
بسم الله الرحمن الرحيم

MSK- II MODULE
SKILL LAB /Physiology PRACTICAL
FIRST YEAR MBBS BATCH 50
DETERMINATION OF TOTAL PLATELET COUNT

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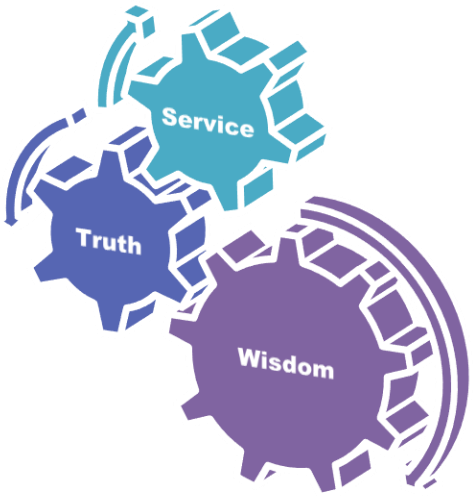
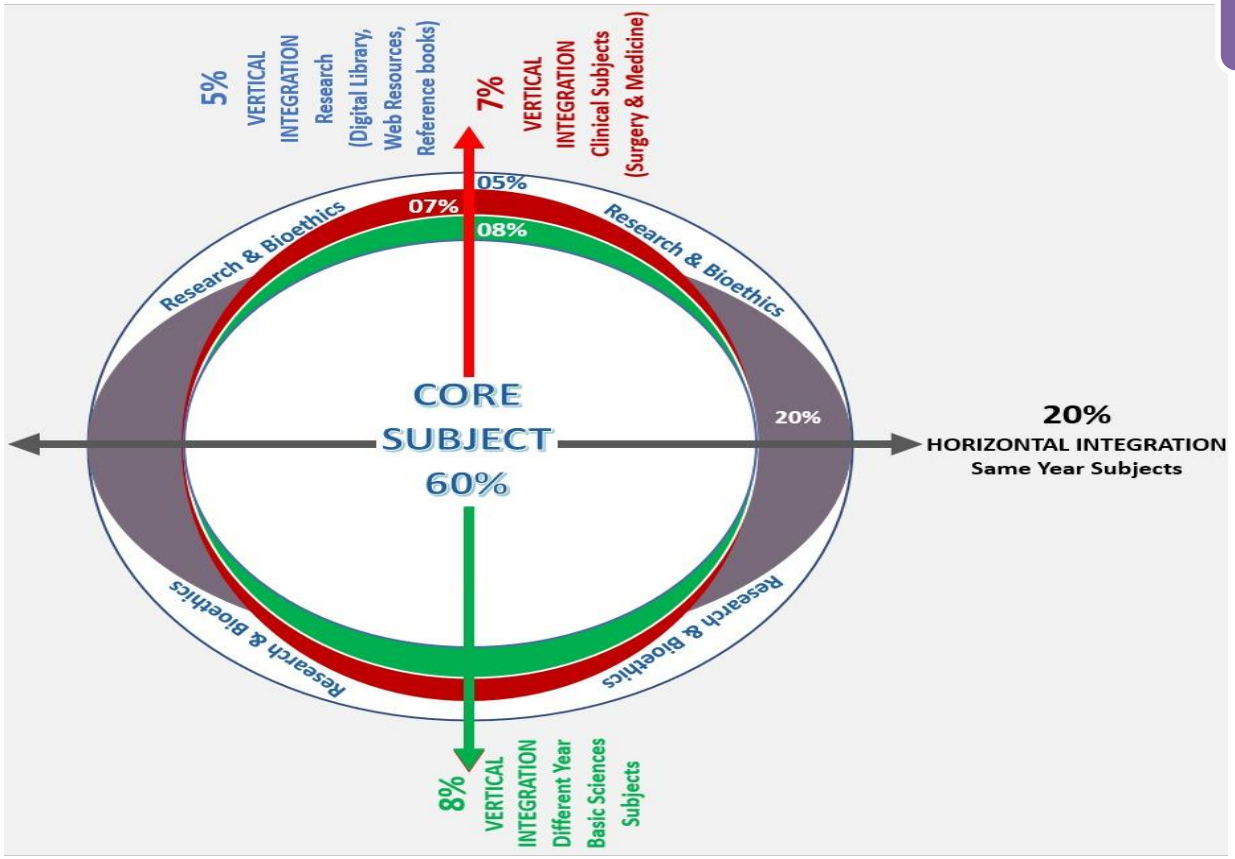
Motto



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

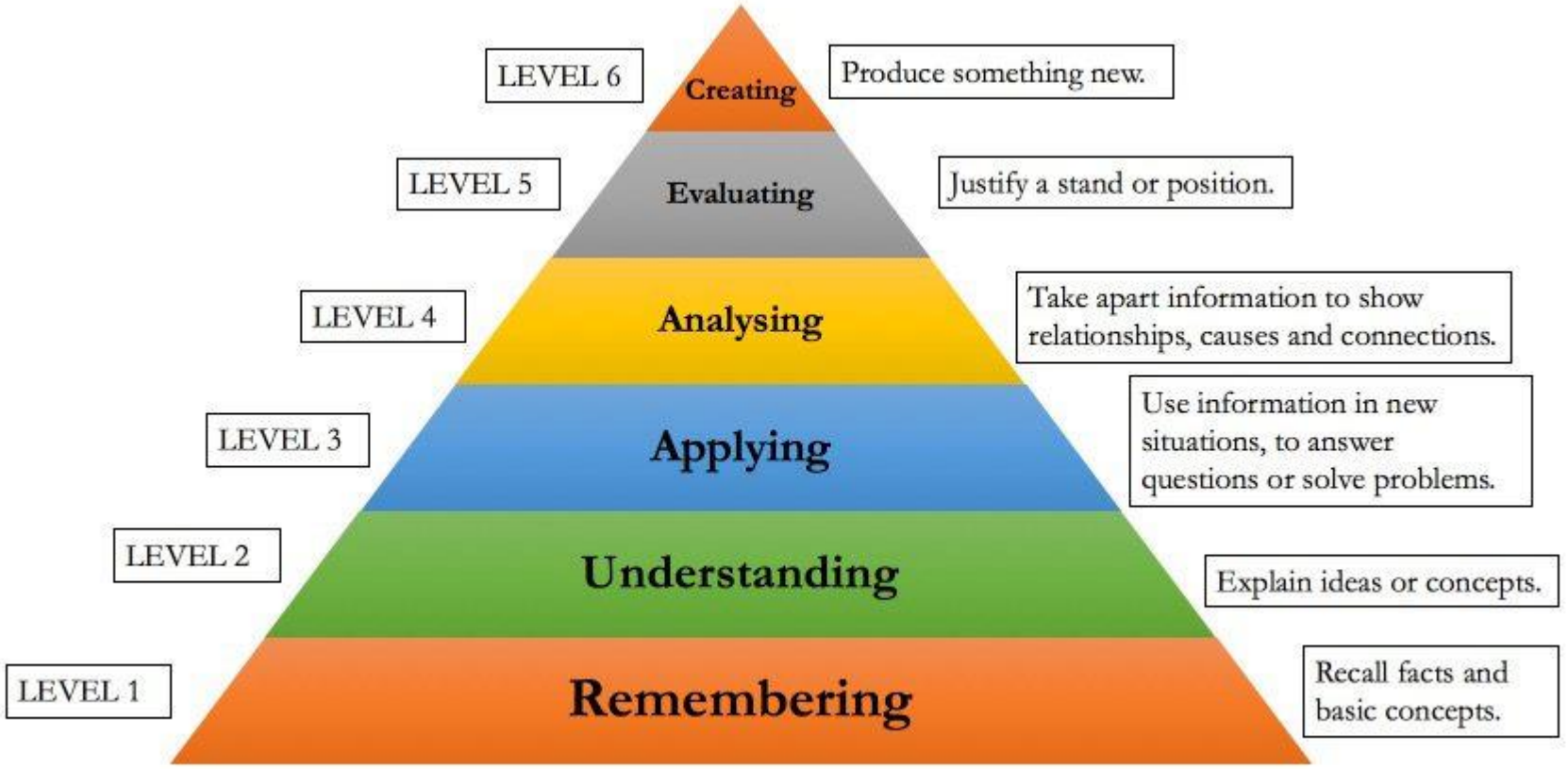
7% VERTICAL INTEGRATION
Clinical integration

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



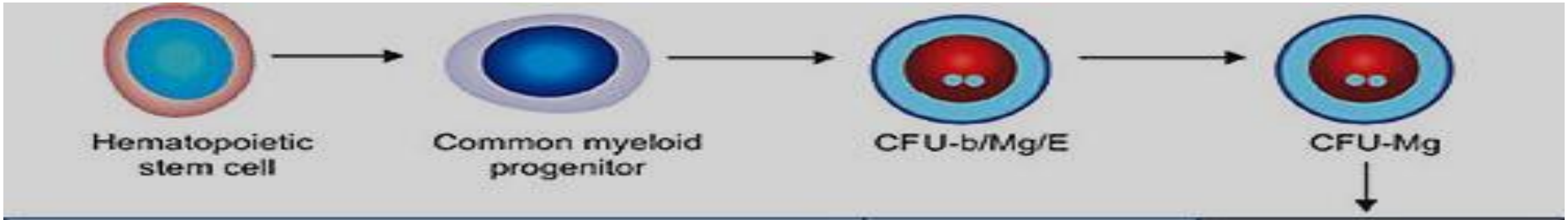
LEARNING OBJECTIVES

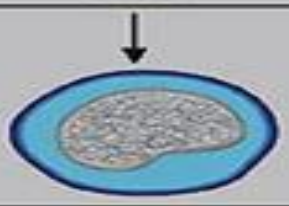
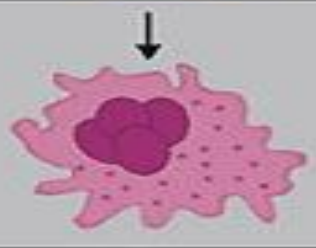
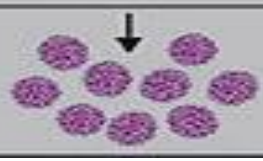
Sr. #	Learning Objective	Domain of Learning
1	To describe the relevance of doing Total Platelet count.	C1
2	To perform step by step the total platelet count practical by Direct method.	P3
3	To describe the composition of Reese Ecker Fluid, Formal citrate	C1
4	To perform step by step the total platelet count practical from a given sample of blood by Reese ecker fluid.	P3
5	To calculate Normal Platelet count and clinicals related to destruction and decrease in platelet count.	C3
6	To understand its vertical integration with thrombocytopenic purpura and its management.	C3

Horizontal integration

Horizontal integration

Horizontal integration (Anatomy)

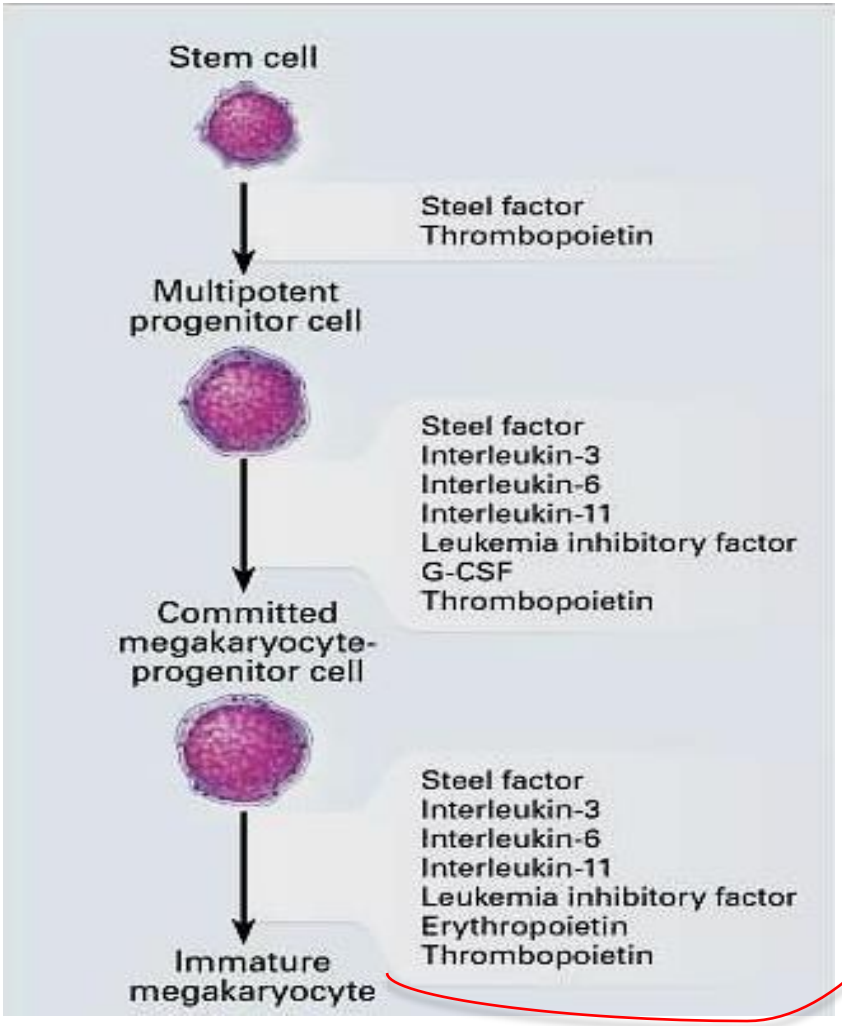


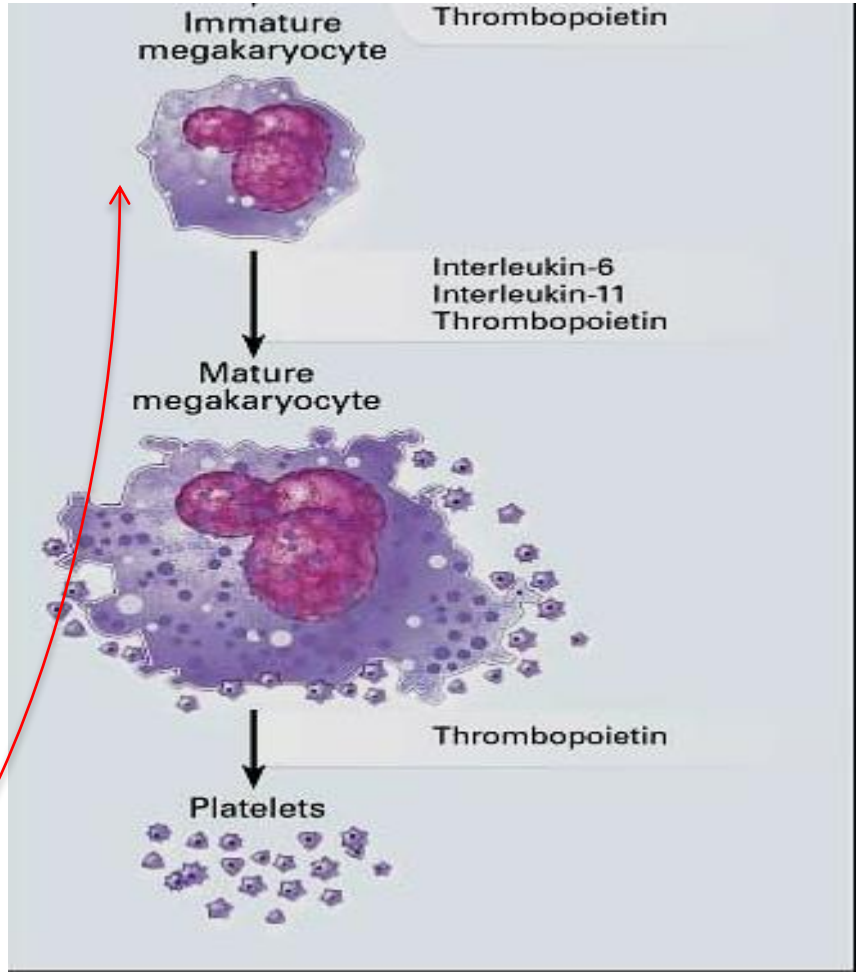
Size: Cytoplasm: Nucleus: Nucleoli:	15-20 µm Basophilic cytoplasm Round Single to multiple nucleoli	Megakaryoblast	
Size: Cytoplasm: Nucleus:	20-30 µm Abundant, less basophilic cytoplasm Oval and elongated	Promegakaryocyte	
Size: Cytoplasm: Nucleus:	35-60 µm, largest cell in the bone marrow Borders irregular and budding of platelets from the cytoplasmic border. Rich in granules. Compact and dense, multi-lobated and pushed to one side.	Megakaryocyte	
Size:	2-3 µm, Anucleated pale blue colored structures with purple granules	Platelets	

Reference:- Google image for Bone Marrow histology

Horizontal integration

Horizontal integration (Biochemistry)





Core Concept

Core Concept

Introduction Of Platelet Physiology

- Platelets are small anucleate cell fragments that circulate in blood playing crucial role in managing vascular integrity and regulating hemostasis.
- > Platelets are also involved in the fundamental biological process of chronic inflammation associated with disease pathology. Platelet indices like mean platelets volume (MPV), platelets distributed width (PDW), and platelet crit (PCT) are useful as cheap noninvasive biomarkers for assessing the diseased states.
- Dynamic platelets bear distinct morphology, where α and dense granule are actively involved in secretion of molecules like GPIIb , IIIa, fibrinogen, vWf, catecholamines, serotonin, calcium, ATP, ADP, and so forth, which are involved in aggregation.
- Differential expressions of surface receptors like CD36, CD41, CD61 and so forth have also been quantitated in several diseases.

Core Concept

DETERMINATION OF Total Platelet Count:-

There are three methods of platelets counting. These include

1. Hemocytometry (direct method)
    a. Formal citrate method.
    b. Reese Ecker method.
2. Study of blood smear (indirect method).
3. Automated counting.

★ In Direct method fresh blood is diluted in a RBC pipette with either formal citrate or with Reese Ecker fluid and the stained platelets are than counted in a hemocytometer.

Core Concept

Method using formal citrate:-

Apparatus and reagents:-

- Microscope
- Hemocytometer
- Cotton swab moist with spirit
- Blood lancet
- Formal citrate as diluting fluid

(Horizontal integration with biochemistry)

Composition of formal citrate (Dilution Fluid):

1. 3:8gm 40% sodium citrate
2. 40% formalin.
3. 100ml of water.

Core Concept

Procedure:-

- Clean the finger pulp with spirit swab. Prick it with pricking needle.
- When blood comes out discard 2 drop and then suck the blood in RBC pipette up to mark 1.
- Add the diluting fluid up to-the mark 101 so the dilution is 100 times.
- Mix the two by rotating the pipette between palms of your hand Clean the Neubauer's slide.
- Put a cover slip on it. Discard 2-3 drops of solution from the stem of the RBC pippette and charge the Neubauer's Chamber and cover n under a petri dish with a moist filter paper on its inside. Wait for 10-15 minutes so the formalin fixates the cells, This is done to prevent the evaporation of formalin.
- Now focus on slide under low and then high power, RBC's and patelets can be differentiated on the basis of their size and shape in the microscopic field.
- RBC's are 7-8 microns in diameter and circular in shape but platelets are 2-3 micron in diameter, they are either rod or coma shaped. They are shining in the center.

Core Concept

Calculation

- No. of platelets in central small square =X
- Area of each small square = 1 x 1 mm2
- Depth of the Neubauer''s chamber = 1/10
- Volume of each small square = 1 x1/10=0.1 mm2
- No. of platelets in 0.1 mm3 of small square = X
- No. of platelets in 1 mm3 =X x 0.1= 10X
- Since dilution is 100 times, so Total platelets count =10X x 100 = 1000X

Core Concept

Precautions

a. Finger should be cleaned properly.

b. Prick should be moderately deep.

c. Avoid squeezing of finger and mixing of tissue fluid with the blood.

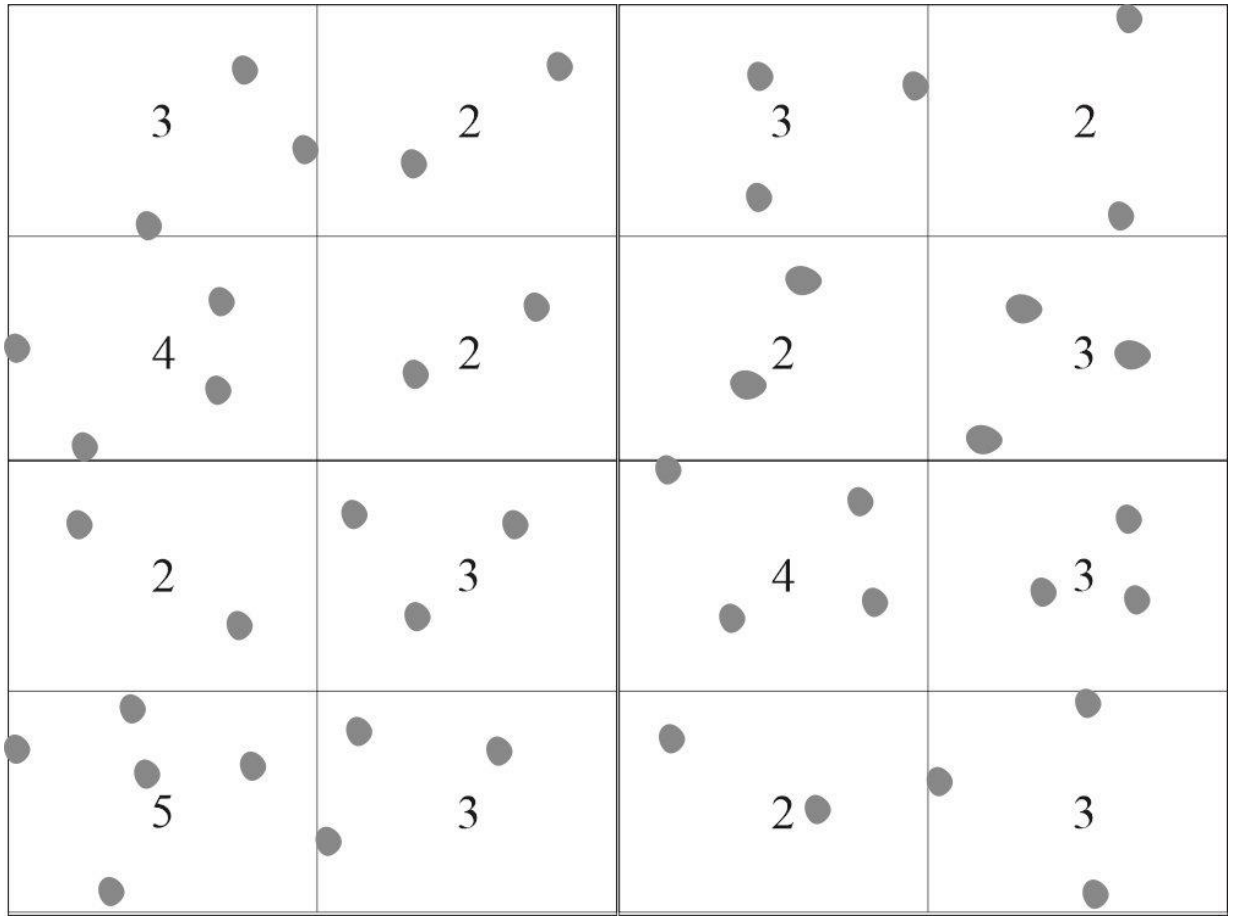
d. Lancet should not be greasy.

e. Diluting fluid should be freshly prepared.

f. If clumping occurs then discardi it and make a new slide.

Core Concept

PROCEDURE



Reference:- Wikipedia image topic platelet count

Core Concept

PROCEDURE 2
USING REESE ECKER FLUID:-

Apparatus and reagents:-

- a. Microscope
- b. Hemocytometer
- c. Cotton swab moist with spirit
- d. Blood lancet
- e. Reese -Ecker fluid

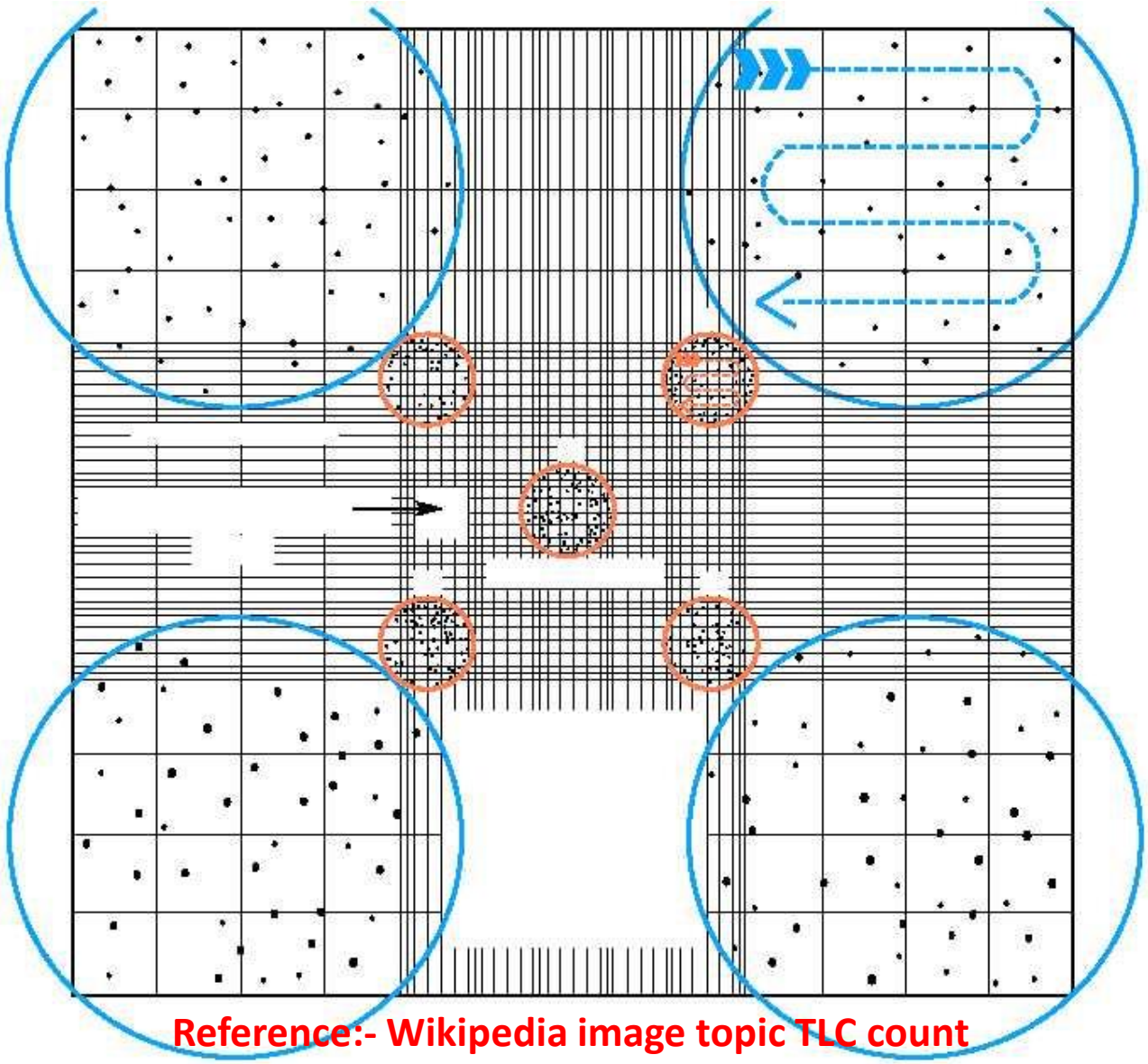
(Horizontal integration with biochemistry)

Reese -Ecker fluid Composition

- Brilliant cresyl blue 0.05g
- Sodium citrate 3.80g
- Formaldehyde (40%) 0.2ml
- Distelled water 100ml

Core Concept

CONTD….



Reference:- Wikipedia image topic TLC count

Core Concept

Procedure:-

- Clean the RBC's pipette and Neubaur chamber.
- Puncture the finger tip and suck the blood exactly upto 0.5 mark of the RBC's pipette.
- Rapidly dilute the blood with Rees Ecker fluid up to mark 101 of the pipette.
- Shake the pipette immediately after dilution for at least 1 minute.
- Discard 2-3 drops of the solution from the pipette.
- Charge the Neubauer's chamber.
- Cover the charged chamber with a petri dish lined with moist filter paper and allow 15 minutes for the platelets to settle in the chamber. (The chamber is covered to prevent evaporation).
- After 15 minutes, count the platelets under high power objective. The platelets are bluish and must be distinguished from debris. Platelets are oval or rounded bodies, normally 2-4 micron in diameter and retractile in nature.
- Count the platelets in 25 medium sized squares
- Enter the results in the squares drawn on paper

Core Concept

Calculation

- The cells are counted in 25 medium size squares.
- The area covered by the 25 medium sized squares is 1 mm2.
- Platelet count = $\frac{\text{Number of platelets X dilution}}{\text{Volume of fluid}}$
- Dilution is 200
- Volume of fluid for 1 mm2 = 1X1/10 = 0.1 mm3

= Number of platelets counted X 200/0.1

= Number of platelets counted X 2000

Core Concept

Precautions

- The glassware must be thoroughly cleaned.
- The diluting fluid must be filtered just before use (to remove stained particles from the stain).
- if venous blood is used, platelets must be counted within 2 hours.

Normal platelet count:-

150000 to 300000 / mm3

Vertical integration

PATHOLOGICAL CAUSES OF DECREASE IN COUNT:

Vertical integration

- Thrombocytopenia (decrease in platelet count) can be caused by various factors, including immune disorders like ITP, drug-induced reactions, bone marrow disorders, viral or bacterial infections, DIC, splenomegaly, SLE, pregnancy, and TTP.
- Bone marrow depression or failure by chloramphenicol and irradiation.
- Bone marrow invasion by leukemias and secondary deposits of malignant disease.
- Periodic thrombocytopenic purpura in which cause is not known.

Vertical integration

CAUSES WHICH INCREASE DESTRUCTION INCLUDE:

- Pathological causes of increased platelet destruction include immune disorders like
- ITP and TTP,
- disseminated intravascular coagulation (DIC),
- drug-induced immune reactions, infections,
- HUS, autoimmune disorders,
- alloimmune disorders,
- hypersplenism,
- certain cancers

IMMUNE THROMBOCYTOPAENIA (ITP)

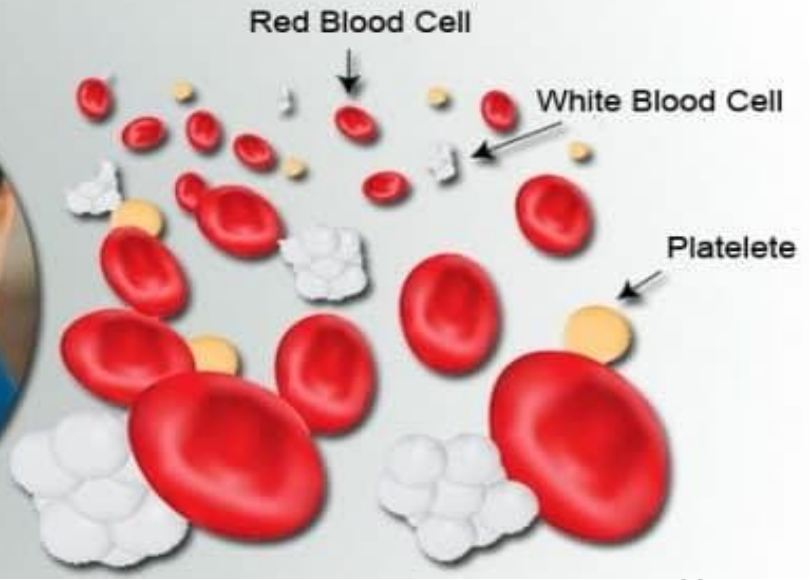
Vertical integration

What is Immune thrombocytopenia Purpura or ITP?

Immune thrombocytopenia Purpora or ITP is a blood disorder with a low blood platelet count.ITP is an autoimmune disorder where your immune system attacks your platelets.

Symptoms :-

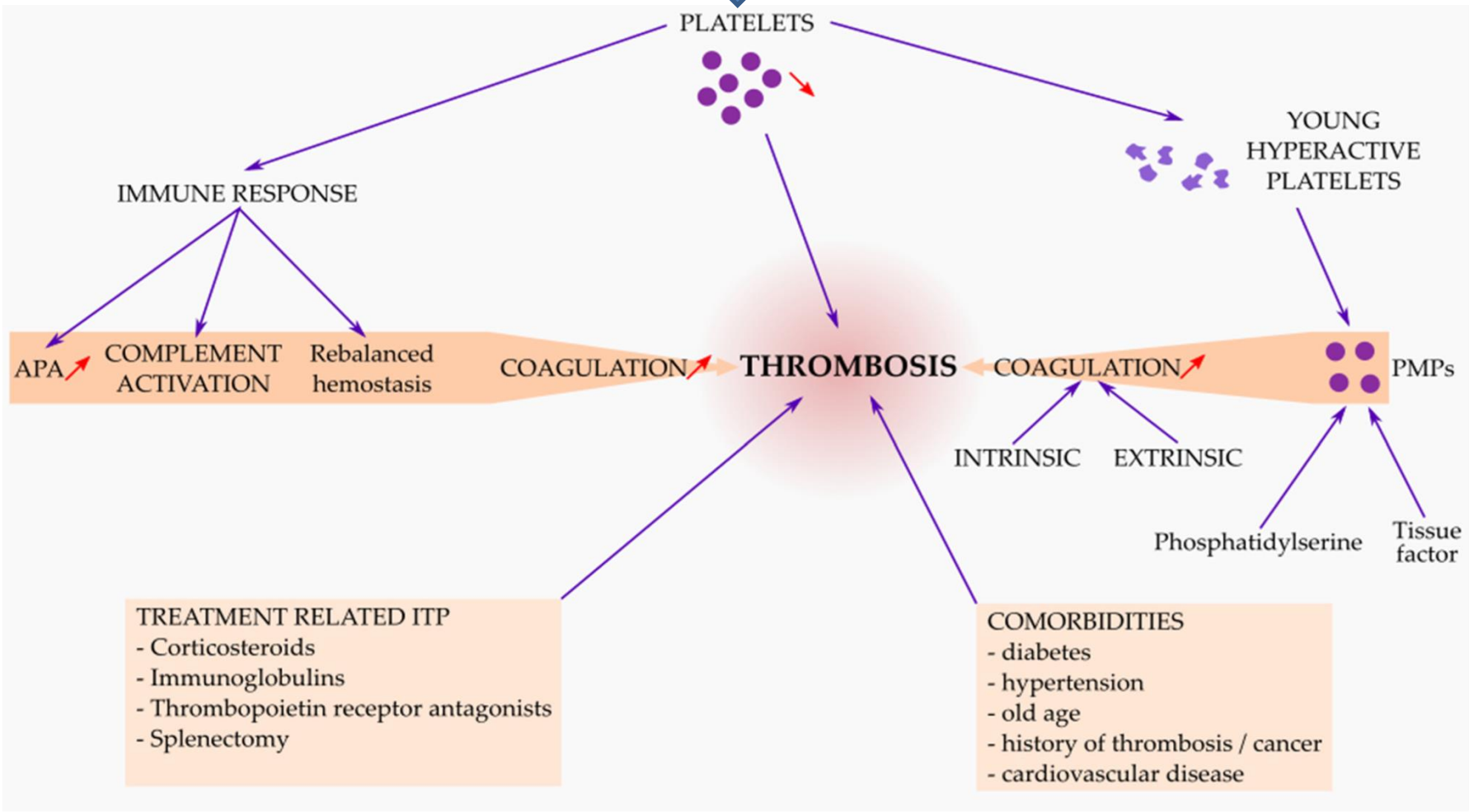
- Excessive bruising.
- Blood in vomit, stool, or urine.
- Heavy menstrual period
- Nosebleed



Reference:- Google Image of Case Based Study

IMMUNE THROMBOCYTOPAENIA (ITP)

Vertical integration



Biomedical Ethics

Components of Medical ethics

> a physician –patient relationship

> a physician – physician relationship

the relationship of the physician to the system of healthcare.

> the relationship of the physician to society

Doctor –Patient Relationship

- Medical ethics has 3 centres :doctor, patient and society.
- Its centre is the doctor –patient relationship.

MORAL DUTIES OF DOCTOR

Vertical integration

- The duty to help cure
- The duty to promote and protect the patient's health.
- The duty to inform
- The duty to confidentiality
- The duty to protects patients life
- The duty to protect the patient's life
- The duty to respect the patient's autonomy
- The duty to protect privacy
- The duty to respect the patients dignity.

Vertical integration

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

Suggested Research Article

Related Research Article

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9356658/

A Comprehensive Review of Thrombocytopenia With a Spotlight on Intensive Care Patients

Monitoring Editor: Alexander Muacevic and John R Adler

Ratnam K Santoshi,[✉1] Reema Patel,[2] Neil S Patel,[3] Varinder Bansro,[4] and Gurdeep Chhabra[5]

Abstract Go to:

Thrombocytopenia is a common entity seen in ICU patients and is associated with increased morbidity such as bleeding and transfusions, and mortality in ICU patients. Various mechanisms such as decreased platelet production, sequestration, destruction, consumption, and sometimes a combination of these factors contribute to thrombocytopenia. An understanding of the mechanism is essential to diagnose the cause of thrombocytopenia and to help provide appropriate management. The management strategies are aimed at treating the underlying disorder, such as platelet transfusion to treat complications like bleeding. Several studies have aimed to provide the threshold for platelet transfusions in various clinical settings and recommend a conservative approach in the appropriate scenario. In this review, we discuss various pathophysiological

Brain Storming

Question & Answer

Brain Storming

Clinical Scenario:-

A 32-year-old female patient presents to the emergency department with complaints of petechiae and easy bruising that started about a week ago. She reports no significant medical history or recent infections. on examination, multiple petechial rashes are noted on her extremities. laboratory investigations reveal a platelet count of 12,000/mm^3, while other blood cell counts are within normal limits. her coagulation profile, liver function tests, and renal function tests are all normal.

Reference:- Google Image of Case Based Study

Brain Storming

Questions Relevant to Vertical Integration

Question 1:-

Based on the patient's clinical presentation and laboratory findings, what is the most likely diagnosis?

ANSWER

Peripheral blood smear: This can help rule out other causes of thrombocytopenia, such as platelet clumping or abnormalities in platelet size or morphology.

Brain storming

QUESTION 2

What further investigations can be performed to confirm the diagnosis?

ANSWER: bone marrow aspiration and biopsy: although not always necessary, these tests may be performed to evaluate the production of platelets and rule out other causes of thrombocytopenia.

QUESTION 3

What are the initial treatment options for this condition?

The initial treatment options for ITP include:
Observation: In patients with mild or asymptomatic thrombocytopenia, observation without active intervention may be appropriate, especially in those without active bleeding or other significant symptoms.

Brain storming

Treatment Of ITP:-

- Corticosteroids: Prednisone is often the initial treatment of choice for ITP.
- Intravenous immunoglobulin (IVIG): IVIG can be administered to quickly raise the platelet count in patients with significant bleeding or those needing rapid platelet increase before surgery. It provides temporary passive immunity and blocks antibody-mediated platelet destruction.
- Platelet transfusion: Platelet transfusion may be considered in cases of severe bleeding or in preparation for invasive procedures, although it is not a definitive treatment for ITP and may have limited efficacy due to rapid platelet destruction.
- Second-line therapies: If the patient fails to respond to initial treatment, other options such as splenectomy, immunosuppressive agents (e.g., azathioprine, mycophenolate mofetil), or thrombopoietin receptor agonists (e.g., romiplostim, eltrombopag) may be considered.

Brain storming

QUESTIONS from practical notebook

QUESTION 1:-

What are the functions of platelets?

Platelets, also known as thrombocytes, have important functions in the body. They play a crucial role in hemostasis by forming blood clots to prevent excessive bleeding. Platelets release clotting factors that initiate coagulation. They also contribute to wound healing by releasing growth factors that aid in tissue repair. Platelets participate in the immune response by interacting with immune cells and releasing immune mediators. Additionally, platelets help maintain blood vessel integrity through the release of substances that regulate blood flow. Overall, platelets are essential for hemostasis, wound healing, immune response, and vascular integrity.

Brain storming

QUESTIONS

Question 2 : What is purpura and what are its causes?

Answer:

Purpura refers to purple or red discoloration of the skin caused by bleeding underneath the skin. It occurs due to various factors that affect blood vessels or platelets. Causes of purpura can include immune disorders like immune thrombocytopenic purpura (ITP) and thrombotic thrombocytopenic purpura (TTP), which involve platelet dysfunction. Other causes include infections, such as meningococcemia, certain medications, blood vessel inflammation (vasculitis), and underlying medical conditions like leukemia or vitamin C deficiency. Purpura can also result from trauma or injury. The specific cause of purpura is crucial for diagnosis and treatment, and medical evaluation is advised for proper assessment.

Brain storming

QUESTIONS

Question 3 : What will be the effect of thrombocytopenia on bleeding time and clotting time?

Answer:

1. Bleeding Time:

Thrombocytopenia typically prolongs bleeding time. Platelets play a crucial role in forming the initial plug to stop bleeding. With a reduced platelet count, the formation of this plug is compromised, leading to prolonged bleeding from minor injuries or cuts.

2. Clotting Time:

The impact of thrombocytopenia on clotting time can vary. While platelets are necessary for primary hemostasis (formation of initial plug), clotting time measures the effectiveness of the coagulation cascade, which involves clotting factors produced by the liver. In some cases, the liver compensates for the low platelet count by increasing clotting factor production, resulting in a relatively normal clotting time. However, if the underlying cause affects both platelet count and clotting factors, clotting time may be prolonged..

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Thank You!