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Original Research Paper

Pathology

ANEMIA: CURRENT LABORATORY DIAGNOSIS APPROACHES

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KEYWORDS :

INTRODUCTION

Anemia, the meaning of which in Greek is "without blood," is a relatively common sign and symptom of various medical conditions. Anemia is not a disease, but is instead the sign of an underlying basic pathological process.

Anemia is defined as a significant decrease in the count of total erythrocyte mass. According to the World Health Organization, anemia is a condition in which the number of red blood cells (Red Blood Cells, and consequently their oxygen carrying capacity) is insufficient to meet the bodys physiologic needs.[1,2] The person's age, gender, residential elevation above sea level (altitude), and different stages of pregnancy changes the specific physiologic requirements of the body.[3] Anemia leads to diminished tissue oxygenation and can worsen the progression of many coexisting diseases.[4]

Anemia causes a decrease in the relative number of circulating red blood cells or hemoglobin, which leads to a consequent decrease in the amount of oxygen delivered to tissues.[5]

In 2010, the World Health Organization (WHO) criteria for diagnosing anemia required hemoglobin levels less than 12 grams per deciliter (g/dl) in premenopausal females and 13 g/dl in postmenopausal females and males of all ages. The journal Blood disagreed with these standards, citing the paucity of WHO data and proposed new thresholds for anemia based on race, gender and age. These proposed standards defined anemia as hemoglobin levels of less than 13.7 g/dl for white men between 20 and 60 years of age, less than 13.2 g/dl for white men older than 60, and white women of all ages were considered anemic at 12.2 g/dl. Although this journal did reference a significant difference in hemoglobin levels in black men and women, no standard levels for the diagnosis of anemia in these populations were proposed. Currently, the majority of the literature utilizes the WHO standards for consistency.

Meaning Of Anemia

Anemia is described as a reduction in the proportion of the red blood cells. Anemia is not a diagnosis, but a presentation of an underlying condition. Whether or not a patient becomes symptomatic depends on the etiology of anemia, the acuity of onset, and the presence of other comorbidities, especially the presence of cardiovascular disease. Most patients experience symptoms related to anemia when the hemoglobin drops below 7.0 g/dL.

Erythropoietin (EPO), which is made in the kidney, is the major stimulator of red blood cell (RBC) production. Tissue hypoxia is the major stimulator of EPO production, and levels of EPO are generally inversely proportional to the hemoglobin concentration. In other words, an individual who is anemic with low hemoglobin has elevated levels of EPO. However, levels of EPO are lower than expected in anemic patients with renal failure. In anemia of chronic disease (AOCD), EPO levels are generally elevated, but not as high as they should be, demonstrating a relative deficiency of EPO.

Normal Hemoglobin (Hgb)-specific laboratory cut-offs will differ slightly, but in general, the normal ranges are as follows:

- 13.5 to 18.0 g/dL in men
- 12.0 to 15.0 g/dL in women
- 11.0 to 16.0 g/dL in children

Varied in pregnancy depending on the trimester, but generally greater than 10.0 g/dL

Epidemiology Of Anemia

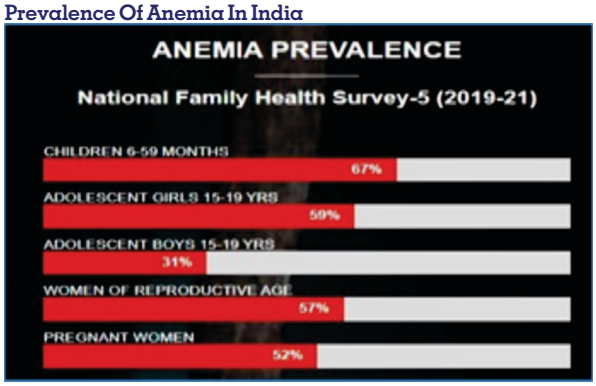
Anemia is an extremely common disease affecting up to one-third of the global population. In many cases, it is mild and asymptomatic and requires no management.

The prevalence increases with age and is more common in women of reproductive age, pregnant women, and the elderly. The prevalence is more than 20% of individuals who are older than the age of 85. The incidence of anemia is 50%-60% in the nursing home population. In the elderly, approximately one-third of patients have a nutritional deficiency as the cause of anemia, such as iron, folate, and vitamin B12 deficiency. In another one-third of patients, there is evidence of renal failure or chronic inflammation.

Classically, mild iron-deficiency anemia is seen in women of childbearing age, usually due to poor dietary intake of iron and monthly loss with the menstrual cycles. Anemia is also common in elderly patients, often due to poor nutrition, especially of iron and folic acid. Other at-risk groups include alcoholics, the homeless population, and those experiencing neglect or abuse.

New-onset anemia, especially in those over 55 years of age, needs investigating and should be considered cancer until proven otherwise. This is especially true in men of any age who present with anemia.[6]

Prevalence Of Anemia In India



Classification Of Anemia

Based on determination of the red blood cell mass, anemia can be classified as either relative or absolute.

Relative anemia is characterized by a normal total red blood cell mass in an increased plasma volume, resulting in a dilution anemia, a disturbance in plasma volume regulation. However, dilution anemia is of clinical and differential diagnostic importance for the hematologist.[7]

Classification of the absolute anemia with decreased red blood cell mass is difficult because the classification has to consider kinetic, morphologic, and pathophysiologic interacting criteria. Anemia of acute hemorrhage is not a diagnostic problem and is usually a genitourinary or gastrointestinal event, not a hematologic consideration.

Initially, anemias should be classified into two groups as diminished production and increased destruction of RBCs. The number of reticulocytes is a remarkable parameter in the materialization of this classification. Then, diagnostic analysis is able to be based upon both morphologic and pathophysiological hallmarks.

Anemias can morphologically be classified into three subgroups as macrocytic, normocytic, and microcytic hypochromic anemias. The classification is based on mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of complete blood count (CBC) and aids the physician to the diagnosis and monitoring of anemias that can be easily cured, such as deficiency of vitamin B12, folic acid, and iron. **Hematocrit :** Proportion of the volume of red cells relative to the volume of blood

Rules of Three: RBC X 3 = Hemoglobin – Hemoglobin X 3 = Hematocrit Packed cell volume (PCV) or Haematocrit (Hct). **Value of Men : 0.45 ± 0.05 l/l** (40-50%). **Value of Women : 0.41 ± 0.05** l/l (38-45 % in non- pregnant women 36-42 % in pregnant women)

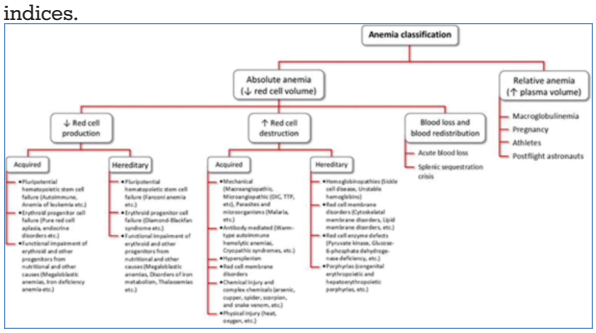
Mean Corpuscular Volume: Dividing the total volume of red cells by the number of red cells. Index for average size of red cells. **Normal range : 92 ± 9 fl 13**

Mean Corpuscular Hemoglobin: Average amount of haemoglobin in each red cell. It is expressed in picograms or pg. **Normal range : 29.5 ± 2.5 pg**

Mean Corpuscular Hemoglobin Concentration (MCHC): This represents the average concentration of haemoglobin in a given volume of packed red cells. **Normal range : 330 ± 15 g/l.** MCHC raised in hereditary spherocytosis. Decreased in hypochromic anaemia. Red cell distribution width, Variation in red cell size. Normal range : 12.8% ± 1.2% Low in B-thalassemia trait. High in iron deficiency anaemia. Normal in anaemia of chronic disease Microcytic/ hypochromic/ Macrocytic/ Normochromic/ Normocytic/ Normochromic Morphologic Categories of Anemia, the nucleus of a small lymphocyte is used as a reference to a normal red cell size.

Reticulocyte Count: Reticulocytes are non- nucleated RBCs that contain RNA. Visualized by staining with supra vital dyes, including new methylene blue or brilliant cresyl blue. Useful in determining response and potential of bone marrow. Normal range is 0.5-2.5% of all erythrocytes.

Pathophysiologic classification is best suited for relating disease processes to potential treatment. In addition, anemia resulting from vitamin- or iron-deficiency states occurs in a significant proportion of patients with normal red blood cell indices.



Morphological Classification of Anemias

Macrocytic Anemia is low/normal reticulocyte count, macrocytosis(oval and round), Elevate in MCV, MCHC, Basophilic stippling, Howell-jolly bodies, Cabot rings, Pancytopenia, Hypersegmented neutrophils, Bone marrow: megaloblastic maturation, sieve like chromatin, nuclear cytoplasmic asynchrony, maturation arrest.

Fe++ deficiency anemia characterized by low hemoglobin and low packed cell volume, low MCV,MCH and MCHC, Microcytosis and hypochromia. Serum ferritin is less than 15 micro gram/dl, Serum iron is low, TIBC is increased and transferrin saturation is less than 10 percent, Free erythrocyte protoporphyrin is increased. Increased soluble transferrin receptor in serum, Bone marrow-micro normoblastic, absence of stainable iron in bone marrow on Perls Prussian blue reaction.

Megaloblastic Anemia Mild to severe anemia characterized by increased MCV and MCH, normal MCHC, Low RBC, Hemoglobin, WBC and PLT counts (fragile cells) due to ineffective hematopoiesis. Low reticulocyte count, Macrocytic ovalocytes and teardrops, Marked anisocytosis and poikilocytosis.

Schistocytes/microcytes is due to RBC breakage upon leaving the BM Erythroid hyperplasia, low M:E ratio (1:1) and Iron stores increased.

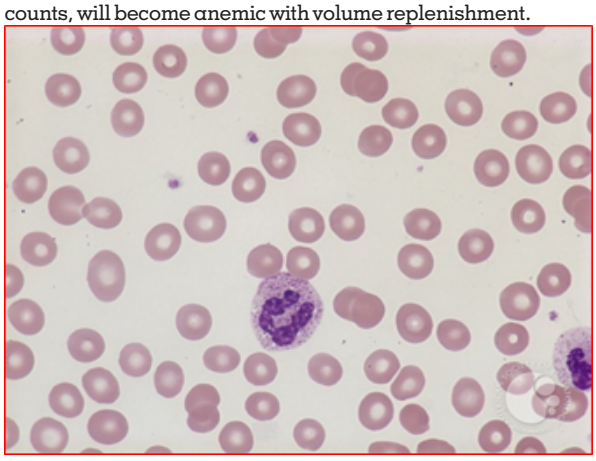
Macrocytic Ovalocytes Blood NRBC Blood Howell- Jolly body Teardrop Schistocyte Stippled RBC and Cabot Ring Giant Platelet Pap bodies Hypersegmented Neutrophil >5 lobes Megaloblastic anemia

Anemia Of Chronic Disease

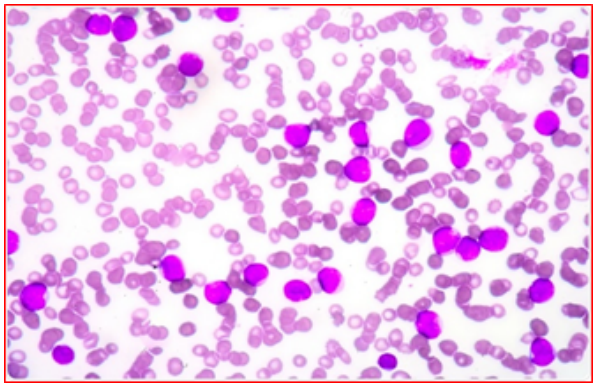
Normocytic anemia with ineffective erythropoiesis (reduced reticulocyte count) **Normochromic** results from Chronic inflammation (like rheumatologic disease) where cytokines released by inflammatory cells cause macrophages to accumulate iron and not transfer it to plasma or developing red cells (iron block anemia) leads to Inflammation to malignancy causes Bone marrow suppression which elevated EPO, decreased serum iron, decreased total iron binding capacity and normal or raised ferritin, increased marrow storage iron and ESR high

Normochromic, normocytic anemia with effective

erythropoiesis increased reticulocyte count, acute blood loss very acutely, with hypovolemia, may have normal blood counts, will become anemic with volume replenishment.



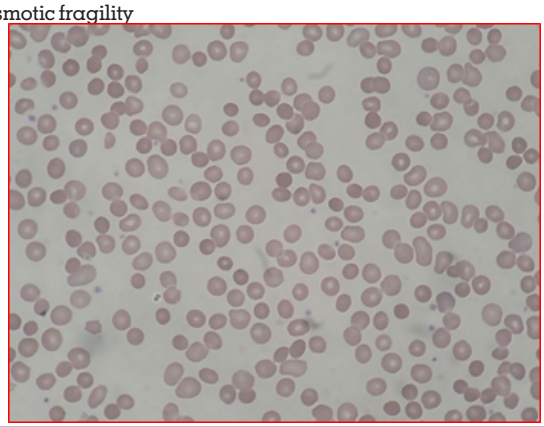
Aplastic anemia is pancytopenia caused by bone marrow failure, decreased production of all cell lines and replacement of marrow with fat.



Inherited Fanconis anaemia, Dyskeratosis congenital and Acquired anemia by Idiopathic, drugs like NSAIDs, chloramphenicol, benzene,parvo virus, hepatitis and EB virus.
Hemolytic anemia will have increased reticulocyte production cannot keep pace with loss of RBCs peripherally. Abnormality intrinsic to red cells, Managed by specific therapy in nutritional anemias.

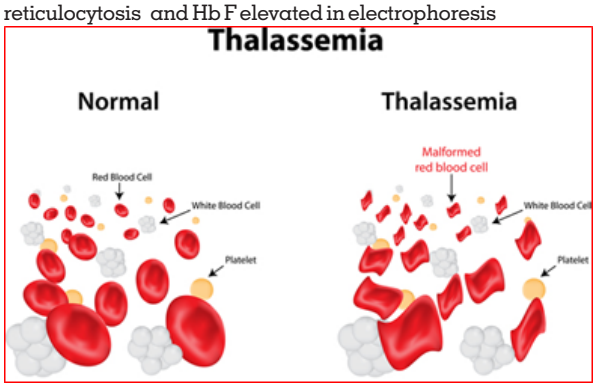
1. Hereditary spherocytosis
2. Thalassamia
3. Sickle cell anaemia
4. Glucose -6-phosphate dehydrogenase deficiency

Hereditary spherocytosis, Inherited defect in the red cell membrane cytoskeleton (spectrin, ankyrin or band 3) leading to the formation of spherocytic red cells, autosomal dominant disorder range from mild to moderate anaemia signified by intermittent jaundice, splenomegaly, pigment gall stones, peripheral smear micro spherocytes, screening test like osmotic fragility

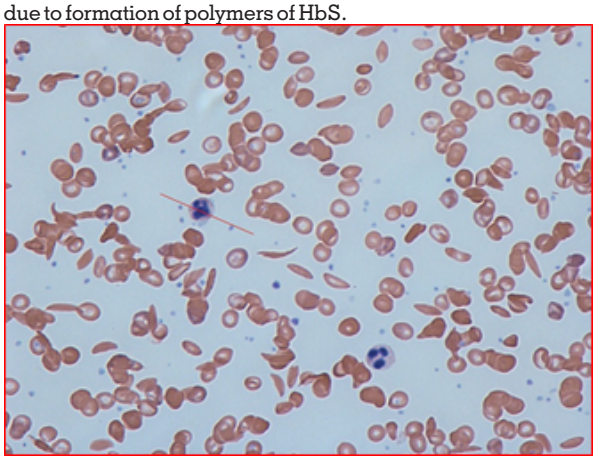


Hereditary spherocytosis

Thalassemia is condition of decreased or absent globin chains. Alpha and beta thalassemias are types with microcytic hypochromic, target cells, basophilic stippling reticulocytosis and Hb F elevated in electrophoresis



Sickle cell anaemia, Presence of Hb S with Point mutation in 6th place of beta chain substitution of valine for glutamic acid, on deoxygenation the sickle cells are formed are also caused due to chronic hemolytic anaemia, vaso- occlusive crisis, Aplastic crisis, Hemolytic crisis and infections, shows sickling test is positive. **Sickling test**: When red cells containing HbS are subjected to deoxygenation, the become sickle-shaped while cells that do not contain HbS remain normal. Certain reducing chemical agents such as 2% sodium metabisulphite or sodium dithionite can deprive red cells of oxygen. Solubility test: Small amount of blood is added to a solution that contains high-phosphate buffer, a reducing agent (sodium dithionite) and saponin. Red cells are haemolysed and HbS, if present, is reduced by dithionite. Reduced HbS forms insoluble polymers, which refract light, and solution becomes turbid. A reader scale is held at the back of the tube; in negative test lines will be clearly seen since HbA is soluble in phosphate buffer, while lines will not be seen in positive test due to formation of polymers of HbS.



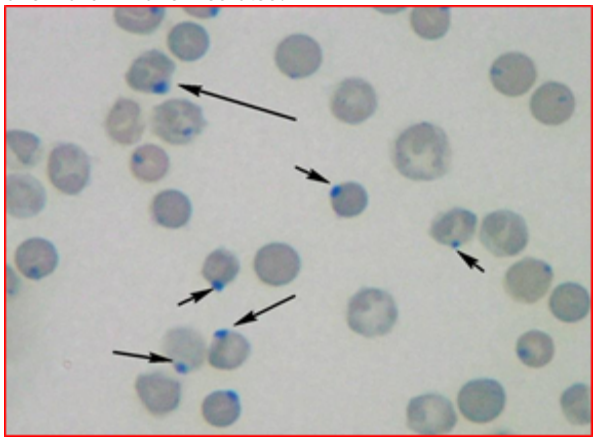
Sickle cell anemia

Glucose-6-phosphate dehydrogenase deficiency is X linked disorder with reduced activity of G6PD, inability to remove H2O2 and accumulated H2O2 leads to oxidation of hemoglobin with precipitation of globin chains, Heinz bodies are seen red cells with heinz bodies destroyed in spleen (extravascular hemolysis). G6PD is Asymptomatic, caused due to neonatal jaundice, acute hemolytic anaemia, chronic hemolytic anaemia, On peripheral smear shows polychromasia, fragmented red cells, spherocytes, bite cells, half ghost cells and Biochemical findingows s shincreased bilirubin, hemoglobinemia and hemoglobinuria. Screening tests done by fluorescent spot test, methemoglobin reduction test and dye decolorisation test.

Glucose 6 Phosphate Dehydogenase deficiency

Immune hemolytic anaemia is warm antibody-persons over 50 years with mild jaundice and splenomegaly, red cells

coated with IgG, spherocytes, autoimmune disorders, lymphoma, cold antibody, acrocyanosis, IgM, cold agglutinin disease, Paroxysmal cold hemoglobinuria (PCH), Coomb's test is detects presence of either antibody on RBC or of antibody in serum, helpful in determining if a hemolytic anemia is immune-mediated.



Other clinical classification are Microcytic hypochromic anaemia-iron deficiency, Macrocytic hyperchromic-megaloblastic anaemia, Normochromic normocytic-hemolytic anaemia, Pancytopenia-megaloblastic and aplastic anemias.

Macrocytic Anemia where in MCV > 94 MCHC > 31, caused by Megaloblastic dyspoiesis, Vitamin B12 deficiency : Pernicious anemia, Folic acid deficiency : Nutritional megaloblas- tic anemia, Sprue, Other malabsorption, Inborn errors of metabolism : Orotic aciduria. Abnormal DNA synthesis : Chemotherapy, Anticonvulsant, Oral contraceptives

Microcytic Hypochromic Anemia is when MCV < 80 MCHC < 31 caused due to Fe deficiency anemia : Chronic blood loss, Inadequate diet, Malabsorption, Increased demand, Abnormal globin synthesis : Thalassemia with or without Hemoglobinopathies, Abnormal porphyrin and heme synthesis : Pyridoxine responsive anemia and Other abnormal Fe metabolism.

Normocytic Normochromic Anemia is where MCV 82 - 92 MCHC > 30 caused due to blood loss, increased plasma volume : Pregnancy, Overhydration, Hemolytic anemia : depend on each cause and Hypoplastic marrow : Aplastic anemia, RBC aplasia, Infiltrate BM : Leukemia, Multiple myeloma, Myelofibrosis, Abnormal endocrine : Hypothyroidism, Adrenal insufficiency, and Kidney disease / Liver disease / Cirrhosis

Etiology Of Anemia

Anemia has multiple etiologies that can be attributed to one of 3 processes:

1. Decreased production of red blood cells (RBCs): As RBCs have a limited lifespan of 90 to 120 days, hematopoiesis must be an ongoing process to keep pace with this natural attrition. Any process disruptive to hematopoiesis can cause a net loss of RBC mass over time, leading to anemia.

2. Increased destruction of RBCs: Any process that either destroys RBCs or significantly shortens the lifespan of the cell in such a fashion that hematopoiesis cannot keep up with destruction will cause anemia.

3. Loss of blood: Any loss of blood, microscopic or macroscopic that exceeds hematopoiesis will result in anemia.

The above processes can be further subdivided into their specific causative etiologies.

a. Frank loss of blood via trauma, bleeding from an organ or visceral system: oto-laryngological, gastrointestinal, genitourinary, gynecological.

b. Lack of a nutritional substrate for hematopoiesis including iron, vitamin B12, or folate, or generalized malnutrition.

c. Chronic disease and/or chronic inflammation. Common culprits include chronic hepatic or renal disease, cancer, chronic infection, and collagen vascular disease.

d. Genetic illness: Common syndromes include but are not limited to the thalassemia, hemoglobinopathies, and enzyme abnormalities of the glycolytic pathways. Less common genetic syndromes include Fanconi anemia, abetalipoproteinemia, and hereditary xerocytosis.

e. Infectious etiologies include bacterial, viral and protozoan infections. Of note, malaria is a major global infectious cause of anemia.

f. Drug and chemical exposures are common etiologies for bone marrow suppression and resultant anemia.

g. Primary or idiosyncratic **bone marrow suppression**.

h. Autoimmune disease.

Further the etiology of anemia can be understood by **hypoproliferative** (corrected reticulocyte count <2%) or **hyperproliferative** (corrected reticulocyte count >2%).

Hypoproliferative anemias are further divided by the mean corpuscular volume into microcytic anemia (MCV<80 fl), normocytic anemia (MCV 80-100 fl), and macrocytic anemia (MCV>100 fl).

Hypoproliferative Microcytic Anemia (MCV<80 fl)

- Iron deficiency anemia
- Anemia of chronic disease (AOCD)
- Sideroblastic anemia (may be associated with an elevated MCV as well, resulting in a dimorphic cell population)
- Thalassemia
- Lead poisoning

Hypoproliferative Normocytic Anemia (MCV 80-100 fL)

- Anemia of chronic disease (AOCD)
- Renal failure
- Aplastic anemia
- Pure red cell aplasia
- Myelofibrosis or myelophthisic processes
- Multiple myeloma

Macrocytic anemia can be caused by either a hypoproliferative disorder, hemolysis, or both. Thus, it is important to calculate the corrected reticulocyte count when evaluating a patient with macrocytic anemia. In hypoproliferative macrocytic anemia, the corrected reticulocyte count is <2%, and the MCV is greater than 100 fl. But, if the reticulocyte count is > 2%, hemolytic anemia should be considered.[8,9]

Hypoproliferative Macrocytic Anemia (MCV>100 fL)

- Alcohol
- Liver disease
- Hypothyroidism
- Folate and Vitamin B12 deficiency
- Myelodysplastic syndrome (MDS)
- Refractory anemia (RA)
- Refractory anemia with ringed sideroblasts (RA-RS)
- Refractory anemia with excess blasts (RA-EB)
- Refractory anemia with excess blasts in transformation
- Chronic myelomonocytic leukemia (CMML)
- Drug-induced
- Diuretics
- Chemotherapeutic agents
- Hypoglycemic agents
- Antiretroviral agents
- Antimicrobials
- Anticonvulsants

Hemolytic anemia Hemolytic anemia (HA) is divided into extravascular and intravascular causes.

Extravascular hemolysis: red cells are prematurely removed from the circulation by the liver and spleen. This accounts for a majority of cases of HA

- Hemoglobinopathies (sickle cell, thalassemias)
- Enzyemopathies (G6PD deficiency, pyruvate kinase deficiency)
- Membrane defects (hereditary spherocytosis, hereditary elliptocytosis)
- Drug-induced

Intravascular hemolysis: red cells lyse within the circulation, and is less common.

- PNH
- AIHA
- Transfusion reactions
- MAHA
- DIC
- Infections
- Snake bites/venom

Pathology Of Anemia

Red Blood Cells (RBC)

RBCs are released from the bone marrow as reticulocytes. The reticulocytes have a network of ribosomal RNA (rRNA) and over a period of 24 hours mature into adult RBCs. The relative reticulocyte count can be used to gauge whether the bone marrow is responding appropriately to anemia by increasing production. The RBC contains 2 alpha and 2 beta chains and a single heme moiety that reversibly binds oxygen. Although there are multiple possible genetic variants leading to an alteration in the configuration of these chains, most do not lead to clinical consequences. However, sickle cell disease and thalassemia variants of alpha and beta chains are causes of anemia. Genetic variants in the cell membrane, cell metabolism, and cell morphology are additional causes of anemia.

Bone Marrow

The bone marrow requires approximately 21 days transitioning a pluripotent stem cell to a reticulocyte released into circulation. The initial stimulus for reticulocyte production is the renal release of erythropoietin, and continued erythropoietin is required for the transformation of a pluripotent stem cell into a proerythroblast. This initial stage takes approximately 10 to 15 days. The next step is iron-dependent and takes 3-4 days, during which iron is added to the proerythroblast, forming a heme moiety and completing the formation of the reticulocyte.

Significant bone marrow related causes of anemia include:

- Lack of substrates such as iron, vitamin B12 or folate required for the production of healthy reticulocytes.
- Direct suppression of the bone marrow's function secondary to medications, toxins, infections or radiation exposure.
- Replacement of the bone marrow by neoplasm or fibrosis.

Kidney

The kidneys have a dual role in the pathophysiology of anemia. Firstly, they are responsible for the production of 90% of the erythropoietin needed to stimulate bone marrow transformation of pluripotent stem cells to proerythroblasts. Interference with erythropoietin production and release will result in anemia. Secondly, acute anemia associated with acute blood loss results in hypotension, which causes the stimulation of stretch receptors, which in turn sends signals to parts of the brain via the glossopharyngeal and vagus nerve that lead to several downstream effects, including antidiuretic hormone (ADH), also known as arginine vasopressin (AVP) or vasopressin secretion. In response, the kidney reabsorbs water, in turn leading to decreased renal perfusion. In direct response to the decreased renal perfusion, the renin-angiotensin system becomes activated, leading to increased vascular tone and stimulation of aldosterone and resultant increased intravascular volume.

Central Nervous System (CNS)

The medulla, cerebral cortex, and pituitary gland coordinate the response to acute blood-loss anemia and the resultant volume changes by increasing sympathetic tone and secreting ADH.

The pathophysiology of anemia varies greatly depending on the primary cause. For instance, in acute hemorrhagic anemia, it is the restoration of blood volume with intracellular and extracellular fluid that dilutes the remaining red blood cells (RBCs), which results in anemia. A proportionate reduction in both plasma and red cells results in falsely normal hemoglobin and hematocrit.

RBC are produced in the bone marrow and released into circulation. Approximately 1% of RBC are removed from circulation per day. Imbalance in production to removal or destruction of RBC leads to anemia.[12,13,14]

The main mechanisms involved in anemia are:

1. Increased RBC destruction

Blood loss

- Acute- hemorrhage, surgery, trauma, menorrhagia
- Chronic- heavy menstrual bleeding, chronic gastrointestinal blood losses (in the setting of hookworm infestation, ulcers, etc.), urinary losses (BPH, renal carcinoma, schistosomiasis) [12]

Hemolytic anemia

- Acquired- immune-mediated, infection, microangiopathic, blood transfusion-related, and secondary to hypersplenism
- Hereditary- enzymopathies, disorders of hemoglobin (sickle cell), defects in red blood cell metabolism (G6PD deficiency, pyruvate kinase deficiency), defects in red blood cell membrane production (hereditary spherocytosis and elliptocytosis)

2. Deficient/defective erythropoiesis

- Microcytic/Normocytic/ normochromic/Macrocytic

Clinical picture of anemia

- History of obvious bleeding- per rectum or heavy menstrual bleeding, black tarry stools, hemorrhoids
- Thorough dietary history
- Consumption of nonfood substances
- Bulky or fatty stools with foul odor to suggest malabsorption
- Thorough surgical history, with a concentration on abdominal and gastric surgeries
- Family history of hemoglobinopathies, cancer, bleeding disorders
- Careful attention to the medications taken daily

1. Symptoms Of Anemia

Classically depends on the rate of blood loss. Symptoms usually include the following:

- Weakness
- Tiredness
- Lethargy
- Restless legs
- Shortness of breath, especially on exertion, near syncope
- Chest pain and reduced exercise tolerance- with more severe anemia
- Pica- desire to eat unusual and nondietary substances
- Mild anemia may otherwise be asymptomatic

2. Signs of anemia
Skin may be cool to touch
Tachypnea
Hypotension (orthostatic)
HEENT
- Pallor of the conjunctiva
- Boxcars' or 'sausaging' of retinal veins: suggestive of hyperviscosity which can be seen in myelofibrosis
- Jaundice- elevated bilirubin is seen in several hemoglobinopathies, liver diseases and other forms of hemolysis
- Lymphadenopathy: suggestive of lymphoma or leukemia
- Glossitis (inflammation of the tongue) and cheilitis (swollen patches on the corners of the mouth): iron/folate deficiency, alcoholism, pernicious anemia

Abdominal exam
- Splenomegaly: hemolysis, lymphoma, leukemia, myelofibrosis
- Hepatomegaly: alcohol,
- Scar from gastrectomy: decreased absorptive surface with the loss of the terminal ileum leads to vitamin B12
- Scar from cholecystectomy: Cholesterol and pigmented gallstones are commonly seen in sickle cell anemia are hereditary spherocytosis

Cardiovascular
- Tachycardia
- Systolic flow murmur
- Severe anemia may lead to high output heart failure

Neurologic exam: Decreased proprioception/vibration: vitamin B12 deficiency

Skin:
- Pallor of the mucous membranes/nail bed or palmar creases: suggests hemoglobin < 9 mg/dL
- Petechiae: thrombocytopenia, vasculitis
- Dermatitis herpetiformis (in iron deficiency due to malabsorption- Celiac disease)
- Koilonychia (spooning of the nails): iron deficienc

Rectal and pelvic exam: These examinations are usually overlooked and underperformed in the evaluation of anemia. If a patient has heavy rectal bleeding, one must evaluate for the presence of hemorrhoids or hard masses that suggest neoplasm as causes of bleeding.

Diagnosis Of Anemia

The current laboratory testing and imaging that may be pertinent in the evaluation of anemia include:

1. Complete blood count (CBC):
Includes hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

2. Reticulocyte Count:
Serves as an estimate of bone marrow red blood cell output.

3. Iron profile:
Includes serum iron, ferritin and total iron-binding content (TIBC).

4. Peripheral blood smear:
Microscopic evaluation of red blood cell morphology.

5. Serum creatinine:
Serves to assist in the evaluation of renal function.

6. Thyroid function tests:
Includes thyroxine (T4) and thyroid-stimulating hormone level (TSH).

7. Coagulation screen:
Includes activated partial thromboplastin time (APTT), prothrombin time/international normalized ratio (PT/INR), and thrombin time (TT).

8. Liver function tests (LFT):
LFT panels may vary but should include calcium, transaminases, total protein, bilirubin, albumin, and alkaline phosphatase.

Additional tests that may provide information about the liver function include lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), 5'- nucleotidase.

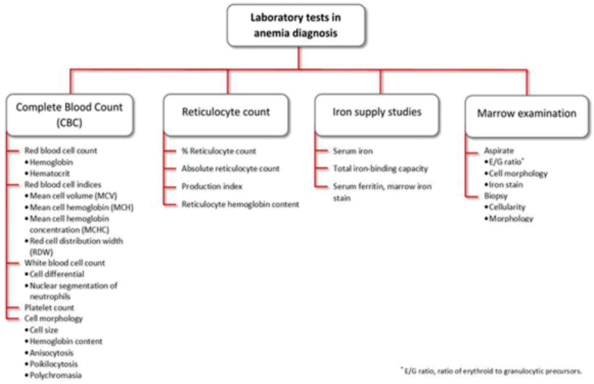
9. Hemolysis profile:
The profile contains haptoglobin, lactate dehydrogenase (LDH), and indirect bilirubin.

10. Macrocytosis profile:
The profile contains vitamin B-12, folate, methylmalonic acid, and homocysteine.

11. Hemoglobin electrophoresis:
Evaluates the hemoglobin amino acid chains.

12. Abdominal sonogram:
Evaluates the size of the spleen size

13. Bone marrow analysis:
Hematology consult is required to obtain this.



A comprehensive laboratory evaluation is required for definitive diagnosis and treatment for any anemia, the various tests for the diagnosis of anemia are done with routine hematological tests such as Complete Blood Count and reticulocyte counts as well as studies of iron status that serve as a leaping point to the diagnosis. When the diagnosis of specific anemic conditions is confirmed, a large number of other specific tests are used.

Complete Blood Count

The blood counts included hemoglobin (Hb) concentration, white blood cell (WBC) count, and manual platelet count. The mean corpuscular volume (MCV), CBC parameters such as Hb concentration, Hct, RBC count, MCV, MCHC, WBC count, platelet count, and other parameters related to formed elements of blood are measured and red blood cell distribution width (RDW) which plays an important role in the diagnosis, treatment, and monitoring of the anemic.[15]

Hemoglobin Concentration

Determination of Hb is a part of CBC. Hemoglobin is intensely colored, and this property has been used in methods for estimating its concentration in the blood. Erythrocytes contain a mixture of hemoglobin, oxyhemoglobin, carboxyhemoglobin, methemoglobin, and minor amounts of other forms of hemoglobin.[4]

Monitoring the response to treatment of anemia and to

evaluate polycythemia, Hb concentration is used to screen for diseases associated with anemia and to determine the severity of anemia[16]

Decreased Hb levels are seen in anemia. Hb must be evaluated along with the RBC and Hct. In iron deficiency, hemoglobinopathies, pernicious anemia, liver disease, hypothyroidism, hemorrhage (chronic or acute), hemolytic anemia (caused by transfusions, reactions to chemical or drugs, infectious and physical agents), and various systemic diseases (Hodgkin's disease, leukemia), decrease in Hb levels can be observed.

Variations in Hb levels occur after hemorrhages, transfusions, and burns (Hb and Hct are both high during and immediately after hemorrhage). Hb and Hct supply valuable information in an emergency situation.[17]

Excessive fluid intake, pregnancy, and drugs, which cause increase in plasma volume and decrease the Hb values, are interfering factors. Drugs such as methyldopa and extreme physical exercise can give rise to increased Hb levels. In addition, people living in high altitudes have increased Hb concentration, Hct, and RBC count[17].

Red blood cell count

The quantification of the percentage of microcytic and hypochromic RBCs has proved its clinical usefulness in the differential diagnosis of microcytic anemia.[18]

RBC count has been recognized as the most efficient single classical measurement in the differential diagnosis of microcytic anemia.[19]

Iron-deficient erythropoiesis is characterized by the production of RBC with a decrease in Hb content, so a high percentage of hypochromic cells are present.

In β-thalassemia cases, increased RBC count is a characteristic as a result of chronic increase in erythropoiesis. Therefore, MCV and MCH are lower in beta thalassemia than in iron deficiency anemia.[20]

Hematocrit

The word hematocrit, also called packed cell volume (PCV), means 'to separate blood,' which underscores the mechanism of the test, because the plasma and blood cells are separated by centrifugation.[21]

Decreased Hct values are an indicator of anemia, in which there is a reduction in the Hct. An Hct ≤30% means that the patient is severely anemic. Decreased values also occur in leukemias, lymphomas, Hodgkin's disease, adrenal insufficiency, chronic diseases, acute and chronic blood loss, and hemolytic reactions (transfusions, chemical, drug reactions)

Increased Hct values are observed in erythrocytosis, polycythemia vera, and shock (when hemoconcentration rise).[7]

Red blood cell

The size and hemoglobin content of erythrocytes (red blood cell indices) [22]

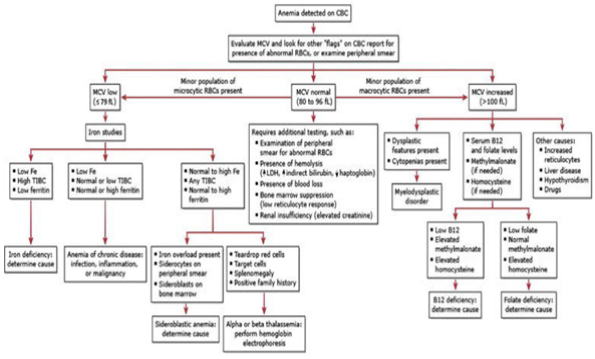
Some red blood cell parameters (for instance, RBC count, Hb concentration, MCV, RDW) are directly measured, while the others (e.g., Hct, MCV, MCHC) are derived from these primary measurements[23]

These measurements are provided by any of the common automated instruments. Automated instruments are not only fast but also extremely accurate. The coefficient of variation (measurement error) of an automated counter is usually less than 2%, and each of the major measurements, including the hemoglobin level, red blood cell count, and mean corpuscular volume, can be standardized independently with commercial red blood cell and hemoglobin standards[7,21,23]

Mean corpuscular volume (MCV)

MCV has been used to guide the diagnosis of anemia in patients, for example, testing patients with microcytic anemia for iron deficiency or thalassemia and those with macrocytic anemia for deficiency of folate or vitamin B12 [7,24]

The reference value of MCV ± 2 SD is 90 ± 9 fL and generally coincides with the peak of the Gaussian distribution of RBC size. Although MCV is both accurate and highly reproducible, errors may be introduced by RBC agglutination, distortions in cell shape, the presence of very high numbers of WBCs, and sudden osmotic swelling[17]



* CBC: complete blood count; MCV: mean corpuscular volume: RBCs: red blood cells; Fe: iron; TIBC: total iron-binding capacity (trasferrin); LDH: lactate dehydrogenase

Flowchart to follow in the diagnosis of anemia according to MCV

Mean corpuscular hemoglobin (MCH)

MCH, the amount of hemoglobin per red blood cell, increases or decreases in parallel with MCV and generally provides similar diagnostic information. Because this parameter is affected by both hypochromia and microcytosis, it is least sensitive as MCV in detecting iron deficiency states[26]

The reference value of MCH is 32 ± 2 pg. This is an excellent measure of the amount of hemoglobin in individual red blood cell. Patients with iron deficiency or thalassemia who are unable to synthesize normal amounts of hemoglobin show significant reductions in the MCH [17,27]

An increase of MCH is associated with macrocytic anemia; a decrease of MCH is associated with microcytic anemia.

Mean corpuscular hemoglobin concentration (MCHC)

MCHC is not used frequently for diagnostic purpose, but is primarily useful for quality control purposes, such as detecting sample turbidity. Because MCHCs are average quantities in the blood with mixed-cell populations, it is difficult for these red blood cell indices to detect abnormalities in the blood[17]

The reference value of MCHC is 33 ± 3 g/dL. The principal purpose of MCHC is to detect patients with hereditary spherocytosis who has very small, dense spherocytes in the circulation. These spherocytes represent cells that have lost considerable intracellular fluid because of a membrane defect.

Decreased MCHC indicates that packed RBCs (a unit volume) contain less Hb than normal. MCHC is decreased in hypochromic anemia (MCHC < 30 g/dL) observed in iron deficiency, microcytic anemias, chronic blood loss anemia, and some thalassemias.

Increased MCHC levels (RBCs cannot accommodate more than 37 g/dL Hb) occur in spherocytosis, in newborns and infants.

Red blood cell distribution width (RDW)

RDW is an estimate of the variance in the volume within the population of red blood cells[17] RDW, provided by automated counters, is an index of the distribution of RBC volumes. RDW is derived from pulse height analysis and can be expressed as an SD (fL) or as a coefficient of variation (%) of the red cell volume. Automated counters use two methods to calculate RDW[20]

The first is referred to as RDW-CV. RDW-CV is the ratio of the width of the red blood cell distribution curve at 1 SD divided by MCV (normal RDW-CV = 13 ± 1%)

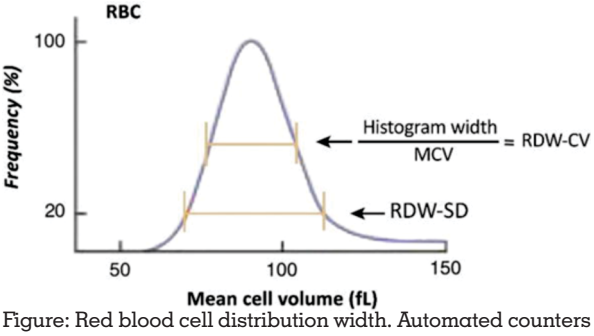


Figure: Red blood cell distribution width. Automated counters provide measurements of the width of the red blood cell distribution curve. RDW-CV is calculated from the width of the histogram at 1 SD from the mean divided by MCV.

The RDW can be used to distinguish thalassemia (normal RDW) from iron deficiency anemia (high RDW). Also, it can be used to distinguish chronic disease anemia (normal RDW) from early iron deficiency anemia (elevated RDW).

RDW increases in iron deficiency anemia, vitamin B12 or folate deficiency (pernicious anemia), abnormal Hb (S, S-C, or H), S-β thalassemia, immune hemolytic anemia, marked reticulocytosis, and posthemorrhagic anemia.

Stained Peripheral Blood Smear

Peripheral blood smears can provide important additional information about RBC morphology in anemia and are easily prepared manually using glass slides. The hematology laboratory usually examines a peripheral blood smear if the patient's indices are abnormal, Automated instruments ensure accurate RBC counts and indices and WBC counts and differentials in both healthy and diseased individuals [18,28]

The peripheral blood smear complements the automated counter measurements of MCV and MCH. Visible changes in cell diameter, shape, and hemoglobin content can be used to distinguish both microcytic and macrocytic cells from normocytic/normochromic RBCs.

The patterns of some abnormal RBCs	Comment
Macrocyte	Larger than normal (>8.5 m diameter)
Microcyte	Smaller than normal (<7 m diameter)
Hypochromic	Less hemoglobin in the cell. Enlarged area of central pallor
Spherocyte	Loss of central pallor, stains more densely, often microcytic. Hereditary spherocytosis and certain acquired hemolytic anemias
Target cell	Hypochromic with central "target" of hemoglobin. Liver disease, thalassemia, Hb D, and postsplenectomy
Leptocyte	Hypochromic cell with a normal diameter and decreased MCV. Thalassemia
Elliptocyte	Oval to cigar shaped. Hereditary elliptocytosis, certain anemias (particularly vitamin B_{12} and folate deficiency)
Stomatocyte	Slit-like area of central pallor in erythrocyte. Liver disease, acute alcoholism, malignancies, hereditary stomatocytosis, and artifact
Acanthocyte	Five to ten spicules of various lengths and at irregular intervals on surface of RBCs
Echinocyte	Evenly distributed spicules on surface of RBCs, usually 10–30. Uremia, peptic ulcer, gastric carcinoma, pyruvate kinase deficiency, and preparative artifact
Sickle cell	Elongated cell with pointed ends. Hb S and certain types of Hb C

Various forms and interpretations of RBCs observed in the peripheral blood smear examination

In clinical cases, the variation such as staining, color, shape, and inclusion bodies in the blood smear of RBCs is not only an indication of RBC abnormalities but also a diagnosis of diseases.

Reticulocyte count

Reticulocyte count is an essential component of CBC and has a substantial role in initially classifying any anemia. Reticulocytes are newly formed red blood cells with residual strands of nuclear material called "reticulin" that remain following extrusion of the nucleus from bone marrow normoblasts[29]

The reticulocyte is a young red blood cell containing residual ribosomal RNA that can be stained with a supravital dye such as acridine orange or new methylene blue [4]. The reticulocyte count can be used in differentiation of the patients with a functionally normal marrow response to anemia/hypoxia and those with a failed marrow response. Whenever the reticulocyte production index (RPI) increases to levels greater than three times normal in response to an anemia (hematocrit <30%), it can be assumed that the patient has normal renal function with an appropriate erythropoietin response and a normal erythroid marrow with an adequate supply of key nutrients (iron, folic acid, and vitamin B12) [20,24]

Reticulocytosis, increased RBC production, occurs when the bone marrow is replaced, is lost, or has prematurely destroyed cells. Identifying reticulocytosis is important for the recognition of other clinic conditions such as hidden chronic hemorrhage or unrecognized hemolysis (thalassemia, sickle cell anemia). Reticulocyte levels increase in hemolytic anemia, immune hemolytic anemia, primary RBC membrane problems, hemoglobinopathy, RBC enzyme deficits, and malaria.

Increased reticulocyte count after hemorrhage (3 to 4 days) or after treatment of anemias can be used as an index for an effective treatment. In iron deficiency anemia, reticulocytes may increase to more than 20% after sufficient doses of iron. A proportional increase in reticulocytes can also be seen when pernicious anemia is treated by transfusion or vitamin B12 therapy.

If there is not enough erythrocyte production in the bone marrow, the reticulocyte count decreases in untreated iron deficiency anemia and aplastic anemia, untreated pernicious anemia, anemia of chronic disease, radiation therapy, endocrine problems, tumor in the marrow (bone marrow failure), myelodysplastic syndromes, and alcoholism.

Some other laboratory tests are useful to define the physiologic defects responsible for anemia. Indirect serum

bilirubin and lactic dehydrogenase (LDH) levels increase in patients with increased hemolysis and in ineffective erythropoiesis. Indirect bilirubin levels correlate with RBC turnover rate. Serum LDH is exceedingly responsive to increased rates of RBC destruction (because of the excess levels of LDH 1 in RBCs)[28]

Reticulocyte hemoglobin content (CHr or Ret-He) measurement demonstrates Hb synthesis in marrow precursors. Ret-He also reflects the early stages of iron deficiency. Ret-He is defined as an auxiliary parameter in the differential diagnosis of anemias.

New red blood cell and reticulocyte indices

Current high-end automated cell counters measure unique properties of mature red blood cells and reticulocytes on a cell-by-cell basis, not just as population averages. This results a plethora of new indices that are in many cases specific to an instrument manufacturer, presenting diagnostic opportunities but also a confusing nomenclature and a potential lack of comparability. Some examples of parameters that have been studied include hypochromic erythrocytes (HypoHe%), percentage microcytic red blood cells (MicroR%), reticulocyte hemoglobin equivalent (Ret-He), reticulocyte hemoglobin content (CHr), red blood cell size factor (RSf), low hemoglobin density (LHD%), and fragmented red blood cells (FRCs)[30,31,32]

Ret-He demonstrates the real-time information on the synthesis of young RBCs in the bone marrow. Other available parameters are the percentage of RBCs with Hb content equivalent ≤17 pg (HypoHe%) and the percentage of RBCs with a volume of <60 fL (MicroR%), which reflects the subpopulation of mature RBCs exhibiting evidence of insufficient iron content[17]

The CHr may be a better predictor of depleted marrow iron stores than traditional serum iron parameters in nonmacrocytic patients and is a more sensitive predictor of iron deficiency than hemoglobin for screening infants and adolescents for iron deficiency[33,34,35,36]

Schistocytes or FRC is also used as new red blood cell indices. Nevertheless only a few studies have been published on this parameter, but concerns have been expressed for false positivity in the presence of hypochromic samples. Schistocytes are elevated in thrombotic microangiopathies[1]

Marrow examination

Bone marrow examination has a special place in the cause of anemia since it is the organ of blood production [20]. The marrow examination is of greatest value in patients who fail to show an appropriate increase in the reticulocyte production index in response to anemia. A sample of the marrow can easily be obtained by needle aspirate or biopsy to evaluate overall cellularity, the ratio of erythroid to granulocytic precursors (E/G ratio), and cellular morphology. The assessment of the bone marrow is the gold standard in iron deficiency. The presence of the mineral in reticuloendothelial cells is the key to the diagnosis[37]

Tests Of Iron

Iron supply tests (serum iron level, transferrin iron-binding capacity, and serum ferritin level) play an important role in the initial differential diagnosis of an anemia. They are essential components to the marrow iron stain whenever a marrow aspirate is performed[11,12]

- Serum iron levels. This is serum iron (SI) measurement which reflects an amount of iron bound to transferrin. The reference range of SI level is 50–150 g/dL for an individual. The proliferative capacity of the erythroid marrow and its ability to synthesize hemoglobin are assessed by serum iron level.
- Total iron-binding capacity (TIBC). The amount of iron which is bound to transferrin is called TIBC. Actually, it is equivalent to measuring the level of transferrin. The reference value of TIBC is 300–360 g/dL. TIBC increases in excess of 360 g/dL in patients with severe iron deficiency.
- Serum ferritin level. Ferritin is a spherical protein and is used clinically to evaluate total body storage iron (body iron stores). A normal adult male has a serum ferritin level of between 50 and 150 µg/L, reflecting iron stores of 600–1000 mg. Serum ferritin levels decrease when the iron stores are depleted. Levels below 10–15 µg/L indicate iron deficiency due to exhaustion of iron store[18]

Other measurements

For the diagnosis of specific hematopoietic disorders, there are some other laboratory tests, special assays are:

Hypoproliferative anemias

a. Cytometric assay of CD59/CD55 levels (paroxymal nocturnal hemoglobinuria)
b. Chromosomal analysis (leukemias)
c. Marrow aspirate/biopsy special stains
d. Trichrom stain, silver stain for reticulin (myelofibrosis)

Maturation disorders

a. Serum vitamin B_{12} level (vitamin B_{12} deficiency)
b. Serum RBC folate level (folic acid deficiency)
c. Hb electrophoresis (abnormal hemoglobins)
d. Hb A_2 level-HPLC (β-thal)
e. Hb F level-HPLC (β-thal)
f. RBC protoporphyrin level (iron deficiency)
g. Brillant aresyl blue stain

Hemolytic anemias

a. Hb electrophoresis and HPLC (hemoglobinopathies)
b. Coombs test (autoimmune hemolytic anemia)
c. Cold aglutinin titer (autoimmune hemolytic anemia)
d. Haptoglobin level (hemolysis)
e. G6PD screen (G6PD deficiency)

Approach To Anemia Includes Identification Of The Type Of Anemia

1. Complete blood count (CBC) including differential
2. Calculate the corrected reticulocyte count = percent reticulocytes x (patient's HCT/normal HCT)

For normal HCT, use 45% in men and 40% in women

If result > 2, suggests hemolysis or acute blood loss, while results < 2 suggests hypoproliferation.

3. After calculating the reticulocyte count, check the MCV.

MCV (<80 fl)

- Iron deficiency- decreased serum iron, percent saturation of iron, with increased total iron-binding capacity (TIBC), transferrin levels, and soluble transferrin receptor
- Lead poisoning- basophilic stippling on the peripheral blood smear, ringed sideroblasts in bone marrow, elevated lead levels
- AOCD- may be normocytic
- Thalassemia- RBC count may be normal/high, low MCV, target cells, and basophilic stippling are on peripheral smear. Alpha thalassemia is differentiated from beta-thalassemia by a normal Hgb electrophoresis in alpha thalassemia. Elevated Hgb A2/HgbF is seen in the beta-thalassemia trait.
- Sideroblastic anemia- elevated serum iron and transferrin with ringed sideroblasts in the bone marrow

MCV (90-100fl)

- Renal failure: BUN/Creatinine
- Aplastic anemia- ask for drug exposure, check for

infections (EBV, hepatitis, CMV, HIV), test for hematologic malignancies and paroxysmal nocturnal hemoglobinuria (PNH)
- Myelofibrosis/myelophthisis- check bone marrow biopsy
- Multiple myeloma- serum and urine electrophoresis
- Pure red cell aplasia- test for Parvovirus B19, exclude thymoma

MCV (>100 fl)
- B12/folate levels- B12 and folate deficiency can be differentiated by an elevated methylmalonic and homocysteine level in B12 deficiency and only an elevated homocysteine level in folate deficiency. Methylmalonic levels are relatively normal.
- MDS- hyposegmented PMNs on peripheral smear, bone marrow biopsy
- Hypothyroidism- TSH, free T4
- Liver disease- check liver function
- Alcohol- assess alcohol intake
- Drugs

Steps To Evaluate For Hemolytic Anemia
1. Confirm the presence of hemolysis- elevated LDH, corrected reticulocyte count >2%, elevated indirect bilirubin and decreased/low haptoglobin
2. Determine extra vs. intravascular hemolysis-

Extravascular
- Spherocytes present
- Urine hemosiderin negative
- Urine hemoglobin negative

Intravascular
- Urine hemosiderin elevated
- Urine hemoglobin elevated

3. Examine the peripheral blood smear
- Spherocytes: immune hemolytic anemia (Direct antiglobulin test DAT+) vs. hereditary spherocytosis (DAT-)
- Bite cells: G6PD deficiency
- Target cells: hemoglobinopathy or liver disease
- Schistocytes: TTP/HUS, DIC, prosthetic valve, malignant HTN
- Acanthocytes: liver disease
- Parasitic inclusions: malaria, babesiosis, bartonellosis

4. If spherocytes +, check if DAT is +
- DAT(+): Immune hemolytic anemia (AIHA)
- DAT (-): Hereditary spherocytosis

Other investigations that might be warranted include esophagogastroduodenoscopy for the determination of an upper GI bleed, colonoscopy for the determination of a lower GI bleed, and imaging studies if malignancy, or internal hemorrhage is suspected. If a menstruating woman has heavy vaginal bleeding, evaluate the presence of fibroids with a pelvic ultrasound.

Treatment Of Anemia
Management depends primarily on treating the underlying cause of anemia.

Anemia due to acute blood loss
Treat with IV fluids, crossmatched packed red blood cells, oxygen. Always remember to obtain at least two large-bore IV lines for the administration of fluid and blood products. Maintain hemoglobin of > 7 g/dL in a majority of patients. Those with cardiovascular disease require a higher hemoglobin goal of > 8 g/dL.

Anemia due to nutritional deficiencies
Oral/Intra venous iron, B12, and folate.

Oral supplementation of iron is by far the most common method of iron repletion. The dose of iron administered depends on the patient's age, calculated iron deficit, the rate of correction required, and the ability to tolerate side effects. The most common side effects include metallic taste and gastrointestinal side effects such as constipation and black tarry stools. The hemoglobin will usually normalize in 6-8 weeks, with an increase in reticulocyte count in just 7-10 days.

IV iron may be beneficial in patients requiring a rapid increase in levels. Patients with acute and ongoing blood loss or patients with intolerable side effects are candidates for IV iron.

3) Anemia due to defects in the **bone marrow and stem cells**, conditions such as aplastic anemia require bone marrow transplantation.

Anemia due to chronic disease
Anemia in the setting of renal failure, responds to erythropoietin. Autoimmune and rheumatological conditions causing anemia require treatment of the underlying disease.

Anemia due to increased red blood cell destruction
Hemolytic anemia caused by faulty mechanical valves will need replacement.
Hemolytic anemia due to medications requires the removal of the offending drug.
Persistent hemolytic anemia requires splenectomy.
Hemoglobinopathies such as sickle anemia require blood transfusions, exchange transfusions, and even hydroxyurea to decrease the incidence of sickling.
DIC, which is characterized by uncontrolled coagulation and thrombosis, requires the removal of the offending stimulus. Patients with life-threatening bleeding require the use of antifibrinolytic agents.

Complication of anemia
Anemia, if undiagnosed or left untreated for a prolonged period of time can lead to multiorgan failure and can even death.

Pregnant women with anemia can go into premature labor and give birth to babies with low birth weight.

Anemia during pregnancy also increases the risk of anemia in the baby and increased blood loss during pregnancy can lead to post partum hemorrhage.

The cardiovascular system is the most commonly affected in chronic anemia. Myocardial infarction, angina, and high output heart failure are common complications.
Other cardiac complications include the development of arrhythmias and cardiac hypertrophy.

Severe iron deficiency is associated with restless leg syndrome and esophageal webs.

Severe anemia from a young age may lead to impaired neurological development in the form of cognitive, mental, and developmental delays.[38,39]

Conclusion
There is no single optimal marker or test combination in the differential diagnosis of anemias. The use algorithms as a tool in determination of anemias in order to reduce the laboratory tests and accurately diagnose the underlying causes in patients with anemia, remarkable progress has been made in the procedures and algorithms in the differential diagnosis of anemias.

CBC is the main procedure for investigating anemia. The percentage of microcytic RBCs is considered in the first step. In

the second step, MCV, RDW, and RBC count should be examined. It is advocated that innovative algorithms, including parameters reflecting hemoglobinization of RBCs and reticulocytes, are integrated to improve the differentiation between anemias. Subsequently, new algorithms, including conventional as well as innovative hematological parameters, were assessed for subgroups with microcytic erythropoiesis.

Nowadays automated reticulocyte counts provide new parameters to evaluate marrow activity. It is therefore important to establish accurate and reliable criteria for both identifying the specific causes of anemia and evaluating the impact of intervention strategies.

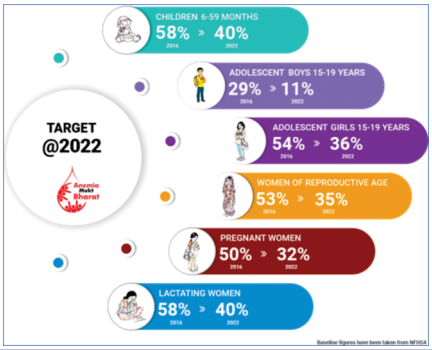
Complete Blood Count is the most sensitive measure in the routine use to obtain the information about the presence and severity of anemia. For the evaluation of anemia, there are some essential basic laboratory tests such as CBC, reticulocyte count, blood smear morphology changes, iron balance studies, and bone marrow morphology reports. Severity of the hematocrit/hemoglobin changes in MCV, RDW, and blood smear morphology are the first parameters to evaluate anemia. These help to define the anemia as normocytic, microcytic, or macrocytic. Reticulocyte index defines the adequacy of the erythropoietin and red blood cell production response. Bone marrow examination can also provide information about proliferative response and whether there is any defect in precursor maturation. Iron studies should also be included in the investigation of anemia, identification of the cause of anemia by the practitioner with the support of laboratory data is an important step to diagnose, treat, and monitor the underlying pathological process.

A brief view of Anemia mukt bharath

Anemia mukt bharath is a initiative to achieve no anemia in the country, the program aims to study the prevalence of anemia in various population, idenfication of risk individuals, and considering them as beneficiaries and to study and implement the intervention in short and long term to achieve no anemia and to develop a nation free from anemia.

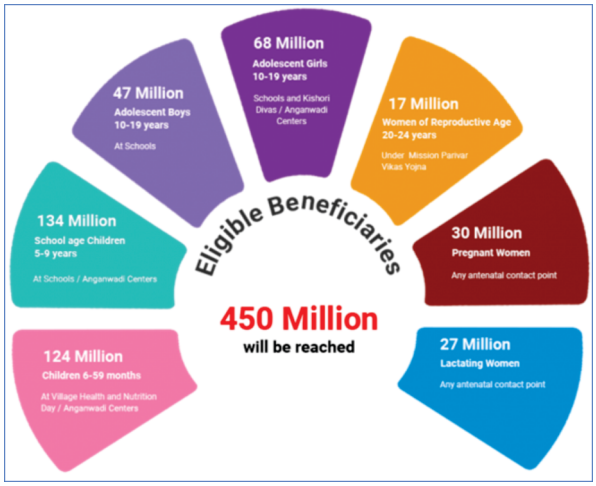
The Logo of Anemia Mukt Bharath

TARGET GROUP IN ANEMIA MUKT BHARAT



6X6X6 STRATEGY

ELIGIBLE BENEFICIARIES



SIX INTERVENTION

1. Prophylactic Iron and Folic Acid supplementation
2. Deworming
3. Intensified year-round Behaviour Change Communication Campaign (Solid Body, Smart Mind) including ensuring delayed cord clamping in newborns
4. Testing of anemia using digital methods and point of care treatment
5. Mandatory provision of Iron and Folic Acid fortified foods in government-funded health programmes
6. Addressing non-nutritional causes of anemia in endemic pockets, with special focus on malaria, haemoglobinopathies and fluorosis.

The present project is a challenge task as the project study the anemia in brief considering meaning, causes, classification, diagnosis, treatment, complication of anemia following which the national milestone in decreasing anemia by national program Anemia Mukt Bharath. As a student of laboratory

technician in the present projects aimed to understand the current approaches in diagnosis of anemia.

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