
بسم الله الرحمن الرحيم

Blood & Immunity Module
SKILL LAB /Physiology PRACTICAL
FIRST YEAR MBBS BATCH 50
DETERMINATION OF CLOTTING TIME (CT)

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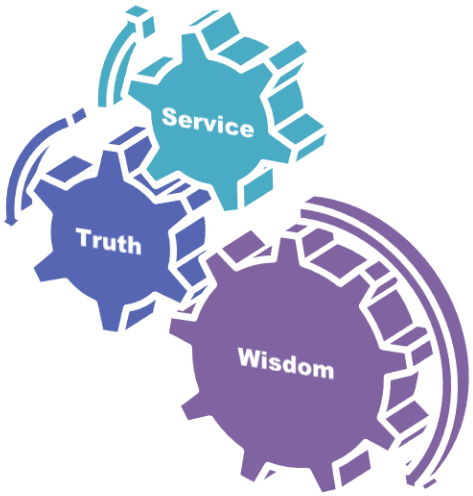
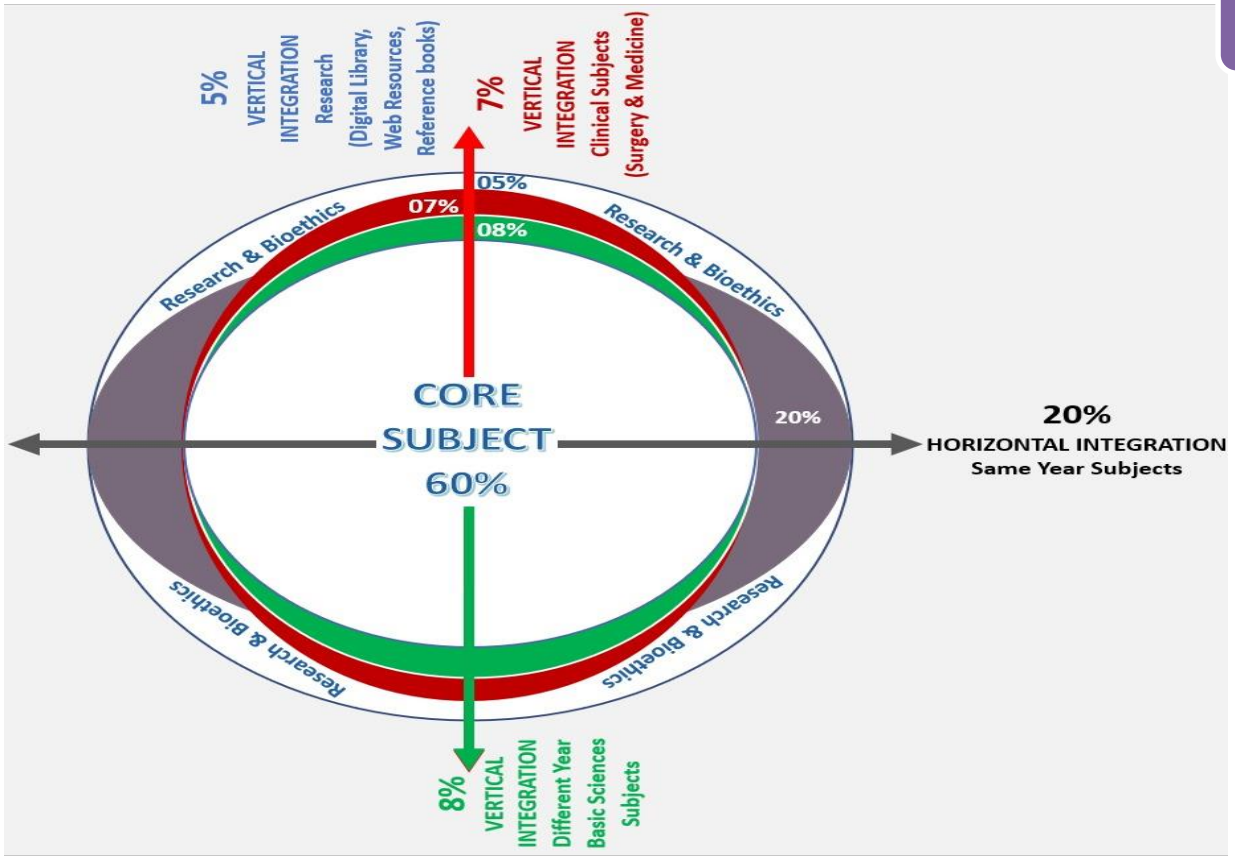
Motto



Vision; The Dream/Tomorrow

- To impart evidence based research oriented medical education
- To provide best possible patient care
- To inculcate the values of mutual respect and ethical practice of medicine

Professor Umar Model of Integrated Lecture



60% CORE SUBJECT

20% HORIZONTAL INTEGRATION Physiology biochemistry

8% VERTICAL INTEGRATION Pathology pharmacology

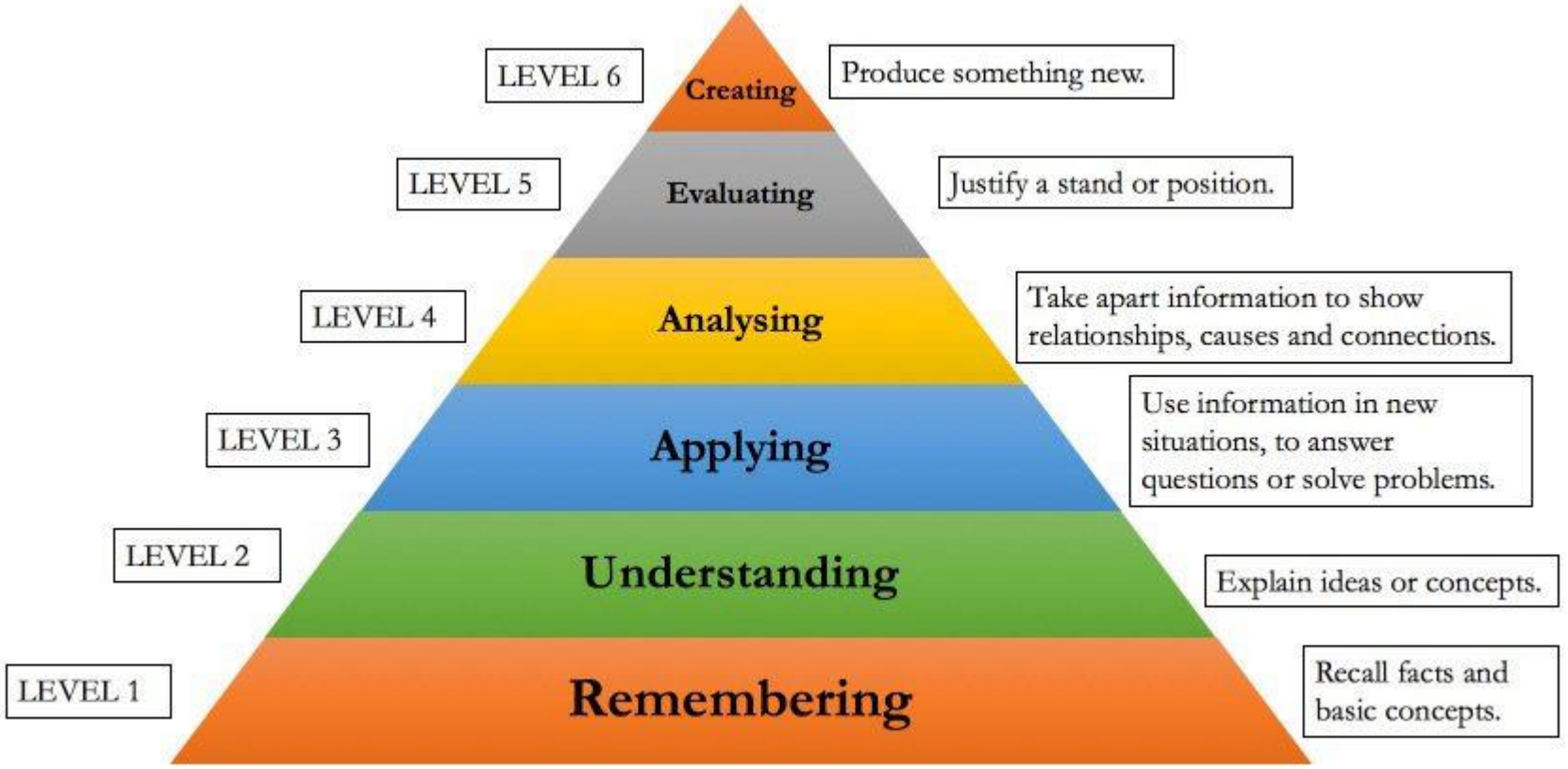
7% VERTICAL INTEGRATION Clinical integration

5% VERTICAL INTEGRATION Research, professionalism Ethics Digital library

BLOOM'S TAXONOMY : DOMAINS OF LEARNING

Sr. #	Domain of learning	Abbreviation	Levels of the domain	Meaning
1	cognition	C	C1	Recall / Remembering
2			C2	Understanding
3			C3	Applying / Problem solving
4	Psychomotor	P	P1	Imitation / copying
5			P2	Manipulation / Follows instructions
6			P3	Precision / Can perform accurately
7	Attitude	A	A1	Receiving / Learning
8			A2	Respond / Starts responding to the learned attitude
9			A3	Valuing / starts behaving according to the learned attitude

BLOOM'S TAXONOMY OF THE COGNITIVE DOMAIN



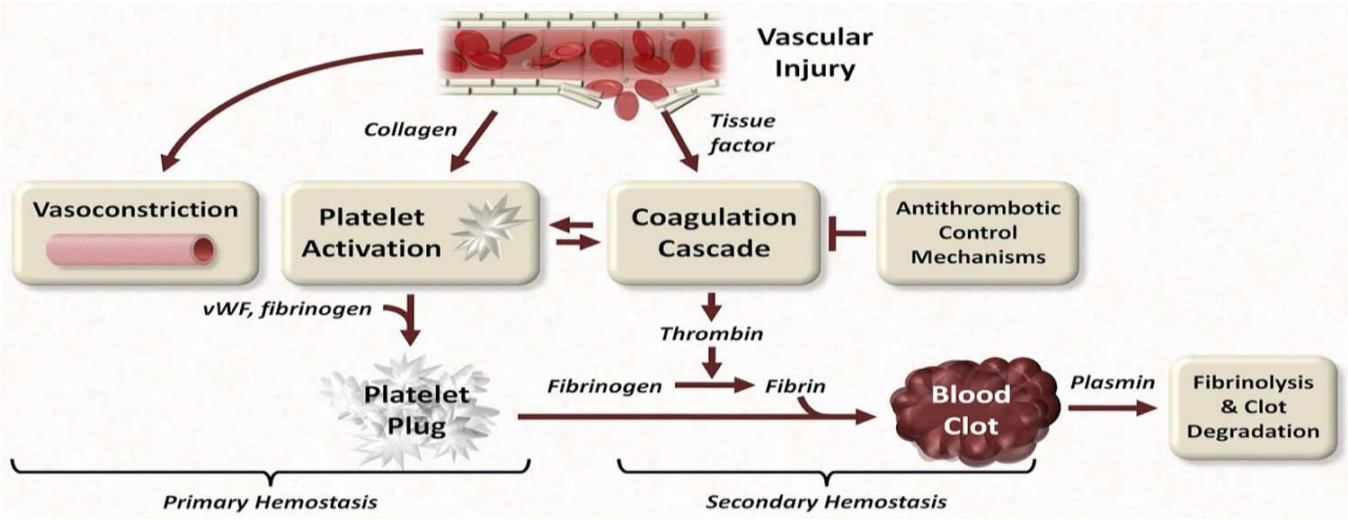
LEARNING OBJECTIVES

Sr. #	Learning Objective	Domain of Learning
1	To describe the relevance of clotting time.	C1
2	To perform step by step the determination of clotting time.	P3
3	To understand the reason of calculating clotting time.	C2
4	To explain different methods to determine clotting time.	C2
5	To correlate Clinically with different clotting and bleeding disorders.	C3
6	To diagnose and investigate hemophilia patients.	C3

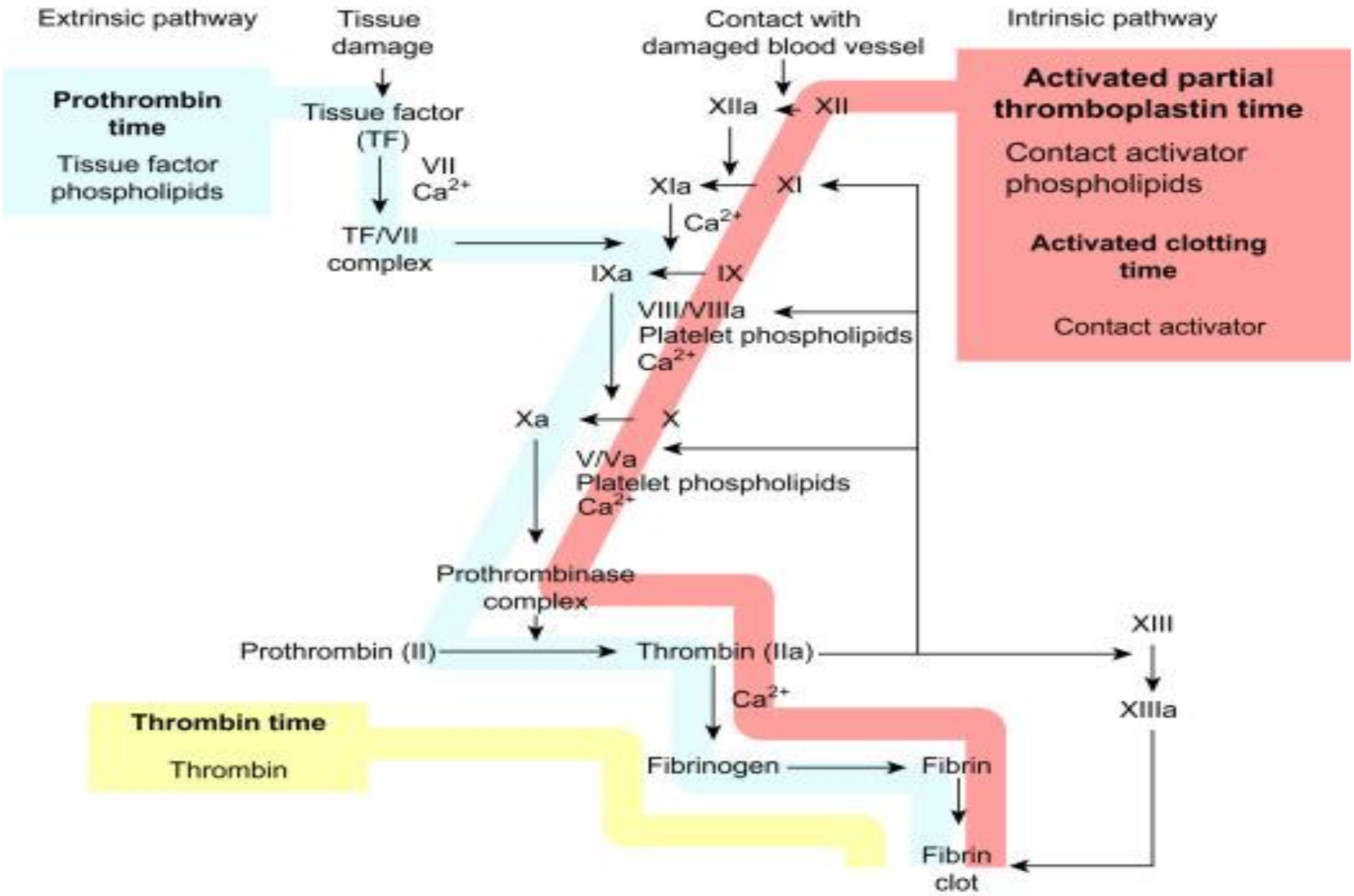
Horizontal integration

Biochemistry

- The overwhelming majority of clotting factors are manufactured principally in hepatocytes.
- Hepatocytes are responsible for providing the body with clotting factors XIII, XII, XI, X, IX, VII, V, II, and I.
- Clotting factors VIII (antihemophilic factor A) and III (tissue factor) originate from endothelial cells.
- Whereas clotting factor IV (calcium ion) is freely available in plasma.
- Megakaryocytes produce the body's platelets and also contribute to the production of factor V.



Reference:- Wikipedia image of hemostasis biochemistry



Reference:- Wikipedia image of hemostasis biochemistry

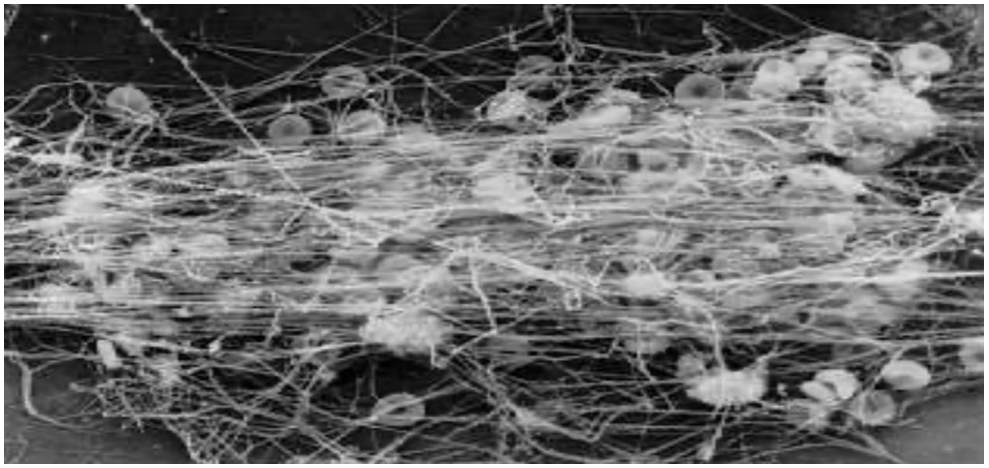
Core Concept

Core Concept

Introduction:-

- Hemostasis is the body's physiologic response to vascular endothelial injury, which results in a series of processes that attempt to retain blood within the vascular system through the formation of a clot. Clotting time is the time interval between pricking of a vein or shedding of blood till it clots.
- First functional platelet evaluation test
- Used to detect defects in primary hemostasis
- Used as a screening test for vascular disorders as well as platelet function test.
- There are three methods to calculate clotting time (CT).

1. Capillary tube method
2. Lee and White method
3. Slide method



Core Concept

Capillary Tube Method:

After pricking the finger pulp with lancet blood is taken into non-heparinized capillary tube. The duration of time that it takes for the blood to clot (as detected by the appearance of fibrin string) is called clotting time. Immediately after filling the test tube should be held in the palms to maintain temperature.

Apparatus Required:

1. A blood lancet
2. Cotton swabs moist with spirit
3. Glass capillary tube

Core Concept

Procedure:-

1) Sterilize the finger tip with spirit
2) Get a deep finger prick with lancet and allow the blood to flow freely without squeezing.
3) Discard the first 2 -3 drops and allow a large drop to form. Fill the capillary tube with blood by dipping one of its open ends in the drop. The blood rises and fills the tube by capillary action. Note the time.
4) Start the stop watch.
5) After 2 minutes start breaking off 1cm bit of the tube from one end, and then repeat it after every 30 seconds, and look for the formation of fibrin threads between the broken ends. The end point is reached when fibrin threads bridge a gap between the broken end. Note the time.

Core Concept

Precautions

I. Explain the procedure to the subject.
II. The finger tip should be cleaned with alcohol before pricking.
III. The puncture must be deep and blood should flow spontaneously.
IV. Immediately after filling the test tube should be held in the palms to maintain temperature.

Normal Value of CT

2-6 minutes (by capillary tube method).
5-12 minutes (by Lee & white method).
2-8 minutes (by slide method)

2. Lee and White (Test Tube Method):

This is more reliable and sensitive than the capillary method, but it needs special arrangements for venepuncture and a temperature -controlled water bath.

When blood obtained by a clean venepuncture is put in glass tube, clotting mechanisms are activated and soon a clot is formed. The time taken by the blood to clot in this way is called whole blood clotting time (CT). It is in sensitive and non specific test. It will be prolonged only in severe hemophilia.
when the factors are as low as 1%. It is sometimes used as a bed side procedure to screen for a heparin effect and circulating anticoagulants.

Core Concept

Apparatus:-

- Three glass test tubes 75X12mm (10mm-bore).
- b. Water bath at 37°C.
- c. Metal rack.
- A disposable plastic syringe.
- Normal saline.
- Three stop watches.

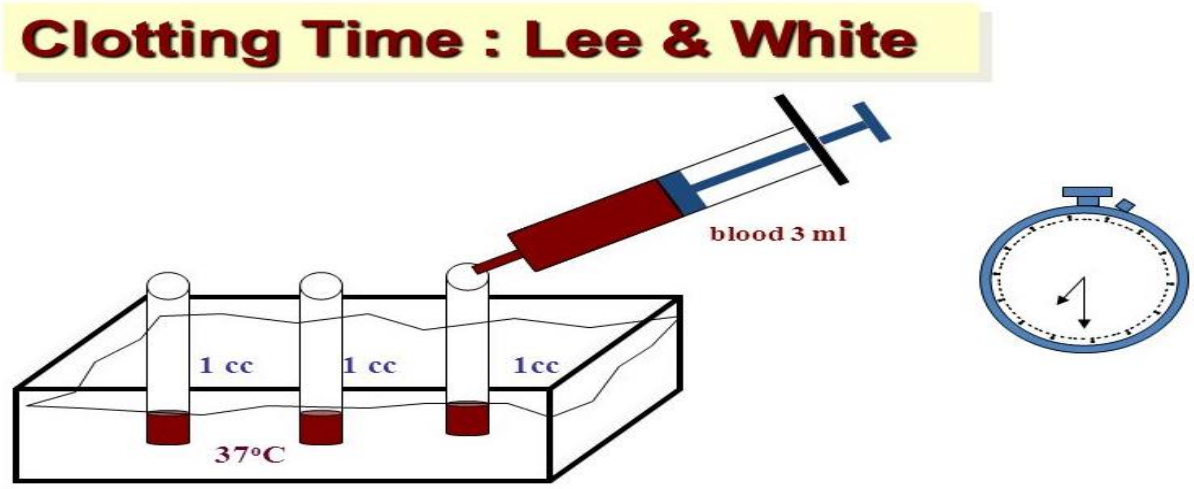
Significance:-

Clotting time is prolonged in diseases in which clotting factors are deficient. If the CT is more than 10 minutes, the patient should be subjected detailed investigation to identify the missing coagulation factor.

Core Concept

Procedure:-

- Rinse the test tubes with normal saline, and put them in a metal rack kept in the water bath at 37°C. Draw 3mlof blood by a clean venepuncture, and start the stop watch when blood enters the disposable plastic syringe.
- Remove the needle and gently add 01ml blood in each of the three test tubes.
- Initially tilt the tube after 5 minutes and then after every 30 seconds to see whether the blood has clotted or not.
- When the blood clots in a tube stop the stopwatch for that tube. Take the mean of the 3 readings as a result.



Core Concept

Precautions

I. One important source of error is inappropriate volume of blood taken for the test (less than 1 ml gives a shorter CT).

II. The temperature of the tubes should be maintained at 37°C.

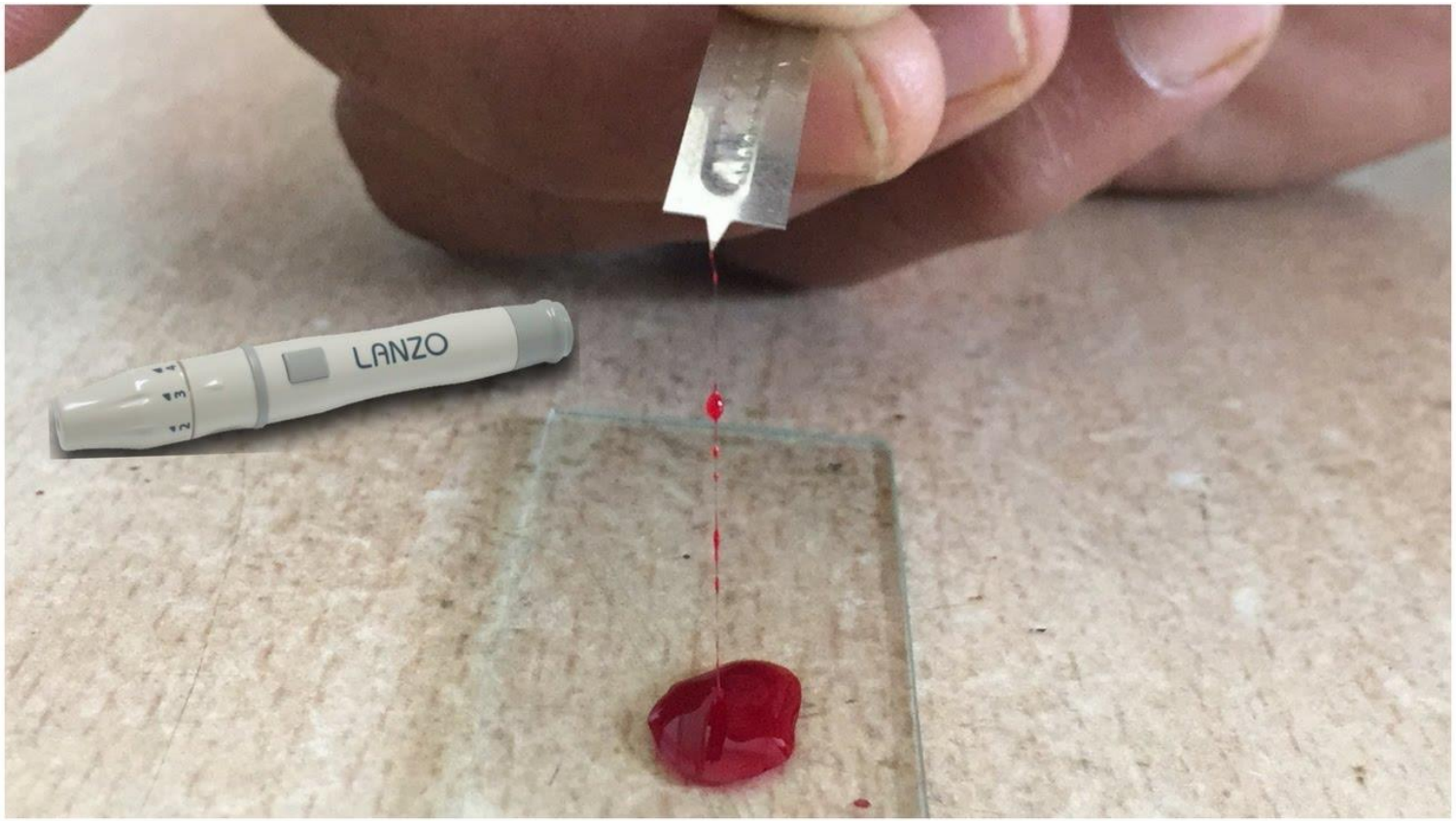
III. The test tube should be of the specified bore (10mm) otherwise the results may vary.

IV. While collecting blood, air bubbles should not enter the syringe . Presence of air bubbles shortens the CT.

Core Concept

3. Slide Method:-

In this method a drop of blood is taken on a clean glass slide. Pass a needle or a common pin through this blood. Repeat the procedure after 30 seconds until the formation of fibrin threads which adhere to common pin is accomplished. Note the clotting time.



Vertical integration

Vertical integration Pediatrics

Bleeding Disorders

Hemophilia

An inherited bleeding disorder caused by deficiency of coagulation factors. (the most common inherited bleeding disorders).

> **HEMOPHILIA A** – inherited deficiency of factor viii (8); an x-linked recessive disorder. factor 8 deficiency is the most common.

> **HEMOPHILIA B** – inherited deficiency of factor ix (9); also called christmas disease; an x-linked recessive disorder.

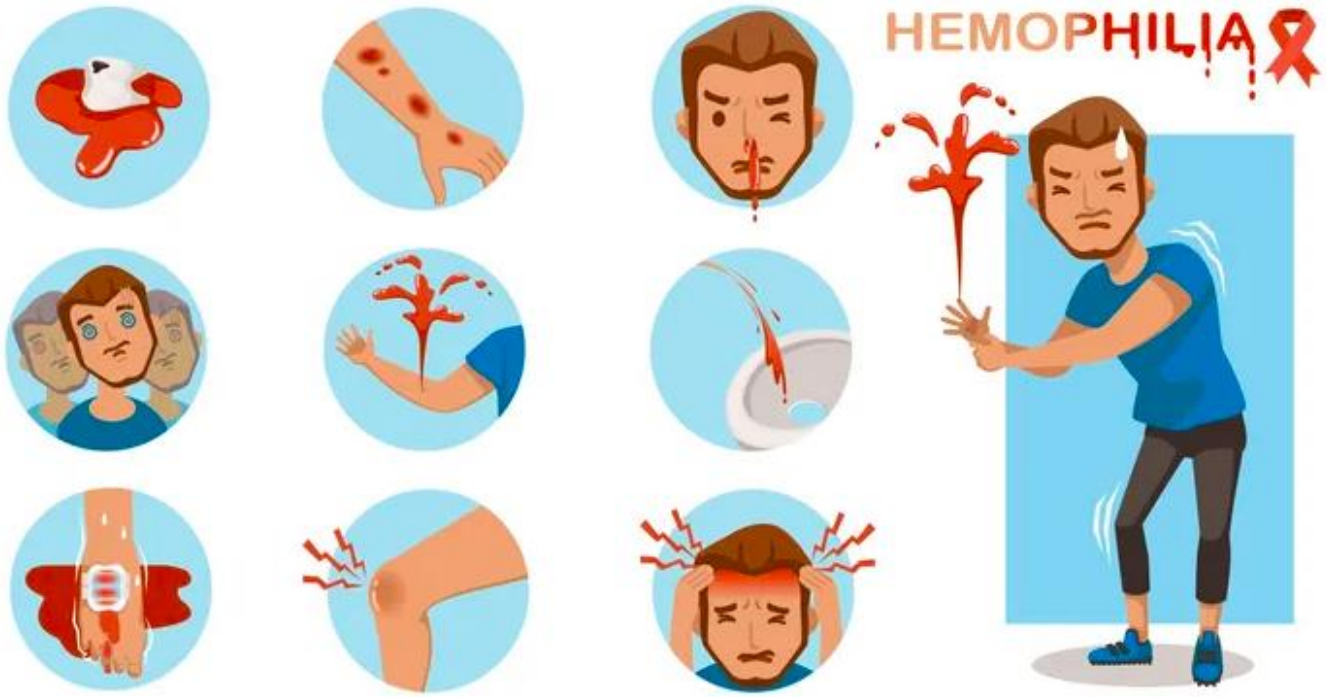
> **HEMOPHILIA C** – inherited deficiency of factor xi (11); also called rosenthal syndrome; variable pattern of inheritance but mainly an autosomal recessive disorder.

Hemophilia patients present with severe pain in their joint (hemarthrosis), we do a baseline factor activity level, greater than 40% is considered normal factor level. baseline factor activity level, <1 is considered severe, >40% is considered normal factor level.

SIGNS & SYMPTOMS

HEMOPHILIA

- hematomas
- hemarthrosis
- bruising
- bleeding (mucosal, GI, GU)
- Any type of coagulation factor deficiency may lead to deep bleeding.



INVESTIGATIONS

HEMOPHILIA

- Bleeding time = normal
- Clotting Time = increased
- PT = Normal
- APTT = prolonged 2-3 times increased
- Low levels of Factor IX and Factor VIII

TREAT ASAP!!! DO NOT WAIT FOR LABS

Hemophilia A

Factor VIII
Each IU ↑ increases plasma levels 2%
Replacement:
- 25 IU/kg for minor/moderate
- 50 IU/kg for major

↓

Adjuncts

Cryoprecipitate
- Contains VW factor and factor VIII
- Dose = 1 bag/6kg of body weight

TXA – antifibrinolytic to stabilize clot formation
- Dose = 10mg/kg IV

Hemophilia B

Factor IX
Each IU ↑ plasma levels 1%
Replacement:
- 50 IU/kg for minor/moderate
- 100 IU/kg for major

↓

Adjuncts

FFP – contains factor IX
- Watch for volume overload!
- Dose = 15ml/kg

TXA – antifibrinolytic to stabilize clot formation
- Dose = 10mg/kg IV

List of Clotting Disorders Table

Coagulation disorders	*Congenital*: Haemophilia A, Haemophilia B (Christmas disease) and Von Willebrand's disease. *Acquired*: Anticoagulants, liver disease, DIC, vit K deficiency.
Vascular defects	*Congenital*: Connective tissue disease (Ehlers-Danlos syndrome), Osler-Weber-Rendu syndrome. *Acquired*: Senile purpura, infections, steroids, scurvy.
Platelet defects **1. Platelet number defects**	Decrease marrow production: Aplastic anaemia, marrow infiltration (leukaemia, myeloma), marrow suppression (cytotoxic drugs, radiotherapy). Excessive destruction: immune thrombocytopenic purpura (ITP), SLE, CLL, heparin treatment, viruses. Thrombotic thrombocytopenic purpura (TTP), sequestration (as in hypersplenism).
2. Platelet structural defects	Myeloproliferative disease, increase urea, Von Willebrand's disease, Bernard-Soulier (giant platelet) syndrome, alcoholism, drug-induced (Aspirin, NSAID).
Fibrinolytic defects	DIC and streptokinase treatments.

REFERENCE = **Robins Pathology Basics, 10th Edition**

Biomedical Ethics

BREAKING BAD NEWS

The aim for any health-professional is to use their skills to deliver bad news clearly, honestly and sensitively in order that patients can both understand and feel supported.

BREAKING BAD NEWS

Respect the people to whom the information is being given by listening and watching them at all stages and being responsive to their wishes and reactions, which will be diverse.

It is important to realize that the environment and healthcare professionals' behavior will have a profound influence upon the patient and family in all respects.

6 STEPS STRATEGY:-

Setting up and starting. Mentally rehearse and arrange for privacy.

Perception. Elicit the patient's perspective.

Invitation. Ask the patient what they would like to know.

Knowledge. Provide information in small pieces.

Emotions. Recognize and empathize with the patient's emotions.

Strategy and summary. Set out a medical plan of action.

Codes of Ethics for Laboratory

Testing laboratories have an obligation to adhere to high ethical standards in order to provide with the accurate and reliable test results needed to meet the requirements and reduce uncertainty in results.

- Always wear white coat in lab
- Handle the glassware gently
- Wait for your turn while working in groups
- Use gloves while handling chemicals
- Do not waste reagents or other lab supplies

Brain Storming

Question & Answer

Brain Storming

Clinical Scenario

7 year old boy brought into the pediatric clinic with complaint of excessive bleeding from a small cut on his knee. According to the parents, he fell while playing in the park, resulting in a minor abrasion. However, despite applying pressure, the bleeding seemed difficult to control and persisted for an unusually long time. This incident raised suspicion of a bleeding disorder, as there was no significant trauma to cause such prolonged bleeding. There is a family history of hemophilia on the maternal side. He has been otherwise healthy child with no significant medical issues in the past.

Question Related to Scenario

Question 1 : What clinical features and family history might raise suspicion of hemophilia in a pediatric patient presenting with bleeding after a minor injury?

Answer:

Clinical features and family history that might raise suspicion of hemophilia in a pediatric patient presenting with bleeding after a minor injury include:

- Prolonged bleeding after minor cuts, bruises, or injuries.
- Easy bruising or spontaneous bleeding without apparent cause.
- Family history of hemophilia or a known bleeding disorder, especially on the maternal side.
- History of bleeding complications after surgical procedures.

Question Related to Scenario

Question 2 : What laboratory investigations would be essential to confirm the diagnosis of hemophilia in this young patient?

Answer:

Essential laboratory investigations to confirm the diagnosis of hemophilia in this young patient would include:

- **Complete Blood Count (CBC)** to assess hemoglobin, hematocrit, platelet count, and white blood cell count.
- **Coagulation Studies:** Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) to screen for bleeding disorders.

Question Related to Scenario

Question 3：What are the key components of the treatment plan for a pediatric patient diagnosed with Hemophilia A?

Answer:

Treatment plan for a pediatric patient diagnosed with Hemophilia A.

Clotting Factor Replacement Therapy: The child will receive intravenous administration of Factor VIII concentrates to replace the missing clotting factor and control bleeding episodes. The treatment regimen will depend on the severity of hemophilia.

Bleeding Management Education: The family will be taught how to manage bleeding episodes at home, including applying pressure and using appropriate dressings for minor cuts and injuries.

Prophylaxis: In severe cases, prophylactic Factor VIII replacement therapy may be considered to prevent spontaneous bleeding and joint damage.

Physical Activity and Joint Protection: The child will be advised on safe physical activities, and the family will learn joint protection techniques to minimize the risk of bleeding into the joints.

Suggested Research Article

Related Research Article

https://ojrd.biomedcentral.com/articles/10.1186/s13023-017-0620-6

Research | Open Access | Published: 07 April 2017

Autosomal recessive inherited bleeding disorders in Pakistan: a cross-sectional study from selected regions

Arshi Naz ✉, Muhammad Younus Jamal, Samina Amanat, Ikram Din ujjan, Akber Najmuddin, Humayun

Abstract

Background

Autosomal recessive bleeding disorders (ARBDs) include deficiencies of clotting factors I, II, V, VII, X, XI, XIII, vitamin K dependent clotting factors, combined factor V & VIII, Von Willebrand Disease (vWD) type 3, Glanzmann's thrombasthenia (GT) and Bernard–Soulier syndrome. Patients with primary bleeding disorders from all the major provincial capitals of Pakistan were screened for ARBDs. Prothrombin (PT), activated partial thromboplastin time (APTT), bleeding time (BT) and fibrinogen levels were measured. Cases with isolated prolonged APTT were tested for factors VIII and IX using factor assays This was followed by FXI:C level assessment in cases with normal FVIII and FIX levels. vWD was screened in

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REFERENCES

BOOKS:-

1. Professor Saqib Sohail Manual of physiology.
2. Guyton and Hall text book of medical physiology, 14th edition
3. Human Physiology by Lauralee Sherwood, 9th edition
4. Ganong's Review of Medical Physiology, 26th Edition
5. Robins Pathology Basics, 10th Edition
6. Davidson Principles and Practice of Medicine.

Research:-

https://ojrd.biomedcentral.com/articles/10.1186/s13023-017-0620-6

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Thank You!