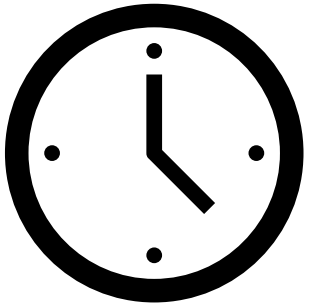






Physiology of the Blood and Lymphoreticular System

Lab (2) – Medical and Dental Students – 2026/2027

- Osmotic Fragility Test
- Reticulocyte Count
- CBC Interpretation

Dr. Abdullah Al-Jaouni

Osmotic Fragility Test

Osmotic Fragility Test – What to Know

- **Osmotic Effects on RBCs**:
  - **Isotonic:** Fluid equilibrium → No net water movement.
  - **Hypertonic:** Water **efflux** → Cell shrinks/Collapses.
  - **Hypotonic:** Water **influx** → Cell swells → **Hemolysis**.

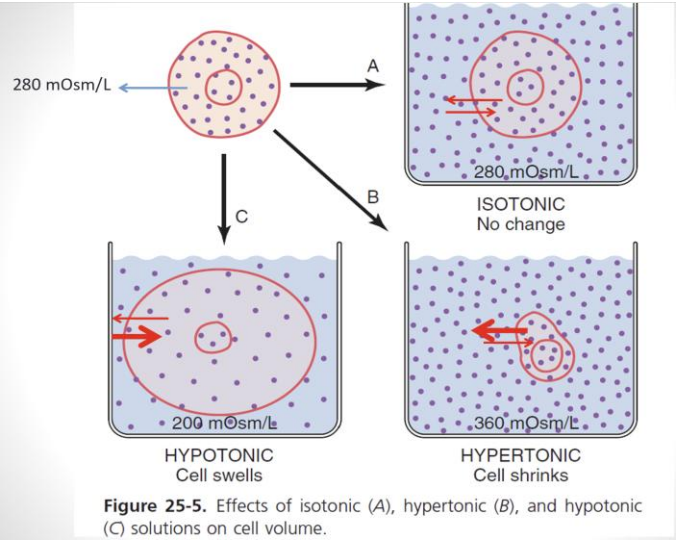
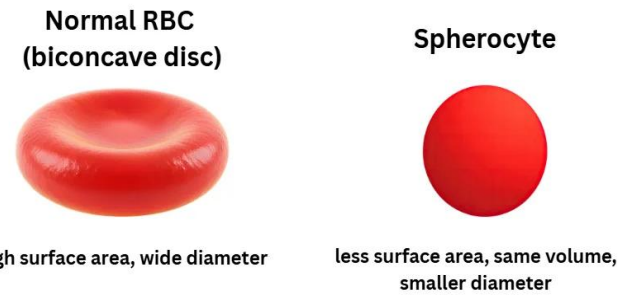


Figure 25-5. Effects of isotonic (*A*), hypertonic (*B*), and hypotonic (*C*) solutions on cell volume.



surface area ↓ → sphere → diameter ↓, volume ~ same

- **Test Principle & Determinants**:
  - **Definition:** Measures RBC resistance to hemolysis across increasingly dilute (hypotonic) saline solutions.
  - **Fragility Determinants:**
    - Surface Area-to-Volume Ratio (SA/V)
    - Membrane Composition & Integrity

- **Clinical Utility**:
  - Decreased SA/V ratio (e.g., spherical cells) limits water accommodation → High fragility and osmotic lysis.
  - **Primary Indication:** Diagnostic test for **Hereditary Spherocytosis**.

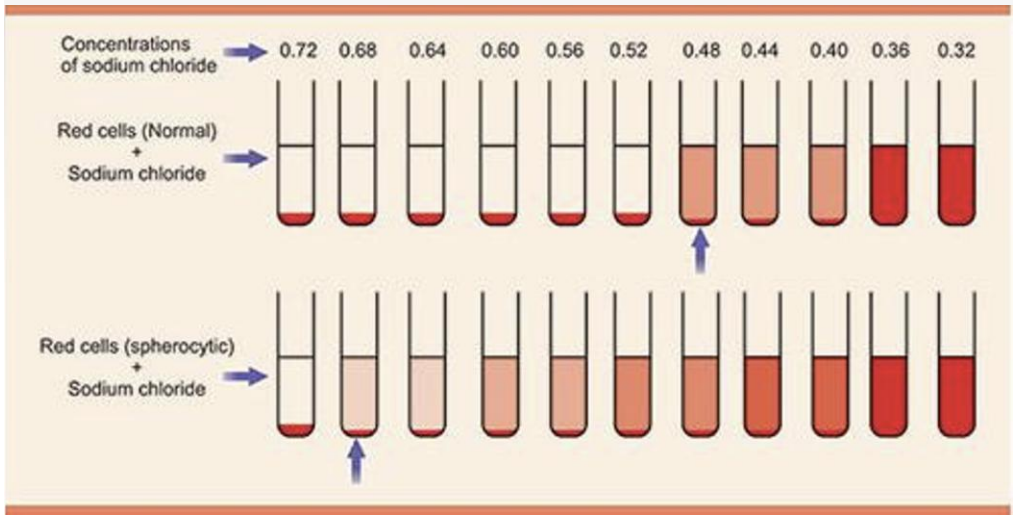
Osmotic Fragility Test – The Procedure

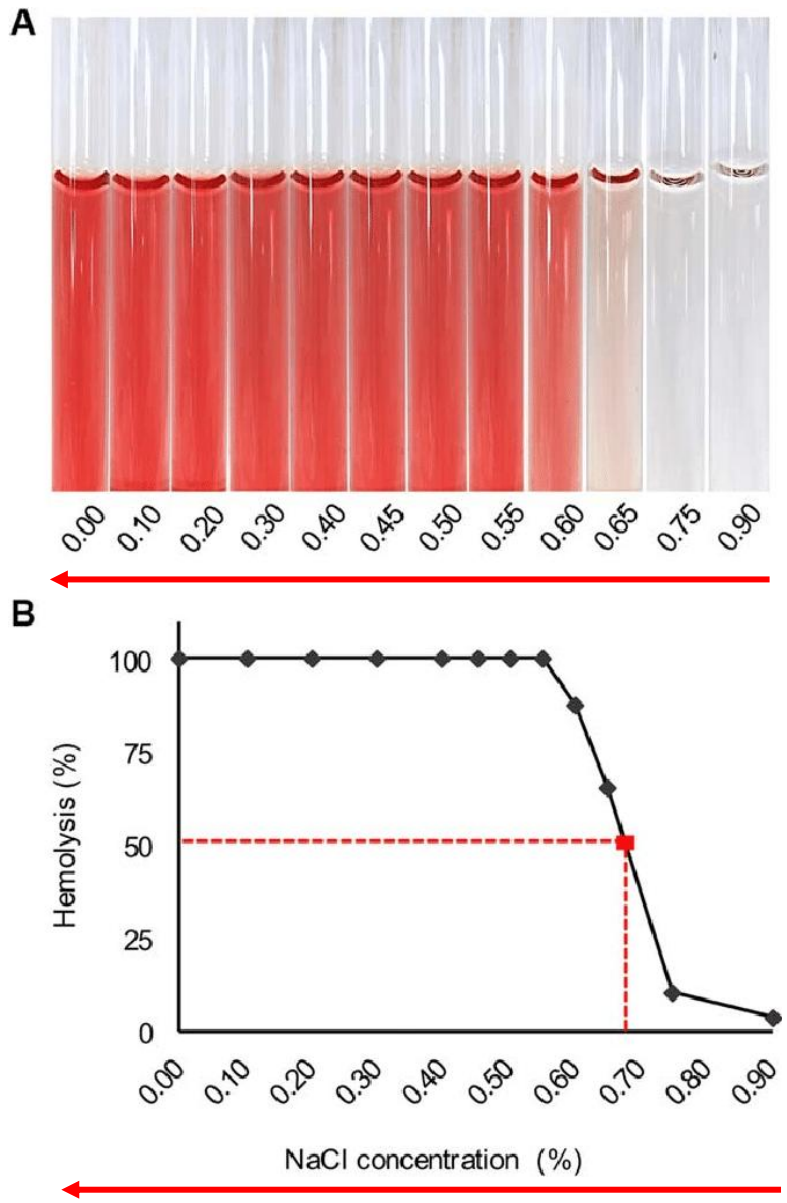
Steps:

1. Put labeled centrifuge tubes in a rack.
2. Prepare NaCl solutions of different concentrations starting from 0.9% NaCl till 0.2% NaCl (aka more hypotonic each time).
3. Add 10 ml of each solution to a different tube then add one drop of blood to each tube.
4. Shake each tube well and allow them to stand for 20 minutes.
5. After 20 minutes, the tubes are centrifuged for 10 minutes.
6. Transfer supernatant fluid from each tube into spectrophotometer cuvettes The absorbance is then measured at 540 nm and used to calculate the percentage of hemolysis for each solution.
7. The results are plotted against the NaCl concentrations, this yields an osmotic fragility curve which is then compared to a standard curve.

Findings:

- Normal: Hemolysis begins at ~ 0.5% NaCl (0.48%). Hemolysis is completed at ~ 0.3% NaCl (0.36%).
- Abnormal: Hemolysis begins at ~ 0.7% NaCl (0.68%).




A
0.00
0.10
0.20
0.30
0.40
0.45
0.50
0.55
0.60
0.65
0.75
0.90
B
100
75
50
25
0
Hemolysis (%)
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
NaCl concentration (%)

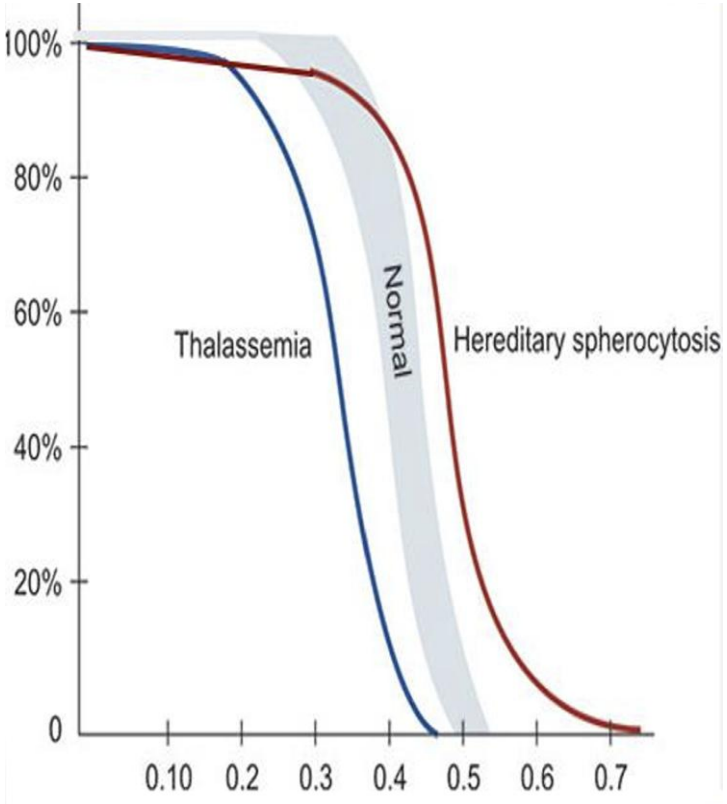
Osmotic Fragility Test – Interpretation and Clinical Significance

Increased red cell fragility (increased susceptibility to hemolysis) is seen in:

- **Hereditary spherocytosis**.
- Autoimmune hemolytic anemia.
- Toxic chemicals, poisons, infections, and some drugs.
- Severe burns.
  - These cells have a low surface area: volume ratio

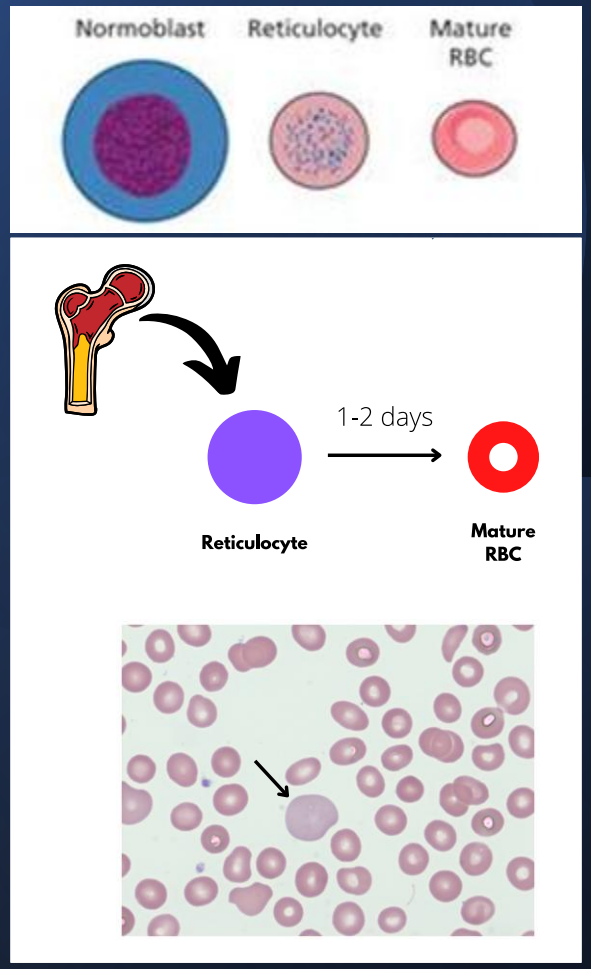
Decreased red cell fragility (increased resistance to hemolysis) maybe seen in:

- Thalassemia.
- Iron deficiency anemia.
  - These cells have a high surface area: volume ratio



Reticulocyte Count

Reticulocyte Count – What to Know



Reticulocytes are the immediate precursor of RBCs.

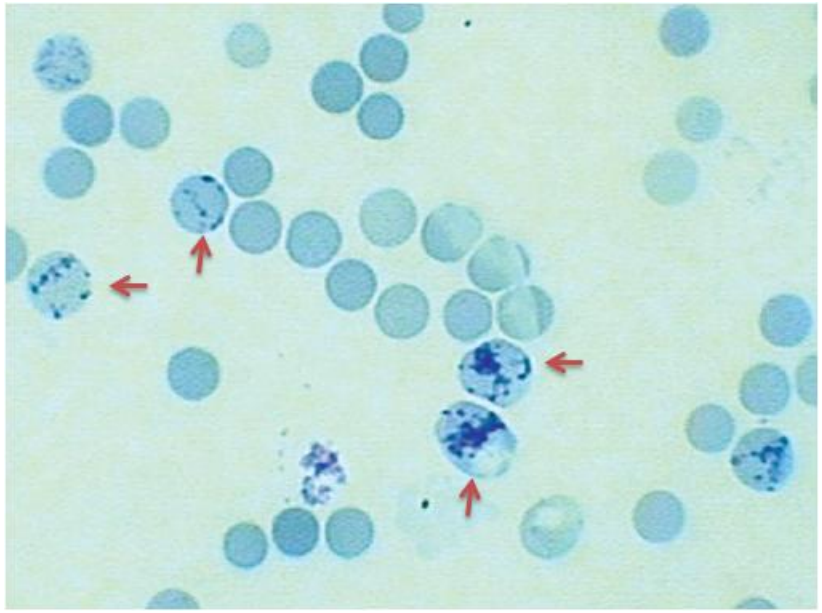
They contain a small amount of **basophilic material**, mainly remnants of the Golgi apparatus and mitochondria.

Following their release into the blood stream they mature within 1-2 days into mature RBCs.

They normally make 0.5 - 2% of all RBCs.

Reticulocyte count is used to estimate the degree of effective erythropoiesis.

Their number increases in cases of bleeding and RBC hemolysis and decreases in cases of bone marrow failure



Reticulocyte Count – The Procedure

1. Add a drop of a **supravital stain** (new methylene blue) to a drop from the patient's blood.
2. Mix properly.
3. Prepare a thin smear of the stained blood specimen.
4. Examine under microscopy.
5. Count reticulocytes, which appear (1) larger than RBCs and (2) contain dark blue dots and curved linear structures in their cytoplasm which represent remnants of the Golgi apparatus and mitochondria. **500-1000 RBCs should be counted and the number of reticulocytes noted**. The count is expressed as a percentage. Normal range is 0.5% - 2%.
6. The percentage can be used to calculate the absolute reticulocyte count (ARC) .
   ARC = (RBC count x reticulocyte%)/100.
   Normal range is 25,000 to 75,000/mm^3.

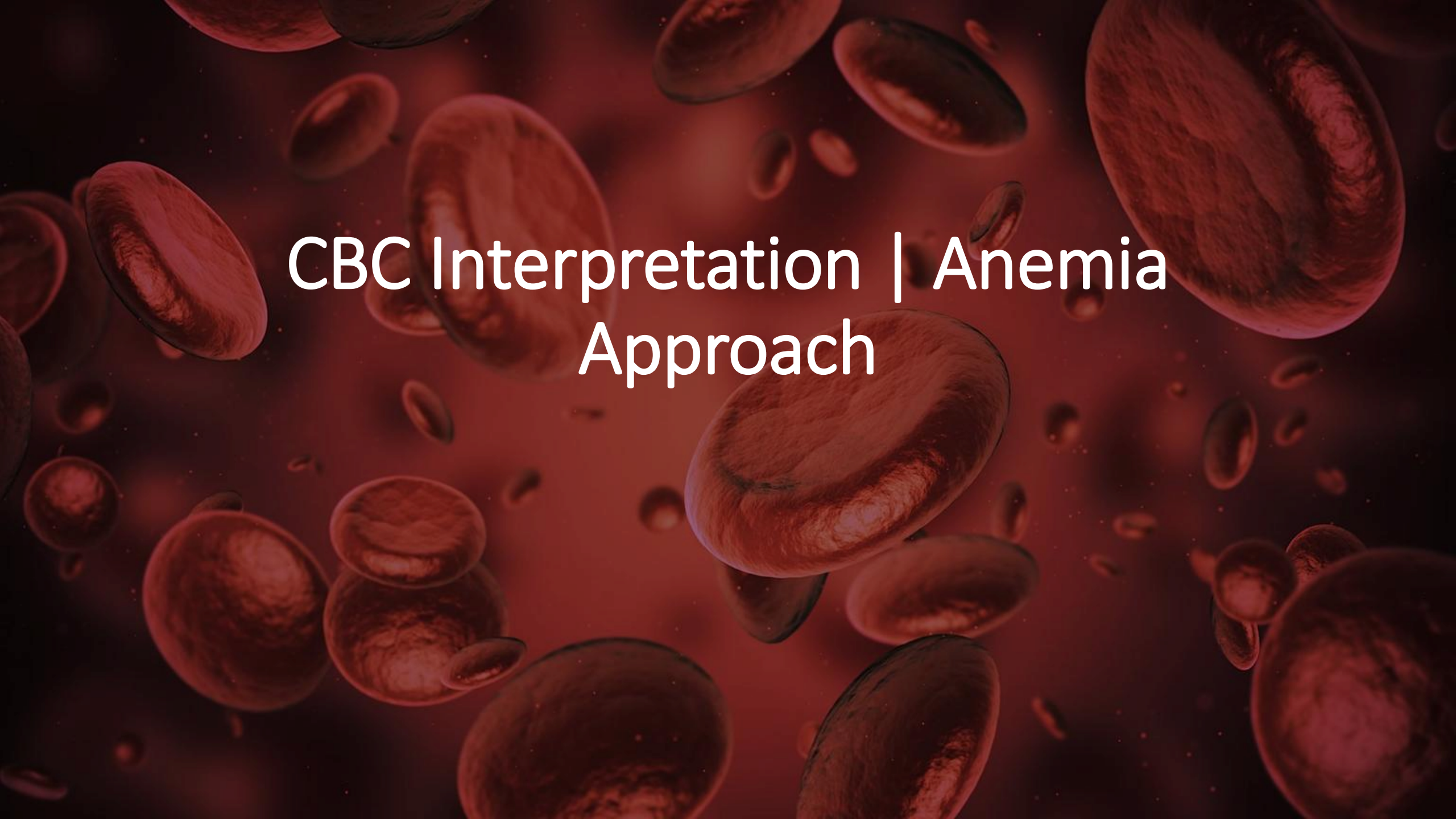
Reticulocyte Count – Interpretation and Clinical Significance

Condition associated with Reticulocytosis:

- Hemolytic anemias.
- Acute/Recent blood loss.
- Following treatment of nutritional anemias.

Condition associated with Reticulocytopenia:

- Iron deficiency anemia.
- Aplastic anemia.
- Bone marrow failure.



CBC Interpretation | Anemia Approach

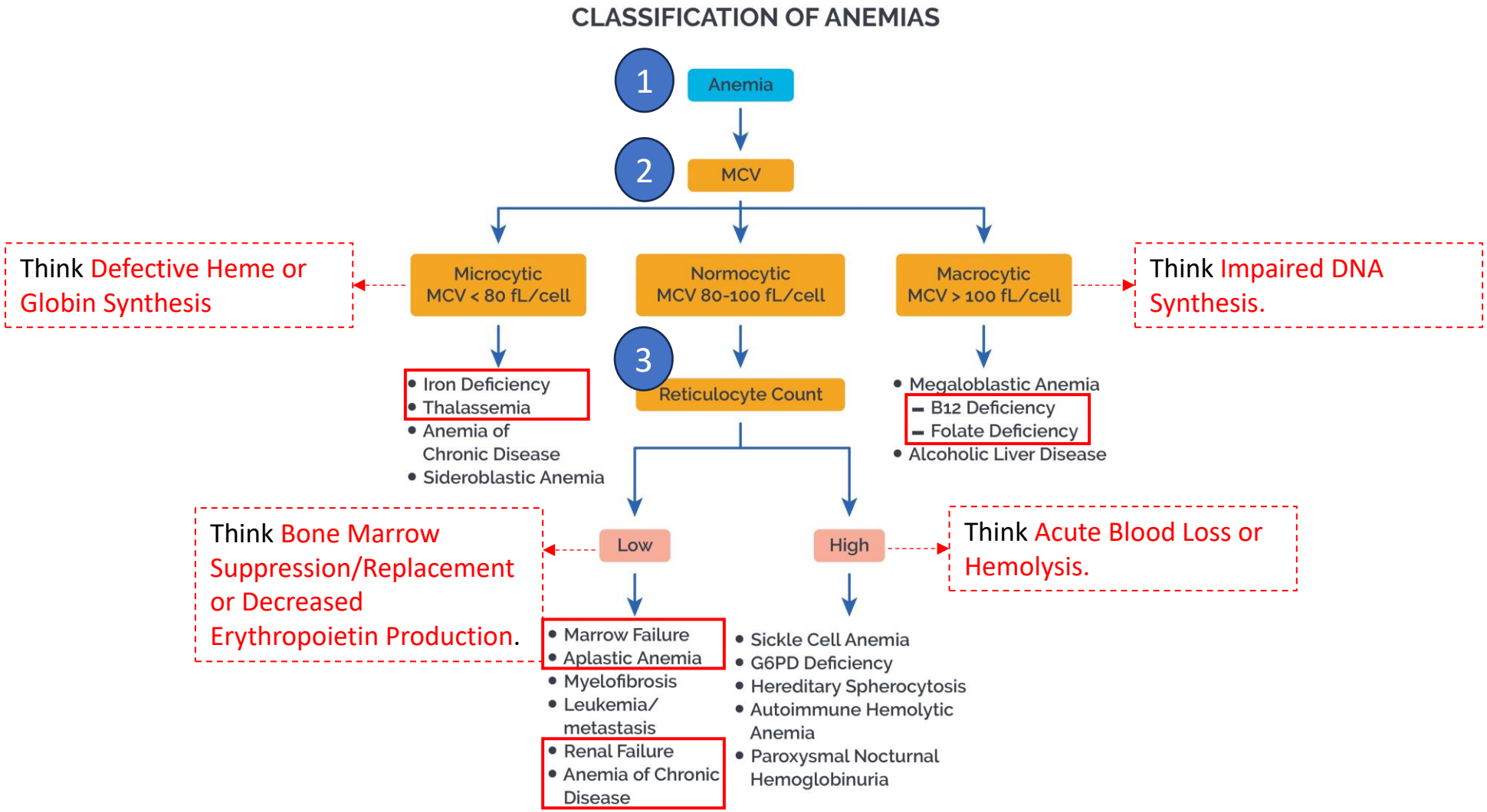
Hb, MCV, MCHC, RBC Count, Reticulocyte Count – Where to Look First?

Anemia is defined as:

- **Hemoglobin** <13.5 g/dL (men) or <12.0 g/dL (non-pregnant women).

Causes of anemia can be classified, pathophysiologically, to:

- Decreased or Impaired RBC Production due to Defective Heme or Globin Synthesis.
- Decreased or Impaired RBC Production due to Bone Marrow Suppression/Replacement or Decreased Erythropoietin Production.
- Increased RBC loss due to Acute Blood Loss or Hemolysis.
- Decreased or Impaired RBC Production due to Impaired DNA Synthesis.


CLASSIFICATION OF ANEMIAS
1
Anemia
2
MCV
Microcytic
MCV < 80 fL/cell
Normocytic
MCV 80-100 fL/cell
Macrocytic
MCV > 100 fL/cell
Think Defective Heme or Globin Synthesis
Think Impaired DNA Synthesis.
3
Reticulocyte Count
• Iron Deficiency
• Thalassemia
• Anemia of Chronic Disease
• Sideroblastic Anemia
• Megaloblastic Anemia
– B12 Deficiency
– Folate Deficiency
• Alcoholic Liver Disease
Low
High
Think Bone Marrow Suppression/Replacement or Decreased Erythropoietin Production.
Think Acute Blood Loss or Hemolysis.
• Marrow Failure
• Aplastic Anemia
• Myelofibrosis
• Leukemia/ metastasis
• Renal Failure
• Anemia of Chronic Disease
• Sickle Cell Anemia
• G6PD Deficiency
• Hereditary Spherocytosis
• Autoimmune Hemolytic Anemia
• Paroxysmal Nocturnal Hemoglobinuria

Let’s Practice

…

(CBC Report (*))

PATIENT NAME	MRN / DOB	AGE / GENDER	ORDERING PHYSICIAN
DOE, JANE M.	#9834120 / 04-12-1994	32 YRS / Female	Dr. A. Vance, MD

CLINICAL PRESENTATION (TEACHING VIGNETTE)

32-year-old female presenting with a 4-month history of progressive fatigue, exertional dyspnea, and heavy menorrhagia (7–8 days).

COMPLETE BLOOD COUNT (CBC) WITH RETICULOCYTE COUNT

TEST PARAMETER	RESULT	FLAG	UNITS	REFERENCE
Erythrocyte Indices & Red Cell Panel				
Red Blood Cell Count (RBC)	**3.10**		$x10^{12}/L$	3.80 - 5.10
Hemoglobin (Hgb)	**8.2**		g/dL	12.0 - 15.5
Hematocrit (Hct / PCV)	**26.0**		%	36.0 - 46.0
Mean Corpuscular Volume (MCV)	**68.0**		fL	80.0 - 100.0
Mean Corpuscular Hgb (MCH)	**21.0**		pg	27.0 - 33.0
MCH Concentration (MCHC)	**29.5**		g/dL	32.0 - 36.0
Red Cell Distribution Width (RDW)	**17.8**		%	11.5 - 14.5
Reticulocyte Evaluation (Bone Marrow Response)				
Reticulocyte Percentage	**0.8**		%	0.5 - 2
Absolute Reticulocyte Count	**24.8**		$x10^{9}/L$	
Leukocyte & Platelet Panel				
White Blood Cell Count (WBC)	**6.4**		$x10^{3}/\mu L$	4.5 - 11.0
Platelet Count (Plt)	**460**		$x10^{3}/\mu L$	150 - 450

Can Other Parameters Support One Diagnosis Over the Other?

- In microcytic anemias, IDA may have HIGH RDW whereas Thalassemia does not.
- Thalassemia may present with HIGH RBC Count compared to IDA.
- In Hereditary Spherocytosis, HIGH MCHC could support the diagnosis.
- Pancytopenia (aka Low WBCs and Low PLT along with Anemia) is suggestive of Aplastic Anemia or Bone Marrow Infiltration.

Thank You