine and other smooth muscle tissues

Ree-Sternberg cells – characteristic cells found in Hodgkin's

prostatitis – inflammation of the male gland, the prostate

lymphoma

prostatovesiculitis – inflammation of the prostate and seminal

transmission index – a measurement of the passage of light

vesicle

refractory anemia – a deficiency of red blood cells that does not

proteases – enzymes that digest proteins

readily yield to treatment

protein C – a plasma protein that functions as a potent natural

refractory anemia with excess blasts (RAEB-1 and RAEB-2) –

anticoagulant

a type of myelodysplastic syndrome

protein S – a plasma protein that functions as a potent natural

refractory anemia with ring sideroblasts (RARS) – a type of

anticoagulant

myelodysplastic syndrome characterized by a ring like deposit

prothrombin – factor II

of iron around the cell nucleus

prothrombin group – blood coagulation factors II, VII, IX, and X

regimen – a schedule of treatment

prothrombin time (PT) – a laboratory coagulation testing method

Reiter's disease – a disease of males characterized in part by migratory

protooncogenes – antecedents of oncogenes that act as central regulatory

polyarthritis

regulators of growth in normal cells

relapse – to reoccur

pseudo-Pelger-Huët anomaly – a false form of this anomaly. See

relative number – a comparative evaluation of cells that can be

Pelger-Huët anomaly

expressed in percentage

pseudopods – cytoplasmic extrusions that resemble pseudopods

relative polycythemia – increases in erythrocytes result from conditions

PT – prothrombin time

conditions not related to increased erythropoietin production

punctate stippling – a type of dark-staining granules in red blood

remission – a period in which the signs and symptoms of a disease,

cells

such as leukemia, subside

purines – an organic family that forms the nucleic acid bases

restriction endonuclease – bacterial enzyme that recognizes short

purpura – extensive areas of red or dark-purple discoloration on

palindromic sequences of DNA and cleaves the DNA near this

the skin

“restriction site”; each enzyme is named for the bacteria from

pyknocytes – an abnormal red blood cell shape

which it has been isolated

pyknosis – contraction of a cell's nucleus that produces a dark,

restriction fragment length polymorphism (RFLP) – alteration in
dense appearance

DNA fragment size caused by a change such as a deletion; rela—
pyrimidine analog – a compound that can be substituted or a
tively stable and can be detected with nucleic acid probes; i close
pyrimidine base to interrupt protein synthesis in actively mitotic
on the chromosome to a disease-producing gene, it can be used
cells

as a marker for this disease

pyrimidines – an organic family that forms the nucleic acid bases

reticulate hemoglobin content – an immature form in reticulo—

pyruvate kinase deficiency – a red blood cell enzyme deficiency

cyte development, immature reticulocyte reaction

reticulocyte – the last stage of the immature erythrocyte. It is

Q

cell lacks a nucleus and is found in both the bone marrow and

qualitative – a difference in type rather than quantity

peripheral blood

quality assessment – see quality control

reticuloendothelial system (RES) – see mononuclear phagocyte system

quality control (QC) – a process that monitors the accuracy and
reticuloendotheliosis – increased growth and development (hyper—
reproducibility of patient results through the use of control spec—
plasias) of the reticuloendothelial system

imens, meeting acceptable standards

retrovirus – reverse the normal process of converting DNA to RNA

rheumatoid arthritis – a form of arthritis most commonly seen in

R

young adults

RA – see rheumatoid arthritis

ribonucleic acid – RNA

macrophages – cells of the body used

ribosomes – cellular organelles that occur both on the surface of the

range – the difference between the highest and lowest measure—

rough endoplasmic reticulum and free in the cytoplasm. They

ments in a series

are associated with cellular protein synthesis

Glossary

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Richter's cells – cells that are similar to lymphocytes

shift reticulocytes (also called stress reticulocytes) – young reticu—

ring sideroblasts – iron deposits encircling immature erythro—

cytes prematurely released from the bone marrow

cytes, particularly metarubricytes (normoblasts)

sickle cell disease – results from the substitution of a valine for glu—

RNA – ribonucleic acid

tamic acid at the sixth position on the beta chain of the hemo—

Romanowsky stain – any stain containing methylene blue and/

globin molecule. In homozygous form (SS), causes sickle cell

or its products of oxidation, and a halogenated fluorescein dye,

anemia

usually eosin B or eosin Y

sickle cells – abnormally shaped red blood cells

root term – the part of a medical term that usually refers to an

sideroblastic anemia – a disorder of iron utilization in which the

anatomical structure

body has adequate iron but is unable to incorporate it in hemo—

rouleaux formation – the appearance of erythrocytes that resem—

globin synthesis

bles a stack of coins

sideroblasts – iron-containing red blood cells

RPI – reticulocyte production index. A measurement of erythro—

siderosome – iron-saturated telolysosomes

poietic activity when “stress” reticulocytes are present

siderotic granules – Pappenheimer bodies

R proteins – one of the binding proteins capable of binding cobala—

signet ring cell – a type of macrophage formed from small vacuoles

min (vitamin B12)

that use one or two large vacuoles that push the nucleus against

rRNA – ribosomal RNA; component of ribosomes that serve as

the side of the cell membrane in a pleural effusion

scaffold for polypeptide synthesis

Singer and Nicholson’s fluid mosaic model – this model explains

rubriblast – the earliest specific red blood cell precursor. Also

the arrangement of the components of the cell membrane into a

referred to as pronormoblast

bilaminar layer of phospholipids, with protein molecules inter—

rubricyte – polychromatophilic normoblast

dispersed as either integral or peripheral units

Russell bodies – round, glassy, transparent bodies that may be seen

single nucleotide polymorphism (SNP) – a region of DNA that
in plasma cells

differs in only a single DNA nucleotide

sinusoids – specialized capillaries found in locations such as the
S

bone marrow, spleen, and liver

S phase – the period during the cellular division cycle in which

size distribution histogram – a display of the distribution of cell
DNA is replicated

volume and frequency. Each channel on the x-axis represents size

safety data sheets (SDS), formerly material safety data sheets

in a liter (L). The y-axis represents the relative number of cells

(MSDS) – U.S. government established guidelines for chemical

SLE – abbreviation for systemic lupus erythematosus

safety

small-bowel stricture – a narrowing of the intestine

sample – a subset of a population

Smoldering leukemia – see MPD

sarcoidosis – a granulomatous, a nonmalignant

smudge cells – a natural artifact seen on peripheral blood smears

Schilling test – an assay to determine the cause of vitamin B12 deficiency that represents the bare nuclei of leukocytes (e.g., lymphocytes).
(cobalamin) deficiency

Increased numbers are seen in CLL

schistocytes – an abnormal red cell shape

sodium citrate – a blood anticoagulant that is frequently used in a

Schüster's dots – red particles seen in erythrocytes containing concentration of 3.2% or coagulation studies

malarial (*Plasmodium vivax*) parasites

soluble transferrin receptor – an indicator of iron deficiency

scleroderma – a chronic disorder characterized by progressive

Southern blot – hybridization technique invented by E.M. Southern

collagenous fibrosis of many organs and systems

in which DNA is digested with restriction enzymes, separated by

Sea-Blue histiocytosis – a disorder with the presence of abnormal electrophoresis, transferred to a solid matrix, and hybridized to macrophages

a labeled probe

secondary lymphoid tissue – lymph nodes, spleen, and Peyer's

spherocytes – dense, ball-shaped red blood cells

patches in the intestine

spermatozoa – male reproductive cells, sperm

secondary polycythemia – an increased concentration of erythro—

spiculate erythrocytes – abnormally shaped red blood cells

cytes in the blood

splenic infarction – tissue necrosis of the spleen

sedimentation rate – erythrocyte sedimentation rate

splenomegaly – an extremely enlarged spleen

segmented neutrophils – see neutrophils

sprue – a chronic form of malabsorption syndrome

seminal fluid – liquid produced by the male reproductive system

ssDNA – single-stranded DNA

sepsis – an infection-induced syndrome defined as the presence of

standard – a highly purified substance of a known composition

two or more of the following features of systemic inflammation:

standard deviation – the square root of the variance

fever or hypothermia, leukocytosis or leukopenia, tachycardia

standard precautions – precautionary methods used to impede

and tachypnea, or supranormal minute ventilation

transmission of infectious microorganisms

septic arthritis – joint inflammation caused by microorganisms

standards – published criteria or acceptable practices or results

septicemia – the presence of pathogenic microorganisms in the blood

stasis – stopping of bleeding

seronegativity – lack of antibodies demonstrated in an assay

stat – immediately

serotonin – a vasoconstrictor produced from tryptophan that stimulates

statistic – any numerical value describing a sample

smooth muscle

stem cells – undifferentiated, immature cells

serous – producing or containing serum

stem term – root term

serum – straw-colored fluid that is present after blood clots

stenosis – the narrowing of a vessel. In a blood vessel, the lumen

serum electrophoresis – separation of serum proteins by electrical

decreases the flow of blood if stenosis exists

methods

stercobilinogen – fecal urobilinogen

Sézary cell – a large lymphocyte with a nucleus that occupies most

sterile body fluids – watery fluids in the body that lack

o the cell

microorganisms

740

Glossary

Stokes Shift – difference in nanometer measure between peak excita—

thrombosis – clotting or the presence o a clot

tion and peak wavelengths when using a uorochrome in an assay

thrombosthenin – actomyosin

stomatocytes – an abnormally shaped red blood cell

thromboxane A₂ – a short-lived substance that acilitates the

stress reticulocytes – see shif reticulocytes

release o platelet granular contents, induces other platelets to

stroma – the structural protein o an erythrocyte. Remains a er the

aggregate, and stimulates vasoconstriction

erythrocyte has been washed ree o hemoglobin and appears as

thrombus – a clot

a ghost cell or shadow when viewed under the microscope

thymus – a primary organ in the reticuloendothelial system

Stuart actor – actor X

TIBC – total iron-binding capacity

subacute leukemia – see MPD

tissue actors – membrane lipoproteins

subarachnoid space – the space between the arachnoid and pia

titer – the strength or concentration of a solution

mater layers of the meninges of the brain

toxic granulation – abnormally dark granulation seen in band and

subsets – subgroups of a sample

segmented neutrophils or monocytes

subanaphilic – having an affinity for Sudan stain

trait – a physical expression

suffix – the ending of a medical term

transcobalamin – a specific globulin protein that is involved in the

soluble hemoglobin – a sulfur-containing hemoglobin variant

physiological mechanism of vitamin B12

supernatant – the fluid above the solid portion in a centrifuged or

transferrin – a beta-globulin glycoprotein that binds iron and

settled mixture

transports it back to the bone marrow for hemoglobin synthesis

symptomatic – a deviation from usual function or appearance

transcription – process in which mRNA translates DNA into RNA

syndrome – a set or group of symptoms that occur together

nucleotide sequences

synovial fluid – joint fluid

translocation – a kind of chromosomal aberration in which a segment

of one chromosome breaks away from its normal location

and attaches to another, nonhomologous, chromosome

systemic lupus erythematosus (SLE) – a multisymptom disorder

that can affect practically every organ of the body

transudates – an abnormal accumulation of fluid (effusion) usually

caused by a systemic disease

traumatic – severe, dramatic

disorders, for example, systemic lupus erythematosus (SLE)

trend – a statistical observation of movement of points on a quality

control chart

T

trisomy – a chromosomal alteration in which a third chromosome

exists with a homologous pair of chromosomes

T lymphocytes or T cells – cells responsible for the cellular immune

tRNA – transfer RNA; small RNA molecules that interact with

response and involved in the regulation of antibody reactions

amino acids and mediate their correct insertion into a growing

polypeptide chain

tanycytes – body fluid cells

Taq DNA polymerase – thermostable DNA polymerase used to

trophoblasts – the peripheral cells of the blastocyst

polymerize new DNA strands; used in the PCR procedure

tumor-suppressing genes – genes that direct cell growth in normal

target cells – abnormal red blood cells with the appearance of a

cells

target with a bull's eye

tunica adventitia – the layer of a blood vessel that consists of fibrous

Tay-Sachs disease – a rare, inherited disease that progressively

connective tissue innervated with autonomic nerve endings

destroys nerve cells in the brain and spinal cord. This disease

tunica intima – the smooth surface of endothelium in a blood

has disrupted synthesis of an enzyme located in lysosomes

vessel

tear drop cells – abnormally shaped red blood cells

tunica media – the thickest layer of a blood vessel. It is composed

of telolysosomes – iron-rich dense bodies in a cell

of smooth muscle and elastic fibers

telomeres – repetitive DNA sequences capped by specific proteins

turbidity – cloudiness

at extremities of linear chromosomes

telophase – the final stage in cellular division (mitosis)

U

thalassemias – a hemoglobin defect where insufficient globulin is

synthesized or the formation of normal hemoglobin molecules

ultrastructure – cellular organelles that can be viewed with electron

thoracentesis – piercing of the thorax (chest cavity) for the purpose

of microscopy

of removing fluid

unconjugated bilirubin – bilirubin not bound to protein

thrombin – a blood coagulation factor (factor IIa) that is the active—

urobilinogen – an end product of bilirubin breakdown

activated form of prothrombin

thrombocytes – blood platelets

V

thrombocythemia – an increase in platelets in the blood

vaccines – live or attenuated bacteria or viruses administered to

thrombocytopenia – a severe decrease in circulating platelets

prevent infection

thrombocytosis – an increase in the number of circulating platelets

vacuolate lymphocytes – vacuolation may be seen in variant lymphocytes (thrombocytes)

phocytes or as a reaction to radiation and chemotherapy

thrombomodulin – an endothelial cell-associated, lipoprotein

variance – the position of each observation (test) in relationship

to factor that assists thrombin in the activation of protein C

to the mean

thrombophilia – a condition of increased likelihood of forming

variant lymphocytes – atypical lymphocytes. Downey cells, blood clots

reactive or transformed lymphocytes, lymphocytoid or plasma—

thromboplastin – blood coagulation factor III

macrocytoid lymphocytes, and virocytes. These cells may be

thrombopoietin – a hormone believed to be of renal origin that is

found in infectious mononucleosis, viral pneumonia, and

secreted in response to the need for platelets

viral hepatitis

Glossary

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vasa vasorum – small networks of blood vessels that supply nutri—

W

vents to the tissues of the wall of a blood vessel

Waldenström's primary macroglobulinemia – neoplastic proliferation—

vascular integrity – the resistance to vessel disruption

tion of the lymphocyte plasma cell system

vasoconstriction – contraction of the blood vessel wall

warfarin – a blood anticoagulant with the brand name, Coumadin

vasoocclusion – a blockage in a blood vessel

Western blot – hybridization technique similar to the Southern blot

VCS – volume, conductivity, scatter

in which the electrophoresed sample is protein and the detector

veins – collecting vessels that return blood to the heart

is immunoglobulin

venipuncture – a medical procedure to withdraw a sample of circu—

Wiskott-Aldrich syndrome – a blood coagulation disorder characterized by bleeding from a vein

terized by extremely small platelets (thrombocytes)

ventricles – cerebral ventricles are hollow spaces in the brain

World Health Organization (WHO) Classification – the organization

venules – microscopically sized veins

tion of blood abnormalities by a recognized medical organization

VH – variable region of the immunoglobulin heavy chain gene

Wright stain – a Romanowsky-type blood stain

locus

viscosity – thickness

X

vitamin B12 – cobalamin

vitamin B

xanthochromia – yellow color

12 deficiency – see pernicious anemia

von Willebrand's disease – a genetic disorder producing a deficiency and defect of blood coagulation factor VIII and defective

factor VII and defective

Z

platelet function

zymogen – an inactive blood-clotting actor

I N DEX

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