Sunday 04 June 2023

CLINICAL PATHOLOGY

* PERIPHERAL BLOOD SMEAR:-

Importance of PBS: PBS (Peripheral blood film) is m. imp, valuable & frequently asked question in hematology lab. It provides the following inform:-

① Red cell morphology:-

Morphological features of RBCs like size, shape, colour, the of inclusions — imp. for diagnosis of Anaemias & other hematological disorders.

Variation in size of RBCs.	shape of RBCs.
① Normocyte [RBC-N, Normocytic normochromic Anaemia	① Normal - biconcave disc shape
② Microcyte (<6μ) [IDA, Thallasemia	② Poikilocytosis - severe anemia (diff. shapes)
③ Macrocyte - Megaloblastic anemia.	③ Spherocytes (sphere) → hereditary spherocytosis, Auto-immune hemolytic anemia
④ Anisocytosis - severe type of Anemia (variation in size)	④ Target cells (leptocytes): · Thallasemia, · sickle cell anemia.
	⑤ Sickle cells

⑥ Schizocytes (fragmented cells) – microangiopathic hemolytic anemia (DIC).

⑦ Elliptocyte (ovalocyte) – hemolytic anemia.
oval shaped

⑧ Pencil shaped – IDA. ⑨ Tear drop & pear shaped cell – B.M. infiltration.

Column of RBCs.

Normochromic	Hypochromic	Polychromasia
cell c 1/3 central pallor	> 1/3 central pallor	purple stained cell.
Normal RBCs	IDA & Thalassemia	Hemolytic anemia

② WBC disorders & DLC:
Quantitative & qualitative changes in WBCs suggests helps in diagnosis of hematological & non hematological disorders.

③ Platelet count & morphology:
useful in the diagnosis of bleeding disorders.

④ Cross check the CBC parameters (complete blood count): helps in cross checking the CBC parameters, derived from automated cell counters.

CBC / Hemogram → (i) Hb.
(ii) TLC
(iii) TEC
(iv) TPC
(v) DLC
(vi) Hematocrit / PCV.
(vii) Reticulocyte count
(viii) PBS — RBCs morphology
Platelet "
Hemoparasites
Any atypical / abnormal cell.

(5) Detection of blood parasites (Hemoparasites)

Hemoparasites include
(i) malarial parasite i.e. Plasmodium
(ii) microfilaria.
(iii) trypanosomes.
(iv) Leishmania donovani
(v) babesiosis.

RETICULOCYTE COUNT:

Reticulocytes → immature, non nucleated RBCs
- released from B.M.
- slightly larger than mature RBCs.

continue to synthesize Hb, after loss of nucleus.

Normal Reticulocyte count: expressed in terms of % of total RBCs

N - 0.5-2.5%

(Q10) Importance of Reticulocyte counting:

* ↑ Reticulocyte count seen in:

(1) Destruction / loss of RBCs
normal functioning B.M.:
(i) Hemolytic anemia.
(ii) hemolytic crisis
(iii) hemorrhage

(2) During therapy of deficiency Anemia & Appropriate substance:

* ↓ Reticulocyte count is seen in:
due to bad erythropoietic activity.

(1) B.M suppression:
(i) Aplastic anemia.
(ii) Aplastic crisis due to parvovirus
(hereditary spherocytosis & sickle cell disease)

(2) Deficiency Anaemia:
(i) ~~IDA~~ IDA
(ii) M.A.

Following Pt in
(i) IDA
(ii) F.A → def. anemia
(iii) Vit B_{12}
* highest count is seen on 6/7th day & indicates BM response to hemarrhage.

(3) primary & 2° polycythemia.

(2) Anemia due to organ failure [Renal failure hepatic disease.

(4) Pure Red cell Aplasia.

(5) Falconi Anemia.

(6) Myelofibrosis.

* In short, Reticulocyte count is a significant test during investigations of various hematological disorders.
It
(i) can almost accurately reflect hematopoetic activity.
(ii) It can help in diagnosis & differential diagnosis of anemias & other diseases.
(iii) It can assess the therapeutic response of different anemias.

- Reticulocytosis - can be a cause of peripheral macrocytosis & normoblastic hematopoesis; as commonly seen in hemorrhagic disorders.

May 2 June 2022

SIN

HEMATOCRIT (HCT) / Packed Cell Vol (PCV) / Erythrocyte volume Fraction.

*Defn: is the ratio of vol of RBCs : whole blood.

- It indicates the relative volume of RBCs & plasma.

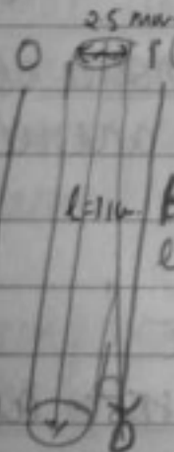
e.g. In anaemia → RBCs ↓ ⇒ ↓ hematocrit

*Method of Estimation of HCT/PCV:

(i) Macromethod using Wintrobe's tube.
(ii) Micromethod using capillary tube
(iii) automated analyzer.

*Wintrobe's method:

Wintrobe's tube is special thick walled glass tube — ℓ — (11cm)
d (internal) — (2.5mm)
capacity — (1ml)



Calculated upside → down
- (0 → 100) — ESR
- (100 → 0) — PCV estimation ↓ descending order

- Anticoagulants used — EDTA
  - dried heparin
  - double oxalate.

• Principle: Anticoagulated whole blood is centrifuged at std. speed. RBC > WBC, platelets, plasma settle down at the bottom & heavier vol. of RBC — indicate the hematocrit

• Different layers:

— Upper layer: clear straw coloured fluid
↳ plasma.

— Middle layer: thin Red grey layer.
↳ platelets & WBC.
L/w ↳ plasma
RBC.

— Lower layer: RBC (packed).

• Normal Range of PCV / HCT: expressed in % (e.g. 45%) or decimal fraction (0.45).
⇓
useful for estimating absolute values like MCV, MCH

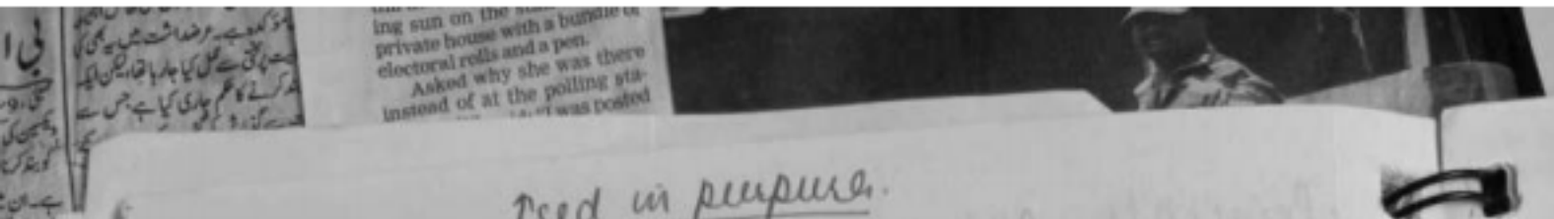
Adult ♂ - 38-47%
Adult ♀ - 36-46%
Infant - 45-70% (cord blood)

HB (g-dl) × 3 = HCT

PCV.
↑ ↓

① Polycythemia vera rubra
② secondary polycythemia

• Anemia.



used in purpura.

SIN

Bleeding Time (BT)

(i) Normal Range: (2-9 min)

(ii) Defn: time interval b/w oozing of blood from cut/injury till stoppage of bleeding.

(iii) Method: (i) Duke's method (obsolete)
(ii) Ivy's method
(iii) Template method - method of choice

Template Method for B.T:-
Template: disposable blade, fitted on to a holder.
made up of plastic, used for test.
≠ recommended as routine pre-operative screening test.

• BT - usually ≠ prolonged in pt. c coagulation factor deficiencies.

(iii) Prolonged B.T. is found in:
(i) Platelet disorder - ~~PLT~~ (TPC < 50k/mm³)
• Quantative: Thrombocytopenia.
• Qualitative: VW disease.

Test in hemophilia

Coagulen / Clotting Time (CT)
(4-11 min)

(i) time interval ~~from~~ b/w oozing of blood from cut / injury till formation of clot.

(ii) Lee-White Method (obsolete now.)

(iv) Disadvantages:

(a) Not sensitive: — One of the oldest tests & ≠ used now
↳ fails to detect mild/moderate coagulens defects.

↳ obsolete now & ≠ recommended as screening Test.

PTT - more sensitive test for assessment of coagulation cascade

(b) Misleading: —
↳ Normal value may be obtained in mild → moderate Hemophilia A & B.
≠ exclude major factor deficiency

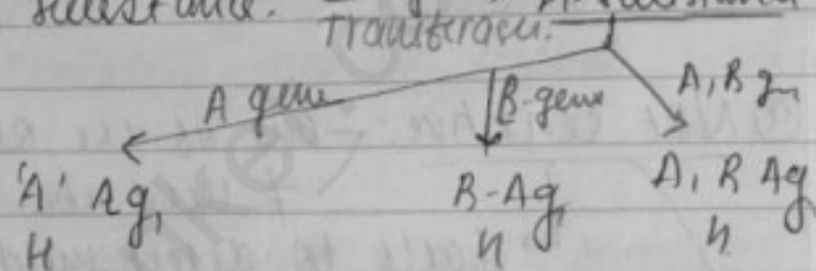
Thurs. 09 June 2022

Bombay Blood Group

↳ special type of blood group, where there is no H-substance, expression over RBCs.

* H is the basic precursor Ag for both A & B Ag. So, it is one of the Ag of ABO blood grp. types.

Basic precursor substance. $\xrightarrow[\text{Transferase}]{\text{H-gene}}$ H-substance



Precursor substance	Genes (inf)	'Ag' expressed over RBCs	Blood Grp
H	A	A, H	A
	B	B, H	B
	A, B	A, B, H	AB
	(-ve)	H	O

*Usually, RBCs from all persons contain H-Ag on their surface, so, in the pres/-nce of A & B gene, H Ag will transform into, A, B, AB or H Ag (No change)

• Depending on these ④ types of ABO blood grps are categorised.

*In case of Bombay blood grp ⇒ no H gene
⇓
∴ no H-substance / 'H' Ag

∴ So even in the presence of A/B gene, no A/B Ag will be expressed over RBCs surface.
∴ RBC will not express any form of ABO Ag (A, B or H).
During forward/classical blood grouping, there will be no clumping of test blood cells with A/B sera. So, the result will mimic 'O' blood group.

*Problem arises in preformed serum Abs.
In ABO blood grp. system, plasma will contain preformed Abs against (not) Ag.
So, [Blood grp A ⟶ Anti-B
" B ⟶ Anti-A
" AB ⟶ no Abs
" O ⟶ Anti A, B.
] ← capable of Agglutinating
as all blood grp contain (H) Ag — so, no Anti-H agglutinins

*But Bombay blood grp will have Anti-H Abs in their serum. So, they show transfusion rxn with all other blood grps of ABO system i.e., A, B, AB, O.
•This agglutinating serum Abs - demonstrated by reverse blood grouping.

*Importance of Bombay Blood grp:
① Bombay blood grp pt. can accept blood only from Bombay blood grp donor.
Why ⇒ ∴ donor serum becomes different

(ii) A register maintaining Bombay blood grp. carrier-must be maintained at state & national level, for collection of blood, when necessary.

ABO Blood Grouping Technique

② type (R2)

- Forward (cell typing) — Ag on RBC - detected
- Reverse (serum typing) — Ab in serum/plasma - detected

- Reverse blood Grouping: (usually done by tube method)

Procedure:

Patient serum
⊕
Reagent (RBCs) of known blood grp (supplied / prepared)
↓↓
Incubate — 5 min at room temp
↓↓
centrifugation
↓↓
sedimentation of RBCs at the bottom of test tube
↓↓
Resuspension of sedimented RBCs by gentle tapping

- clumps of RBCs suspended in medium ⊕ve test (anti RBC Ab test)
- Uniform suspension of RBCs ⊖ve test (Serum Ab -ve)

- Separate tubes for ⊕ve & ⊖ve controls are used.

- By reverse blood grouping, serum Ab against RBC surface Ag can be detected.
- Routine forward blood grping can only detect Ag present on the surface of RBCs, using known Anti A/B Ab.
↳ This method can miss out anti RBC Ab present in serum of 'ABO' blood grp. which may lead to transfusion hazards.
Ex. A Bombay (hh) blood grp — I'd as 'O' blood grp (∵ no ABO Ag on the surface of RBCs).
But,
if Bombay (hh) blood grp ⇒ transfused to 'O' blood grp
↓↓
Hemolysis
(∵ Anti 'H' Ab present in recipient's serum).

* Reverse blood grouping — demonstrates the presence of serum Ab (anti H Ab) against all types of RBCs of ABO system.
∴ transfusion hazards can be avoided.

*Other advantages of reverse blood grouping are:—
(i) Easier i'd of weakly reactive Ag (like A1).
(ii) A method of double checking, to rectify any flaw of forward grouping, as mentioned above
(iii) to detect serum anti RBC Ab, which may be missed during usual grouping.
(iv) v. imp. in cases, where there will be pathological change in antigenicity of RBCs (e.x. colonic carcinoma)

Transfusion Related disease can be avoided

- A lot of infective diseases can be transmitted during blood transfusions like:-
  - (i) Bacterial: Syphilis.
  - (ii) Viral – Hepatitis; B, C, D & HIV-I & II
  - (iii) Parasitic – Malaria

- For transmission to occur, the donor must be a sufferer / carrier of the disease.
- Nowadays, adequate tests are available for the detection of infection in donor's blood & unless seen to be devoid of all contaminants ⇒ ≠ used for transfusion.

⇒ Problem arises when blood is collected during incubation period of infection.

↳ usual screening test may ≠ ⊕ve.

Ex- During early phase of HIV infection

↓

ELISA may be (-ve)

↓

ELISA ⊖ve case.

↓

further examined for the
presence of P-24 antigenemia

↓

will be ⊕ve even during earlier phase of infection.

→ In this way, almost all infected carriers can be detected, irrespective of the duration of disease.

- Rare blood samples are destroyed healthy, disease free samples.
  ↓
  preserved for future blood transfusion.

→ So, safe blood banking procedure, transfusion related diseases can be avoided.

Q10 Blood component therapy is beneficial than whole blood transfusion.

- After collection of blood donor, blood is divided into different components by centrifugation.

Whole blood.

↓ 1st centrifugation

↓ Packed RBC / Red cell concentrate

↓ Platelet rich plasma.

↓ 2nd centrifugation

↓ Platelet concentrate

↓ Platelet poor plasma / Fresh plasma

↓ S/N

FFP

↓ Cryoprecipitate

- Insoluble plasma pr.
- Fibrinogen
- Factor VIII
- VWF

* These components are stored in appropriate cond" & used for transfusion to suitable recipient a/c need.

The main advantage of component therapy, over whole blood transfusion are:

(I) A single unit of blood can be used for benefit of 7-1 pt. So, it is cost effective.
Ex. RBC can be transfused to an anemic pt.
& plasma to burn pt.

(II) It can ensure that only the required component are transfused. Ex - During tt of anaemia, transfusion of whole blood ⇒ vol. overload ⇒ heart failure.
∴ Packed RBC transfusion is free from all these hazards.

(III) Component seperation helps to maintain the potency of individual components for longer period. Ex - whole blood $\xrightarrow[\text{after 48 hrs}]{2-6^\circ C}$ no platelet.

- platelet concentrate $\xrightarrow[\text{preserved}]{\text{properly}}$ can be used for (5-6 days)
- Cryoppt & FFP can be used for more longer period

(IV) Component seperat - ↓s the wastage of donated blood. ~~there~~ at least one/2 components > single unit chance of whole blood utilisation.

So, component seperation should be encouraged in blood banking.

(RIN)

Fresh Frozen Plasma (FFP)

→ prepared by freezing the plasma.

→ contains ← plasma proteins & all coagulation factors

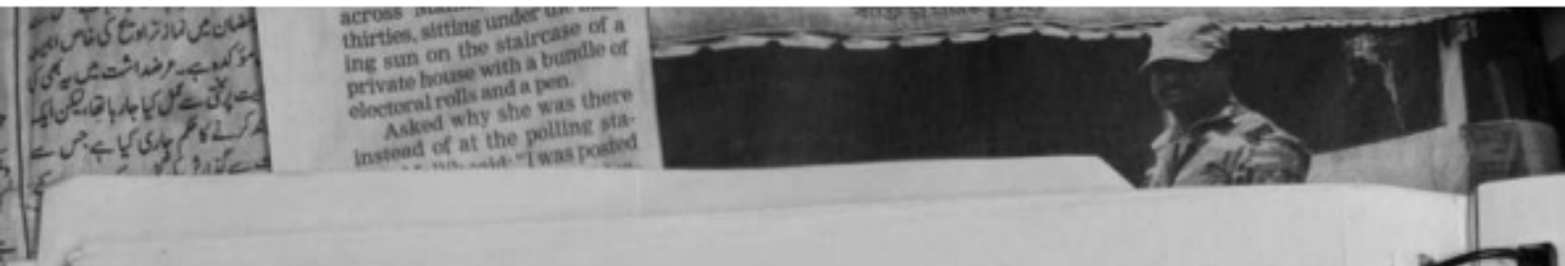
that, include. ① Albumin
② Protein C
③ Protein S.
④ Antithrombin III
⑤ VWF

(PTB)

★ It is indicated in replacement of coagulation factors, in acquired coagulation factor deficiency.

★ Indications for FFP:

(i) Pt. on anticoagulant drug therapy (ex. Coumarin)
(ii) vit K deficiency
(iii) Antithrombin deficiency
(iv) coagulopathy of liver disease.
(v) Microangiopathic hemolytic Anemia, includy TTP, hemolytic uremic syndrome, HELLP syndra
(vi) DIC. ✓



(81N)

TRANSFUSION REACTIONS.

→ Blood transfusion is useful & life saving when performed ~~c~~ caution & ~~c~~ clear indication.

- St. (~2-4% of cases), unfavourable complications can occur, in spite of ⌐ precaution & preventive measures, which are k/a transfusion reactions.

Complications of blood transfusion / Transfusion reactions.

Infectious complications

→ some diseases transmitted by transfusion are.

① Hepatitis (HBV, HCV, HDV)
② HIV (AIDS)
③ Cytomegalovirus
④ Malaria.
⑤ Syphilis.

Non-infectious complications.

Immediate Rxn

Immunological

① Hemolytic transfusion rx.
② febrile non-hemolytic rx.
③ Allergic rx
④ Anaphylactic rx.
⑤ TRALI
Transfusion related acute lung injury.

Non immunological.

① circulatory overload
② Air embolism.

Delayed RXn

Immunological.

① Alloimmuniz-ation
② hemolytic transfusion rxn (mostly asymptomatic).
③ transfusion-associated graft-versus-host disease.

Non immuno

① Fe-overlo-ad transfu
② hemo-siderosis
③ Thrombo-phlebitis.