4CEUINC CE BROKER #: 50-2256

Last Rev: 7/12/23

FLORIDA LABORATORY COMBO-23 PART II

4CEUINC COURSE CODE: 24F23-II
CONTACT HOURS: 13
COURSE LEVEL: Intermediate
CE BROKER #: 20-987620

Provider Reports CE/CME 24 HOURS cebroker

- - COURSES ARE REVIEWED EVERY 2 YEARS - -

* ALL SECTIONS MUST BE COMPLETED TO RECEIVE CREDIT *			
	TITLE	**CATEGORY**	**HRS**
1	Potassium Regulation and Associated Disorders	Chemistry	1.5
2	Case Report: Rhabdomyolysis Associated Acute Kidney Injury Following Physical Violence	Chemistry	0.5
3	Bleeding Disorders Associated with Abnormal Platelets	Hematology	1.5
4	Human Parvovirus B19 Infection in Renal Transplant Recipient	Hematology	0.5
5	Risks, Complications, and Reactions Associated with Transfusions	Blood Banking	1
		Immunohematology	1
6	Monkeypox: A Detailed Review	Microbiology	2
7	RNA & DNA A Basic Review	Molecular Pathology	2.5
8	Vaccines: FDA's Oversight Role	Serology	2
	TOTAL HOURS		13

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COURSE OBJECTIVES

At the end of this course you will be able to:

See each course section for specific course objectives. PART II Course Titles Included:

1.) Potassium and Associated Disorders

2.) Rhabdomyolysis Associated Acute Kidney Injury Following Physical Violence

3.) Bleeding Disorders Associated with Abnormal Platelets

4.) Human Parvovirus B19 Infection in Renal Transplant Recipient

5.) Risks, Complications, and Reactions Associated with Transfusions

6.) Monkeypox: A Detailed Review

5.) RNA & DNA: A Basic Review

6.) Vaccines: FDA's Oversight Role

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FINAL QUIZ: FLORIDA LABORATORY COMBO-23 PART II

CE Broker Provider #: 50-2256 | **CE Broker Course ID:** 20-987620 | **Contact Hours:** 13

☑ "Review Page Number" references correspond to the numbers in the lower right corner of each page.

SECTION 1: POTASSIUM REGULATION AND DISORDERS - 1.5 Hours Chemistry

1.) _____ of the total body potassium is located within the intracellular compartment. (Review Page 1)

- **A.** Less than 24%
- **B.** Less than 58%
- **C.** Over 98%

2.) The kidney is responsible for maintaining the total body potassium content by matching intake with excretion. (Review Page 2)

- **A.** True
- **B.** False

3.) _____ may be seen in lactic acidosis. (Review Page 4)

- **A.** Hyperkalemia
- **B.** Hypokalemia
- **C.** Pseudohyperkalemia

4.) About 70% of potassium is excreted in the urine with the remaining 30% excreted via the stool. (Review Page 6)

- **A.** True
- **B.** False

5.) There are several factors that influence principal cells to secrete potassium, including low potassium diet, high potassium diet, angiotensin II, high serum potassium, aldosterone, luminal flow rate, extracellular pH and high Na delivery. (Review Page 9)

- **A.** True
- **B.** False

6.) Aldosterone has the ability to signal the kidney to cause sodium retention without potassium secretion in states of volume depletion. (Review Page 10)

- **A.** True
- **B.** False

7.) As many as ___ of hospitalized patients are found to have hypokalemia, but only _____ of this is deemed to be clinically significant. (Review Page 12)

A. 5%, 10%
B. 34%, 80%
C. 20%, 4-5%

8.) Hypokalemia impairs insulin release and induces insulin resistance which worsens glycemic control in diabetic patients. (Review Page 15)

A. True
B. False

9.) Hyperkalemia can be the result of psuedohyperkalemia, potassium redistribution from intracellular fluid to extracellular fluid, and imbalances between potassium intake and excretion. (Review Page 19)

A. True
B. False

10.) About one-third of patients with platelet counts of 500–1000 × 10−9 have psuedohyperkalemia. (Review Page 20)

A. True
B. False

11.) Pouring off blood from an EDTA tube into another serum or plasma tube will cause an erroneous increase in the potassium result. (Review Page 26)

A. True
B. False

12.) Transporting the tubes in a pneumatic tube system without a cushion barrier can lead to hemolysis of a blood specimen. (Review Page 29)

A. True
B. False

SECTION 2: Rhabdomyolysis Associated Acute Kidney Injury – 0.5 hrs. Chemistry

13.) Physical violence can lead to serious and, rarely, fatal injuries. In addition to head injury, injuries of the musculoskeletal system and internal organs are an important cause of assault-related morbidity. (Review Page 1)

A. True
B. False

14.) Highly elevated levels creatinine phosphokinase (CPK), with values from _____ times the upper limit of normal is the most specific laboratory parameter for the diagnosis of rhabdomyolysis. (Review Pg. 2)

A. 2 to 5
B. 5 to 10
C. 10 to 15

15.) Acute Kidney Injury (AKI) is said to occur in _____ of cases with rhabdomyolysis, and is found to have a fatal outcome when there is coexisting multi-organ failure. (Review Pg. 3)

A. 2 to 5%
B. 10 to 55%
C. 70 to 85%

SECTION 3: Bleeding Disorders Associated with Abnormal Platelets – 1.5 hrs. Hematology

16.) Platelets perform their tasks in ensuring hemostasis in __ stages. (Review Pg. 1)

A. two
B. four
C. seven

17.) Platelet dysfunctions can be hereditary or acquired. (Review Pg. 2)

A. True
B. False

18.) Glanzmann Thrombasthenia (GT) has an increasing incidence in populations where marriage between close relatives is an accepted tradition. (Review Pg. 3)

A. True
B. False

19.) A normal platelet count on a routine blood smear can exclude the diagnosis of GT. Because patients with GT usually do not show any abnormalities in the number of platelets. (Review Pg. 5)

A. True
B. False

20.) In GT, Patients with severe bleeding cases should continue to receive platelet transfusions for _____ until bleeding ceases and wound healing occurs in operated cases. (Review Pg. 6)

A. 12 hr.
B. 48 hr.
C. 72 hr.

21.) The first successful bone marrow transplantation in GT was performed in a 4-year-old child with anti-GPIIb/IIIa antibodies in _____. (Review Pg. 9)

A. 1969
B. 1985
C. 2005

22.) Bernard-Soulier Syndrome (BSS) is a rare autosomal dominant platelet dysfunction that is characterized by a low levels, absence, or dysfunction of the GpIb/V/IX complex on the platelet surface. (Review Pg. 9)

A. True
B. False

23.) The platelets of BSS cases lack or have a dysfunctional _____ receptor. (Review Page 10)

A. GPIb-IX-VII
B. GPIb-IX-α
C. GPIb-IX-V

24.) When ruling out BSS, GT, _____, von Willebrand disease, May-Hegglin anomaly, and other inherited giant platelet disorders, for example, gray platelet syndrome are among the differential diagnoses. (Review Page 11)

A. hemophilia
B. acute fatty liver of pregnancy (AFLP)
C. idiopathic thrombocytopenic purpura (ITP)

25.) Since GT and BSS are rare diseases, diagnosis of patients can be delayed. (Review Pg. 12)

A. True
B. False

26.) According to Table 1 _____ has/have a normal platelet count. (Review Pg. 19)

A. Bernard-Soulier Syndrome (BSS)
B. Glanzmann Thrombasthenia (GT)
C. neither BSS, nor GT

27.) As noted in the last case report on pages 31 & 32, it's important to differentiate BSS and idiopathic thrombocytopenia (ITP) because patients often receive unnecessary treatment, including medications and splenectomies when improperly diagnosed with ITP. (Review Page 32)

A. True
B. False

SECTION 4: Human Parvovirus B19 Infection in Renal Transplant Recipient – 0.5 hrs Hematology

28.) _____ occurs during viremia, but hemoglobin levels do not decline. (Review Pg. 2)

A. Normal reticulocyte count
B. Reticulocytosis
C. Reticulocytopenia

29.) Although the B19-associated anemia can improve spontaneously, intravenous gammaglobulin is usually necessary in the majority of patients. (Review Page 3)

A. True
B. False

SECTION 5: Risks, Complications, and Reactions Associated with Transfusions – 1 hr BB/1 hr Immunohem

30.) A transfusion reaction that requires "Major intervention required to prevent death, including, vasopressors, intubation, transfer to ICU, etc." would be classified as: (Review Page 1)

A. Severe
B. Life-Threatening
C. Death (fatal)

31.) If a donor had an antibiotic in their system when they donated blood that the recipient is allergic to, it's possible for the recipient to have an anaphylactic reaction. (Review Page 3)

A. True
B. False

32.) Hemolytic transfusion reactions are almost *never* preventable. (Review Page 5)

A. True
B. False

33.) A Delayed Hemolytic Transfusion Reaction (DHTR) can occur up to _____ after the conclusion of the transfusion. (Review Page 8)

A. 72 hours
B. 12 days
C. 28 days

34.) Febrile non-hemolytic transfusion reactions (FNHTR) can be caused by _____ in the plasma of the blood component being transfused. (Review Page 11)

A. Platelets
B. Cytokines
C. macrophages

35.) One definition of "massive transfusion" could be defined as transfusing more than the patient's entire blood volume in a 24-hour period. (Review Page 14)

A. True
B. False

36.) Whenever possible, fresh blood <_____ old should be used during a massive transfusion to prevent hyperkalemia. (Review Page 15)

A. 12 hours
B. 7 days
C. 20 days

37.) If metabolic acidosis is caused by a massive transfusion, the blood gas pH would most likely be: (Review Page 16)

A. >7.45
B. 8.00
C. <7.35

38.) It's estimated that TACO occurs in 1 in _____ transfusions, with a higher incidence in perioperative orthopedic settings. (Review Page 19)

A. 200
B. 400
C. 700

39.) _____ are responsible for Transfusion-Associated Graft vs. Host Disease. (Review Page 21)

A. T-lymphocytes
B. B-lymphocytes
C. Natural Killer Cells

40.) Antibodies directed toward Human Leukocyte Antigens (HLA) or Human Neutrophil Antigens (HNA) have been implicated in TRALI. (Review Page 22)

A. True
B. False

41.) _____ are the most likely cause of bacterial contamination in a transfusion. (Review Pg 26)

A. WBCs
B. RBCs
C. Platelets

42.) According to Appendix 1 Dyspnea can be caused by TACO. (Review Page 28)

A. True
B. False

SECTION 6: Monkeypox: A Detailed Review - 2.5 hrs. Microbiology

43.) Monkeypox is considered a Zoonotic disease. (Review Slide 3)

A. True
B. False

44.) Monkeypox was first discovered in_____. (Review Slide 6)

A. 1938
B. 1958
C. 1985

45.) The initial patient in the 2022 outbreak was _____. (Review Slide 10)

A. a Canadian resident who had recently traveled to Australia
B. a U.S. resident who had recently adopted a pet rodent
C. a British resident who had recently traveled to Nigeria

46.) At the beginning of the 2022 outbreak a disproportionate number of men who have sex with men (MSM) was identified. (Review Slide 13)

A. True
B. False

47.) A Person Under Investigation meets one of the epidemiologic criteria AND has fever or new rash AND at least one other sign or symptom with onset 21 days after last exposure meeting epidemiologic criteria. (Review Pg. 15)

A. True
B. False

48.) Papules consist of dried serum, blood, or white blood cells. (Review Slide 18)

A. True
B. False

49.) Monkeypox can be spread through Respiratory Droplets. (Review Slide 21)

A. True
B. False

50.) Monkeypox has _____ steps of progression in the disease. (Review Slide 25)

A. 2
B. 3
C. 5

51.) During the incubation period a person has severe symptoms. (Review Slide 27)

A. True
B. False

52.) The monkeypox rash goes through _____ stages. (Review Slide 30)

A. 3
B. 4
C. 6

53.) The patient remains contagious throughout all phases of the rash stage. (Review Slide 34)

A. True
B. False

54.) Before sending testing in the U.S., consult with your local State Health Department. (Review Slide 38)

A. True
B. False

55.) As a distinguishing feature, smallpox patients get swollen lymph nodes. (Review Slide 42)

A. True
B. False

56.) Treatments are for the purpose of controlling a monkeypox outbreak and lessening its duration. (Review Slide 45)

A. True
B. False

57.) For purposes of infection control, fans, vacuum cleaners, dry dusting, or sweeping should be avoided around the patient. (Review Slide 53)

A. True
B. False

SECTION 7: RNA & DNA: A Basic Review - 2.5 hrs. Microbiology

58.) DNA's building blocks, collectively called nucleotides, are: (Review Pg. 1)

A. A (adenine), U (uricil), C (cytosine), and G (guanine)
B. A (adenine), T (thymine), C (cytosine), and G (guanine)
C. A (adenine), Tr (tyrosine), C (cytosine), and G (guanine)

59.) Long strings of nucleotides form genes, and groups of genes are packed tightly into structures called _____. (Review Pg. 3)

A. a nucleus
B. a base
C. chromosomes

60.) Eggs and sperm are considered _____ cells because of the number of chromosome sets they have. (Review Pg. 6)

A. haploid
B. diploid
C. triploid

61.) The first major step in making a protein is _____. (Review Pg. 9)

A. splicing
B. Meiosis
C. transcription

62.) According to Figure 11, the DNA segments that do contain protein-making instructions are known as introns (pictured as blue areas on the graphic). (Review Pg. 12)

A. True
B. False

63.) All states test newborns for PKU, the genetic disease known as phenylketonuria. (Review Pg 14)

A. True
B. False

64.) Ribosomes are among the largest and most intricate structures in the cell, with human ribosomes having between _____ different proteins. (Review Pg. 15)

A. 15 and 20
B. 40 and 50
C. 70 and 80

65.) Science now has the ability to attach a piece of every gene in a genome to a postage stamp-sized glass slide. This ordered series of DNA spots is called _____. (Review Pg. 19)

A. computerized DNA series
B. DNA microarray
C. serial gene display

66.) The chemical units of RNA are like those of DNA, except that RNA has the nucleotide uracil (U) instead of ____. (Review Pg. 21)

A. thymine (T)
B. adenine (A)
C. cytosine (C)

67.) Chromatin consists of long strings of DNA spooled around a compact assembly of proteins called _____. (Review Pg. 27)

A. protamines
B. cadherin
C. histones

68.) In human and mouse telomeres the nucleotide repeated sequence is _____. (Review Pg. 32)

A. TTGGGG
B. TTAGGG
C. TTAAAG

69.) Unlike chromosomal DNA, which is inherited from both parents, humans get all their mitochondrial DNA from their fathers. (Review Pg. 34)

A. True
B. False

SECTION 8: Vaccines: FDA's Oversight Role - 2 hr. Serology

70.) The US Food and Drug Administration (FDA) is the regulatory body that has oversight for the safety, effectiveness, and overall quality of all vaccines that are distributed in the United States. (Review Pg. 1)

A. True
B. False

71.) Research moves to the _____when the vaccine is ready to be tested on humans. (Review Pg. 3)

A. Research and Discovery
B. Pre-Clinical Stage
C. Clinical Development Stage

72.) In Phase 2 of vaccine development, _____. (Review Pg. 4)

A. a small group of ~25 participants is tested
B. varying doses of the vaccine are tested
C. only participants with diabetes are included

73.) In phase 3 clinical trials, the vaccine is generally administered to ___ of volunteers. (Review Pg. 5)

A. 100s
B. 1000s
C. millions

74.) As Phase 3 ends, a company seeking permission to distribute and market a vaccine for use in the United States would submit a BLA to the FDA. (Review Pg. 6)

A. True
B. False

75.) Prescribing information for a vaccine is based on scientific data that are submitted by the manufacturer in the BLA and determined by the FDA to be satisfactory to support the approved indication(s), usage, dosing, and administration. (Review Pg. 7)

A. True
B. False

76.) Regular use of the vaccine in the general population is considered _____. (Review Pg. 8)

A. Phase 3
B. Phase 4
C. Phase 5

77.) Patients or healthcare providers may self-report problems or side effects through the Vaccine Adverse Event Reporting System (VAERS). (Review Pg. 9)

A. True
B. False

78.) Although most vaccines take years to develop, there is no predetermined timeline for bringing a vaccine to market under special circumstances. (Review Pg. 10)

A. True
B. False

79.) The HHS declaration to support an EUA must be based on one of _____ types of determinations of threats or potential threats by the Secretary of HHS, Homeland Security, or Defense. (Review Pg. 11)

A. two
B. four
C. six

- - END OF QUIZ - -

Last Rev: 7/12/23

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- - 3rd Party Material

Florida Laboratory Combo-23 PART II: SECTION 1

Potassium Regulation and Disorders

CONTACT HOURS: 1.5
COURSE LEVEL: Basic
CE BROKER: Automatically Reported

Provider Reports CE/CME 24 HOURS ce broker

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NOTE: Although we submitted this course for 2 contact hours, ASCLS P.A.C.E. has only approved 1.5 hours, since they have deemed the videos as supplemental and not included in the total time.

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COURSE OBJECTIVES

At the end of this course you will be able to:

1. Recall the physiology and homeostasis of potassium in the body.
2. Discuss Hypokalemia, its etiology, symptoms, and treatment.
3. Discuss Hyperkalemia, its etiology, symptoms, and treatment.
4. Discuss potassium testing and list the different factors that can affect the test results.

RIGHTSHOLDER:

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Dr. Lance Miller, PhD (Videos)
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ARTICLE INFORMATION

The Main article is an Open Access article:

- ✓ CITED: Srinivasa, V. "Potassium Disorders". Fluid and Electrolyte Disorders, edited by Usman Mahmood, IntechOpen, 2019. DOI: 10.5772/intechopen.86848. For easier reading, it has been reformatted from its original version.

Potassium Videos:

- ✓ Potassium Videos may be accessed directly by visiting Dr. Lance Miller's YouTube account.

Quiz:

- ✓ If you prefer, the quiz may be printed so that you can work offline. When you're done, return to the Take Quiz link and enter your answers for grading.

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NOTE: The original article has been reformatted for easier reading. Video links & testing sections have been added for supplemental information.

POTASSIUM TESTING SECTION

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VIDEO LINKS

NOTE: Video links in this course remain the property of the creator.

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THE MAIN BODY OF THIS COURSE IS A 3RD PARTY ARTICLE

4CEUINC is providing the quiz and associated contact hours.

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Table of Contents

INTRODUCTION

Potassium is the major intracellular cation in the human body. Over 98% of the total body potassium is located within the intracellular compartment. In healthy adults, the total intracellular content of potassium is equivalent to 3000–3500 mmol. Approximately 70% of this amount is found in skeletal muscle with lesser amounts in bone, red blood cells, liver, and skin. The extracellular compartment contains 1–2% of the total body potassium. This uneven distribution of total body potassium is the result of an electro- genic pump, Na+, K+ ATPase. This pump transports three sodium ions extracellularly in exchange for transporting two potassium ions intracellularly. This mechanism creates a ratio that determines the cell membrane potential. Maintenance of this potassium ratio and membrane potential is vital for normal nerve conduction and muscular contraction. Potassium is abbreviated K+.

Video 1: Introduction to Potassium Regulation

INTRODUCTION TO POTASSIUM REGULATION | **Source:** © Dr. Lance Miller, PhD

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POTASSIUM PHYSIOLOGY AND HOMEOSTASIS

The kidney is responsible for maintaining the total body potassium content by matching intake with excretion. Insulin and catecholamines are primarily responsible for the regulation and distribution of potassium between the intracellular and extracellular compartments [21].

Other factors that can alter the distribution of potassium between compartments include acidbase disorders, plasma osmolarity and exercise. The following section describes the effects of these factors in causing transcellular shifts of potassium.

Video 2: Cellular Regulation

CELLULAR REGULATION | **Source:** © Dr. Lance Miller, PhD

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TRANSCELLULAR SHIFTS

Insulin and Catecholamines

After a meal, postprandial release of insulin shifts dietary potassium from the extracellular compartment into the intracellular compartment. This trans-cellular shift is mediated by insulin binding to cell surface receptors, which stimulates glucose uptake in insulin-responsive tissues via the glucose transporter protein, GLUT 4.

Furthermore, insulin activates the Na+, K+ ATPase pump via increased intracellular CAMP production. This increases cellular uptake of potassium, thereby lowering serum potassium. In contrast to insulin, the effect of potassium regulation by catecholamines is dependent on which adrenergic receptor subtype is activated.

Activation of the beta 2 receptor triggers Na+, K+ ATPase, which induces cellular potassium uptake causing a fall in serum potassium. Activation of the alpha 1 receptor has the opposite effect, causing inhibition of Na+, K+ ATPase preventing cellular uptake and causing elevated serum potassium levels. These effects have important pharmacological implications. Drugs that block beta 2 receptors tend to increase serum potassium. Likewise, drugs that block the alpha 1 receptors can lower serum potassium.

Aldosterone

Aldosterone alters the distribution of potassium between the extracellular and intracellular compartments. The Na+, K+ ATPase pump is activated by aldosterone and causes cellular uptake of potassium. In the absence of altered renal potassium excretion, hypokalemia can result.

Aldosterone can also increase potassium excretion via the kidneys and to some degree by the gastrointestinal tract. Details on the actions of aldosterone in the renal tubule are further later in this course.

Hyperglycemia / Hyperosmolality

Hyperglycemia and hyperosmolarity cause water movements from the intracellular to the extracellular compartment. This movement is responsible for solvent drag which transports potassium out of the cell. Additionally, cell shrinkage occurs and increases intracellular potassium concentration. There is feedback inhibition of the Na/K ATPase pump which decreases cellular uptake of potassium, thus normalizing intracellular potassium. This creates a concentration gradient that allows for potassium exchange between compartments.

Metabolic Acidosis

Metabolic acidosis is associated with abnormal serum potassium. Acidosis caused by inorganic anions such as NH4Cl and HCl can result in hyperkalemia. The mechanism behind this is not understood. Organic acids such as lactic acid generally do not cause potassium shifts between compartments. Hyperkalemia may be seen in lactic acidosis; this is the result of tissue ischemia causing cellular death and release of intracellular potassium into the extracellular fluid.

Exercise

Exercise has multiple effects on potassium. Contraction of skeletal muscle during heavy exercise results in the release of potassium. This in turn signals catecholamine release which stimulates alpha 1 adrenergic receptors to cause potassium to shift out

of cells. The increase in extracellular potassium further induces arterial vasodilation in normal blood vessels, thereby increasing skeletal blood flow. Catecholamine release during exercise also activates beta 2 adrenoreceptors which increase skeletal muscle uptake of potassium, regulating potassium and minimizing exercise-induced hyperkalemia.

DIETARY INTAKE

Video 3: Intake and Absorption

INTAKE AND ABSORPTION | **Source:** © Dr. Lance Miller, PhD

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According to international dietary guidelines, the recommended dietary intake of potassium should be 90–120 mmol/day, or ~4700g. [3, 20]

Potassium is absorbed through the gastrointestinal tract and is distributed amongst the intracellular and extracellular fluid compartments. Dietary intake varies worldwide; the western diet provides 50–100 mmol of potassium daily [3, 21].

Foods that are rich in potassium include many fruits and vegetables.

After a potassium-rich meal, increases in extracellular potassium are negated by rapid cellular uptake that allows for elimination in the urine over a period of 6–8 h.

About 90% of potassium is excreted in the urine with the remaining 10% excreted via the stool. Potassium homeostasis is controlled by the changes in renal potassium excretion. The following section describes the basic physiology of renal potassium excretion.

RENAL POTASSIUM SHIFTS

Evolving concepts in renal potassium excretion involves the recognition of reactive and predictive systems [16].

The reactive system comprises of a negative and a forward system. The negative system consists of a negative feedback loop that modulates renal potassium, on the basis of plasma potassium and serum aldosterone levels [16].

High plasma potassium concentrations or elevated serum aldosterone levels increase urinary potassium excretion bringing plasma potassium concentration back to physiologic range. The forward system describes an unidentified potassium-sensing gut factor that increases urinary potassium excretion, in response to a high potassium diet before an increase in plasma potassium concentration, or changes in plasma aldosterone levels occur [4, 5, 7]. In addition to these systems, a circadian rhythm of potassium excretion has been proposed, for instance, the predictive system which is independent of potassium intake and activity. In studies measuring urinary potassium excretion, it has been observed that urinary potassium excretion is the lowest in the night and early mornings and highest from noon to early afternoon [16].

RENAL POTASSIUM HANDLING

Serum potassium is almost completely ionized and not bound to plasma proteins. It is filtered through the glomerulus. Approximately 65–70% of potassium filtered through

glomeruli is reabsorbed in the proximal tubule. Less than 10% of the filtered load reaches the distal nephron.

Potassium reabsorption in the proximal tubule primarily occurs through paracellular pathways.

Sodium reabsorption across the tubule allows for fluid absorption to occur. As a result of this process, solvent drag occurs which permits potassium reabsorption. In addition, the electrical voltage within the tubular lumen gradually becomes more positive as fluid flows down the tubule.

This change in voltage provides an additional force favoring potassium reabsorption through the paracellular pathway, which is of low resistance.

Video 4: Potassium Reabsorption

POTASSIUM REABSORPTION | **Source:** © Dr. Lance Miller, PhD

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Video 5: Potassium Secretion

POTASSIUM SECRETION | **Source:** © Dr. Lance Miller, PhD

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In the loop of Henle, both secretion and absorption occur. Potassium is secreted in the descending loop in deep nephrons and is reabsorbed in the ascending loop through the action of the Na+, K+ 2Cl− cotransporter. The majority of the potassium reabsorbed by this protein is recycled back into the tubular lumen by the renal outer medullary potassium channel (ROMK), an ATP-dependent apical potassium channel that transports potassium out of cells. Modest net absorption of potassium occurs as a result of this process. The site and regulation of renal potassium excretion predominantly occurs in the distal tubule and collecting duct.

The distal nephron, which comprises the distal tubule and collecting duct, has both reabsorptive and secretory functions. Potassium excretion primarily occurs here.

There are several cell types within the epithelium of the distal tubule and collecting ducts. The most important of these cell types are the principal cells, which approximate 70% of cells and the intercalated cells. Both cell types are located within the collecting duct. Principal cells are primarily located within the cortical collecting duct and intercalated cells are dispersed throughout the entire length of the collecting duct.

Potassium secretion is by principal cells, which involves uptake of potassium from the interstitium by Na+, K+ ATPase and secretion into the tubular lumen through potassium channels: ROMK and BK also known as maxi-K.

ROMK and BK are both permeable to potassium and are regulated by different mechanisms [3].

There are several factors that influence principal cells to secrete potassium. These factors include low potassium diet, high potassium diet, angiotensin II, high serum potassium, aldosterone, luminal flow rate, extracellular pH and high Na delivery.

Sodium delivery to the distal tubule is the major regulator of potassium excretion. High sodium delivery stimulates potassium secretion. It achieves this in two ways. Firstly, increased sodium delivery causes increased sodium entry via epithelial sodium channels (ENaC), which depolarizes the apical membrane causing an increase in the electrochemical gradient, promoting outward flow potassium through the potassium channels. Secondly, the more sodium delivered to the tubule, the more sodium is pumped out by Na+, K+ ATPase and more potassium is pumped in [3].

This potassium is then secreted across the apical membrane of principal cells into the luminal fluid by apical potassium channels.

At low dietary loads of potassium, there is no secretion by either channel. The body is conserving potassium. ROMK channels are sequestered into intracellular vesicles. BK channels are closed [3]. In normal concentrations of potassium, ROMK channels secrete potassium whereas BK channels remain closed. In conditions where there is high potassium secretion, for example, high potassium diet, both ROMK and BK channels are open [3].

Angiotensin II is an inhibitor of potassium secretion; its mode of action is to decrease activity of ROMK, thereby limiting potassium flux into the tubular lumen. The intercalated cells are subdivided into type A which are numerous, type B which are limited in number and non-A and non-B cells.

The intercalated cells, particularly type A, reabsorb potassium. Type A intercalated cells reabsorb potassium via the H+, K+ ATPase, located within the apical membrane which actively takes up potassium from the lumen in exchange for hydrogen ions. Potassium can then enter the tubular interstitium across the basolateral membrane via potassium channels. In conditions of low potassium, potassium depletion increases H+, K+ ATPase expression resulting in increased active potassium reabsorption and decreased potassium excretion.

An important regulator of potassium in the distal nephron is the enzyme with no lysine kinases (WNK kinases). WNK kinases activate sodium reabsorption in the distal tubule and inhibit the ROMK channel [16, 22].

As a result of this, there is decreased sodium delivery to the collecting duct, and coupled with this is decreased ROMK expression leading to decreased potassium secretion [16, 22].

WNK kinase activity is sensitive to chloride and potassium concentrations [16, 22].

ALDOSTERONE PARADOX

Aldosterone has the ability to signal the kidney to cause sodium retention without potassium secretion in states of volume depletion but can also stimulate potassium secretion without sodium retention in the hyperkalemic state [6].

In humans, aldosterone is the major mineralocorticoid. It promotes sodium absorption and potassium excretion by binding to mineralocorticoid receptors located in the distal tubules and collecting ducts. Aldosterone increases Na+, K+ ATPase activity in the basolateral membrane which is responsible for sodium reabsorption across the luminal membrane. This increases the electronegativity of the lumen which increases the electrical gradient and potassium permeability. In states of volume depletion, the renin-angiotensin-aldosterone axis is activated and causes renal sodium absorption restoring

extracellular fluid volume without a demonstrable effect on renal potassium excretion. In the presence of hyperkalemia, release of aldosterone increases urinary potassium excretion, thereby restoring serum potassium levels to normal. This effect, however, does not result in sodium renal retention.

DISORDERS OF POTASSIUM

HYPOKALEMIA

Video 6: Potassium Regulation - Hypokalemia

Potassium Regulation: Hypokalemia | **Source:** © Dr. Lance Miller, PhD

Click Link to View Video on YouTube: https://www.youtube.com/watch?v=ha2dXO55tC0

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Epidemiology

Hypokalemia is defined as serum potassium concentration levels of <3.5 mmol and is a common electrolyte disturbance amongst hospitalized patients [6].

As many as 20% of hospitalized patients are found to have hypokalemia, but only 4–5% of this is deemed to be clinically significant [6, 13, 22].

There are no significant differences in its prevalence amongst males and females [6].

Etiology

Redistribution

About 2% of the total body potassium is within the extracellular compartment. Consequently, small shifts of potassium from the extracellular compartment to the intracellular compartment can cause hypokalemia. Additionally, glycogenesis during total parenteral nutrition or enteral hyperalimentation causes insulin release which shifts potassium into cells. Furthermore, the sympathetic nervous system is involved in the activation of the beta 2 receptors causing intracellular shift of potassium. Stimulation of beta 2 receptors can also occur in thyrotoxicosis.

A rare cause of redistribution-induced hypokalemia is hypokalemic periodic paralysis. In this condition, flaccid paralysis and muscular weakness occur during the night or early mornings, typically after ingestion of a large carbohydrate meal.

Renal Potassium Losses

Renal potassium losses are the most common cause of hypokalemia.

Drugs are common causes of renal potassium loss:

Thiazide and loop diuretics block sodium reabsorption in the distal convoluted tubule and loop of Henle, respectively. Reabsorption does not occur proximal to the collecting duct, thereby increasing sodium delivery to the principal cells of the collecting duct. This stimulates sodium uptake and at the same time promotes potassium secretion causing potassium loss resulting in hypokalemia.

High dosage of penicillin is thought to cause hypokalemia by increased sodium

delivery to the collecting duct and principal cells which result in urinary potassium secretion [22].

The antifungal agent amphotericin directly increases collecting duct secretion of potassium. This is achieved by its direct action of binding to collecting duct cells and forming pores which result in potassium loss.

The mechanism of action for aminoglycosides causing hypokalemia is not completely understood [22]. It is postulated that ROMK is activated by aminoglycosides causing urinary potassium secretion [22].

Cisplatin, an antineoplastic agent can cause both hypokalemia and hypomagnesemia.

Hypokalemia is related to hypomagnesemia. Magnesium mediates inhibition of ROMK. In states that where there is magnesium deficiency, ROMK inhibition is lost enabling potassium excretion [22].

Coupled with this is inhibition of Na+, K+ ATPase pump caused by low magnesium, causing potassium to be excreted via K channels particularly in the thick ascending limb [22].

Toluene is thought to lead to potassium wasting by causing renal tubular acidosis (RTA) [22].

Licorice and herbal cough mixtures contain glycyrrhizic and glycyrrhetinic acids. They are thought to exert mineralocorticoid effects leading to hypokalemia2 [2].

Bicarbonaturia results from metabolic alkalosis, distal RTA or treatment with proximal RTA.

Increased distal tubular bicarbonate delivery increases potassium secretion.

Magnesium deficiency can cause high potassium excretion and potassium deficiency. Under ideal conditions, intracellular magnesium inhibits the apical ROMK channel. In magnesium deficiency, the ROMK channel is not inhibited by magnesium resulting in increased potassium excretion.

Magnesium deficiency should be suspected when potassium replacement does not correct the hypokalemia.

Intrinsic renal potassium transport defects are rare. Barterrs, Gittlemanns and Liddles are such conditions. A review of these conditions is not described here.

Similarly, detailed descriptions of genetic defects that result in elevated levels of aldosterone, glucocorticoid remediable aldosteronism, congenital adrenal hyperplasia and syndrome of apparent mineralocorticoid excess, are not described in great detail here (**See Table 1**).

Table 1: Causes of Renal Potassium Losses

Drugs	Hormones	Renal tubular defects	Genetic defects
• Thiazide diuretics • Loop diuretics • Penicillins; Piperacillin-Tazobactam • Amphotericin B • Aminoglycosides • Cisplatin • Toluenes • Herbal cough mixtures	• Aldosterone	• Bartter syndrome • Gitelman syndrome • Liddle syndrome	• Glucocorticoid-remediable aldosteronism • Syndrome of apparent mineralocorticoid excess • Congenital adrenal hyperplasia

Extra-Renal Potassium Losses

The skin and gastrointestinal tract excrete small amounts of potassium. Excessive sweating or chronic diarrhea can cause potassium losses. Likewise, vomiting or nasogastric suction can cause hypokalemia although gastric fluids contain only 5–8 mmol/l of potassium. This is associated with concomitant metabolic alkalosis and intravascular volume depletion which cause secondary hyperaldosteronism and increases urinary potassium loss.

Pseudohypokalemia

Pseudohypokalemia occurs when serum potassium decreases artifactually after phlebotomy.

Acute leukemia is the most common cause. Abnormal leucocytes take up potassium when blood is stored in collection vial for a prolonged period of time at room temperature. Rapid separation of plasma and storage at 4°C are used for diagnosis.

Clinical features: the clinical manifestations of hypokalemia are proportionate to the degree and duration of serum potassium reduction.

Symptoms are often not present until serum potassium is below 3.0 mmol/L.

A potentiating factor such as digoxin can predispose hypokalemic patients to have cardiac arrhythmias because of altered resting membrane potential.

Cardiac

Epidemiological studies have linked hypokalemia and low potassium diet with an increased prevalence of hypertension.

Potassium deficiency can increase blood pressure. Mechanisms that have been proposed to be responsible for this effect include sodium retention with subsequent increased intravascular volume and endogenous vasoconstriction which sensitizes the vasculature.

Electrocardiographic (ECG) changes with cardiac arrhythmias can be seen. Common ECG changes are U waves and ST segment depression along with T wave flattening.

Hormonal

Hypokalemia impairs insulin release and induces insulin resistance which worsens glycemic control in diabetic patients.

Muscular

Hypokalemia can lead to skeletal muscle weakness and increases sensitivity to develop exertional rhabdomyolysis by reducing skeletal muscle blood flow. Furthermore, hypokalemia hyperpolarises skeletal muscle reducing muscle contraction.

Renal

Hypokalemia can lead to significant disturbances in renal function.

Reduced medullary blood flow and increased renal vascular resistance may result in hypertension, tubulointerstitial and cystic changes, acid base disturbances and damage to the renal concentrating mechanisms [22].

Potassium deficiency can cause tubulointerstitial fibrosis which is seen in the outer medulla. The duration of hypokalemia determines the degree of damage. Prolonged hypokalemia may result in renal failure. Furthermore, chronic potassium deficiency causes renal hypertrophy that can lead to renal cyst formation particularly during increased mineralocorticoid use [22].

Hypokalemia increases renal ammonia production.

Metabolic alkalosis is associated with hypokalemia and occurs because of increased renal net acid secretion as a result of increased ammonia excretion [22].

Additionally, it can also cause increased urinary potassium secretion resulting in hypokalemia.

In cases of severe hypokalemia, respiratory muscle weakness may arise leading to the development of respiratory acidosis and if severe, respiratory acidosis.

Severe potassium depletion can cause polyuria, with urinary outputs measuring 2–3 L.

Increased thirst and nephrogenic diabetes insipidus are factors potentiating the severity of polyuria. Nephrogenic diabetes insipidus is a result of decreased expression of water transporter aquaporin 2 (AQP2) and urea transporter proteins UT-A1, UT-A3, and UT-B which take part in urine concentration mechanisms and water reabsorption [22].

Nervous System

Cramps, paresthesia, paresis, and ascending paralysis are typical features of neurological involvement.

Treatment

Treatment approach is dependent on the severity of hypokalemia and the presence of symptoms. Treatment should include reducing the amount of potassium lost, replenishing potassium stores, assessing for potential toxicities, and determining the cause so that future episodes can be prevented [6, 22].

Short-term risks of hypokalemia are cardiovascular arrhythmias and neuromuscular weakness which can be life-threatening and require urgent treatment in the form of intravenous potassium usually 5–10 mmol over 15–20 min [22].

Urgent treatment for hypokalemia however is rarely required [14].

It should be noted that the body responds to potassium losses, by shifting potassium from the ICF compartment to the ECF compartment, minimizing change in extra-cellular potassium. With potassium replacement, potassium is shifted back into the ICF. The degree or magnitude of potassium deficiency can be masked. The amount of potassium required to replace the potassium lost is greater than predicted change in extra-cellular volume [6, 22].

The severity of hypokalemia determines the administration of either intravenous or oral potassium. Patients presenting with potassium levels of 2.5–3.5 mmol represent mild

to moderate hypokalemia and can be treated with oral potassium supplements. Severe hypokalemia defined as potassium levels of <2.5 mmol should be treated with intravenous potassium [6, 22].

Hypokalemia is associated with magnesium deficiency. Magnesium is important for potassium uptake and for maintenance of intracellular potassium levels particularly in the myocardium [1].

Intravenous Potassium: Intravenous potassium infusions can cause pain if given peripherally via a small vein. The maximum rate of potassium administration peripherally is 10 mmol/h [1, 6, 22].

In cases where more rapid replacement is necessary, potassium infusion rates >10 mmol/h can be administered but require central access, electrocardiograph monitoring and frequent monitoring of serum potassium [1, 6, 22].

Oral Potassium: Oral potassium supplements can take the form of potassium chloride or effervescent tablets. Potassium chloride tablets contain 8 mmol of potassium per tablet, as opposed to effervescent tablets which contain 14 mmol per tablet (**Table 2**).

Table 2: Treatment of Hypokalemia

Hypokalemia	Treatment
Mild (3.0–4.0 mmol)	Oral potassium: • Effervescent tablets1–2 tabs bd (14–28 mmol) • Potassium chloride tablets 1–2 tabs bd (8–16 mmol) • IV potassium; 60 mmol/24 h
Moderate (2.5–3.0 mmol)	Oral requirements; total requirements are 96 mmol/day [2]. IV potassium infusion; 90 mmol/24 h [2]
Severe (<2.5 mmol)	IV potassium infusion: 5–10 mmol/h

HYPERKALEMIA

Video 7: Potassium Regulation – Hyperkalemia

Potassium Regulation: Hyperkalemia | **Source:** © Dr. Lance Miller, PhD

Click Link to View Video on YouTube: https://youtu.be/1abWu-hp_zM

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Epidemiology

Hyperkalemia occurs frequently amongst patients with chronic kidney disease, diabetes and heart failure and patients using RAAS inhibitors (renin-angiotensin-aldosterone) or NSAIDS (non-steroidal anti-inflammatories). Less than 1% of normal healthy adults develop hyperkalemia [22]

Etiology

Hyperkalemia can be the result of pseudohyperkalemia, potassium redistribution from intracellular fluid to extracellular fluid and imbalances between potassium intake and excretion. In this section, a brief description of each cause is given.

Pseudohyperkalemia

Release of potassium from erythrocytes after phlebotomy occurs. Free hemoglobin is released into plasma from damaged erythrocytes and is reported as hemolysis. In the presence of hemolysis, reported plasma potassium is not representative of the actual plasma potassium. Treatment should not be initiated and repeat measurement of plasma potassium must take place.

Ischemia from difficult phlebotomy or exercise of limb in the presence of tourniquet can lead to abnormally increased potassium values. Potassium can also be released from other cellular elements present in blood during clotting particularly, with severe leukocytosis (>70,000/cm3) or thrombocytosis. About one-third of patients with platelet counts of 500–1000 × 10−9 have pseudohyperkalemia [22].

Diagnosis of pseudohyperkalemia is made by measuring serum/plasma potassium.

Redistribution

Hyperglycemia from insulin deficiency and hyperosmolarity are important causes of potassium movement from the intracellular fluid to the extracellular fluid. Moreover, medications such as beta 2 adrenoreceptor antagonists, RAAS inhibitors and mineralocorticoid receptor blockers are common agents that can cause hyperkalemia.

Potassium Intake

In general, excessive dietary intake does not cause chronic hyperkalemia because the kidney can excrete ingested potassium.

There are other factors that contribute to hyperkalemia when renal potassium excretion is impaired.

Impaired Potassium Excretion

In patients with decreased kidney function, there is impaired potassium excretion.

In chronic kidney disease, renal potassium secretion from distal nephrons is preserved until the glomerular filtration rate is reduced to 10–20 ml/min [22]. Medications can affect potassium excretion. A list of medications and their effects is described in Table 3.

Hyperkalemia may occur in obstructive uropathy. This is in part due to decreased Na+, K+ ATpase expression and activity. It can persist for months or years after the obstruction is relieved [22]. This is thought to be due to a persistent defect in the collecting duct, where secretion is impaired.

Aldosterone deficiency is not responsible.

Table 3: Pharmacological agents causing hyperkalemia.

Class	Class example	Mechanism
Potassium-containing drugs	Potassium chloride	Increased potassium intake
Beta adrenergic blockers	Propranolol, metoprolol, and atenolol	Inhibition of renin release
Angiotensin-converting enzyme (ACE) inhibitors	Ramipril, perindopril, and lisinopril	Inhibition of angiotensin I to angiotensin II
Angiotensin receptor blockers	Irbesartan, losartan, and candesartan	Inhibition of angiotensin I receptor by angiotensin II
Direct renin inhibitors	Aliskiren	Inhibition of renin activity resulting in decreased angiotensin II production
Heparin	Heparin sodium	Inhibition of aldosterone synthase, rate-limiting enzyme for aldosterone synthesis
Aldosterone receptor antagonists	Spironolactone and eplerenone	Block aldosterone receptor activation
Potassium-sparing diuretics	Amiloride and triamterene	Block collecting duct apical ENaC channel, decreasing gradient for K secretion.
NSAIDS and COX-2 inhibitors	Ibuprofen	Inhibition of prostaglandin stimulation of collecting duct potassium secretion. Inhibition of renin release
Digitalis glycosides	Inhibition of Na^+, K^+ ATPase necessary for collecting duct K secretion and regulation of K distribution into cells.	Digoxin
Calcineurin inhibitors	Inhibition of Na^+, K^+ ATPase necessary for collecting duct K secretion.	Cyclosporine and tacrolimus

Class Example and Action description for digoxin and CNI need to be reversed, for eg action of drug for digoxin under class example and class example digoxin is under action of drug, this also applies FOR CNI.

CLINICAL MANIFESTATIONS

Hyperkalemia may be asymptomatic or cause life threatening arrhythmias.

Cardiac

Hyperkalemia decreases the transmembrane potassium gradient. This results in cell membrane depolarization, slowing of ventricular conduction and decrease in the duration of the action potential. These changes result in electrocardiogram (ECG) manifestations including peaked T waves, broadening of QRS complexes, loss of p wave and ventricular fibrillation which can lead to asystole. Changes in plasma potassium may not result in ECG changes. ECG has been described to be a poor tool for detecting hyperkalemia with a sensitivity of 34–40% [9–12, 15].

Neuromuscular

Neuromuscular effects include paresthesia, weakness and paralysis. Deep tendon reflexes may be depressed or absent. Sensory findings are absent.

Gastrointestinal

Nausea, vomiting and diarrhea can occur but are less encountered.

DIAGNOSIS

Transtubular potassium gradient (TTKG) can help distinguish renal causes of hyperkalemia from non-renal causes.

It is a measurement of net potassium secretion by the collecting duct after correcting for changes in urinary osmolality.

The formula is as follows Eq. (1):

$$\mathrm{TTKG} = \frac{\text{urine potassium} \cdot \text{urine osmolality}}{\text{plasma potassium} \cdot \text{plasma osmolality}}$$

Table 4: Interpretation of TTKG

TTKG	Indication
<5–7	Suggest aldosterone deficiency or resistance
6–12	Normal
>10	Suggest normal aldosterone action and extra renal cause of increased potassium.

Table from Comprehensive Clinical Nephrology 6th Edition. 2019.

Effects on the Cardiac System

Calcium given by the parenteral route does not produce changes in extracellular potassium but stabilizes cell membrane potential by ameliorating the effects of hyperkalemia on myocardial conduction system and depolarization [22] (**Tables 4 and 5**).

Table 5: Treatment of Hyperkalemia

Medication	Dose	Route of administration	Time of onset	Mechanism
Calcium gluconate Calcium chloride	Calcium gluconate 10% Calcium chloride 10 mls	Intravenous	1–3 min	Cell membrane stabilisation
Insulin with dextrose	10 units IV with 50 mls of 50% dextrose	Intravenous	30 min	Cellular potassium uptake
Beta 2 adrenergic agonist	Salbutamol 15–20 mg	Nebuliser	30 min	Cellular potassium uptake
Sodium polystyrene sulfonate	30 g–60 g	Oral	>2 h	Potassium removal by potassium binding resins
*Sodium bicarbonate**	25–100 mls 8.4% $NaHCO_3$ over 5–15 minutes	Intravenous	within 60 min	Transcellular shift by alkalinisation Bicarbonate affecting H/K exchange; pushes potassium back into cells.

*Sodium bicarbonate can be considered if acidemia is present; pH <7.2.

**Hemodialysis is the most effective method of removal of potassium. Acute hemodialysis is indicated when hyperkalemia is life threatening and is refractory to medical treatment. The more severe the hyperkalemia is, the more rapid reduction of plasma potassium is required, until serum potassium is <6.0 mmol/L.

Responses occur within a few minutes and duration of action is between 30 and 60 min.

Although there are no clinical studies assessing efficacy, it has been accepted for the treatment of hyperkalemia when life threatening ECG changes are present or when cardiac arrest occurs. Life-threatening ECG changes include absent P waves, broad QRS complexes and sine-wave pattern.

The dose of calcium gluconate is higher than calcium chloride because it requires liver metabolism to release calcium.

Cellular Uptake of Potassium

Insulin and beta 2 adrenergic agonists stimulate cellular uptake of potassium. Insulin achieves this by binding to insulin receptors located on skeletal muscle. The duration of action for insulin can last for 4–6 h. Glucose is co-administered to prevent hypoglycemia.

Beta 2 receptor adrenergic agonists can be administered via inhalation and subcutaneous or intravenous routes. Tachycardia is a significant complication of therapy particularly at high doses required to treat hyperkalemia (2–8 times higher given for bronchodilation).

It has been reported that up to 25% of patients with hyperkalemia do not respond to beta 2 agonist therapy [17, 19].

Potassium Removal

Reducing total body potassium involves decreased oral intake, enhanced fecal and urinary potassium excretion and dialysis.

In terms of dietary intake, limited amounts of citrus fruits, potatoes, tomatoes and salt products should be ingested. Hemodialysis is the most effective mode of removal of potassium. In patients with advanced renal failure, the ability of the distal nephron to excrete potassium is reduced. In these patients, hemodialysis is the preferred mode of removal.

Oral potassium binding resins are other agents used in the treatment of hyperkalemia. This is best observed in patients with chronic hyperkalemia. Sodium polystyrene sulfonate and calcium polystyrene sulfonate are common agents used. They exchange sodium and calcium, respectively, for potassium in the gastrointestinal tract. It can be administered orally or rectally as a retention enema. Furthermore, polystyrene sulfonates have been reported to cause constipation, intestinal necrosis and colonic perforation. Consequently, newer agents have been developed and are being evaluated in clinic trials.

Sodium zirconium cyclosilicate (ZS-9) is an oral cation exchanger designed to trap monovalent cations in the gastrointestinal tract. Its framework structure is full of micropores that allow selectivity of trapping potassium ions in exchange for sodium and hydrogen. Clinical trials have demonstrated its success in lowering plasma potassium levels within 24 h. The onset of action is 1 h following the first dose. Dose has varied from 2.5 to 10 g. Dose-dependent edema is a notable side effect. It should be given 2 h apart from oral medications with gastric pH dependence. It binds potassium throughout the gastrointestinal tract. The bioavailability is 7 h after the onset of action after the first dose. Location of potassium binding is predominantly in the distal colon.

Long-term effects on mortality are still yet to be confirmed. In May 2018, the FDA approved ZS-9 for the treatment of hyperkalemia. It is known as Lokelma in the USA.

Patiromer is another new agent that binds potassium in the lumen of the gastrointestinal tract. It consists of a polymer anion (the active moiety patiromer) and a calcium-sorbitol complex.

Clinical trials have shown a reduction in plasma potassium levels but there are some side effects that have been observed. Hypomagnesemia has been reported in patients taking this agent.

Its use in patients with cardiac arrhythmia has been questioned, as hypomagnesemia can be associated with cardiac arrhythmias. It can also cause gastrointestinal side effects, for example, mild to moderate constipation. Its brand name is Veltessa.

POTASSIUM TESTING

Testing the potassium level is an important part of a patient's wellness check. Several types of potassium tests exist including RBC potassium, plasma potassium, serum potassium, urine potassium, and fecal potassium, with serum potassium being the most common.

Normal Serum Levels: 3.5 – 5.2 mmol/L

Please note: Normal ranges can vary slightly from lab to lab. Critical values should be promptly called to the physician or nursing staff so that the patient can receive prompt care.

As already discussed, many clinical factors can affect potassium levels including diet, pH, medications (especially diuretics), disease states, kidney function, etc. In the sections below, we will discuss some of the most common factors that affect potassium test results during and after collection.

SPECIMEN INTEGRITY FACTORS THAT AFFECT POTASSIUM

Potassium levels are highly susceptible to many outside factors and interferences, which can affect the test result. Below are some of the more common factors associated with collection.

List 1a: Causes of Possible Erroneous Potassium Results During Blood Collection	**Affect**
▪ Drawing blood above or too close to an active IV line causing specimen dilution	↓
▪ Drawing blood above or too close to an active IV line running potassium	↑
▪ Pouring off blood from an EDTA tube into another serum or plasma tube.	↑
▪ Leaving the tourniquet on for > 1 minute, causing hemoconcentration	↑
▪ Fist clenching or pumping during blood collection (Fig. 3b)	↑
▪ Hemolysis (see next section for causes)	↑
List 1b: Causes of Possible Erroneous Potassium Results After Blood Collection	**Affect**
▪ Aggressive mixing, handling, or transport (esp. pneumatic transport) of the tubes	↑
▪ Storage temperature after collection - cold and hot - esp. during offsite transport	↑↓
▪ Analysis delay after collection (should be < 2 hrs.)	↑
▪ Delay in testing, while the specimen remains unspun [23]	↑
▪ Multiple or excessive centrifugation of a tube (rare occurrence – e.g.: Multiple Myeloma)	↑

NOTE: Some factors will be more variable for specimens collected offsite and then transported to the Lab facility.

Hemolysis

Hemolysis is among the most frequent causes of laboratory error, which can affect test results. According to a previous CAP survey on reasons for specimen rejection in the chemistry department, hemolysis came in at #1, accounting for 59.6% of the rejected specimens.

Fig. 1: Red Blood Cell Hemolysis

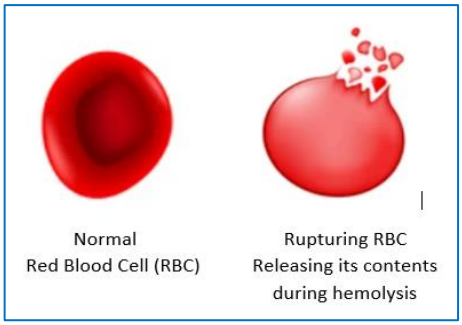
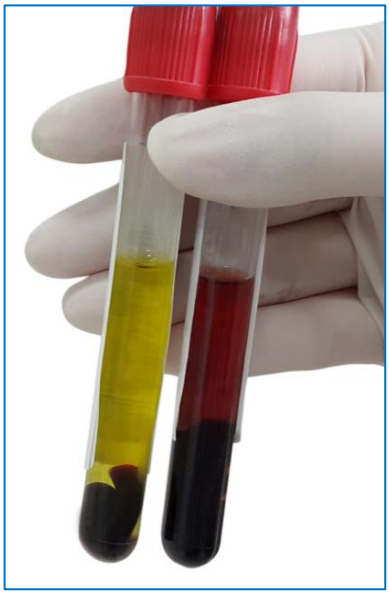


Fig. 2: Normal Serum vs. Hemolysis



Hemolysis occurs when the Red Blood Cell membrane is ruptured, causing the cell to release its hemoglobin and other internal contents. The hemoglobin that's released will cause the serum or plasma portion of the blood to visually appear tinged as pink or red in color, depending on the number of cells that have been ruptured; while other cell contents, such as potassium and other analytes, will affect those tests.

Hemolysis can occur in vivo or in vitro. In vivo hemolysis occurs within the body and has already occurred prior to the blood collection process, while in vitro hemolysis occurs after or during the collection process.

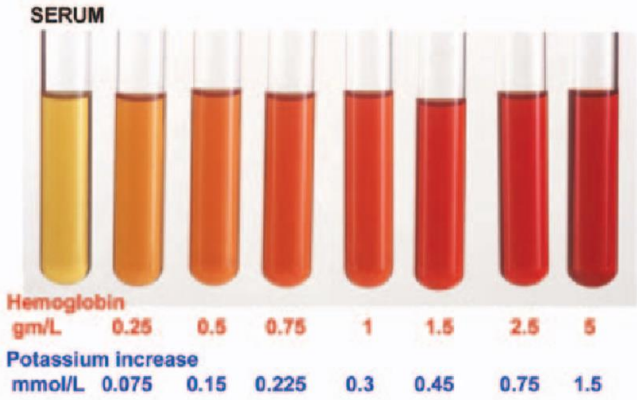


Figure 3a: shows the more free hemoglobin released from RBCs during hemolysis, the more it will raise the K^+ level.

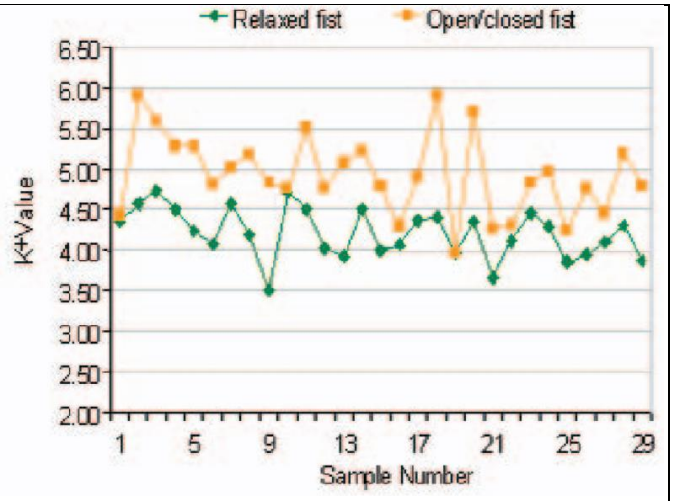


Figure 3b: affect on serum K+ levels during blood draw with a relaxed fist (green) and pumping your hand (yellow).

1. Baer, D M et al. "Investigating elevated potassium values." *MLO: medical laboratory observer* 38 11 (2006): 24, 26, 30-1 .

Regardless of the type of hemolysis present, potassium levels will be affected. Fig. 3a shows that the higher the hemoglobin level is in the hemolyzed specimen, the larger the increase in the potassium value. Since potassium plays a critical role in the body, most importantly the heart, it's important that the test results are accurate. For this reason, when a specimen is hemolyzed enough to affect the test result, a recollect will be requested. For specimens that are unable to be recollected, or in patients that have some other in vivo hemolytic condition such as hemolytic anemia, the result may need to be reported with a note added alerting the physician to the high degree of hemolysis which will falsely elevate the potassium result.

Causes of Hemolysis

Let's take a look at the reasons hemolysis may occur. As discussed, hemolysis can occur in the body prior to blood collection, or it can occur during or after blood collection. Since 92% of potassium is intracellular, anything that disrupts a cell's membrane, most importantly RBCs, will affect the potassium result.

List 2: Examples of In Vivo Hemolysis Causes:

NOTE: this is not an all-inclusive list

Infections & Toxins

- Septicemia with certain bacteria
- Malaria
- Tickborne: Babesia, Bartonella
- Bites from: certain snakes, spiders

Other

- Fresh water drowning
- Mechanical heart valve, ECMO, etc.
- Transfusion reaction

Certain Medications [27]

Non-Immune

- Antibiotics: Nitrofurantoin, Rifampin, Dapsone
- Antiviral: Ribavirin
- Antimalarial: Primaquine
- Sulfasalazine

Immune

- Antibiotics: Penicillin, Cephalosporins
- Alpha-Methyldopa
- Quinine / Quinidine
- NSAIDS

Disease States

- Glucose-6-Phosphate Dehydrogenase
- Autoimmune Hemolytic Anemia (AIHA)
- Hereditary Spherocytosis
- Disseminated Intravascular Coagulation
- Thalassemia

NOTE: Items that appear in this list do not always cause hemolysis, however they may in some circumstances.

Hemolysis can also occur in vitro during or after blood collection. Collection technique, operator error, temperature control, and handling of the specimens are the most common causes that contribute to this type of hemolysis.

List 3: Examples of In Vitro Hemolysis Causes:

NOTE: this is not an all-inclusive list.

During Specimen Collection

- Alcohol not dry before venipuncture
- Leaving the tourniquet on for >1min
- Inserting the needle bevel side down
- Moving the needle around in the vein
- Using a needle that's too small
- Missing tubes too aggressively

Syringe Collection

- Pulling too forcefully on the plunger during collection -or- pushing the blood to forcefully into the tubes from the syringe

After Specimen Collection

- **CENTRIFUGING:** Not centrifuging the specimen within 1 hr of collection, separating the serum or plasma from the cells -or- centrifuging the tube multiple times
- **TEMPERATURE:** Exposing the tubes to hot, cold, or fluctuating temperatures before testing. IE: transport from an offsite draw center in a car's trunk, excessive time in an outdoor specimen box, etc.
- **TRANSPORT:** Transporting the tubes in a pneumatic tube system without a cushion barrier -or- placement in a vehicle's trunk for transport from an offsite draw center without proper cushioning

NOTE: Items that appear in this list do not always cause hemolysis, however there's a high likelihood.

Videos 8a, b, c, d: Discussing Hemolysis

Potassium: Preventing Hemolysis | **Source:** © Video Creators

Click Links to View Videos on YouTube:

GREINER BIO-ONE: Hemolysis - Video 8a | Hemolysis in Collection - Video 8b | Hemolysis in Transport - Video 8c

PHLEBOTOMY SOLUTIONS: Preventing Hemolysis - Video 8d

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CONCLUSION

Potassium is a critical analyte that helps regulate many functions in the human body. Over 98% of the total body potassium is located within the intracellular compartment of cells, while 2% is in the extracellular space. When any shift in this balance occurs, it can cause either hypokalemia or hyperkalemia to occur, along with related symptoms. Potassium is also easily susceptible to pseudohyperkalemia when hemolysis occurs in the specimen; therefore, proper collection technique is important.

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3. Greiner Bio-One: https://www.youtube.com/@greinerbio-onenorthamerica1399/videos

CE BROKER #: 50-2256

- - 3rd Party Material

Florida Laboratory Combo-23 PART II: SECTION 2

Rhabdomyolysis Associated Acute Kidney Injury Following Physical Violence

CATEGORY: Chemistry
CONTACT HOURS: 0.5
COURSE LEVEL: Basic
CE BROKER: Automatically Reported

Provider Reports CE/CME 24 HOURS ce broker

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COURSE OBJECTIVES

At the end of this course you will be able to:

1. Discuss Acute Kidney Injury (AKI) as a complication of Rhabdomyolysis.
2. Recall the Lab Values that are commonly affected when Rhabdomyolysis is present.

RIGHTSHOLDER:

Author: Dr. Thivaharan, et al

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Clinical Medical Reviews and Case Reports

CASE REPORT

Rhabdomyolysis Associated Acute Kidney Injury Following a Physical Violence

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Abstract

Introduction: Physical violence can lead to serious and, rarely, fatal injuries. In addition to head injury, which is the leading cause of death and long-term disability, injuries of the musculoskeletal system and internal organs are important cause of assault-related morbidity. This paper discusses such rare complication of an interpersonal violence - rhabdomyolyis associated with Acute Kidney Injury (AKI).

Case: A 37-year-old man who claims to be assaulted by a group of people, presented with focal contusions and extensive grazed abrasions over the trunk and limbs. Injuries to brain and other visceral organs were excluded. Serum creatinine and urea were elevated significantly, along with increase in C-reactive protein and liver enzymes. Urinalysis contained red cells and leukocyte esterase, following which rhabdomyolysis was diagnosed. He developed oliguric AKI, and haemodialysis was initiated. He was discharged after eleven days of hospitalization, following improvement in renal functions.

Discussion: Rhabdomyolysis is a common cause for oliguric renal failure, and can be traumatic or non-traumatic. Rhabdomyolysis has specific clinical and laboratory parameters, but still requires high level of suspicion, for timely diagnosis. Highly elevated levels creatinine phosphokinase (CPK) is the most specific parameter for the diagnosis of rhabdomyolysis. Myoglobinuria, elevated levels of lactase dehydrogenase and transaminases are also considered valuable markers of rhabdomyolysis. AKI is the commonest systemic complication of rhabdomyolysis and various causative mechanisms have been explained.

Conclusion: Rhabdomyolysis requires high index of suspicion when acute kidney injury and altered metabolite levels are suspected in a patient with major or minor muscle injuries, in order to prevent complications or death.

Keywords

Rhabdomyolysis, Acute kidney injury, Creatinine phosphokinase, Muscle injuries

Introduction

Forensic pathologists, even though deal mainly with postmortem examinations, they also come across a wide variety of clinical cases, in their day-to-day practice. Patients with a history of physical violence/ assault predominate the number of cases seen per day. History of assault can vary from domestic violence to blunt and sharp force trauma inflicted upon one another during a brawl.

Physical violence can lead to serious and, rarely, fatal injuries. In addition to head injury, which is the leading cause of death and long-term disability, injuries of the musculoskeletal system and internal organs are important cause of assault-related morbidity [1]. Such instances can lead to immediate death or delayed death due to complications.

One should be mindful of the rare complications of trauma acquired by a victim, as high level of suspicion and expertise is required to diagnose such conditions. Some of such life-threatening conditions are handlebar hernias [2], traumatic abdominal wall hernias and evisceration [3], fatal thromboembolic complications [4], genital trauma [5], and fat embolism [6] to name a few.

Utmost caution in analysis of findings and

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formulating the medico legal conclusions should be practiced when such rare sequelae are encountered, because the categorization of hurt which plays a major role on the punishment and/or compensation of the offence during the medico-legal examination can be challenged by the court of law. Therefore, a detailed history and examination, considering the possible differential diagnosis, making relevant referrals in order to solicit specialist opinion becomes vital.

This paper discusses such rare complication of an interpersonal violence - rhabdomyolyis associated with Acute Kidney Injury.

Case

A 37-year-old man was brought to the hospital by a known person, who claims that this man was assaulted by a group of people, few hours prior to the admission. His past medical and surgical history was not known as the person who brought in the patient was not aware of it.

On admission, the patient was drowsy with the Glasgow Comas Scale (GCS) being 12/15, with eyes opening to pain (4), inappropriate responses through discernible words (3) and motor response to painful stimulus (5). Blood pressure was 90/60 mmHg and heart rate was 88 beats/min. Physical examination showed multiple scalp haemotomas, focal contusions over the trunk and limbs and extensive grazed abrasions on the back of the chest. A fracture of the left leg and foot was suspected as there was gross swelling noted over the areas. His breath smelled of alcohol at the time of admission. Both lung fields were clear with equal air entry.

His non-contrast CT Brain and FAST scan (Focused Assessment with Sonography in Trauma) were unremarkable, and thereby injuries to brain and visceral organs were excluded. Fracture of the leg and foot were also excluded after relevant radiological investigations. Laboratory studies showed serum creatinine of 88 µmol/L rising up to 446 µmol/L within 24 hours of admission (normal being 70-115 µmol/L), serum urea 100 mg/dL (normal range 8-50 mg/dL), blood Urea Nitrogen 42.43 mg/dL, C-reactive protein 208.45 mg/L, transaminase (aspartate aminotransferase, 4,393 U/L; and alanine aminotransferase, 2,491 U/L), along with marked elevation of serum creatinine phosphokinase (CPK) level 4984 U/L (normal range being 38-145 U/L). Serum potassium level was 6.3 mmol/L and sodium level was 133 mmol/L. Full blood count revealed a neutrophilic white cell count of 31,190 and haemoglobin level of 14.4 g/dL which dropped to 7.8 g/dL in the following three days. Complete Urine Analysis (CUA) contained haemoglobin 3+, 27 red blood cells per high power field and was positive for leukocyte esterase. His Arterial Blood Gas Analysis revealed metabolic acidosis. Blood picture shows many polychromatic cells and occasional fragmented cells, and was concluded that non-immune haemolysis or Disseminated Intravascular Coagulation cannot be excluded.

A diagnosis of rhabdomyolysis was made. Shortly after admission, the patient's condition worsened and he developed oliguric acute kidney injury (AKI), and a cycle of haemodialysis was initiated.

Further history revealed that the patient has neither comorbidities nor past history of rhabdomyolysis.

In the following days, his renal functions improved and hemodialysis was discontinued. He was discharged after 11 days of hospital stay.

Discussion

This 37-year-old male who was brought into the hospital following an assault by a group of people, presented with altered level of consciousness, in addition to being under the influence of alcohol. There were extensive grazed abrasions and contusions involving his trunk and limbs. His FAST scan and NCCT_Brain were negative for any internal organ injuries. He gradually developed oliguria and his serum creatinine levels increased by many folds within 24 hours of admission. His liver enzymes, CRP and serum potassium levels were elevated, with a more than 35-fold increase of CPK. His white cell count was high and was predominantly neutrophillic and his urine contained red blood cells. His Arterial Blood Gas analysis revealed a metabolic acidosis.

Rhabdomyolysis is a common cause for oliguric renal failure, and can be traumatic [7] or non-traumatic [8] in origin, such as vigorous physical workouts, seizures, insect bites, drugs and toxins like alcohol, opioids and statins, infections, certain autoimmune diseases, endocrinopathies like hyper- or hypothyroidism, muscular dystrophies, metabolic disorders, hypokalaemia and septicaemia [9].

Rhabdomyolysis has specific clinical and laboratory parameters, but still requires high level of suspicion, so that the diagnosis is not missed. Patients with rhabdomyolysis may present with obvious muscle injuries or in the absence of injuries with muscle tenderness, localized or diffused pain, weakness and asymptomatic features like fatigue, nausea, vomiting, increased temperature, tachycardia and red or brown coloured urine [10]. Highly elevated levels creatinine phosphokinase (CPK), with values from 5 to 10 times the upper limit of normal is the most specific laboratory parameter for the diagnosis of rhabdomyolysis. Presence of myoglobinuria which is usually confirmed by the presence of red cells is also a reliable marker for the diagnosis of rhabdomyolysis. Elevated levels of lactase dehydrogenase and transaminase levels are also considered valuable markers of rhabdomyolysis [11]. But studies have shown the creatinine phosphokinase is more specific than other markers in diagnosing rhabdomyolysis [12]. Among the

radiological investigations MRI is said to be superior to ultrasonography or CT in identifying affected muscles [13].

Acute Kidney Injury (AKI) is the most common recognized systemic complication of rhabdomyolysis. AKI is said to occur in 10 to 55% of cases with rhabdomyolysis, and is found to have a fatal outcome when there is co-existing multi-organ failure [14]. Arrhythmias, Acidosis, Compartment syndrome, Disseminated Intravascular Coagulation (DIC) and volume depletion a found to be other possible complications of rhabdomyolysis [15].

This case emphasizes the importance of bearing in mind the remote possibility of rhabdomyolysis in a patient with unexplained renal compromise and altered levels of metabolites. In clinical set-up, frequently Acute Kidney Injury following a trauma is thought to be a result of shock and the presence of dark urine is considered to be due to haematuria caused by bladder injuries. In this patient the highly elevated CPK, which is almost 35 times higher than the upper limit of normal is the most specific parameter to diagnose rhabdomyolysis, in association with elevated transaminase levels. Lactase dehydrogenase and urine myoglobin levels were not evaluated. But positive red cells and haemoglobin in the urine can be considered an indicator of myoglobinuria. Taking into account the clinical picture of the patient, his leg and foot swelling, could be sign of soft tissue oedema as a result of rhabdomyolysis and acute kidney injury. The rapid rise of serum creatinine levels in this case, is valid evidence of acute onset of diminishing renal functions. In the meantime, patient denies similar symptoms or symptoms indicative of kidney disease prior to the assault.

Rhabdomyolysis commences when the muscle contents are released into the circulation following an acute muscle injury [16]. These cellular contents from the damaged muscles lead to increased anion gap metabolic acidosis, hyperkalaemia, hyperphosphotaemia and hyperuriceamia [9] - two of these are observable in this case. Hypocalcaemia is thought to occur in cases of rhabdomyolysis, as the calcium is known to get deposited in damaged muscles, thereby causing a reduction in serum levels.

The main cause of AKI with a high mortality is rhabdomyolysis [17] with an incidence of 10-40%. In this case the rapid rise of serum creatinine levels from 88 µmol/L rising up to 446 µmol/L within a day is due to the release of muscle constituents into the blood stream and reduced clearance due to diminishing renal functions [18]. Any compromised blood is compensated by an intravascular fluid shift, which usually takes at least 24-72 hours after the initial insult [19]. This explains the delayed drop in haemoglobin levels in this case. The very high neutrophilic counts are likely a result of the response to muscle injury and inflammation [20].

Various mechanisms have been explained to be causing AKI due to rhabdomyolysis and they are renal vasoconstriction, formation of intratubular casts, direct toxic effects by myoglobin, renal ischaemia secondary to muscular vasoconstrictors, injury by the free radicals and disseminated intravascular coagulopathy [21,22]. Excessive leakage of extracellular fluid into injured muscle cells prompted by Renin-Angiotensin-Aldosterone system results in renal vasoconstriction [23]. Casts are formed when myoglobin is filtered through the glomerular basement membrane. During water absorption the concentration of myoglobin rises, and in the presence of acidic urine they accumulate and form obstructive casts. Free iron is formed when myoglobin fractions haeme ions, which in turn catalyze to form free radicals that are nephrotoxic. Substrates released from damaged muscle cells activate the coagulation cascade that can further enhance tubular obstruction. Interestingly, myoglobin is known to be less nephrotoxic in the absence of hypovolaemia and acidic urine.

To establish the diagnosis of rhabdomyolysis as the sole cause of AKI in this case with a vague history, it is important that the managing team excludes other possible causes such as pre-existing renal pathology, history of substance and alcohol abuse, usage of medications like statins and comorbidities such as seizures and endocrinopathies.

Once the managing medical team establishes the diagnosis of AKI due to rhabdomyolysis, it becomes an essential responsibility of the forensic pathologist to determine if the muscle injuries are a result of intentional trauma. In this case, an accidental fall, like one following a seizure can be excluded as the injuries are not confined to the anatomical prominences of the body. In the same manner, self-infliction of these injuries can be ruled out, as there are multiple injuries on the inaccessible sites of the body, such as the back aspect of the chest. Therefore, the causative agent of these injuries can be determined to be those caused by intentional violence.

Categorization of injuries in the medico-legal examination is of paramount importance in any case. But it is crucial in cases like this, not only because the diagnosis is principally made by the treating physician but also because these are very rare instances encountered by most of us, especially by the judicial system. If this patient was not attended to on time and if he had not been managed extensively to the level of subjecting him to haemodialysis he would have succumbed to his injuries. In other words, these injuries would definitely result in death in the absence of prompt and proper medical care. Therefore, the injuries were ‘fatal in the ordinary course of nature’ [24].

Conclusion

Rhabdomyolysis requires high index of suspicion

when acute kidney injury and altered metabolite levels are suspected in a patient with major or minor muscle injuries. Therefore, forensic experts should always bear in mind the possibility of rhabdomyolysis and its fatal complications, when handling a case of physical violence, consisting of muscle trauma.

Compliance with Ethical Standards and Consent

This case report is about a clinical case scenario of a victim of physical abuse, who was eventually diagnosed with rhabdomyolysis associated with acute kidney injury. The examination was carried with informed written consent of the patient and his anonymity is maintained throughout the manuscript. This manuscript has not been submitted to any other journals, and is the original work of the authors and has not been published elsewhere.

Conflict of Interest

The authors declare that they have no conflict of interest.

Adherence to National and International Regulations

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Material

No objections in sharing data.

Competing Interests

The authors declare that they have no competing interests.

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HEMATOLOGY

- - 3rd Party Material

Florida Laboratory Combo-23 PART II: SECTION 3

Bleeding Disorders Associated with Abnormal Platelets

CATEGORY: Hematology
CONTACT HOURS: 2
COURSE LEVEL: Intermediate
CE BROKER #: Automatically Reported

- - COURSES ARE REVIEWED EVERY 2 YEARS - -

NOTE: Although we submitted this course for 2 contact hours, ASCLS P.A.C.E. has only approved 1.5 hours, since they have deemed the videos throughout the course as supplemental and not included in the total time.

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COURSE OBJECTIVES

At the end of this course you will be able to:

1. Briefly describe the normal steps in platelet activation and hemostasis.
2. Recall what Glanzmann Thrombasthenia (GT) is, its clinical manifestations, symptoms, and cause.
3. Recall what Bernard-Soulier Syndrome (BSS) is, its clinical manifestations, symptoms, and cause.
4. Discuss the case reports for GT and BSS, their clinical presentations and treatments.

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PLATELET UNITS OF MEASURE

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Platelet results can be reported as either: [platelet result #] x 10^3 /µl -or- [platelet result #] x 10^9 /L

The unit of measure chosen can vary from lab to lab or from country to country depending on which they choose, however the platelet result # before the unit of measure will remain the same.

ARTICLE

Chapter

Bleeding Disorders Associated with Abnormal Platelets: Glanzmann Thrombasthenia and Bernard-Soulier Syndrome

Muhammet Mesut Nezir Engin

Abstract

Platelets, the smallest cells in the blood, are associated with hemostasis, bowel formation, tissue remodeling, and wound healing. Although the prevalence of inherited platelet disorders is not fully known, it is a rare disease group and is encountered in approximately between 10000 and 1000000. Glanzmann thrombasthenia (GT) and Bernard-Soulier syndrome (BSS) are more frequently observed in inherited platelet disorders. In GT, the platelet aggregation stage due to deficiency or dysfunction of the platelet GPIIb/IIIa complex cannot take place. BSS is a platelet adhesion disorder due to the absence or abnormality of GPIb/IX complex on the platelet surface. If there is bleeding after easy bruising, mucous and oral cavities, menorrhagia, tooth extraction, tonsillectomy, or other surgical interventions, inherited platelet dysfunction should be considered if the platelet count is normal while the bleeding time is long. Firstly, other causes should be investigated by making differential diagnosis of GT and BSS. In this chapter, the definition, etiology, historical process, epidemiology, genetic basis, pathophysiology, clinical findings, diagnosis, differential diagnosis, and the follow-up and treatment approach of GT and BSS will be reviewed according to the current medical literature.

Keywords: Glanzmann thrombasthenia, Bernard-Soulier syndrome, thrombocyte function disorder, thrombocyte transfusion, rFVIIa

1. Introduction

Platelets, the smallest cells in the blood, are associated with hemostasis, bowel formation, tissue remodeling, and wound healing. Platelets perform their tasks in ensuring hemostasis in four stages: platelet adhesion, activation of platelet, platelet aggregation, and platelet procoagulant activity. When a damage occurs on the vascular endothelial surface, platelets bind to the collagen, fibronectin, von Willebrand factor, thrombospondin, and fibrinogen in the endothelial substrate with the glycoprotein receptors they carry on their surface. In this way, platelet adhesion takes place. Binding of platelet receptors to their respective ligands causes activation of the platelet. This activation occurs as a result of the change in the cytoskeleton system due to intracellular calcium. By importing the impulse from outside the cell, platelet α-granules secrete their contents. The released ADP causes structural

	Glanzmann thrombasthenia	Bernard-Soulier syndrome
Genetic mutation	17q21 chromosome. ITGA2B or ITGBS genes	GPIbα, GPIbβ, and GPIX genes
Pathophysiology	Deficiency or dysfunction of the platelet GPIIb/IIIa complex	Deficiency or dysfunction of the platelet GpIb/V/IX complex
Affected platelet function	Aggregation	Adhesion

Table 1.
Comparison of genetic mutation, pathophysiology, and affected platelet function status of Glanzmann thrombasthenia and Bernard-Soulier syndrome.

change in GPIIb/IIIa on the platelet surface. Fibrinogen binds two or more platelets via GPIIb/IIIa receptors that are structurally altered, resulting in platelet aggregation. After aggregation of platelets, platelet plugs are formed at the damage site. Activation of platelets leads to changes in phospholipids on their surface. These phospholipids enable the activation of some clotting factors and perform platelet procoagulant activity [1–6].

The problem in any of the functions of platelets creates a tendency for the primary hemostatic plug not to form and therefore to bleed. Platelet dysfunctions can be hereditary or acquired. Although the prevalence of inherited platelet disorders is not fully known, it is a rare disease group and is frequently encountered in approximately between 10000 and 1000000. Glanzmann thrombasthenia (GT) and Bernard-Soulier syndrome (BSS) are more frequently observed in inherited platelet disorders.

In GT, the platelet aggregation stage due to deficiency or dysfunction of the platelet GPIIb/IIIa complex cannot take place. BSS is a platelet adhesion disorder due to the absence or abnormality of GPIb/IX complex on the platelet surface [1, 7, 8] (**Table 1**).

If there is bleeding after easy bruising, mucous and oral cavities, menorrhagia, tooth extraction, tonsillectomy, or other surgical interventions, inherited platelet dysfunction should be considered if the platelet count is normal while the bleeding time is long. Firstly, other causes should be investigated by making differential diagnosis of GT and BSS [1, 7]. In this chapter, the definition, etiology, historical process, epidemiology, genetic basis, pathophysiology, clinical findings, diagnosis, differential diagnosis, and treatment approach of GT and BSS will be reviewed according to the current medical literature.

2. Glanzmann thrombasthenia

2.1 Definition

GT is an autosomal recessive congenital bleeding disorder characterized by a lack of platelet aggregation due to defect and/or deficiency of α IIbβ 3 integrin. Integrin is a platelet fibrinogen receptor, necessary for platelet aggregation and hemostasis. Patients with this disorder often experience lifelong bleeding episodes involving mucocutaneous membranes [9–13].

2.2 History

This disease was first described by Swiss pediatrician Eduard Glanzmann in 1918 as "hereditary hemorrhagic thrombasthenia." Braunsteiner and Pakesch, on the other hand, reviewed platelet dysfunctions in 1956, after which they identified thrombasthenia as a hereditary disease characterized by normal size platelets that did not spread

to the surface and did not support clot retraction. The diagnostic characteristics of GT including the absence of platelet aggregation as a primary feature, were reported in 1964 by Caen et al. has been clearly identified by the classical report on 15 French patients. Those patients without platelet aggregation and no clot retraction were later called type I disease patients and those with absent aggregation but residual clot retraction were called type II disease patients; variant disease was first identified in 1987 [8, 10, 14–16].

2.3 Etiology

GT is an autosomal recessive disease with mutations containing the 17q21 chromosome, especially the ITGA2B or ITGB3 genes. GT results when a patient is homozygous for the same mutation or is a compound heterozygote for different mutations. GT is usually caused by decreased or absent expression of αIIb or β3, abnormalities in protein folding, transport of the integrin subunit causing post-translational defective processing or decreased surface expression, or abnormalities affecting protein function. Other defects change the integrin function by altering the ligand binding pocket (interface between αIIb and β3) that changes the cytoplasmic domain and affects the binding of regulators or locks integrin in active form [8, 9, 12, 13, 17–20].

2.4 Epidemiology

GT has an increasing incidence in populations where marriage between close relatives is an accepted tradition. The prevalence is estimated to be approximately 1:1,000,000 in the general population. Research shows that women are slightly more frequently affected than men. For example, when 177 patients with GT in Paris were examined, 102 (58%) of the patients were shown to be women. In addition, 12 patients were in the USA, 55 were in Israel and Jordan, and 42 were in South India. Some patients may have mild symptoms and are never detected to have GT, so the true prevalence may be higher than reported. Type I is the most common subtype and accounts for about 78% of patients with GT type II and type III (functional variant in receptor) and accounts for about 14% and 8% of cases [8, 9, 12].

2.5 Genetic basis

The ITGA2B and ITGB3 genes are found on chromosomes 17q21.31 and 17q21.32, respectively, and are independently expressed. Due to autosomal recessive inheritance, compound heterozygosity is common, except for selected ethnic groups, where homozygosity is more likely due to kinship. A higher percentage of pathogenic variants occur in ITGA2B compared to ITGB3, which consists of 15 exons with 788 amino acids, probably because this gene is larger with 30 exons encoding 1039 amino acids. There is a constantly updated database on the Internet http://sinaicentral.mssm.edu/intranet/research/glanzmann: currently, it contains a list of 558 mutations that lead to GT. In addition, when the data in the database were examined, it was found that 269 patients had homozygous mutations. This shows us that consanguineous marriage is an important feature in the heredity of this disease. Some researchers described that pathogenic, nonsense missense, and splice site variants are commonly observed and large deletions and duplications are rarely observed. Pathogenic missense variants cause the disruption of subunit biosynthesis megakaryocytes or prevention of the exit of pro-αIIbβ3 complexes from endoplasmic reticulum to Golgi device or the cell surface mature complexes. Most of the genetic variants affect the β-propeller region of αIIb and domains of β3 of the epithelial growth factor [20–23].

2.6 Pathophysiology

The main mechanism in the pathophysiology of GT is the qualitative or quantitative disorder of the autosomal recessive platelet surface receptor of GPIIb/IIIa (ITG αIIbβ3). As a result, it results in erroneous platelet aggregation and reduced clot retraction. ITG αIIbβ3 is a large heterodimeric cell transmembrane receptor consisting of a larger αIIb and a smaller P3 subunit. These subunits were not covalently attached to permit bidirectional signal between the cell membrane and extracellular matrix when initiating intracellular signaling pathways. It contains cytoplasmic and transmembrane domains that act as the junction point for intracellular signal molecules and proteins. The activation of ITG αIIbβ3 is provided by the B3 subunit consisting of large disulfide epidermal growth factor (EGF) domains. Calcium binding sites for complex formation and platelet-platelet adhesion are found on the p-propeller region of the αIIb subunit. The receptor head function consisting of binding fibrinogen, VWF, vitronectin, and fibronectin is necessary for platelet communications by regulation of cell migration, platelet aggregation and adhesion, and the formation of a thrombus [24].

The ITGA2B gene, located on chromosome 17q21.31, encodes the platelet GPαIIb, while the gene encoding the glycoprotein subunit IIIa is found in chromosome ITGB3, 17q21.32. Both subunits are collected from the precursors of the endoplasmic reticulum by further processing in the Golgi apparatus. Nurden et al. examined more closely the p-propeller ectodomain mutations of the αIIb subunit. Nurden et al. concluded that a large series of mutations affecting the β-propeller field interrupts calcium binding and has numerous harmful effects on αIIbβ3 expression and function, and causes different types of GT [21, 25, 26].

Homozygous or heterozygous mutations in both gene locations determine the severity of abnormality seen in GT. Mutations can stop subunit production, prevent complex formation, and/or inhibit intracellular trade. When complex build-up is prevented, the subunits of αIIb or β3 are now broken. Now based on the expression and functionality of the subunits, GT is classified into three types: <5% of αIIbβ3 now specifies type I GT; now 5–20% of αIIbβ3 is type II GT; and rarely >20% of residual αIIbβ3 with dysfunctional features make up the variant type GT. Acquired GT is usually the result of autoantibody attack on platelet αIIbβ3 or isoanorbons that inhibit proper function. The production of autoantibodies has been associated with multiple hematological conditions, including immune thrombocytopenic purpura, non-Hodgkin lymphoma, multiple myeloma, myelodysplastic syndrome, hairy cell leukemia, and acute lymphoblastic leukemia, as well as platelet transfusions [21, 24, 26].

2.7 Clinical manifestations

The most common symptoms of bleeding are purpura, nosebleeds (60–80%), gingival bleeding (20–60%), and menorrhagia (60–90%). Gastrointestinal bleeding in the form of melena or hematochezia is found in 10–20% and intracranial hemorrhage is developed in 1–2%. Mucocutaneous bleeding may occur spontaneously or following minimal trauma. Epistaxis is the most common cause of severe bleeding especially in children. Menorrhagia is quite common in affected women, and there is a higher risk of serious bleeding during menarche due to the prolonged estrogenic effect on the proliferative endometrium that occurs during anovulatory cycles. Bleeding complications during pregnancy are rare; however, there is a high risk of obstetric bleeding during and after birth. Hematuria and spontaneous hemarthrosis have been described in some cases, but are generally not part of the bleeding phenotype. Currently, specific cuts could

not be identified to define a positive bleeding score. Although the types of bleeding are consistent among individuals, the degree of bleeding is quite variable. The severity of bleeding (except for menorrhagia and pregnancy-related bleeding) decreases with age [8, 20].

2.8 Diagnosis

The diagnosis of GT is often not noticed, because many platelet disorders share common clinical and laboratory features. GT should be remembered in the differential diagnosis of medical history (insidious or bleeding episodes or severe bleeding after minor trauma), family history (consanguinity). In order to diagnose GT, it is necessary to choose the appropriate laboratory tests. A normal platelet count on a on a routine blood smear does not exclude the diagnosis of GT. Because patients with GT usually do not show any abnormalities in the number of platelets, complete blood count may be normal or show iron deficiency. Prothrombin time and activated partial thromboplastin time may be normal if bleeding time is prolonged; further investigations should be done [24].

Let us examine the laboratory methods in detail.

2.8.1 Complete blood count

In the evaluation of peripheral blood smear with light microscopy, normal platelet count and normal granular size should be. If the bleeding is severe and/or chronic, patients may have a red cell distribution width that increases with low hemoglobin, microcytosis, and secondary iron deficiency. Other abnormalities of the complete blood count (CBC) suggest an alternative diagnosis [20].

2.8.2 Coagulation screening tests

Prothrombin time (PT), activated thromboplastin time (aPTT), and fibrinogen values are usually normal unless a patient is evaluated in a significant acute bleeding environment and there is no evidence of consumption coagulopathy [20].

2.8.3 Platelet function screening tests **(View Video 1 - Page 18)**

Platelet function analyzer PFA-100 provides a measure of platelet function under reduced platelets. Very long closing times (>300 s) show GT but are heat-specific. Some other disorders such as severe von Willebrand disease, Bernard Soulier syndrome, and afibrinogenemia may produce the same result. A normal PFA-100 reveals a very high negative predictive value for GT and generally excludes this diagnosis [27].

2.8.4 Platelet light transmission aggregometry **(View Video 2 - Page 18)**

Light transmission aggregometry (LTA) is widely accepted as the gold standard diagnostic tool for evaluating platelet function. Although this test provides specific data, LTA is very time-consuming and dependent on staff and requires the use of experienced laboratories [24].

2.8.5 Whole blood impedance aggregometry

Although it can be performed using whole blood samples and lower volumes, there is insufficient evidence to support equivalent sensitivity and reproducibility compared to LTA [28].

The best way to fully diagnose GT is by mutation analysis. The genomic DNA sequence of 45 exons containing the αIIb and 3 unit should be investigated together with the junctions of the ITGB3 and ITGA2B gene and established mutations should be confirmed by a second DNA sample analysis. Genetic analysis is clinically useful for confirming the diagnosis, identifying carriers at risk, reproductive risk counseling for a particular couple/family, and definitive prenatal or preimplantation genetic diagnosis. Consequently, the diagnosis of GT involves the presence of normal platelet count (typically at the lower end of normal), long bleeding time, and long PFA time [24].

2.9 Differential diagnosis

Leukocyte adhesion deficiency type III, RASGRP2-related platelet dysfunction, BSS, Hermansky-Pudlak syndrome, von Willebrand disease, Medich platelet syndrome, Scott syndrome, and Acquired Glanzmann thrombasthenia are among the differential diagnoses [20].

2.10 Treatment

A gradual treatment standard is applied in GT treatment. The first treatment for mild bleeding is local measures including local compression, cauterization, stitching, or ice therapy. The treatment applied in case of unresponsiveness to these treatments or in heavier bleeding is antifibrinolytic therapy first, followed by platelet transfusion, and recombinant active factor VII (rFVIIa) if bleeding persists. Platelet concentrates may be single-donor and HLA-matched due to the risk of developing alloantibodies against the platelet glycoproteins, αIIbβ3, or αIIbβ3, and/or the HLA antigens. Platelet concentrates may be repeatedly transfused. If HLA-matched platelets are not found, patients should be given leukocyte-reduced platelets. This has been shown to reduce the rate of HLA immunization. Patients with severe bleeding cases should continue to receive platelet transfusion for 48 h until bleeding ceases and wound healing occurs in operated cases. These patients should be trained to avoid over-the-counter drugs that increase the risk of bleeding, such as nonsteroidal anti-inflammatories and aspirin products. Prescription drugs that may affect hemostasis should be carefully monitored [9, 24, 25, 29, 30].

Let us examine the treatment of GT according to the frequently observed conditions.

2.10.1 Treatment of minor to moderate bleeding

Local measures and/or antifibrinolytic drugs can stop mild to moderate bleeding. Local measures include compression, gelatin sponges, fibrin sealants, and topical thrombin. Antifibrinolytic agents include epsilon aminocaproic acid and tranexamic acid. Since both agents can be given orally or intravenously, they have been used successfully in the treatment of nosebleeds, bleeding gums, and menorrhagia, as well as prophylaxis before tooth extraction and other minor surgical procedures. Antifibrinolytic agents, such as tranexamic acid, can be used as a mouthwash for gingival bleeding. Antifibrinolytic use in cases of hematuria should be avoided due to the risk of a clot in the urinary tract and should be used with caution in patients undergoing procedures at high risk of thrombosis [26].

2.10.2 Treatment in epistaxis

One of the most common bleeding symptoms in GT patients is epistaxis. Local compression to epistaxis, application of tampons to the nose, topical thrombin,

antifibrinolytics, and a combination of these may respond. If bleeding persists, further treatments with platelets transfusion and/or rFVIIa should be given. Antifibrinolytic agents, nasal cautery, rFVIIa, and nasal packing with synthetic materials may be used to control bleeding. If these treatments fail nasal packing with salt pork strips may be successfully used for life-threatening nasal hemorrhage in a child with GT [6, 31].

2.10.3 Treatment of menorrhagia

Antifibrinolytic agents should be first-line therapy to control menorrhagia. If it fails, hormone supplementation either progesterone alone or progesterone with estrogen may be given. A continuous estrogen-progestin oral contraceptive agent or intramuscular depot medroxyprogesterone acetate regimen given every 3 months in women with GT has been used successfully. It can be tried on hormonal intrauterine devices to reduce bleeding. Severe menorrhagia, which may be seen in many women with GT, can be treated with high-dose conjugated estrogen intravenously for 24–48 h and later by following with a combination of high doses of oral estrogen-progestin. Intensive menstrual bleeding does not always respond to typical treatment. rFVIIa has been utilized with anecdotal success in GT when anti-fibrinolytics and platelet transfusions did not control excessive menorrhagia. In addition, surgical treatments such as hysterectomy or endometrial ablation in treatment-resistant severe menorrhagia are therapeutic options [24].

2.10.4 Treatment of postpartum hemorrhage

Pregnant women with GT have high complications and are best managed in a specialized center with a multidisciplinary team. Although most complications are associated with bleeding and occur during delivery, treatment of pregnant GT patients should start in the prenatal period. According to the recommendations in the guidelines, platelet transfusions, or rFVIIa in combination with an antifibrinolytic can be used as a prophylaxis for vaginal delivery. A systematic review of 35 pregnant women with GT showed that hemorrhage during or after delivery is common and severe, and occurred up to 20 days postpartum. If patients were not given any platelet transfusions as prophylaxis, they were more likely to experience postpartum hemorrhage (63% versus 38%). The use of rFVIIa as prophylaxis was documented in three pregnancies, and it did not prevent hemorrhage in those cases. A study showed that maternal platelet alloantibodies were documented in 16 pregnancies, and plasma exchange successfully reduced the alloantibody titer in one case. Four of the 16 cases resulted in neonatal deaths, 3 of which resulted from intracranial hemorrhage between 24- and 31-weeks' gestation. One study reported successful use of rFVIIa for permanent postpartum hemorrhage. In one study, the patient was followed up with the diagnosis of GT and 18 units of random platelet concentrates, 6 units of apheresis platelet concentrates, and 2 units of erythrocyte suspension were given in the peripartum period. Although various forms of treatment have been reported about the treatment of obstetric bleeding occurring during and after birth of women pregnant with GT, there is no consensus on the most appropriate treatment. Further studies on this subject are needed [21, 26, 32].

2.10.5 Role of transfusions in the therapy of GT

Platelet transfusion allows partial correction of functional defect in patients with GT. Platelet transfusion is the standard prophylaxis when local precautions and/or antifibrinolytics cannot control bleeding and the patient is undergoing

major surgery. It is not uncommon for patients with severe bleeding after trauma or delivery to require multiple platelet transfusions. Multiple platelet transfusions can be performed if necessary. An important risk associated with platelet transfusion is the possibility of developing isoantibodies. Up to 30% of patients develop anti-GPIIb/IIIa or anti-HLA antibodies after platelet transfusion. Platelet alloimmunization can lead to relative or absolute platelet refractory, causing rapid destruction of platelets and therapeutic failure of platelet transfusions. For this reason, platelet transfusions should be reserved for only major surgeries, life-threatening bleeding, and significant bleeding that does not respond to the above measure. When possible, platelet concentrates should be single-donor derived and HLA-matched. If HLA-matched platelets are not available, patients should be given leukocyte-reduced platelets because this has been shown to reduce the rate of HLA immunization. Transfusions in women of reproductive age should ideally be avoided as the antibodies can cross the placenta and affect the fetus [13, 21, 22, 33–35].

2.10.6 Use of rFVIIa in GT

Treatment of rFVIIa in a GT patient was successfully used for severe and uncontrolled bleeding in a 2-year-old child in 1996 for the first time. The worldwide use of rFVIIa continued afterward, and it was observed that most patients with GT were effective in successfully controlling bleeding. But it was also observed that it was not effective in all GT patients. The mechanism of rFVIIa is not fully delineated. It is thought are poorly attached to the surface of platelets and increase the activation of factor IX and X, thereby increasing thrombin production. Increased amount of thrombin increases platelet adhesion and supports platelet aggregation, including those not containing GPIIb/IIIa [6, 25, 33].

High success rates and relatively low risks associated with the use of rFVIIa as a treatment or prevention of bleeding in GT patients have yielded good results, especially in those who are refractory to platelet transfusion or have antiplatelet antibodies. HLA-compatible platelets have been used in the past and have been recommended as prophylaxis for major surgical procedures, including cesarean section. rFVIIa can be used to completely prevent platelet transfusion, which will reduce the risk of platelet alloimmunization in case of life-threatening bleeding when local measures and antifibrinolytics fail. The optimal dosage for use in GT patients has not been established. However, the recommended dose is bolus injections of 90 mcg/kg intravenously 3 times a day or every 2 h until bleeding stops, followed by one or more maintenance doses [6, 8, 21, 24, 33, 36, 37].

The adverse or thromboembolic events have not been reported in patients given the rFVIIa bolus. The incidence of thrombotic events is not known in GT patients treated with rFVIIa. Controlled clinical trials are needed to further assess risk [26, 36].

A UK study showed that rFVIIa was successful in 71% of patients treated within 12 h of onset, but only after 12 h, only 18% of patients responded to rFVIIa. Therefore, rFVIIa should be administered as early as possible in bleeding episodes. Minor surgeries in GT patients have been successfully treated by rFVIIa prophylaxis without the need for platelet transfusion. rFVIIa prophylaxis used is recommended by the United Kingdom Hemophilia Centre Doctors' Organization for minor surgical prophylaxis including dental extractions [6, 26, 33].

2.10.7 Other treatments

Desmopressin (DDAVP) causes VWF, FVIII, and tissue plasminogen activator to be released into the plasma. Although DDAVP is successful in treating other platelet disorders, there is little data to support its use in GT patients [26].

Rituximab (anti-CD20) is a human-mouse chimeric monoclonal antibody that targets the B cell CD20 antigen. Successful treatment has been reported for acquired GT patients. Multiple case reports have demonstrated the efficacy of rituximab in patients with treatment-resistant GT and bleeding symptoms or ecchymosis [38].

Bevacizumab (Avastin) is an anti-VEGF antibody used in combination with chemotherapy in various cancers. A single case report in the literature documented success using bevacizumab in a patient with type I GT who had severe, recurrent GI bleeding due to angiodysplasia. The patient was resistant to platelet transfusion, tranexamic acid, and embolization, but responded to bevacizumab [25].

Hematopoietic stem cell transplantation (HSCT) provides a treatment for patients with severe, recurrent bleeding episodes and resistant cases to platelet transfusion due to platelet alloantibodies. There is currently no clearly defined algorithm for transplantation in GT, and HSCT is rarely used for GT. The first successful bone marrow transplantion in GT was performed in a 4-year-old child with anti-GPIIb/IIIa antibodies in 1985. It has been reported in the literature that successful stem cell transplantation has been performed in 19 severe GT patients [26, 33, 39].

2.10.8 Future therapy

Gene therapy is very promising for GT patients to provide a treatment with significant progress using different techniques, vectors, and model organisms [40–42].

3. Bernard-Soulier syndrome

3.1 Definition

BSS is a rare autosomal recessive platelet dysfunction that is characterized by a low levels, absence, or dysfunction of the GpIb/V/IX complex on the platelet surface. BSS thrombocytopenia <20,000/mm^3 is characterized by decreased platelet adhesion, abnormal prothrombin consumption, and low-surviving large platelets. Mucocutaneous hemorrhages such as purpura, epistaxis, oral mucosa bleeding, GIS bleeding, and menorrhagia are generally seen in BSS as in other platelet function disorders [43, 44].

3.2 History

BSS with autosomal recessive transition was first described by Bernard and Soulier in 1948 as congenital bleeding disorder characterized by thrombocytopenia and large platelets [45].

3.3 Etiology

Mutations in GP1BA [GPIbα], GP1BB (GPIbß), and GP9 (GPIX) cause BSS. There of the four genes encode for the subunita of the GP Ib-IX-V complex. This key platelet receptor constituted of four subunits, GPIbα, GPIbß, GPIX, and GP5 (GPV), which included in the ratio 2:4:2:1 in endoplasmic reticulum. They because mature in Golgi apparatus before localizing at the cell surface. The GPIb-IX-V complex can attach to von Willebrand factor, fitting together like a lock and its key. Von Willebrand factor is located on the inside surface of blood vessels when there is an injury. These platelets form clots, plugging holes in the blood vessels to help stop bleeding. Due to the specified conditions occurring in BSS, clot formation is impaired and excessive bleeding occurs [46–50].

3.4 Epidemiology

The incidence of BSS is estimated to be 1 in 1 million live births, but is likely to be higher since it is often misdiagnosed [50]. In a study with 97 BSS patients in Iran, consanguineous marriage was reported in 81% of the cases' families [51].

3.5 Genetic basis

BSS occurs as a result of homozygous or compound heterozygous mutations that affect the expression of genes encoding GPIbα, GPIbß, and GPIX proteins. Two types of mutations have been reported in the GP Ib-IX-V complex. The first one is biallelic mutations, often homozygous mutations. It is characterized by a severe decrease or absence of the GP Ib-IX-V complex. To date, more than 50 biallelic mutations have been identified in the GPIbα, GPIbß, and GP9 gene. In a few cases, there is a compound heterozygous mutation. Most of the mutations identified are missense and nonsense mutations. Most BSS mutations occur in the GPIbα gene, and most of these mutations lead to a decrease in GPIbα expression on the platelet surface, and some to a loss of function. GPIbα is connected to GPIbß by disulfide bond. These are connected by noncovalent bonds with GPIX and GPV. GPV is the proteolytic subunit in this complex, and its extracellular part is destroyed by GPIbα-bound thrombin activates platelets. As a result, mutations in GP1BA, GPIbß, and GP9 in humans generally lead to a decrease in the total expression of the GP Ib-IX-V complex on the platelet surface and the disease occurs [6, 43, 44, 50, 52].

3.6 Pathophysiology

Platelets play a critical role in normal primary hemostasis and clot formation. There are specific GP receptors on the platelet membrane, which function in platelet adhesion, activation, and aggregation. The GPIb-IX-V receptor complex is responsible for platelet adhesion through its interaction with von Willebrand factor on the exposed subendothelium. The GPIb-IX-V receptor complex is composed of four transmembrane polypeptide subunits-disulfide-linked alpha and beta subunits of GPIb, and noncovalently bound subunits GPIX and GPV. The platelets of BSS cases lack or have a dysfunctional GPIb-IX-V receptor. This results in defective adhesion to the subendothelium. The dysfunctional platelets found in BSS can result from one of several different glycoprotein mutations such as missense, nonsense, or deletion mutations of the GPIbα, GPIbβ, or GPIX genes [53].

3.7 Clinical manifestations

As with other inherited platelet disorders, BSS can manifest with a tendency to bleed in early childhood. Mucocutaneous bleeding is seen predominantly. Easy bruising, purpura, epistaxis, bleeding gums, menorrhagia, and excessive bleeding after surgery or trauma are common symptoms. Menorrhagia is an important problem for female BSS patients. Prolonged menstruation may be the first symptom to help diagnose BSS in some patients. Although the severity of bleeding is associated with a genetic defect that affects receptor function and platelet count, it is highly variable in patients with the same mutations. Although bleeding sites are well defined for BSS, it is difficult to predict the severity of bleeding in patients with BSS. In some cases, no serious bleeding is observed and diagnosis may not be established until adulthood. Other genetic differences and acquired conditions affecting hemostasis are thought to affect the severity of bleeding in these patients,

studies related to this need to be done. Heterozygotes may not have signs of bleeding, but giant platelets may appear in peripheral blood smear [6, 43, 46, 50, 54, 55].

3.8 Diagnosis

Although thrombocytopenia is generally observed in BSS, the number of platelets is variable. The platelet count typically ranges from 30 to 200 × 10^3/μL. Giant platelets are seen in peripheral blood smear (**Figure 1**). In order to the differential diagnosis of other giant platelet syndromes, leukocyte counts and morphology should be carefully examined. Skin bleeding time and PFA-100 closure time are found to be prolonged. Routine coagulation tests should be found normal. Prothrombin consumption and thrombin generation tests are found markedly decreased because of the defective binding of FXI and thrombin. Results of platelet aggregation studies are pathognomonic for BSS. In vitro platelet aggregation studies characteristically indicate that aggregation with ristocetin failed and responded slowly with low doses of thrombin. Flow cytometric analysis of platelet also show characteristic for BSS normal binding with CD41 (GPIIb) and CD61 (GPIIIa) antibodies, but defective binding with CD42a (GPIX), CD42b (GP Ib), CD42c (GP Ib), and CD42d (GPV) antibodies suggest BSS. Immunoblotting after separating components of the GP Ib-IX-V complex with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) may describe the defective fragments but needs specialized interpretation. Also, in recent years, most families are offered molecular genetic testing to identify which gene carries the mutations [6, 53, 56–59].

3.9 Differential diagnosis

GT, idiopathic thrombocytopenic purpura (ITP), von Willebrand disease, May-Hegglin anomaly, and other inherited giant platelet disorders, for example, gray platelet syndrome are among the differential diagnoses [52, 53].

3.10 Treatment

BSS treatment is generally supportive. Platelet transfusion is used to treat when surgery is needed or when there is a risk of life-threatening bleeding. The patient may develop antiplatelet antibodies due to the presence of glycoproteins Ib/IX/V,

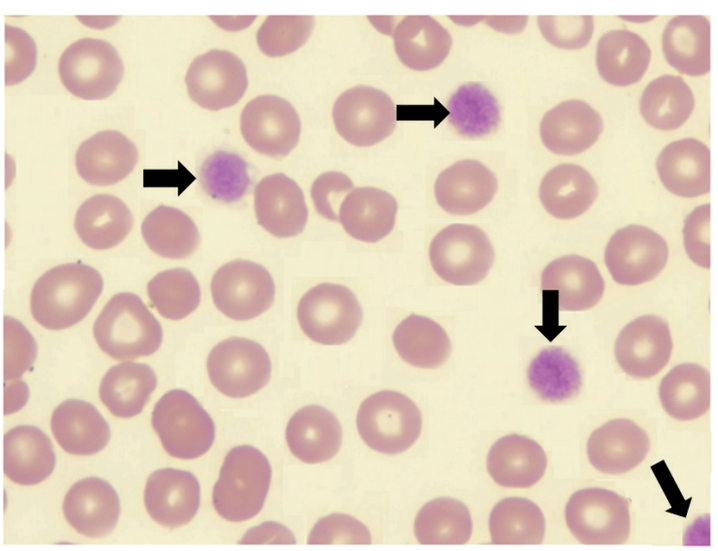

Figure 1.
Giant platelet appearance in peripheral blood smear in Bernard-Soulier syndrome.

which are present on the transfused platelets but absent from the patient's own platelets. Although some publications have suggested that patients should receive platelets from human leukocyte antigen-matched donors in order to avoid alloimmunization, this is not currently a widely accepted strategy. Antifibrinolytic agents such as p-aminocaproic acid or tranexamic acid may be useful for mucosal bleeding. rFVIIa has been reported to reduce bleeding times in patients with BSS. Desmopressin has been found to shorten bleeding episodes for some patients. A test dose should be used to determine those patients who will benefit. Stem cell transplantation has been successfully used to treat two children with BSS who had severe, life-threatening bleeding episodes. Transplantation should be considered in severe disorders when the patients have developed antiplatelet antibodies. Patients with BSS should be counseled about the importance of preventing even minor trauma as well as avoiding aspirin-containing medications and other platelet antagonists [52, 53, 60].

4. General recommendations for GT, BSS, and other inherited diseases

1. Should pay attention to dental health by brushing your teeth regularly.
2. Avoid sports activities with potential trauma (wrestling, boxing etc.).
3. Should not use aspirin or NSAIDS that affect platelet function.
4. Oral contraceptives should be considered in patients with hypermenorrhoea.
5. It should be vaccinated against hepatitis A and B since blood products may be required.
6. The patient should carry a small information card describing the condition, blood group, and what to do in an emergency.

5. Conclusion

Genetic defects of the blood platelet membrane glycoproteins, GPIIb-IIIa (CD41/CD61) and GPIb-IX-V (CD42) are the origin of several rare bleeding disorders, the best known of which are GT and BSS. GT results in defective or absence of GPIIbIIIa. As a result of this, patients with GT are unable to undergo platelet aggregation, a critical step in stemming blood flow. Either gene can be affected and mutations leading to lack of expression or to expression of poorly functional forms have been identified. BSS occurs due to defective or absence of GPIb-IX-V. As a result of this, platelets from patients with BSS are unable to adhere to the damaged vessel wall at high-shear stress and also have a reduced platelet response to thrombin.

Since GT and BSS are rare diseases, diagnosis of patients can be delayed. When diagnosed early, patients will be able to prevent bleeding that may occur due to protective measures. If there is bleeding after easy bruising, mucous and oral cavities, menorrhagia, tooth extraction, tonsillectomy, or other surgical interventions, GT or BSS should be considered among the differential diagnoses. Although GT cannot be diagnosed with routine laboratory tests, BSS is suspected in the presence of thrombocytopenia and giant platelet. Detailed examination is required for a definitive diagnosis. Treatment includes local measures, platelet infusion, rFVIIa,

and other treatments. Although there is no permanent treatment for now, research is still ongoing. For this, it is more important for patients to avoid situations that may increase their tendency to bleed.

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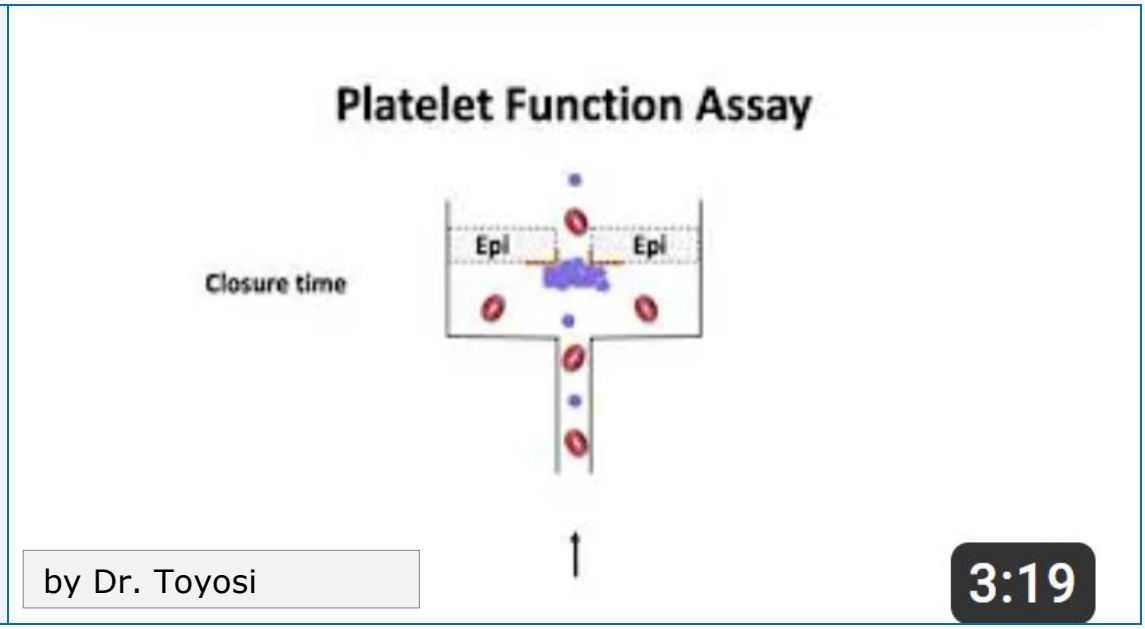

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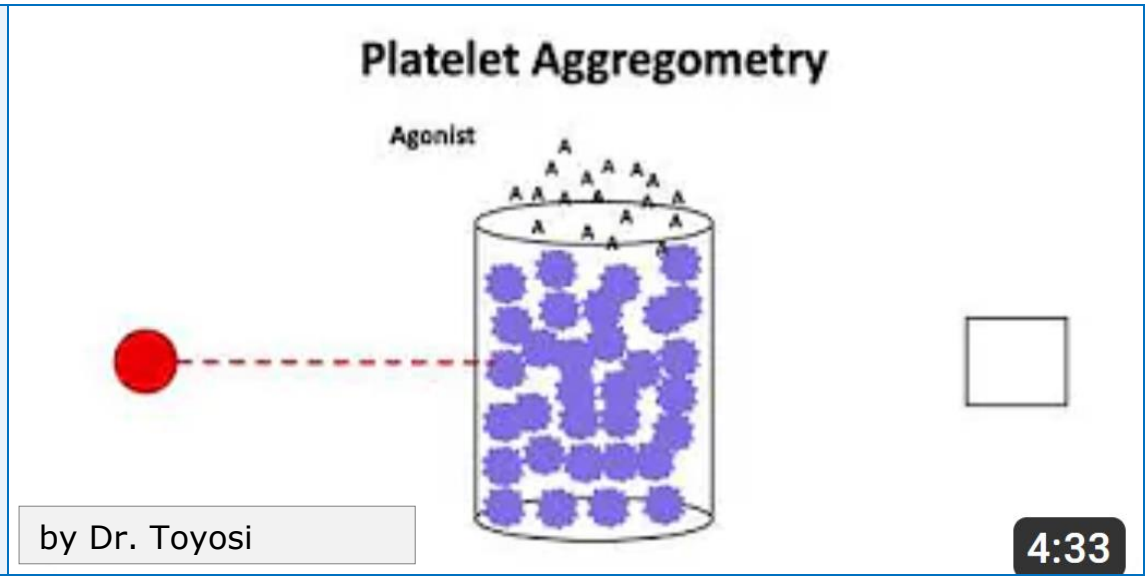

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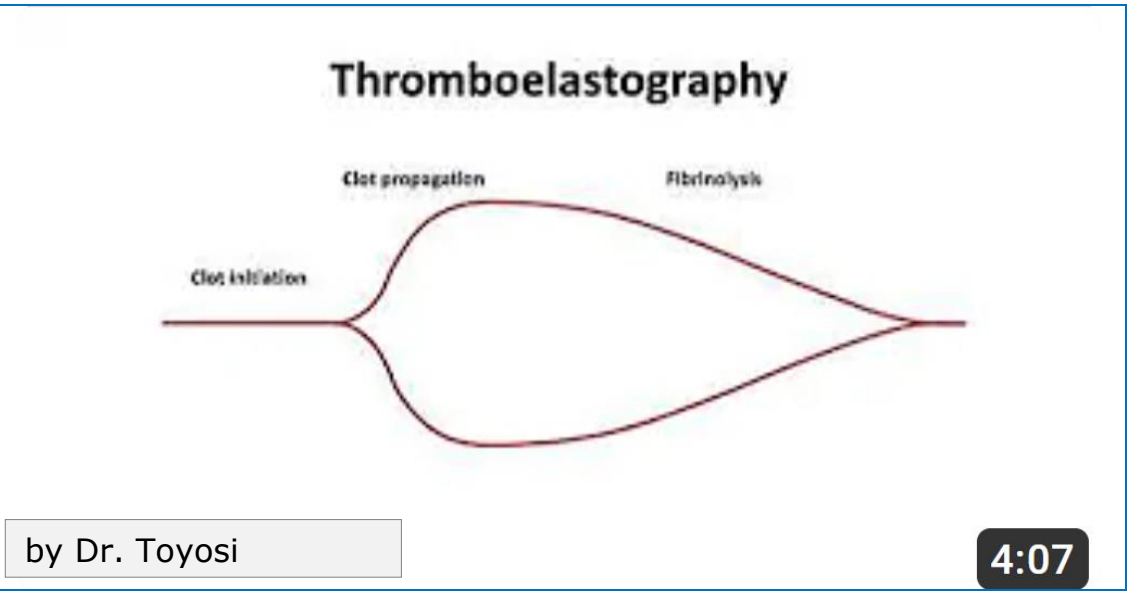

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Table 1: Comparison of Inherited Platelet Disorders

DISORDER	DEFECT	CLINICAL MANIFESTATIONS
☑ Bernard-Soulier Syndrome	Defect of adhesion due to a lack of GP Ib/IX/V [vWF receptor]	▪ Thrombocytopenia ▪ Large platelets on smear
☑ Glanzmann Thrombasthenia	Defect of aggregation due to a lack of GP IIb/IIIa [fibrinogen receptor]	▪ Normal platelet count ▪ Single isolated platelets without platelet clumping on smear
MYH9-Related Disorder	Defect of cytoplasmic structure and cell mobility due to mutation in non-muscle myosin heavy chain IIA	▪ Thrombocytopenia ▪ Large platelets on smear, as well as granulocyte inclusions – Dohle-like bodies ▪ May also present with sensorineural hearing loss, cataracts, and renal failure
Grey Platelet Syndrome (storage pool deficiency)	Absence of platelet α granules (contains vWF, factor V, and fibrinogen)	▪ Thrombocytopenia ▪ Large, gray platelets on smear ▪ Associated with myelofibrosis & splenomegaly

vWF = von Willebrand Factor **Info Source:** GrepMed © Strong Medicine / Dr. Eric Strong

Figure 2: Platelet Aggregometry Comparing Glanzmann Thrombasthenia & Bernard-Soulier Syndrome to Other Platelet Disorders

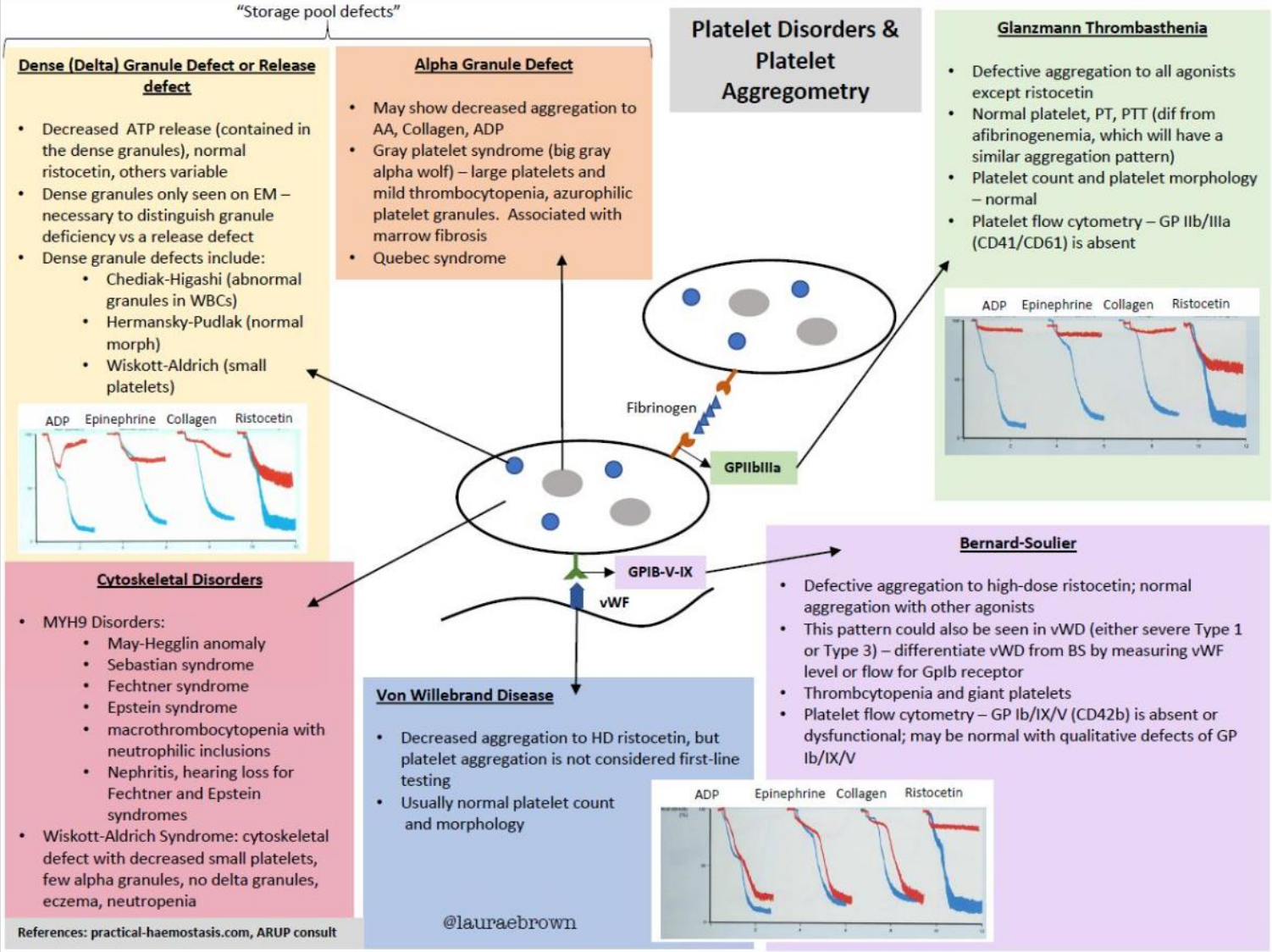

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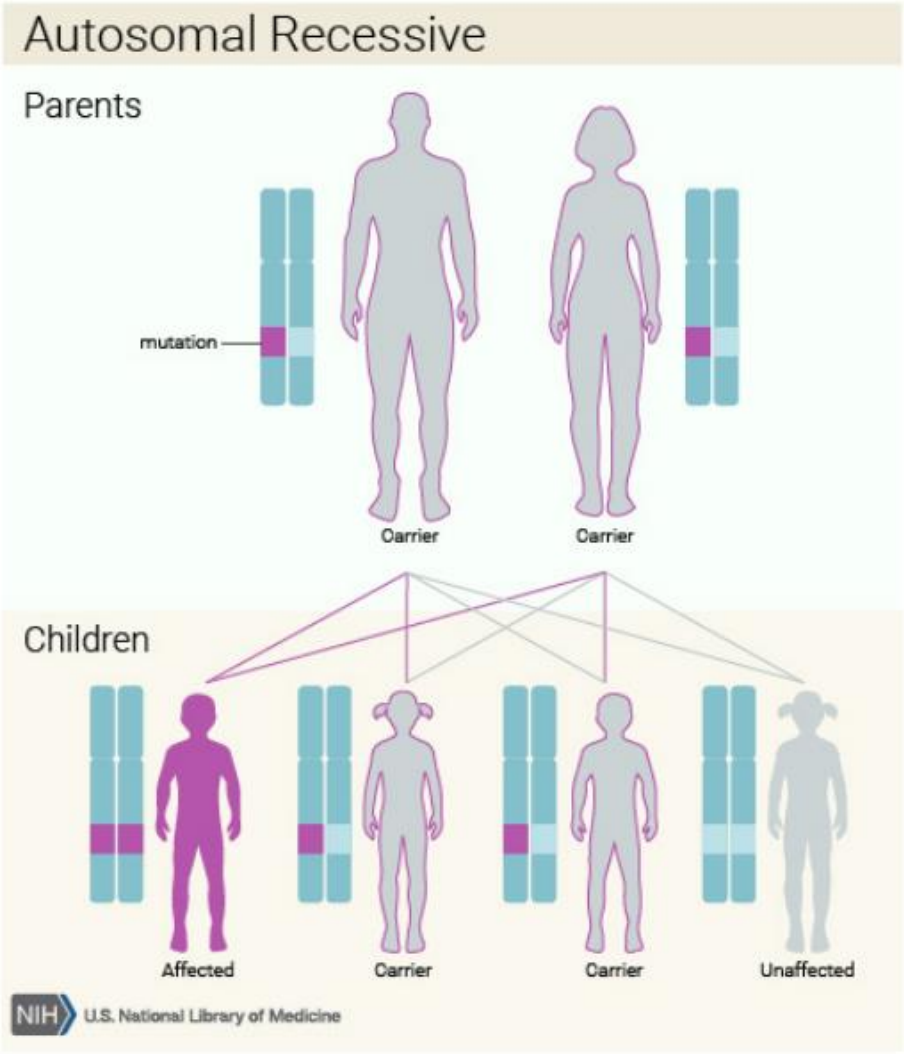
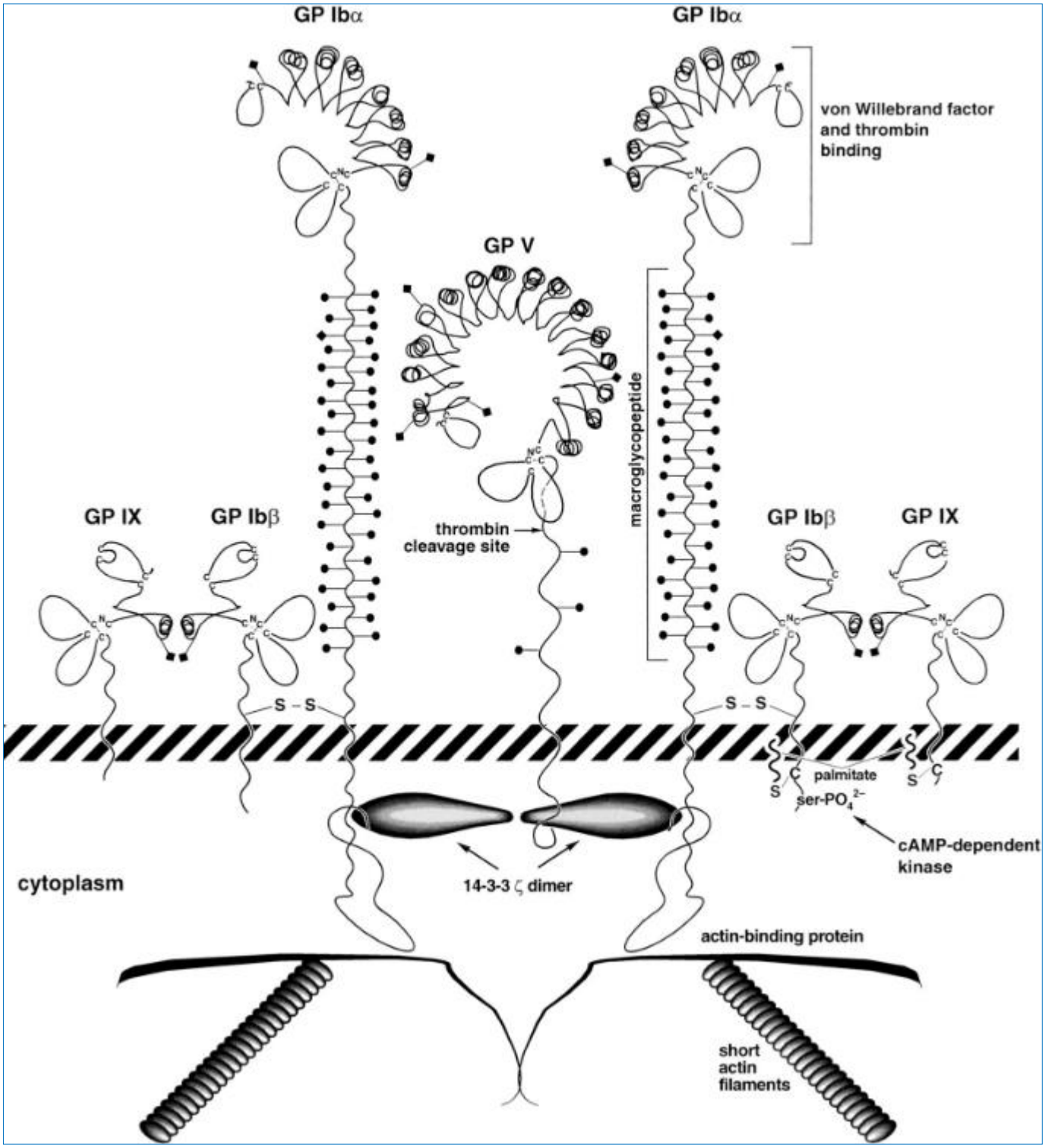
Figure 3: Autosomal Recessive Pattern



Both Glanzmann Thrombasthenia and Bernard-Soulier Syndrome are inherited autosomal recessive disorders. With an autosomal recessive pattern, both copies of the gene in each cell have mutations. Parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, however they typically do not show signs and symptoms of the condition themself. As shown above, the offspring of two parents that each carry one copy of the genetic mutation, have a 25% chance of inheriting the disease. To inherit the condition the patient must inherit one copy of the affected gene from each patent, as shown in purple above.

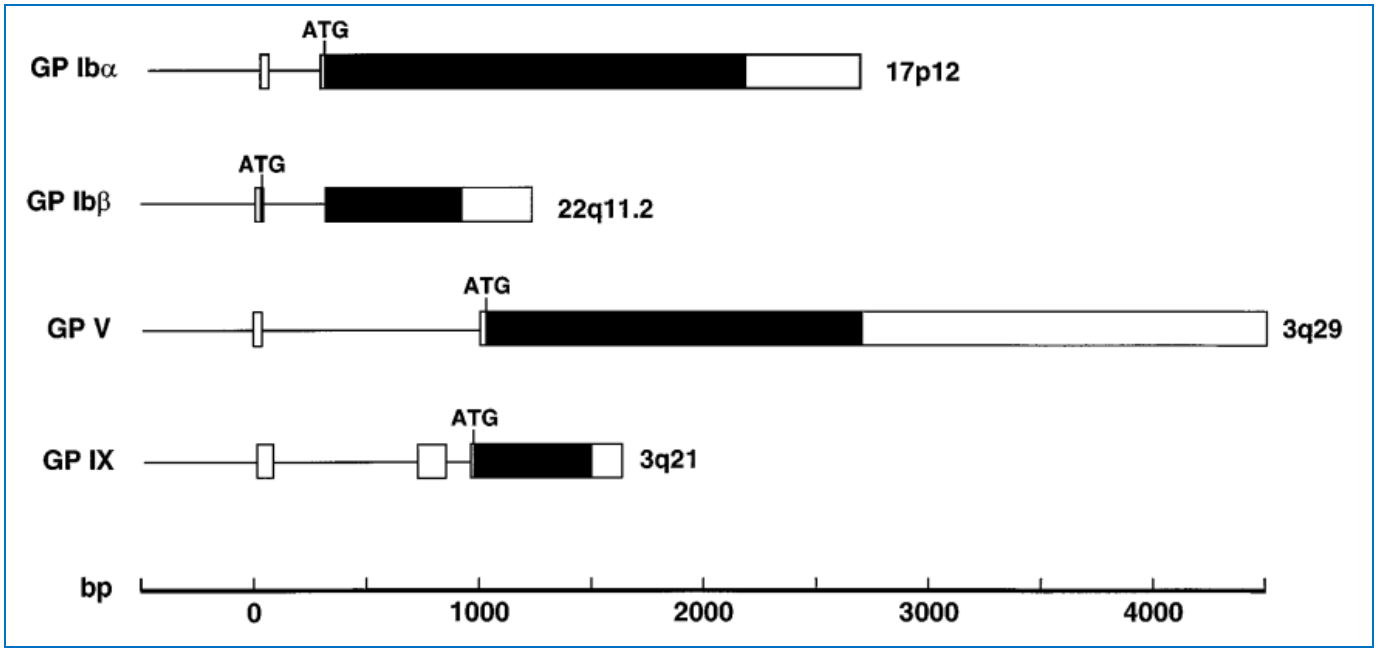
Figure 4: Key Structural Features of the GP Ib-IX-V Complex seen in Bernard-Soulier



Source: ASH Publications https://ashpublications.org/blood/article/91/12/4397/260887/Bernard-Soulier-Syndrome

Schematic view of the platelet GP Ib-IX-V complex. Key structural features of the complex are shown. The leucine-rich repeats of the four polypeptides are drawn based on the structure determined for the porcine ribonuclease inhibitor, a protein made up entirely of leucine-rich repeats.32 The depicted polypeptide arrangement is based on the published stoichiometry determined by monoclonal antibody binding17-19 and on the associations determined for the polypeptides.47,112 A caveat about this depiction: the quantity of GP V on the platelet surface has only been determined using 2 GP V monoclonal antibodies,18,20 which could lead to overestimates or underestimates of true polypeptide number. In addition, no quantitation has ever been performed to indicate that every GP V molecule on the platelet surface is associated with the complex. Complexes of greater complexity having the same stoichiometry are also possible.22,82 Diamonds on stalks represent N-linked carbohydrates and circles on stalks represent O-linked carbohydrate.

Figure 5: Structures of the Genes Encoding the 4 Polypeptides of the GP Ib-IX-V Complex



Source: ASH Publications https://ashpublications.org/blood/article/91/12/4397/260887/Bernard-Soulier-Syndrome

Structures of the genes encoding the 4 polypeptides of the GP Ib-IX-V complex with exons shown as boxes, introns as the lines between boxes, and open reading frames in black. The position of the ATG start codon is also indicated.

Figure 6: Algorithm for Determining the Genetic Basis of Bernard-Soulier Syndrome.

Source: ASH Publications https://ashpublications.org/blood/article/91/12/4397/260887/Bernard-Soulier-Syndrome

CASE REPORTS

CASE REPORTS

GLANZMANN THROMBASTHENIA

As discussed previously, Glanzmann thrombasthenia (GT) is a rare inherited blood clotting disorder characterized by impaired platelet function, caused by a defect in aggregation due to a lack of GP llb/llla. In contrast with Bernard-Soulier Syndrome, GT patients have a normal platelet count. Patients with GT may experience menorrhagia, easy bruising, purpura, epistaxis, and gingival bleeding. An extended medical history (including family members), light transmission aggregometry, platelet function analyzer, and flow cytometry remain the gold standard for the diagnosis of GT. Studies have shown that antifibrinolytic therapy such as tranexamic acid, aminocaproic acid, recombinant factor VII, and platelet transfusions are the beneficial therapies for a patient with GT.

A RARE CASE REPORT OF GLANZMANN THROMBASTHENIA

A 28-year-old man presented with complaints of malena (black tarry stools), epigastric pain, and generalized weakness. After evaluation, he was found to have anemia for which he received a transfusion of packed red blood cells (PRBC). Additionally, an abdominal ultrasound showed a mass of increasing size, while a computed tomography (CT) showed an intra-abdominal hemorrhage. Magnetic resonance imaging (MRI) was also ordered and showed a perihepatic hematoma. Later, the patient also developed black-colored stools and an occasional cough. An Endoscopy was performed with normal results. A colonoscopy showed a sigmoid ulcer, so biopsies were performed. The biopsies showed multiple fragments of large intestinal mucosa with muscularis mucosa included in one of them. The lamina propria was mildly edematous and contained a few congested blood vessels and focal lymphoid aggregates. The normal crypt architecture was preserved. There was no cryptitis or crypt abscess, and no granuloma or parasites seen. There were no features to suggest inflammatory bowel disease, dysplasia, or malignancy. Coagulation testing was performed and was suggestive of Glanzmann thrombasthenia. The patient was treated accordingly with stool softeners, antibiotics, packed red blood cell (PRBC) transfusion, tranexamic acid, and iron supplements. His serial hemoglobin levels were monitored and there was no significant blood loss. The patient improved symptomatically and is being discharged in stable condition.

Discussion

After an extensive patient history and diagnosis of the bleeding episode, this patient was managed with PRBC transfusion, tranexamic acid, and iron supplements. During the diagnostic phase, it's important to take an extensive family history since GT is hereditary; it also mimics several other platelet conditions.

As shown, GT can be managed with appropriate treatment. Allogenic bone marrow transplantation should be considered in patients with severe disease or who are unresponsive to treatment. It should be noted that several reports suggest that platelet transfusion should be avoided except in cases of severe bleeding, as it may lead to platelet antibody development.

1. Somy, C, Preenumol, T, and Roshni, P. CASE REPORT: A rare case report on Glanzmann thrombasthenia. NJPPP Vol 9, Issue 11, 1291-1292, 2017

CASE OF TWO SIBLINGS WITH GLANZMANN THRIOMBASTHENIA

Two siblings, a 14-year-old female and an 8-year-old male, reported to the dental clinic with the chief complaint of bleeding gums. The older sibling had a history of bleeding for the past 5 days, while the younger sibling had bleeding for 4 days. The children were reported to be well until bleeding spontaneously started while brushing their teeth. Both children live in a remote village where there was no access to health care.

On examination, a blood clot was seen extending across several teeth in the female [Fig. 1a], whereas the male also had a blood clot extending across several teeth on the opposite side of the mouth. The removal of the clot initiated fresh bleed from the gingiva [Fig. 1b].

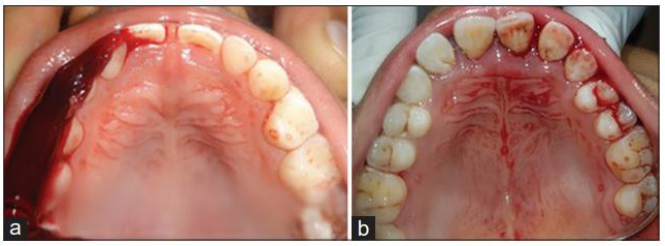


Figure 1: (a) Blood clot extending from primary lateral incisor to permanent first molar in younger sibling. (b) Fresh bleeding after removal of clot in older sibling

During the history, it was reported that the female patient had a history of purpuric patches since birth, as well as prolonged bleeding after vaccination, with recurrent episodes of mucocutaneous bleeding. The parents did not take the situation seriously or associate the signs and symptoms with being a serious illness. Blood test investigations further revealed the female sibling had anemia. Both siblings had normal platelet counts, prolonged bleeding times, and abnormal platelet aggregation tests.

Both siblings were referred to a more advanced medical facility, where a more extensive family history was taken and additional testing run; including genetic testing where they were diagnosed with Glanzmann Thrombasthenia. A pedigree chart was made which showed three other relatives were also suffering from the same disorder. [see discussion section]

After consultation with a pediatrician, treatment was planned for both siblings. Both patients were hospitalized just in case any future bleeding episode would occur. During hospitalization, the older sibling received a transfusion of 20 platelet concentrates and 10 packed RBCs for her anemia, after which her hemoglobin (Hgb) level rose from 4 gm/dl to 9 gm/dl. Both patients remained in the hospital for 5 days to observe for bleeding and to receive much needed dental work. For the female patient, the pediatricians recommended one unit of platelet transfusion 2 hours preoperatively and one unit postoperatively as blood loss was anticipated. She also received topical tranexamic acid to help stop any bleeding. The male patient had a tooth extracted and was treated with a local anesthesia infiltration of 2% lidocaine in a 1:200,000 dilution of adrenaline. Hemostasis was achieved by compression of the socket and ice application.

Discussion

Pediatric dentists often come across different medical conditions when children are brought for dental management. Any episode of excessive bleeding after extraction or cleaning of primary teeth, unexplained and spontaneous bleeding from the gingiva, and mucous membranes should alert the dentist about the possibility of bleeding disorders, which should be investigated by a specialist.

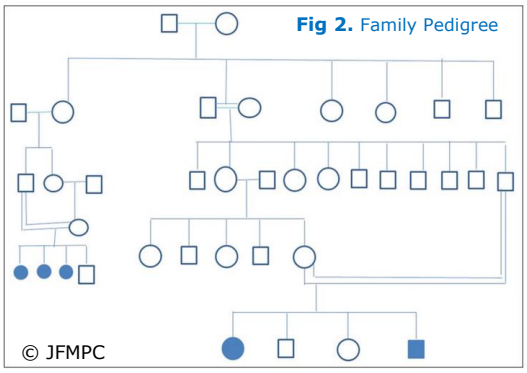


Fig 2. Family Pedigree

GT is typically present from birth, with signs and symptoms that can vary greatly from person to person, as shown in the two siblings in this case report. After taking an extended family history, it was discovered that the siblings were the offspring of a consanguineous marriage. Consanguineous marriage (where cousins marry) is common in some cultures, often leading to genetic abnormalities. Since patients often require multiple transfusions throughout their life, measures to avoid platelet alloimmunization should be taken, which is best accomplished by leukocyte-depleted blood products and the use of human leukocyte antigen (HLA)-matched platelets.

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INFANT WITH SUSPECTED GLANZMANN THROMBASTHENIA – DIFFERENTIAL DIAGNOSIS

This short vignette describes an infant presenting with mini nosebleeds, bleeding gums, petechiae and purpura. This discussion reviews a brief introduction, diagnosis, and treatment. It also includes an 11-minute Podcast discussing this case (link at the very bottom of the page). You can access the information at the links directly below.

FULL WEB PAGE WITH PODCAST: https://step1.medbullets.com/hematology/111032/glanzmann-thrombasthenia

PODCAST ONLY: https://www.listennotes.com/search/?ocid=1cc2cfe38cce4cce9db751a89652ee01&q=glanzmann+thrombasthenia (11:34)

BERNARD-SOULIER SYNDROME

Bernard-Soulier Syndrome (BSS) is a rare hereditary autosomal recessive bleeding disorder of platelet function, caused by a qualitative or quantitative defect in the membrane glycoprotein Ib-IX-V complex, a primary platelet adhesion receptor. It presents with a low platelet count (thrombocytopenia) and giant platelets on a blood smear. It is marked by the inability of the platelets to interact with the von Willebrand factor (VWF), which acts as a bridge between the subendothelial matrix and platelets, rendering the platelets unable to adhere to vessel walls during injury, leading to an increased bleeding tendency. The clinical manifestations are variable and include purpura, epistaxis, gingival bleeding, menorrhagia, occasional gastrointestinal bleeding, hematoma, or hematuria.

BSS CASE REPORT DURING PREGNANCY

A 21-year-old Caucasian female from southern Brazil, with a known history of Bernard-Soulier Syndrome (BSS), presented to her obstetric appointment at 33 weeks gestation. According to her medical history, she was diagnosed with BSS at the age of 5. Suspicion was due to a positive family history for the syndrome and during diagnosis, a Ristocetin test showed no platelet aggregation along with thrombocytopenia, suggestive of her diagnosis. To date she denies having any bleeding history, except for a few episodes of gingival hemorrhages, where no medical intervention was needed. She also denied receiving platelet transfusions or any other treatment for this syndrome during her lifetime.

At this appointment, her physical exam was unremarkable, with the exception that she had severe thrombocytopenia (30 × 10^3 platelets/μL), with presence of giant platelets on the peripheral blood smear. She also had a positive Group B Streptococcus swab test, while her other routine laboratory tests were unremarkable for any abnormal pathology.

In order to monitor her platelet count and coagulation profile, she was hospitalized and after a multidisciplinary consultation, it was decided that she would undergo an elective cesarean section at 37 weeks. Due to a family history of bleeding during surgery, precautions were taken to ensure that platelets, packed RBCs, and factor VII would be available if needed during the C-section. Tranexamic acid was prescribed at a dosage of 10 mg/kg three times a day on the day prior to surgery and for three days thereafter. During her hospital stay, she received four doses of intramuscular Dexamethasone.

At the gestational age of 37 weeks and 4 days, she underwent a cesarean section under general anesthesia. Her platelet count just prior to the procedure was 35 × 10^3/μL, so special attention regarding hemostatic care during the operation was given. This was necessary to prevent any excessive or unnecessary bleeding, with major intraoperative and postoperative hemorrhage as the primary concern. The patient remained hemodynamically stable throughout the procedure with minimal blood loss, estimated to be ~700 ml. A healthy baby girl was delivered during the procedure.

Soon after the procedure, the platelet count was 56 × 10^3/μL and the coagulation tests were normal. In total, the patient received the following platelet concentrates: 6 U on the day before the procedure, 9 U 1hr. before the procedure and 6 U on the first and second postoperative days. The patient did not experience any major bleeding in the postpartum period and the surgical wound healing occurred as expected, without complications. Tranexamic acid was maintained for 24 hours post-procedure. She was then discharged at 4 days postpartum with a platelet count of 70 × 10^3 platelets/μL and hemoglobin of 11.5 mg/dL. Table 1 shows the platelet count and values of coagulation testing performed during pregnancy and puerperium (6 week period post childbirth).

The first platelet count from the newborn was 53 × 10^3 platelets/μL and the hemoglobin was 19.1 g/dL. She did not present any episodes of bleeding in the neonatal period and was discharged with her mother. The diagnostic suspicion was of BSS in the autosomal dominant form (OMIM 153670), based on family history and thrombocytopenia. She is currently being followed as an outpatient with a hematology specialist.

Table 1: Maternal Platelet Counts During Pre- and Postpartum Periods

Gestational age	Platelet counts	Coagulation tests (INR, aPTT)
33w + 4d—prenatal consult and hospitalization	6000 (manual evaluation)	Values within normal limits
34w + 5d—hospitalization	30,000 (manual evaluation)	Values within normal limits
35w + 4d—hospitalization	5000 (manual evaluation)	Values within normal limits
36w + 4d—hospitalization	15,000 (manual evaluation)	Values within normal limits
37w + 4d—after receiving 6 IU of platelets the previous day and before cesarean section	36,000 (manual evaluation)	Values within normal limits
37w + 4d—during cesarean section and after receiving 9 IU of platelets one hour before the procedure	58,000 (manual evaluation)	Values within normal limits
37w + 4d—after the procedure and after receiving 6 IU of platelets postoperatively	54,000 (manual evaluation)	Values within normal limits
First day of puerperium	48,000 (manual evaluation)	Values within normal limits
Second day of puerperium	50,000 (manual evaluation)	Values within normal limits
Third day of puerperium	70,000 (manual evaluation)	Values within normal limits
Fourth month of puerperium	25,000 (manual evaluation)	Values within normal limits

INR: international normalized ratio; aPTT: activated partial thromboplastin time; w: weeks; d: days.

Discussion

With BSS, women are at risk for postpartum hemorrhage and may need an emergency hysterectomy. The newborn also has an increased risk of severe bleeding, such as intracranial bleeding caused by the thrombocytopenia. The course of the disease varies in each pregnant woman and also within the same patient during different pregnancies. The most common occurrence is intrapartum and postpartum bleeding, rarely occurring in the antepartum period. As postpartum hemorrhage is common, follow-up should be done for 6 weeks in the puerperium period. Therefore, it is still not clear whether cesarean section is preferable over vaginal birth in women with such bleeding disorders. Through careful monitoring and treatment, bleeding episodes can often be prevented or minimized.

1. Vilaverde Perez, A, et al. "Bernard-Soulier Syndrome in Pregnancy: A Case Report". Open Journal of Obstetrics and Gynecology, Vol.9 No.6, 2019

BSS CASE REPORT MISDIAGNOSED AS IMMUNE THROMBOCYTIC PURPURA

A Saudi Arabian girl, previously healthy until the age of 5, when she initially presented for medical examination with petechiae all over face, chest, arms, abdomen, and legs. She was not on any medications, and other systemic reviews were unremarkable.

She presented again at 7 years old and at examination she was conscious, alert, oriented, and not in distress. No enlarged lymph nodes were palpable in any part of her body, her abdomen was not distended, her spleen and liver were not palpable, and other systemic examinations were unremarkable.

Laboratory screenings were performed with the following results: **CBC** (Hgb: 11.7, RBC: 4.19, WBC: 11.2, Platelets: 80). **Blood film:** many large and giant platelets were seen. **Serologic examinations** for Human Immunodeficiency Virus and hepatitis B and C were performed and were all negative. In addition, ANA and direct coombs tests were also negative. With the exception of a low platelet count and abnormally large platelets, all other results fell within the normal limits.

The patient was diagnosed with idiopathic thrombocytopenia (ITP) and received IVIG, after which she was discharged in good condition.

Following this initial presentation, she had multiple admissions with the same complaints of petechiae and low platelet count. She received IVIG multiple times, responding with a platelet count increase of at least by 30,000 × 10^3/μL, however, after two to four weeks the platelet count would drop again, at times even below 10,000 × 10^3/μL. There was no response to steroids, however, she did respond to anti-D once for four weeks. She also received four doses of rituximab with no response through 16 weeks. Bone marrow aspiration and biopsy were performed with a result of normal cellular marrow, with normal megakaryocytes content. No pathology was reported on the marrow to explain the thrombocytopenia.

She was again diagnosed as chronic ITP with frequent admission and poor response to treatment. Due to logistical issues, platelet aggregation and flow cytometry were not performed. Her doctor arranged an appointment for follow-up and genetic analysis to rule out Bernard-Soulier syndrome. The result of Molecular genetic analysis of the genes

GP1BA, GP1BB, GP9 showed a presence of a homozygous deletion of 39 nucleotides in the exon 2 of GP1BA. At this point, she was diagnosed with Bernard-Soulier Syndrome.

Discussion

Bernard-Soulier Syndrome is a rare, autosomal recessive platelet function disorder which is commonly mistaken for idiopathic thrombocytopenic purpura (ITP). It's important to differentiate the two because patients often receive unnecessary treatment, including medications and splenectomies when improperly diagnosed with ITP.

It's often difficult to differentiate the two, since both present with low platelet counts. The platelet appearance on a peripheral blood smear may not be of much value either, since large platelets can be seen whenever platelet turnover is increased [2]. Since BSS is a genetic abnormality, the only definitive way to differentiate the two is to run a molecular genetic analysis and to take an extended family history.

ITP is an acquired disease that's typically self-limiting in most patients, with improvement within 6 months. In ~20-30% of children however, ITP can become chronic, lasting >12 months. Since BSS is an inherited genetic condition, thrombocytopenia is present from birth. By taking an extended family history, the physician can confirm that thrombocytopenia and bleeding issues are present in more than one family member during a BSS diagnosis, while ITP is typically only present in one family member since it is acquired. BSS is also commonly seen in cultures where consanguineous marriage exists, allowing cousins to marry. Extended family histories will help point the physician toward genetic testing in the diagnostic phase.

1. Ahmed Wasfi, L, et al. A Case Report Of Bernard-Soulier Syndrome In Differential Diagnosis of Immune Thrombocytopenic Purpura. International Journal of Advanced Research, 6(1), 120-130, 2018.

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COURSE OBJECTIVES

At the end of this course you will be able to:

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2. Recall the transmission route.
3. Discuss this case report and how renal transplantation may have played a role in infection.

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CASE REPORT Open Access

Human parvovirus B19 infection in a renal transplant recipient: a case report

Michelle Teodoro Alves[1,2], Sandra Simone Vilaça[3], Maria das Graças Carvalho[2], Ana Paula Fernandes[2], Luci Maria Sant' Ana Dusse[2] and Karina Braga Gomes[1,2*]

Abstract

Background: Parvovirus B19 presents tropism for human erythroid progenitor cells, causing chronic anemia in organ transplant recipients, due to their suppressed humoral and cellular responses. Diagnosis may be achieved through serological tests for detection of anti-B19 antibodies. However, renal transplant recipients are not routinely tested for parvovirus B19 infection, since there is scanty data or consensus on screening for B19 infection, as well as for treatment or preventive management of transplanted patients.

Case presentation: Herein we report a kidney transplant recipient, who was unresponsive to treatment of severe anemia, and presented hypocellular hematopoietic marrow, megaloblastosis and hypoplasia of erythroid lineage with larger cells with clear nuclei chromatin and eosinophilic nuclear inclusions. This patient was seropositive for Epstein-Barr and Cytomegalovirus infections and negative for anti-parvovirus B19 IgM and IgG antibodies, although symptoms were suggestive of parvoviruses infection. A qualitative polymerase chain reaction testing for B19 in serum sample revealed positive results for B19 virus DNA.

Conclusion: This case report suggests that the diagnostic process for parvovirus B19 in renal transplant recipients should include a polymerase chain reaction assay to detect B19-DNA, since specific serological tests may be unreliable given their impaired humoral responses. These results also indicate the importance of considering parvovirus B19 infection in the differential diagnosis of persistent anemia in transplanted patients.

Keywords: Parvovirus B19, Anemia, Renal transplant, Antibodies

Background

The parvovirus (erythrovirus) B19 is a common human infection worldwide. The clinical manifestations of B19 infection depend on the host's haematological status and immune responses [1]. In immunocompetent individuals, B19 causes the erythema infectiosum, also known as "fifth" disease. Classically, erythema infectiosum affects children who develop rash, fever and malaise, while in adults it may be associated with acute symmetrical polyarthropathy. B19 infection during pregnancy is associated with hydrops fetalis. In patients with chronic haemolytic anaemia, it correlates with transient aplastic crisis. In addition, it may also cause chronic anemia and pure red cell aplasia in immunocompromised patients [1,2].

The cellular receptor for B19 is a globoside (P antigen), present in erythroid precursor cells. The virus infects, replicates in, and then lyses erythroid progenitor cells [1]. This direct effect on erythroid cells manifests characteristically as pure red cell aplasia on bone marrow examination, revealing the presence of giant pronormoblasts, which can help the diagnostic process. B19 infection depends on mitotically active cells and susceptibility to infection increases in the erythroid precursors with differentiation [3]. Therefore, tissue distribution of the blood group P antigen helps to explain the extreme tropism of B19 for erythroid cells and the effects on hematopoiesis and bone marrow failure. Patients that do not present the P antigen in their erythrocytes are, therefore, resistant to infection by this pathogen [4].

Transmission of B19 infection occurs either by the respiratory route, vertically from mother to fetus, through

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transfusion, blood-derived products or transplantation. In immunocompetent patients, B19 infection is characterized by fever, chills and myalgia, which are followed by rash and joint symptoms [5].These later symptoms are associated to the appearance of specific antiviral antibodies. An effective immune response limits intense viremia in approximately 5 days. B19 specific immunoglobulin M (IgM) may persist for up to 6 months. Specific IgG is detectable about 2 weeks following infection and remains for years. Low reticulocytopenia occurs during viremia, but hemoglobin levels do not decline. In patients with chronic hemolytic disorders, transient aplastic crisis may occur during infection, since reticulocytopenia results in decreased hemoglobin levels. Nevertheless, the anemia is transitory resulting from development of specific antibodies against B19 antigens.

In immunocompromised patients, unable to mount humoral or cellular responses, B19 infection persists and may cause chronic anemia or erythroid bone marrow aplasia. Morphologically, bone marrow aspirates show giant proerythroblasts, large eosinophilic nuclear inclusion bodies, and cytoplasmic vacuolization [6].

Currently, diagnosis is based on detection of B19 IgG and IgM antibodies or B19 DNA in blood or tissue samples by polymerase chain reaction (PCR). A simple dot blot hybridization assay also detects infection; however the sensitivity of B19 detection is greatly improved by PCR. Immunohistochemistry is a specific alternative and may complement diagnosis in cases of placental or fetal infection [7-9].

Case presentation

This case report describes to a Brazilian woman, 42 years-old, who presented with a renal failure and was submitted to haemodialysis for five years, before a kidney transplant, which occurred in 2007. After transplantation, the therapeutic regimen of immunosuppression included prednisone (5 mg daily), tacrolimus (5 mg daily) and azathioprine (50 mg daily). Dosage of serum tacrolimus was 5.8 ng/mL.

The donor's B19 status for this recipient was unknown. In December 2010, the patient developed significant anemia, which was resistant to erythropoietin (1,119.0 mUI/mL) and, eventually, required blood transfusion. After transfusion, the patient's hemoglobin was 6.8 g/dL and her hematocrit was 20.2%.

In April 2011, she presented cutaneous mucosa paleness, fatigue after minimal effort, arthropathy and malaise. She presented at the Hospital Felício Rocho, Belo Horizonte, MG, Brazil. Levels of hemoglobin and hematocrit were 3.6 g/dL and 10.3%, respectively. She received a transfusion of 600 mL of erythrocytes. Reticulocyte count was 7,200/mm^3, leukocytes 4,100/mm^3 and platelet 220,000/mm^3. Dosage of serum creatinine was 2.3 mg/dL, iron (152 mcg/dL), transferrin saturation (89.9%), folate level (20.0 ng/mL), ferritin (938.6 ng/mL) and vitamin B12 (238.0 pg/mL), which did not suggest a nutritional or iron deficient anemia. Other laboratory investigations revealed she was seropositive for anti-Epstein-Barr (high IgG levels – 477.0 U/mL) and Cytomegalovirus (IgG positive) and negative for anti-hepatitis B, anti-hepatitis C and anti-HIV antibodies.

At this time point, a bone marrow aspirate revealed hypocellular for red and white cells and platelets. Besides, there were dysplasia and megaloblastosis in the erythrocytic series, which were attributed to azathioprine associated with tacrolimus toxicity.

A bone marrow biopsy was also obtained and showed severe hypoplasia of elements of the erythroid lineage, presence of larger cells with clear nuclei chromatin and eosinophilic nuclear inclusions, suggesting inclusions caused by B19. Nonetheless this evidence has indicated B19 infection, IgM and IgG assays were negative. However, as the symptoms and bone marrow biopsy were suggestive of B19 infection, a qualitative PCR testing for parvovirus B19 was performed, revealing the presence of this virus.

The woman received 5 doses of intravenous gammaglobulin, 400 mg/Kg body weight daily, which improved the symptoms. A new evaluation revealed an important increase in hemoglobin, from 3.6 to 12.6 g/dL.

Discussion

In transplanted patients, parvovirus B19 infection is transmitted through the donor organ or contaminated blood products, during transfusion. Increased susceptibility due to immunosuppressive therapy is likely to favor establishment of infection [5,10]. B19 is not frequently regarded as a cause of anemia in immunosuppressed patients, although anemia without previous blood loss or reticulocytopenia should alert for potential B19 infection. Pure red cell aplasia and severe chronic anemia are also manifestations of B19 infection in organ transplant recipients and is directly related to the virus tropism for human erythroid precursor cells. Persistence of virus in bone marrow leads to prolonged suppression of erythropoiesis [10-12].

Although many cases of B19 infection in renal transplanted patients and various infection-related complications have been reported, only a few studies have been performed to evaluate the incidence of active B19 infection in anemic transplanted patients. Reported incidences of this infection vary from 23 to 31.1% of the cases. Given the prevalence of B19 infection in the general population (approximately 85% of older adults) and the increased susceptibility of transplanted patients to viral infection, it may be admitted that B19 infection is under-reported in this population [11,13-15].

This wide range of prevalence values reflects differences in definition of infection, in patient selection and in sensitivities of diagnostic methods. The diagnostic tests to detect anti-B19 IgM antibodies based on μ-capture sandwich enzyme immunoassay, display sensitivity of 89.1%and specificity of 99.4% [16]. The enzyme immunoassay for detection of IgG antibodies is reported to have a sensitivity of 98.6% and specificity 100% [17]. PCR and real-time PCR improve the sensitivity of detection of B19 infection, and many clinical laboratories use these molecular assays to complement the serologic diagnosis. Regarding the sensitivity of molecular methods, the Nested-PCR results in a thousand fold improved sensitivity when compared to conventional PCR. Since the qualitative detection of the DNA is not useful to confirm recent infection, real-Time PCR quantitative assays for viral DNA may be applied to differentiate acute from chronic infection [9,18,19]. Therefore, the interpretation of diagnostic test results is not always straightforward.

Moreover, in immunocompromised patients, false negative results may occur as due to depressed immune responses [15]. Egbuna *et al.* [11] described three transplanted patients with inadequate response to recombinant human erythropoietin treatment, under immunosuppressive therapy. One patient had negative serological tests for parvovirus B19, but a positive PCR, demonstrating the in-ability of the patient to mount a detectable effective anti-viral humoral response. Geetha *et al.* [12] performed a review of the literature for relevant articles of parvovirus B19 related anemia in solid organ transplant recipients, published between 1974 and 1999. Among the 14 cases reported, in which both serological and molecular tests were applied, all of them had positive PCR for B19. How-ever, the IgM test was negative in two cases; the IgG test was negative in one and both IgM and IgG were negative in two cases. In the remaining nine cases, both serological and molecular tests were positive. Cavallo *et al.,* [13] described 48 renal transplant recipients with anemia. Eleven patients (23%) were positive for B19 DNA. How-ever, ten were seropositive and one seronegative for the virus.

The case reported herein presented no evidence of hemolysis, blood loss or nutritional deficiency. The immunosuppression consisted of prednisone, azathioprine and tacrolimus. Persistent anemia and reticulocytopenia were observed and erythroid aplasia was established, even with erythropoietin use. Bone marrow biopsy revealed giant proerythroblasts and intranuclear inclusions, suggesting a chronic B19 infection, which may have been reactivated because of the immunosuppression. Furthermore, positive serological tests, i.e. anti-Epstein-Barr and anti-Cytomegalovirus, which are commonly seen in B19 disease, were also positive in this woman.

Organ transplant recipients are in risk of symptomatic B19 virus infections. However, the diagnosis may be complicated by low titer viremia and the absence of detectable humoral and/or cellular immune response, due to immunosuppressive therapy [13,15]. In the present case, despite the evidence of parvovirus B19 infection, the specific IgM and IgG were negative, requiring a qualitative B19 PCR in serum, which is more sensitive, to confirm the diagnostic hypothesis. These serological findings suggest that B19 infection has been acquired from the donor of the transplanted kidney and that immunosuppression did not allow the development of the patient's antibody response. Nevertheless, the hypothesis of reactivation of B19 infection cannot be ruled out, since the donor or the patient's infection status, previously to the transplant, is unknown.

Transplanted patients are immunossupressed to avoid host to graft reactions. However, the optimal treatment for B19 infection requires reduction of intravenous immunosuppressive drugs and administration of gammaglobulin, since immunosuppression may alter host defense mechanisms, leading to reactivation of the B19 virus or impaired immune responses, when infection is transmitted through the organ grafting. Although the B19-associated anemia can improve spontaneously, intravenous gammaglobulin is usually necessary in the majority of patients. However, it is still unknown whether the virus is completely eliminated after this treatment [12,20].

Conclusion

Parvovirus B19 infection should be considered for the differential diagnosis of persistent anemia non responsive to erythropoietin, aplastic crisis and other opportunist infections in transplanted patients. The true incidence of this infection may be underestimated, because B19 serology may not be routinely searched in transplanted patients. Since serological tests may fail to detect B19 infection in immunosuppressed patients, addition of a polymerase chain reaction assay to detect B19 DNA should be considered to improve sensitivity and to guide adequate treatment.

Consent

Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MTA reviewed the patient's medical record, analyzed data from the literature and wrote the article. SSV followed up the patient during the hospitalization and reviewed the manuscript. MGC, APF and LMSD analyzed data from the

literature and reviewed the manuscript. KBG was the major contributor in writing the manuscript. All authors read and approved the final manuscript.

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CE BROKER #: 50-2256

Florida Laboratory Combo-23 PART II: SECTION 5

RISKS, COMPLICATIONS, AND REACTIONS ASSOCIATED WITH TRANSFUSIONS

CONTACT HOURS: 2
COURSE LEVEL: Basic
CE BROKER: Automatically Reported

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COURSE OBJECTIVES

At the end of this course you will be able to:

1.) Discuss the current trends in blood safety.
2.) Describe the causes & clinical presentation of Allergic reactions triggered by transfusing a blood product.
3.) Describe the causes and clinical presentations of the different types of hemolytic transfusion reactions.
4.) Identify the cause of a Hypotensive transfusion reaction.
5.) List the various complications associated with a Massive Transfusion.
6.) Describe the causes and clinical presentation of Post Transfusion Purpura.
7.) Describe the causes and list the clinical differences between TACO, TAD, TAGvHD, TRALI, and TRIM.
8.) Identify the various causes of a Transfusion Transmitted Infection (TTI).

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BLOOD SAFETY

According to a report from the FDA, the blood supply is safer today than at any time in history. Due to advances in donor screening, improved testing, automated data systems, and changes in transfusion medicine practices, the risks associated with blood transfusion continue to decrease.

Overall, the number of transfusion related fatalities reported to the FDA remains small in comparison to the total number of transfusions administered. In 2021, for example, there were approximately 15.1 million blood components transfused and during that same period, there were 29 reported transfusion related and potentially related fatalities, with 44 reported in 2019, and 31 in 2018.

In addition to fatalities as the end result, there are many other types of transfusion reactions that can occur to the patient during and after administration. It's important for blood bank and laboratory personnel to familiarize themselves with each possible reaction that can occur to patients receiving blood or blood products, since the post-transfusion lab workup will be dependent on the type of reaction that has occurred. In addition, front-line medical personnel may seek guidance from the lab or the blood bank regarding follow-up procedures once a reaction has occurred.

CLASSIFYING THE SEVERITY OF THE TRANSFUSION REACTION

Medically speaking, transfusion reactions are typically classified by their severity and fall under one of 5 categories including:

Not-determined: The severity or definitive cause of the reaction is unknown or not stated.
Non-severe: Medical intervention given, but lack of treatment would not result in permanent injury or impairment.
Severe: Hospitalization or prolonged hospitalization required & is directly attributed to the transfusion. Intervention is necessary to prevent permanent damage or impairment.
Life-threatening: Major intervention required to prevent death, including, vasopressors, intubation, transfer to ICU, etc.
Death: The recipient died as a result of the adverse transfusion reaction.

ALLERGIC REACTION

An allergic reaction can result when there's an interaction between an allergen in the transfused blood with an existing antibody(s) in the patient's blood. In some instances, infusion of antibodies from the donor may be involved. The reaction may present as mild or life-threatening.

MILD ALLERGIC REACTION

Mild allergic reactions can occur at any time during or shortly after the transfusion. On average mild allergic reactions occur at a rate of about 1 in 100 transfusions.

CAUSES

The exact cause is unclear; however, it's suggested that the reaction is precipitated by factors present in the plasma portion of the blood.

CLINICAL PRESENTATION

Minor allergic reactions usually resolve themselves after treatment or slowing down the transfusion rate. Reactions are generally mild and include hives (urticaria) which can appear as minimal or widespread and may be accompanied by itching.

MANAGEMENT

Mild allergic reactions can be managed by stopping the transfusion, then administering 25-50mg of Benadryl by mouth or IV. After a few minutes the transfusion can be restarted as long as the hives involve <2/3 of the body surface and there are no symptoms suggesting a more severe allergic reaction.

If multiple or future transfusions need to be administered, the patient can be pre-medicated with Benadryl or corticosteroids in an effort to minimize the reaction.

Plasma depleted RBCs and platelets or washed RBCs and platelets may also be ordered in an effort to minimize the patient's reaction to the antigen present.

ANAPHYLACTIC REACTION

Anaphylaxis is a severe, life-threatening reaction to a blood transfusion. Transfusion induced anaphylaxis is rare and accounts for approximately 3% of transfusion associated fatalities.

CAUSES

Although the vast majority of anaphylactic reactions are unexplained, other causes are varied and can include:

- Passive transfer of IgE (food, drugs, etc.), which triggers a release of mast cells causing major inflammation and anaphylaxis to occur
- Transfusing an allergen, such as penicillin or aspirin, (which was taken by the blood donor) to a patient who has an allergy to those substances
- Antibodies to certain serum proteins, such as, IgG, albumin, haptoglobin, □-1-antitrypsin, transferrin, C3, C4, etc.
- Transfusing anti-IgA to an IgA deficient patient

CLINICAL PRESENTATION

Anaphylactic reactions can occur quickly and can occur within 1 to 45 minutes after the start of the infusion. Although it may start with hives just like a mild reaction, it quickly intensifies and can include symptoms, such as:

- Hypotension (low blood pressure)
- Shortness of breath
- Hoarseness
- Wheezing
- Edema
- Swelling of lips, tongue, throat
- Swelling around the eyes
- Maculopapular rash
- Chest pain
- Anxiety
- Nausea & Vomiting
- Death may occur if severe

Definitive

To classify the reaction as 'definitive' for anaphylaxis, two or more of the symptoms above must occur during or within 4 hours of the transfusion.

Probable

To classify the reaction as 'probable' for anaphylaxis, any 1 of the following must occur during or within 4 hours of the transfusion:

- Edema of the conjunctiva
- Swelling of the lips, tongue, throat
- Hives and itching
- Swelling around the eyes
- Maculopapular rash

SEVERITY

Anaphylaxis can be classified as a severe or life-threatening reaction, but can be fatal even with supportive treatment.

TREATMENT

The transfusion should be stopped at the first signs of anaphylaxis! Benadryl should be administered to the patient if hives are present. If the disease progresses to additional symptoms, epinephrine, corticosteroids, vasopressors, and other supportive care should be administered quickly. Manual respiratory ventilation may also be required if the patient goes into respiratory failure.

ACUTE HEMOLYTIC TRANSFUSION REACTION (AHTR)

An acute hemolytic transfusion reaction (AHTR) is the rapid destruction of red blood cells that occurs during, immediately after, or within 24 hours of a transfusion when a patient is given an incompatible blood type. The recipient's body immediately begins to destroy the donated red blood cells resulting in fever, pain, and sometimes severe complications such as kidney failure.

Hemolytic transfusion reactions are almost always preventable and are caused by a breakdown in safety procedures and checklists before and during the transfusion.

CAUSES:

Acute hemolytic transfusion reactions can be associated with ABO incompatibility, other blood group alloantibodies, or rarely when group O platelets with high titers of anti-A/anti-B and are transfused in a non-group O patient. It should be noted that in addition to ABO, there are 29 blood groups systems that may cause incompatibility.

ABO-incompatibility:

- ABO-incompatibility due to clerical error or error in patient identification
  - This is the most common cause of morbidity from transfusion
  - Half of all errors are due to administering properly labelled blood to the wrong patient.
- Errors as a result of lab testing or improper labelling

RBC Alloantibodies (Non-ABO related)

- Patient previously immunized through prior transfusion or pregnancy
- Causes of reactions include:
  - Clerical error during antibody screening
  - Transfusing uncrossmatched blood to patient who is alloimmunized
  - Patient's RBC alloantibodies below detectable levels
  - Laboratory fails to identify detectable levels resulting in a lab error

Table 2: Antibodies Associated with AHTR, DHTR, DSTR

Anti-A	Anti-B	Anti-A,B	Anti-C	Anti-c	Anti-D	Anti-E	Anti-e	Anti-Fy^a
Anti-Fy^b	Anti-Jk^a	Anti-Jk^b	Anti-K	Anti-k	Anti-M	Anti-S	Other	

CLINICAL PRESENTATION

When a patient presents within 24 hours of transfusion with signs and symptoms compatible with an AHTR a medical investigation should take place. A reaction is classified as definitive, probable, or possible depending on the signs and symptoms that the patient presents with.

DEFINITIVE REACTION

In order to classify it as a 'definitive' AHTR reaction, the patient would present with ANY of these signs or symptoms:

- Back or flank pain
- Chills or rigors
- Nosebleed
- Fever
- Disseminated Intravascular Coagulation (DIC)
- Hematuria with visual hemolysis
- Anuria or oliguria
- Hypotension
- Pain and/or oozing at the IV site
- Renal failure

In addition, the patient must have 2 or more of the following:

- Decreased haptoglobin
- Decreased fibrinogen
- Elevated hemoglobin
- Hemoglobinuria
- Elevated bilirubin
- Elevated LDH
- Spherocytes on peripheral smear
- Red plasma discoloration

Plus, either:

Immune Mediated -

- Positive DAT for anti-IgG or anti-C3
- **AND** a positive elution test with alloantibody present on the transfused RBCs

OR

Non-Immune Mediated -

- Serologic testing is negative and physical cause, such as, chemical, osmotic, thermal, etc is confirmed.

PROBABLE REACTION

A 'probable' reaction meets the signs and symptoms for acute hemolysis **and either**:

Immune Mediated -

- Physical cause is excluded, but serologic evidence in not sufficient

OR

Non-Immune Mediated -

- Physical cause is suspected, and serologic testing is negative.

POSSIBLE CAUSE

AHTR is suspected, but symptoms, test results, or information is not sufficient to meet the criteria defined above.

SEVERITY

The severity of the AHTR can be classified by any one of the five severity levels, depending on the symptoms and stability of the patient.

MANAGEMENT

Management should always follow the guidelines of your facility; however, they usually include:

- Stopping transfusion immediately
- Check for clerical or identification errors
- Notify the blood bank & return blood bag and tubing for follow-up testing
- Order urine & appropriate blood specimens for lab testing.
- Administer appropriate patient care

DELAYED HEMOLYTIC TRANSFUSION REACTION

A delayed hemolytic transfusion reaction (DHTR) occurs when the recipient develops antibodies to red blood cell antigen(s) between 24 hours and 28 days after a transfusion. Symptoms are usually milder than in acute hemolytic transfusion reactions and may even be absent. DHTR is diagnosed with appropriate laboratory testing.

CAUSES

Several causes of delayed hemolytic transfusion reaction exist, including:

- Transfusion-transmitted Babesia or malaria
- Common antigens, including, E, Jk^a, c, Fy^a, K[37]
- See Table 2 for complete list of causative antibodies*
- Formation of antibodies in the recipient to transfused RBC alloantigens in the unit. This usually happens when the patient's antibodies are below the level of detection on the antibody screen.

CLINICAL PRESENTATION

A patient should be considered for a DHTR when they present between 25 hours and 28 days post-transfusion with signs of hemolytic anemia, including:

- Elevated bilirubin
- Low hemoglobin
- Elevated LDH
- Spherocytes on peripheral smear
- Increased reticulocytes
- Positive antibody screen
- Positive direct anti-globulin test

NOTE: Patients may be asymptomatic in some cases, or present with symptoms similar to an AHTR; symptoms do not have to meet the case definitions below to be considered.

As with acute transfusion reactions, DHTR is classified as definitive, probable, or possible.

DEFINITIVE

A '**definitive**' DHTR reaction has occurred when the patient develops a positive direct anti-globulin test (DAT) within the appropriate timeframe.

AND EITHER

- Positive elution test with alloantibody present on the transfused cells **OR**
- Newly identified RBC alloantibody in the patient's serum

AND EITHER

- Inadequate rise of post-transfusion hemoglobin or a rapid fall of hemoglobin back to pre-transfusion levels **OR**
- Unexplained spherocytes on peripheral smear.

PROBABLE

Newly identified RBC alloantibody that occurs during the appropriate timeframe for a DHTR, but with incomplete laboratory evidence to meet the definitive criteria above.

POSSIBLE

DHTR is suspected, but symptoms, test results, or information is not sufficient to meet the criteria defined above.

SEVERITY

As with AHTR, the severity of the reaction falls under one of the five previously listed categories: not-determined, non-severe, severe, life-threatening, and death.

MANAGEMENT

Management includes treating the patient according to their presenting symptoms. Future blood transfusions should be compatible with any antibody that the patient carries, even in very low quantities.

HEMOLYSIS RELATED TO RBC ALLOANTIBODIES

Hemolysis may also occur for other reasons and should be considered when determining AHTR and DHTR. Most of the reactions are benign, however, life-threatening hemolysis and renal failure may rarely occur. Causes may include:

- Transfusion of outdated RBCs
- Overheating or freezing of RBCs by improper transport or storage
- Medical device malfunction – cell saver, blood warmer, etc.
- Use of hypotonic IV solutions during blood administration
- Rapidly transfusing RBCs by use of pressure through a small-bore needle

DELAYED HEMOLYTIC TRANSFUSION REACTION

A delayed serologic transfusion reaction (DSTR) occurs when a recipient develops new antibodies against red blood cells between 25 hours and 28 days after a transfusion without clinical symptoms or laboratory evidence of hemolysis. Clinical symptoms rarely occur with DSTR. See Table 2 for list of the most common antibodies that cause reactions.

FEBRILE NON-HEMOLYTIC TRANSSFUSION REACTION

Febrile non-hemolytic transfusion reactions are the most common reaction reported after a transfusion. FNHTR is characterized by fever and/or chills in the absence of RBC hemolysis which occurs in the patient during or up to 4 hours after a transfusion. These reactions are generally mild and respond quickly to treatment. Fever can be a symptom of a more severe reaction with more serious causes, however, and should be fully investigated. The incidence of this type of reaction is typically 1 in 300 for RBCs and 1 in 20 for platelets transfused.

NOTE: Pathogen contamination should be ruled out, especially bacterial contamination.

CAUSES

- Antibodies in the patient that are reacting to antigens in the donor blood, usually on the white blood cells
- Soluble factors, such as cytokines, in the plasma of the blood component being transfused
- Bacterial contamination

CLINICAL PRESENTATION

Occurs during or up to 4 hours after transfusion is complete, with either a fever of ≥38°C/100.4°F and a change of at least 1°C/1.8°F from the pre-transfusion value OR chills/rigors are present in the patient.

MANAGEMENT

This type of reaction is easily managed with acetaminophen. If the patient develops severe rigor (shivering or shaking) during transfusion, IV Demerol may be given.

Pre-transfusion medication, including acetaminophen and Benadryl, is typically not helpful. There are mixed reactions on pre-medicating with corticosteroids, plasma-depleted units, washed RBCs, or using fresh blood.

HYPOTENSIVE TRANSFUSION REACTION

A hypotensive transfusion reaction is a drop in systolic blood pressure which occurs soon after a transfusion begins. Symptoms should respond quickly to stopping the transfusion and administering supportive treatment.

Hypotension can also be a symptom of a more severe anaphylactic reaction and should be fully investigated.

CAUSES

Post-transfusion hypotension can be initiated by:

- Bradykinin - believed to play a major role.
  - Bradykinin, a peptide, is an inflammatory mediator that causes blood vessels to dilate.
- Angiotensin-converting enzyme (ACE) is the main enzyme responsible for degrading bradykinin and some patients have a genetic abnormality which results in a decrease of bradykinin degradation.

CLINICAL PRESENTATION

The majority of hypotensive episodes occur with administration of platelet transfusions; the majority of those patients were on ACE inhibitors. In addition to hypotension the patient may also experience additional symptoms including nausea, vomiting, and shortness of breath. This type of reaction is rarely fatal.

DEFINITIVE DIAGNOSIS

Once all other adverse reactions with hypotension are ruled out, a 'definitive' diagnosis can be made when:

Adults ≥18 yrs	Infants, Children Adolescents	Neonates
Drop in systolic blood pressure (BP) of ≥30mmHg and a systolic BP of ≤80mmHg	>25% drop in systolic BP from baseline	>25% drop in baseline value

TREATMENT

Early detection is important, so when blood is administered the patient should be monitored with vital signs for the first 15 minutes and then a minimum of every 15 minutes after. If hypotension occurs, the transfusion should be stopped and not restarted. Supportive treatment should be provided to the patient as needed.

Other diagnoses should be considered, such as, acute hemolytic transfusion reaction, sepsis, TRALI, and allergic reactions.

COMPLICATIONS OF MASSIVE TRANSFUSION

There may be medical circumstances, such as trauma or uncontrolled hemorrhage, where the patient must receive massive amounts of blood. A massive transfusion is defined as either transfusing more than the patient's entire blood volume in a 24 hour period OR more than 10 units of RBCs. Although necessary and life-saving in some instances, massive transfusion carries its own set of risks for the patient and is considered an independent risk factor for multi-organ failure.

The occurrence and severity of complications are typically dependent on how fast units are infused, the number of units transfused, the patient's health status, as well as their previous transfusion history. Some of the complications associated with massive transfusion are:

HYPOTHERMIA

Hypothermia from rapid infusion of cold blood can cause a cardiac arrhythmia to occur, therefore, it's critical for a blood warmer to be used during administration. The risk of clinically important hypothermia significantly increases after 5 or more units are transfused.

MORTALITY

Mortality is inversely related to core body temperature:

Celsius Temperature	Fahrenheit Temperature	Mortality
<34°C	~ 93.2°F	40%
<33°C	~ 91.4°F	69%
<32°C	~ 89.6°F	100%

CONSEQUENCES

Hypothermia has additional consequences on the body including:

- Hypotension
- Reduced clearance of citrate
- Platelet dysfunction
- Cardiac arrhythmias
- Decreased cardiac output
- Decreased coagulation factor activity

Hypothermia is a major risk factor in trauma death due to the fact that it contributes to halting the normal coagulation cascade, preventing blood from clotting normally.

HYPOCALCEMIA/HYPOMAGNESEMIA/CITRATE TOXICITY

Although citrate is routinely metabolized by the liver, the citrate anticoagulant used in blood components can build up in the patient's blood over time during a massive transfusion, since the liver's ability to function normally may be compromised.

In the body, citrate binds ionic calcium and magnesium, which can cause hypocalcemia, hypomagnesemia, and metabolic alkalosis, which occurs from increased bicarbonate (a metabolite of citrate). Clinical symptoms may include hypotension, tetany, elevated pulmonary artery pressure, paresthesia, and cardiac arrhythmias.

When hypocalcemia develops, IV calcium chloride is administered. In theory a patient exhibiting a normal body temperature that is not in shock can tolerate up to 20 units before calcium supplementation is necessary. Careful monitoring of the calcium and magnesium levels should be performed.

HYPERKALEMIA

Transfusion associated hyperkalemia is a concern with massive transfusions, since potassium is released over time from the RBCs into the plasma of a donor unit. The older the unit, the higher the potassium concentration in many instances. This may become a critical issue if a patient is receiving multiple units of ageing donor blood.

Whenever possible, fresh blood <7 days old should be used during massive transfusions. In addition, lab testing should be ordered 1 hour after the unit's infused and routinely thereafter for ongoing transfusions to check for hyperkalemia.

DILUTIONAL COAGULOPATHY

Massively transfused patients can present with dilutional coagulation abnormalities from the disproportionate number of RBCs transfused. The number of RBC units administered does not accurately predict the need for FFP and platelet transfusion, so frequent lab testing is needed to properly monitor each patient. As a general rule of thumb with massive transfusions:

- 50% of the patients will develop an INR >2.0
- 33% will develop thrombocytopenia with a platelet count of <50,000

METABOLIC ACIDOSIS

This is a rare complication associated with the acid pH of the blood products transfused but may also be an indicator of lactic acidosis in patients with tissue hypoperfusion. Typically, metabolic alkalosis is seen due to bicarbonate production from citrate metabolism in the donor units. Generally, lab values may appear as follows:

Condition	Blood Gas	Anion Gap	K+	CL-	CO2
Metabolic Acidosis	pH <7.35	Normal or ↑	↑	↑	↓
Metabolic Alkalosis	pH >7.45	--	↓	↓	↑

NOTE: Values may change for electrolytes as noted above, but may not necessarily fall into an abnormal range

LABORATORY TESTING

Since transfusing massive quantities of blood components can alter a patient's baseline lab values quickly, routine testing should be performed and at a minimum should include:

- CBC with manual differential
- PT with INR
- PTT
- Fibrinogen
- Magnesium
- Chemistry panel, including calcium
- Arterial blood gas

Depending on the patient's symptoms and diagnosis, additional testing may be required.

POST TRANSFUSION PURPURA (PTP)

Post-transfusion purpura (PTP) is a rare but potentially fatal condition that occurs when a transfusion recipient develops antibodies against platelets, resulting in rapid destruction of both the transfused platelets and the patient's own platelets resulting in a severe decline in the platelet count. PTP usually occurs 5-12 days after a transfusion and is more common in women than in men. Without treatment, thrombocytopenia usually lasts for approximately 2 weeks.

CAUSES

PTP can occur when transfusion of platelet antigen-positive RBCs, platelets, or plasma is administered to a patient who is lacking that same platelet antigen. Seventy five percent of the cases occur in a Human Platelet Antigen-1b (HPA-1b) homozygous patient who is transfused with HPA-1a positive blood products. Statistically, 3% of the North American population are homozygous HPA-1b, but only 28% appear to be able to form anti-HPA-1a.

CLINICAL PRESENTATION

PTP is 5 times more likely to occur in a female, since many women have been previously sensitized through pregnancy.

DEFINITIVE

A 'definitive' reaction for PTP occurs when alloantibodies against HPA or other specific platelet antigens are detected in the patient after thrombocytopenia is detected and the thrombocytopenia decreases to less than 20% of the pre-transfusion count.

PROBABLE

A 'probable' reaction for PTP is similar to a 'definitive' reaction with the exception that the platelet count only decreases to levels between 20% and 80% of the pre-transfusion count.

POSSIBLE

In a 'possible' reaction for PTP is suspected but laboratory findings are not sufficient to meet the criteria.

SEVERITY

PTP can range in severity from non-severe to death depending on the patient's presentation. Mortality is approximately 8% and usually occurs from an intracranial bleed.

TREATMENT

Treatment therapy is IVIG for 2 days. The platelet count should increase 4 days after the start of therapy.

For prevention of future reactions, patients should be tested for platelet-specific antibodies and can receive antigen-negative platelet and RBC transfusions. Washed RBCs do not appear able to prevent PTP from occurring.

TRANSFUSION ASSOCIATED CIRCULATORY OVERLOAD (TACO)

Transfusion-associated circulatory overload (TACO) occurs when the volume of blood or blood components transfused cannot be effectively processed quickly enough by the recipient. It's currently estimated that 1 in 700 transfusions results in TACO; with an incidence much higher in perioperative orthopedic settings among elderly patients, with an incidence estimated at 1 in 100 transfusions.

CAUSES

TACO can occur due to an excessively high infusion rate and/or volume, or due to an underlying heart or kidney condition.

CLINICAL PRESENTATION

Symptoms may include difficulty breathing, cough, cyanosis, tachycardia, hypertension, and fluid in the lungs. Patients will also exhibit elevated central venous pressure (CVP), evidence of left side heart failure, X-ray confirmed pulmonary edema, and an elevated brain natriuretic peptide (BNP) blood test.

TACO can occur at any age, although patients over 70 years of age are particularly vulnerable.

DEFINITIVE

A 'definitive' diagnosis for TACO can be made when 3 or more of the signs and symptoms above are seen within 6 hours of transfusion.

SEVERITY

TACO can range in severity from non-severe to fatal when the fluid overload is so great it shuts down the kidneys and heart or creates a severe electrolyte imbalance.

MANAGEMENT

TACO is managed by stopping the transfusion while oxygen and diuretics are administered to the patient. The transfusion may be restarted at a much slower rate if the patient is stable enough to continue.

TRANSFUSION ASSOCIATED DYSPNEA (TAD)

Transfusion associated dyspnea (TAD) is the onset of respiratory distress within 24 hours of transfusion that cannot be defined as TACO, TRALI, or an allergic reaction.

When this occurs, the transfusion should be stopped, and the patient's vital signs should be recorded. After confirming the patient's identification, the blood unit, along with the tubing should be returned to the blood bank for further testing.

TRANSFUSION ASSOCIATED GRAFT VS. HOST DISEASE

Transfusion-associated graft vs. host disease (TA-GvHD) is a rare complication of a transfusion that occurs in immunocompromised or immunocompetent patients.

CAUSES

TA-GvHD occurs when donor T-lymphocytes (the "graft") introduced by the blood transfusion rapidly increase in number in the recipient (the "host") and then attack the recipient's own cells.

CLINICAL PRESENTATION

Symptoms include fever, a characteristic rash, enlargement of the liver with liver dysfunction, and diarrhea that occurs between 2 days and 6 weeks post transfusion. Though very rare, this inflammatory response is difficult to treat and often results in death.

Pancytopenia usually occurs as the reaction progresses, exposing the patient to overwhelming infections often resulting in a mortality of >90%.

DEFINITIVE

A 'definitive' diagnosis of TA-GvHD occurs when the above symptoms are present along with the characteristic appearance on a skin or liver biopsy.

PROBABLE

A 'probable' diagnosis for TA-GvHD occurs when all of the above criteria are met, but a biopsy is negative or is not performed.

MANAGEMENT

Treatment is largely ineffective and relies on immunosuppressive therapy.
It's critical for at risk patients who need a blood transfusion to receive irradiated products. Patients should also carry a card identifying them as someone who needs these special requirements.

TRANSFUSION RELATED ACUTE LUNG INJURY (TRALI)

Transfusion-related acute lung injury (TRALI) is a serious but rare reaction that occurs when fluid builds up in the lungs but is not related to excessive volume of blood products transfused. TRALI typically occurs with plasma containing products, such as FFP or Platelet concentrate.

CAUSES

The mechanism of TRALI is not well understood but is thought to be associated with the presence of antibodies in donor blood. Antibodies directed toward Human Leukocyte Antigens (HLA) or Human Neutrophil Antigens (HNA) have been implicated. Women who have had more than one child can develop these antibodies through exposure to fetal blood and transfusion of blood components obtained from these donors is thought to carry a higher risk of inducing immune-mediated TRALI. Previous transfusion or transplantation can also lead to donor sensitization. To be at risk of TRALI via this mechanism, the blood recipient must express the specific HLA or neutrophil receptors to which the implicated donor has formed antibodies.

CLINICAL PRESENTATION

Symptoms include acute respiratory distress with no other explanation for lung injury such as pneumonia, trauma, or aspiration. TRALI occurs during or within 6 hours of transfusion, although the majority of the cases present within 1-2 hours of the start of the transfusion. In addition to the lung symptoms, an acute, transient leukopenia may also occur. **Figure 1** below shows a lung biopsy from a patient with TRALI showing microscopic damage, while **Figure 2** shown the change in lung status.

Seventy two percent of TRALI cases require mechanical ventilation, with death occurring in 5-10% of those patients. For patients that do survive, TRALI usually resolves itself within 24-72 hours. TRALI is a leading cause of transfusion-related death as reported to the FDA.

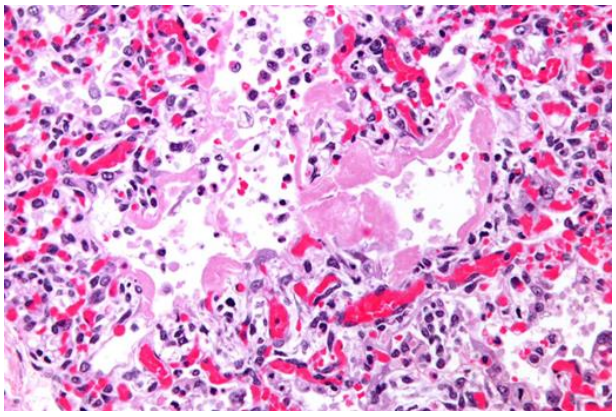


FIGURE 1.

H&E-stained lung biopsy showing diffuse alveolar damage from TRALI.

DEFINITIVE REACTION

A '**definitive**' diagnosis of TRALI is fairly straightforward and includes all of the following:

- No previous evidence of acute lung injury (ALI) prior to transfusion
- ALI evidence develops during or within 6 hours of transfusion
- Hypoxemia with an oxygen saturation of <90% on room air
- X-ray evidence of lung infiltrates – see Figure 2 below
- No evidence of circulatory overload as in TACO

TRALI, especially mild cases, may present with similar symptoms to TACO.

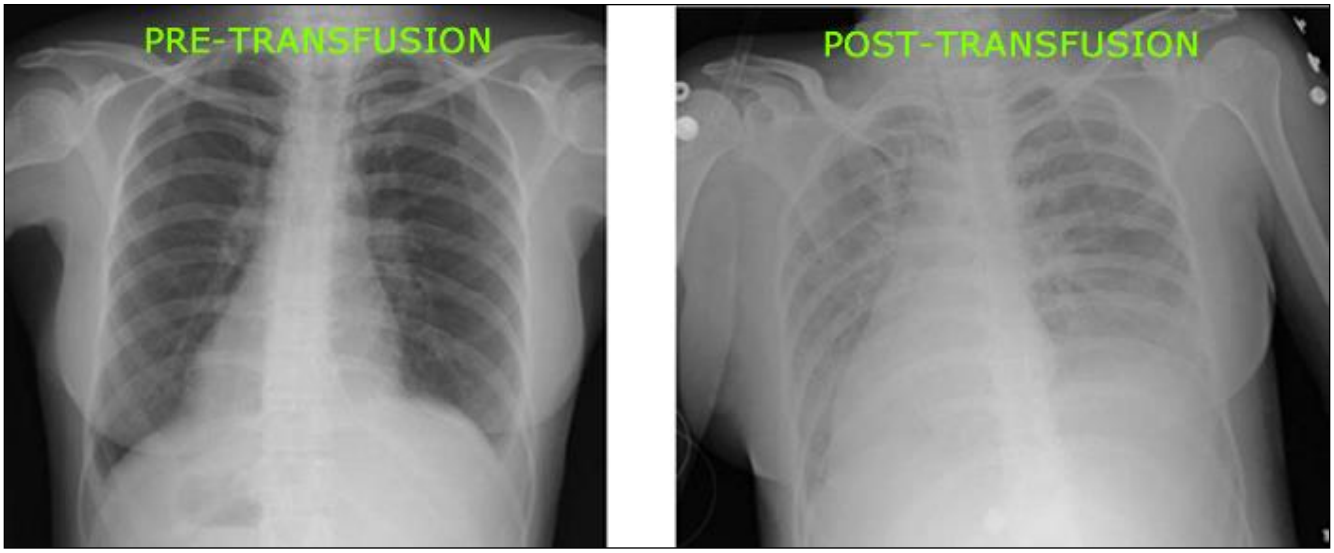
SEVERITY

Although the severity of TRALI can range from non-severe to fatal, it is most always a severe, life-threatening complication when it occurs.

MANAGEMENT

Patient management should include symptom treatment, including mechanical ventilation when necessary.

Figure 2. X-ray of patient with TRALI



Source: Wikipedia

TRANSFUSION RELATED IMMUNOMODULATION (TRIM)

Transfusion related immunomodulation (TRIM) refers to the transient depression of the immune system following transfusion of blood products. It is well established that allogenic transfusion suppresses the immune system of the patient and can increase the risk of infection or recurrence of cancer, albeit slightly in some patients.

It has been recognized as more common in patients who have had multiple miscarriages and those who have undergone a kidney transplant.

TRANSFUSION TRANSMITTED INFECTION (TTI)

PLEASE NOTE: This course only touches on brief highlights of TTI, since it is a rather detailed topic in itself.

A transfusion-transmitted infection (TTI) occurs when an infection from bacteria, parasites, viruses, or other potential pathogens are transmitted to the patient through a contaminated blood product.

CAUSES

- Skin contaminants from the donor may enter the blood bag if the skin is not sterilized properly
- Unrecognized, low-level infection in the donor
    - Antigen or antibody levels may have been too low to detect, especially for viruses, if the patient was still in the "window period" when they donated.
- Contamination during handling of the blood product or transfusion preparation

PREVENTION

- Careful patient donor preparation of the puncture site should be done
- Detailed donor history, especially travel history, should be taken
- Depending on the product, refrigerate or freeze the blood products immediately (except platelets)
- Initially sequester a small amount of blood (~40ml) during collection to lessen the risk of skin contamination
- Leukoreduction techniques may help prevent or reduce some intracellular infections, such as CMV

CLINICAL PRESENTATION

Clinical presentation will depend on the causative organism, but may include:

- Bacterial Sepsis Symptoms – chills, fever, hypotension, nausea, rapid heartbeat, difficulty breathing, DIC, etc.
    - Symptoms may come on rapidly, or if the bacterial load is low, may take hours to days to escalate.

- Viral symptoms – viral infections may not show symptoms for days to weeks after transfusion and symptoms will vary according to the causative pathogen.
- Tick-borne Infections – Each has their own presentation of symptoms, but usually takes days to weeks for symptoms to show up.

MANAGEMENT

Management will depend on the organism causing the infection.

INFECTION TYPES

BACTERIA

- Bacterial infections are the most common type of TTI and accounts for up to 10% of all transfusion-associated fatalities.
- Although any blood product can be contaminated, platelets are the most likely cause since they are incubated at room temperature where bacteria can multiply easily.
    - 1 in 2,000-3,000 platelet transfusions results in infection
- More serious morbidity and mortality tends to occur with gram-negative infections

COMMON CONTAMINANTS

Gram-Positive Organisms	Gram-Negative Organisms
▪ Staph aureus ▪ Staph epidermidis ▪ Streptococcus sp. ▪ Bacillus sp.	▪ E. coli ▪ Klebsiella pneumonia ▪ Pseudomonas sp ▪ Serratia marcescens ▪ Yersinia enterocolitica

VIRUSES

Although the blood supply is tested for the most common viruses, such as hepatitis, it's still possible to transmit them in a blood product transfusion, especially during the 'window period'.

- Cytomegalovirus (CMV)
- Enterovirus spp.
- Epstein Barr (EBV)
- Hepatitis A
- Hepatitis B
- Hepatitis C
- Dengue fever
- Human Immunodeficiency Virus 1 (HIV-1)
- Human Immunodeficiency Virus 2 (HIV-2)
- Human Parvovirus B-19
- Human T-Cell Lymphotropic Virus-1 (HTLV-1)
- Human T-Cell Lymphotropic Virus-2 (HTLV-2)
- West Nile Virus (WNV)

RISKS

Donating blood during the "window period" of a viral infection runs the risk that the infection may not be detected on the blood screening tests. The outcomes of transfusion-related viral transmission can vary as follows:

VIRUS	OUTCOME
HIV	Chronic infection with progressive loss of CD4+ lymphocytes leading to opportunistic infections, immune system dysfunction, and direct viral effect on multiple organ systems
HCV	80% of recipients develop chronic HCV. 30% develop severe progressive hepatitis with long-term consequences of cirrhosis and risk of hepatocellular carcinoma
HBV	The vast majority of cases resolve by developing immunity. In <5% of cases, chronic infection occurs with the likelihood of chronic liver disease. Rarely, HBV presents with acute fulminant hepatitis.
HTLV	Long-term consequences of transfusion transmitted HTLV remain unclear, but the virus is associated with the development HTLV associated lymphomas and myelopathy in the endemic form.

Source: www.transfusionmedicine.ca

PARASITES

Parasites that can be transmitted via blood include:

- Babesiosis (Babesia spp.)
- Chagas disease (trypanosoma cruzi)
- Malaria (Plasmodium spp.)

OTHER

Other rare types of pathogens may also cause infection, such as, prions, rickettsia, spirochetes, etc. and can include:

- Rocky Mountain Spotted Fever
- Ehrlichiosis (Ehrlischia)
- Treponema pallidum (Syphilis)
- Variant Creutzfeldt-Jakob Disease

APPENDIX 1: TABLE OF POSSIBLE REACTIONS CATEGORIZED BY SYMPTOMS

SYMPTOM	POSSIBLE CAUSE
Fever	• Febrile Non-Hemolytic Transfusion Reaction (FNHTR) • Acute Hemolytic Transfusion Reaction • Bacterial Sepsis or Blood Product Contamination
Dyspnea (difficulty breathing)	• Transfusion Related Acute Lung Injury (TRALI) • Transfusion-associated Circulatory Overload (TACO)
Urticaria (hives) or Other Allergic Reactions or Anaphylaxis	• Anaphylaxis • Minor Allergic Reaction, including Urticaria
Hypotension	• Bradykinin-Mediated Reaction
Hemolysis After Transfusion	• Hemolysis not related to RBC Alloantibodies • Delayed Hemolytic Transfusion Reactions (DHTR)
Cytopenia After Transfusion	• Transfusion-Related Alloimmune Neutropenia • Transfusion-Related Alloimmune Thrombocytopenia • Post-Transfusion Purpura (PTP) • Transfusion-Associated Graft vs Host Disease (TA-GvHD)
Symptoms of Bacterial, Virus, Parasite, and Prion Infections	• Viral Pathogen Cause • Parasitic Pathogen Cause • Prion pathogen Cause • Bacterial Pathogen Cause • Other Infectious Transfusion-Transmitted Agents

Source: Adapted from "Clinical Guide to Transfusion" Chapter 10

CE BROKER #: 50-2256

Florida Laboratory Combo-23 PART II: SECTION 6

MONKEYPOX: A DETAILED REVIEW

CONTACT HOURS: 2.5
COURSE LEVEL: Basic

CE BROKER: Automatically Reported

Provider Reports CE/CME 24 HOURS ce broker

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COURSE OBJECTIVES

At the end of this course you will be able to:

1.) Recall the history of monkeypox outbreaks.

2.) Describe the routes of transmission.

3.) List the signs & symptoms associated with monkeypox including the stages of the rash.

4.) Recall the testing options for diagnosis.

5.) Recall the isolation procedure and steps for infection control.

RIGHTSHOLDER:

Author: Deborah L. Buckley, MBA, MT(ASCP)

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Introduction

Section I

IMPORTANT NOTE: Recently the CDC has begun to refer to Monkeypox as MPox. Since this course was written just prior to the name change, all name references in this course are to Monkeypox.

Introduction

About the Disease

1 - About the Disease

Monkeypox is a rare viral disease caused by infection with the monkeypox virus.

Monkeypox is a double-stranded DNA type of Orthopoxvirus. Orthopoxviruses are a genus of viruses that belong to the Poxviridae family. Vertebrates, including mammals, humans, and arthropods, serve as natural hosts to the virus.

There are 12 viral species within this genus, with 5 associated diseases:

Viral Species

- Abatino macacapox virus
- Akhmeta virus
- Camelpox virus
- Cowpox virus
- Ectromelia virus
- Monkeypox virus
- Raccoonpox virus
- Skunkpox virus
- Taterapox virus
- Vaccinia virus
- Variola virus
- Volepox virus

Diseases

- Smallpox
- Cowpox
- Horsepox
- Camelpox
- Monkeypox

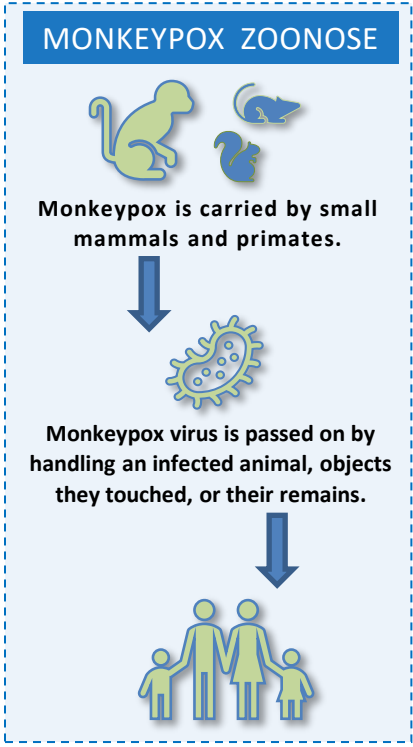
Introduction

Fast Facts

2 - Fast Facts About the Disease

- Monkeypox is considered a Zoonotic disease. Zoonotic diseases or zoonoses are a general term used for a group of infectious diseases that can be transmitted from an animal host to a human.
- Monkeypox is in the same family as smallpox and shares a lot of the same characteristics and symptoms. Compared to smallpox however, monkeypox is clinically less severe.
- Monkeypox is endemic to certain parts of Africa.
- To date, monkeypox has rarely occurred outside of Africa.
- There are two *strains of monkeypox: West African and Congo Basin. The Congo Basin strain causes a more severe illness with a higher death rate of up to 10%, while the death rate for the West African strain sits around 1%.

*Strain can also be referred to as clade or variant.



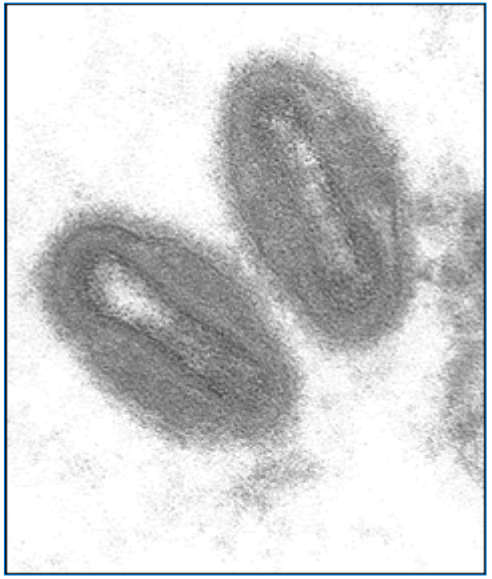
Introduction

Fast Facts

2 - Fast Facts About the Disease

- Monkeypox is most commonly transmitted from small mammals and primates to humans through bushmeat, contact with the animal and their surroundings, or bites and scratches from them.
- Monkeypox can be transmitted from human to human in situations where there's close skin to skin contact, sharing a small space for extended periods of time through respiratory droplets, and sharing or touching contaminated items.
- Monkeypox and Chickenpox are both pox illnesses caused by viruses but are not related. Monkeypox belongs to the Poxviridae family, while Chickenpox is part of the Herpesviridae family. For this reason, the Chickenpox vaccine has no affect on monkeypox.

Monkeypox Under Electron Microscope



SOURCE: Wikipedia

History of Monkeypox

Section II

1 - History of the Disease

Monkeypox was first discovered in 1958 when two outbreaks of a pox-like disease occurred in colonies of monkeys that were being kept for research. The disease was given the name monkeypox since they were the first animal to be diagnosed, although it is believed that monkeys are not the primary source of the virus.

In 1970 during an intense campaign dedicated to eliminating smallpox, the first human case of monkeypox was discovered inside Africa in the Democratic Republic of Congo. Since that date, monkeypox has been reported in humans in other central and western African countries.

Prior to 2022, rare human monkeypox cases had occurred outside of Africa, including cases in the United States, Israel, Singapore, and the United Kingdom. These cases were linked to both international travel and imported animals.

2 - History of the Disease in the U.S.

2003 Outbreak from Imported Mammals

In April 2003, forty-seven probable and confirmed cases of monkeypox were reported in six states including Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin. All people in this outbreak became ill after having contact with pet prairie dogs – there was no human to human spread. This was the first time that human monkeypox was reported outside of Africa.

After an investigation it was determined that the pet prairie dogs, which originated at a small animal vendor in Illinois, became infected after being housed near other small mammals that were imported from Ghana. After testing, it was found that some of those imported animals had active monkeypox infections.

The Centers for Disease Control (CDC) took several measures to control the outbreak, including restrictions on importing African rodents.

2 - History of the Disease in the U.S.

July 2021 Travel-Associated Case

On July 15, 2021, the CDC and the Texas Department of Health Services confirmed a case of human monkeypox in a U.S. citizen who traveled from Nigeria. Through flight records, the CDC, state, and local health officials identified more than 200 people who had possible contact with the patient. Those contacts were asked to monitor their health for 21 days. After 21 days had passed no additional cases were identified.

November 2021 Travel-Associated Case

On November 16, 2021, the CDC and the Maryland Department of Health confirmed a case of human monkeypox in a U.S. citizen who traveled from Nigeria. After careful monitoring of possible contacts for 21 days, no additional cases were identified.

2 - History of the Disease in the U.S.

May 2022 – Origin Unknown

- On May 18, 2022, the CDC and the Massachusetts Department of Public Health confirmed a case of human monkeypox in a U.S. citizen who had recently returned from Canada. At the present time, its unclear how the patient became infected.
- On May 21, 2022, a 2nd positive case was identified in New York City. At the present time, its unclear how the patient became infected.
- On May 22, 2022, a 3rd case was reported in Broward County FL that was related to international travel. **Additional U.S. cases have been reported**

These initial cases are part of a larger outbreak that started at the beginning of May 2022 that is currently affecting multiple nations worldwide. **Scroll ½ way down this page to find the US case map.**

3 - 2022 Worldwide Outbreak

The initial index case diagnosed during the ongoing outbreak was confirmed on May 6, 2022, in a British resident who had recently traveled to Nigeria. While in Nigeria, the patient became ill and developed a rash which appeared on April 29, 2022. The patient did not seek treatment and therefore was not diagnosed while in Nigeria, returning back to London on May 4 while infected with monkeypox. Detailed contact tracing was initiated, including those that were on the plane, and told to isolate for a minimum of 21 days.

Contact tracing was later extended to Scotland where on May 14, 2022, a small number of people were ordered to self-isolate following close contact with the initial patient.

Polymerase chain reaction (PCR) testing of the index patient's sample revealed the infection to be of the West African strain of monkeypox, which is the less deadly of the two known monkeypox variants.

History

Worldwide Outbreak

3 - 2022 Worldwide Outbreak

On May 14, 2022, two additional cases of monkeypox were confirmed in London in two people who lived in the same household. On May 17, four additional patients tested positive - 3 from London and 1 from North East England, who travelled to London. All of these patients have denied any link to the index patient or to international travel, suggesting that wider community transmission was taking place in the London area.

Additional early cases included fourteen confirmed in Portugal on May 18, with an additional six cases suspected. On the same date, cases were confirmed in Spain, Canada, and the U.S. In the days following, additional cases were confirmed in Sweden, Belgium, Italy, the Canary Islands, Austria, Switzerland, and Greece.

*Due to the fast initial spread, it is anticipated that additional cases in additional countries will occur.

History

Worldwide Outbreak

3 - 2022 Worldwide Outbreak

The image to the right shows the initial locations where monkeypox was confirmed or suspected up through May 24, 2022.

It is highly anticipated by both the WHO and the CDC that additional cases are likely to be diagnosed in the coming months and continue to spread to additional countries.

First Countries to Report Cases During the 2022 Outbreak (Date: 052422)

SOURCE: dailymail.co.uk

4 - What's Different About the 2022 Worldwide Outbreak?

This outbreak was the first time that **community transmission** of monkeypox was recorded outside of Africa. As previously mentioned, there were patients in the London area with no link to the initial case or to international travel who tested positive.

Another unique finding is that a **disproportionate number** of men who have sex with men (MSM) have been infected. Prior to the 2022 outbreak, monkeypox was not considered to be sexually transmitted, however, the rapid spread of the virus between sexual partners in the initial stages of this outbreak has prompted a discussion that it should be considered.

After careful contact tracing, it appears that some of the initial cases can be linked back to two locations: An event in the Canary Islands and a bath house in Madrid, Spain. Many of the initial cases who visited these locations sought treatment for what was initially believed to be an STD, prompting a new transmission route.

It should be noted that there have been other routes of transmission during this outbreak outside of MSM. That was notable because it's the first time sexual transmission has been documented.

NOTE: **Due to the ongoing outbreak, this information is subject to change as information gets updated.**

Terminology

Section III

1 - Common Case Terminology

PERSON UNDER INVESTIGATION:

Persons under investigation (PUI) are individuals who are reported as suspicious but have not been tested in a Laboratory Response Network (LRN) laboratory. This includes cases that health departments have been consulted on due to clinician concern.

POSSIBLE CASE:

Meets one of the epidemiologic criteria **AND** has fever or new rash **AND** at least one other sign or symptom with onset 21 days after last exposure meeting epidemiologic criteria.

Source: CDC

Terminology

Common Terms

1 - Common Case Terminology

PROBABLE CASE:

1. Meets one of the epidemiologic criteria **AND** has a new rash with or without fever **AND** at least one other sign or symptom with onset 21 days after last exposure meeting epidemiologic criteria **AND**
2. Demonstration of detectable levels of anti-orthopoxvirus IgM antibody during the period of 4 to 56 days after rash onset

CONFIRMED ORTHOPOXVIRUS CASE:

1. Meets possible case definition **AND**
2. Demonstration of orthopoxvirus DNA by polymerase chain reaction testing of a clinical specimen OR demonstration of presence of orthopoxvirus using immunohistochemical or electron microscopy testing methods.

Source: CDC

Terminology

Common Terms

1 - Common Case Definition Terminology

CONFIRMED MONKEYPOX CASE:

1. Meets possible case definition **AND**
2. Demonstration of presence of monkeypox virus DNA by polymerase chain reaction testing or Next-Generation sequencing of a clinical specimen OR isolation of monkeypox virus in culture from a clinical specimen.

INCUBATION PERIOD:

In medicine the incubation period is the time between the moment of exposure to an infectious agent – in this case monkeypox - until signs and symptoms of that disease begin to appear.

Source: CDC

2 - Common Rash Terminology

Definitions listed in the chronological order which they appear during the illness:

ENANTHEM:

Enanthem or enanthema is a rash or small spots that appear on the mucous membranes in the mouth and is characteristic of patients with viral infections.

MACULE:

Macules are flat, nonpalpable lesions usually < 10 mm in diameter. They present as a change in skin color and are not raised or depressed compared to the skin surface.

PAPULE:

Papules are elevated lesions usually < 10 mm in diameter that can be felt or palpated.

Source: Merck Manual

2 - Common Rash Terminology

Definitions listed in the chronological order which they appear during the illness:

VESICLES:

Vesicles are small, clear, fluid-filled blisters < 10 mm in diameter.

PUSTULES:

Pustules are vesicles that contain pus.

SCABS:

Scabs consist of dried serum, blood, or white blood cells. Crusting can occur when inflammatory or infectious skin diseases start healing.

Source: Merck Manual

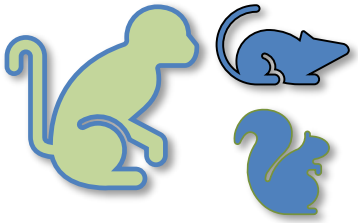
Transmission

Section IV

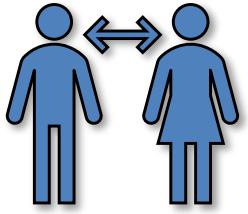
1 - General Transmission

Monkeypox is spread when you come into contact with the virus through infected animals or humans, respiratory droplets, or contaminated objects.

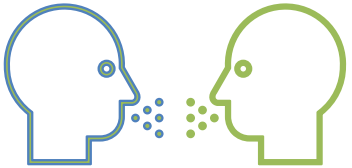
The virus can gain entry into the body through broken skin (even if not visible), the respiratory tract, or the mucous membranes (eyes, nose, mouth, vagina, or rectum).



Infected Animals



Direct Contact



Respiratory Droplets

Contaminated Objects

Transmission

Animal to Human

2 - Animal to Human Transmission

Monkeypox is most commonly transmitted to humans from small mammals and primates.

Animal-to-human transmission occurs mainly through broken skin and direct contact. This can occur from:

- Virus entering through existing skin breaks
- Bites & scratches from the infected animal
- Direct contact with an infected animal's blood, bodily fluids, or pox lesions.
- Touching or kissing the animal
- Touching or cleaning the animal's surroundings, food bowls, bedding, etc.
- Eating wild bushmeat in an endemic area.

NOTE: Inoculation through bites and scratches tend to cause a more severe disease course than through other routes of infection.

Source: CDC

Transmission

Animal to Human

3 - Human to Human Transmission

When monkeypox is transmitted from human to human it has multiple routes of entry, although respiratory droplets are thought to be the most common.

Transmission can occur through skin breaks, respiratory tract, and mucous membranes by:

- Prolonged close contact with an infected person without adequate respiratory protection.
- Direct contact with an infected person's blood, bodily fluids, or pox lesions.
- Sharing, touching, or cleaning an infected patient's surroundings, utensils, bedding, laundry, etc.

NOTE: The 2022 outbreak was the first time sexual transmission was documented.

Source: CDC

Disease Progression

Section V

Disease Progression

Progression Steps

1 - Progression of the Disease:

1. Incubation Period
2. Prodrome Symptoms
3. Monkeypox Rash

1. After exposure to the virus there is an **incubation period** where the patient feels fine and does not display symptoms. A person is not contagious during this stage.
2. After the incubation period passes, the person will slowly start to experience what is known as **prodrome**. Prodrome is a vague set of early symptoms such as fever, fatigue, etc. A person may sometimes be contagious during this period.
3. The appearance of the **monkeypox rash** is the last stage of the disease. In this stage the rash lesions progress through several stages before scabs form. A person is contagious from the onset of the oral enanthem through the scab stage.

NOTE: Once all the scabs fall off and a new layer of skin appears, the patient is no longer contagious.

Signs & Symptoms

Section VI

Signs & Symptoms

Time to Symptoms

1 - Monkeypox Incubation Period

Incubation Period: After viral exposure the incubation period for monkeypox is reported to typically fall between 7 and 14 days, although it can span from 5 to 21 days. For this reason, anyone with possible exposure should isolate for at least 21 days.

Incubation Period Fast Facts

- Incubation period averages 7–14 days but can range from 5–21 days.
- During the incubation period a person **does not have symptoms** and may feel fine.
- A person is not contagious during this period.

Once the incubation period passes, symptoms of the disease will begin to appear.

Source: CDC

Signs & Symptoms

General

2 - General Signs & Symptoms

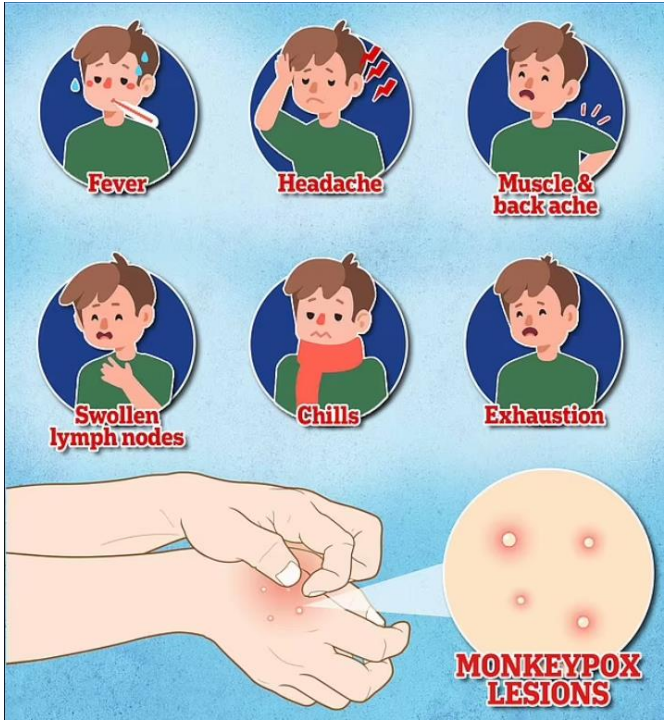
In humans, the signs & symptoms of monkeypox present very similar to smallpox but with a milder clinical course.

Initial symptoms are like most other viral illnesses, however, eventually the telltale monkeypox rash starts to appear.

The disease typically lasts 2 – 4 weeks.

Viral Monkeypox Symptoms

- Fever
- Headache
- Muscle and Backache
- Swollen Lymph Nodes
- Chills and Shivering
- Fatigue
- Rash



SOURCE: dailymail.co.uk

3 - Prodrome

As the patient moves into the prodrome phase, they will start to experience symptoms. Every patient is different in how pronounced the symptoms will be, as some will feel quite ill, while others will barely notice them. A person **may sometimes be contagious** during this period. Its likely that this is the phase many unknowingly pass the disease on to others.

Initial Symptoms

- Fever is the first symptom to appear
  - Measured as ≥100.4° F (>38° C)
  - Can be accompanied by shivering and chills
- Malaise / Fatigue
- Body Aches / Muscle pain
- Headache (not always present)
- Sore throat and cough (occasionally occurs)

Lymphadenopathy (swollen glands)

- Lymph nodes may swell in the neck, armpits, or groin
- Swelling is not always uniform, but typically starts in the neck and works its way down the body
- Occurs 1-2 days before rash appears
- Lymphadenopathy is a distinguishing feature when diagnosing monkeypox, as smallpox patients do not experience lymphadenopathy.

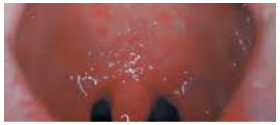
Signs & Symptoms

Monkeypox Rash

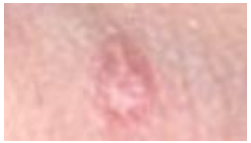
4 - Monkeypox Rash Stages

- Occurs 1 - 2 days after Lymphadenopathy begins
- Rash goes through 6 stages
- Hypo & hyperpigmentation and pitting & scarring can occur after healing

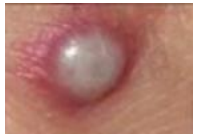
The Monkeypox Rash Goes Through 6 Stages Before Healing is Complete:



1. Oral Enanthem



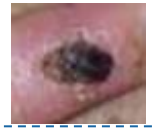
3. Papules



5. Pustules

2. Macules

4. Vesicles



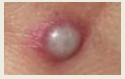
6. Scabs

Signs & Symptoms

Lesion Stages

4 - Monkeypox Rash Stages - Lesions in the Order that They Appear

Source: CDC

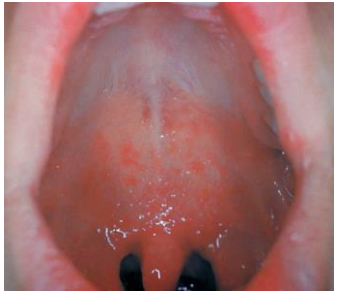
Lesion	Stage	Duration	Characteristics
	Enanthem		The first lesions to develop are on the tongue and in the mouth.
	Macules	1-2 days	• Following the enanthem, a macular rash appears on the skin, starting on the face and spreading to the arms and legs and then to the hands and feet, including the palms and soles. • The rash typically spreads to all parts of the body within 24 hours becoming most concentrated on the face, arms, and legs (centrifugal distribution).
	Papules	1-2 days	By the 3rd day of rash, lesions have progressed from macular (flat) to papular (raised).
	Vesicles	1-2 days	By the 4th to 5th day, lesions have become vesicular (raised and filled with clear fluid).
	Pustules	5-7 days	• By the 6th to 7th day, lesions have become pustular (filled with opaque fluid) –sharply raised, usually round, and firm to the touch (deep seated). • Lesions will develop a depression in the center (umbilication). • The pustules will remain for approximately 5 to 7 days before beginning to crust.
	Scabs	7-14 days	• By the end of the second week, pustules have crusted and scabbed over. • Scabs will remain for about a week before beginning to fall off.

Signs & Symptoms

Monkeypox Rash Photos

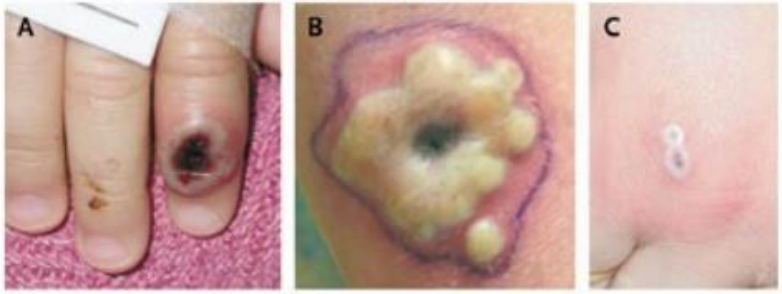
5 - Photos of Monkeypox Rash

Oral Enanthem



SOURCE: Wikipedia

Inoculation Type Lesions (entry through cuts, scratches, bites)

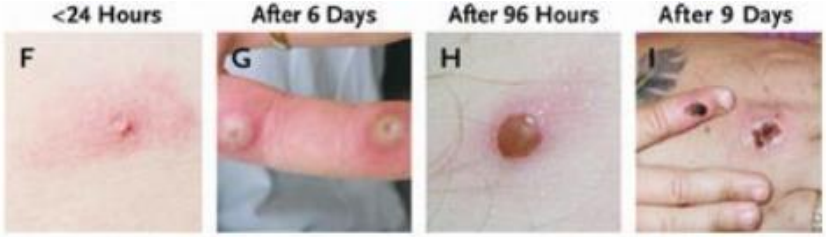


SOURCE: New England Journal of Medicine (NEJM)

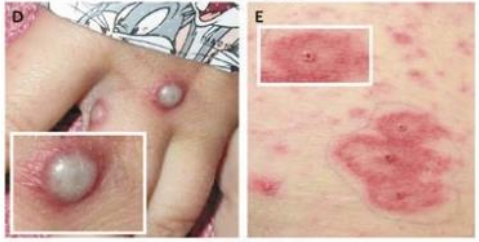
A. From infected Prairie Dog bite
B. Infected Prairie Dog Scratch
C. Preexisting cat scratch exposed to Monkeypox

Lesions in Various Stages of Healing

SOURCE: NEJM



Disseminated Lesions SOURCE: NEJM

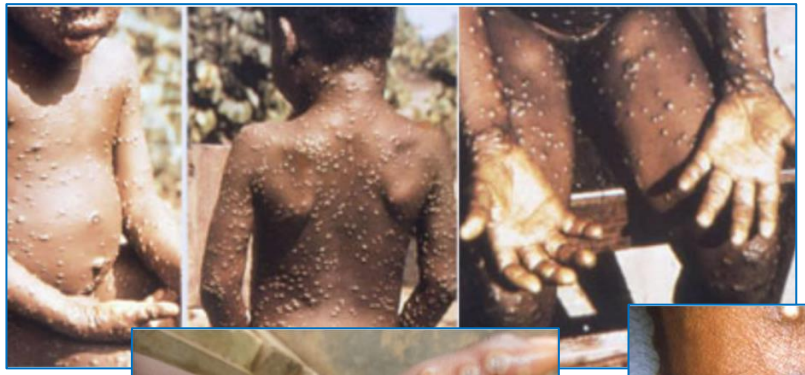


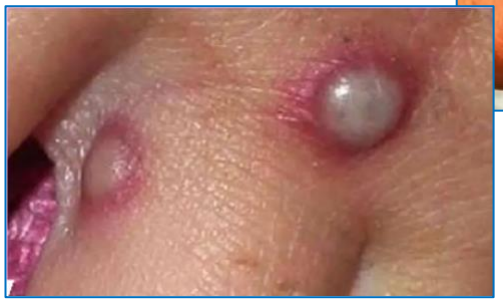
D. Pustules
E. Macules turning into Papules

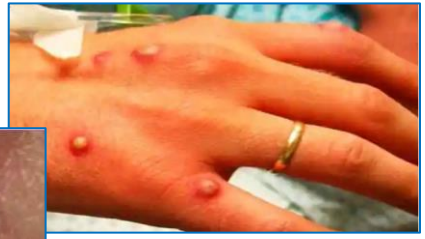
Signs & Symptoms

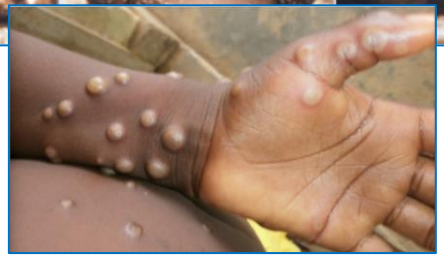
Monkeypox Rash Photos

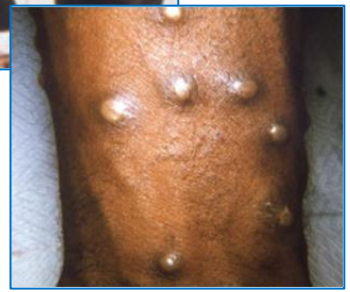
5 - Photos of Monkeypox Rash







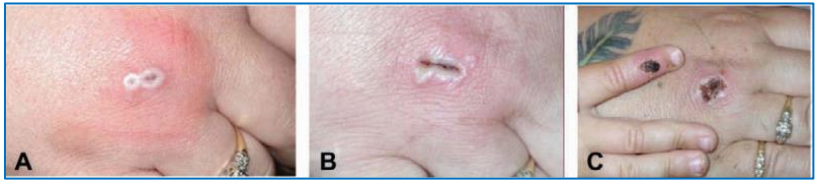




SOURCE: CDC

A. Day 1
B. Day 3
C. Day 9

Preexisting cat scratch exposed to Monkeypox



SOURCE: Healthcare Epidemiology (Dec 2015)

Signs & Symptoms

Fast Facts

6 - Monkeypox Lesion Fast Facts

- Monkeypox lesions are well circumscribed, deep seated, and often develop an umbilication (raised dot) in the center.
- Lesions are relatively uniform in size and develop at the same stage.
- Disseminated rash is described as centrifugal, with lesions are found more often on the face and extremities.
- Lesions are found on the palms and the soles.
- Lesions are described as painful until they scab over and become itchy.
- The patient remains contagious throughout all phases of the rash stage.
- Once all lesions have crusted, those crusts have separated, and a fresh layer of healthy skin has formed underneath, the patient is no longer contagious.
- Hypo & hyperpigmentation and pitting & scarring can occur after healing.

Diagnosis

Section VII

Diagnosis

Specimen Collection

1 - Specimen Collection

Confirming a diagnosis of monkeypox requires collecting samples of the patient's lesions. Safety during collection remains the #1 priority for the collector.

Collection Safety & PPE

- Collect in a closed room with minimal personnel
- Use plastic transport containers, NOT glass
- Do not collect near a fan or AC vent
- Have the patient wear a well-fitting N-95 mask
- Collection PPE: fluid proof gown, gloves, face/ eye protection (full face shield preferable), and an N-95 or higher respirator.
- Disinfect all surfaces & floor appropriately after collection using an approved product .

Sample Collection

- Before collection, consult with your local health department, CDC, or governing health body
- Collect & transport according to their instruction
- Specimens: Throat swabs, lesion fluid, lesion roofs, scab material, or biopsies
- Swabs: can be dry swabs or placed in viral transport media – swab area vigorously
- Two lesions of the same stage (from different locations) should be placed in one collection tube
- Refrigerate (2-8^{o}C) or freeze (-20^{o}C) within 1 hour

Diagnosis

Specimen Transport & Handling

2 - Specimen Transport & Handling

All specimen types from monkeypox patients must be clearly labeled as a biohazard and packaged appropriately for transport, whether transporting long distance or within the same facility. **Never use a pneumatic tube system!**

BSL 3 or BSL4 Testing Labs for Monkeypox Identification

- Performs the monkeypox testing. In the US, this would be a Laboratory Resource Network (LRN) lab or CDC lab. BSL3 and higher labs are equipped to manipulate the monkeypox virus.
- Specimens must be handled with proper PPE for a BSL3 level organism within appropriate hoods.
- It's recommended that testing personnel should be vaccinated with the smallpox vaccine.

BSL 2 Testing Labs for Routine Lab Testing of Inpatients (most hospital labs are BSL2)

- Limit # of personnel handling routine specimens – smallpox vaccinated if possible. Wear full PPE!!
- Specimens should be manipulated in a Class II biosafety cabinet (hood). Proper disinfection must take place!
- Centrifuging may create aerosols & should be performed under a hood or in a sealed system.

Helpful Links: CDC - Lab Info | CDC BMBL | WHO - Interim Guidance | WHO Biosafety Manual | APHL (2003)

Diagnosis

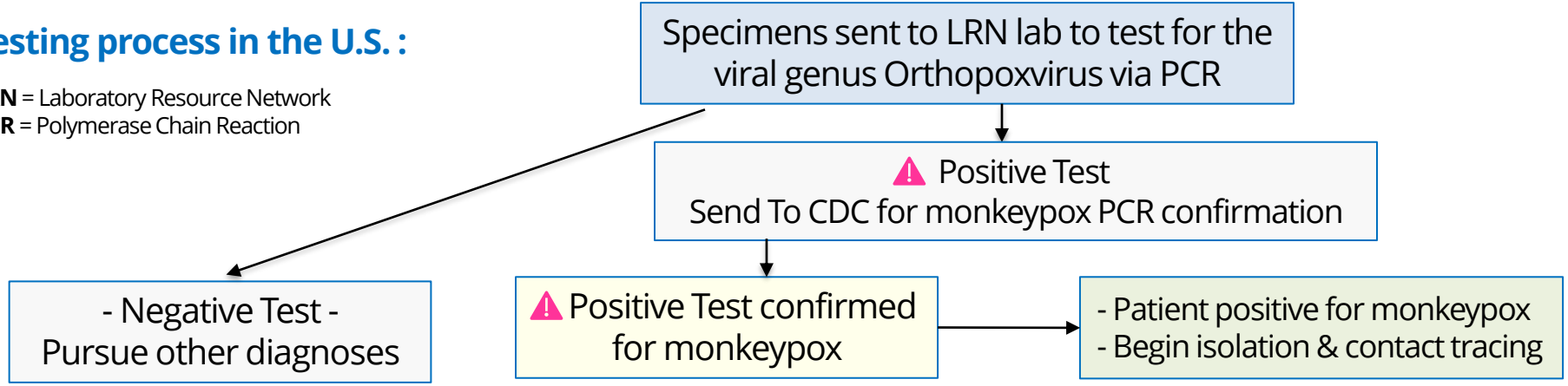
Specimen Testing

3 - Specimen Testing Flow Chart

U.S. : Before sending testing, consult with your local State Health Department. Alert state & CDC of possible case.
Worldwide : Consult with your local health authority on testing protocols. Alert the appropriate agencies of possible case.

Testing process in the U.S. :

LRN = Laboratory Resource Network
PCR = Polymerase Chain Reaction



NOTE: Protocols are subject to change. Be sure to check with your facility & local health authorities for updates.

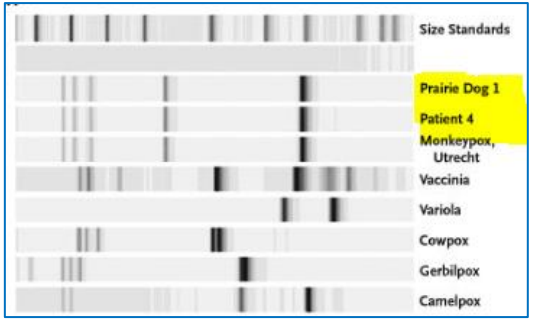
Diagnosis

Specimen Testing

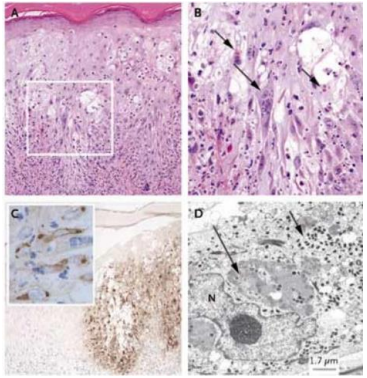
4 - Monkeypox Testing Examples

IMAGE SOURCE: NEJM

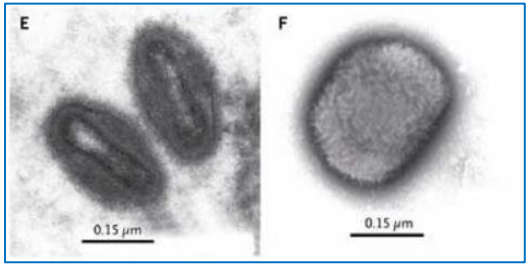
Although PCR is currently the gold standard for testing, there are other test methods some of which are only available in research facilities. Methodologies include PCR, histology & immunohistochemistry (IHC) tissue staining, electron microscopy, and cell culture.



PCR (example from 2003 outbreak)



Tissue Histology & IHC



Electron Microscopy

Differential Diagnosis

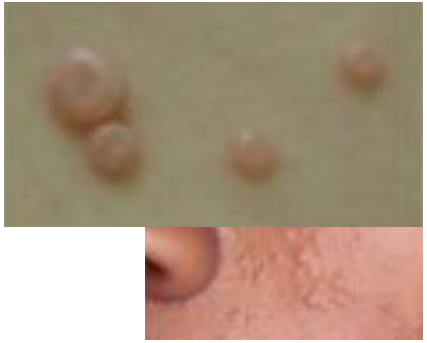
Section VIII

Differential Diagnosis

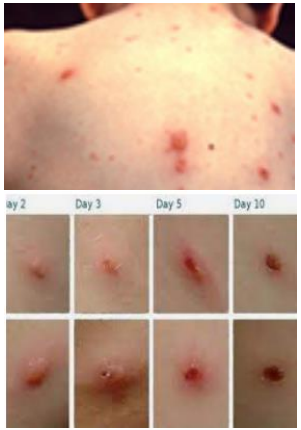
Monkeypox vs. Visually Similar Illnesses

1 - Rashes Visually Similar to Monkeypox

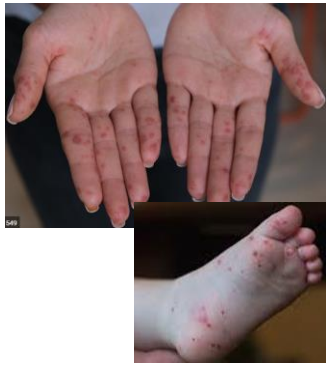
There are several common illnesses that can be easily to confused with monkeypox, the most important being smallpox. Here are a few examples below:



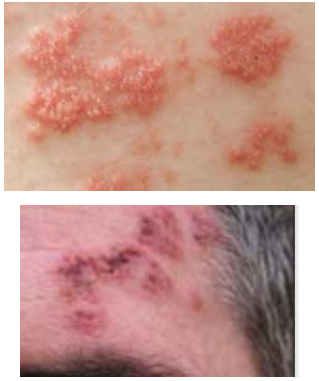
Molluscum contagiosum



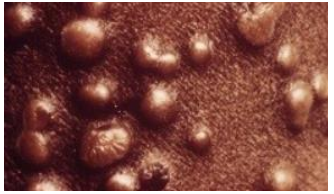
Chickenpox



Hand, Foot, Mouth



Shingles



Smallpox

Monkeypox

Differential Diagnosis

Monkeypox vs. Smallpox

2 - Identifying Differences Between Monkeypox and Smallpox

In humans, the signs & symptoms of monkeypox present in a very similar manner to smallpox so it's important to be able to distinguish differences between the two while tests are pending.

The table below lists a few differences that help identify the type of illness.

	Monkeypox	Smallpox
Incubation Period	Avg: 7-14 days | Range: 5-21 days	Avg: 10-14 days | Range: 7-19 days
Severity of Disease	Moderate	Severe
Swollen Lymph Nodes	✓ Yes	⊘ No

Treatment

Section IX

Treatment

General Information

1 - General Treatment

No proven or specific treatment is available at this time to treat or cure Monkeypox.

General supportive care is provided to the patient to alleviate their symptoms, manage any immediate needs, and prevent long-term complications.

Supportive Care Options for Monkeypox

- Fever reducers
- Fluids (possibly intravenous)
- Good nutrition
- Sterile coverings for skin lesions
- Antibiotics for secondary infection of skin lesions
- Physical therapy, if needed, for extensive scarring from the skin rash

2 - Treatments for Control of Outbreaks

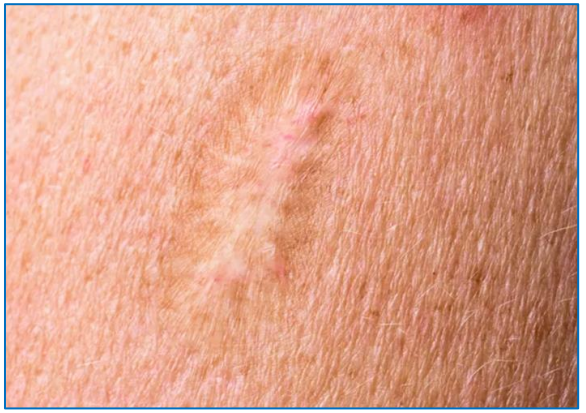
For purposes of controlling a monkeypox outbreak and lessening its duration, the smallpox vaccine, antivirals, and vaccinia immune globulin (VIG) can be used.

Monkeypox and Smallpox Vaccine

Because monkeypox virus is closely related to the virus that causes smallpox, the smallpox vaccine can help to protect people from getting monkeypox. Since routine smallpox vaccination in the U.S. stopped in 1972, most of the population younger than 55 years old are not vaccinated against smallpox.

Although no longer routinely administered, there are currently two vaccines that can help to control Monkeypox outbreaks if they occur.

Smallpox Vaccine Scar



SOURCE: Huffpost

2 - Treatments for Control of Outbreaks

Monkeypox and Smallpox Vaccine (cont'd)

1. One vaccine, JYNNEOSTM (aka: Imvamune or Imvanex), has been licensed in the U.S. to prevent both monkeypox and smallpox. Past data from Africa suggests that smallpox vaccine is at least 85% effective in preventing monkeypox. Experts also believe that vaccination after a monkeypox exposure may help prevent the disease or make it less severe.

2. ACAM2000, which contains a live vaccinia virus, is licensed for immunization in people who are at least 18 years old and at high risk for smallpox infection. It can be used in people exposed to monkeypox if used under an expanded access investigational new drug protocol.

At this time, the smallpox vaccine is currently not available to the general public.

2 - Treatments for Control of Outbreaks

Antiviral Medication

Although data is not available on the effectiveness of using antivirals in treating human cases of monkeypox, the medications below have proven activity against poxviruses.

1. Cidofovir and Brincidofovir (CMX001).
2. Tecovirimat (ST-246)

Vaccinia Immune Globulin (VIG)

Data is not available on the effectiveness of VIG in treatment of monkeypox. Use of VIG must be administered under an Investigational New Drug (IND) application.

VIG can be considered for prophylactic use in an exposed person with severe T-cell immuno-deficiency for which smallpox vaccination following exposure to monkeypox is contraindicated.

Prevention

Section X

1 - General Prevention Measures

A number of measures can be taken to prevent infection with monkeypox virus:

- Take a full history on all patients, including travel history.
- Be aware of any unusual rashes that patients may have.
- Use proper PPE when caring for infected patients.
- Always practice proper hand hygiene and infection control measures, using the correct disinfectants capable of killing the virus.
- Isolate infected patients from others.
- Avoid contact with animals that could harbor the virus
- Avoid contact with any objects, that have been in contact with an infected animal or human.
- Take extra precautions when traveling to an endemic area.

Proper Hand Hygiene is Important

SOURCE: CDC

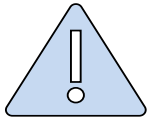
Isolation & Infection Control

Section XI

Isolation & Infection Control

Duration

1 - Duration of Patient Isolation



The goal of isolation is to break the cycle of disease spread to others.

Exposure to Monkeypox: If someone has an exposure to a patient with known monkeypox or one under suspicion for monkeypox, they should isolate for up to 21 days – the full length of the incubation period. Isolation can end early if the person they were exposed to tests negative.

Diagnosed with Monkeypox: For individuals diagnosed with monkeypox, isolation precautions should be continued until all lesions have fully resolved and a fresh layer of skin has formed.

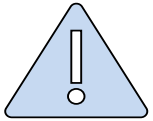
Leaving isolation prior to healed lesions forming, results in the possibility of infecting others through contamination of your surroundings from lesion fluid, scab material, and respiratory droplets. Special care should be taken around immunocompromised individuals, including:

- **Patients with immunodeficiency:** HIV, Congenital Immune Deficiency, etc.
- **Chronic Diseases:** Diabetes, Cancer, etc.
- **Immunosuppressive therapy:** Chemotherapy, Steroids, Radiation, etc.

Isolation & Infection Control

2 - Infection Control at Home

Patients with a milder disease course can isolate and recover at home. The main goal is to protect others in the household from exposure to the virus.



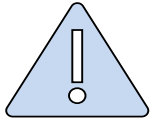
Patients should:

- Isolate in a separate closed room away from others, including pets
- Do not have visitors in the home
- Do not touch or share objects
- Have food and other needs delivered, leaving them just outside the door
- Do not use fans, vacuum cleaners, or other devices that disturb air flow
- Wear a mask to prevent respiratory spread or have others wear them if you can't
- Wear clean gloves & other PPE if you must leave the isolation room
- Clean and disinfect surfaces with an approved cleaner
- Cover lesions at all times with a sterile dressing to avoid spread.
- Bandages & PPE are considered hazardous waste and should never be placed in the regular trash!
- Consult with the local health department on proper contaminated waste disposal.

Source: CDC

3 - Infection Control in Healthcare Settings

Healthcare settings should follow the guidelines put forth by the CDC in the Guidelines for Isolation Precautions.



STANDARD PRECAUTIONS:

- Applies to patient care and the patient
- Alert all staff of the patient's status
- Label & bag all specimens appropriately
- Fans, vacuum cleaners, dry dusting, or sweeping should be avoided

WASTE MANAGEMENT:

- Waste Mgmt. should be performed in accordance with U.S. DOT Hazardous Materials Regulations HMR; 49 CFR 171-180
- Waste handling storage & disposal should be in accordance with state & local regs.

PATIENT PLACEMENT:

- Patients should be placed in a single-person room
- Keep door closed
- Perform intubation & other respiratory procedures in an airborne isolation room
- Private bathroom
- Limit movement outside of room

PERSONAL PROTECTIVE EUIPMENT:

- Gloves, gowns, eye protection, NISOH approved N-95 respirator

ENVIRONENTAL INFECTION CONTROL:

- Disinfect using an EPA-registered hospital-grade disinfectant with an emerging viral pathogen claim
- Laundry should be gently and promptly contained.
- Guidelines for Env. IC is found here
- Guidelines to prevent Transmission of Infectious Agents is found here

Source: CDC

Conclusion

Section XII

Conclusion

1 - Monkeypox Conclusion

Monkeypox is a zoonotic virus typically endemic to certain parts of Africa. Until the 2022 outbreak, cases had only rarely been diagnosed outside of the African continent. Cases are typically associated with travel to an endemic area or contact with an animal or human that has the disease. What's unusual about the 2022 outbreak is that it's the first time sexual transmission has been documented and the first time community spread has occurred outside of Africa.

If you suspect a patient may have monkeypox you must promptly contact your local health department or health authority governing your area or country.

U.S. Contact Numbers:

CDC Emergency Operations Center - 770-488-7100 | **State Health Department** Contacts

References

Section XIII

References

Section XII

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- **JOURNAL OF INFETIOUS DISEASES:** https://academic.oup.com/jid/article/194/6/773/864712?login=false (Accessed May 27, 2022)
- **BBC:** https://www.bbc.com/news/health-61551164 (Accessed May 25, 2022)
- **CDC:** https://www.cdc.gov/poxvirus/monkeypox/index.html (Accessed May 25, 2022)
- **CDC:** https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html (Accessed May 25, 2022)
- **CDC:** https://www.cdc.gov/poxvirus/monkeypox/lab-personnel/lab-procedures.html#Clinical%20Pathology,%20Molecular%20Testing,%20and%20Analysis
- **CDC:** https://www.cdc.gov/poxvirus/monkeypox/outbreak/current.html (Accessed May 25, 2022)
- **CDC:** https://www.cdc.gov/poxvirus/monkeypox/pdf/Monkeypox-lab-process.pdf (Accessed May 29, 2022)
- **DAILY MAIL:** https://www.dailymail.co.uk/news/article-10839877/Monkeypox-outbreak-Europes-biggest-100-cases-reported.html (Accessed May 27, 2022)
- **THE HILL:** https://thehill.com/policy/healthcare/3496363-cdc-warns-doctors-to-watch-for-monkeypox/ (Accessed May 26, 2022)
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Section XII

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Florida Laboratory Combo-23 PART II: SECTION 7

RNA & DNA: A Basic Review

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COURSE LEVEL: Basic
CE BROKER #: Reported Automatically

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COURSE OBJECTIVES

At the end of this course you will be able to:

1. Describe the structure of DNA and recall how it replicates itself.
2. Describe the processes of meiosis, transcription, and translation.
3. Describe the structure of ribosomes and recall what they do.
4. Describe what a DNA microarray is and what it can do.
5. List the differences between RNA and DNA.
6. Define the terms microRNA, RNA interference, gene expression, chromatin, and imprinting.
7. Define what a telomere is and what its function is.
8. Describe what mitochondrial DNA is and where it comes from.

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How Genes Work

People have known for many years that living things inherit traits from their parents. That common-sense observation led to agriculture, the purposeful breeding and cultivation of animals and plants for desirable characteristics. Firming up the details took quite some time, though. Researchers did not understand exactly how traits were passed to the next generation until the middle of the 20th century.

Now it is clear that **genes** are what carry our traits through generations and that genes are made of **deoxyribonucleic acid (DNA)**. But genes themselves don't do the actual work. Rather, they serve as instruction books for making functional molecules such as **ribonucleic acid (RNA)** and **proteins**, which perform the chemical reactions in our bodies.

Proteins do many other things, too. They provide the body's main building materials, forming the cell's architecture and structural components. But one thing proteins can't do is make copies of themselves. When a cell needs more proteins, it uses the manufacturing instructions coded in DNA.

The DNA code of a gene—the sequence of its individual DNA building blocks, labeled A (adenine), T (thymine), C (cytosine) and G (guanine) and collectively called **nucleotides**—spells out the exact order of a protein's building blocks, **amino acids**.

Occasionally, there is a kind of typographical error in a gene's DNA sequence. This mistake—which can be a change, gap or duplication—is called a **mutation**.

Genetics in the Garden

The monk Gregor Mendel first described how traits are inherited from one generation to the next.

In 1900, three European scientists independently discovered an obscure research paper that had been published nearly 35 years before. Written by Gregor Mendel, an Austrian monk who was also a scientist, the report described a series of breeding experiments performed with pea plants growing in his abbey garden.

Mendel had studied how pea plants inherited the two variant forms of easy-to-see traits. These included flower color (white or purple) and the texture of the peas (smooth or wrinkled). Mendel counted many generations of pea plant offspring and learned that these characteristics were passed on to the next generation in orderly, predictable ratios.

When he cross-bred purple-flowered pea plants with white-flowered ones, the next generation had only purple flowers. But directions for making white flowers were hidden somewhere in the peas of that generation, because when those purple-flowered

>>>Continued on botton of next page <<<

A mutation can cause a gene to encode a protein that works incorrectly or that doesn't work at all. Sometimes, the error means that no protein is made.

But not all DNA changes are harmful. Some mutations have no effect, and others produce new versions of proteins that may give a survival advantage to the organisms that have them. Over time, mutations supply the raw material from which new life forms evolve.

Beautiful DNA

Up until the 1950s, scientists knew a good deal about heredity, but they didn't have a clue what DNA looked like. In order to learn more about DNA and its structure, some scientists experimented with using X rays as a form of molecular photography.

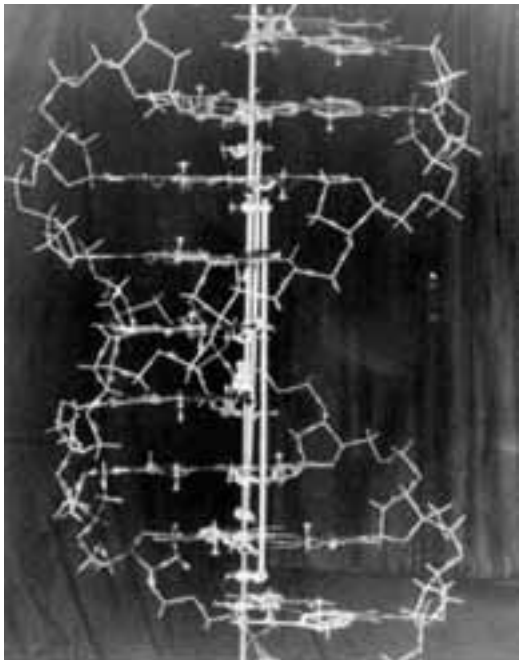
Rosalind Franklin, a physical chemist working with Maurice Wilkins at King's College in London, was among the first to use this method to analyze genetic material. Her experiments

plants were bred to each other, some of their offspring had white flowers. What's more, the second-generation plants displayed the colors in a predictable pattern. On average, 75 percent of the second-generation plants had purple flowers and 25 percent of the plants had white flowers. Those same ratios persisted, and were reproduced when the experiment was repeated many times over.

Trying to solve the mystery of the missing color blooms, Mendel imagined that the reproductive cells of his pea plants might contain discrete "factors," each of which specified a particular trait, such as white flowers. Mendel reasoned that the factors, whatever they were, must be physical material because they passed from parent to offspring in a mathematically orderly way. It wasn't until many years later, when the other scientists unearthed Mendel's report, that the factors were named genes.

Early geneticists quickly discovered that Mendel's mathematical rules of inheritance applied not just to peas, but also to all plants, animals and people. The discovery of a quantitative rule for inheritance was momentous. It revealed that a common, general principle governed the growth and development of all life on Earth.

FIGURE 2:



COLD SPRING HARBOR LABORATORY ARCHIVES

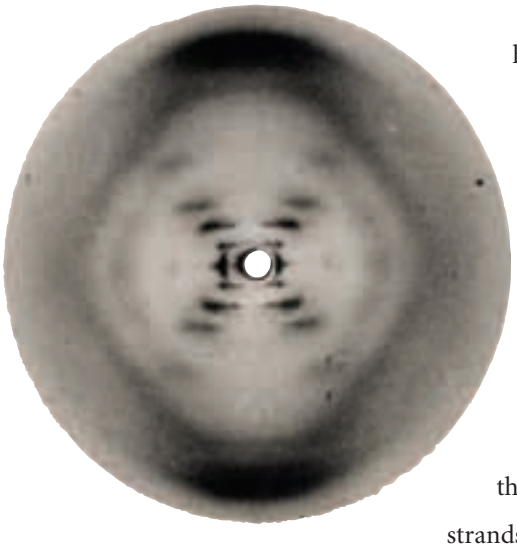
▲ In 1953, Watson and Crick created their historic model of the shape of DNA: the double helix.

produced what were referred to at the time as "the most beautiful X-ray photographs of any substance ever taken."

Other scientists, including zoologist James Watson and physicist Francis Crick, both working at Cambridge University in the United Kingdom, were trying to determine the shape of DNA too. Ultimately, this line of research revealed one of the most profound scientific discoveries of the 20th century: that DNA exists as a double helix.

The 1962 Nobel Prize in physiology or medicine was awarded to Watson, Crick and Wilkins for this work. Although Franklin did not earn a share of the prize due to her untimely death at age 38, she is widely recognized as having played a significant role in the discovery.

FIGURE 1:



▲ Rosalind Franklin's original X-ray diffraction photo revealed the physical structure of DNA.

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The spiral staircase-shaped double helix has attained global status as the symbol for DNA. But what is so beautiful about the discovery of the twisting ladder structure isn't just its good looks. Rather, the structure of DNA taught researchers a fundamental lesson about **genetics**. It taught them that the two connected strands—winding together like parallel handrails—were complementary to each other, and this unlocked the secret of how genetic information is stored, transferred and copied.

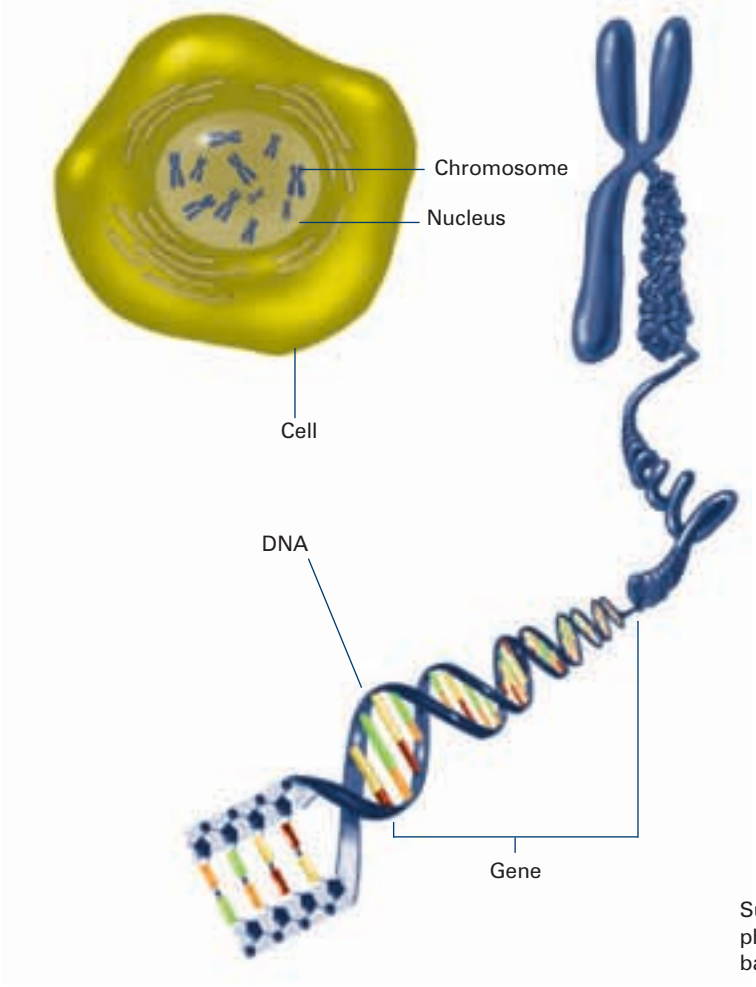
In genetics, complementary means that if you know the sequence of nucleotide building blocks on one strand, you know the sequence of nucleotide building blocks on the other strand: A always matches up with T and C always links to G (see next page).

Long strings of nucleotides form genes, and groups of genes are packaged tightly into structures called **chromosomes**. Every cell in your body except for eggs, sperm and red blood cells contains a full set of chromosomes in its **nucleus**.

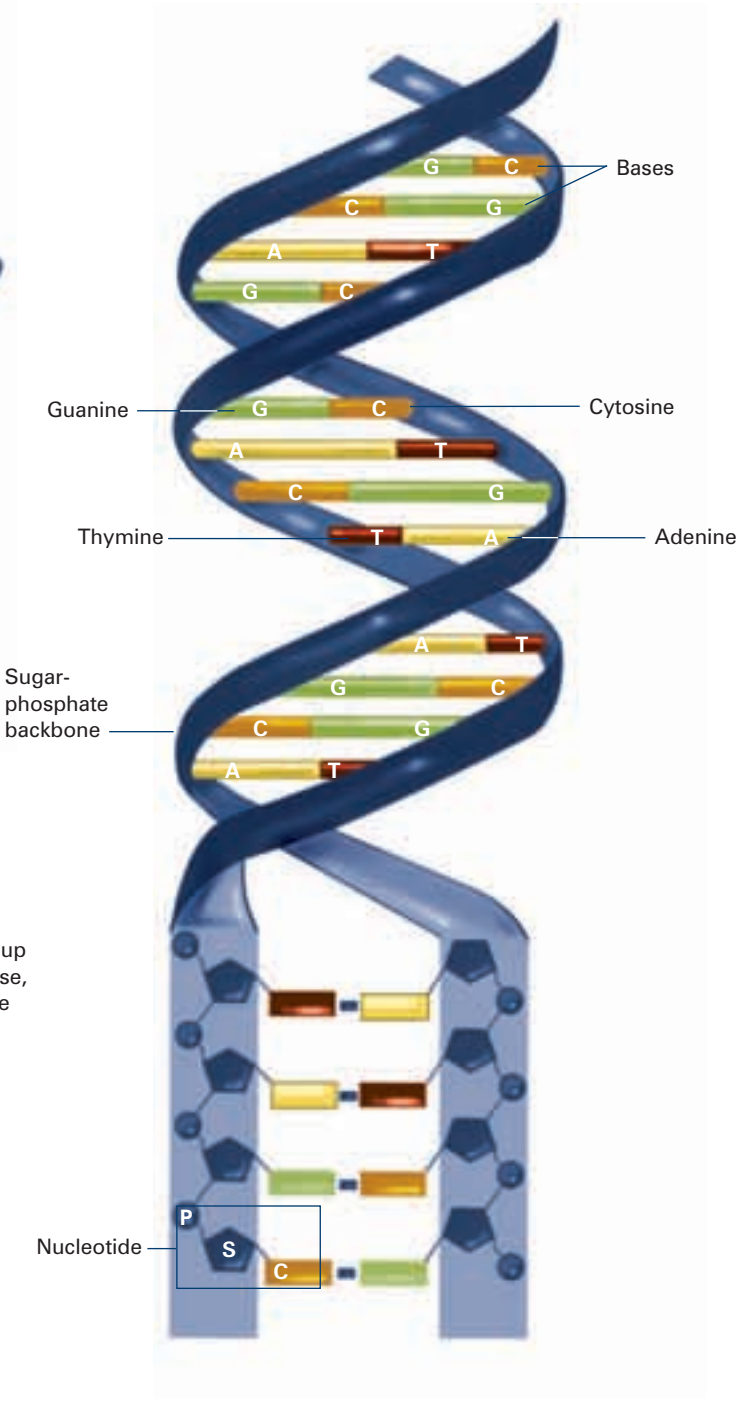
If the chromosomes in one of your cells were uncoiled and placed end to end, the DNA would be about 6 feet long. If all the DNA in your body were connected in this way, it would stretch approximately 67 billion miles! That's nearly 150,000 round trips to the Moon.

FIGURE 3:

DNA Structure



The long, stringy DNA that makes up genes is spooled within chromosomes inside the nucleus of a cell. (Note that a gene would actually be a much longer stretch of DNA than what is shown here.)



DNA consists of two long, twisted chains made up of nucleotides. Each nucleotide contains one base, one phosphate molecule and the sugar molecule deoxyribose. The bases in DNA nucleotides are adenine, thymine, cytosine and guanine.

FIGURE 5:

▲ Humans have 23 pairs of chromosomes. Male DNA (pictured here) contains an X and a Y chromosome, whereas female DNA contains two X chromosomes.

CYTOGENETICS LABORATORY, BRIGHAM AND WOMEN'S HOSPITAL

Copycat

It's astounding to think that your body consists of trillions of cells. But what's most amazing is that it all starts with one cell. How does this massive expansion take place?

As an embryo progresses through development, its cells must reproduce. But before a cell divides into two new, nearly identical cells, it must copy its DNA so there will be a complete set of genes to pass on to each of the new cells.

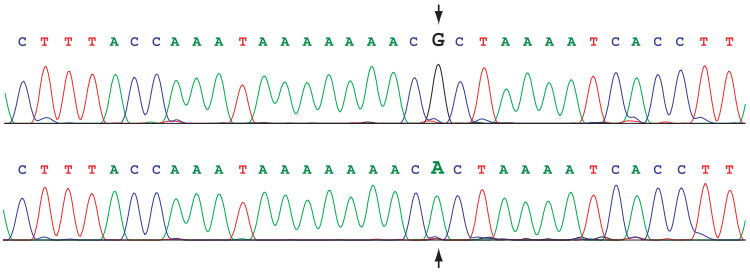
To make a copy of itself, the twisted, compacted double helix of DNA has to unwind and separate its two strands. Each strand becomes a pattern, or template, for making a new strand, so the two new DNA molecules have one new strand and one old strand.

The copy is courtesy of a cellular protein machine called **DNA polymerase**, which reads the template DNA strand and stitches together the complementary new strand. The process, called **replication**, is astonishingly fast and accurate, although occasional mistakes, such as deletions or duplications, occur. Fortunately, a cellular spell-checker catches and corrects nearly all of these errors.

Mistakes that are not corrected can lead to diseases such as cancer and certain genetic disorders. Some of these include Fanconi anemia, early aging diseases and other conditions in which people are extremely sensitive to sunlight and some chemicals.

DNA copying is not the only time when DNA damage can happen. Prolonged, unprotected sun exposure can cause DNA changes that lead to skin cancer, and toxins in cigarette smoke can cause lung cancer.

FIGURE 4:



▲ When DNA polymerase makes an error while copying a gene's DNA sequence, the mistake is called a mutation. In this example, the nucleotide G has been changed to an A.

It may seem ironic, then, that many drugs used to treat cancer work by attacking DNA. That's because these chemotherapy drugs disrupt the DNA copying process, which goes on much faster in rapidly dividing cancer cells than in other cells of the body. The trouble is that most of these drugs do affect normal cells that grow and divide frequently, such as cells of the immune system and hair cells.

Understanding DNA replication better could be a key to limiting a drug's action to cancer cells only.

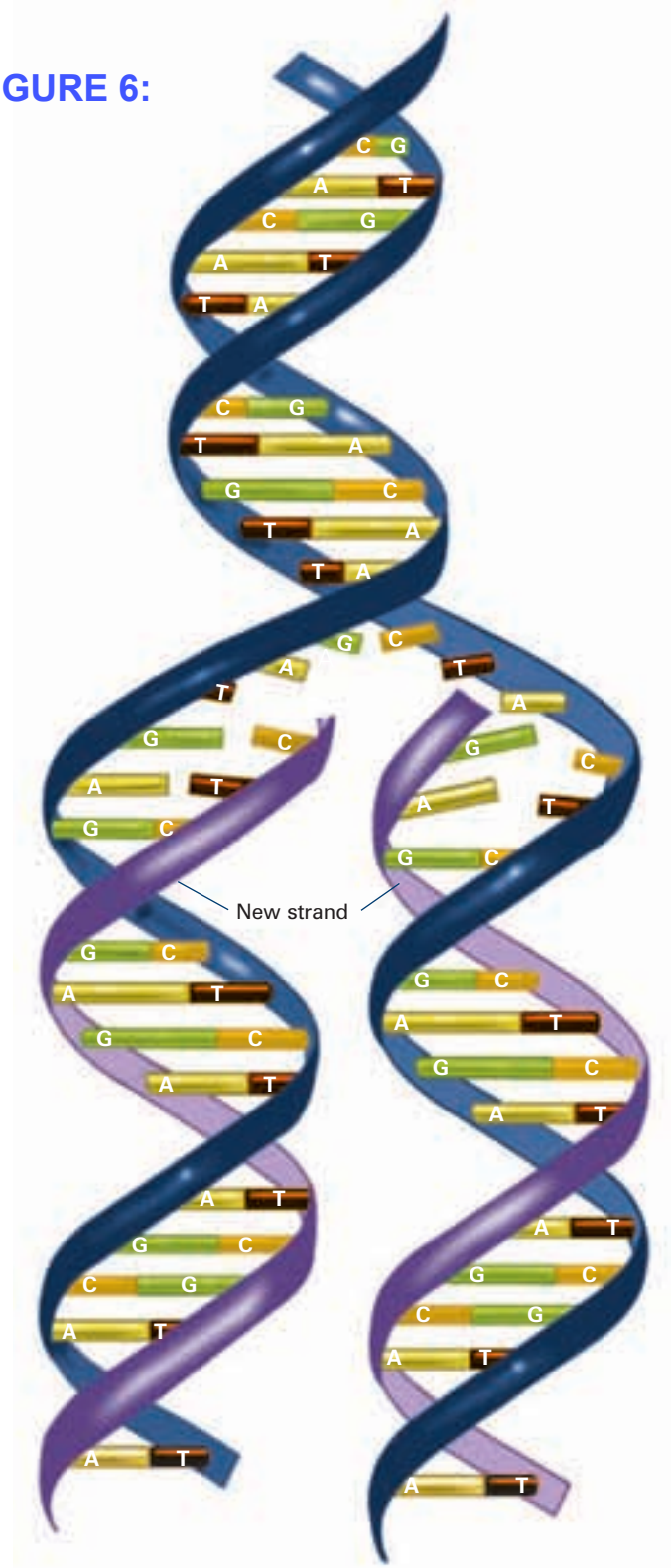
Let's Call It Even

After copying its DNA, a cell's next challenge is getting just the right amount of genetic material into each of its two offspring.

Most of your cells are called **diploid** ("di" means two, and "ploid" refers to sets of chromosomes) because they have two sets of chromosomes (23 pairs). Eggs and sperm are different; these are known as **haploid** cells. Each haploid cell has only one set of 23 chromosomes so that at fertilization the math will work out: A haploid egg cell will combine with a haploid sperm cell to form a diploid cell with the right number of chromosomes: 46.

Chromosomes are numbered 1 to 22, according to size, with 1 being the largest chromosome. The 23rd pair, known as the sex chromosomes, are called X and Y. In humans, abnormalities of chromosome number usually occur during **meiosis**, the time when a cell

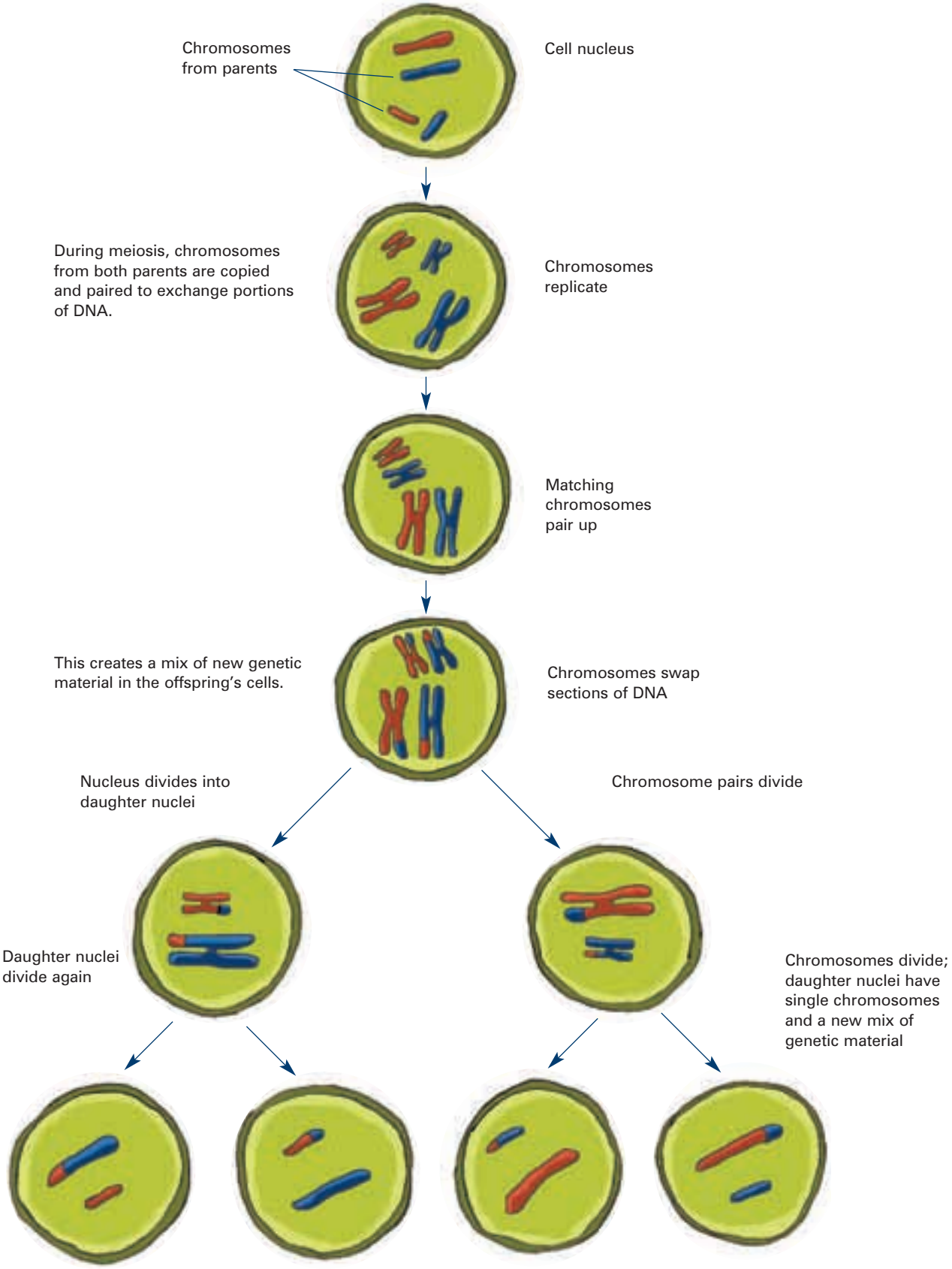
FIGURE 6:



▲ During DNA replication, each strand of the original molecule acts as a template for the synthesis of a new, complementary DNA strand.

FIGURE 7:

Meiosis



reduces its chromosomes from diploid to haploid in creating eggs or sperm.

What happens if an egg or a sperm cell gets the wrong number of chromosomes, and how often does this happen?

Molecular biologist Angelika Amon of the Massachusetts Institute of Technology in Cambridge says that mistakes in dividing DNA between daughter cells during meiosis are the leading cause of human birth defects and miscarriages. Current estimates are that 10 percent of all embryos have an incorrect chromosome number. Most of these don't go to full term and are miscarried.

In women, the likelihood that chromosomes won't be apportioned properly increases with age. One of every 18 babies born to women over 45 has three copies of chromosome 13, 18 or 21 instead of the normal two, and this improper balancing can cause trouble. For example, three copies of chromosome 21 lead to Down syndrome.

To make her work easier, Amon—like many other basic scientists—studies yeast cells, which separate their chromosomes almost exactly the same way human cells do, except that yeast do it much faster. A yeast cell copies its DNA and produces daughter cells in about $1\frac{1}{2}$ hours, compared to a whole day for human cells.

The yeast cells she uses are the same kind bakeries use to make bread and breweries use to make beer!

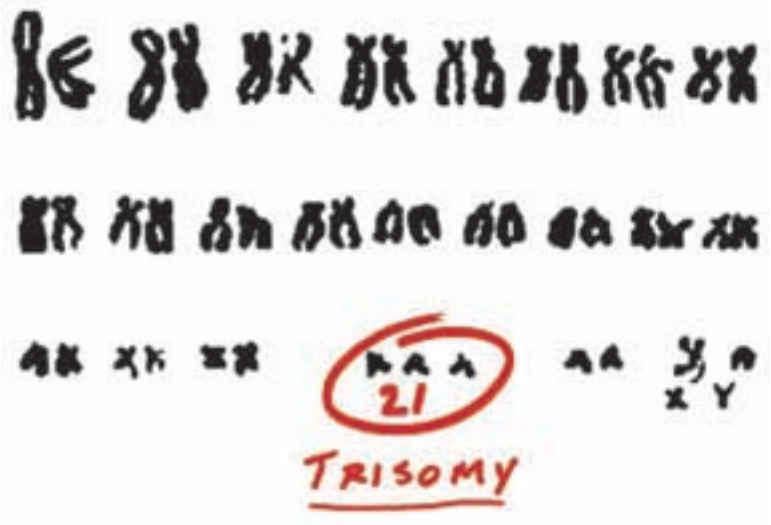
Amon has made major progress in understanding the details of meiosis. Her research shows how, in healthy cells, gluelike protein complexes called cohesins release pairs of chromosomes at exactly the right time. This allows the chromosomes to separate properly.

These findings have important implications for understanding and treating infertility, birth defects and cancer.

Getting the Message

So, we've described DNA—its basic properties and how our bodies make more of it. But how does DNA serve as the language of life? How do you get a protein from a gene?

FIGURE 8:



▲ Trisomy, the hallmark of Down syndrome, results when a baby is born with three copies of chromosome 21 instead of the usual two.

There are two major steps in making a protein. The first is **transcription**, where the information coded in DNA is copied into RNA. The RNA nucleotides are complementary to those on the DNA: a C on the RNA strand matches a G on the DNA strand.

The only difference is that RNA pairs a nucleotide called uracil (U), instead of a T, with an A on the DNA.

A protein machine called **RNA polymerase** reads the DNA and makes the RNA copy. This copy is called messenger RNA, or mRNA, because it delivers the gene's message to the protein-producing machinery.

At this point you may be wondering why all of the cells in the human body aren't exactly alike, since they all contain the same DNA. What makes a liver cell different from a brain cell? How do the cells in the heart make the organ contract, but those in skin allow us to sweat?

Cells can look and act differently, and do entirely different jobs, because each cell "turns on," or expresses, only the genes appropriate for what it needs to do.

That's because RNA polymerase does not work alone, but rather functions with the aid of many helper proteins. While the core part of RNA polymerase is the same in all cells, the helpers vary in different cell types throughout the body.

You'd think that for a process so essential to life, researchers would know a lot about how transcription works. While it's true that the basics are clear—biologists have been studying gene transcribing by RNA polymerases since these proteins were first discovered in 1960—some of the details are actually still murky.

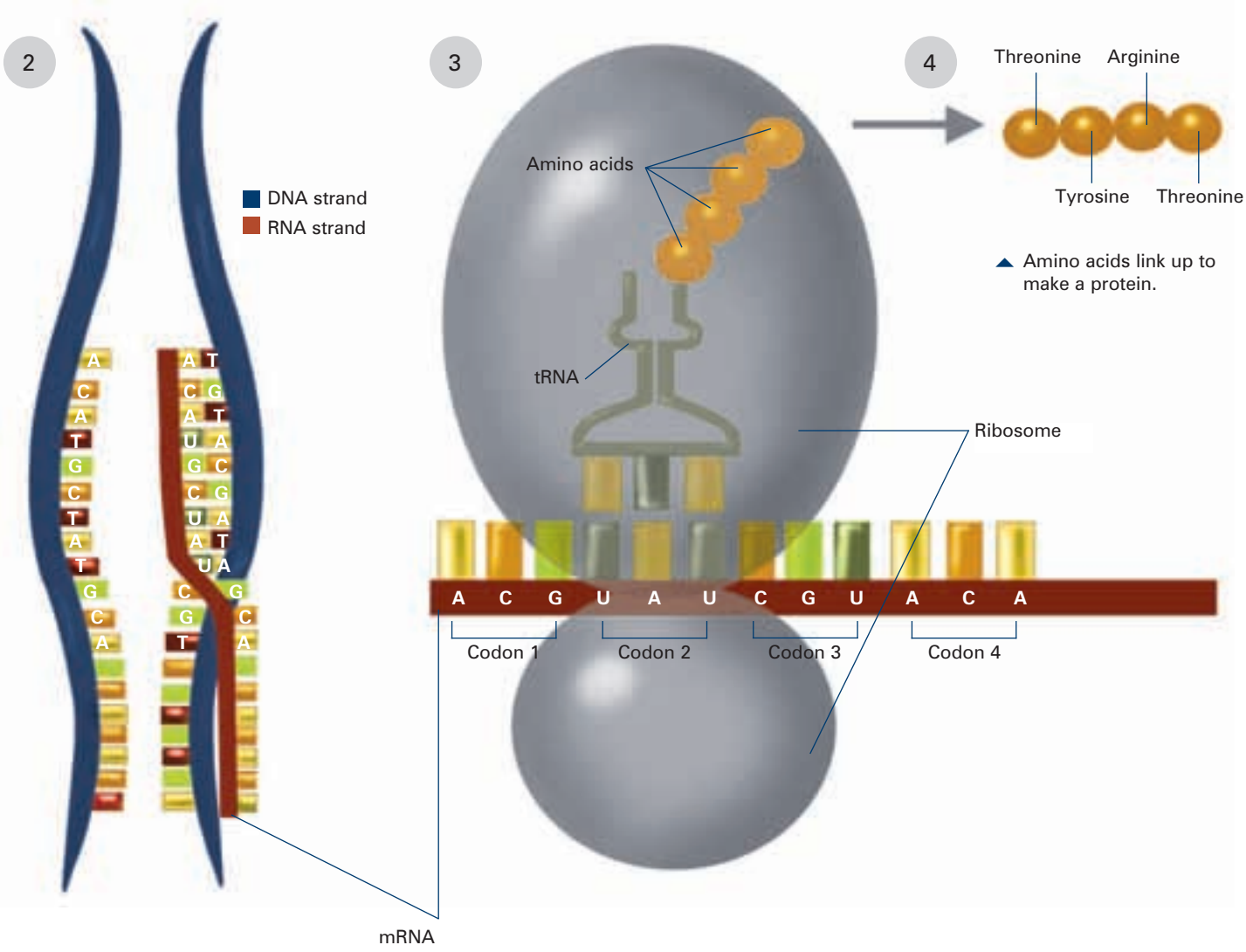
FIGURE 9:

▲ RNA polymerase transcribes DNA to make messenger RNA (mRNA).

The biggest obstacle to learning more has been a lack of tools. Until fairly recently, researchers were unable to get a picture at the atomic level of the giant RNA polymerase protein assemblies inside cells to understand how the many pieces of this amazing, living machine do what they do, and do it so well.

But our understanding is improving fast, thanks to spectacular technological advances. We have new X-ray pictures that are far more sophisticated than those that revealed the structure of DNA. Roger Kornberg of Stanford University in California used such methods to determine the structure of RNA polymerase. This work earned

FIGURE 9 (continued)

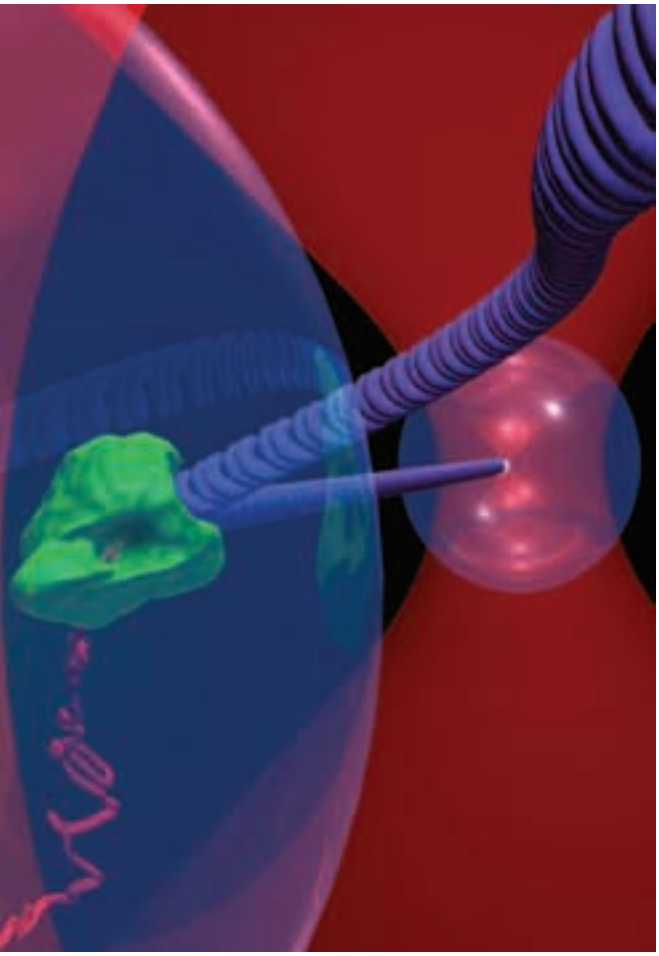


▲ The mRNA sequence (dark red strand) is complementary to the DNA sequence (blue strand).

▲ On ribosomes, transfer RNA (tRNA) helps convert mRNA into protein.

▲ Amino acids link up to make a protein.

FIGURE 10:



▲ RNA polymerase (green) and one end of a DNA strand (blue) are attached to clear beads pinned down in two optical traps. As RNA polymerase moves along the DNA, it creates an RNA copy of a gene, shown here as a pink strand.

STEVEN BLOCK

him the 2006 Nobel Prize in chemistry. In addition, very powerful microscopes and other tools that allow us to watch one molecule at a time provide a new look at RNA polymerase while it's at work reading DNA and producing RNA.

For example, Steven Block, also of Stanford, has used a physics technique called optical trapping to track RNA polymerase as it inches along DNA. Block and his team performed this work by designing a specialized microscope sensitive enough to watch the real-time motion of a single polymerase traveling down a gene on one chromosome.

The researchers discovered that molecules of RNA polymerase behave like battery-powered spiders as they crawl along the DNA ladder, adding nucleotides one at a time to the growing RNA strand. The **enzyme** works much like a motor, Block believes, powered by energy released during the chemical synthesis of RNA.

Nature's Cut-and-Paste Job

Several types of RNA play key roles in making a protein. The gene transcript (the mRNA) transfers information from DNA in the nucleus to the **ribosomes** that make protein. Ribosomal RNA forms about 60 percent of the ribosomes. Lastly, transfer RNA carries amino acids to the ribosomes. As you can see, all three types of cellular RNAs come together to produce new proteins.

But the journey from gene to protein isn't quite as simple as we've just made it out to be. After transcription, several things need to happen to mRNA before a protein can be made. For example, the genetic material of humans and other **eukaryotes** (organisms that have a nucleus) includes a lot of DNA that doesn't encode proteins. Some of this DNA is stuck right in the middle of genes.

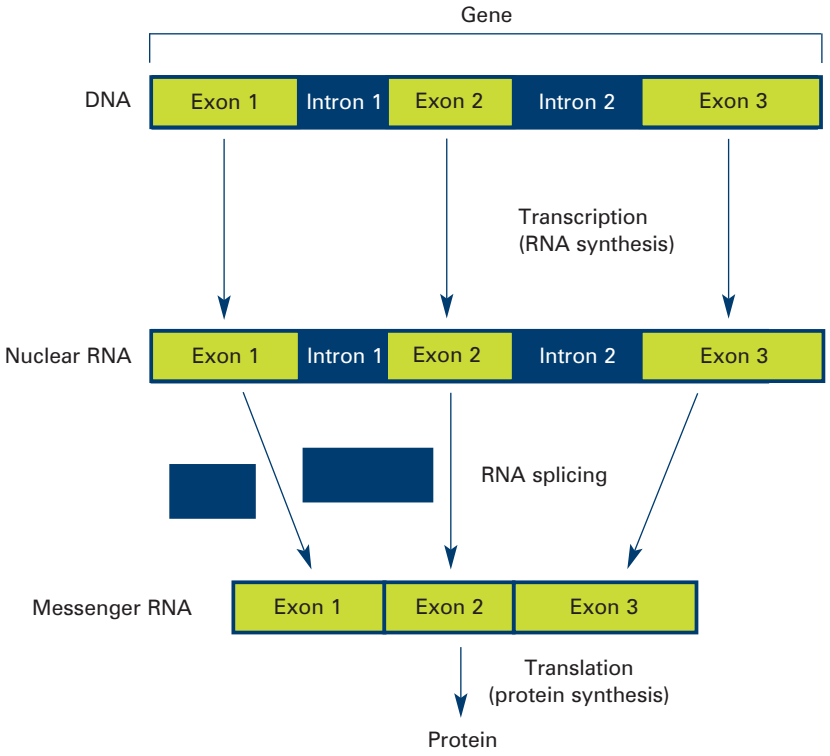
To distinguish the two types of DNA, scientists call the coding sequences of genes **exons** and the pieces in between **introns** (for intervening sequences).

If RNA polymerase were to transcribe DNA from the start of an intron-containing gene to the end, the RNA would be complementary to the introns as well as the exons.

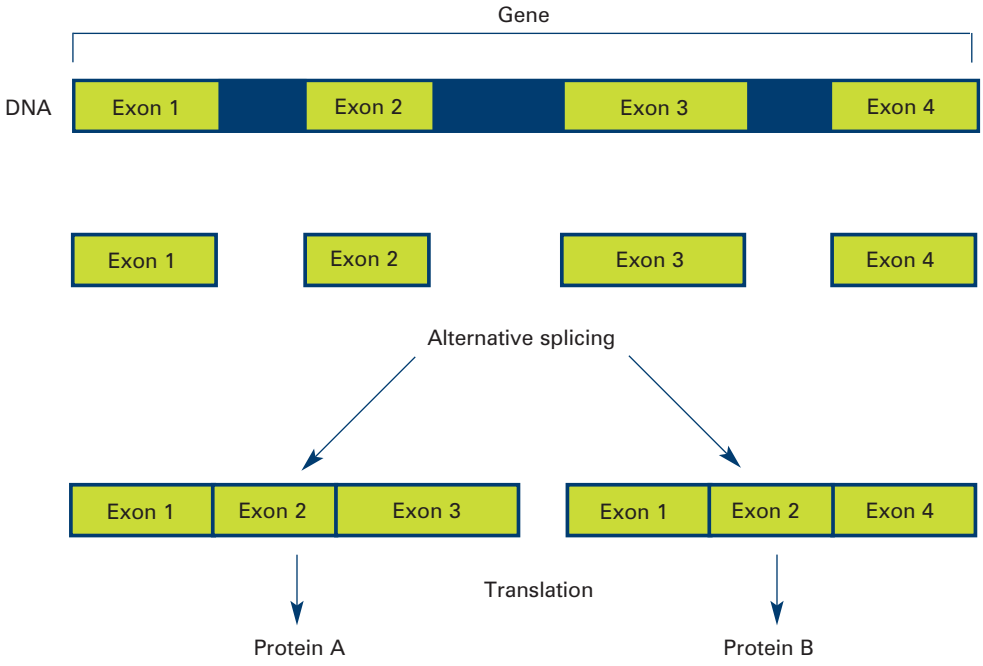
To get an mRNA molecule that yields a working protein, the cell needs to trim out the intron sections and then stitch only the exon pieces together (see drawing, next page). This process is called **RNA splicing.**

FIGURE 11:

RNA Splicing



Genes are often interrupted by stretches of DNA (introns, blue) that do not contain instructions for making a protein. The DNA segments that do contain protein-making instructions are known as exons (green).



Arranging exons in different patterns, called alternative splicing, enables cells to make different proteins from a single gene.

Splicing has to be extremely accurate. An error in the splicing process, even one that results in the deletion of just one nucleotide in an exon or the addition of just one nucleotide in an intron, will throw the whole sequence out of alignment. The result is usually an abnormal protein—or no protein at all. One form of Alzheimer's disease, for example, is caused by this kind of splicing error.

Molecular biologist Christine Guthrie of the University of California, San Francisco, wants to understand more fully the mechanism for removing intron RNA and find out how it stays so accurate.

She uses yeast cells for these experiments. Just like human DNA, yeast DNA has introns, but they are fewer and simpler in structure and are therefore easier to study. Guthrie can identify which genes are required for splicing by finding abnormal yeast cells that mangle splicing.

So why do introns exist, if they're just going to be chopped out? Without introns, cells wouldn't need to go through the splicing process and keep monitoring it to be sure it's working right.

As it turns out, splicing also makes it possible for cells to create more proteins.

Think about all the exons in a gene. If a cell stitches together exons 1, 2 and 4, leaving out exon 3, the mRNA will specify the production of a particular protein. But instead, if the cell stitches together exons 1, 2 and 3, this time leaving out exon 4, then the mRNA will be translated into a different protein (see drawing, page 15).

By cutting and pasting the exons in different patterns, which scientists call alternative splicing, a cell can create different proteins from a single gene. Alternative splicing is one of the reasons why human cells, which have about 20,000 genes, can make hundreds of thousands of different proteins.

All Together Now

Until recently, researchers looked at genes, and the proteins they encode, one at a time. Now, they can look at how large numbers of genes and proteins act, as well as how they interact. This gives them a much better picture of what goes on in a living organism.

Already, scientists can identify all of the genes that are transcribed in a cell—or in an organ, like the heart. And although researchers can't tell you, right now, what's going on in every cell of your body while you read a book or walk down the street, they can do this sort of "whole-body" scan for simpler, single-celled organisms like yeast.

Using a technique called genome-wide location analysis, Richard Young of the Massachusetts Institute of Technology unraveled a "regulatory code" of living yeast cells, which have more than 6,000 genes in their genome. Young's technique enabled him to determine the exact places where RNA polymerase's helper proteins sit on DNA and tell RNA polymerase to begin transcribing a gene.

Since he did the experiment with the yeast exposed to a variety of different conditions,

GENETICS AND YOU: *Nursery Genetics*

While most genetic research uses lab organisms, test tubes and petri dishes, the results have real consequences for people. Your first encounter with genetic analysis probably happened shortly after you were born, when a doctor or nurse took a drop of blood from the heel of your tiny foot.

Lab tests performed with that single drop of blood can diagnose certain rare genetic disorders as well as metabolic problems like phenylketonuria (PKU).

Screening newborns in this way began in the 1960s in Massachusetts with testing for PKU, a disease affecting 1 in 14,000 people. PKU is caused by an enzyme that doesn't work properly due to a genetic mutation. Those born with this disorder cannot metabolize the amino acid phenylalanine, which is present in many foods. Left untreated, PKU can lead to mental retardation and neurological damage, but a special diet can prevent these outcomes. Testing for this condition has made a huge difference in many lives.

Newborn screening is governed by individual states. This means that the state in which a baby is born determines the genetic conditions for which he or she will be screened. Currently, states test for between 28 and 54 conditions. All states test for PKU.

Although expanded screening for genetic diseases in newborns is advocated by some, others question the value of screening for conditions that are currently untreatable. Another issue is that some children with mild versions of certain genetic diseases may be treated needlessly.

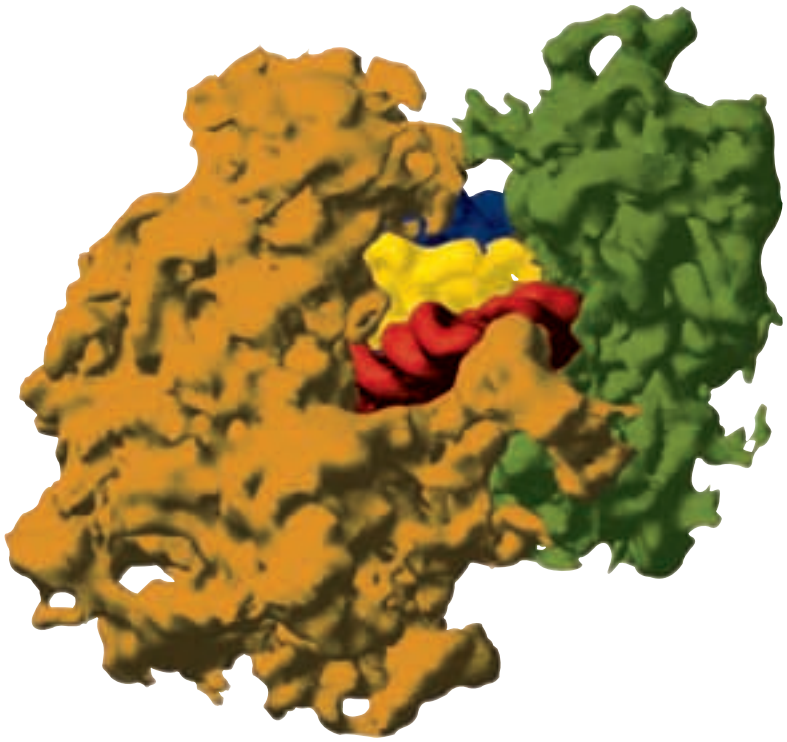
In 2006, the Advisory Committee on Heritable Disorders in Newborns and Children, which assists the Secretary of the U.S. Department of Health and Human Services, recommended a standard, national set of newborn tests for 29 conditions, ranging from relatively common hearing problems to very rare metabolic diseases.

Young was able to figure out how transcription patterns differ when the yeast cell is under stress (say, in a dry environment) or thriving in a sugary-rich nutrient solution. Done one gene at a time, using methods considered state-of-the-art just a few years ago, this kind of analysis would have taken hundreds of years.

After demonstrating that his technique worked in yeast, Young then took his research a step forward. He used a variation of the yeast method to scan the entire human genome in small samples of cells taken from the pancreases and livers of people with type 2 diabetes. He used the results to identify genes that aren't transcribed correctly in people with the disease.

This information provides researchers with an important tool for understanding how diabetes and other diseases are influenced by defective genes. By building models to predict how genes respond in diverse situations, researchers may be able to learn how to stop or jump-start genes on demand, change the course of a disease or prevent it from ever happening.

FIGURE 12:



▲ A ribosome consists of large and small protein subunits with transfer RNAs nestled in the middle.

RIBOSOME STRUCTURE COURTESY OF JAMIE CATE, MARAT YUSUPOV, GULNARA YUSUPOVA, THOMAS EARNEST AND HARRY NOLLER. GRAPHIC COURTESY OF ALBION BAUCOM, UNIVERSITY OF CALIFORNIA, SANTA CRUZ.

Found in Translation

After a gene has been read by RNA polymerase and the RNA is spliced, what happens next in the journey from gene to protein? The next step is reading the RNA information and fitting the building blocks of a protein together. This is called **translation**, and its principal actors are the ribosome and amino acids.

Ribosomes are among the biggest and most intricate structures in the cell. The ribosomes of bacteria contain not only huge amounts of RNA, but also more than 50 different proteins. Human ribosomes have even more RNA and between 70 and 80 different proteins!

Harry Noller of the University of California, Santa Cruz, has found that a ribosome performs several key jobs when it translates the genetic code of mRNA. As the messenger RNA threads through the ribosome protein machine, the

ribosome reads the mRNA sequence and helps recognize and recruit the correct amino acid-carrying transfer RNA to match the mRNA code. The ribosome also links each additional amino acid into a growing protein chain (see Figure 9).

For many years, researchers believed that even though RNAs formed a part of the ribosome, the protein portion of the ribosome did all of the work. Noller thought, instead, that maybe RNA, not proteins, performed the ribosome's job. His idea was not popular at first, because at that time it was thought that RNA could not perform such complex functions.

Some time later, however, the consensus changed. Sidney Altman of Yale University in New Haven, Connecticut, and Thomas Cech, who was then at the University of Colorado in Boulder, each discovered that RNA can perform work as complex as that done by protein enzymes. Their "RNA-as-an-enzyme" discovery turned the research world on its head and earned Cech and Altman the 1989 Nobel Prize in chemistry.

Noller and other researchers have continued the painstaking work of understanding ribosomes. In 1999, he showed how different parts of a bacterial ribosome interact with one another and how the ribosome interacts with molecules involved in protein synthesis. These studies provided near proof that the fundamental mechanism of translation is performed by RNA, not by the proteins of the ribosome.

FIGURE 13:

▲ Some first-aid ointments contain the antibiotic neomycin, which treats infections by attacking ribosomes in bacteria.

RNA Surprises

But which ribosomal RNAs are doing the work? Most scientists assumed that RNA nucleotides buried deep within the ribosome complex—the ones that have the same sequence in every species from bacteria to people—were the important ones for piecing the growing protein together.

However, recent research by Rachel Green, who worked with Noller before moving to Johns Hopkins University in Baltimore, Maryland, showed that this is not the case. Green discovered that those RNA nucleotides are not needed for assembling a protein. Instead, she found, the nucleotides do something else entirely: They help the growing protein slip off the ribosome once it's finished.

Noller, Green and hundreds of other scientists work with the ribosomes of bacteria. Why should you care about how bacteria create proteins from their genes?

One reason is that this knowledge is important for learning how to disrupt the actions of disease-causing microorganisms. For example, antibiotics like erythromycin and neomycin work by attacking the ribosomes of bacteria, which are different enough from human ribosomes that our cells are not affected by these drugs.

As researchers gain new information about bacterial translation, the knowledge may lead to more antibiotics for people.

New antibiotics are urgently needed because many bacteria have developed resistance to the current arsenal. This resistance is sometimes the result of changes in the bacteria's ribosomal RNA. It can be difficult to find those small, but critical, changes that may lead to resistance, so it is important to find completely new ways to block bacterial translation.

Green is working on that problem too. Her strategy is to make random mutations to the genes in a bacterium that affect its ribosomes. But what if the mutation disables the ribosome so much that it can't make proteins? Then the bacterium won't grow, and Green wouldn't find it.

Using clever molecular tricks, Green figured out a way to rescue some of the bacteria with defective ribosomes so they could grow. While some of the rescued bacteria have changes in their ribosomal RNA that make them resistant to certain antibiotics (and thus would not make good antibiotic targets) other RNA changes that don't affect resistance may point to promising ideas for new antibiotics.

An Interesting Development

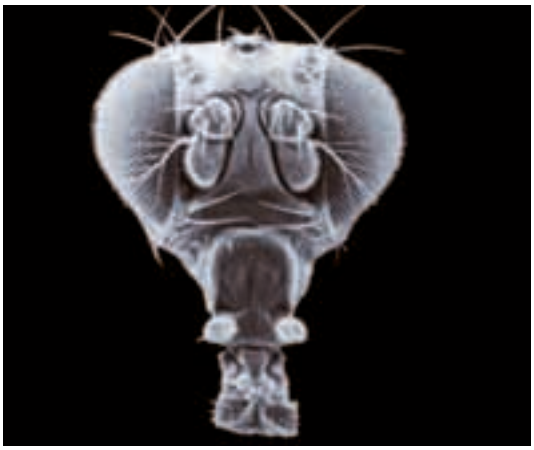
In the human body, one of the most important jobs for proteins is to control how embryos develop. Scientists discovered a hugely important set of proteins involved in development by studying mutations that cause bizarre malformations in fruit flies.

The most famous such abnormality is a fruit fly with a leg, rather than the usual antenna, growing out of its head (see page 21). According to Thomas C. Kaufman of Indiana University in Bloomington, the leg is perfectly normal—it's just growing in the wrong place.

In this type of mutation and many others, something goes wrong with the genetic program that directs some of the cells in an embryo to follow developmental pathways, which are a series of chemical reactions that occur in a specific order. In the antenna-into-leg problem, it is as if the cells growing from the fly's head, which normally would become an antenna, mistakenly believe that they are in the fly's thorax, and therefore ought to grow into a leg. And so they do.

Thinking about this odd situation taught scientists an important lesson—that the proteins made by some genes can act as switches. Switch genes are master controllers that provide each body part with a kind of identification card. If a protein that normally instructs cells to become an antenna is disrupted, cells can receive new instructions to become a leg instead.

FIGURE 14:



▲ Normal fruit fly head.

FLYBASE; R. TURNER

▲ Fruit fly head showing the effects of the *Antennapedia* gene. This fly has legs where its antennae should be.

Scientists determined that several different genes, each with a common sequence, provide these anatomical identification card instructions. Kaufman isolated and described one of these genes, which became known as *Antennapedia*, a word that means "antenna feet."

Kaufman then began looking a lot more closely at the molecular structure of the *Antennapedia* gene. In the early 1980s, he and other researchers made a discovery that has been fundamental to understanding evolution as well as developmental biology.

The scientists found a short sequence of DNA, now called the **homeobox**, that is present not only in *Antennapedia* but in the several genes next to it and in genes in many other organisms. When geneticists find very similar DNA sequences in the genes of different organisms, it's a good clue that these genes do something so important and useful that evolution uses the same sequence over and over and permits very few changes in its structure as new species evolve.

Researchers quickly discovered nearly identical versions of homeobox DNA in almost every non-bacterial cell they examined—from yeast to plants, frogs, worms, beetles, chickens, mice and people.

Hundreds of homeobox-containing genes have been identified, and the proteins they make turn out to be involved in the early stages of development of many species. For example, researchers have found that abnormalities in the homeobox genes can lead to extra fingers or toes in humans.

The Tools of Genetics: Mighty Microarrays

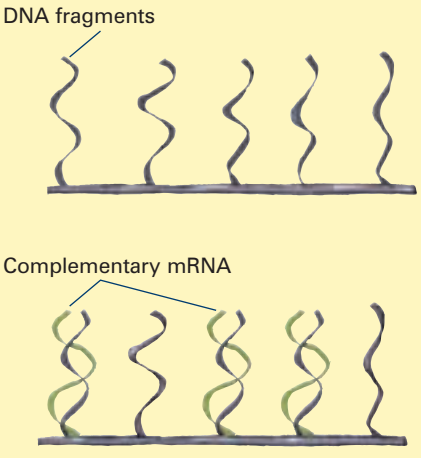
We now have the ability to attach a piece of every gene in a **genome** (all of an organism's genes) to a postage stamp-sized glass microscope slide. This ordered series of DNA spots is called a DNA **microarray**, a **gene chip** or a **DNA chip**.

Whichever name you prefer, the chip could also be called revolutionary. This technology has changed the way many geneticists do their work by making it possible to observe the activity of thousands of genes at once.

In recent years, microarrays have become standard equipment for modern biologists, but teachers and students are using them, too. The Genome Consortium for Active Teaching program (www.bio.davidson.edu/GCAT) provides resources and instructions for high school and college students to do gene-chip experiments in class.

Microarrays are used to get clues about which genes are expressed to control cell, tissue or organ function. By measuring the level of RNA production for every gene at the same time, researchers can learn the genetic programming that makes cell types different and diseased cells different from healthy ones.

The chips consist of large numbers of DNA fragments distributed in rows in a very small space. The arrays are laid out by robots that can



DNA fragments are attached to glass or plastic, then fluorescently tagged molecules are washed over the fragments.

Some molecules (green) bind to their complementary sequence. These molecules can be identified because they glow under fluorescent light.

▼ The resulting pattern of fluorescence indicates which genes are active.

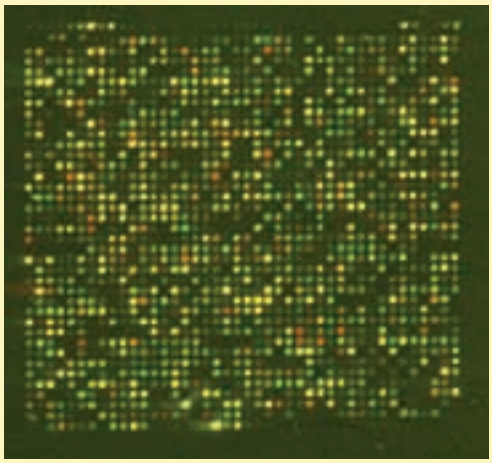
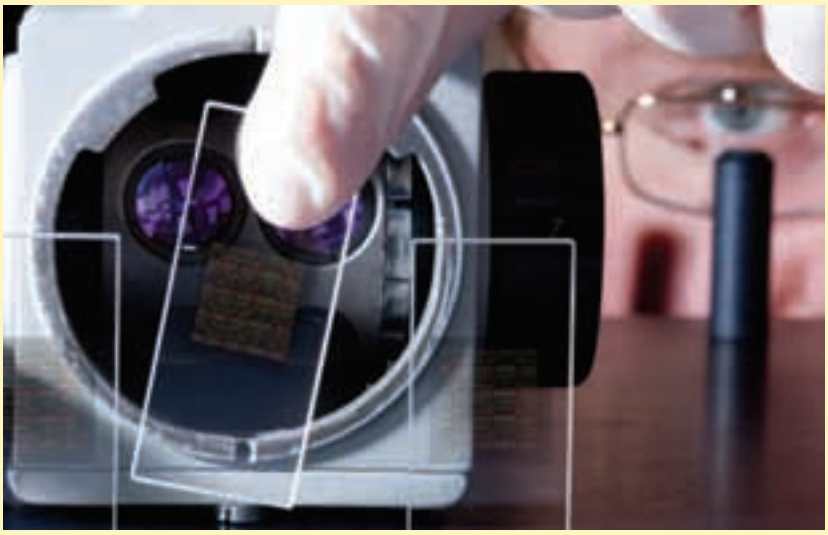


FIGURE 15:

FIGURE 16:



position DNA fragments so precisely that more than 20,000 of them can fit on one microscope slide.

Scientists isolate mRNA from cells grown under two conditions and tag the two sources of RNA with different colors of fluorescent molecules. The two colors of RNA are then placed on the chip, where they attach to complementary DNA fragments anchored to the chip's surface.

Next, a scanner measures the amount of fluorescence at each spot on the chip, revealing how active each gene was (how much mRNA each gene produced). A computer analyzes the patterns of gene activity, providing a snapshot of a genome under two conditions (*e.g.*, healthy or diseased).

In December 2004, the U.S. Food and Drug Administration cleared the first gene chip for medical use. The Amplichip CYP450™, made by Roche Molecular Systems Inc. of Pleasanton, California, analyzes variations in two genes that play a major role in the body's processing of many widely prescribed drugs. This information can help doctors choose the proper dose of certain medicines for an individual patient.

Got It?

Why are some infections hard to treat with antibiotics? What are some things researchers might do to solve this public health problem?

How does DNA work as a form of information storage?

How can 20,000 human genes provide the instructions for making hundreds of thousands of different proteins?

What newborn tests does your area hospital routinely do?

RNA and DNA Revealed: New Roles, New Rules

For many years, when scientists thought about heredity, DNA was the first thing to come to mind. It's true that DNA is the basic ingredient of our genes and, as such, it often steals the limelight from RNA, the other form of genetic material inside our cells.

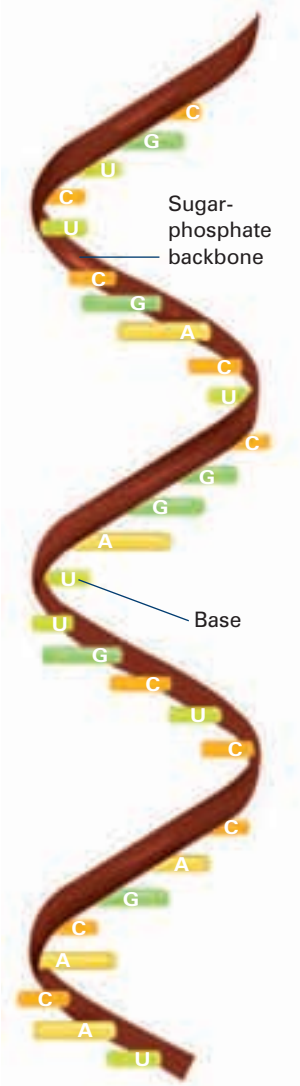
But, while they are both types of genetic material, RNA and DNA are rather different.

The chemical units of RNA are like those of DNA, except that RNA has the nucleotide uracil (U) instead of thymine (T). Unlike double-stranded DNA, RNA usually comes as only a single strand. And the nucleotides in RNA contain ribose sugar molecules in place of deoxyribose.

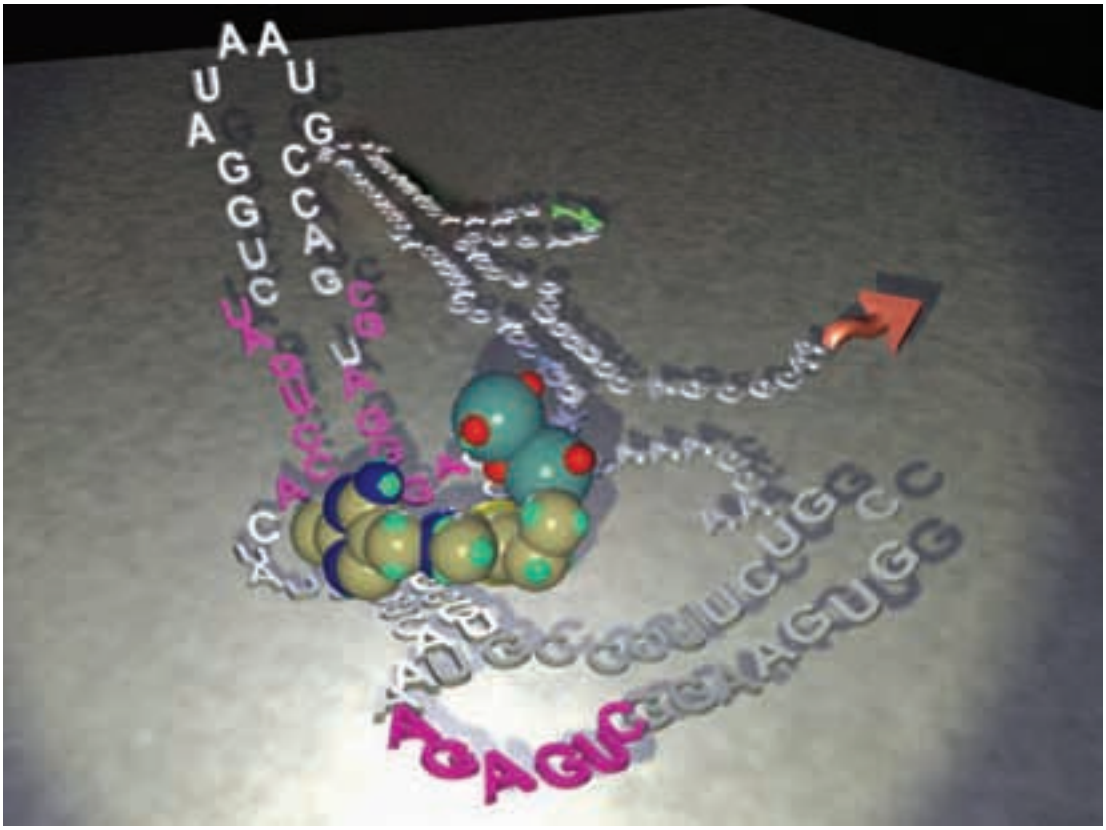
RNA is quite flexible—unlike DNA, which is a rigid, spiral-staircase molecule that is very stable. RNA can twist itself into a variety of complicated, three-dimensional shapes. RNA is also unstable in that cells constantly break it down and must continually make it fresh, while DNA is not broken down often. RNA's instability lets cells change their patterns of protein synthesis very quickly in response to what's going on around them.

Many textbooks still portray RNA as a passive molecule, simply a "middle step" in the cell's gene-reading activities. But that view is no longer accurate. Each year, researchers unlock new secrets about RNA. These discoveries reveal that it is truly a remarkable molecule and a multi - talented actor in heredity.

FIGURE 17:



▲ Ribonucleic acid (RNA) has the bases adenine (A), cytosine (C), guanine (G) and uracil (U).



RONALD BREAKER

FIGURE 18:

Riboswitches are RNA sequences that control gene activity. The riboswitch shown here bends into a special shape when it grips tightly onto a molecule called a metabolite (colored balls) that bacteria need to survive.

Today, many scientists believe that RNA evolved on the Earth long before DNA did. Researchers hypothesize—obviously, no one was around to write this down—that RNA was a major participant in the chemical reactions that ultimately spawned the first signs of life on the planet.

RNA World

At least two basic requirements exist for making a cell: the ability to hook molecules together and break them apart, and the ability to replicate, or copy itself, from existing information.

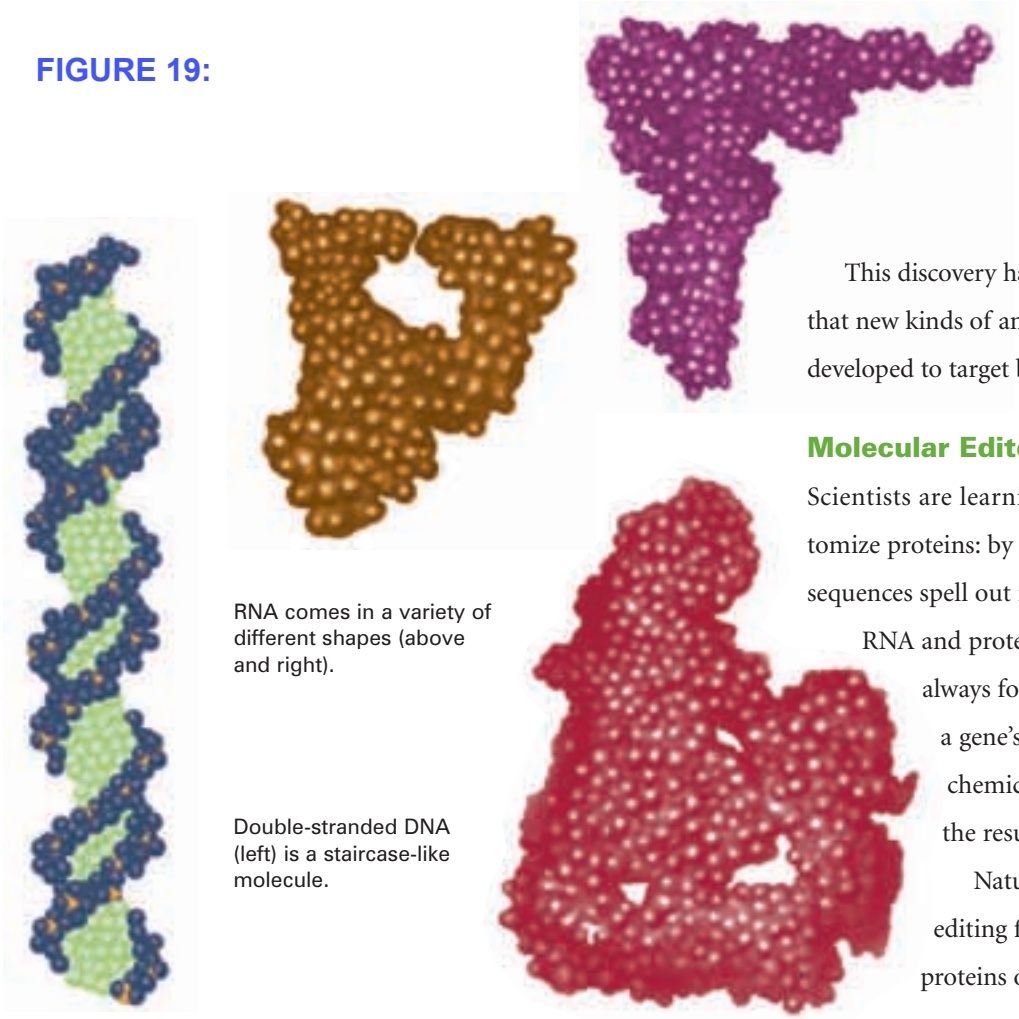
RNA probably helped to form the first cell. The first organic molecules, meaning molecules containing carbon, most likely arose out of random collisions of gases in the Earth's primitive atmosphere, energy from the Sun, and heat from naturally occurring radioactivity. Some scientists think that in this primitive world, RNA was a critical molecule because of its ability to lead a double life: to store information and to conduct chemical reactions. In other words, in this world, RNA served the functions of both DNA and proteins.

What does any of this have to do with human health? Plenty, it turns out.

Today's researchers are harnessing some of RNA's flexibility and power. For example, through a strategy he calls directed evolution, molecular engineer Ronald R. Breaker of Yale University is developing ways to create entirely new forms of RNA and DNA that both work as enzymes.

Breaker and others have also uncovered a hidden world of RNAs that play a major role in controlling gene activity, a job once thought to be performed exclusively by proteins. These RNAs, which the scientists named riboswitches, are found in a wide variety of bacteria and other organisms.

FIGURE 19:



RNA comes in a variety of different shapes (above and right).

Double-stranded DNA (left) is a staircase-like molecule.

This discovery has led Breaker to speculate that new kinds of antibiotic medicines could be developed to target bacterial riboswitches.

Molecular Editor

Scientists are learning of another way to customize proteins: by RNA editing. Although DNA sequences spell out instructions for producing RNA and proteins, these instructions aren't always followed precisely. Editing a gene's mRNA, even by a single chemical letter, can radically change the resulting protein's function.

Nature likely evolved the RNA editing function as a way to get more proteins out of the same number of

Small But Powerful

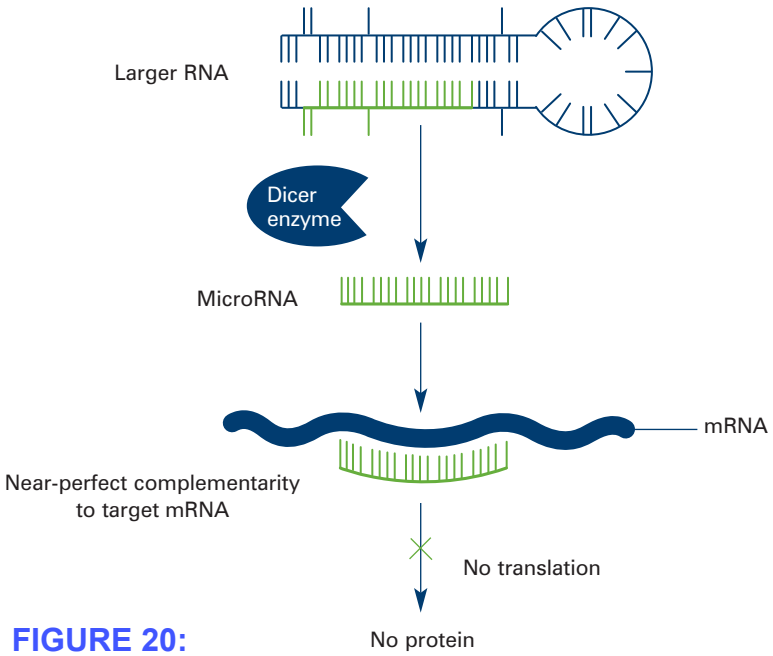


FIGURE 20:

The enzyme Dicer generates microRNAs by chopping larger RNA molecules into tiny Velcro®-like pieces. MicroRNAs stick to mRNA molecules and prevent the mRNAs from being made into proteins.

Recently, molecules called **microRNAs** have been found in organisms as diverse as plants, worms and people. The molecules are truly "micro," consisting of only a few dozen nucleotides, compared to typical human mRNAs that are a few thousand nucleotides long.

What's particularly interesting about microRNAs is that many of them arise from DNA that used to be considered merely filler material (see PDF page #'s 14-17 "Getting the Message" section).

How do these small but important RNA molecules do their work? They start out much bigger but get trimmed by cellular enzymes, including one aptly named Dicer. Like tiny pieces of

genes. For example, researchers have found that the mRNAs for certain proteins important for the proper functioning of the nervous system are particularly prone to editing. It may be that RNA editing gives certain brain cells the capacity to react quickly to a changing environment.

Which molecules serve as the editor and how does this happen? Brenda Bass of the University of Utah School of Medicine in Salt Lake City studies one particular class of editors called adenosine deaminases. These enzymes "retype" RNA letters at various places within an mRNA transcript.

They do their job by searching for characteristic RNA shapes. Telltale twists and bends in folded RNA molecules signal these enzymes to change the RNA sequence, which in turn changes the protein that gets made.

Bass' experiments show that RNA editing occurs in a variety of organisms, including people. Another interesting aspect of editing is that certain disease-causing microorganisms, such as some forms of parasites, use RNA editing to gain a survival edge when living in a human host. Understanding the details of this process is an important area of medical research.

FIGURE 21:

AMY PASQUINELLI

Worms with a mutated form of the microRNA let-7 (right) have severe growth problems, rupturing as they develop.

Velcro®, microRNAs stick to certain mRNA molecules and stop them from passing on their protein-making instructions.

First discovered in a roundworm model system (see ~~*Living Laboratories*, page 49~~), some microRNAs help determine the organism's body plan. In their absence, very bad things can happen. For example, worms engineered to lack a microRNA called let-7 develop so abnormally that they often rupture and practically break in half as the worm grows.

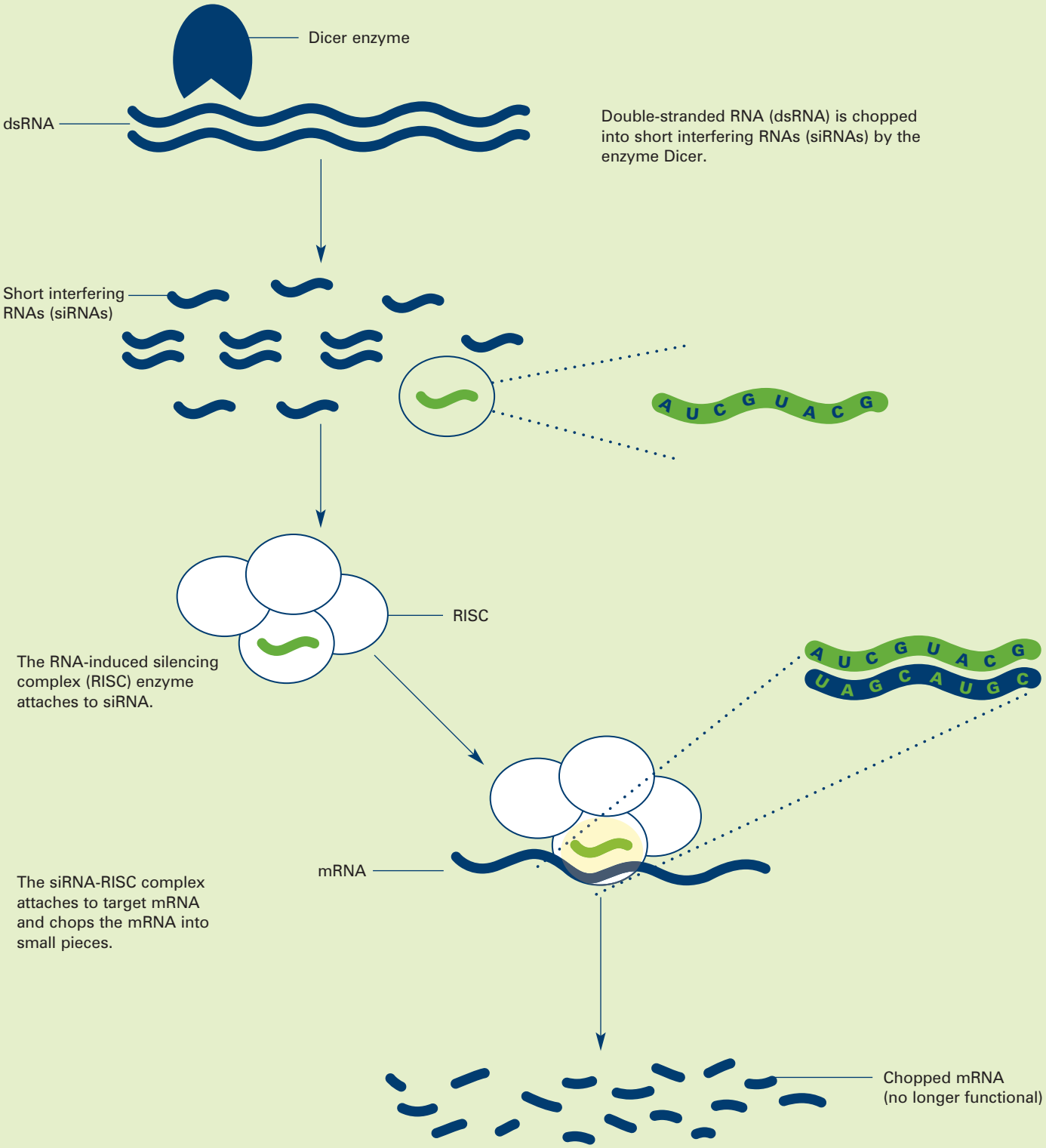
Perhaps it is not surprising that since microRNAs help specify the timing of an organism's developmental plan, the appearance of the microRNAs themselves is carefully timed inside a developing organism. Biologists, including Amy Pasquinelli of the University of California, San Diego, are currently figuring out how microRNAs are made and cut to size, as well as how they are produced at the proper time during development.

MicroRNA molecules also have been linked to cancer. For example, Gregory Hannon of the Cold Spring Harbor Laboratory on Long Island, New York, found that certain microRNAs are associated with the severity of the blood cancer B-cell lymphoma in mice.

Since the discovery of microRNAs in the first years of the 21st century, scientists have identified hundreds of them that likely exist as part of a large family with similar nucleotide sequences. New roles for these molecules are still being found.

FIGURE 22:

RNA Interference (RNAi)



Healthy Interference

RNA controls genes in a way that was only discovered recently: a process called **RNA interference**, or **RNAi**. Although scientists identified RNAi less than 10 years ago, they now know that organisms have been using this trick for millions of years.

Researchers believe that RNAi arose as a way to reduce the production of a gene's encoded protein for purposes of fine-tuning growth or self-defense. When viruses infect cells, for example, they command their host to produce specialized RNAs that allow the virus to survive and make copies of itself. Researchers believe that RNAi eliminates unwanted viral RNA, and some speculate that it may even play a role in human immunity.

Oddly enough, scientists discovered RNAi from a failed experiment! Researchers investigating genes involved in plant growth noticed something strange: When they tried to turn petunia flowers purple by adding an extra "purple" gene, the flowers bloomed white instead.

This result fascinated researchers, who could not understand how adding genetic material could somehow get rid of an inherited trait. The mystery remained unsolved until, a few years later, two geneticists studying development saw a similar thing happening in lab animals.

The researchers, Andrew Z. Fire, then of the Carnegie Institution of Washington in Baltimore and now at Stanford University, and Craig Mello of the University of Massachusetts Medical School in Worcester, were trying to block the expression of genes that affect cell growth and tissue formation in roundworms, using a molecular tool called antisense RNA.

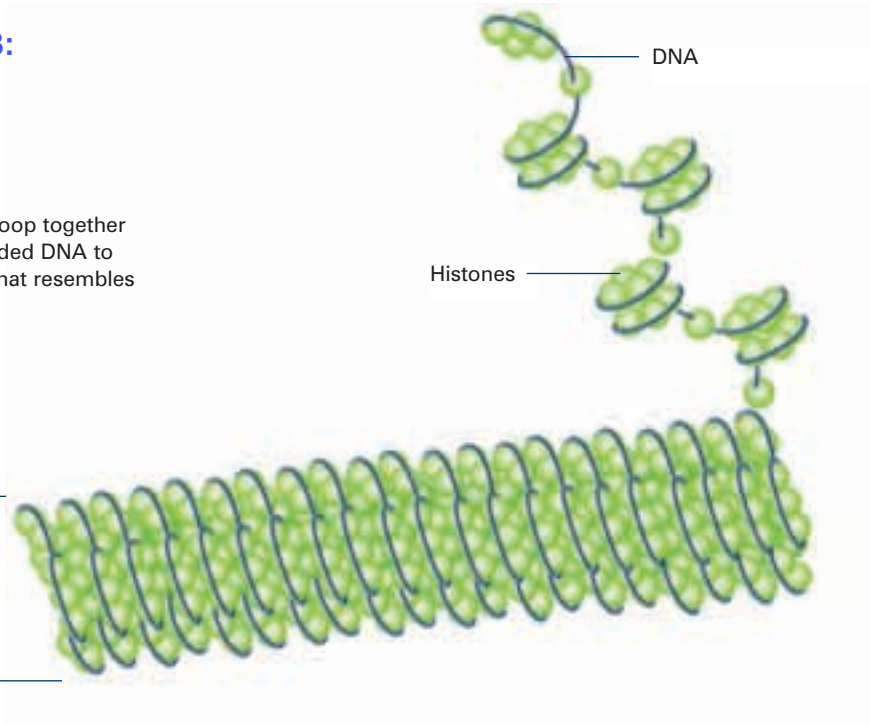
To their surprise, Mello and Fire found that their antisense RNA tool wasn't doing much at all. Rather, they determined, a double-stranded contaminant produced during the synthesis of the single-stranded antisense RNA interfered with **gene expression**. Mello and Fire named the process RNAi, and in 2006 were awarded the Nobel Prize in physiology or medicine for their discovery.

Further experiments revealed that the double-stranded RNA gets chopped up inside the cell into much smaller pieces that stick to mRNA and block its action, much like the microRNA pieces of Velcro discussed above (see drawing, page 28).

Today, scientists are taking a cue from nature and using RNAi to explore biology. They have learned, for example, that the process is not limited to worms and plants, but operates in humans too.

Medical researchers are currently testing new types of RNAi-based drugs for treating conditions such as macular degeneration, the leading cause of blindness, and various infections, including those caused by HIV and the herpes virus.

FIGURE 23:



Histone proteins loop together with double-stranded DNA to form a structure that resembles beads on a string.

Dynamic DNA

A good part of who we are is "written in our genes," inherited from Mom and Dad. Many traits, like red or brown hair, body shape and even some personality quirks, are passed on from parent to offspring.

But genes are not the whole story. Where we live, how much we exercise, what we eat: These and many other environmental factors can all affect how our genes get expressed.

You know that changes in DNA and RNA can produce changes in proteins. But additional control happens at the level of DNA, even though these changes do not alter DNA directly. Inherited factors that do not change the DNA sequence of nucleotides are called **epigenetic** changes, and they too help make each of us unique.

Epigenetic means, literally, "upon" or "over" genetics. It describes a type of chemical reaction that can alter the physical properties of DNA without changing its sequence. These changes make genes either more or less likely to be expressed (see Figure 24).

Currently, scientists are following an intriguing course of discovery to identify epigenetic factors that, along with diet and other environmental influences, affect who we are and what type of illnesses we might get.

Secret Code

DNA is spooled up compactly inside cells in an arrangement called **chromatin**. This packaging is critical for DNA to do its work. Chromatin consists of long strings of DNA spooled around a compact assembly of proteins called **histones**.

One of the key functions of chromatin is to control access to genes, since not all genes are turned on at the same time. Improper expression of growth-promoting genes, for example, can lead to cancer, birth defects or other health concerns.

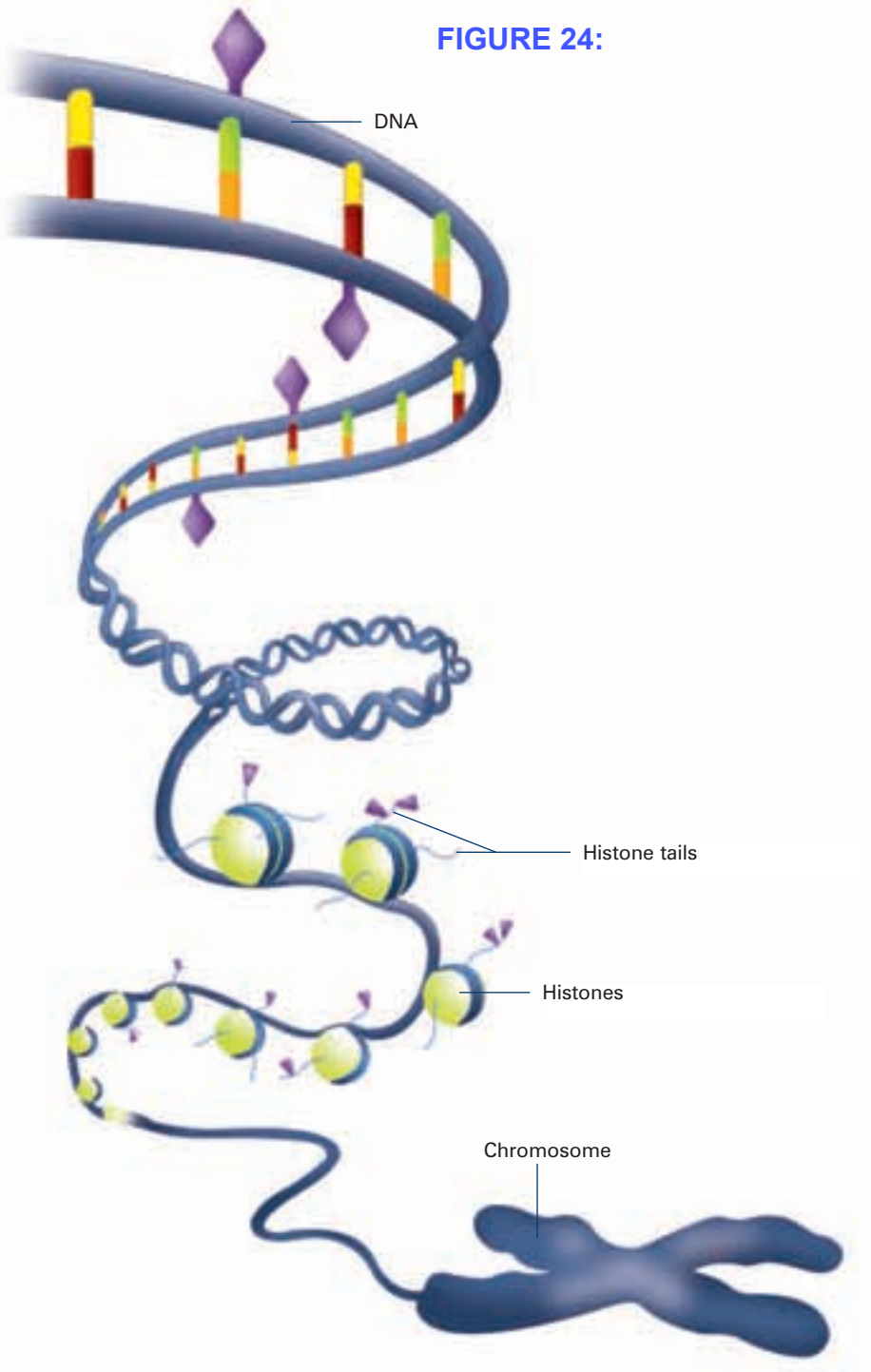
Many years after the structure of DNA was determined, researchers used a powerful device known as an electron microscope to take pictures of chromatin fibers. Upon viewing chromatin up close, the researchers described it as "beads on a string," an image still used today. The beads were the histone balls, and the string was DNA wrapped around the histones and connecting one bead to the next.

Decades of study eventually revealed that histones have special chemical tags that act like switches to control access to the DNA. Flipping these switches, called epigenetic markings, unwinds the spooled DNA so the genes can be transcribed.

The observation that a cell's gene-reading machinery tracks epigenetic markings led C. David Allis, who was then at the University of Virginia Health Sciences Center in Charlottesville and now works at the Rockefeller University in New York City, to coin a new phrase, the "histone code." He and others believe that the histone code plays a major role in determining which proteins get made in a cell.

Flaws in the histone code have been associated with several types of cancer, and researchers are actively pursuing the development of medicines to correct such errors.

FIGURE 24:



The "epigenetic code" controls gene activity with chemical tags that mark DNA (purple diamonds) and the "tails" of histone proteins (purple triangles). These markings help determine whether genes will be transcribed by RNA polymerase. Genes hidden from access to RNA polymerase are not expressed.

GENETICS AND YOU: *The Genetics of Anticipation*

Occasionally, unusual factors influence whether or not a child will be born with a genetic disease.

An example is the molecular error that causes Fragile X syndrome, a rare condition associated with mental retardation. The mutation leading to a fragile X chromosome is not a typical DNA typing mistake, in which nucleotides are switched around or dropped, or one of them is switched for another nucleotide. Instead, it is a kind of stutter by the DNA polymerase enzyme that copies DNA. This stutter creates a string of repeats of a DNA sequence that is composed of just three nucleotides, CGG.

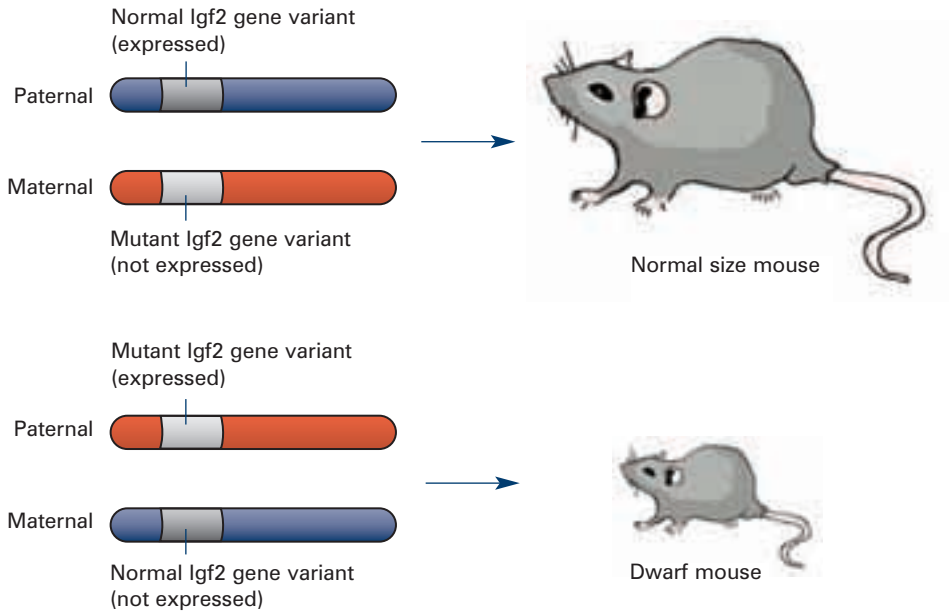
Some people have only one repeat of the CGG nucleotide triplet. Thus, they have two copies of the repeat in a gene, and the extra sequence reads CGGCGG. Others have more than a thousand copies of the repeat. These people are the most severely affected.

The number of triplet repeats seems to increase as the chromosome is passed down through several generations. Thus, the grandsons of a man with a fragile X chromosome, who is not himself affected, have a 40 percent risk of retardation if they inherit the repeat-containing chromosome. The risk for great-grandsons is even higher: 50 percent.

Intrigued by the evidence that triplet repeats can cause genetic disease, scientists have searched for other examples of disorders associated with the DNA expansions. To date, more than a dozen such disorders have been found, and all of them affect the nervous system.

Analysis of the rare families in which such diseases are common has revealed that expansion of the triplet repeats is linked to something called genetic **anticipation**, when a disease's symptoms appear earlier and more severely in each successive generation.

FIGURE 25:



Igf2 is an imprinted gene. A single copy of the abnormal, or mutant, form of the Igf2 gene (red) causes growth defects, but only if the abnormal gene variant is inherited from the father.

Battle of the Sexes

A process called **imprinting**, which occurs naturally in our cells, provides another example of how epigenetics affects gene activity.

With most genes, the two copies work exactly the same way. For some mammalian genes, however, only the mother's or the father's copy is switched on regardless of the child's gender. This is because the genes are chemically marked, or imprinted, during the process that generates eggs and sperm.

As a result, the embryo that emerges from the joining of egg and sperm can tell whether a gene copy came from Mom or Dad, so it knows which copy of the gene to shut off.

One example of an imprinted gene is insulin-like growth factor 2 (Igf2), a gene that helps a mammalian fetus grow. In this case, only the father's copy of Igf2 is expressed, and the mother's copy remains silent (is not expressed) throughout the life of the offspring.

Scientists have discovered that this selective silencing of Igf2 and many other imprinted genes occurs in all placental mammals (all except the platypus, echidna and marsupials) examined so far, but not in birds.

Why would nature tolerate a process that puts an organism at risk because only one of two copies of a gene is working? The likely reason, many researchers believe, is that mothers and fathers have competing interests, and the battlefield is DNA!

The scenario goes like this: It is in a father's interest for his embryos to get bigger faster, because that will improve his offspring's chances of survival after birth. The better an individual's

chance of surviving infancy, the better its chance of becoming an adult, mating and passing its genes on to the next generation.

Of course mothers want strong babies, but unlike fathers, mothers provide physical resources to embryos during pregnancy. Over her lifetime, a female is likely to be pregnant several times, so she needs to divide her resources among a number of embryos in different pregnancies.

Researchers have discovered over 200 imprinted genes in mammals since the first one was identified in 1991. We now know that imprinting controls some of the genes that have an important role in regulating embryonic and fetal growth and allocating maternal resources. Not surprisingly, mutations in these genes cause serious growth disorders.

Marisa Bartolomei of the University of Pennsylvania School of Medicine in Philadelphia is trying to figure out how Igf2 and other genes become imprinted and stay silent throughout the life of an individual. She has already identified sequences within genes that are essential for imprinting. Bartolomei and other researchers have shown that these sequences, called insulators, serve as "landing sites" for a protein that keeps the imprinted gene from being transcribed.

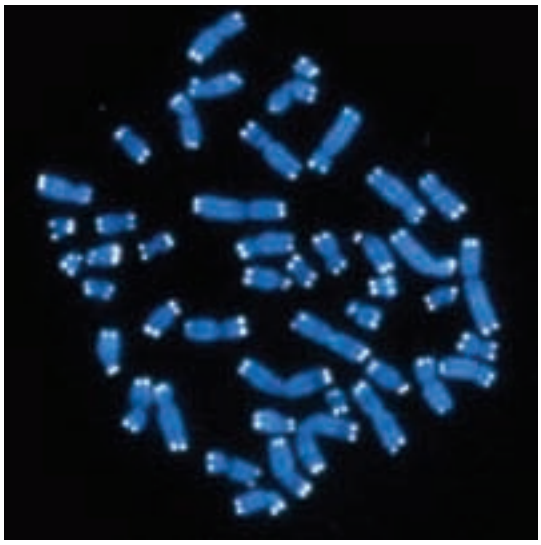
Starting at the End

When we think of DNA, we think of genes. However, some DNA sequences are different: They don't encode RNAs or proteins. Introns, described in Chapter 1, are in this category.

Another example is **telomeres**—the ends of chromosomes. There are no genes in telomeres, but they serve an essential function. Like shoelaces without their tips, chromosomes without telomeres unravel and fray. And without telomeres, chromosomes stick to each other and cause cells to undergo harmful changes like dividing abnormally.

Researchers know a good deal about telomeres, dating back to experiments performed in the 1970s by Elizabeth Blackburn, a basic researcher who was curious about some of the fundamental events that take place within cells.

FIGURE 26:



HESED PADILLA-NASH AND THOMAS RIED

Telomeres, repeated nucleotide sequences at the tips of chromosomes, appear white in this photo.

FIGURE 27:

Molecular biologist Carol Greider discovered the enzyme telomerase. This license plate, which was on her car when she worked at Cold Spring Harbor Laboratory on Long Island, New York, advertises her research interest!

At the time, Blackburn, now at the University of California, San Francisco, was working with Joseph Gall at Yale University. For her experimental system, she chose a single-celled, pond-dwelling organism named *Tetrahymena*. These tiny, pear-shaped creatures are covered with hairlike cilia that they use to propel themselves through the water as they devour bacteria and fungi.

Tetrahymena was a good organism for Blackburn's experiments because it has a large number of chromosomes—which means it has a lot of telomeres!

Her research was also perfectly timed, because methods for sequencing DNA were just being developed. Blackburn found that *Tetrahymena's* telomeres had an unusual nucleotide sequence: TTGGGG, repeated about 50 times per telomere.

Since then, scientists have discovered that the telomeres of almost all organisms have repeated sequences of DNA with lots of Ts and Gs. In human and mouse telomeres, for example, the repeated sequence is TTAGGG.

The number of telomere repeats varies enormously, not just from organism to organism but in different cells of the same organism and even within a single cell over time. Blackburn reasoned that the repeat number might vary if cells had an enzyme that added copies of the repeated sequence to the telomeres of some but not all chromosomes.

With her then-graduate student Carol Greider, now at Johns Hopkins University, Blackburn hunted for the enzyme. The team found it and Greider named it telomerase. Blackburn, Greider and Jack Szostak of Harvard Medical School in Boston shared the 2009 Nobel Prize in physiology or medicine for their discoveries about telomeres and telomerase.

As it turns out, the telomerase enzyme consists of a protein and an RNA component, which the enzyme uses as a template for copying the repeated DNA sequence.

What is the natural function of telomerase? As cells divide again and again, their telomeres get shorter. Most normal cells stop dividing when their telomeres wear down to a certain point, and eventually the cells die. Telomerase can counteract the shortening. By adding DNA to telomeres, telomerase rebuilds the telomere and resets the cell's molecular clock.

The discovery of telomerase triggered new ideas and literally thousands of new studies. Many researchers thought that the enzyme might play important roles in cancer and aging. Researchers were hoping to find ways to turn telomerase *on* so that cells would continue to divide (to grow extra cells for burn patients, for example), or *off* so that cells would stop dividing (to stop cancer, for instance).

So far, they have been unsuccessful. Although it is clear that telomerase and cellular aging are related, researchers do not know whether telomerase plays a role in the normal cellular aging process or in diseases like cancer.

Recently, however, Blackburn and a team of other scientists discovered that chronic stress and the perception that life is stressful affect telomere length and telomerase activity in the cells of healthy women. Blackburn and her coworkers are currently conducting a long-term, follow-up study to confirm these intriguing results.

The Other Human Genome

Before you think everything's been said about DNA, there's one little thing we didn't mention: Some of the DNA in every cell is quite different from the DNA that we've been talking about up to this point. This special DNA isn't in chromosomes—it isn't even inside the cell's nucleus where all the chromosomes are!

So where is this special DNA? It's inside **mitochondria**, the organelles in our cells that produce the energy-rich molecule adenosine triphosphate, or ATP. Mendel knew nothing of mitochondria, since they weren't discovered until late in the 19th century. And it wasn't until the 1960s that researchers discovered the mitochondrial genome, which is circular like the genomes of bacteria.

In human cells, mitochondrial DNA makes up less than 1 percent of the total DNA in each of our cells. The mitochondrial genome is very small—containing only about three dozen genes. These encode a few of the proteins that are in the mitochondrion, plus a set of ribosomal RNAs used for synthesizing proteins for the organelle.

Mitochondria need many more proteins though, and most of these are encoded by genes in the nucleus. Thus, the energy-producing capabilities of human mitochondria—a vital part of any cell's everyday health—depend on coordinated teamwork among hundreds of genes in two cellular neighborhoods: the nucleus and the mitochondrion.

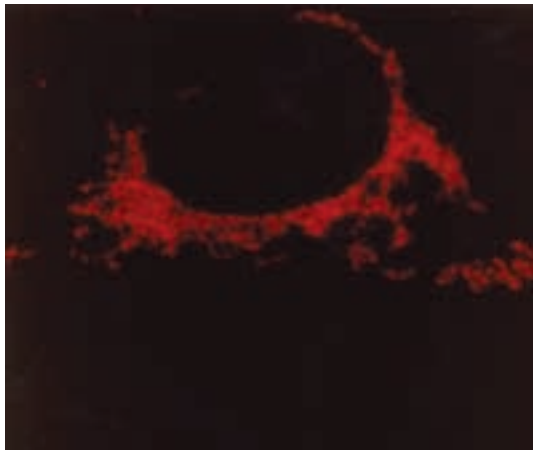
FIGURE 28:

Mitochondrial DNA gets transcribed and the RNA is translated by enzymes that are very different from those that perform this job for genes in our chromosomes. Mitochondrial enzymes look and act much more like those from bacteria, which is not surprising because mitochondria are thought to have descended from free-living bacteria that were engulfed by another cell over a billion years ago.

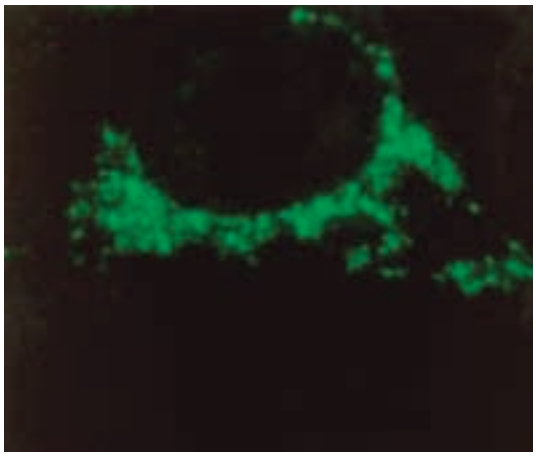
Scientists have linked mitochondrial DNA defects with a wide range of age-related diseases including neurodegenerative disorders, some forms of heart disease, diabetes and various cancers. It is still unclear, though, whether damaged mitochondria are a symptom or a cause of these health conditions.

Scientists have studied mitochondrial DNA for another reason: to understand the history of the human race. Unlike our chromosomal DNA, which we inherit from both parents, we get all of our mitochondrial DNA from our mothers.

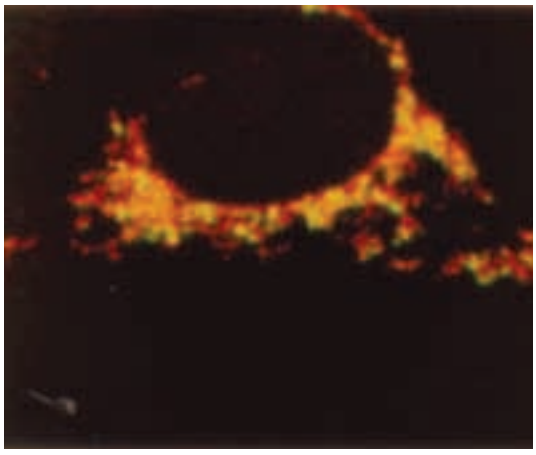
Thus, it is possible to deduce who our maternal ancestors were by tracking the inheritance of mutations in mitochondrial DNA. For reasons that are still not well understood, mutations accumulate in mitochondrial DNA more quickly than in chromosomal DNA. So, it's possible to trace your maternal ancestry way back beyond any relatives you may know by name—all the way back to "African Eve," the ancestor of us all!



Mitochondria (labeled with a red dye) are scattered throughout the cytoplasm of this human cancer cell.



The cell has also been treated with a dye that colors the mitochondrial DNA green.

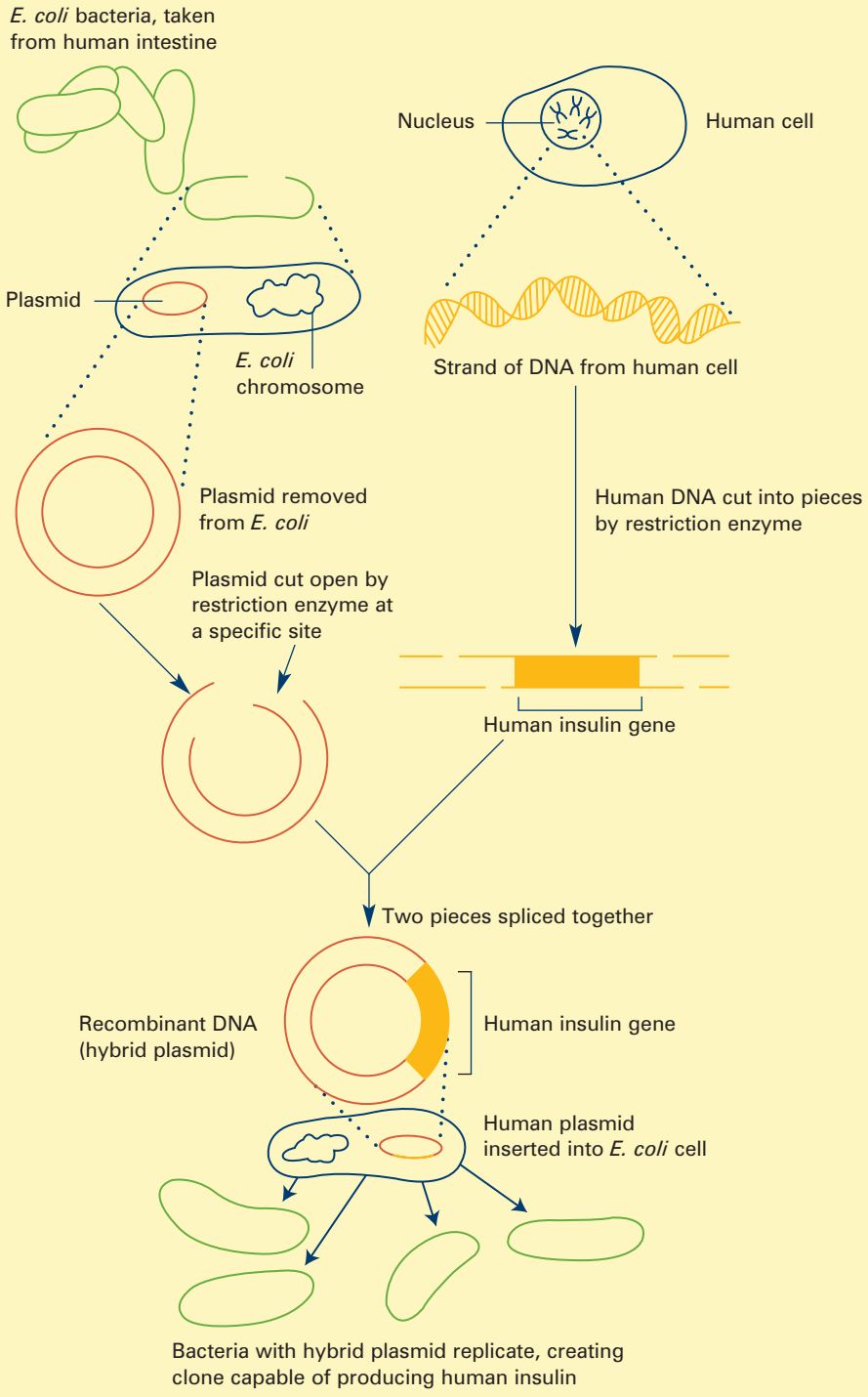


A computerized overlay of these two images of the same cell shows that mitochondria and its DNA appear together (yellow regions).

ALISON DAVIS

FIGURE 29:

The Tools of Genetics: Recombinant DNA and Cloning



Recombinant DNA. To splice a human gene (in this case, the one for insulin) into a plasmid, scientists take the plasmid out of an *E. coli* bacterium, cut the plasmid with a restriction enzyme and splice in insulin-making human DNA. The resulting hybrid plasmid can be inserted into another *E. coli* bacterium, where it multiplies along with the bacterium. There, it can produce large quantities of insulin.

FIGURE 30:

Scientists in Scotland were the first to clone an animal, this sheep named Dolly. She later gave birth to Bonnie, the lamb next to her.

ROSLIN INSTITUTE, EDINBURGH

In the early 1970s, scientists discovered that they could change an organism's genetic traits by putting genetic material from another organism into its cells. This discovery, which caused quite a stir, paved the way for many extraordinary accomplishments in medical research that have occurred over the past 35 years.

How do scientists move genes from one organism to another? The cutting and pasting gets done with chemical scissors: enzymes, to be specific. Take insulin, for example. Let's say a scientist wants to make large quantities of this protein to treat diabetes. She decides to transfer the human gene for insulin into a bacterium, *Escherichia coli*, or *E. coli*, which is commonly used for genetic research (see *Living Laboratories*, page 46). That's because *E. coli* reproduces really fast, so after one bacterium gets the human insulin gene, it doesn't take much time to grow millions of bacteria that contain the gene.

The first step is to cut the insulin gene out of a copied, or "cloned," version of the human DNA using a special bacterial enzyme from bacteria called a restriction endonuclease. (The normal role of these enzymes in bacteria is to chew up the DNA of viruses and other invaders.) Each restriction enzyme recognizes and cuts at a different nucleotide sequence, so it's possible to be very precise about DNA cutting by selecting one of several hundred of these enzymes that cuts at the desired sequence. Most restriction endonucleases make slightly staggered incisions, resulting in "sticky ends," out of which one strand protrudes.

The next step in this example is to splice, or paste, the human insulin gene into a circle of bacterial DNA called a plasmid. Attaching the cut ends together is done with a different enzyme (obtained from a virus), called DNA ligase. The sticky ends join back together kind of like jigsaw puzzle pieces. The result: a cut-and-pasted mixture of human and bacterial DNA.

The last step is putting the new, **recombinant DNA** back into *E. coli* and letting the bacteria reproduce in a petri dish. Now, the scientist has a great tool: a version of *E. coli* that produces lots of human insulin that can be used for treating people with diabetes.

So, what is cloning? Strictly speaking, it's making many copies. However, the term is more commonly used to refer to making many copies of a gene, as in the *E. coli* example above. Researchers can also **clone** entire organisms, like Dolly the sheep, which contained the identical genetic material of another sheep.

Got It?

Besides the sequence of nucleotides in genes, what are some other changes to DNA and RNA that can affect our health and who we are?

Can you imagine treat ments—other than vaccines and current medicines—crafted from genetic information and new molecular tools?

How is cloning a gene different from cloning an animal or a person? How do researchers use gene cloning to study health and disease?

Do you have any recurring illnesses in your extended family?

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VACCINES: FDA'S OVERSIGHT ROLE

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COURSE OBJECTIVES

At the end of this course you will be able to:

1.) Briefly recall the FDA's role in vaccine development.

2.) List the Phases in the Clinical Development Process and what happens in each stage.

3.) Discuss special circumstances in vaccine development, such as an EUA, and when they can be used.

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> A **Vaccine** is a biological preparation that provides the recipient acquired immunity to a specific infectious disease.

FDA'S OVERSIGHT ROLE IN VACCINES COMING TO MARKET

The US Food and Drug Administration (FDA) is the regulatory body that has oversight for the safety, effectiveness, and overall quality of all vaccines that are distributed in the United States.

Since vaccines are given to millions of people of all age ranges, it's one of the FDA's top priorities that they are demonstrated to be safe and effective before distribution.

Vaccine development is a complex process, so the FDA maintains a robust system of providing scientific and regulatory advice to the vaccine developers, as well as performing evaluations to determine the safety and effectiveness of those vaccines. The FDA's Center for Biologics Evaluation and Research (CBER) ensures that the FDA's rigorous scientific and regulatory processes are followed by those who pursue the development of vaccines.

STEPS IN THE FDA APPROVAL PROCESS

Figure 1: Basic Steps in the FDA Vaccine Approval Process

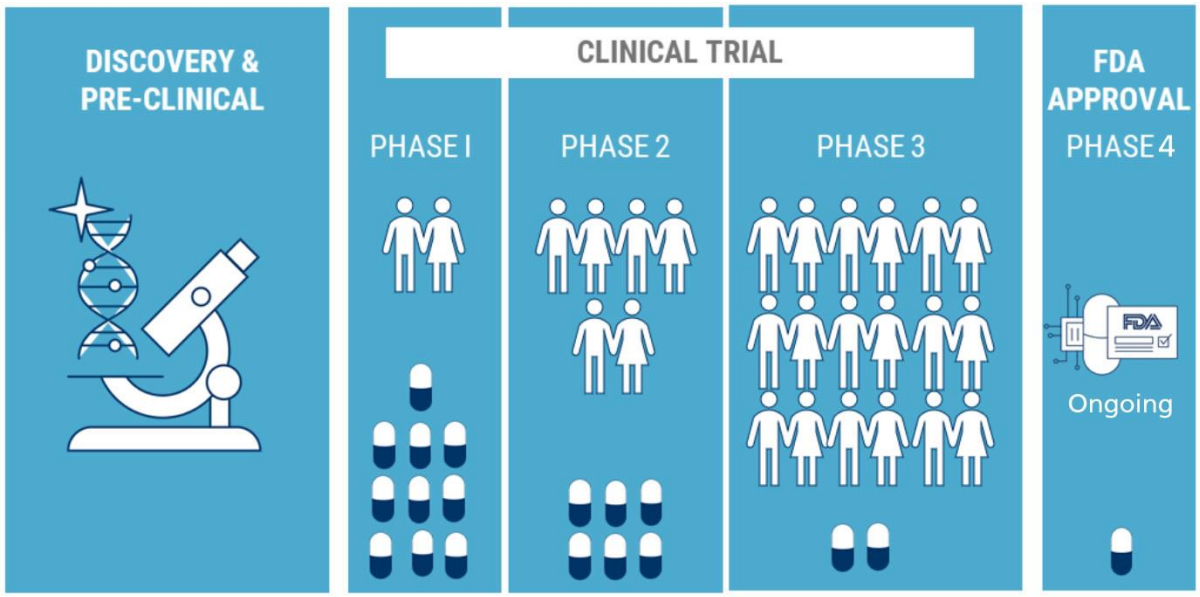

Figure 2: Detailed Steps in the FDA Vaccine & Drug Approval Process

PHASE 1	PHASE 2	PHASE 3
Purpose Safety and dosage	**Purpose** Efficacy and side effects	**Purpose** Efficacy and monitoring adverse reactions
Study Participants 20–100 healthy volunteers or people with the disease/condition	**Study Participants** Up to several hundred people with the disease/ condition	**Study Participants** 300–3,000 volunteers who have the disease or condition
Length of Study Several months	**Length of Study** Several months to 2 years	**Length of Study** 1 to 4 years
Approximately 70% of drugs move to the next phase	Approximately 33% of drugs move to the next phase	Approximately 25–30% of drugs move to the next phase

NOTE: Although most vaccines and drugs follow the rigorous process in this graphic, the COVID-19 vaccine was release quickly under an Emergency Use Authorization (EUA) due to the urgency of the situation.

RESEARCH AND DISCOVERY

The first step in vaccine development is the Research and Discovery Stage. Here scientists develop a plan for a vaccine based on how the infectious organism causes disease.

Once a rationale for their plan is in place, the scientists then conduct laboratory research to test their idea for that vaccine candidate to confirm whether their idea will work. This initial step is typically conducted on animals. If the scientific findings are thought to have practical applications in humans, the research moves forward.

PRE-CLINICAL STAGE

Before a vaccine can be tested on humans, researchers perform additional laboratory testing on animals to obtain information about how the vaccine works, whether it produces the expected results, and whether it's likely to be safe in humans. These tests are known as the Pre-clinical phase.

CLINICAL DEVELOPMENT

Research moves to the Clinical Development Stage when the vaccine is ready to be tested on humans. To reach this stage, the vaccine company will compile the results from all their pre-clinical and discovery testing, as well as the manufacturing technology that will be used to produce the vaccine and submit that to the FDA in what is known as an Investigational New Drug application or IND.

Once the IND is received, the FDA evaluates the pre-clinical data to determine whether the testing was conducted according to Good Laboratory Practices. In this evaluation, the FDA conducts an assessment of the product, its quality, safety, and technology to manufacture it, to determine whether it is safe to move into human trials. If it's determined it can move forward, it will move into the Clinical Development Stage, which typically consists of three pre-market phases under the oversight of the FDA.

> **Good Laboratory Practices** are a set of principles intended to ensure the quality and integrity of non-clinical laboratory testing intended to support research for products regulated by the government.

Clinical Development phases typically progress sequentially, although it's not uncommon for phases of development to overlap at some points in the process. The phases include the following:

PHASE 1

Phase 1 is the initial phase tested on human volunteers. Emphasis during this phase is on product safety and generally includes 20–100 volunteers who have not previously been exposed to the disease being studied and who are generally otherwise healthy.

Studies in this phase are used to determine whether there are adverse reactions with increasing doses and, if possible, to gain early information about how well the vaccine works to induce an immune response in people.

PHASE 2

Provided that no safety concerns are observed in phase 1 studies, the trial moves on to phase 2. Phase 2 randomized-controlled studies include:

- A larger group of participants (typically in the 100s)
- Varying dosages of the vaccine are tested
- Participants have various health statuses
- Multiple demographic groups

These studies provide additional safety information on common short-term side effects and risks, examines the relationship between the dose administered and the immune response, and provides initial information regarding the effectiveness of the vaccine and its ability to generate an immune response. Standardized and validated tests are used to evaluate the immune responses.

In this phase, the vaccine studies typically include:

- A control group of those who received the vaccine
- A placebo group who receives another substance, such as saline

So as not to affect the volunteer's reporting of possible side effects, most trials conducted are what is known as a Double-Blind Trial. Once phase 2 is complete, people who received the vaccine are compared to those in the control group to assess the vaccine's effectiveness.

> A **Double-Blind Study** is one in which neither the participants nor the patient's doctor know whether the patient has received the actual vaccine or whether they are in the placebo group, only the directors of the study know. This is to prevent bias when reporting side effects.

PHASE 3

In phase 3 clinical trials, the vaccine is generally administered to 1000s of volunteers, which generates critical information on effectiveness and important safety data.

This phase includes additional information about the recipient's immune response and compares those who receive the vaccine to those who receive a placebo. For example, the number of disease cases in the vaccinated group is compared to the number in the control group to see whether the vaccine reduces the incidence of the disease it's trying to prevent. This phase also provides information about the vaccine's longer-term safety and helps identify less common side effects.

Assessment of Manufacturing as a Key Component

While the vaccine is being tested on humans in Clinical Development, the FDA is also assessing information pertaining to the manufacturing of the vaccine and the facility where it will be made. Vaccine manufacturing is complex and the process of making the candidate vaccine for the phase 3 studies in batches called "lots" helps the manufacturer ramp up for

commercial-scale manufacturing. FDA requires vaccine manufacturers to submit data to support manufacturing processes, facilities, product characterization, and demonstration of lot-to-lot consistency. FDA works with the manufacturer to develop a lot release protocol – a template of tests to be conducted on the vaccine- that will be used for each lot of vaccine post-approval. Experienced FDA-investigators carefully examine and evaluate the facility and operation for compliance with FDA regulations.

Once a manufacturing process is developed that ensures that the vaccine can be produced reliably and consistently, and the preclinical and clinical development programs have been successfully completed, companies submit a Biologics License Application (BLA) to the FDA. A BLA is a comprehensive submission that is submitted to the Agency. It includes preclinical and clinical data and information, as well as details of the manufacturing process and facilities.

Seeking Approval

As Phase 3 ends, a company seeking permission to distribute and market a vaccine for use in the United States would submit a BLA to the FDA. The FDA then evaluates the data to determine whether the safety and effectiveness of the vaccine has been satisfactorily demonstrated and whether the manufacturing and facility information assure product quality and consistency. After its evaluation, the FDA decides whether or not to approve (license) the vaccine for use in the United States. If FDA approves the vaccine, the company is permitted to market it in the United States for use in the population for which it is approved.

The FDA makes its decisions based on analysis of the benefits and risks for the intended population who will receive the vaccine, as well as the

disease(s) to be prevented. The FDA's scientific team works together to evaluate all of the scientific data and information included in the BLA and makes the determination whether to give final approval to a vaccine. A typical FDA team is comprised of: physicians, chemists, statisticians, pharmacologists/toxicologists, microbiologists, post-marketing safety experts, clinical study site inspectors, manufacturing and facility inspectors, and labeling and communications experts.

In some cases, the FDA may seek the input of its Vaccines and Related Biological Products Advisory Committee (VRBPAC). This committee is comprised of a panel of outside, independent, technical experts from various scientific and public health disciplines that provide input on scientific data and its public health significance in a public forum. The FDA will consider, but is not bound by, the input received from the VRBPAC when determining whether to approve a vaccine.

Prescribing Information/Labeling

Prescribing information for a vaccine is based on scientific data that are submitted by the manufacturer in the BLA and determined by the FDA to be satisfactory to support the approved indication(s), usage, dosing, and administration. The prescribing information is updated as needed to include the most current information about the vaccine that is available to and reviewed by FDA. The prescribing information does not necessarily address all aspects of vaccine use, such as recommendations that are specific to disease outbreaks, vaccine shortages, and all subpopulations with underlying medical conditions.

PHASE 4

Once a vaccine has passed through phase 3 and is approved by the FDA for use the United States, the oversight for safety and effectiveness doesn't end, but rather continues. Regular use of the vaccine in the general population is considered "phase 4". This is different than the first 3 phases in that the vaccine has already been approved for use, so the FDA monitors its after-market safety record.

Continued Monitoring for Safety and Effectiveness

It's important to note that a vaccine is essentially a drug. Like any drug, vaccines have benefits and risks, and even when highly effective, no vaccine is 100% effective in preventing disease or 100% safe in all individuals. Most side effects of vaccines are usually minor and short-lived. For example, a person may feel soreness at the injection site or experience a mild fever. Serious vaccine reactions are extremely rare, but they can happen and are often picked up in this phase drug monitoring.

Although the vaccine development process and FDA's evaluation are rigorous and comprehensive, there's still a need for ongoing surveillance after FDA-approval to identify uncommon adverse events or long-term complications that may occur, and sometimes to monitor effectiveness over time. In certain cases, as a requirement of approval, the FDA may require the manufacturer to conduct post-marketing studies to further assess known or potential serious risks. These studies are what's known as Phase 4 of development.

Vaccines are closely monitored using various surveillance systems, such as:

- Vaccine Adverse Event Reporting System (VAERS)
- FDA BEST (Biologics Effectiveness and Safety) program
- FDA Sentinel Program

- FDA and Centers for Medicare & Medicaid Services (CMS) partnership
- Centers for Disease Control and Prevention's (CDC) Vaccine Safety Datalink.

Patients or healthcare providers may also self-report problems or side effects through:

- Vaccine Adverse Event Reporting System (VAERS)

MAKE A REPORT TO VAERS OR SEARCH VACCINE DATA

https://vaers.hhs.gov

Lot Release

Lot release is another mechanism that the FDA uses with a real-time system to continuously monitor product quality in the after-market phase. When manufactured, vaccines are generally made in batches called lots. The FDA requires each vaccine manufacturer to submit data to support the demonstration of lot-to-lot consistency.

After approval, the manufacturer must submit the following materials relating to that vaccine lot or "batch":

- **Protocols**: contain the agreed-upon tests
- **Results**: the results of the testing performed by the manufacturer. Testing typically includes assessment of purity, potency, identity, and sterility.
- **Samples**: generally, the manufacturer must submit samples of the vaccine from the lot in question to permit FDA to perform confirmatory testing.

Manufacturers are not permitted to distribute a specific lot of vaccine until the FDA releases it.

SPECIAL CONSIDERATIONS

Although most vaccines take years to develop, there is no predetermined timeline for bringing a vaccine to market under special circumstances.

In public health emergencies, such as a pandemic, the development process may be atypical or expedited. As demonstrated by the response to the COVID-19 pandemic, the U.S. government may coalesce government agencies, international counterparts, academia, nonprofit organizations, and pharmaceutical companies to develop a coordinated strategy to expedite development of the vaccine. In addition, the federal government may decide to make investments in the necessary manufacturing capacity at its own risk, allowing for faster distribution of the vaccine.

Recognizing the urgent need for safe and effective vaccines, the FDA utilizes its various authorities and expertise to facilitate the expeditious development and availability of safe and effective vaccines. Early in a public health crisis, FDA provides clear communication to the pharmaceutical industry pertaining to the scientific data and information needed for safe and effective vaccines and works quickly to provide advice on their proposed development plans and assessment of the data that are generated.

Once certain criteria are met during a public health emergency, manufacturers submit a request to the FDA for Emergency Use Authorization (EUA) to expedite the availability and use of their vaccine.

The COVID-19 vaccine was released under an EUA.

EMERGENCY USE AUTHORIZATION

The Emergency Use Authorization (EUA) authority allows FDA to help strengthen the nation's public health protections against chemical,

biological, radiological, and nuclear (CBRN) threats including infectious diseases, by facilitating the availability and use of medical countermeasures (MCMs) needed during public health emergencies.

Under section 564 of the Federal Food, Drug, and Cosmetic Act (FD&C Act), when the Secretary of HHS declares that an emergency use authorization is appropriate, FDA may authorize unapproved medical products or unapproved uses of approved medical products to be used in an emergency to diagnose, treat, or prevent serious or life-threatening diseases or conditions caused by CBRN threat agents when certain criteria are met, including where there are no adequate, approved, and available alternatives. The HHS declaration to support such use must be based on one of four types of determinations of threats or potential threats by the Secretary of HHS, Homeland Security, or Defense, including:

1. A determination by the Secretary of Homeland Security that there is a domestic emergency, or a significant potential for a domestic emergency, involving a heightened risk of attack with a, chemical, biological, radiological, or nuclear ("CBRN") agent or agents
2. The identification of a material threat by the Secretary of Homeland Security, pursuant to section 319F-2 of the Public Health Service (PHS) Act, sufficient to affect national security or the health and security of United States citizens living abroad
3. A determination by the Secretary of Defense that there is a military emergency, or a significant potential for a military emergency, involving a heightened risk to United States military forces, including personnel operating under the authority of title 10 or title 50
4. Determination by the Secretary that there is a public health emergency, or a significant potential for a public health emergency, that affects, or has a significant potential to affect, national security or the health and security of United States citizens, including those living abroad, and that involves a CBRN agent or agents, or a disease or condition that may be attributable to such agent or agents.

COVID-19: a determination under section 319 of the Public Health Service Act that a public health emergency exists, such as the one issued on January 31, 2020, does not enable FDA to issue EUAs. However, on February 4, 2020 the HHS Secretary determined that there was a public health emergency that had a significant potential to affect national security or the health and security of United States citizens living abroad, and that involves the virus that causes COVID-19. Subsequent HHS declarations supporting the use of EUAs were based on this determination.

CONCLUSION

The US Food and Drug Administration (FDA) is the regulatory body that has oversight for the safety, effectiveness, and overall quality of all vaccines that are distributed in the United States. It is their role to provide the guidance and oversight for the development, testing, manufacturing, and ultimate approval for use of vaccines in the U.S.

They also played a major role in bringing the COVID-19 vaccine to market under an EUA, expediting the process and distribution.

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