UNIVERSITATEA DE STAT DE MEDICINĂ ȘI FARMACIE
„NICOLAE TESTEMIȚANU” DIN REPUBLICA MOLDOVA

Basic semiology of the haematopoiesis and the diagnostic approach to childhood anemia

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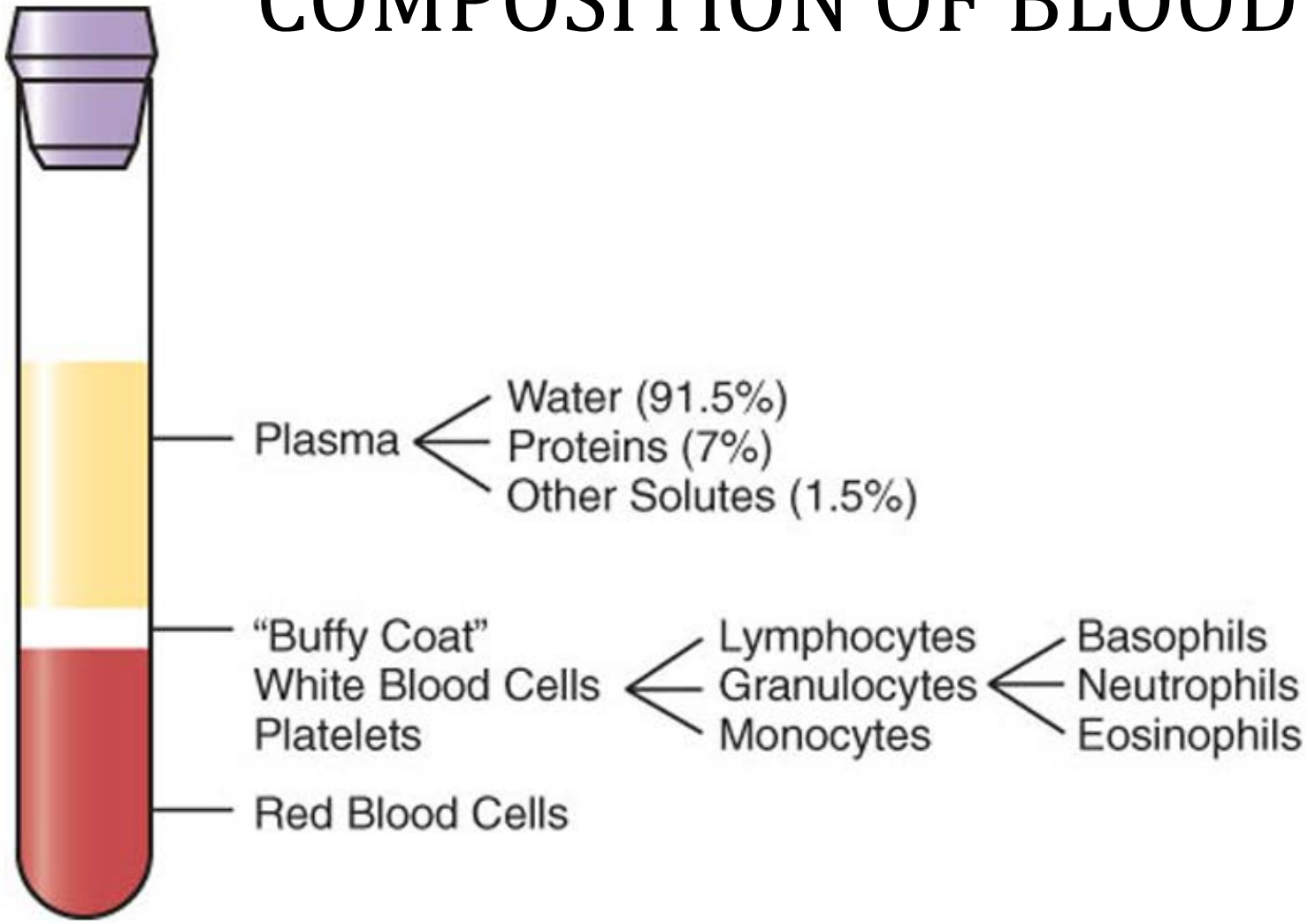
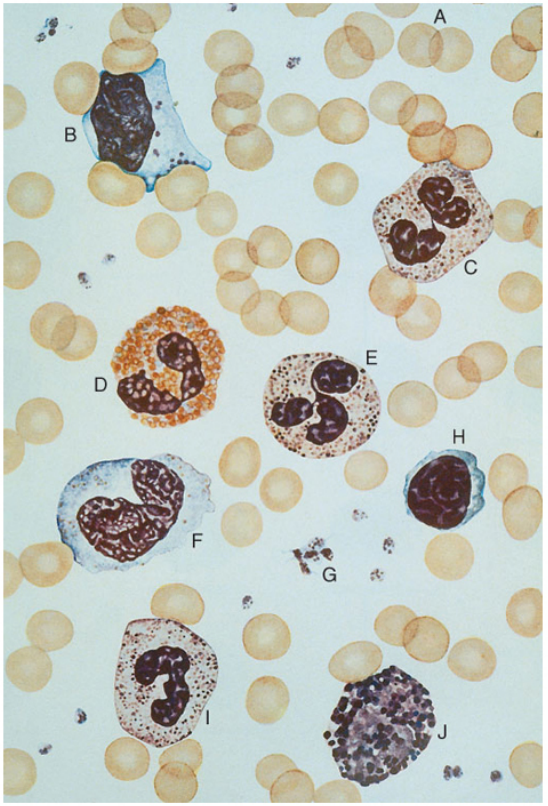
What is hematology?

- Hematology is the study of blood which is composed of plasma (~55%), and the formed elements which are:
  - The erythrocytes (RBCs) (~45%)
    - Contain hemoglobin
    - Function in the transport of O_2 and CO_2
  - The Leukocytes (WBCs) and platlets (thrombocytes) (~1%)
    - Leukocytes are involved in the body's defense against the invasion of foreign antigens.
    - Platlets are involved in hemostasis which forms a barrier to limit blood loss at an injured site.

COMPOSITION OF BLOOD



TYPES OF FORMED ELEMENTS IN THE BLOOD



- Hematology is primarily a study of the formed cellular elements.
- Alterations in the formed elements in the blood are usually a result of disease rather than being the primary cause of disease.
  - In fact, variations in the formed elements in the blood are often the first sign that disease is occurring in the body.
  - The changes caused by disease may be detected by lab tests that measure deviations of the blood constituents from the normal values. These lab test may include:

- RBC count
- WBC count
- Platlet count
- Hematocrit (packed cell volume)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin concentration (MCHC)

- Under normal conditions the production, release, and survival of blood cells is a highly regulated process. Quantitative and/or qualitative hematologic abnormalities may result when there is an imbalance between cell production, release, and/or survival.

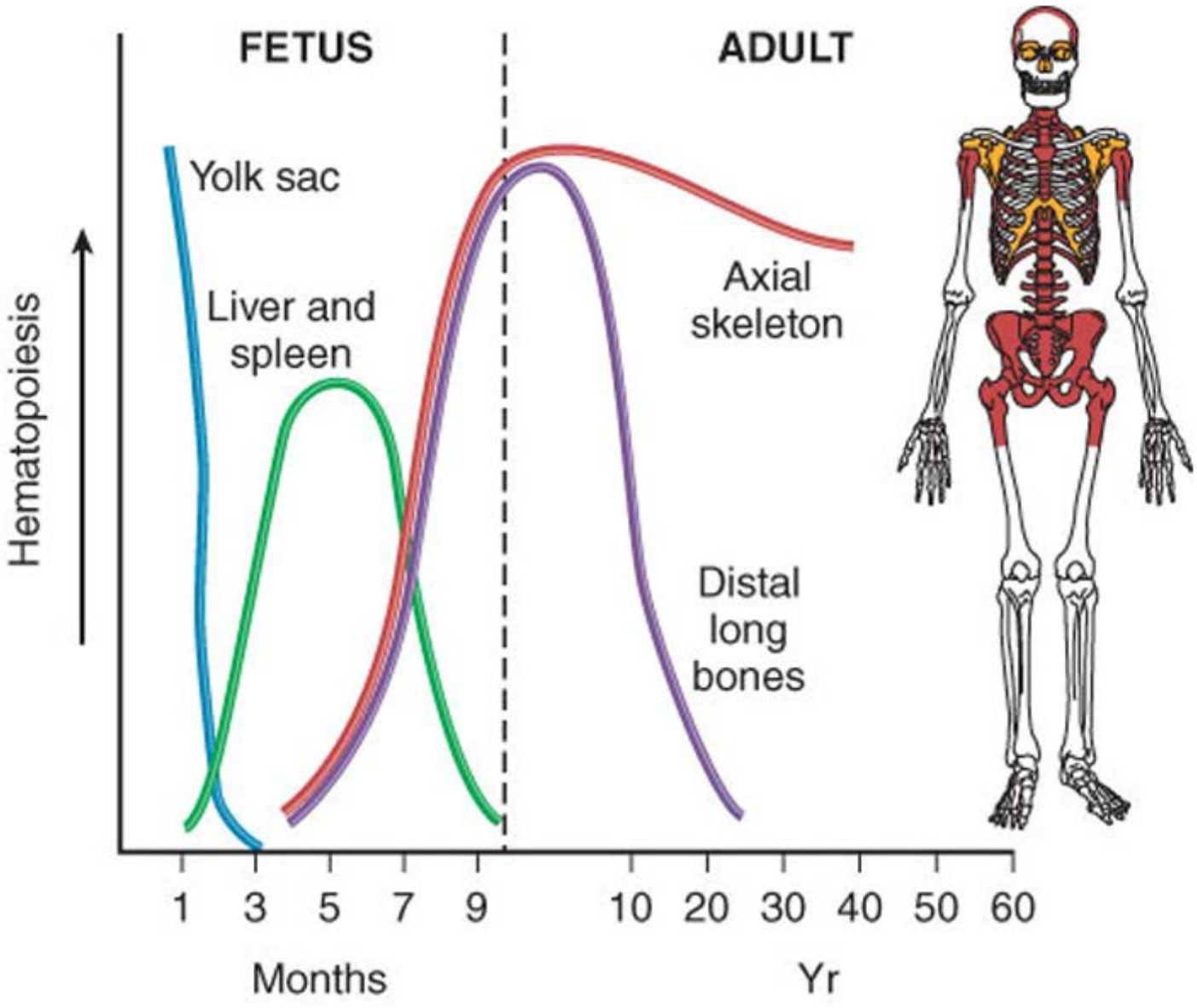
- Age, sex, and geographic location are involved in physiologic changes in normal values of the formed cellular elements
- Pathologic changes in the values of the formed cellular elements occur with disease or injury.
- Normal values for a group are determined by calculating the mean for healthy individuals of the group and reporting the normal range as the mean +/- 2 standard deviations

Hematopoiesis

- A term describing the formation and development of blood cells.
  - Cells of the blood are constantly being lost or destroyed. Thus, to maintain homeostasis, the system must have the capacity for self renewal. This system involves:
    - Proliferation of progeny stem cells
    - Differentiation and maturation of the stem cells into the functional cellular elements.

- Hematopoiesis begins as early as the nineteenth day after fertilization in the yolk sac of the embryo
  - Only erythrocytes are made
  - The RBCs contain unique fetal hemoglobins
- At about 6 weeks of gestation, yolk sac production of erythrocytes decreases and production of RBCs in the human embryo itself begins.

- The fetal liver becomes the chief site of blood cell production.
  - Erythrocytes are produced
  - The beginnings of leukocyte and thrombocyte production occurs
- The spleen, kidney, thymus, and lymph nodes serve as minor sites of blood cell production.
- The lymph nodes will continue as an important site of lymphopoiesis (production of lymphocytes) throughout life, but blood production in the other areas decreases and finally ceases as the bone marrow becomes the primary site of hematopoiesis at about 6 months of gestation and continues throughout life.
  - When the bone marrow becomes the chief site of hematopoiesis, leukocyte and thrombocyte production become more prominent.



Hematopoiesis

- Hematopoiesis in the bone marrow is called medullary hematopoiesis
- Hematopoiesis in areas other then the bone marrow is called extramedullary hematopoiesis
  - Extramedullary hematopoiesis may occur in fetal hematopoietic tissue (liver and spleen) of an older child when the bone marrow cannot meet the physiologic needs of the tissues. This can lead to hepatomegaly and/or splenomegaly (increase in size of the liver and/or spleen because of increased functions in the organs).

- Hematopoietic tissue includes tissues involved in the proliferation, maturation, and destruction of blood cells

- The mononuclear phagocytic system (also called the reticular endothelial system or RES) is involved in cellular destruction and it includes:
  - Circulating blood monocytes
  - Fixed macrophages in the bone marrow, liver, spleen, and lymph nodes
  - Free macrophages
    - These cells are involved in:
      - » Engulfing particulate matter
      - » Processing of antigens for lymphocyte presentation
      - » Removal of damaged or senescent (aged) cells

- Spleen – contains the largest collection of lymphocytes and mononuclear phagocytes in the body. The spleen functions in:
  - Filtering and destruction of senescent (aged) or damaged RBCS – also called culling
  - Removal of particles (are found in some types of anemia) from RBC membranes – also called pitting – this causes a decrease in the surface to volume ratio of the RBC resulting in the formation of spherocytes (more on this later)
  - Enforcing close contact of blood borne antigens with lymphocytes and phagocytic cells – this is more important in children particularly in protection of the host from infections due to enveloped organisms

- Sequestering 1/3 of the platlet mass – in massive splenomegaly this can lead to peripheral thrombocytopenia (decrease in platlets in the peripheral blood)
- After a splenectomy (removal of the spleen), RBC inclusions and abnormal RBC shapes are seen. Culling is taken over by the liver which is less effective in performing all of the splenic functions
- Hypersplenism (splenomegaly) – in a number of conditions the spleen may become enlarged and through an exaggeration of its normal functions of filtering, and destruction and sequestering, it may cause anemia (may be caused by decreased RBCs), leukopenia (decreased WBCs), or thrombocytopenia or combinations of these cytopenias. When all three cell types are decreased this is called pancytopenia.

There are two types of hypersplenism:

- Primary – no underlying disease has been identified
- Secondary – caused by an underlying disorder such as:
  - » Inflammatory diseases
  - » Infectious diseases
  - » Blood disorders that cause compensatory or workload hypertrophy of the organ such as:

    Abnormal blood cells, antibody coated blood cells, hereditary spherocytosis, idiopathic throbocytopenic purpura (ITP)
  - The effects of these are relieved by splenectomy

- Lymph nodes – the lymphatic system is composed of lymph nodes and lymphatic vessels that drain into the left and right lymphatic duct. Lymph is formed from blood fluid that escapes into the connective tissue.
  - Lymph nodes are composed of lymphocytes, macrophages, and a reticular network.
  - They act as filters to remove foreign particles by phagocytic cells
  - As antigens pass through the lymph nodes, they come into contact with and stimulate immunocompetent lymphocytes to proliferate and differentiate into effector cells.

- The structure of the lymph node consists of :
  - An inner area called the medulla which contains plasma cells
  - An outer area called the cortex which contains follicles with B lymphocytes surrounded by T lymphocytes and macrophages

- Thymus – this organ is well developed at birth and increases in size until puberty at which time it starts to decrease in size.
  - It serves as a compartment for the maturation of T lymphocytes into immunocompetent T cells. The hormone thymosin plays a role in this process.
  - The structure of the thymus consists of:
    - An outer area called the cortex which is densely packed with small lymphocytes and macrophages
    - An inner area called the medulla which is less cellular with a few lymphocytes, macrophages, and epithelial cells.

Bone marrow – is located inside spongy bone

- In a normal adult, ½ of the bone marrow is hematopoietically active (red marrow) and ½ is inactive, fatty marrow (yellow marrow).
- The marrow contains both Erythroid (RBC) and leukocyte (WBC) precursors as well as platlet precursors.
- Early in life most of the marrow is red marrow and it gradually decreases with age to the adult level of 50%.
- In certain pathologic states the bone marrow can increase its activity to 5-10X its normal rate.
  - When this happens, the bone marrow is said to be hyperplastic because it replaces the yellow marrow with red marrow.

–This occurs in conditions where there is increased or ineffective hematopoiesis.

–The degree to which the the bone marrow becomes hyperplastic is related to the severity and duration of the pathologic state.

–Pathologic states that cause this include:

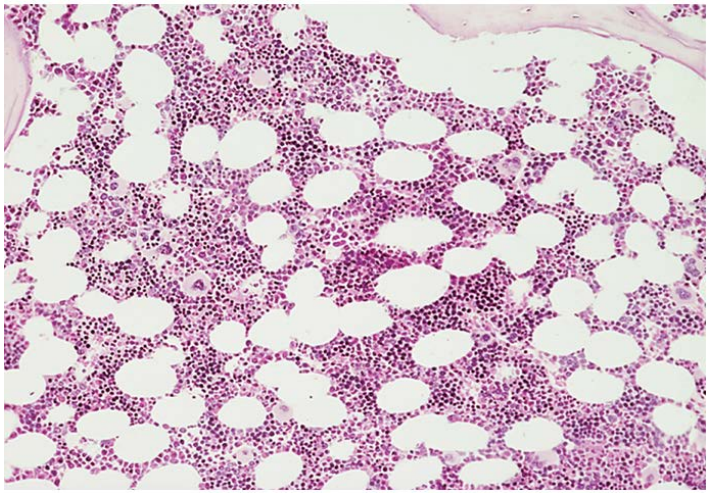
»Acute blood loss in which there is a temporary replacement of the yellow marrow

»Severe chronic anemia – erythropoiesis (RBC production) may increase to the extent that the marrow starts to erode the bone itself.

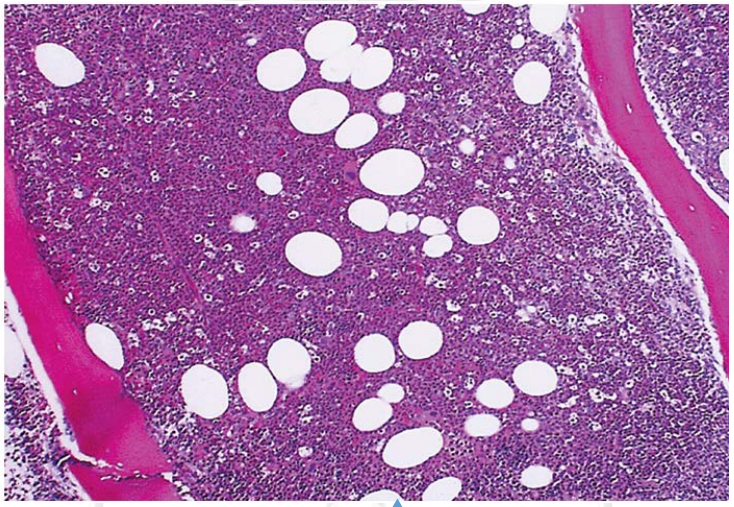
»Malignant disease – both normal red marrow and fatty marrow may be replaced by proliferating abnormal cells.

- The hematopoietic tissue may also become inactive or hypoplastic. This may be due to:
  - Chemicals
  - Genetics
  - Myeloproliferative disease that replaces hematopoietic tissue with fiberous tissue

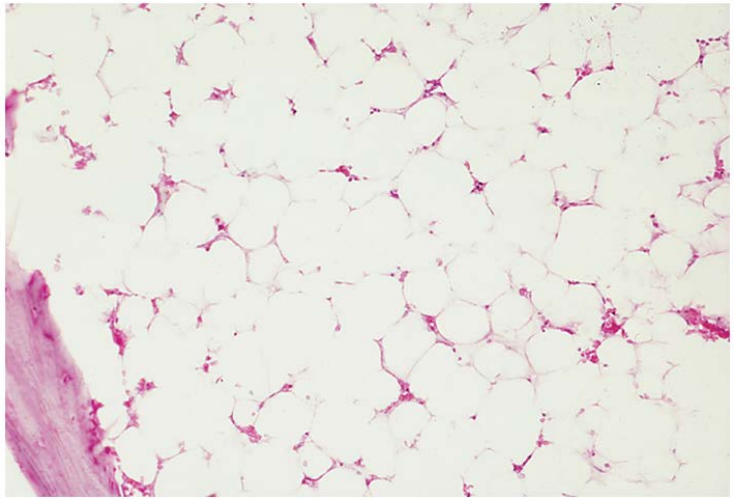
BONE MARROW



Normal



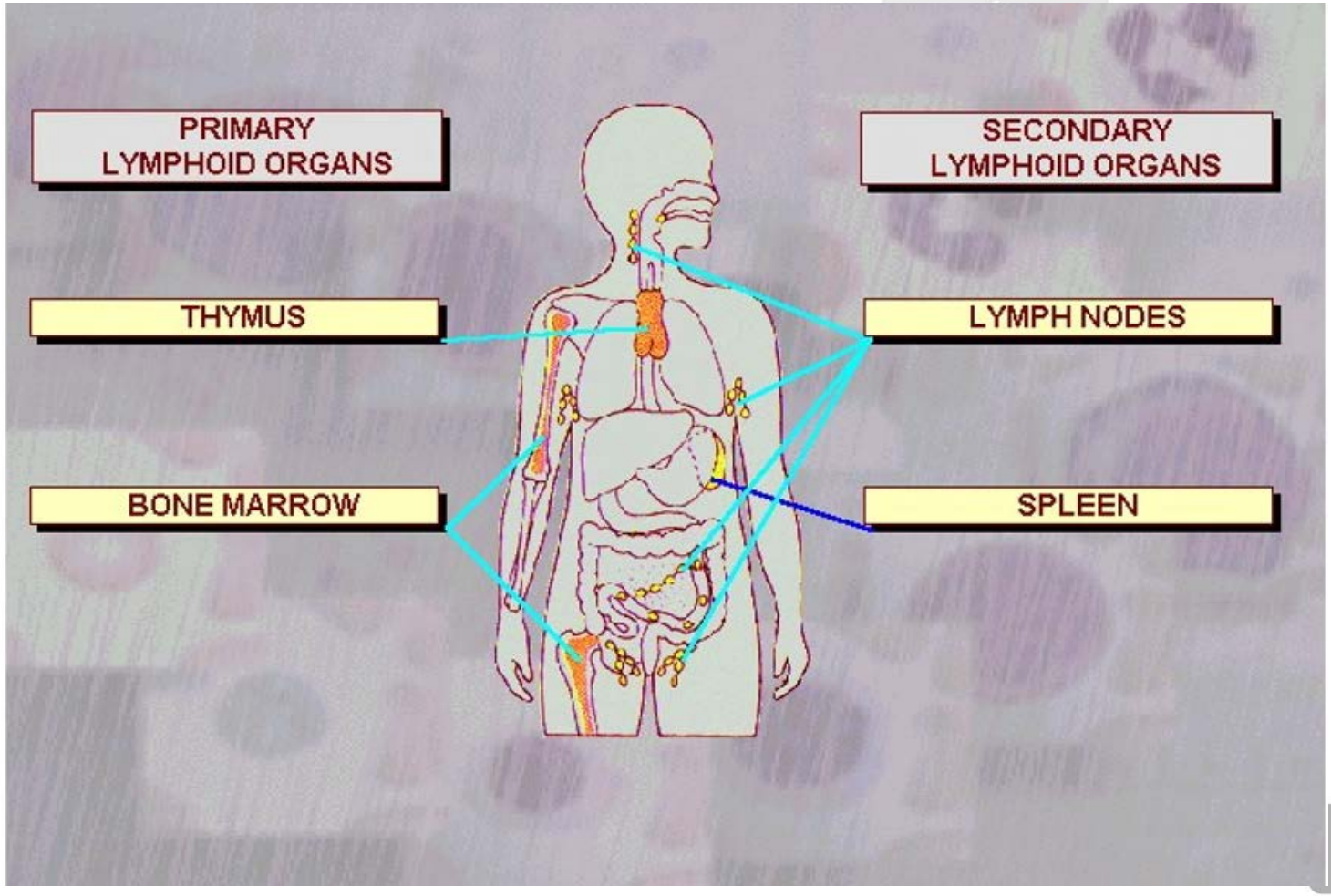
Hyperplastic



Hypoplastic

- Liver – contains phagocytic cells known as Kupffer cells that act as a filter for damaged or aged cells in a manner similar to, but less efficient than the phagocytic cells in the spleen.
  - If the bone marrow cannot keep up with the physiologic demand for blood cells, the liver may resume the production of blood cells that it began during fetal life

Summary of blood forming organs

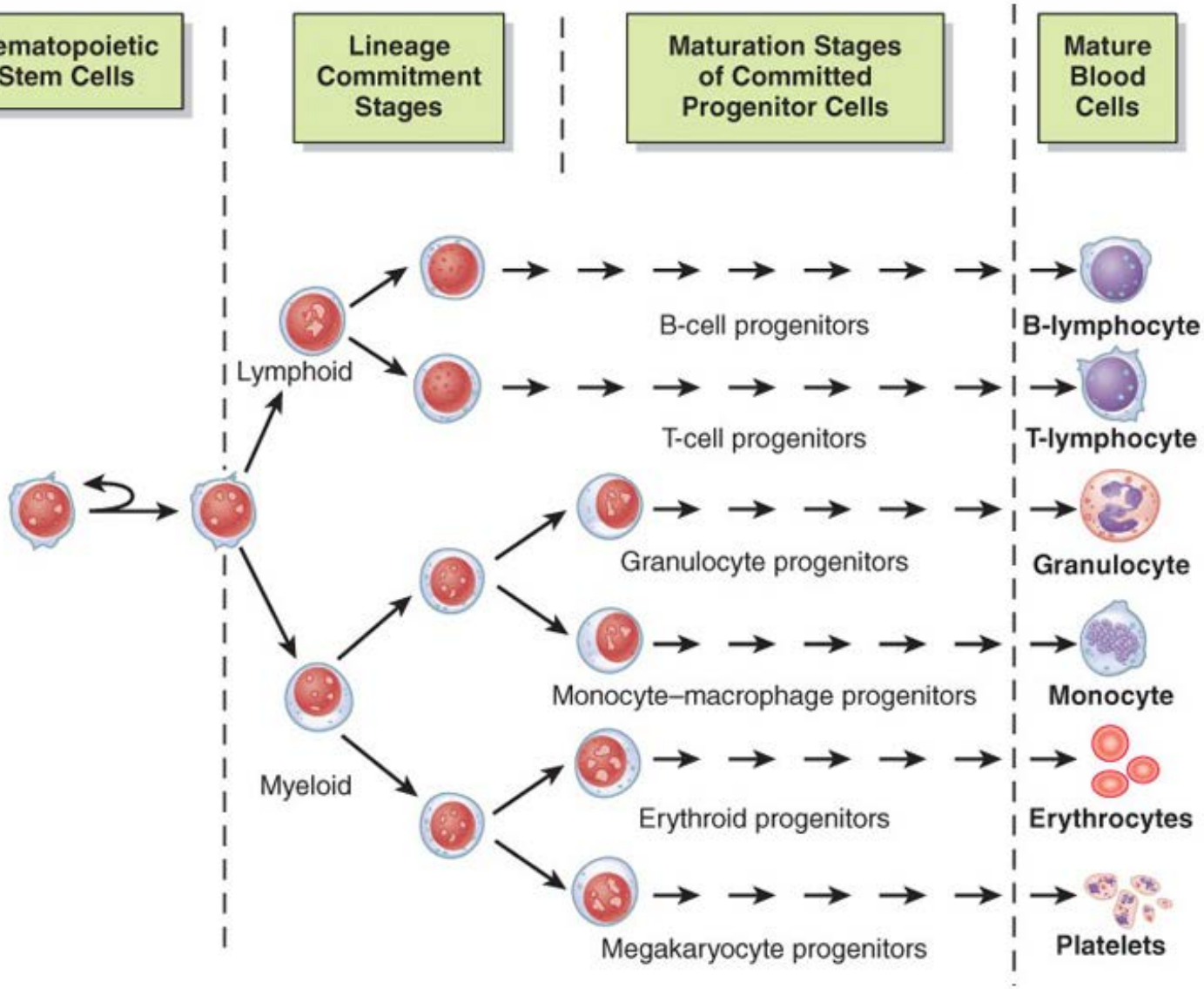


Derivation of blood cells

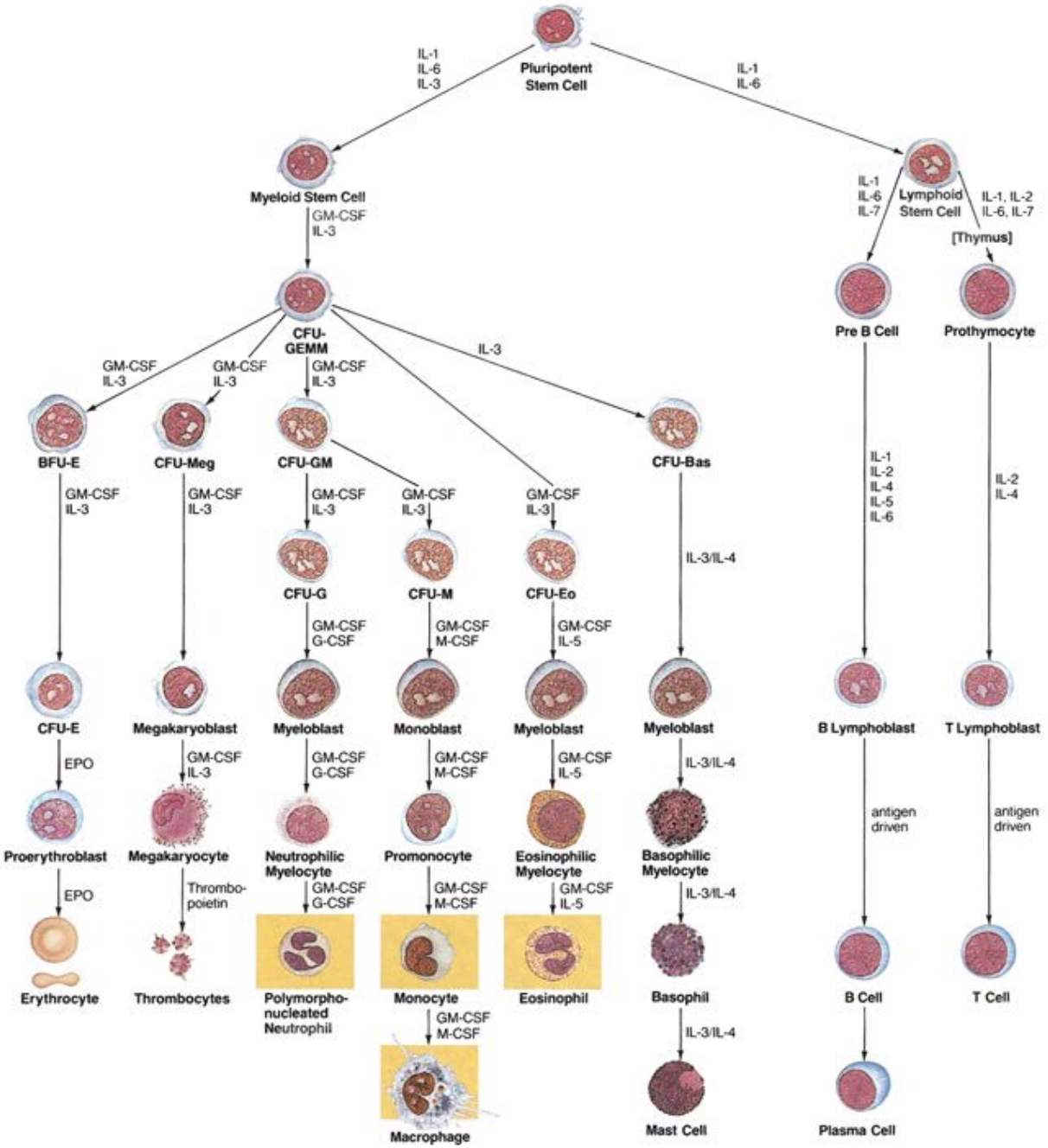
- Mature blood cells have a limited life span and with the exception of lymphocytes, are incapable of self-renewal.
  - Replacement of peripheral hematopoietic cells is a function of the pluripotential (totipotential) stem cells found in the bone marrow
    - Pluripotential stem cells can differentiate into all of the distinct cell lines with specific functions and they are able to regenerate themselves.
    - The pluripotential stem cells provide the cellular reserve for the stem cells that are committed to a specific cell line.

- The committed lymphoid stem cells will be involved in lymphopoiesis to produce lymphocytes
- The committed myeloid stem cell can differentiate into any of the other hematopoietic cells including erythrocytes, neutrophils, eosinophils, basophils, monocytes, macrophages, and platlets.

– Hematopoietic cells can be divided into three cellular compartments based on maturity:
  - Pluripotential stem cell capable of self-renewal and differentiation into all blood cell lines.
  - Committed proginator stem cells destined to develop into distinct cell lines
  - Mature cells with specialized functions



Hematopoiesis



Definition of anemia

- From Greek meaning “without blood”
- Condition where capacity of blood to transport oxygen to tissues is reduced or a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs.
  - decreased hemoglobin, RBC count, and hematocrit
- Anemia is not a disease but a *manifestation* of disease
- Treatment depends on discovering underlying cause

Introduction

- Anemia is estimated to affect 1.6 billion people. The highest prevalence is found in preschool-age children (47.4%), followed by pregnant females (41.8%), non-pregnant females (30.2%), school-age children (25.4%), and males (12.7%).

- Anemia is rarely an isolated disease and is most often a sign of an acquired or inherited disorder.

World Health Organization. The Global Prevalence of Anaemia in 2011. Geneva: WHO, 2015; De Benoist B, 2008

Hematopoiesis

- Red cells are produced in the bone marrow from myeloid progenitor cells, where their production requires a permissive bone marrow micro-environment and adequate substrate (including iron, vitamin B12 and folate) for hemoglobin, protein and DNA synthesis.

- Erythropoiesis is controlled by erythropoietin, which is synthetized by peritubular fibroblasts in the renal cortex in response to reduced oxygen tension.

Basic hematologic lab tests

- Complete blood count (CBC)
  - ✓ Amount of hemoglobin
  - ✓ Number, size, and shape of red blood cells (RBCs)
  - ✓ Number of white blood cells (WBCs) and platelets
  - ✓ +/- automated WBC differential

- Manual differential/manual peripheral smear review Abnormalities that fall outside of established parameters result in manual review

Complete blood count (CBC)

- Hemoglobin (g/dL) – amount of oxygen carrying protein
- Hematocrit (%) – % of blood volume occupied by RBCs
- RBC count (M/uL) – number of RBCs
- MCV (fL) – mean cell volume
- MCH (pg) – mean cell hemoglobin
- MCHC (g/dL) – mean cell hemoglobin concentration
- RDW (%) – red cell distribution width
- WBC (K/uL) – number of WBCs
- Platelet count (K/uL) – number of platelets

Measuring RBC parameters

- Hemoglobin (Hb)
  - measured directly as absorbance of cyanomethemoglobin
- RBC count (RBC)
  - measured directly by impedance
- Hematocrit (Hct)
  - measured by centrifugation; ratio of volume of RBCs to volume of whole blood
  - can also calculate (MCV x RBC)
- MCV
  - measured by mean height of voltage pulses in an impedance counter
  - can also calculate (Hct / RBC)
- MCH = Hb / RBC
- MCHC = Hb / Hct

Age-Specific Normative Red Blood Cell Values

Age	Hemoglobin (g per dL)		Hematocrit (%)		Mean corpuscular volume (fL)	
	Mean	2 SDs below mean	Mean	2 SDs below mean	Mean	2 SDs below mean
26 to 30 weeks' gestation	13.4	11.0	41.5	34.9	118.2	106.7
28 weeks' gestation	14.5	NA	45	NA	120	NA
32 weeks' gestation	15.0	NA	47	NA	118	NA
Full term (cord sample)	16.5	13.5	51	42	108	98
1 to 3 days	18.5	14.5	56	45	108	95
2 weeks	16.6	13.4	53	41	105	88
1 month	13.9	10.7	44	33	101	91
2 months	11.2	9.4	35	28	95	84
6 months	12.6	11.1	36	31	76	68
6 months to 2 years	12.0	10.5	36	33	78	70
2 to 6 years	12.5	11.5	37	34	81	75
6 to 12 years	13.5	11.5	40	35	86	77
12 to 18 years (male)	14.5	13.0	43	36	88	78
12 to 18 years (female)	14.0	12.0	41	37	90	78

Evaluation of the size of RBCs

- MCV (Mean Cell Volume)

  - ✓ Microcytic < 80 fl (cubic micrometers or femtoliters (μm^3 = **fL**))
  - ✓ Normocytic 80-100 fl
  - ✓ Macrocytic > 100 fl

Fundamental questions to be considered

- What is the cause of the anemia?
- What is the urgency for correcting the anemia, i.e. is a blood transfusion or other urgent intervention indicated?

Age-Specific Causes of Anemia

Cause	*Etiology and epidemiology*	*Presentation*	*Indices and other laboratory testing*
Neonatal[7]			
Blood loss	Hemorrhage (placental abruption, subgaleal, traumatic); maternal-fetal and twin-twin transfusion Accounts for 5 to 10 percent of all cases of severe neonatal anemia	Tachypnea, pallor, and mental status change (irritability, poor feeding); >20 percent loss of blood volume results in shock and cardiopulmonary collapse	Anemia with normal indices; reticulocyte count is initially normal, then increases; positive Kleihauer-Betke test in maternal-fetal hemorrhage
Isoimmunization	ABO incompatibility, Rh incompatibility Rh incompatibility occurs in 10.6 per 10,000 live births; 50 percent of these infants develop anemia	Jaundice and mild anemia; infants with severe isoimmunization (e.g., untreated Rh incompatibility) may present with hydrops fetalis	Positive Coombs test; elevated bilirubin level; normocytic anemia with elevated reticulocyte count
Congenital hemolytic anemia	Spherocytosis, G6PD deficiency	Hyperbilirubinemia and moderate jaundice	Low enzyme activity; with hemolysis, smear may show poikilocytosis, reticulocytosis, Heinz bodies, and bite cells (in G6PD deficiency) or spur cells (in pyruvate kinase deficiency)

Age-Specific Causes of Anemia

Cause	*Etiology and epidemiology*	*Presentation*	*Indices and other laboratory testing*
Congenital infection	Parvovirus B19, human immunodeficiency virus, syphilis, rubella, sepsis	Pallor, irritability, and other findings associated with infection (e.g., deafness)	Normocytic anemia with low reticulocyte count
Diamond-Blackfan syndrome	Congenital pure red cell aplasia resulting from increased apoptosis in erythroid precursors Affects 7 per 1 million live births	Neonatal pallor progressing to symptomatic anemia; average age of diagnosis is 3 months; about 30 percent have other abnormalities	Macrocytic anemia with low reticulocyte count
Fanconi anemia	Increased susceptibility of progenitor cells in bone marrow leads to increased apoptosis, progressing to pancytopenia	Average age of diagnosis is 8 years, but associated congenital abnormalities may facilitate early diagnosis (e.g., café-au-lait spots; microsomy; low birth weight; thumb, renal, skeletal, and eye abnormalities)	Microcytic anemia and reticulocytopenia, thrombocytopenia, or leukopenia; DNA sequencing can detect genetic mutations for Fanconi anemia complementation groups

Age-Specific Causes of Anemia

Cause	*Etiology and epidemiology*	*Presentation*	*Indices and other laboratory testing*
Infancy to toddlerhood[2]			
Iron deficiency	Inadequate dietary intake, chronic occult blood loss (excessive cow's milk consumption, inflammatory bowel disease, Meckel diverticulum, parasites) Prevalence is 8 to 15 percent	Usually asymptomatic; severe cases can present with fatigue, pallor, or dyspnea; rarely occurs before 6 months of age; highest risk is at 6 to 36 months of age	Microcytic anemia with elevated RBC distribution width; peripheral smear shows hypochromic microcytes and may show target cells; iron and ferritin levels and iron saturation are low; transferrin level is elevated
Concurrent infection	Bacterial or viral infection leading to cytokine-mediated decrease in iron utilization and RBC production	Presenting symptoms usually result from infectious process	Normocytic or mildly microcytic, low/normal serum iron level with low transferrin level; ferritin level may be elevated because it is an acute phase reactant
Blood loss	Trauma, gastrointestinal bleeding	Tachypnea, tachycardia, pallor, hypotension	Hgb levels may initially be normal, followed by anemia with normal indices

Age-Specific Causes of Anemia

Cause	*Etiology and epidemiology*	*Presentation*	*Indices and other laboratory testing*
Disorder of Hgb structure or synthesis	Thalassemia, sickle cell disease	Anemia in thalassemia may range from mild and asymptomatic to severe, depending on number of heme chains affected; sickle cell disease presents with hemolysis, pain crises, dactylitis, and aplastic crisis; symptoms are rarely present at birth but typically develop in the first year	Microcytic anemia, low RBC distribution width, and low Mentzer index in thalassemia; Hgb electrophoresis may show Hgb F; smear with basophilic stippling; hemolysis, reticulocytosis, and Hgb S on electrophoresis in sickle cell disease
RBC enzyme defects	G6PD deficiency, pyruvate kinase deficiency 10 percent of the black population has G6PD deficiency	Neonatal hyperbilirubinemia and hemolytic anemia when exposed to oxidative stress	Low enzyme activity; with hemolysis smear may show poikilocytosis, reticulocytosis, Heinz bodies, and bite cells (in G6PD deficiency) or spur cells (in pyruvate kinase deficiency)

Age-Specific Causes of Anemia

Cause	*Etiology and epidemiology*	*Presentation*	*Indices and other laboratory testing*
RBC membrane defects	Spherocytosis, elliptocytosis	Hyperbilirubinemia, splenomegaly, gall bladder disease, and aplastic crisis; autosomal dominant, so family history is positive in about 75 percent of patients	Macrocytosis, reticulocytosis, elevated bilirubin and lactate dehydrogenase levels; spherocytes or elliptocytes on smear; osmotic fragility test is commonly done but not specific
Acquired hemolytic anemias	Antibody-mediated hemolysis, drug-induced hemolysis, hemolytic uremic syndrome, disseminated intravascular coagulation	Jaundice, fatigue, dyspnea	Positive Coombs test and spherocytes visible on smear in antibody-mediated hemolysis; schistocytes visible on smear in hemolytic uremic syndrome or disseminated intravascular coagulation
Transient erythro-blastopenia of childhood	Transient immune reaction against erythroid progenitor cells	Anemia after toxin ingestion or viral illness, usually in children 6 months to 3 years of age	Normocytic anemia, initially with reticulocyte count of 0; anemia resolves within 2 months
Leukemia, myelofibrosis	Usually spontaneous, but rates are increased in patients with prior radiation exposure or chemotherapy	Anemia causes pallor, fatigue, and dyspnea; patients with leukemia may present with petechiae, low-grade fever, nonspecific bone pain, gum swelling, or rash	Normocytic anemia with decreased reticulocyte count; leukopenia, leukocytosis, or thrombocytopenia; peripheral smear shows blast cells

Age-Specific Causes of Anemia

Cause	*Etiology and epidemiology*	*Presentation*	*Indices and other laboratory testing*
Lead poisoning	Risk factors include young age, living in a home built before 1970 or in areas where soil is contaminated, and pica (as in iron deficiency)	In addition to anemia, patients may present with abdominal pain, altered mental status, renal disease, and hypertension	Microcytic anemia may be concurrent with iron deficiency; peripheral smear may show basophilic stippling; hemolysis may be present
Late childhood and adolescence[2]			
Iron deficiency	Second peak in iron deficiency occurs in adolescence because of growth spurt, menstruation, and poor dietary iron intake	Pallor, fatigue, dyspnea	Same as for infants and toddlers, above
Chronic disease	Renal disease, liver disease, hypothyroidism, other chronic illnesses	Usually mild and asymptomatic	Normocytic or mildly microcytic, low/normal serum iron level with low transferrin level; ferritin level may be elevated because it is an acute phase reactant

Age-Specific Causes of Anemia

Cause	*Etiology and epidemiology*	*Presentation*	*Indices and other laboratory testing*
Blood loss	Same as for infants and toddlers, above Menstruation in adolescent girls		
Disorders of Hgb synthesis or RBC membrane defects	Same as for infants and toddlers, above		
Acquired hemolytic anemias	Same as for infants and toddlers, above		
Leukemia and other bone marrow disorders	Same as for infants and toddlers, above		

Classification

Based on **RED CELL CHARACTERISTICS -**

- red cell size, chromia and morphology:
  - ✓ microcytic hypochromic
  - ✓ macrocytic normochromic
  - ✓ normocytic normochromic

- morphology:
  - ✓ leuco-erythroblastic
  - ✓ micro-/macroangiopathic

Classification (cont.)

Based on **UNDERLYING MECHANISM** -

- decreased BM production/output:
  - ✓ BM aplasia/infiltrate
  - ✓ ineffective haematopoiesis (e.g. megaloblastic anaemia)
  - ✓ myelodysplastic syndromes, HIV
  - ✓ substrate deficiency
  - ✓ EPO insufficiency

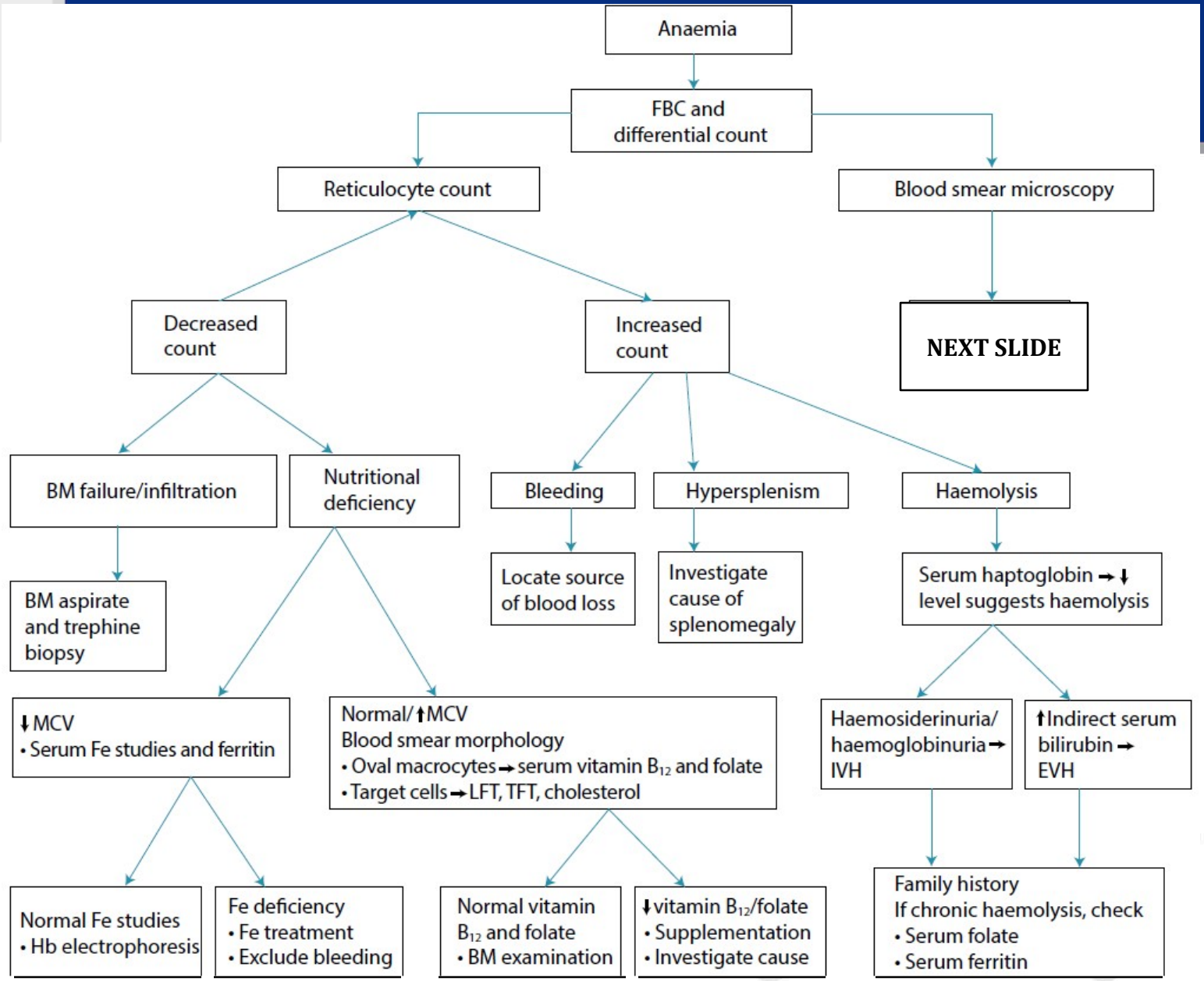
- peripheral loss/destruction:
  - ✓ bleeding
  - ✓ sequestration
  - ✓ haemolysis

Risk Factors for Anemia

Etiology	*Risk factor*	*Comment*
Decreased RBC production	Chronic disease	Renal disease can result in anemia because of decreased erythropoietin levels; hypothyroidism can result in macrocytic anemia because of impaired RBC production; chronic inflammation (as in chronic infection or rheumatologic disease) can lead to cytokine-mediated suppression of erythropoiesis; inflammatory bowel disease or celiac disease can result in anemia because of inflammation and nutrient malabsorption
	Iron deficiency[10]	Pica induced by iron deficiency increases risk of lead ingestion, and lead is absorbed more readily in the presence of iron deficiency; iron levels should be tested in patients with lead poisoning
	Poor diet	Inadequate nutrient intake can cause deficiencies in iron, folate, and vitamins A, B_{12}, and D
	Prematurity	Decreased iron stores and increased demand for catch-up growth can cause iron deficiency; rarely occurs before birth weight is doubled
Increased RBC turnover	Drug use	Primaquine, sulfamethoxazole, and nitrofurantoin (Furadantin) can lead to hemolysis; this is more pronounced in patients with G6PD deficiency but can occur in any patient; phenytoin (Dilantin) can cause megaloblastic anemia
	Ethnicity	African ancestry in sickle cell disease; Mediterranean, Asian, or African ancestry in thalassemia; Sephardic Jewish, Filipino, Greek, Sardinian, or Kurdish ancestry in G6PD deficiency
	Family history	Thalassemia, spherocytosis, and sickle cell disease; family history may include gallstones and jaundice in addition to anemia
	Mechanical heart valves	Mechanical destruction by the valve can cause hemolysis
	Sex	G6PD deficiency and pyruvate kinase deficiency are X-linked and therefore more common in males
	Splenomegaly	Sequestration and increased destruction of RBCs can cause hemolysis
Both	Infection	Infection can precipitate immune-mediated hemolytic anemia or cause hemolytic crises in patients with inherited enzyme defects and sickle cell disease; can cause RBC aplasia (as in parvovirus B19 infection) or result in transient erythroblastopenia of childhood

G6PD = glucose-6-phosphate dehydrogenase; RBC = red blood cell.

ALGORITHM DOR THE DIAGNOSTIC APPROACH TO ANAEMIA



(TFT = thyroid function test; LFT = liver function test; MCV = mean cell volume; IVH = intravascular haemolysis; EVH = extravascular haemolysis; FBC = full blood count; Fe = iron.)

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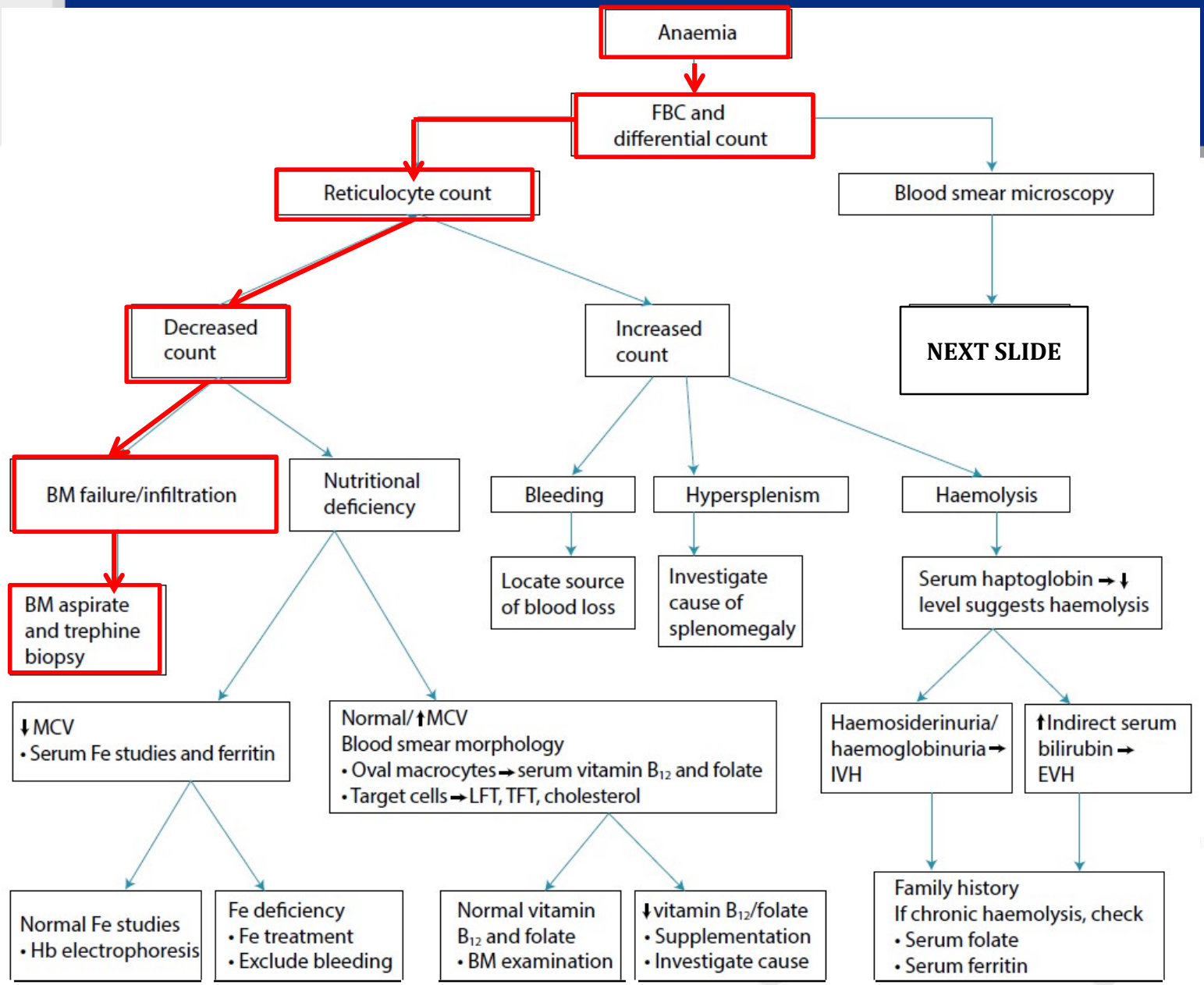

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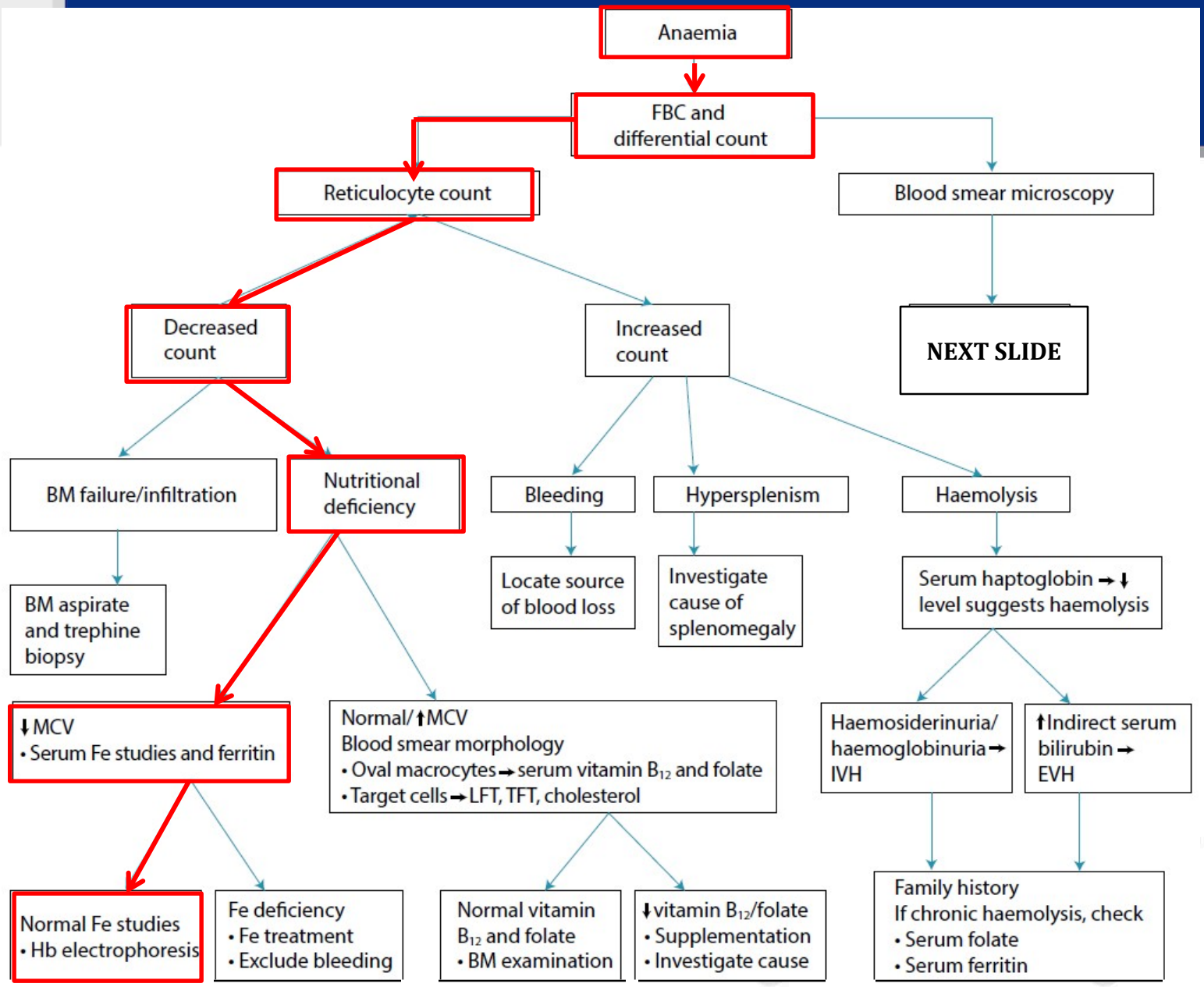

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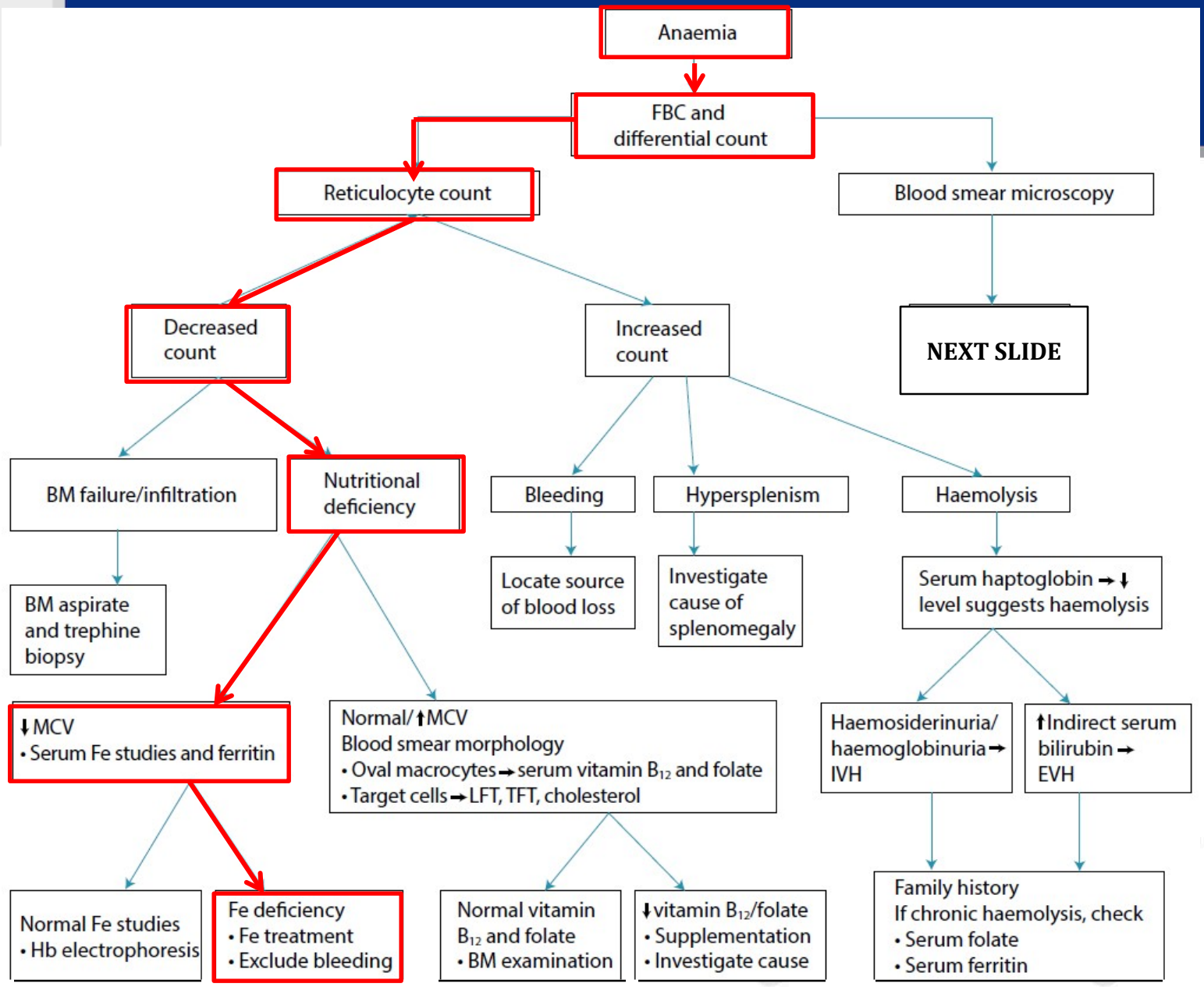
ALGORITHM DOR THE DIAGNOSTIC APPROACH TO ANAEMIA



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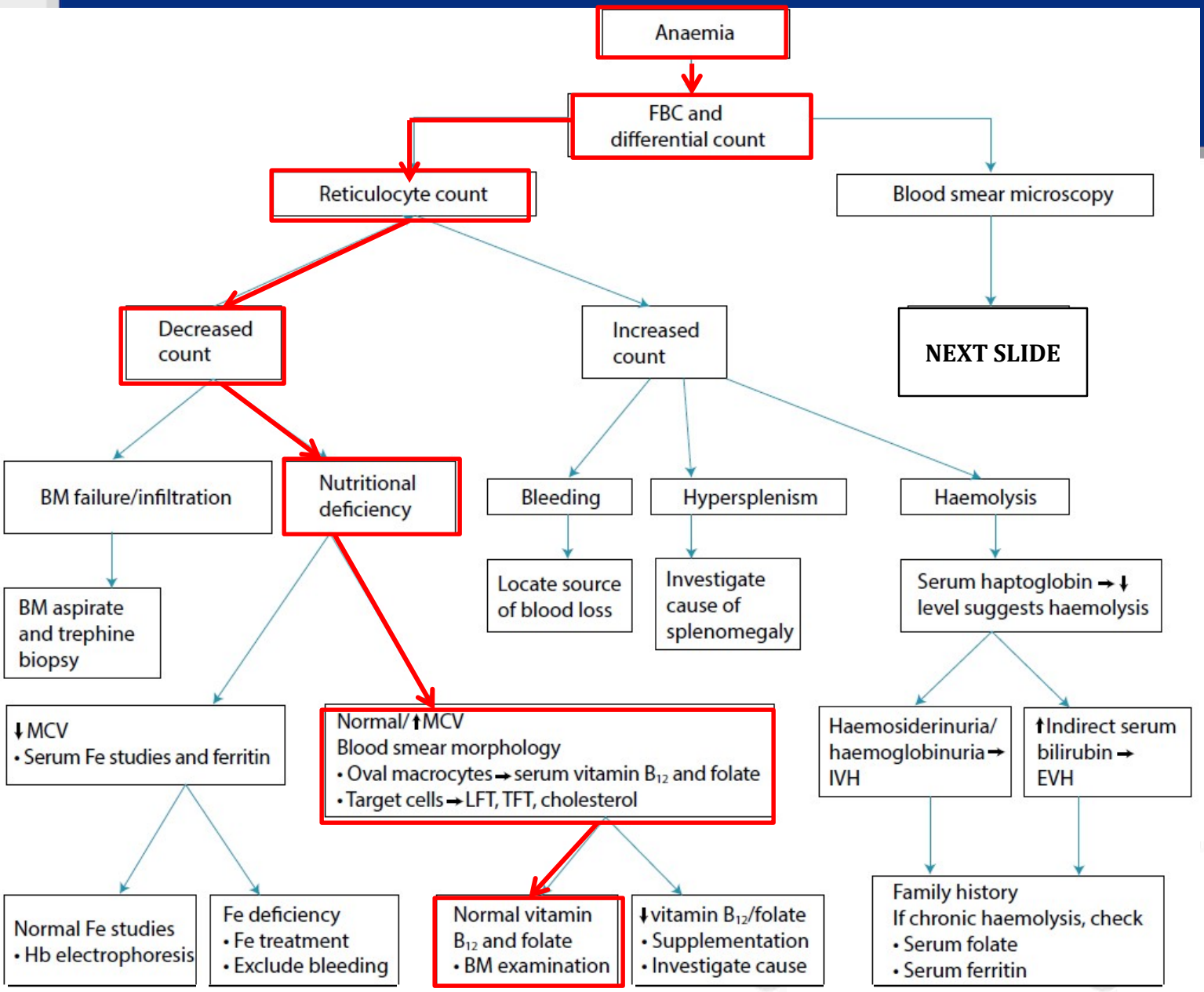

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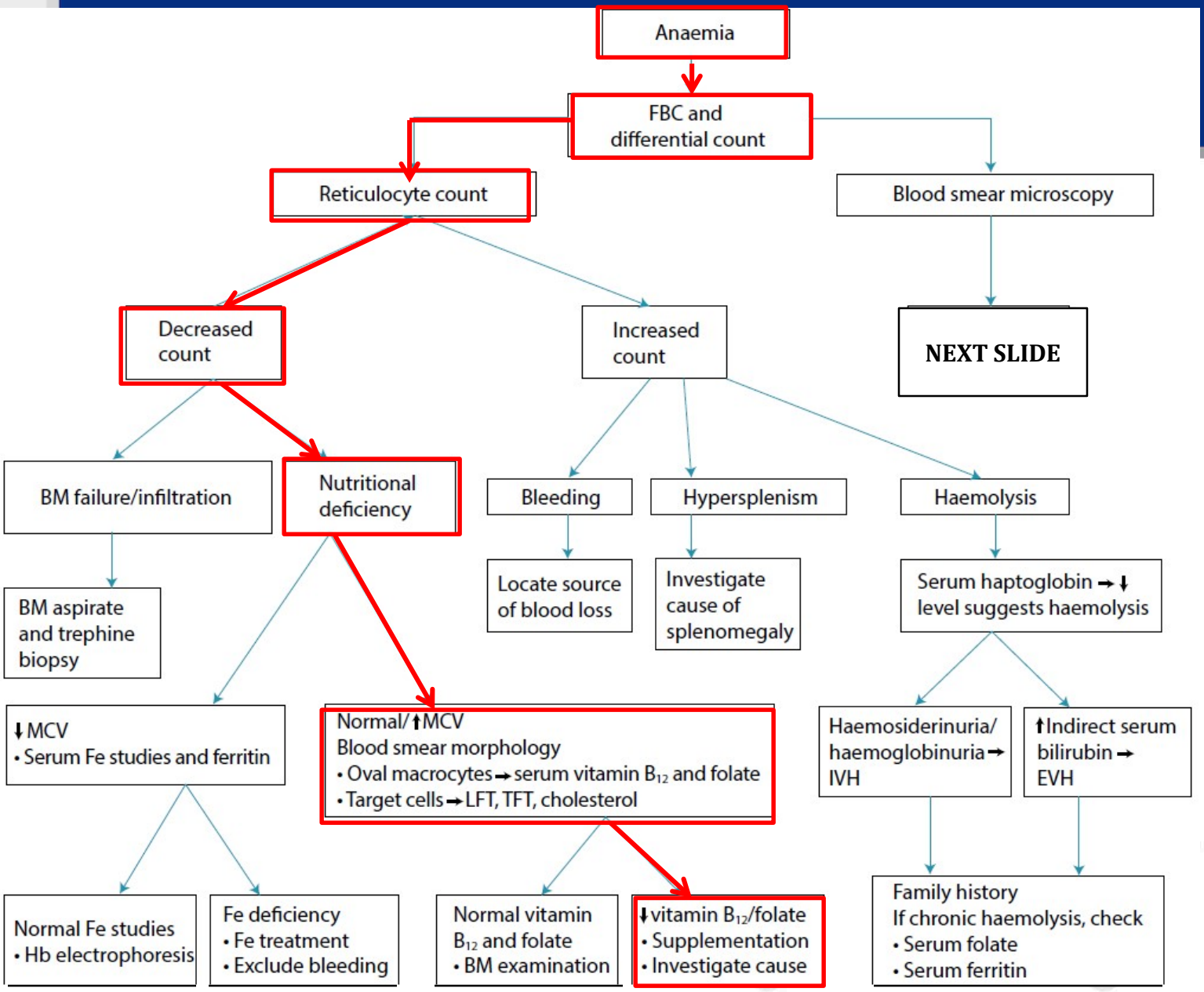

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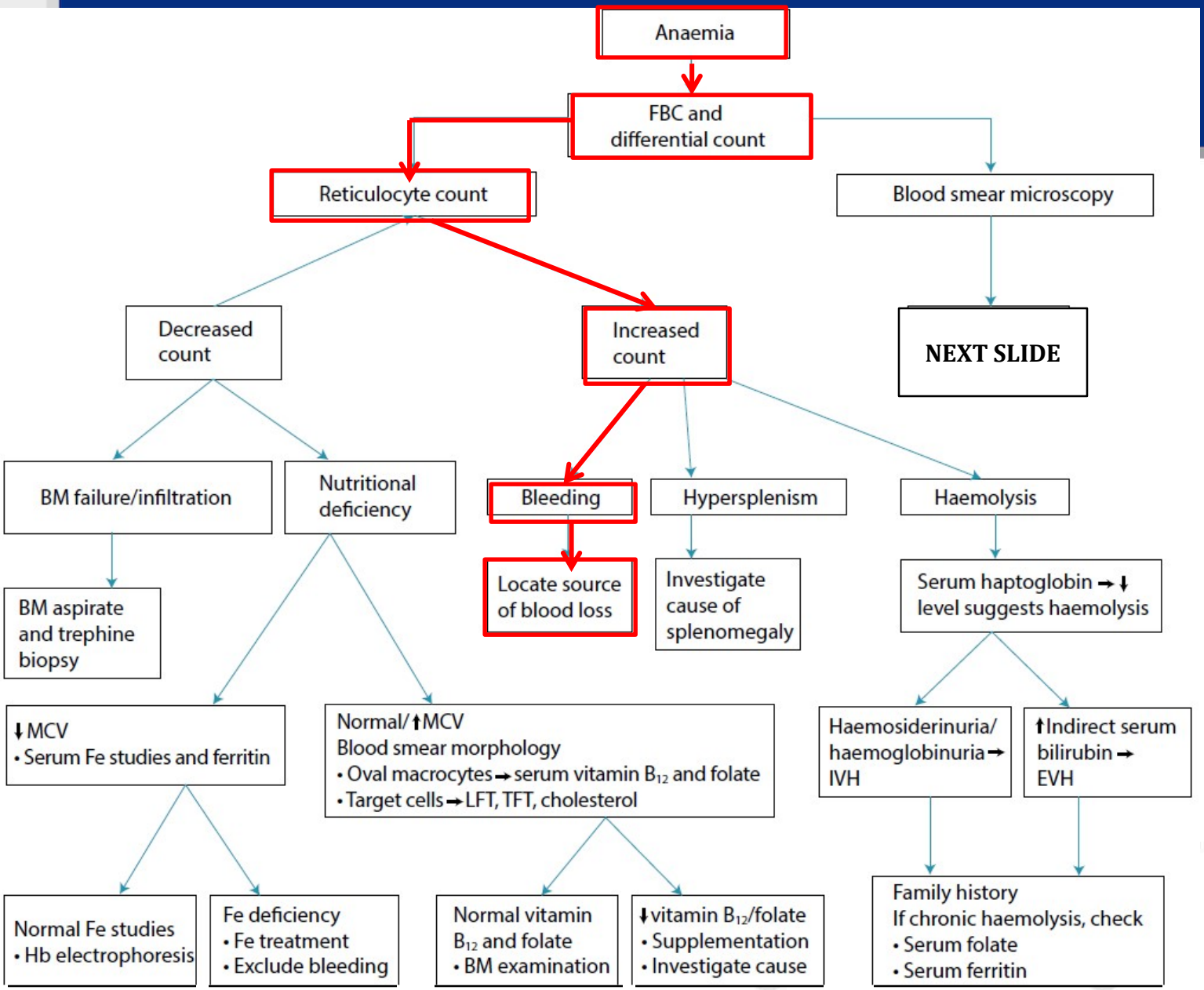

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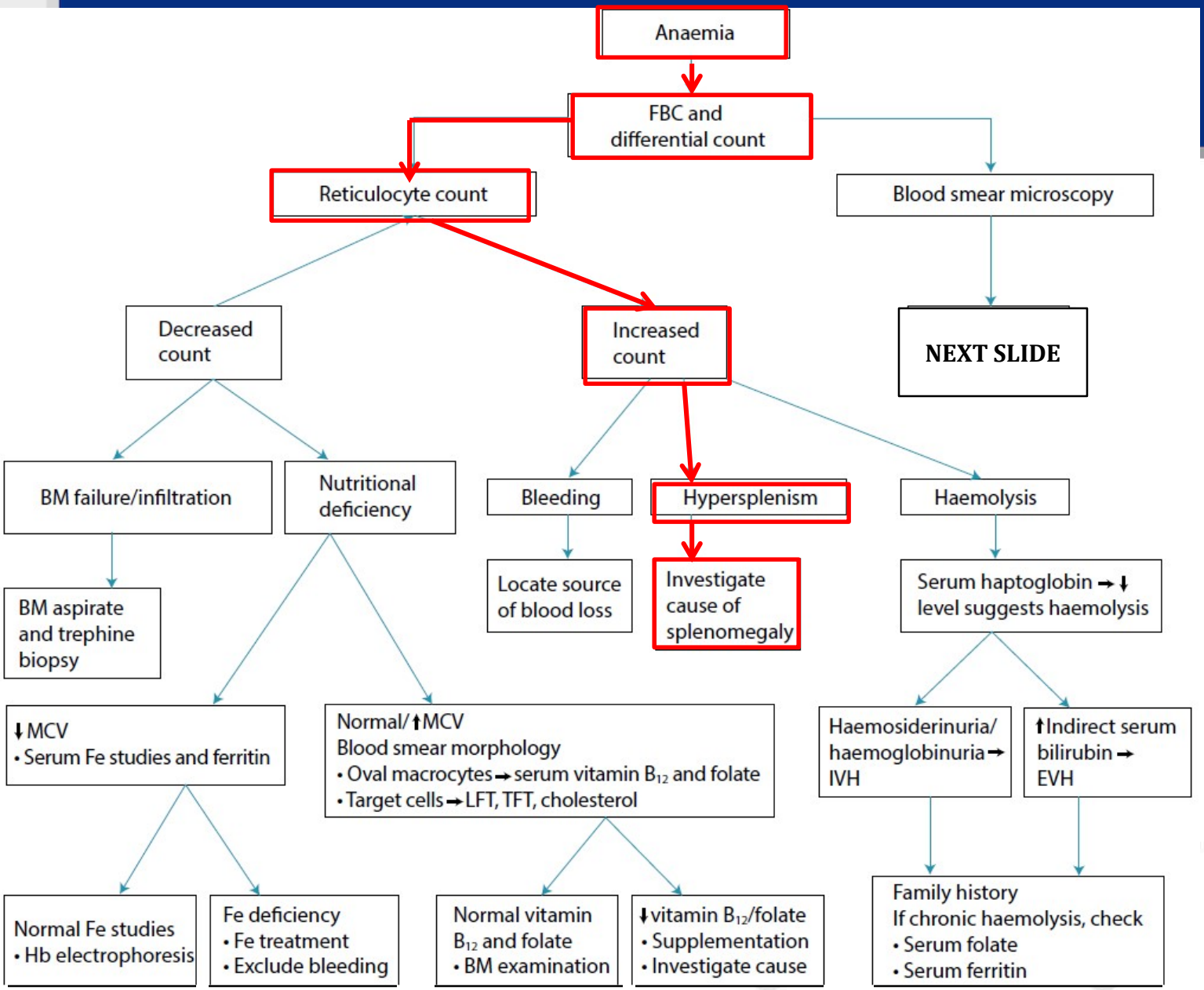

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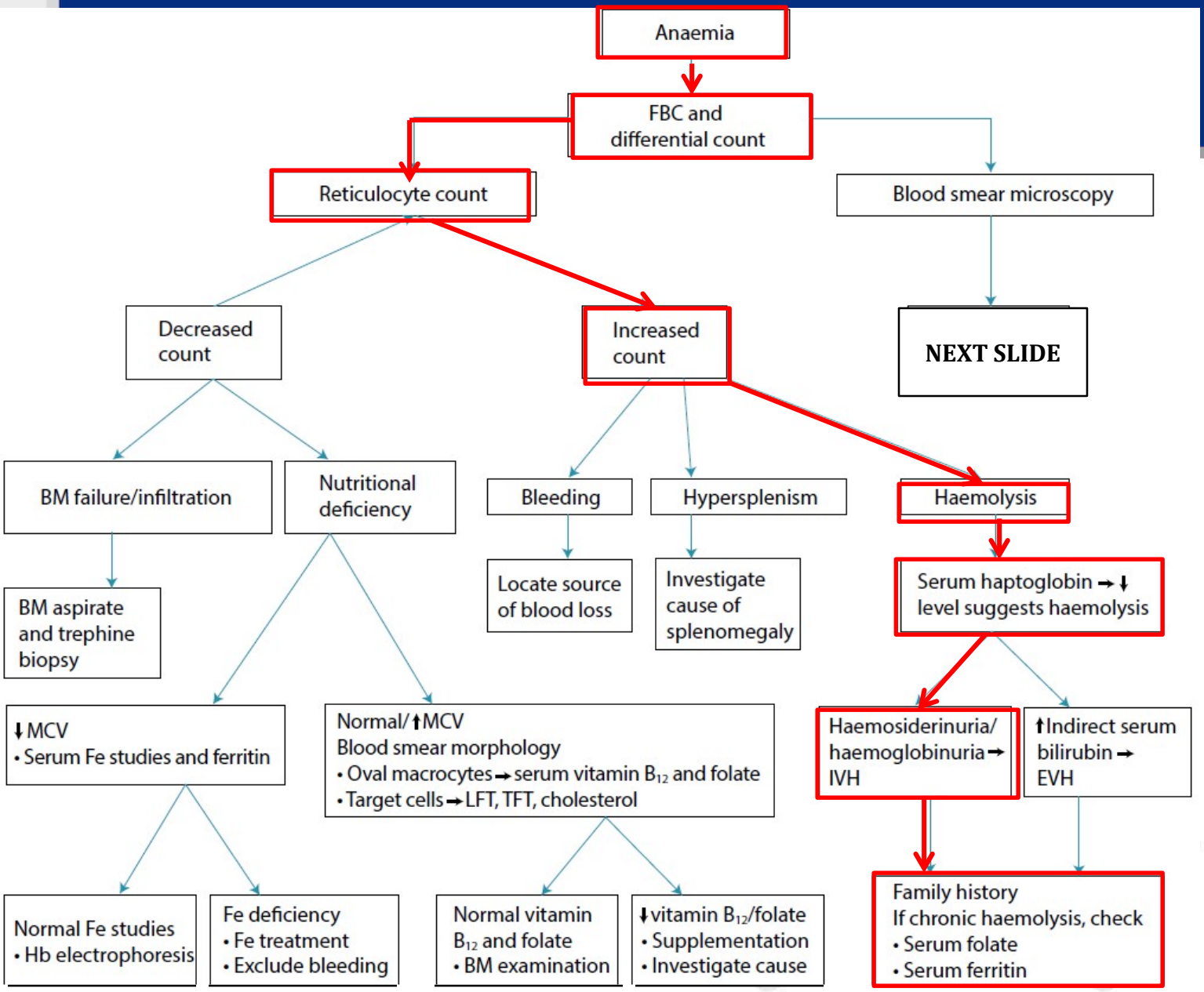

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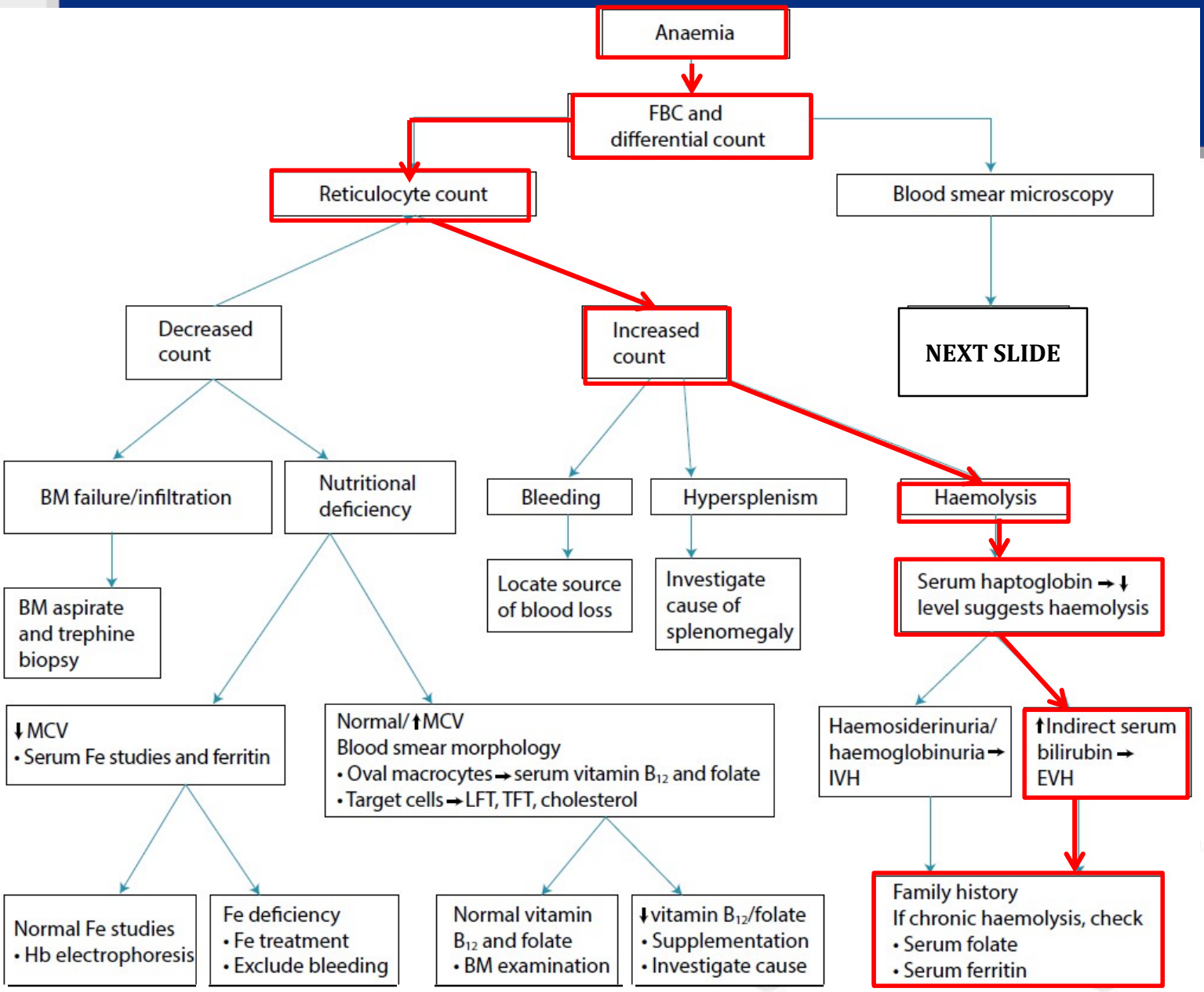

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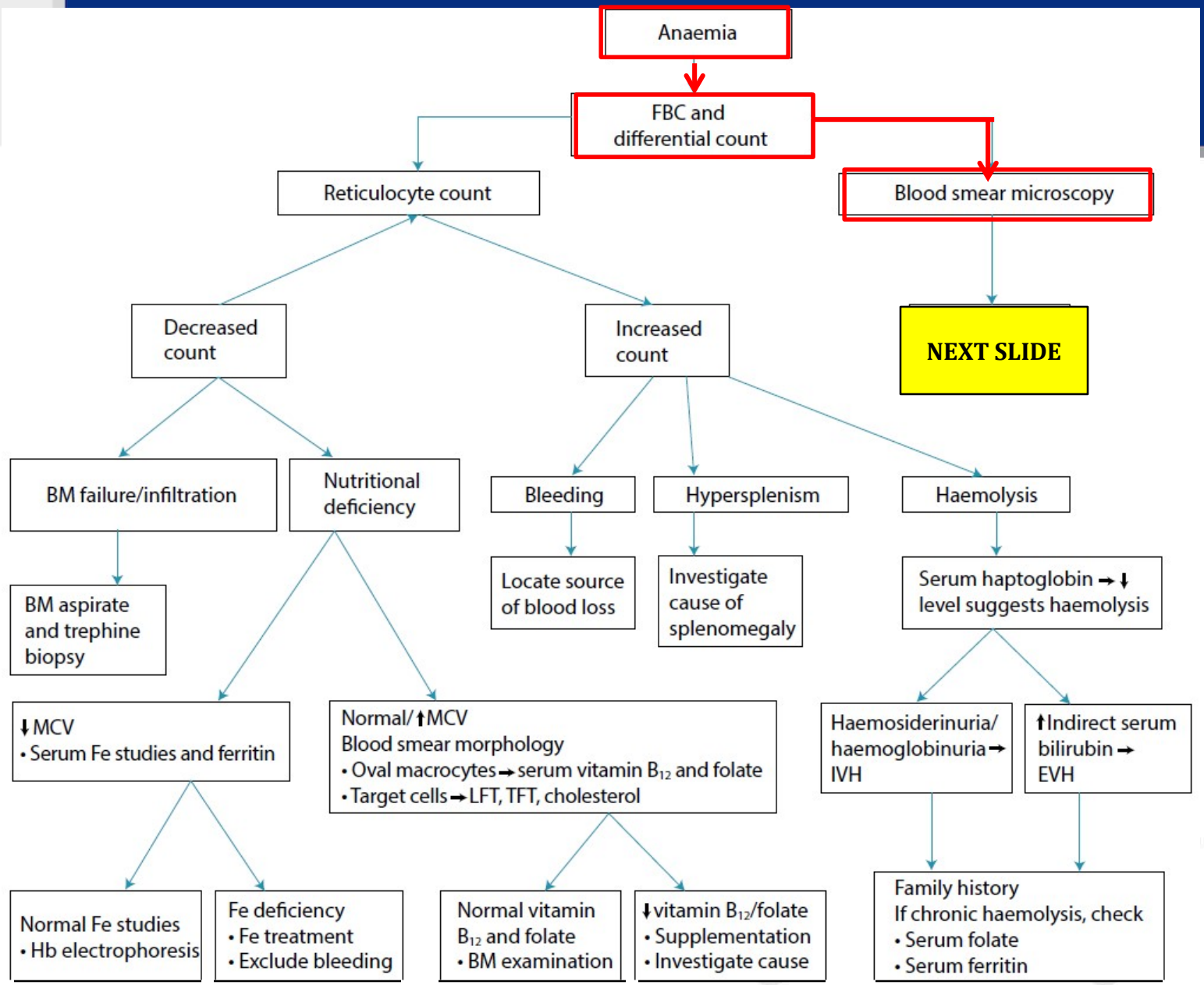

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RED CELL MORPHOLOGICAL CHARACTERISTICS IN THE BLOOD SMEAR

Morphological observation	Significance	Further tests indicated and expected result
Oval macrocytes, teardrops, basophilic stippling, right shift	MA (megaloblastic anaemia)	Serum vitamin B_{12} and folate levels
Hypochromia	Iron deficiency anaemia (also see pencil cells)	Serum ferriin, transferrin and iron levels
Microcytosis	Chronic disorder; thalassaemia trait	Hb electrophoresis/HPLC
Sickle cells	Seen in sickle cell disease	Hb electrophoresis to confirm presence of Hb S, and quantitate Hb F level
Spherocytes	Noted in hereditary spherocytosis, warm AIHA	Coombs test: positive for IgG in warm AIHA Red cell membrane analysis: selected membrane protein abnormalities
Elliptocytes/ovalocytes	Hereditary elliptocytosis/ovalocytosis	Red cell membrane analysis: selected membrane protein abnormalities
Autoagglutination	Cold AIHA	Coombs test: positive for C3d in cold AIHA
Red cell fragmentation	If platelets decreased → microangiopathic haemolysis If platelets normal → macroangiopathic haemolysis	Microangiopathic haemolysis: DIC screen → consumptive coagulopathy U&E: marked renal dysfunction in HUS Altered neurological status: suspect TTP
Malaria	Life-threatening infection	Identify species Monitor FBC and parasite count while on treatment

AIHA = auto-immune haemolytic anaemia; U&E = urea and electrolytes;
DIC = disseminated intravascular coagulation; HUS = haemolytic uraemic syndrome;
HPLC = high-performance liquid chromatography; TTP = thrombotic thrombocytopenic purpura

Presenting complains

- increased tiredness/fatigue
- dyspnoea
- decreased effort tolerance

The severity of symptoms depends on the degree of anaemia and rate of Hb decrease. Therefore, at a given Hb level, anaemia from acute blood loss is likely to manifest more severely than anaemia of insidious onset (weeks to months).

Symptoms during early childhood should remind one of possible inherited forms of anaemia, e.g. thalassaemia.

History

- interrogation of the presenting complaint and duration of the problem
- transfusion history
- dietary history, including pica (craving for unusual food items, generally associated with iron deficiency)
- travel history (to endemic malarial or other infectious areas)
- change in bowel habits
- bleeding (e.g. gastrointestinal and genito-urinary)
- drug history (e.g. anticoagulants, antiplatelet agents, renotoxic agents, anticonvulsants)
- chronic disease (e.g. HIV, tuberculosis)
- surgery (e.g. gastrectomy, small-bowel surgery)
- family history

Clinical examination

- **Skin and mucous membrane**:
  - ✓ pallor is the cardinal clinical sign for anaemia, which should be confirmed by measuring the Hb level
  - ✓ angular stomatitis
  - ✓ glossitis in nutritional deficiencies
  - ✓ koilonychia (spoon-shaped nails) in iron deficiency
  - ✓ premature greying, which often accompanies megaloblastic anaemia
  - ✓ scleral ictus, which indicates possible haemolysis or ineffective erythropoiesis

Clinical examination (cont.)

- **Neuromuscular**:
  - ✓ muscle weakness
  - ✓ headache, lack of concentration, drowsiness, tinnitus
  - ✓ paraesthesias, peripheral neuropathy, ataxia and loss of vibration sense, and proprioception in pernicious anaemia

- **Cardiovascular**:
  - ✓ hyperdynamic circulation with haemic 'flow' murmurs
  - ✓ cardiac failure

Clinical examination (cont.)

- **Infection, malignancy**:

(e.g. lymphoma, leukaemia, metastatic carcinoma)

✓hepatosplenomegaly

✓lymphadenopathy

✓bleeding manifestations (petechiae, purpura, ecchymosis), bone marrow failure

Laboratory testing

- starting point of investigations – **complete blood count**, **differential count** and **reticulocyte count** together with **microscopic blood smear examination**
- reticulocyte count gives an indication of the bone marrow status, i.e. decreased activity versus appropriate response to the anaemia
- **reticulocyte production index (RPI)** provides a more accurate representation of marrow activity than an isolated reticulocyte count, as it corrects for the degree of anaemia and presence of immature reticulocytes in the peripheral blood

Reticulocyte production index

The RPI is calculated as follows:

% reticulocytes × patient haematocrit/45 ÷ reticulocyte maturation time (days) in peripheral blood

The reticulocyte maturation time is calculated as follows:

- haematocrit >40% = 1 day
- 30 - 40% = 1.5 days
- 20 - 30% = 2 days
- <20% = 2.5 days

A decreased RPI signifies a suboptimal bone marrow response for correction of the anaemia

Differential diagnosis for **MICROCYTIC** anaemia includes:

- anaemia of chronic disorder
- thalassaemia trait
- sideroblastic anaemia

Pertinent Findings in Microcytic Hypochromic Anemia

Cause of Anemia	Erythrocyte Count	Red Cell Distribution Width	Anisopoikilo-cytosis	Basophilic Stippling	Bone Marrow Iron
Iron deficiency	Decreased	Increased	Yes	No	Decreased
Thalassemia minor	Normal or increased	Normal	No	Yes	Increased
Sideroblastic anemias					
Hereditary	Decreased	Variable	Variable	Yes	Increased ringed sideroblasts
Acquired	Decreased	Dimorphic population	Yes	Yes	Increased ringed sidero-blasts
Chronic disease	Decreased	Variable	Variable	No	Decreased in siderocytes; increased in RE cells

RE = reticuloendothelial.

Anaemia of chronic disorder

- occurrs frequently in patients with chronic infection, malignancy or autoimmune disorders
- is caused by a combination of functional iron deficiency due to increased hepcidin, leading to iron sequestration (reticuloendothelial iron blockade) and EPO deficiency or resistance
- iron studies show raised ferritin and low TF levels with low or normal TF percentage saturation
- anaemia is usually normocytic and normochromic, but can be microcytic in approximately one-third of cases due to long-standing iron restriction at the macrophage level

! ferritin is an acute-phase protein and may be falsely normal or raised in patients with iron deficiency in the presence of acute inflammation

Thalassemia

- Hemoglobin is a tetramer; with two alpha (α) and two beta (β)
- Due to abnormally low production of alpha or beta-globin chains: named for the chain which is decreased or absent
- **+** - indicates diminished, but some production of globin chain still happens: e.g. β+
- **0** - indicates complete absence of production production of globin chain by gene: e.g. β^0

Thalassemia

- Found most frequently in the Mediterranean, Africa, Western and Southeast Asia, India and Burma

- Distribution parallels that of *Plasmodium falciparum*

Distinguishing features between IDA and thalassemia

- Mentzer index: MCV/RBC <13 favors thalassemia
- England and Fraser Index: MCV – (5 × Hemoglobin)
- The RBC count in thalassemia is more than $5.0x10^6/\mu L$ ($5.0x10^{12}/L$) and in IDA is less than $5.0x10^6/\mu L$ ($5.0x10^{12}/L$)
- MCV usually less than 70 in thalassemia trait, more than 70 in IDA
- The red cell distribution width (RDW) in IDA is more than 17% and in thalassemia trait is less than 17%

Results of Iron Studies in Hypochromic Anemias

Cause of Anemia	Serum Iron Level	Total Iron-Binding Capacity	Percent Saturation	Soluble Serum Transferrin Receptor Level	Bone Marrow Storage Iron
Iron deficiency	Decreased	Increased	Decreased	High	Decreased
Thalassemias	Increased or normal	Decreased or normal	Increased or normal	Variable, may be high	Increased or normal
Sideroblastic anemias	Increased	Decreased or normal	Increased	Variable, may be high	Increased
Chronic disease	Decreased	Decreased	Decreased	Normal	Increased

Differential diagnosis for **MACROCYTIC** anaemia includes:

- Non-megaloblastic
  - Liver disease
  - Myelodysplastic syndrome
  - Increased reticulocyte count
    - ✓ Hemorrhage

- Megaloblastic
  - Vitamin B12 deficiency
  - Folic acid deficiency

Substrate deficiency anemias

Iron deficiency anemia

- Common nutritional deficiency

- Iron facts
  - Body iron:
    - 80% functional (Hgb, myoglobin, cytochromes, etc.)
    - 20% storage
  - Absorption: primarily in the duodenum
  - Transferrin: transports iron in blood
  - Ferritin: storage form of iron
  - Hemosiderin: derived from ferritin, long-term storage of iron

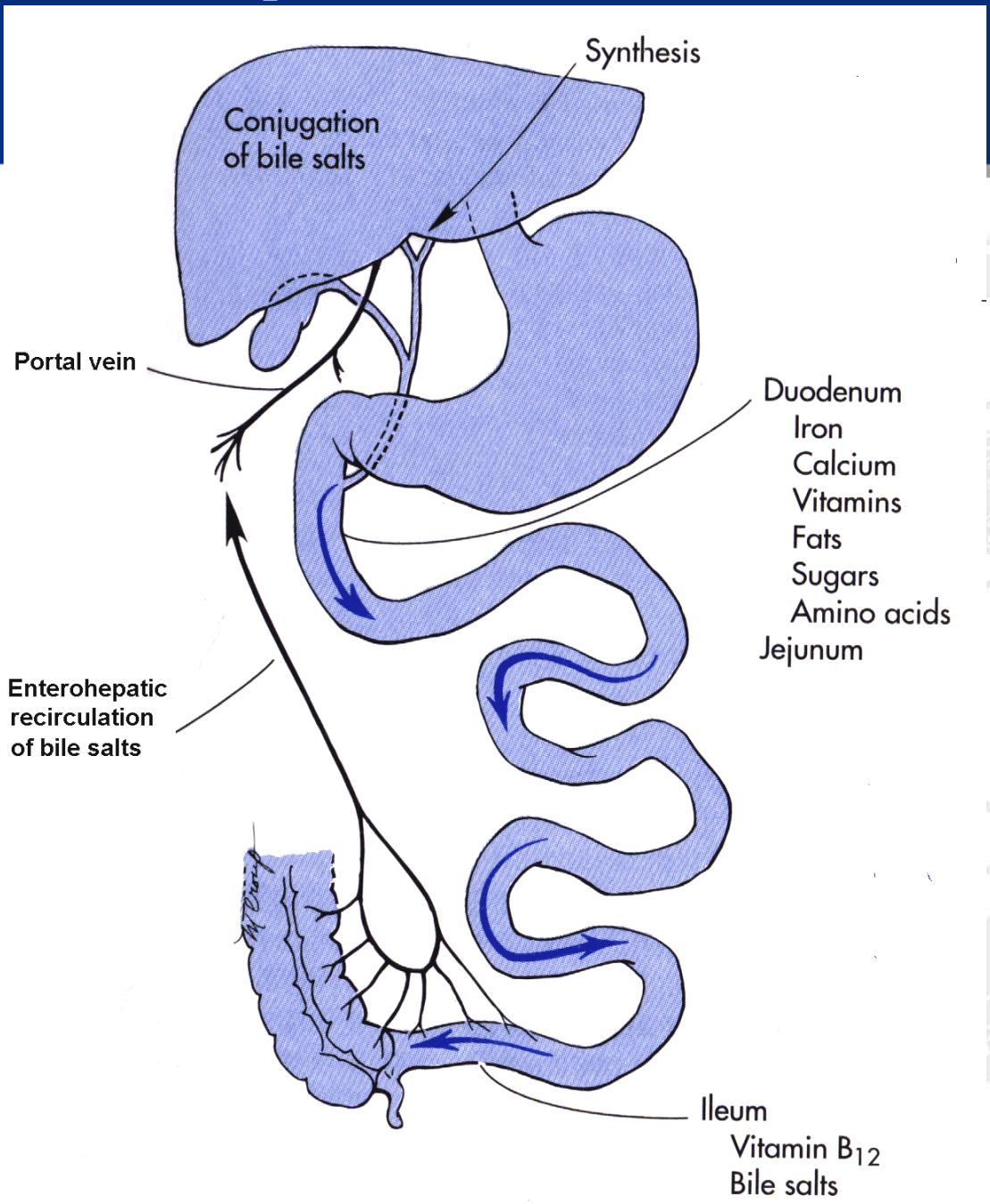
Dietary Iron

- There are 2 types of iron in the diet: haem iron and non-haem iron
- Haem iron is present in Hb containing animal food like meat, liver & spleen
- Non-haem iron is obtained from cereals, vegetables & beans
- Milk is a poor source of iron

Iron absorption

- **Haem iron** is not affected by ingestion of other food items
- It has constant absorption rate of 20-30% which is little affected by the iron balance of the subject
- The haem molecule is absorbed intact and the iron is released in the mucosal cells
- The absorption of **non-haem iron** varies greatly from 2% to 100% because it is strongly influenced by:
  - ✓ the iron status of the body
  - ✓ the solubility of iron salts
  - ✓ integrity of gut mucosa
  - ✓ presence of absorption inhibitors or facilitators

Sites of absorption of iron and vitamin B12



Inhibitors of iron absorption

- Food with polyphenol compounds
  - Cereals like sorghum & oats
  - Vegetables such as spinach and spices
  - Beverages like tea, coffee, cocoa
  - A single cup of tea taken with meal reduces iron absorption by up to 11%
- Some fruits inhibit the absorption of iron although they are rich in ascorbic acid because of their high phenol content e.g strawberry, banana and melon
- Food fermentation aids iron absorption by reducing the phytate content of diet

Inhibitors of iron absorption

- Food containing phytic acid (i.e. bran; cereals like wheat, rice, maize & barely; legumes like soya beans, black beans & peas)
- Cow's milk due to its high calcium & casein contents
- The dietary phenols & phytic acids compounds bind with iron decreasing free iron in the gut & forming complexes that are not absorbed
- Cereal milling to remove bran reduces its phytic acid content by 50%

Promoters of Iron Absorption

- Foods containing **ascorbic acid** like citrus fruits, broccoli & other dark green vegetables because ascorbic acid reduces iron from ferric to ferrous forms, which increases its absorption

- Foods containing **muscle protein** enhance iron absorption due to the effect of cysteine containing peptides released from partially digested meat, which reduces ferric to ferrous salts and form soluble iron complexes

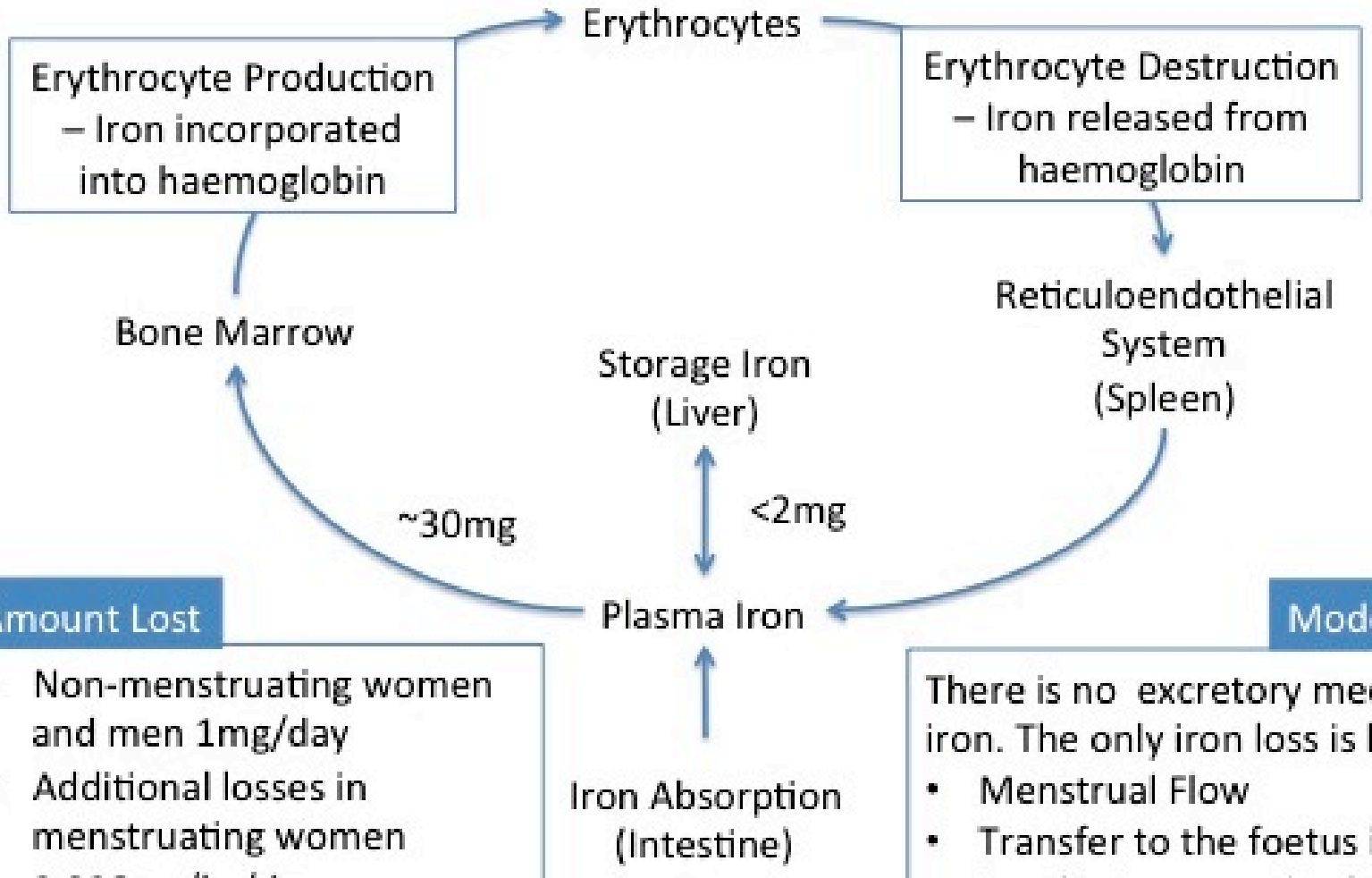
Iron transport

- **Transferrin** is the major protein responsible for transporting iron in the body
- **Transferrin receptors**, located in almost all cells of the body, can bind two molecules of transferrin
- Both transferrin concentration & transferrin receptors are important in assessing iron status

Storage of iron

- Tissues with higher requirement for iron (bone marrow, liver & placenta) contain more transferrin receptors
- Once in tissues, iron is stored as ferritin & hemosiderin compounds, which are present in the liver, reticuloendothelial cells & bone marrow
- The amount of iron in the storage compartment depends on iron balance (positive or negative)
- Ferritin level reflects amount of stored iron in the body & is important in assessing iron deficiency

The Iron Cycle



Amount Lost

- Non-menstruating women and men 1mg/day
- Additional losses in menstruating women 0.006mg/kg/day
- Pregnant Woman loose 3.5mg/day

Modes of Iron Loss

There is no excretory mechanism for iron. The only iron loss is lost as

- Menstrual Flow
- Transfer to the foetus in pregnancy
- Iron lost as a result of desquamation of epithelia
- Sweat and urine iron loss that are of

Role of iron in the body

Iron have several vital functions:

- Carrier of oxygen from lung to tissues
- Transport of electrons within cells
- Co-factor of essential enzymatic reactions:
  - neurotransmission
  - synthesis of steroid hormones
  - synthesis of bile salts
  - detoxification processes in the liver

Daily Iron Requirements for Infants and Young Children

Age	*Daily iron requirement*	*Source*
Up to 4 to 6 months (full-term infants)	0.27 mg	Breast milk or iron-fortified formula
4 to 6 months to 1 year (full-term infants)	11 mg	Breast milk or formula plus iron-rich foods*
1 month to 1 year (premature or low–birth-weight infants)	2 to 4 mg per kg	Iron-fortified preterm formula or iron supplementation (2 mg per kg per day) plus breast milk and iron-rich foods
1 to 3 years	7 mg	Iron-rich foods

**—If a full-term breastfed infant cannot consume adequate iron after 6 months of age, supplementation is necessary (1 mg per kg per day).*

Prevalence of Iron Deficiency Anaemia

Region	0-4yr		5-12yr	
	Women			
South Asia	56%	50%	58%	
Africa	56%	49%	44%	
Latin Am	26%	26%	17%	
Gulf Arabs	40%	36%	38%	
Developed	12%	7%	11%	
World	43%	37%	35%	

Etiology of Iron Deficiency Anaemia

- Iron deficiency is the most common cause of anaemia, occurring in an estimated 15% of the world's population [Means RT, 2013]

- The causes of iron deficiency:
  - ✓ **inadequate iron intake** (e.g. nutritional deficiency, iron malabsorption)
  - ✓ **excessive iron loss** (mostly due to bleeding, menstrual blood losses)
  - ✓ **increased iron demands** (in young children because of the rapid growth)

Lab studies in iron deficiency anemia

- Microcytic, hypochromic anemia
  - decreased MCV, MCH, & MCHC

- Iron studies
  - low serum iron
  - high total iron binding capacity (TIBC, transferrin concentration)
  - low % transferrin saturation
  - low ferritin
  - decreased bone marrow storage iron (hemosiderin)

Treatment of Iron Deficiency Anaemia

- Oral iron supplementation is the mainstay of therapy for IDA

- Iron should ideally be given between meals together with vitamin C to maximise absorption, but it can be administered with food or at reduced doses in patients who experience excessive abdominal side-effects (e.g. abdominal discomfort, nausea or constipation).

- Treatment of IDA aims restoring the Hb to normal, followed by a further 4 - 6 months of therapy to replenish iron stores.

Treatment of Iron Deficiency Anaemia

- Response to oral iron includes:
  - 24-48 hr – subjective improvement in CNS
  - 48-72 hr – reticulocytosis
  - 4-30 days – increase in Hb
  - 1-3 months – repletion of iron stores

- Therapeutic dose: 3-6 mg/kg/day of elemental iron
  - induces an increase in Hb of 0.25-0.4 g/dl per day or 1%/day rise in hematocrit

- Failure of response after 2 weeks of oral iron requires reevaluation for ongoing blood losses, infection, poor compliance or other causes of microcytic anaemia

Treatment of Iron Deficiency Anaemia

- Intravenous iron is necessary when more rapid correction of the Hb level is desired, if iron malabsoprtion is the main cause for the IDA, or if oral iron is not tolerated.

- Blood transfusion is indicated for severe, symptomatic anaemia, especially in patients who are bleeding.

Genetic forms of iron deficiency anaemia

DMT-1 mutations

- DMT1 is a transmembrane protein encoded by the SLC11A2 gene located on chromosome 12.
- The disease is autosomal recessive and the anaemia is present from birth.
- It is involved in iron absorption by the enterocytes in the duodenum and in iron transport from the microsomes to the cytoplasm in the erythroblasts.

Genetic forms of iron deficiency anaemia

DMT-1 mutations

- In case of a mutation affecting the function of DMT-1 iron absorption in the duodenum continues because the absorption of haem iron is not disturbed. (In fact, in meat-eating humans it is estimated that about 2/3 of absorbed iron comes from haem.)
- Thus in humans, a mutation in DMT1 protein will primarily affect iron utilisation and not absorption, leading to a severe microcytic iron deficiency anaemia with increased iron stores.
- To date mutations in the gene encoding DMT1 has been described only in several families.

Genetic forms of iron deficiency anaemia

Mutations in matriptase-2 gene

- Matriptase-2 is an essential regulator of iron homeostasis.
- In mice as well as in humans, mutations in the TMPRSS6 -/- gene lead to severe iron deficiency anaemia.
- This state is characterised by reduced ferroportin expression (shown in the mouse model) and both animals and humans have high hepcidin levels.
- Recent studies have demonstrated that TMPRSS6 (Matriptase-2) is a transmembrane protease suppressor of hepcidin gene expression. *In vitro* studies showed that it acts via hepatic haemojuvelin.

Genetic forms of iron deficiency anaemia

Mutations in matriptase-2 gene

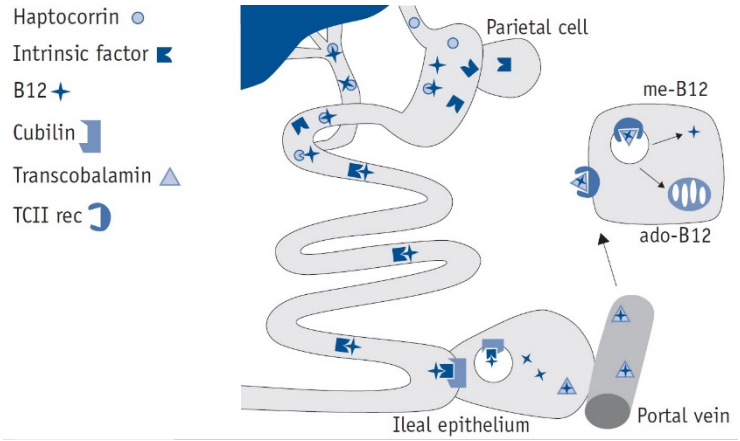
- Mutation in matriptase leads to the IRIDA disease (Iron-Refractory, Iron-Deficiency Anaemia).
- Nine patients have so far been described. All of them presented from birth with a moderate to severe anaemia with severe microcytosis (MCV from 49 to 65 fl), with typical iron deficiency state (low serum iron and serum transferrin saturation, high serum transferrin receptor).
- Oral iron administration is ineffective and response to parenteral iron administration is partial.

Anemia Due to Folate or Vitamin B12 (Cobalamin) Deficiency

- Between 1847 and 1880, Adisson, Biermer and Ehrlich described a severe form of anaemia associated with megaloblastic features and neurological disturbance. Slowly progressive and invariably causing death, it was called pernicious anaemia.
- In 1927 the first effective treatment was administered to patients thanks to the work of Whipple, Minot and Murphy consecrated by a Nobel prize in 1934. A diet containing large quantities of liver allowed correction of anaemia and of neurological signs.
- In the same period, Castle identified a factor produced in the stomach (intrinsic factor) that was found to improve Hb values of patients with pernicious anaemia.
- Intrinsic factor is a 45 kD protein produced by gastric parietal cells with a low affinity for cobalamin. The synthesis of vitamin B12 (cyanocobalamin, 1948) allowed a simple treatment of this otherwise lethal condition.

Vitamin B12 absorption process and biochemical pathways

- Vitamin B12 is released from food, by gastric acid and pepsin and initially is fixed by haptocorrin present in saliva.
- In the duodenum the alkaline environment and proteases release haptocorrin, allowing fixation to intrinsic factor.
- A specific ileal receptor, cubilin, allows specific absorption and transfer of B12 to transcobalamin in the blood circulation.
- Final utilisation by cells is driven by the transcobalamin receptor.



In order to be adequately absorbed, all the following conditions must be achieved:

- sufficient dietary intake,
- release of B12 by acid and pepsin,
- sufficient pancreatic enzyme to free B12 from haptocorrin,
- secretion of normal amounts of intrinsic factor and
- a normal ileal mucosa to bind B12-IF complex.

Once absorbed into body cells, vitamin B12 is used in two important biochemical pathways: methylcobalamin (in conjunction with folic acid) is required for the synthesis of methionine, which in turn allows methylation of DNA and proteins, while adenosylcobalamin is required for the synthesis of succinyl CoA and synthesis of fatty acids.

Anemia Due to Folate or Vitamin B12 (Cobalamin) Deficiency

- Folate and cobalamin required for DNA synthesis
- Deficiency results in megaloblastic anaemia due to impaired DNA replication

- Impaired nuclear development but abundant cytoplasm (nuclear-cytoplasmic asynchrony)
- Large marrow progenitors

- Similar clinical features* in peripheral blood and marrow morphology in folate and cobalamin deficiency

*Exception: Neurologic abnormalities in B12 deficiency

Causes of vitamin B12 and folate deficiencies

Vitamin B_{12} deficiency	Folate deficiency
Dietary deficiency	Dietary deficiency
Ovolactovegetarians	Poor diet (e.g. alcoholics, elderly)
Malabsorption	Malabsorption
Pernicious anaemia	Inflammatory bowel disease
Ileal pathology	Coeliac disease
Crohn's disease/ulcerative colitis	Short-bowel syndrome
Infection, e.g. HIV, TB	Impaired folate metabolism*
Infiltration, e.g. lymphoma surgery	Drugs (e.g. methotrexate, anticonvulsants, antimalarials)
Gastrectomy, ileal resection	Alcoholism
Diphyllobothrium latum (fish tapeworm)	Hypothyroidism
Blind loop syndrome	Excessive folate demand
Drugs (e.g. proton pump inhibitors, metformin)	Chronic haemolytic anaemia
	Pregnancy and lactation (particularly if the diet is poor)

*Serum folate levels may be normal.

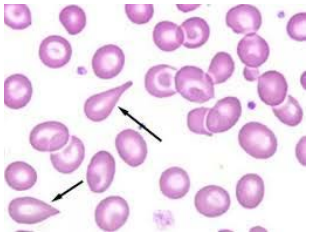
Folate and Cobalamin Deficiency

Clinical and Laboratory Findings

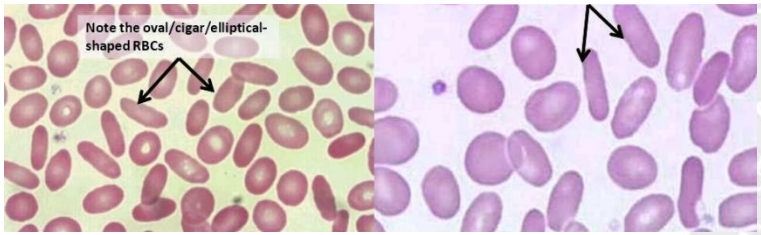
- Non-specific signs and symptoms of anemia
- Macrocytic anemia
- Relatively low reticulocyte count
- Hypersegmentation of neutrophils
- Mild thrombocytopenia and/or neutropenia
- Megaloblastic changes in marrow
- Neurological findings (B12 deficiency only): loss of position sense, ataxia, psychomotor retardation, seizures

Diagnosis of Megaloblastic Anaemia

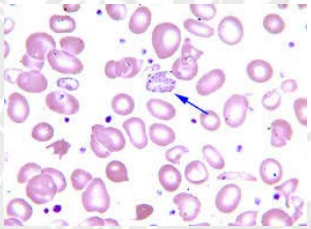
- Low serum levels of vitamin B12 or folate
- Intrinsic-factor antibodies are positive in 50 - 70% of cases
- Characteristic peripheral blood smear morphology
  - ✓ teardrops
  - ✓ macro-ovalocytosis
  - ✓ varying numbers of red cell fragments
  - ✓ basophilic stippling
  - ✓ hypersegmentation (right shift) of neutrophils



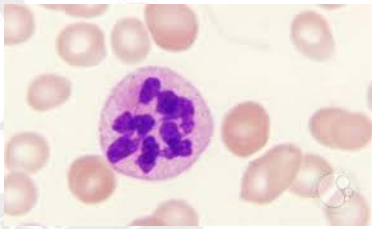
teardrops cells



macro-ovalocytosis

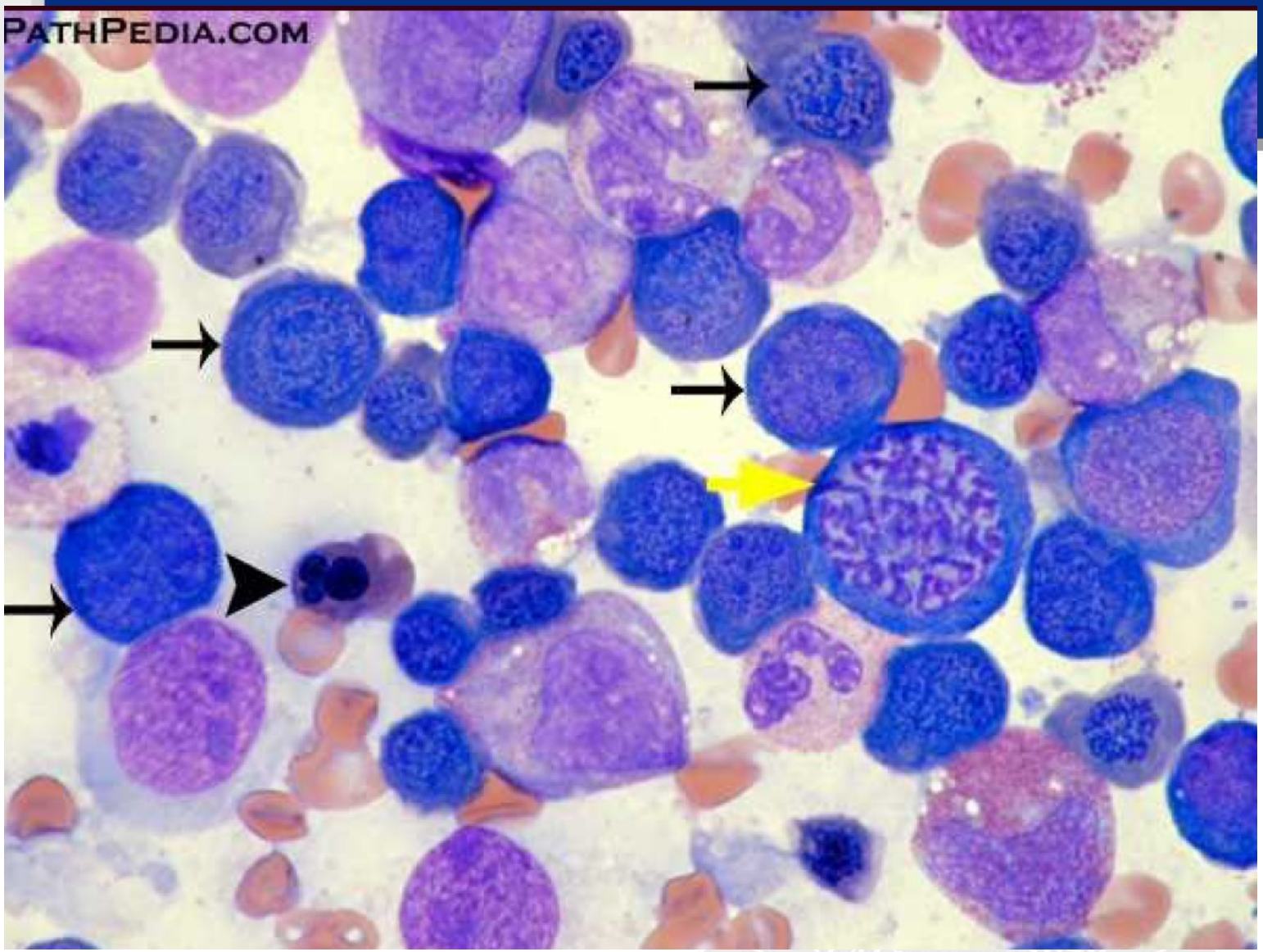


basophilic stippling



hypersegmentation of neutrophils

Bone Marrow Picture of Megaloblastic Anemia



Small black arrows (erythroid hyperplasia),
Yellow arrow (megaloblastic chage) and
Arrohead shows the dysplasia

Therapeutic protocols for nutritional anaemia

Micronutrient deficiency	Suggested therapeutic protocol
Iron	Ferrous sulphate 200 mg orally 3 times daily between meals together with vitamin C for 4 - 6 months
Folate	5 mg orally daily for 2 - 4 months (or while the underlying risk factor persists)
Vitamin B_{12}	1 000 µg daily intramuscularly for 5 - 7 days, followed by 1 000 µg intramuscularly weekly for a month, and then 1 000 µg intramuscularly every 2 months for life

Recommendations for Screening for Anaemia

Recommendations	*High-risk groups*
Screening is recommended at 9 to 12 months of age and again 6 months later for all infants in populations with high rates of iron deficiency, or (in populations with a rate of 5 percent or less) in infants with medical risks or whose diet puts them at risk of iron deficiency	Premature infants Low–birth-weight infants Infants fed low-iron formula Breastfed infants older than 6 months who are not receiving iron supplementation Infants fed non–iron-fortified formula or cow's milk before 12 months of age Breastfed infants older than 6 months without adequate iron supplementation Children who consume more than 24 oz of cow's milk per day Children with special health care needs (e.g., medications that interfere with iron absorption, chronic infection, inflammatory disorders, blood loss)
Screening is recommended for children from low-income or newly immigrated families between 9 and 12 months of age, then 6 months later, then annually from 2 to 5 years of age	Premature infants Low–birth-weight infants Recent immigrants Adolescent girls who follow fad diets or who are obese Adult females
Screening should be considered for preterm and low–birth-weight infants before 6 months of age if they are not fed iron-fortified formula	
Infants and young children with risk factors should be assessed at 9 to 12 months of age, and again 6 months later	
Beginning in adolescence, all nonpregnant women should be screened every 5 to 10 years	
No recommendation for or against screening for iron deficiency anemia in asymptomatic children 6 to 12 months of age	
Screening at 9 to 12 months of age is recommended for high-risk infants	