Diagnostic Parameters in Iron Deficiency Anaemia

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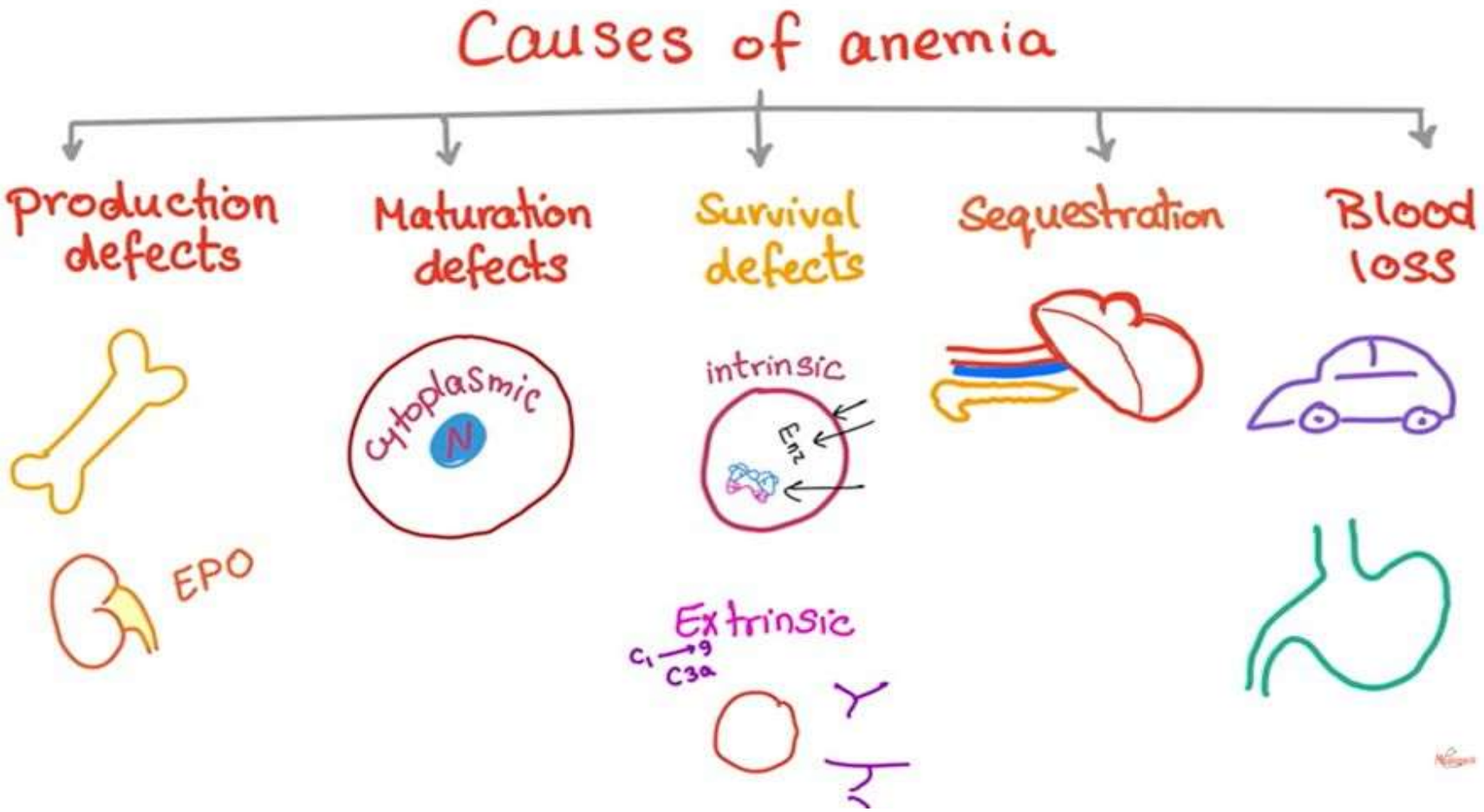
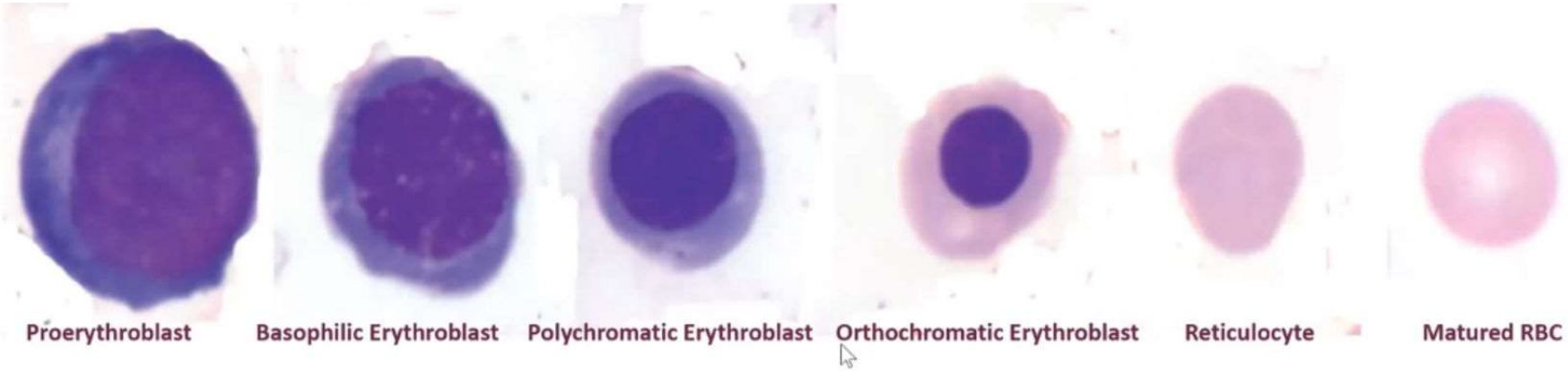
Consultant haematopathologist

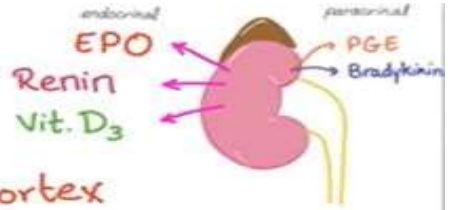
Mechanisms of Anaemia



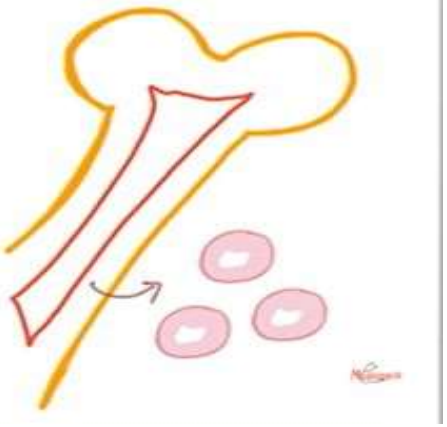


Haematopoeisis

Erythropoietin (EPO) Synonyms: Hemopoietin

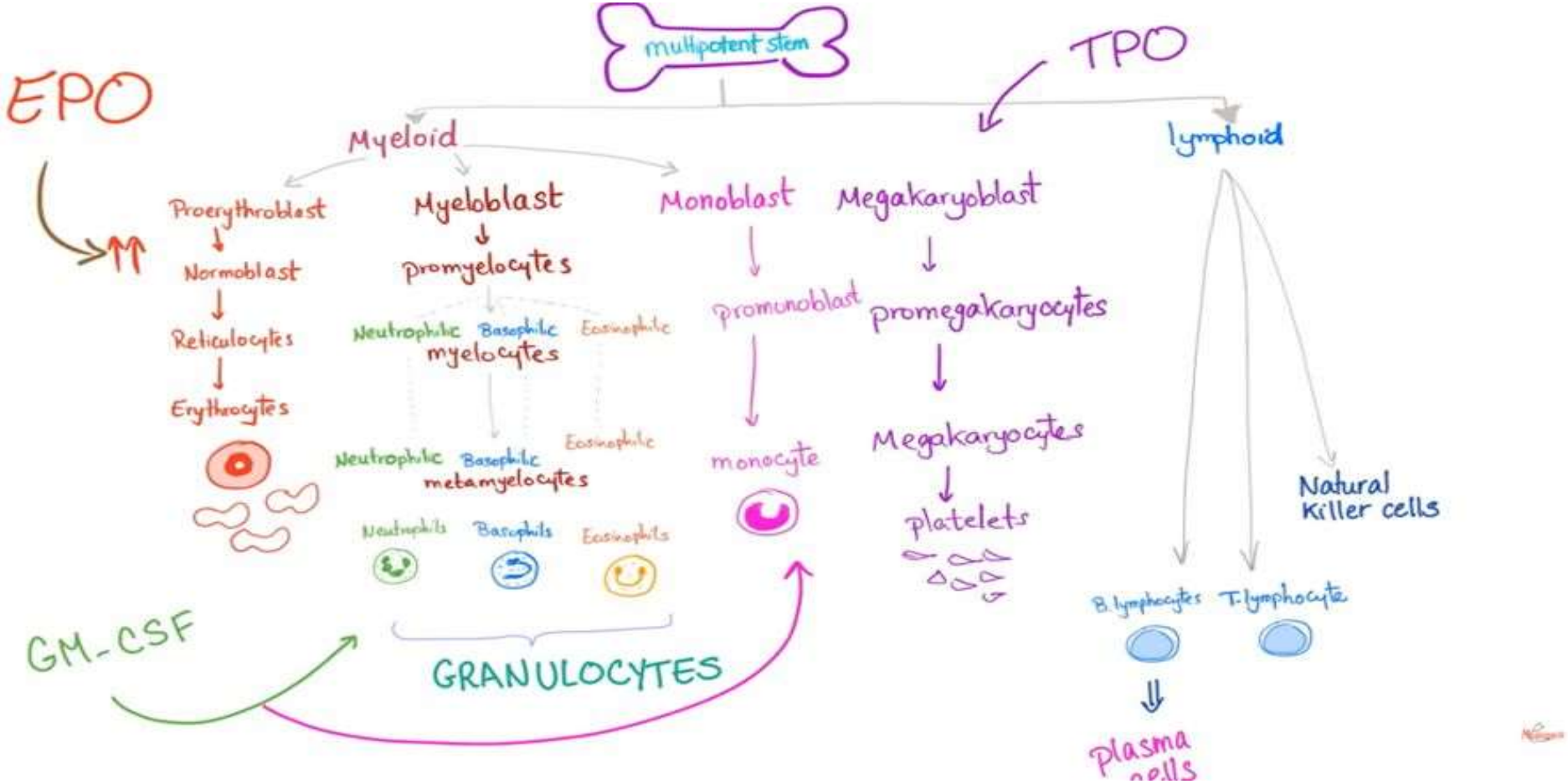


- Nature ? protein (glycoprotein)
- Secreted by ? - Kidney (mainly) Cortex or medulla ? - Cortex
  Where ? - interstitial fibroblasts near the peritubular capillaries (peritubular capillary endothelium)
  - liver (pre-natal, perinatal)
- Function ?
  - 1ry regulator for RBC production (erythropoiesis)
- Stimulus ?
  - Hypoxia
    - ischemia
    - Hypoxemia
    - Hemoglobin abnormalities (Dyshemoglobin)
      - CO poisoning
      - Methemoglobinemia



RBC production-EPO

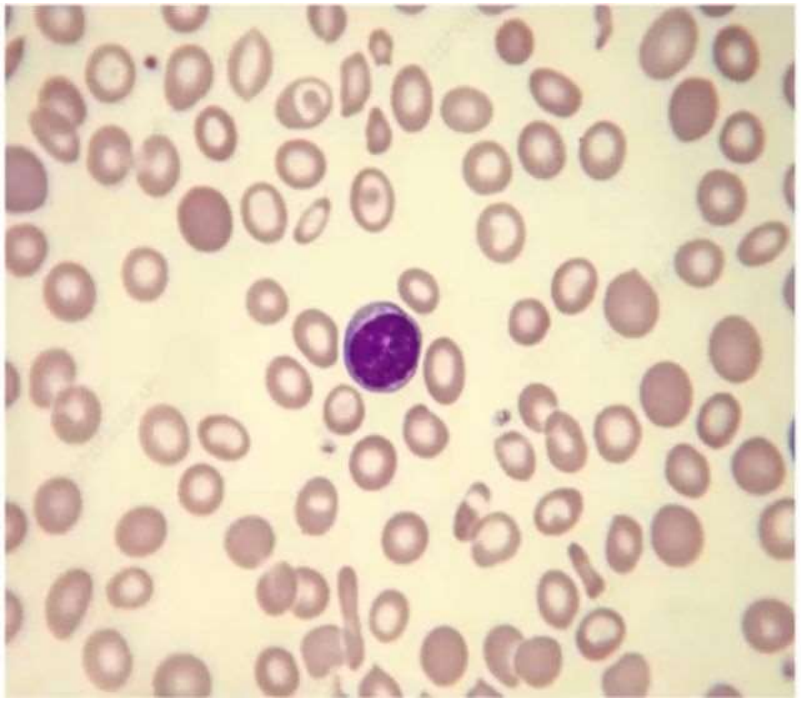
EPO-Erythropoeisis

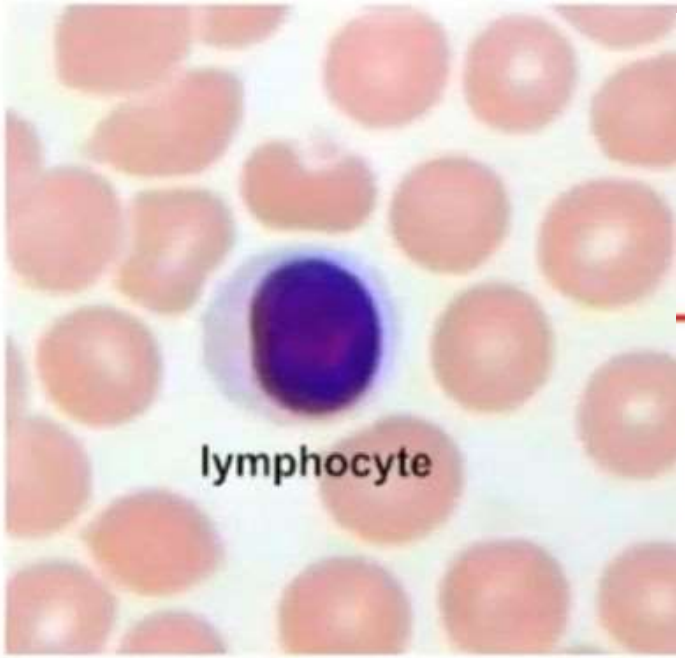


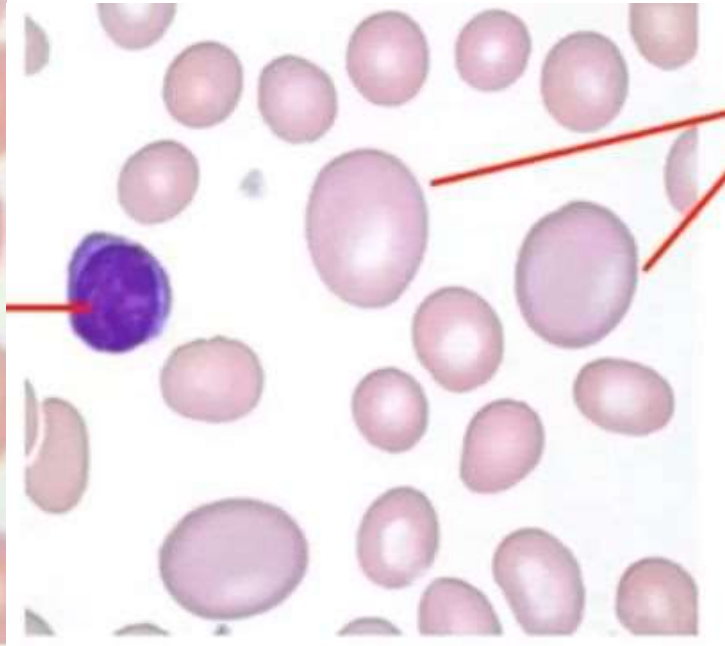
Classification of Anaemia-MCV Model

MCV<80FL	MCV:80-100FL	MCV>80FL
• Thalassaemia • Anaemia of chronic disease • **Iron deficiency Anaemia** • Lead poisoning • Sideroblastic Anaemia	• Depending on Retic count • Hypoproliferative-Bone Marrow Failure • Hyperproliferative-Blood loss or hemolysis	• Folate deficiency • Vitamin B12 deficiency

Macrocytosis versus Microcytosis





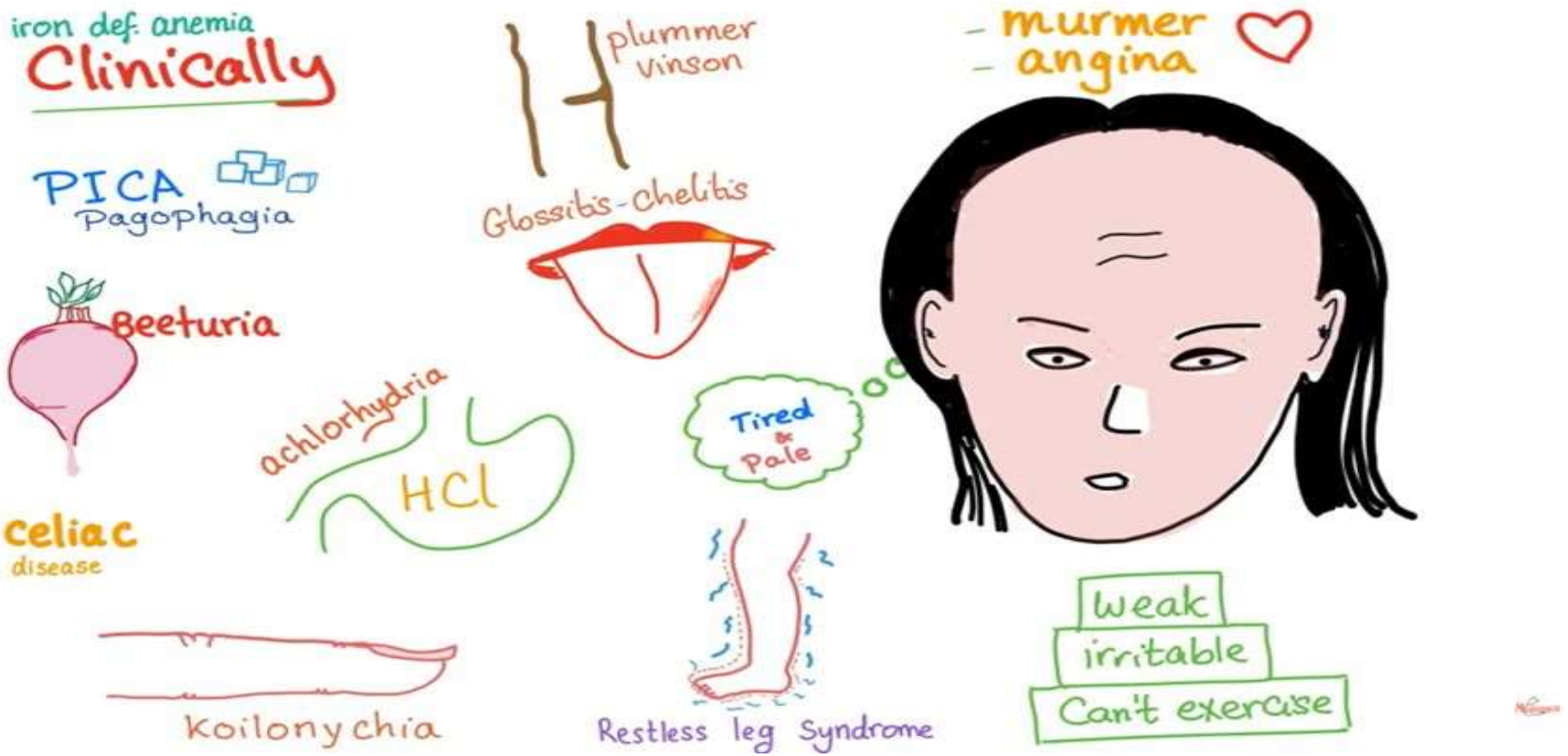


Iron Deficiency Anaemia-prevalence

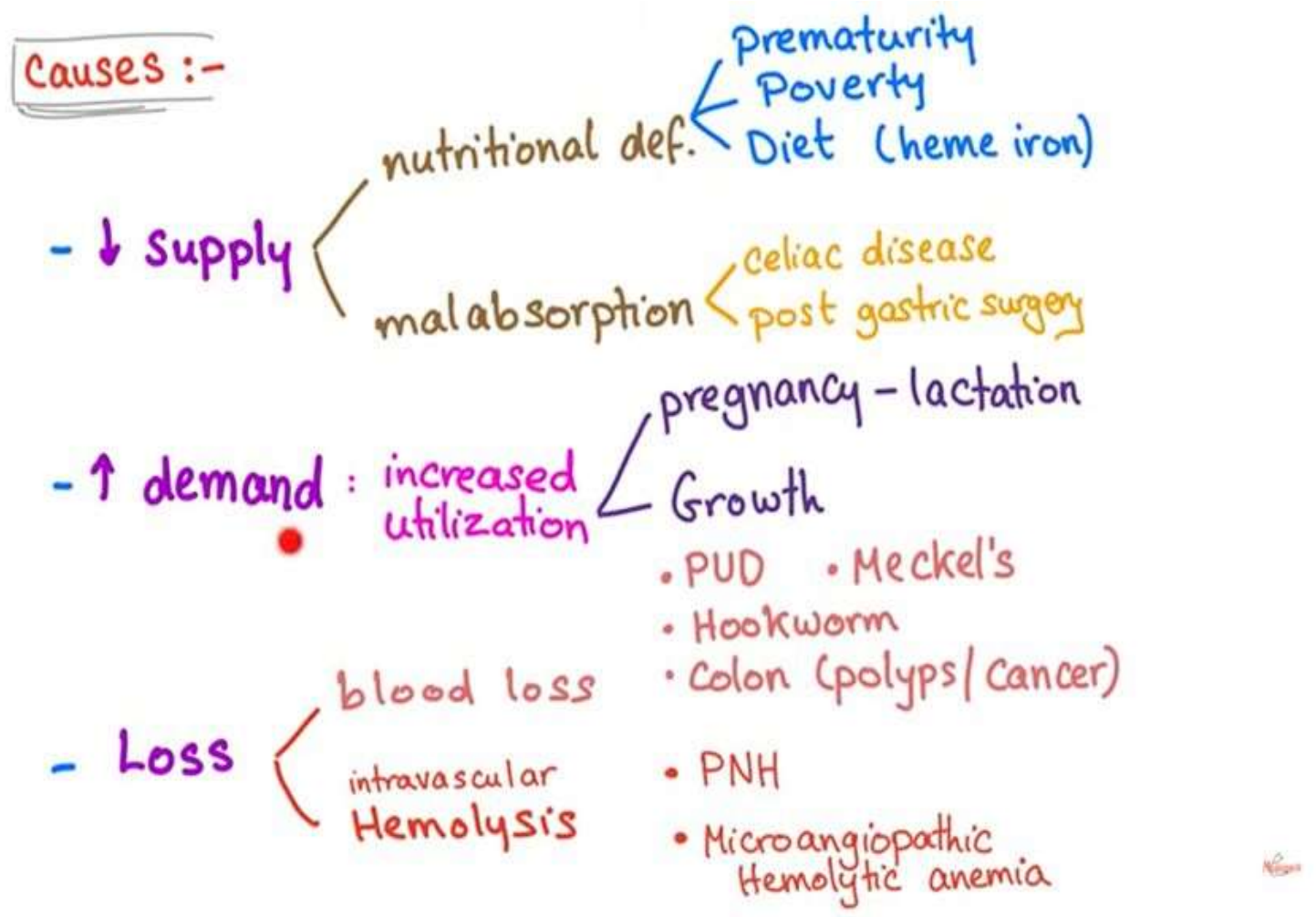
- Most common Anaemia worldwide
- Prevalence of 24.8% affecting 1.62 billion people
- Insidious onset,chronic often asymptomatic

TARGET
PATHOLOGY LABORATORIES
Fast · Accurate · Reliable

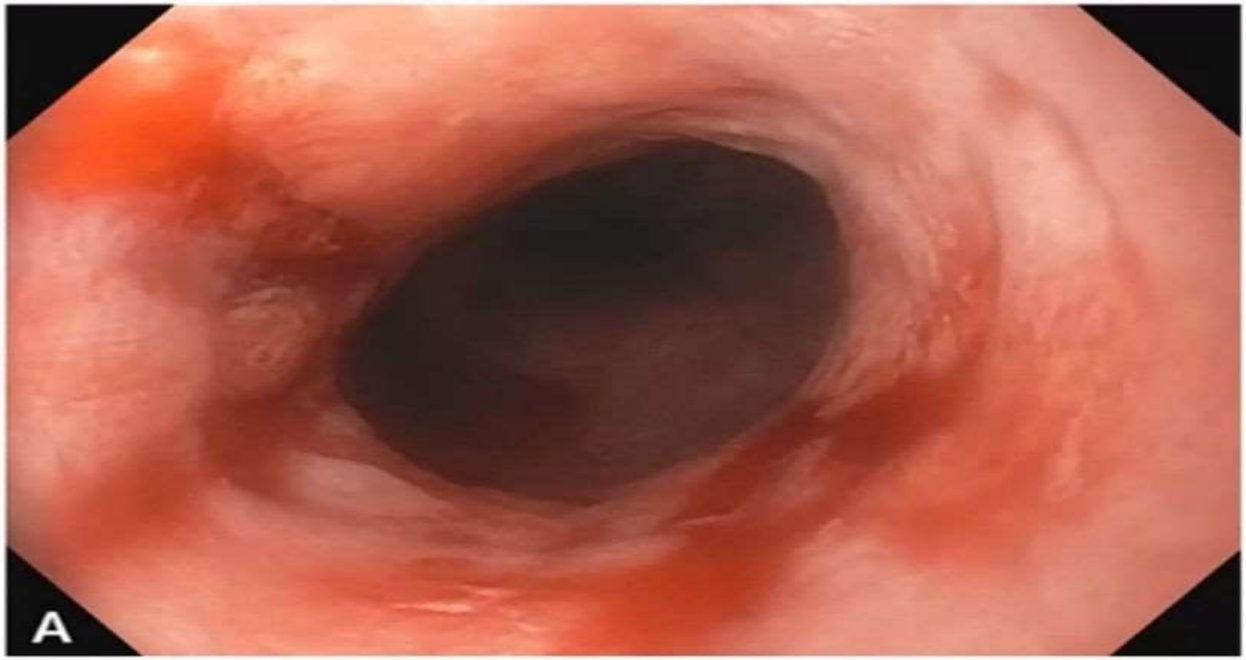
Iron Deficiency Anaemia-clinical presentation



Iron Deficiency- auses

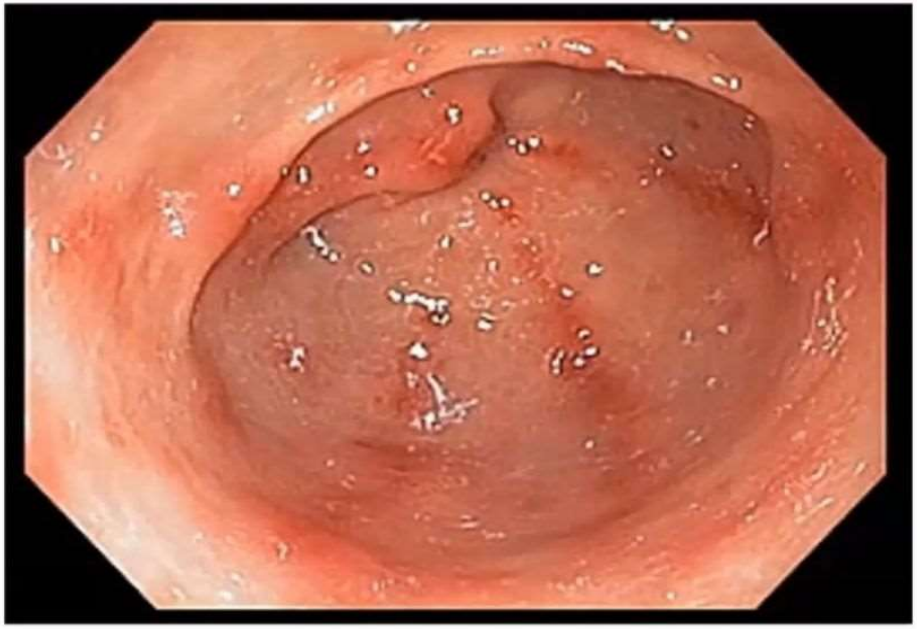


Esophagitis



Endoscopic examination of the esophagus showing esophagitis and bleeding. This may cause retrosternal pain or may be asymptomatic and present as iron deficiency.

Gastritis



In any case of gastritis, look for H. Pylori infection as it has multiple mechanisms of causing iron deficiency anemia.

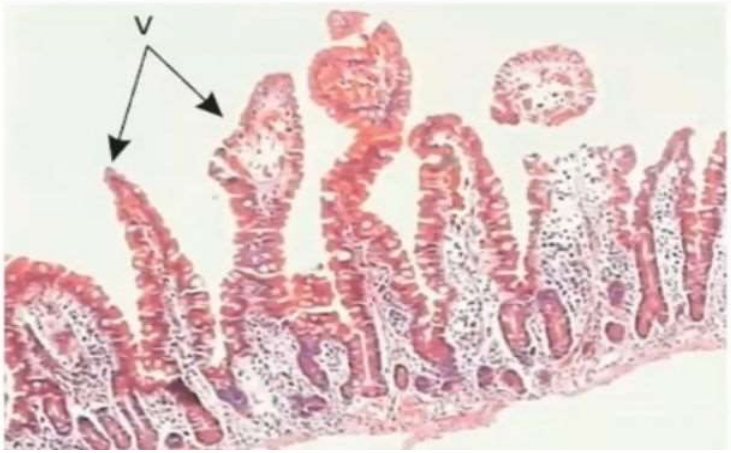
Possible pathogenic mechanisms involved in iron deficiency anemia in patients with *H. pylori* infection:

- It causes occult blood loss secondary to chronic erosive gastritis,
- It decreases iron absorption secondary to chronic gastritis causing hypochlorhydria or achlorhydria,
- It uptakes iron and use it by itself.

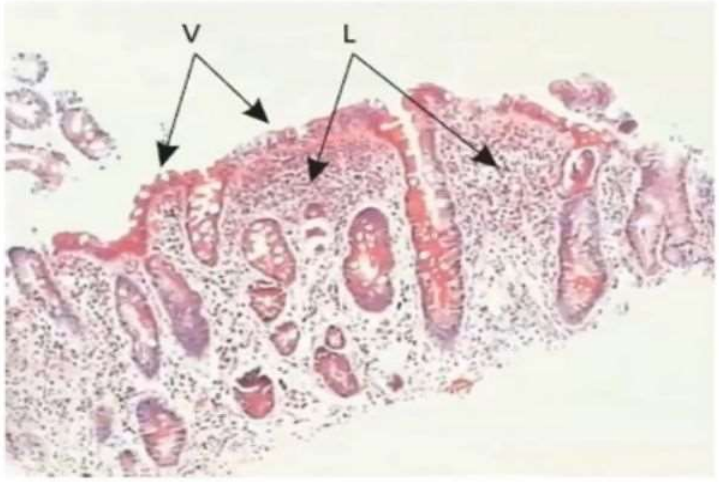
***H. pylori* eradication reverses iron deficiency in patients with asymptomatic gastritis and improves oral iron absorption.**

Small Intestine-coeliac disease

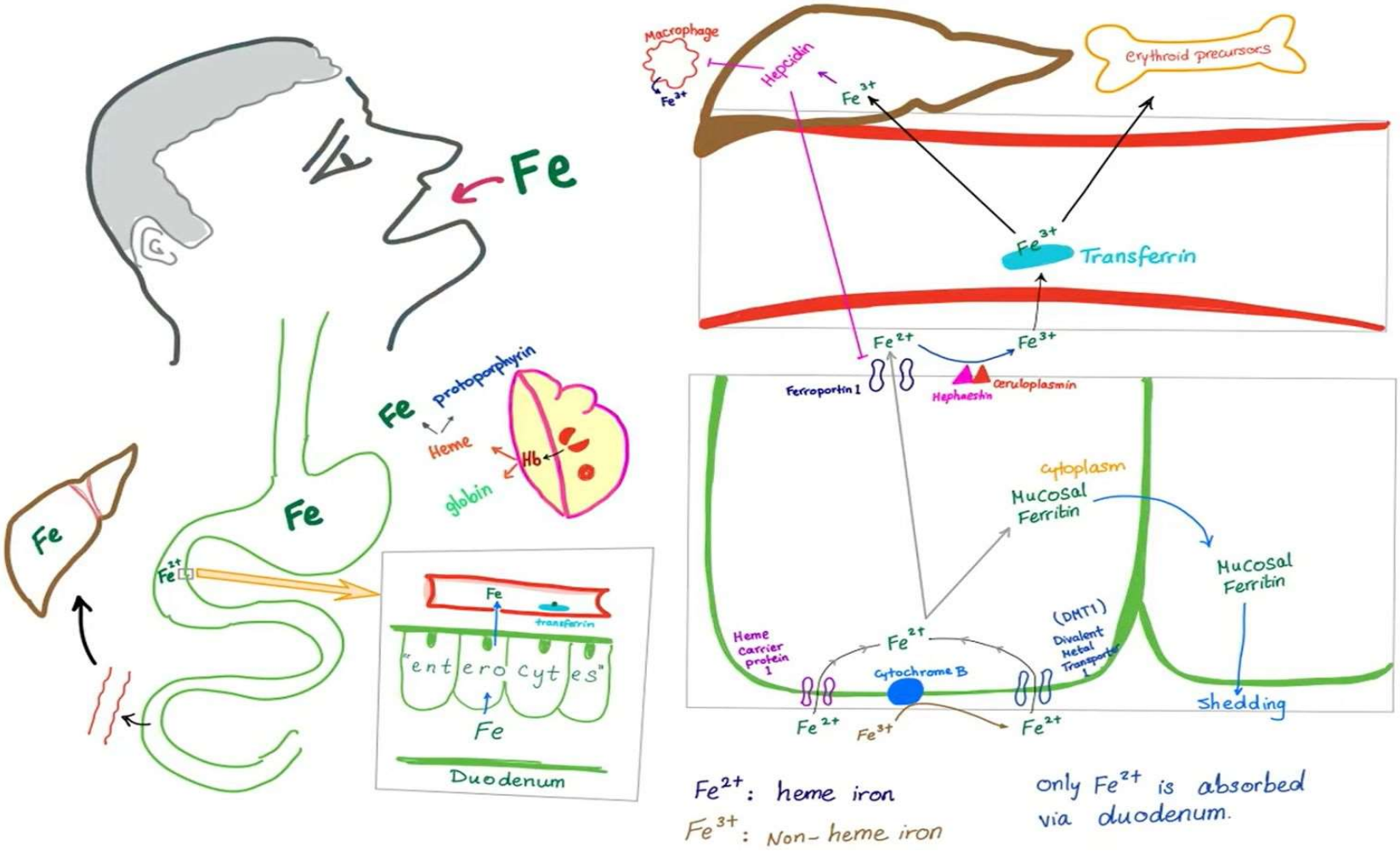
The normal pattern of the villi (V) in the small bowel.



Flattened villi (V) and lymphocyte (L) infiltrate present in coeliac disease.



Iron Absorption

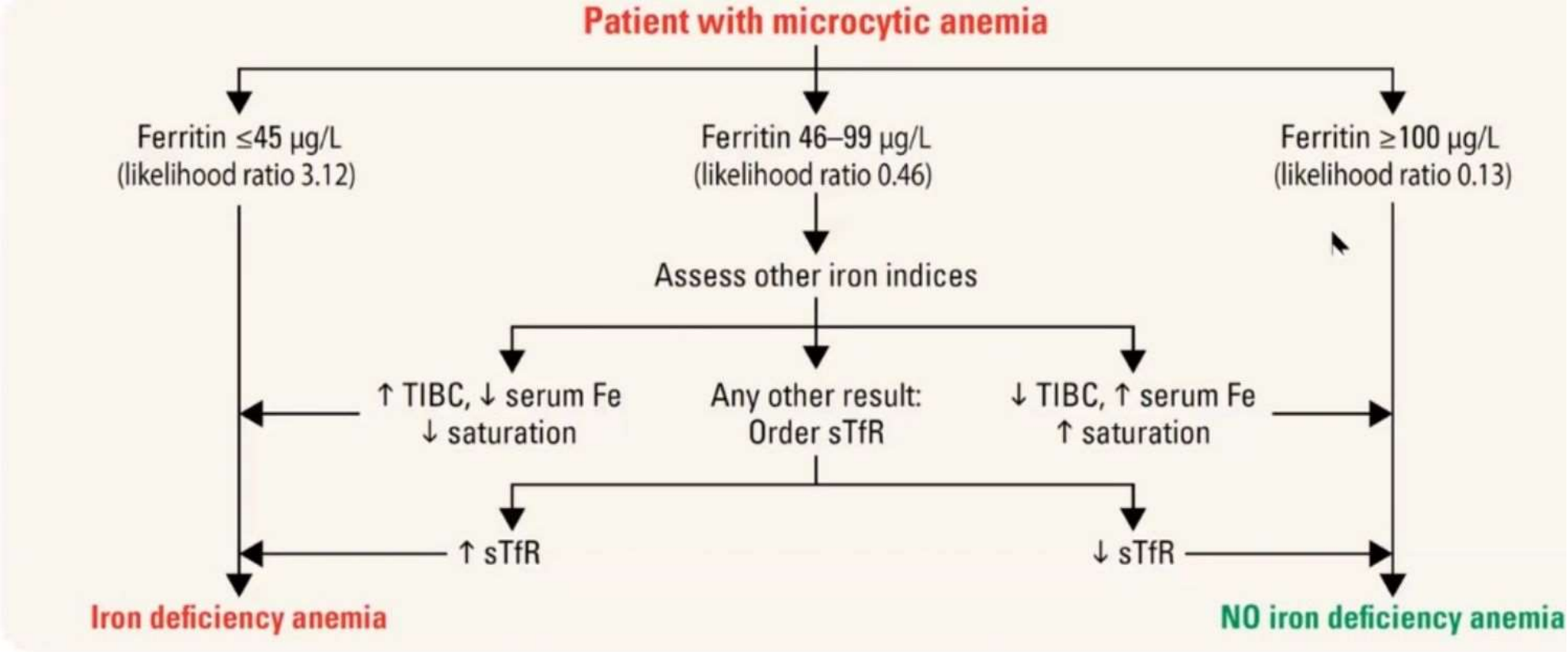


IDA-Laboratory findings

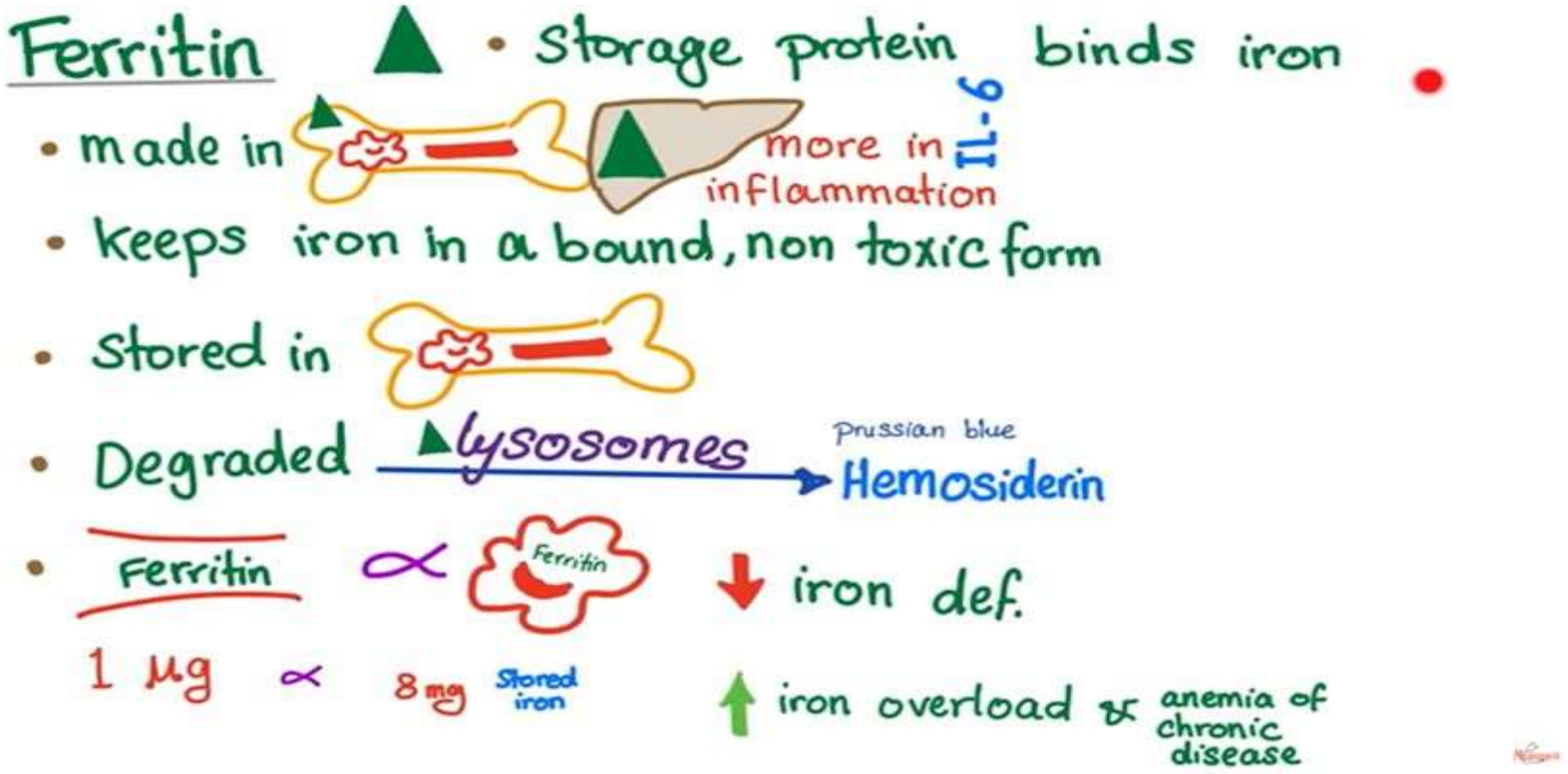
	Patient results	Normal range (female)
Hb	8.0 g/dL	11.5–16.4 g/dL
MCV	62 fL	83–99 fL (μm^3)
MCH	19.0 pg/cell	26.7–32.5 pg/cell
MCHC	30 g/dL	30.8–34.6 g/dL
WBC	5.3 × 10^9/L	4.0–11.0 × 10^9/L (10^3/μL)
Platelets	550 × 10^9/L	140–450 × 10^9/L (10^3/μL)

- Ferritin level
- Iron studies
- Peripheral smear
- BMA

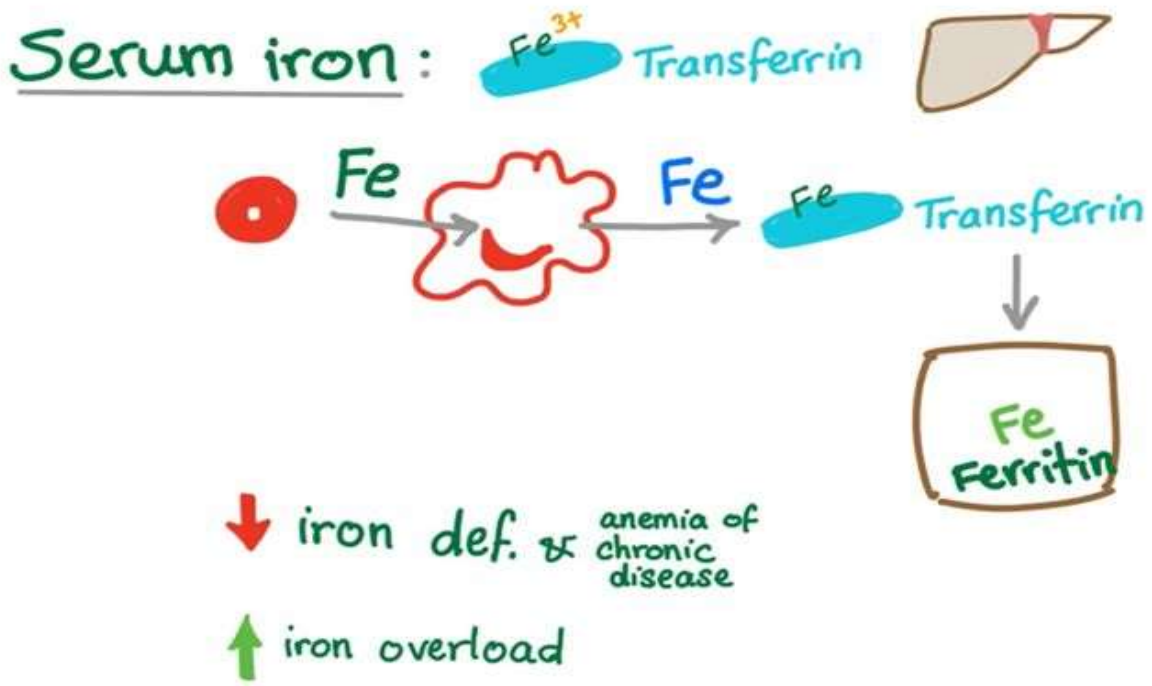
Traditional diagnostic parameters for IDA DX



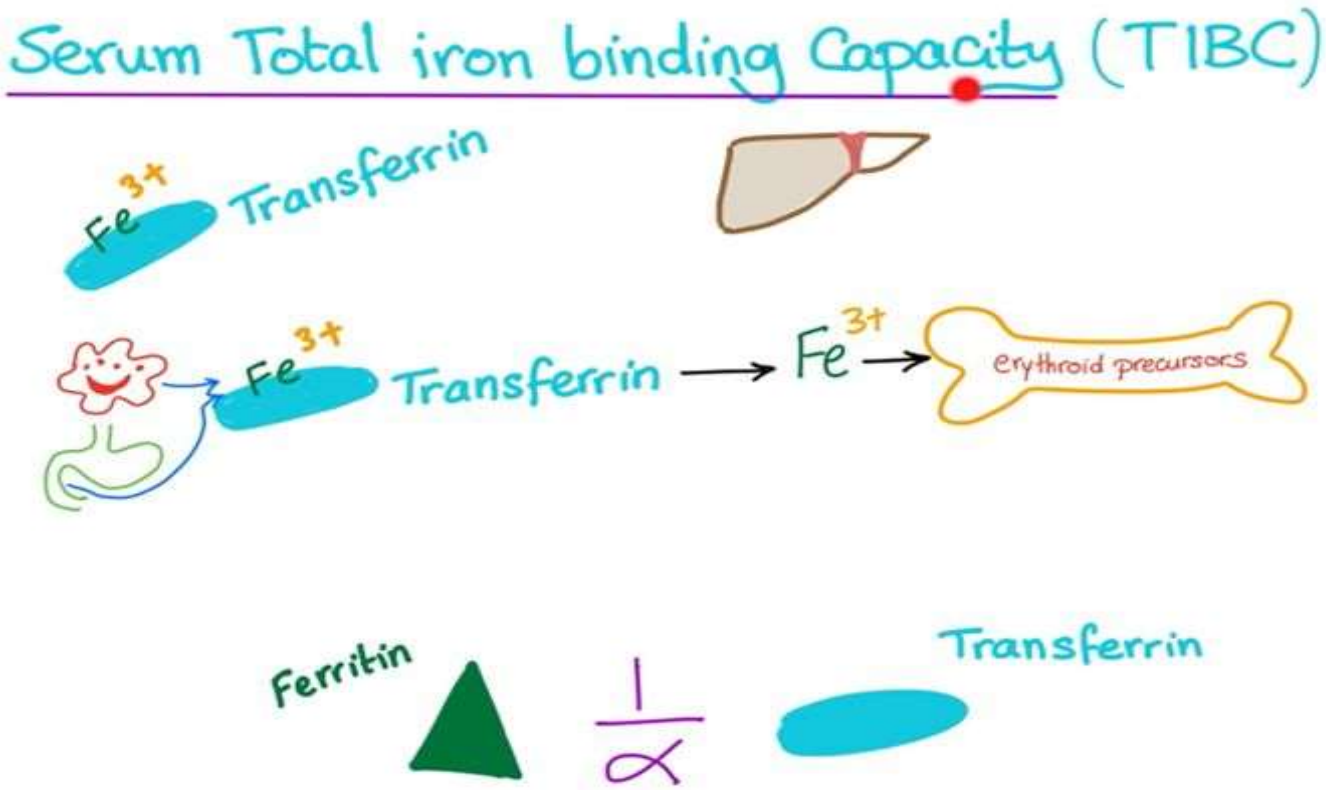
Ferritin


Ferritin
• Storage protein binds iron
• made in
more in inflammation
IL-6
• keeps iron in a bound, non toxic form
• Stored in
• Degraded lysosomes
Prussian blue
Hemosiderin
• Ferritin ∝ Ferritin
iron def.
1 µg ∝ 8 mg Stored iron
iron overload & anemia of chronic disease

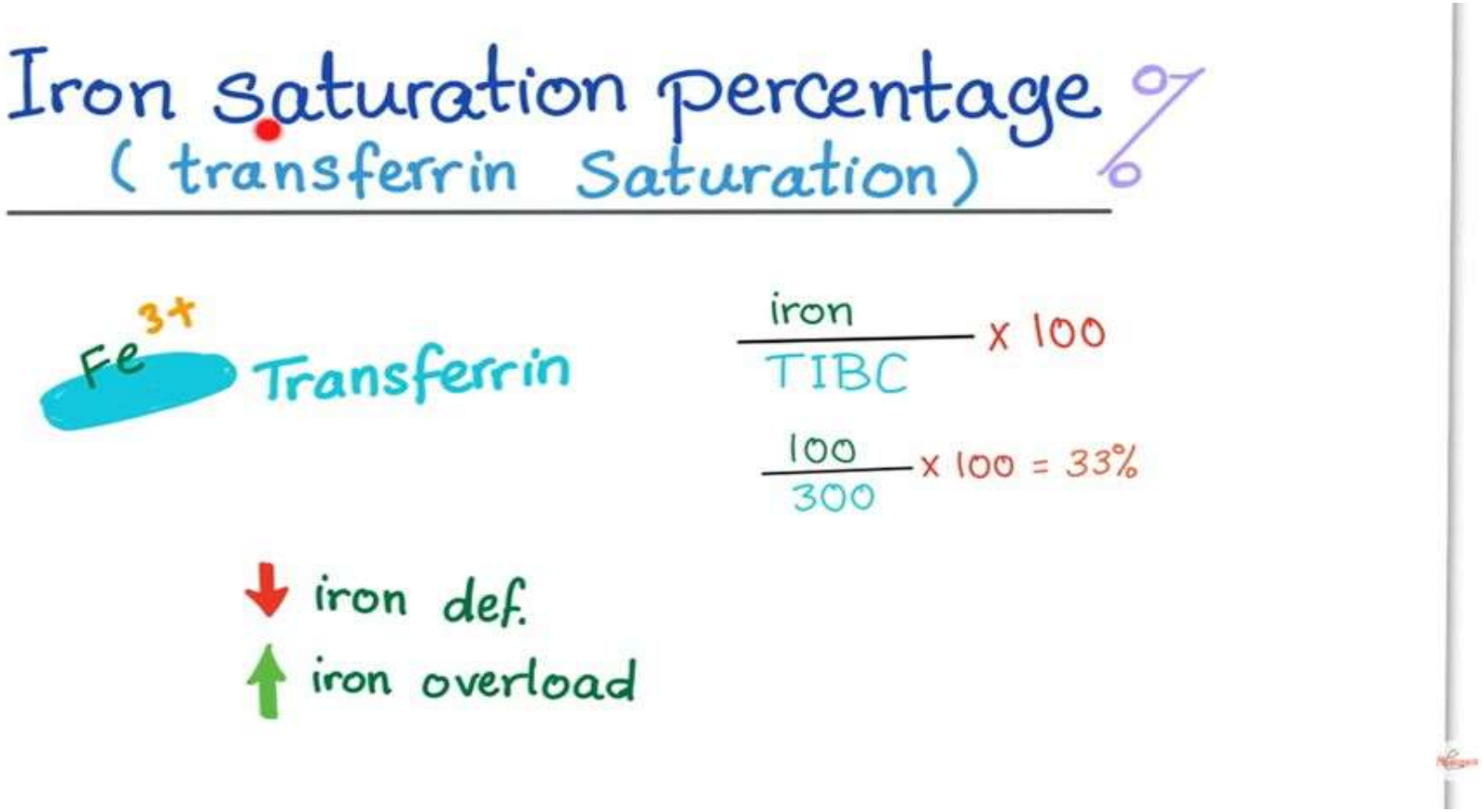
Serum Iron


Serum iron :
Fe3+
Transferrin
Fe
Fe
Fe
Transferrin
Fe
Ferritin
iron def. & anemia of chronic disease
iron overload

TIBC


Serum Total iron binding Capacity (TIBC)
Fe 3+
Transferrin
Fe 3+
Transferrin
Fe 3+
erythroid precursors
Ferritin
1/α
Transferrin

Iron saturation percentage



STFR

Soluble transferrin receptor (STFR) Concentration

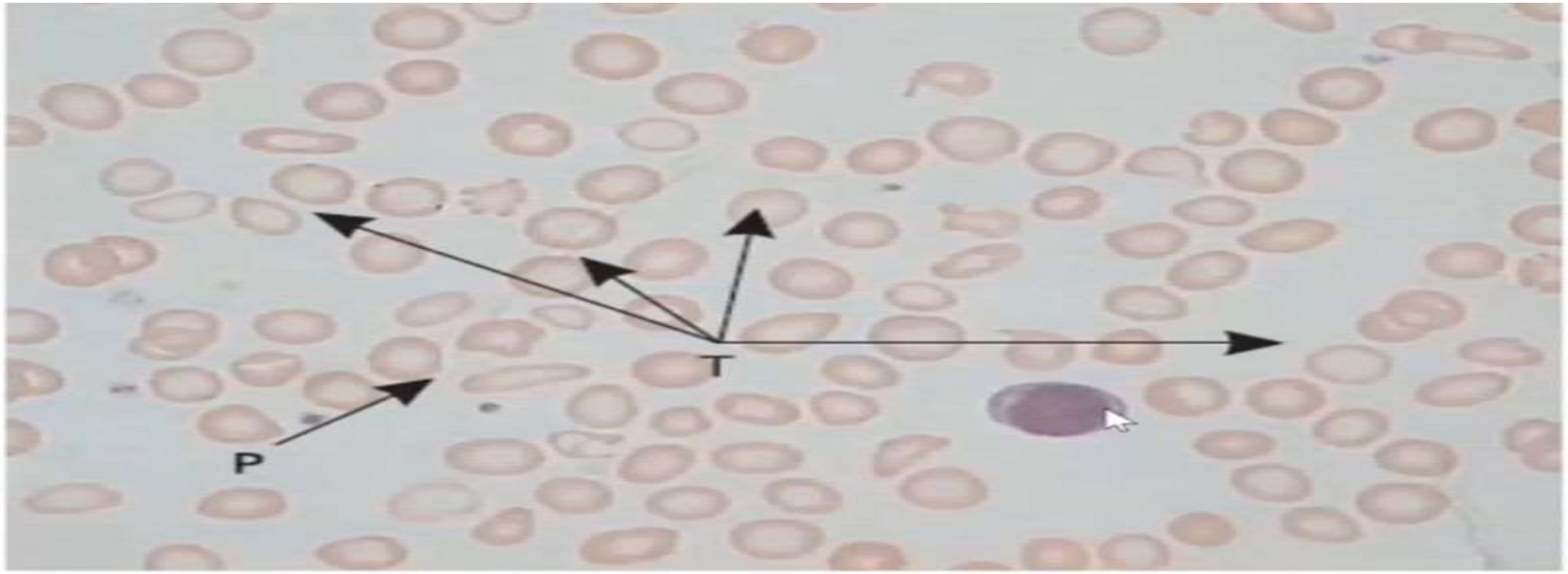
⇑ : iron deficiency anemia

Normal : anemia of chronic disease

FREE ERYTHROCYTE PROTOPORPHYRIN

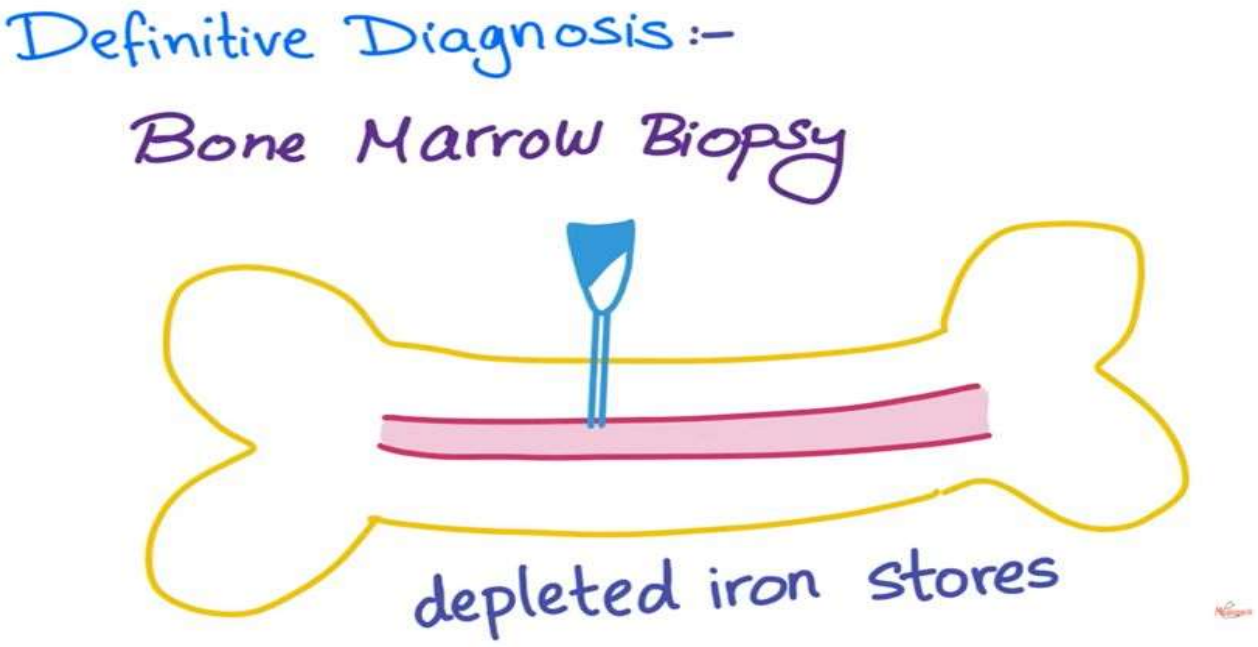
- increased free erythrocyte protoporphyrin (FEP)

Peripheral smear

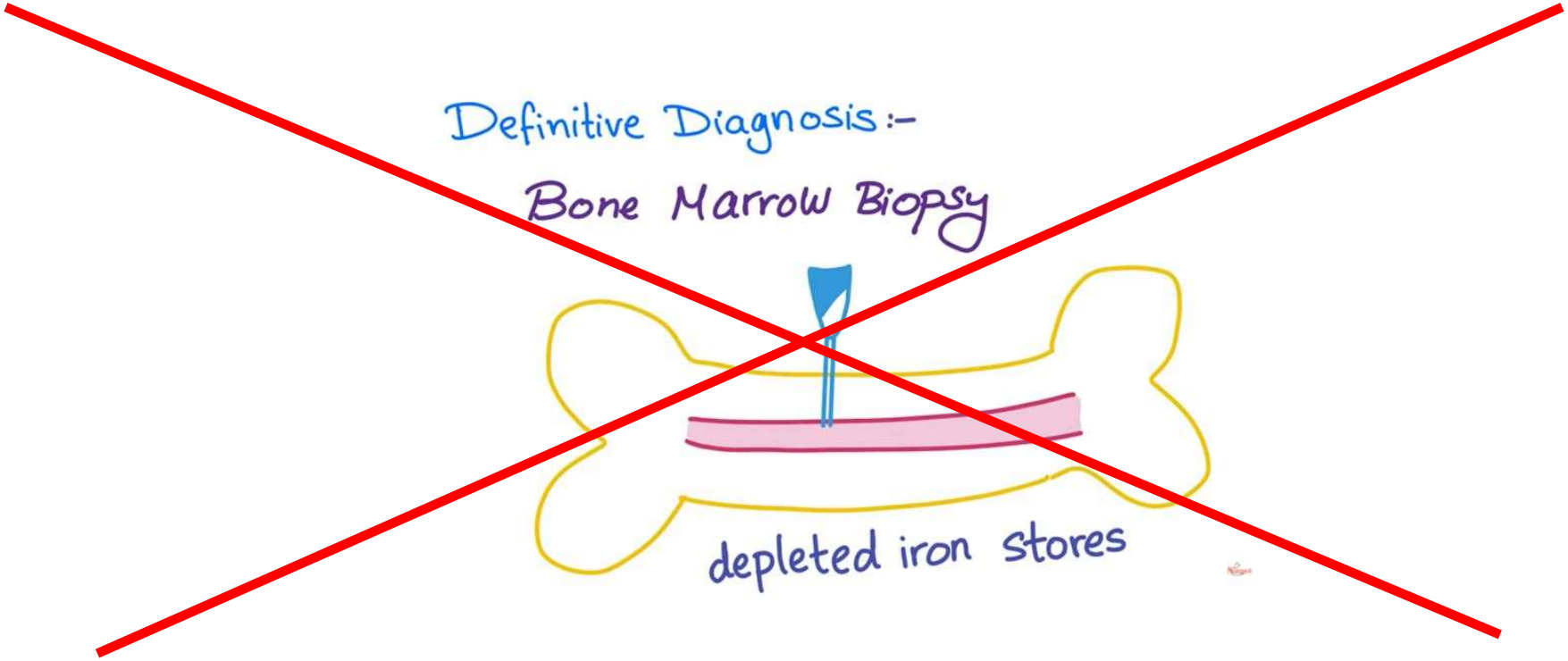


Blood film showing red blood cells that are small and pale. Pencil-shaped cells (P) and target cells (T) are also present.

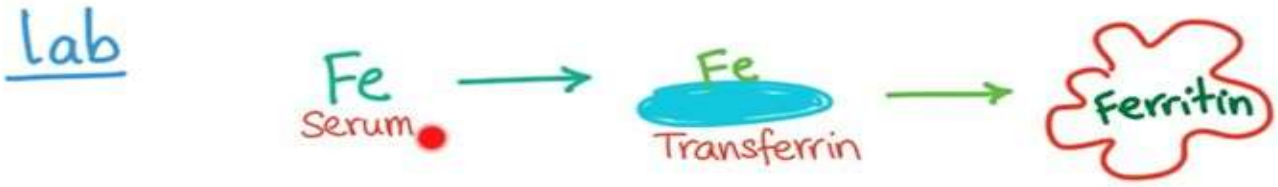
BONE MARROW ASPIRATE



BMA


Definitive Diagnosis :-
Bone Marrow Biopsy
depleted iron stores

Laboratory dx IDA



Hb Hct

MCV

MCH MCHC

Reticulocyte

WBCs

Platelets

Serum iron

Ferritin IL-6

TIBC

% Saturation

STfR ~~IL-6~~

RDW

Case review

- An otherwise healthy 35-year-old male who came to a blood donation campaign was rejected. He was told to ask to be evaluated by a hematologist. He found a friend doctor who sent you his CBC and iron panel through WhatsApp message (shown). What is the most likely diagnosis?

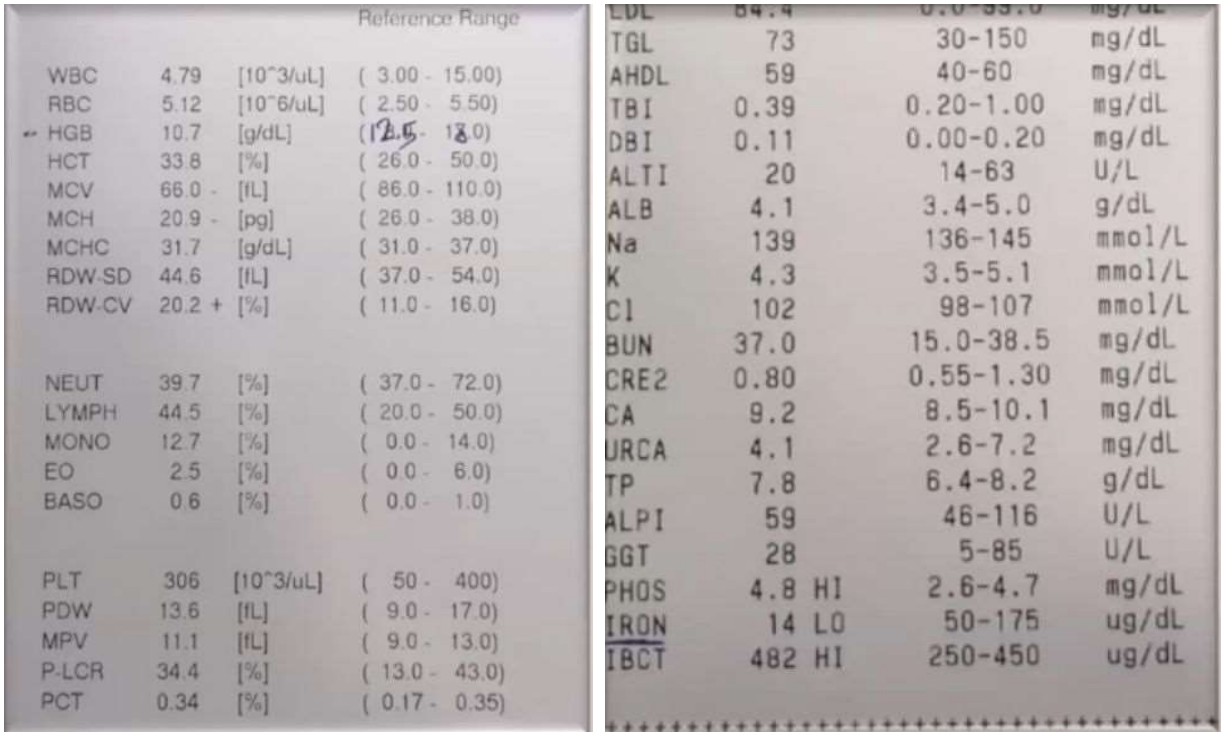
A. Iron deficiency anemia

B. Asymptomatic thalassemia trait

C. Both iron deficiency anemia and thalassemia trait

D. HbH disease

E. He needs to have hemoglobin electrophoresis to get the definitive diagnosis



			Reference Range
WBC	4.79	[10^3/uL]	(3.00 - 15.00)
RBC	5.12	[10^6/uL]	(2.50 - 5.50)
HGB	10.7	[g/dL]	(12.5 - 18.0)
HCT	33.8	[%]	(26.0 - 50.0)
MCV	66.0 -	[fL]	(86.0 - 110.0)
MCH	20.9 -	[pg]	(26.0 - 38.0)
MCHC	31.7	[g/dL]	(31.0 - 37.0)
RDW-SD	44.6	[fL]	(37.0 - 54.0)
RDW-CV	20.2 +	[%]	(11.0 - 16.0)
NEUT	39.7	[%]	(37.0 - 72.0)
LYMPH	44.5	[%]	(20.0 - 50.0)
MONO	12.7	[%]	(0.0 - 14.0)
EO	2.5	[%]	(0.0 - 6.0)
BASO	0.6	[%]	(0.0 - 1.0)
PLT	306	[10^3/uL]	(50 - 400)
PDW	13.6	[fL]	(9.0 - 17.0)
MPV	11.1	[fL]	(9.0 - 13.0)
P-LCR	34.4	[%]	(13.0 - 43.0)
PCT	0.34	[%]	(0.17 - 0.35)

LDL	84.4		mg/dL
TGL	73	30-150	mg/dL
AHDL	59	40-60	mg/dL
TBI	0.39	0.20-1.00	mg/dL
DBI	0.11	0.00-0.20	mg/dL
ALTI	20	14-63	U/L
ALB	4.1	3.4-5.0	g/dL
Na	139	136-145	mmol/L
K	4.3	3.5-5.1	mmol/L
CI	102	98-107	mmol/L
BUN	37.0	15.0-38.5	mg/dL
CRE2	0.80	0.55-1.30	mg/dL
CA	9.2	8.5-10.1	mg/dL
URCA	4.1	2.6-7.2	mg/dL
TP	7.8	6.4-8.2	g/dL
ALPI	59	46-116	U/L
GGT	28	5-85	U/L
PHOS	4.8 HI	2.6-4.7	mg/dL
IRON	14 LO	50-175	ug/dL
IBCT	482 HI	250-450	ug/dL

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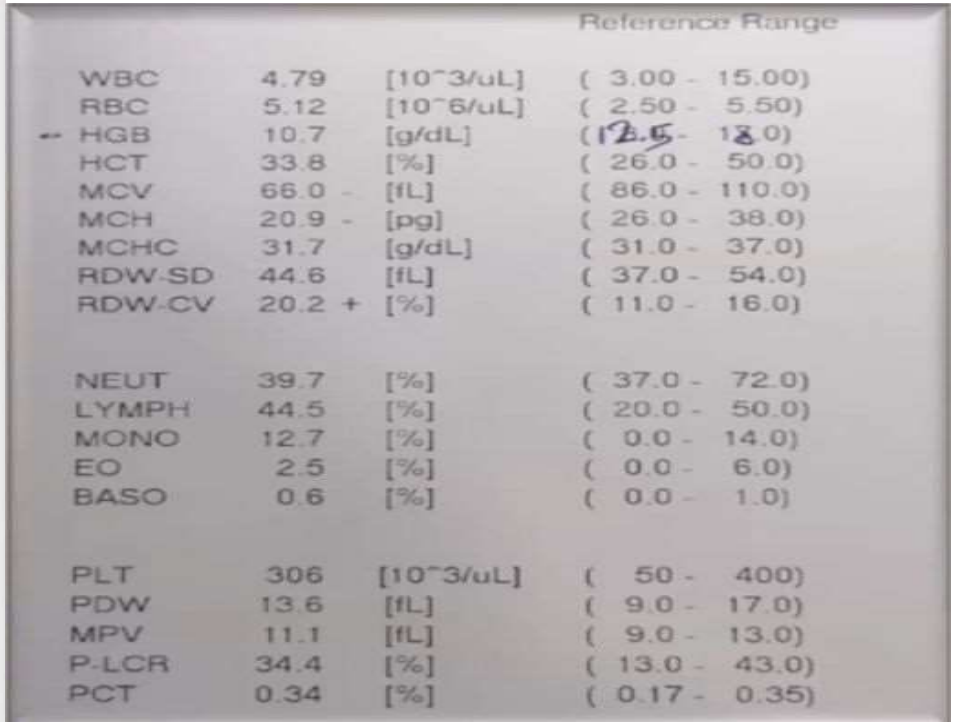

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	PDW	13.6	[fL]	(9.0 - 17.0)
	MPV	11.1	[fL]	(9.0 - 13.0)
	P-LCR	34.4	[%]	(13.0 - 43.0)
	PCT	0.34	[%]	(0.17 - 0.35)

Iron Deficiency versus Thalassaemia

Parameter	Thalassemia	Iron deficiency anemia
RBC count	>5.0 x 10^6/µL (5.0 x 10^{12}/L)	< 5.0 x 10^6/µL (5.0 x 10^{12}/L)
MCV	usually less than 70 in TT	more than 70
RDW	TT is less than 17%	more than 17%
Mentzer index: MCV/RBC	< 13 favors thalassemia	
England and Fraser Index: MCV– (5 x Hemoglobin)		

Mentzer Index and others

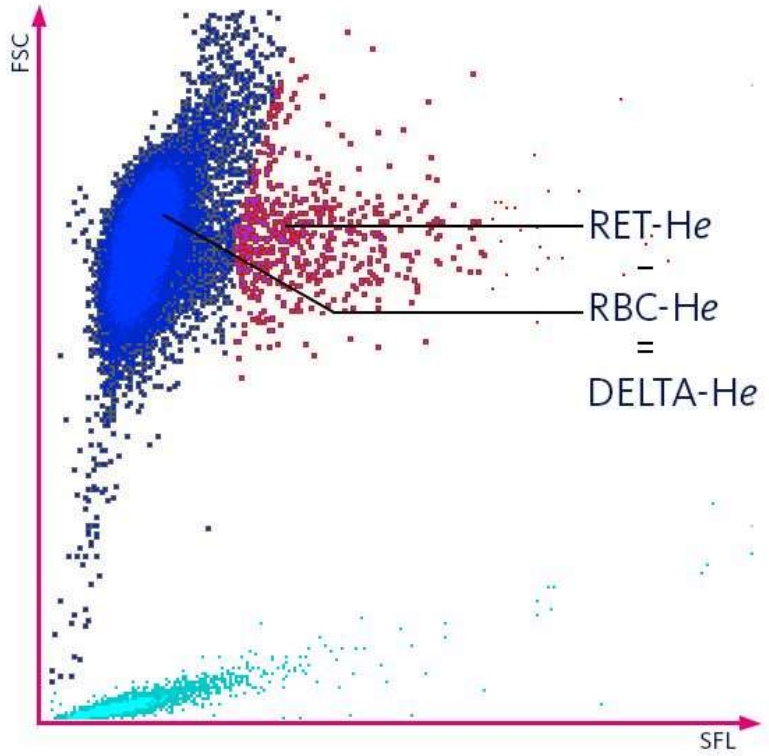
- Several algorithms using conventional red cell indices have been developed to facilitate discrimination of these two diseases. However, several reports using these algorithms have shown appropriate classification in only 30-40% of cases
- Evaluation of new diagnostic algorithms which incorporates reticulocyte parameters demonstrated better performance compared to conventional algorithms increasing sensitivities to 70%
- New generation haematology analyzers enabled the use of more innovative parameters such as **reticulocyte-haemoglobin measurement**

RET-HE

- Ret-He (reticulocyte haemoglobin equivalent) gives the Hb content of the freshly produced red blood cells and thus offers real-time information of iron supply during the course of erythropoiesis.
- Ret He provides information on availability of Functional Iron
- Useful to differentiate between the two most common anaemias (iron deficiency anaemia and anaemia of chronic disease (ACD) and between IDA and Thalassaemia
- Monitoring therapy of chronic infections or tumours
- Monitoring erythropoietin therapy and iron substitution

SYSMEX XN1000

- The SYSMEX XN 1000 evaluates the maturation of reticulocytes by quantitating the fraction of reticulocytes within low-middle and high fluorescence intensity regions. The IRF is the sum of the fraction of high-fluorescence plus middle-fluorescence. The mean forward light scatter intensity of stained reticulocytes (RET-HE) is measured in the reticulocyte channel of the Sysmex XN 1000 (Sysmex Corporation, Kobe, Japan) analyzer.

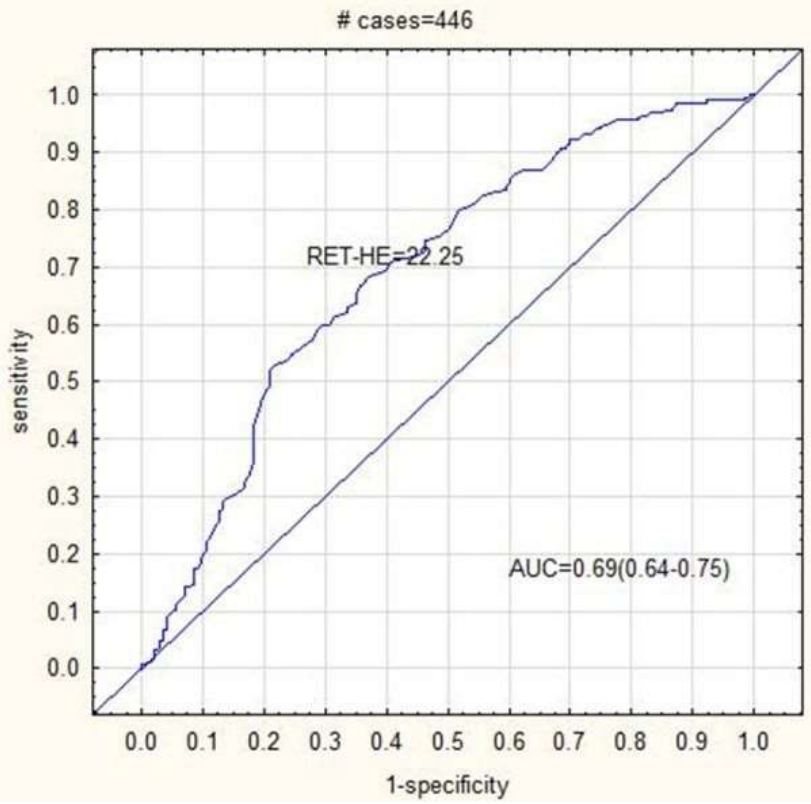


A new parameter for discriminating IDA versus Thalassaemia

- Sedick Q, Elyamany G, Hawsawi H, Alotaibi S, Alabbas F, Almohammadi M, Alahmari HA, Aljasem H, Ferrer AG, Alzahrani AS, AlMoshary M, Alsuhaibani O. **Diagnostic accuracy of reticulocyte parameters on the sysmex XN 1000 for discriminating iron deficiency anaemia and thalassaemia in Saudi Arabia**. Am J Blood Res. 2021 Apr 15;11(2):172-179. PMID: 34079632; PMCID: PMC8165718.

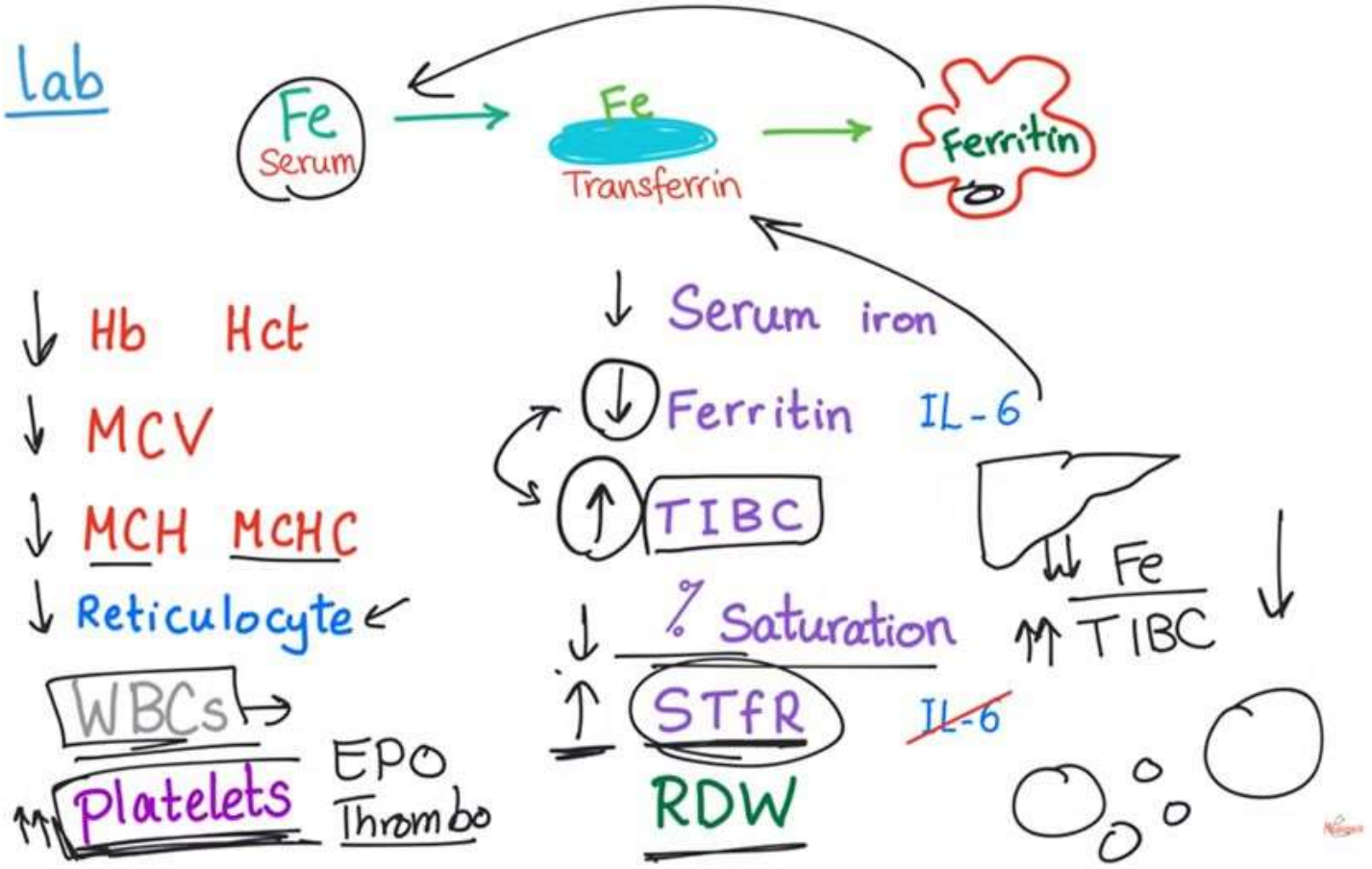
RET-HE

- Reticulocyte parameters most accurate for discriminating IDA from Thalassemia patients was: RET, RET-HE and IRF. The RET-HE had the best statistical significance for IDA patients with AUC = 0.69 for cut off 22.25.



IDA DX-Summary

RET-HE



Iron deficiency Anaemia prevention

- Prevention-consumption of iron rich foods,ascorbic acid
- The WHO recommends iron supplementation to prevent ID/IDA in instances where the prevalence of anaemia is 40% or higher: children 6–23 months (10–12.5 mg elemental iron daily – drops/syrups, three consecutive months in a year), 24–59 months (30 mg elemental iron daily – drops/syrups/tablets, three consecutive months in a year), 5–12 years (30–60 mg elemental iron daily – tablets/capsules, three consecutive months in a year).
- The WHO also recommends iron supplementation in the same setting for menstruating adult women and adolescent girls (nonpregnant females in the reproductive age group) with 30–60 mg elemental iron daily tablets for three consecutive months in a year

Iron Deficiency Anaemia-treatment

- **Management of the underlying cause of IDA should always be considered.**
- **Iron replenishment can occur via three routes:**
- **<u>Oral iron, parenteral oral and transfusion of packed red cells.</u>**

Oral Iron

- Oral ferrous sulfate supplementation-100-200mg of elementary iron or 3-6mg per kilogram body weight of liquid preparation
- Taking vitamin c concurrently will enhance iron absorption
- A systematic review demonstrated that GI side effects were the most problematic with constipation being the most frequent complaint, followed by nausea and diarrhoea.This will have a resultant effect on patient adherence, likely leading to cessation and, thus, inadequate treatment.

Dosing of oral iron

- To adequately replenish iron stores, therapeutic treatment of IDA was initially felt to require 200mg of iron sulphate 2–3 times per day in order to raise Hb by 20g/L over a 4-week period, with treatment continuing for 3 months.
- The daily doses of elemental iron should not be greater than 100mg/day26 as the body can only absorb 10–20mg of iron per day.
- Recent studies indicate that single daily dose (40–60mg) or a slightly higher alternate-day dose (80–100mg) is the preferred dosing regimen in order to reduce the side effects and optimise the proportion of elemental iron

Novel oral iron formulations

- Ferric maltol,is a non-salt oral iron formulation composed of stable ferric iron complexed with a sugar derivative, tri-maltol.licenced in the European Union and the USA as Feraccru and Accrufer
- When absorbed, the maltol ligand remains complexed to iron, which reduces the formation of free iron and facilitates iron transport across the enterocyte
- Increases the bioavailability of iron- lower doses of elemental iron are required to treat IDA compared with the ferrous iron preparations and it has less effects on the gut microbiome

Sucrosomial Iron

- Sucrosomial iron is an innovative oral ironcontaining carrier, in which ferric pyrophosphate is within a phospholipid bilayer membrane forming the ‘sucrosome’, creating a gastroresistant complex
- unique structure protects iron from the acidic environment in the stomach, increases intestinal epithelial absorption and ensures high bioavailability while reducing the risk for potential adverse GI effects

Intravenous iron

- Bypasses the GI tract absorption, avoiding mucosal aggravation and inflammation,less side effects
- Older iv iron preparations such as high-molecular weight dextran iron (**Dexferrum)** have been discontinued due to their unfavourable safety profiles
- The lower molecular weight dextran compounds such as **Cosmofer** still in use, shown to be effective, lower incidence of anaphylactoid reactions
- Ferric derisomaltose (**Monofer**) is an alternative intravenous iron preparation, which is often preferred to Cosmofer
- (**Ferinject)** is a preparation widely used in the UK
- Iron sucrose (**Venofer**) is given by a slow injection of 100–200mg 2–3 times a week

Parenteral Iron Indications

- -Iron refractory defined as absence of response after 4-6 weeks of oral iron treatment
- -Iron intolerance
- -Severe or symptomatic anaemia
- -Gastric or duodenal surgery leads to malabsorption of iron
- -chronic blood loss that cannot be compensated by oral iron
- -active inflammatory bowel disease or end stage kidney disease

Red blood cell transfusion

- Reserved for patients with severe anaemia, haemodynamically unstable and/or have associated comorbid conditions
- A unit of blood contains approximately 200mg of iron,
- Risks are no insignificant-increased incidence of transfusion-related reactions. This includes the risk of Transfusion Related Acute Lung Injury, transmitting infections, both viral and bacterial.