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Laboratory Screening for Anemia: Hematological Assessment and Diagnostic Evaluation-An Updated Review

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Abstract

Background: Anemia is a common hematological condition characterized by reduced hemoglobin concentration or circulating red blood cell mass. It represents an important clinical and public health concern because it can impair tissue oxygenation, worsen underlying diseases, and indicate several nutritional, genetic, infectious, and systemic disorders. Early detection and accurate diagnosis are essential for appropriate management.

Aim: This review aims to provide an overview of anemia screening, focusing on its etiology, epidemiology, pathophysiology, diagnostic investigations, interpretation of laboratory findings, and laboratory quality and safety requirements.

Methods: A narrative review was conducted covering the principal mechanisms and causes of anemia, its distribution across different populations, the physiological regulation of erythropoiesis, and the laboratory approaches used for screening and diagnosis. Relevant investigations, transfusion considerations, quality assurance procedures, and laboratory safety practices were also discussed.

Results: Anemia develops primarily through decreased red blood cell production, increased red blood cell destruction, or blood loss. Its prevalence varies according to geographical, demographic, genetic, nutritional, and socioeconomic factors. Initial screening relies on complete blood count parameters, including hemoglobin, hematocrit, and red blood cell indices. Reticulocyte count, peripheral blood smear, iron profile, vitamin B12, folate, and targeted biochemical investigations support etiological classification. Additional immunological, genetic, imaging, and bone marrow investigations may be required in selected cases. Accurate results depend on appropriate specimen handling, internal and external quality control, and adherence to laboratory safety standards.

Conclusion: Systematic laboratory screening facilitates early anemia detection, identification of underlying causes, and appropriate clinical management.

Keywords: Anemia; Laboratory screening; Complete blood count; Hemoglobin; Iron deficiency; Diagnostic tests; Quality control.

Introduction

Anemia is a common hematological condition encountered in both inpatient and outpatient healthcare settings. It is generally defined as a reduction in circulating red blood cells (RBCs) or hemoglobin concentration below the expected level for an individual's age and sex [1]. Despite its frequent occurrence, anemia remains inadequately investigated and managed in many clinical settings. Although it is often recorded as an independent diagnosis, anemia should be considered a clinical sign rather than a disease in itself. Its presence may indicate an underlying pathological process that requires further investigation to establish the cause and guide appropriate management. Therefore, identifying anemia represents only the first step in clinical assessment, while determining its underlying etiology is essential for selecting suitable interventions. Anemia can also impair tissue oxygenation and contribute to the deterioration of existing medical conditions, potentially increasing the overall clinical burden in affected patients. Despite its considerable impact on health, inconsistencies persist in the formal definition of anemia and the recommendations for its routine screening [2,3]. The clinical presentation of anemia varies according to its severity, duration, underlying cause, and the patient's general health status. Common manifestations include fatigue, generalized weakness, lightheadedness, headache, and pallor. Some patients may also develop jaundice, tachycardia, palpitations, chest pain, shortness of breath, cold extremities, and intermittent claudication. These manifestations are primarily

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associated with reduced oxygen delivery to body tissues and the physiological responses that develop to compensate for decreased oxygen availability. However, symptoms are not uniform among patients. Some individuals may experience considerable functional impairment, whereas others, particularly those with mild or slowly developing anemia, may remain asymptomatic. Consequently, relying exclusively on clinical symptoms may not be sufficient to identify all affected individuals, highlighting the importance of appropriate laboratory assessment.

The reduction in RBC numbers or hemoglobin concentration associated with anemia results in decreased oxygen-carrying capacity and impaired oxygen delivery to peripheral tissues [4]. Nevertheless, the hemoglobin threshold used to establish an anemia diagnosis is influenced by several demographic factors, particularly age, sex, and ethnicity. These variations complicate the development of universally accepted diagnostic criteria and screening recommendations. Furthermore, there is no complete agreement regarding the populations that should undergo routine anemia screening. Recommendations also differ concerning the hemoglobin levels that should prompt treatment and the therapeutic targets that should be achieved. Such differences may reflect variations in medical specialties, patient characteristics, underlying diseases, and clinical objectives [5,6]. In 2010, the World Health Organization (WHO) established widely used diagnostic criteria for anemia. According to these criteria, anemia is defined as a hemoglobin concentration below 12 g/dL in premenopausal women and below 13 g/dL in postmenopausal women and men of all ages [7]. These thresholds have been adopted in many clinical studies and continue to provide a common reference for epidemiological investigations and clinical assessment. However, their applicability across different demographic groups has been questioned.

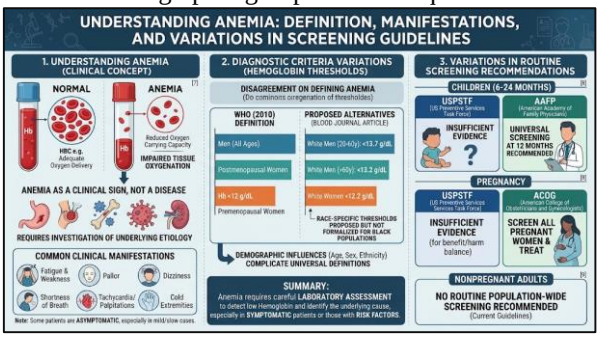


Figure-1: Anemia Overview.

An article published in the journal *Blood* challenged the WHO thresholds, arguing that the available supporting data were limited and proposing alternative hemoglobin cutoffs according to race, sex, and age. Under the proposed criteria, anemia in White men was defined as a hemoglobin concentration below 13.7 g/dL among those aged 20–60 years and below 13.2 g/dL among those older than 60 years. For White women, the proposed threshold was below 12.2 g/dL regardless of age. Although the authors acknowledged differences in hemoglobin concentrations among Black men and women, they did not establish corresponding diagnostic thresholds for these populations. Despite these concerns, most contemporary literature continues to apply WHO criteria to maintain consistency in anemia classification and reporting. Differences in screening recommendations also exist among professional organizations and healthcare authorities. These disagreements are particularly evident in pediatric populations. The United States Preventive Services Task Force (USPSTF) evaluated routine anemia screening in asymptomatic children aged 6–24 months and concluded that available evidence was insufficient to determine whether the potential benefits outweigh the possible harms. In contrast, the American Academy of Family Physicians (AAFP) recommended universal anemia screening at 12 months of age. This recommendation includes measuring hemoglobin concentration and assessing risk factors associated with iron deficiency and iron deficiency anemia (IDA) [8,9]. The difference between these recommendations demonstrates the uncertainty surrounding the optimal screening strategy, even in populations considered at increased risk of nutritional deficiencies.

A similar lack of agreement applies to anemia screening during pregnancy. The USPSTF concluded that evidence was insufficient to determine the effects of routine screening for iron deficiency anemia in asymptomatic pregnant women on maternal health and birth outcomes. It therefore considered the balance between potential benefits and harms undetermined. The AAFP adopted a similar position. Conversely, the American College of Obstetricians and Gynecologists, in Practice Bulletin No. 95, recommended screening all pregnant women for anemia and providing treatment when necessary. These contrasting recommendations reflect differences in the interpretation of available evidence and the clinical priorities of professional organizations. For nonpregnant, otherwise healthy adults, current guidelines do not recommend routine population-wide anemia screening. This absence of a universal recommendation further emphasizes the need to identify appropriate screening populations and establish evidence-based laboratory assessment strategies. Overall, anemia screening should be considered an important component of clinical evaluation, particularly in individuals with relevant symptoms or underlying risk factors. Laboratory investigations are essential not only for detecting reduced hemoglobin concentrations but also for supporting subsequent diagnostic evaluation and identification of the underlying cause.

Etiology and Epidemiology

Anemia is a multifactorial hematological condition that develops through several underlying pathological mechanisms. Its etiology can be broadly classified into three major categories: decreased red blood cell (RBC) production, increased RBC destruction, and blood loss. Understanding these mechanisms is essential for identifying the underlying cause of anemia and selecting appropriate laboratory investigations and treatment strategies. The first mechanism involves decreased RBC production. Under normal physiological conditions, RBCs have an average lifespan of approximately 90–120 days. Therefore, continuous hematopoiesis in the bone marrow is necessary to replace aging and damaged cells and maintain an adequate circulating RBC mass. Any disturbance affecting the production, maturation, or differentiation of erythroid cells may reduce the rate of RBC formation. When RBC production becomes insufficient to compensate for normal cellular turnover, the circulating RBC mass gradually decreases, resulting in anemia [10]. Several conditions can interfere with this process, including nutritional deficiencies, chronic inflammatory diseases, renal impairment, malignancies, and bone marrow disorders. Deficiencies of iron, vitamin B12, and folate are particularly relevant because these nutrients contribute to normal erythropoiesis and RBC maturation. The second mechanism is increased RBC destruction, which occurs when the rate of erythrocyte removal exceeds the bone marrow's ability to replace circulating cells. This process is commonly associated with hemolytic conditions that shorten the normal RBC lifespan. Hemolysis may result from inherited abnormalities affecting hemoglobin structure, RBC membrane integrity, or intracellular enzymes. Acquired causes may include autoimmune reactions, infections, exposure to certain medications or chemicals, and other pathological processes that damage erythrocytes. When the destruction of RBCs exceeds compensatory hematopoiesis, anemia develops. The severity depends on the rate of RBC destruction and the capacity of the bone marrow to increase erythrocyte production [11].

The third major mechanism is blood loss. Anemia may develop following acute or chronic bleeding when the amount of blood lost exceeds the body's capacity to replace circulating RBCs. Acute blood loss can occur following traumatic injuries, surgical procedures, or severe bleeding from internal organs. Chronic blood loss may be less apparent and can result from persistent bleeding within the gastrointestinal, genitourinary, gynecological, or otolaryngological systems. In some cases, blood loss remains microscopic and may not produce obvious clinical manifestations during the early stages. However, prolonged bleeding can progressively reduce RBC mass and deplete iron stores, eventually resulting in iron deficiency anemia. The clinical consequences depend on the volume and duration of blood loss and the patient's ability to compensate through increased hematopoiesis [12]. These three mechanisms encompass a wide range of specific etiological factors. Frank blood loss resulting from trauma or bleeding within different organ systems represents an important cause. Nutritional deficiencies are another major category and include insufficient iron, vitamin B12, or folate intake, as well as generalized malnutrition. Chronic diseases and inflammatory conditions can also contribute to anemia, particularly hepatic and renal disorders, malignancies, chronic infections, and collagen vascular diseases. These conditions may interfere with normal RBC production or survival.

Genetic disorders constitute another important group of causes. These include thalassemia, other hemoglobinopathies, and inherited abnormalities affecting glycolytic enzymes involved in RBC metabolism. Less common inherited conditions include Fanconi anemia, abetalipoproteinemia, and hereditary xerocytosis. Infectious diseases also contribute substantially to the global burden of anemia. Bacterial, viral, and protozoan infections may affect erythrocyte production or survival, while malaria represents a particularly important contributor in endemic regions. Furthermore, certain medications and chemical exposures can suppress bone marrow activity and interfere with RBC production. Primary or idiopathic bone marrow suppression and autoimmune diseases represent additional etiological categories that should be considered during diagnostic evaluation [13]. From an epidemiological perspective, determining the global prevalence of anemia remains challenging because studies do not consistently apply the same diagnostic criteria. The United States uses certain standards that differ from the WHO thresholds commonly applied in international research. Differences in diagnostic definitions, demographic characteristics, genetic backgrounds, and geographical distribution therefore complicate comparisons between populations. These variations should be considered when interpreting prevalence estimates and comparing epidemiological findings across countries. Available estimates suggest that anemia prevalence is relatively similar in the United States, Canada, and northern European countries. Approximately 4% of males and 8% of females in these regions meet the diagnostic criteria for anemia. However, epidemiological information from other parts of the world remains more limited. Although available estimates are not entirely precise, evidence suggests that anemia occurs approximately two to five times more frequently worldwide than in the United States, Canada, and northern Europe [14–16]. This difference reflects the influence of nutritional, infectious, genetic, and socioeconomic factors on anemia distribution.

Several geographical regions have populations with particularly high anemia prevalence. Parts of Africa, India, and the Mediterranean basin have increased rates of sickle cell disease, which contributes to the burden of inherited anemia. Thalassemia is also more common in Mediterranean populations and represents another important genetic cause. In areas where malaria and other protozoal infections are endemic, infection-related anemia and chronic inflammatory processes contribute substantially to disease prevalence. In addition, impoverished regions frequently experience higher rates of nutritional anemia because of inadequate dietary intake, limited food availability, and insufficient access to healthcare services. The distribution of anemia therefore reflects the interaction between inherited susceptibility, nutritional status, infectious diseases, chronic medical conditions, and socioeconomic circumstances. These regional differences highlight the importance of considering local epidemiological patterns when designing anemia screening programs. A laboratory screening approach should not only identify reduced hemoglobin concentrations but also support further investigation of the underlying causes, particularly nutritional deficiencies, inherited disorders, chronic diseases, and ongoing blood loss.

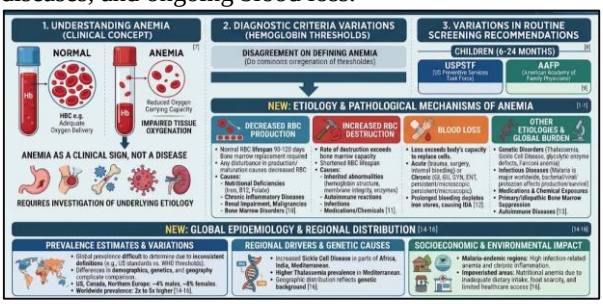


Figure-2: Etiology and epidemiology of anemia.

Pathophysiology

The pathophysiology of anemia involves complex interactions between the bone marrow, kidneys, and central nervous system (CNS), which collectively regulate red blood cell (RBC) production, oxygen transport, and cardiovascular homeostasis. Disruption of any component of this regulatory system can impair erythropoiesis, reduce circulating RBC mass, or accelerate RBC destruction. The physiological response depends on the underlying cause, severity, and rate of anemia development.

Red Blood Cells

RBCs originate in the bone marrow and enter the circulation as reticulocytes, which are immature erythrocytes containing residual ribosomal RNA. Reticulocytes undergo final maturation into adult RBCs within approximately 24 hours. Their relative count provides important information about bone marrow activity and helps determine whether erythropoiesis is responding appropriately to anemia [17]. Mature RBCs contain hemoglobin, a protein composed of two α-globin and two β-globin chains, together with heme groups that reversibly bind oxygen. Genetic variations affecting globin-chain structure or production may interfere with normal hemoglobin function. Although many variants have no significant clinical consequences, abnormalities associated with sickle cell disease and α- or β-thalassemia can cause substantial reductions in RBC survival or production. Other inherited defects affecting the RBC membrane, intracellular metabolism, and cellular morphology may also contribute to anemia [18].

Bone Marrow

The bone marrow is the primary site of erythropoiesis. The development of a pluripotent stem cell into a circulating reticulocyte takes approximately 21 days. This process depends on adequate erythropoietin stimulation and the availability of essential nutrients, particularly iron, vitamin B12, and folate. Erythropoietin stimulates the differentiation of hematopoietic stem cells toward the erythroid lineage, supporting their development into proerythroblasts. This initial developmental phase takes approximately 10–15 days. Subsequent maturation requires iron incorporation into developing erythroid cells for heme synthesis and takes approximately 3–4 days [19,20]. Several pathological conditions can interfere with bone marrow function. These include deficiencies of essential substrates required for RBC formation, direct suppression caused by medications, toxins, infections, or radiation exposure, and replacement of normal marrow tissue by malignant cells or fibrosis. Such disturbances reduce effective erythropoiesis and may result in decreased reticulocyte production.

Kidneys

The kidneys play a central role in regulating RBC production through erythropoietin secretion. They produce approximately 90% of the body's erythropoietin, which stimulates erythroid differentiation and maturation in the bone marrow. Consequently, impaired renal function can reduce erythropoietin production and contribute to decreased RBC formation and anemia [21]. The kidneys also participate in cardiovascular compensation following acute blood loss. Reduced circulating blood volume causes hypotension and activates neural responses through cardiovascular stretch receptors. Signals transmitted through the glossopharyngeal and vagus nerves stimulate central regulatory pathways, resulting in antidiuretic hormone (ADH), also known as arginine vasopressin, secretion. ADH promotes renal water reabsorption and helps preserve circulating volume. In parallel, reduced renal perfusion activates the renin–angiotensin–aldosterone system, increasing vascular tone, promoting sodium and water retention, and supporting intravascular volume restoration [21].

Central Nervous System

The CNS coordinates physiological responses to acute blood loss and reduced tissue perfusion. The medulla, cerebral cortex, and pituitary gland contribute to these responses by regulating sympathetic nervous system activity and ADH secretion. Increased sympathetic tone promotes peripheral vasoconstriction and cardiovascular compensation, while ADH supports fluid retention. These mechanisms help maintain blood pressure and perfusion of vital organs during acute reductions in circulating blood volume [22].

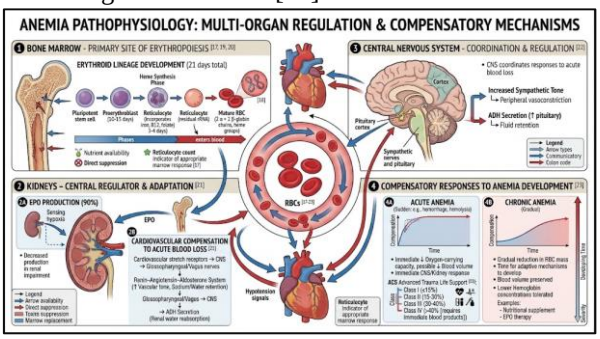


Figure-3: Pathophysiology of Anemia.

Physiological Responses to Acute and Chronic Anemia

The physiological response to anemia depends considerably on its onset and progression. Acute anemia resulting from substantial blood loss or rapid hemolysis produces an immediate reduction in oxygen-carrying capacity and, in cases of hemorrhage, circulating blood volume. The CNS and kidneys initiate compensatory mechanisms to maintain perfusion and intravascular volume. However, these mechanisms have physiological limits [23]. According to the American College of Surgeons' Advanced Trauma Life Support classification, hemorrhage is divided into four classes:

- **Class I** involves blood loss of up to 15% of circulating volume. Vital signs generally remain stable, and specific intervention is usually unnecessary.
- **Class II** involves blood loss of 15–30%. Patients may develop tachycardia, reduced pulse pressure, and peripheral vasoconstriction. Crystalloid administration is generally sufficient, and blood transfusion is usually unnecessary.
- **Class III** involves blood loss of 30–40%, commonly producing hypotension, tachycardia, and signs of shock. Management requires fluid resuscitation and blood transfusion.
- **Class IV** involves blood loss exceeding 40% of circulating volume. This degree of hemorrhage exceeds normal compensatory capacity and requires immediate resuscitation with blood products, crystalloids, and vasopressors when indicated.

In contrast, chronic anemia develops gradually and allows physiological adaptation to reduced RBC mass. Patients may tolerate substantially lower hemoglobin concentrations than those with acute blood loss because circulating blood volume is generally preserved and compensatory mechanisms have time to develop. Management therefore depends on the severity and cause of anemia, the patient's clinical condition, and the need for interventions such as RBC transfusion, nutritional supplementation, or erythropoietin therapy.

Diagnostic Tests

The diagnosis of anemia requires a systematic laboratory and clinical evaluation to establish its presence, identify the underlying cause, and determine the appropriate treatment. The selection of diagnostic investigations depends on the patient's clinical presentation, medical history, physical examination, and initial laboratory findings. Although a complete blood count (CBC) represents the primary investigation for detecting anemia, additional tests are often necessary to differentiate between nutritional deficiencies, impaired erythropoiesis, increased red blood cell (RBC) destruction, chronic diseases, and inherited hematological disorders. The complete blood count is the initial laboratory investigation and provides essential information about hemoglobin concentration, hematocrit, and RBC indices. These indices include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). MCV helps classify anemia according to RBC size as microcytic, normocytic, or macrocytic. MCH and MCHC provide information about the hemoglobin content and concentration within RBCs. These measurements help guide subsequent investigations and narrow the differential diagnosis [22][23][24].

The reticulocyte count provides an estimate of bone marrow activity and its response to reduced circulating RBC mass. An increased reticulocyte count generally indicates an appropriate marrow response, as may occur with blood loss or hemolysis. Conversely, a low or inappropriately normal reticulocyte count suggests inadequate RBC production, which may result from nutritional deficiencies, chronic disease, renal impairment, or bone marrow dysfunction. The relative reticulocyte count can therefore help distinguish between anemia caused by reduced production and anemia associated with increased RBC destruction or blood loss. Assessment of iron status is another important component of anemia screening. The iron profile, which includes serum iron, ferritin, and total iron-binding capacity (TIBC), helps evaluate iron availability and body iron stores. Ferritin reflects stored iron, while serum iron measures circulating

iron. TIBC provides information about the blood's capacity to bind and transport iron. These parameters are particularly useful in identifying iron deficiency anemia (IDA) and evaluating disturbances in iron metabolism. Soluble transferrin receptor measurement may provide additional information when differentiating IDA from anemia of chronic disease, particularly when conventional iron parameters are difficult to interpret [22][23][24].

Peripheral blood smear examination provides a microscopic assessment of RBC morphology and may reveal abnormalities in cell size, shape, color, and distribution. These findings can support the diagnosis of different types of anemia and identify structural abnormalities associated with inherited or acquired hematological disorders. When hemolysis is suspected, a hemolysis profile comprising haptoglobin, lactate dehydrogenase (LDH), and indirect bilirubin helps assess increased RBC destruction. The direct antiglobulin test, also known as the direct Coombs test, detects antibodies or complement components attached to RBCs and assists in identifying immune-mediated hemolytic anemia. Urinalysis may also be useful because the presence of hemoglobinuria or hemosiderinuria can indicate intravascular hemolysis. For patients with macrocytic anemia, additional investigations are required to identify nutritional and metabolic abnormalities. The macrocytosis profile includes vitamin B12, folate, methylmalonic acid, and homocysteine measurements. These tests help identify deficiencies that interfere with normal DNA synthesis and RBC maturation. Vitamin B6 and copper levels may also be considered in patients with atypical presentations or anemia that does not respond to conventional treatment [22][23][24].

Evaluation of renal and endocrine function is important because systemic disorders can interfere with erythropoiesis. Serum creatinine helps assess renal function, particularly in patients with suspected chronic kidney disease. Measurement of erythropoietin levels may provide additional information when investigating anemia associated with renal impairment or suspected bone marrow failure. Thyroid function tests, including thyroid-stimulating hormone (TSH) and thyroxine (T4), help identify thyroid dysfunction that may contribute to anemia. Additional investigations may be selected according to suspected systemic disease. Liver function assessment includes transaminases, bilirubin, albumin, total protein, and alkaline phosphatase. Other markers, such as LDH, gamma-glutamyl transferase, and 5'-nucleotidase, may provide further information about hepatic or hepatobiliary abnormalities. Inflammatory markers, particularly C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), help identify systemic inflammation and support the evaluation of anemia associated with chronic disease. Coagulation investigations, including activated partial thromboplastin time (aPTT), prothrombin time (PT), international normalized ratio (INR), and thrombin time, may be indicated when an underlying bleeding tendency is suspected [22][23][24].

Hemoglobin electrophoresis is useful for investigating inherited hemoglobin disorders, including sickle cell disease and thalassemia. It identifies abnormal hemoglobin fractions and supports the characterization of different hemoglobinopathies. Genetic testing may be required to confirm hereditary conditions such as hereditary spherocytosis and pyruvate kinase deficiency. The glucose-6-phosphate dehydrogenase (G6PD) activity test is another important investigation for detecting an enzymatic deficiency associated with hemolytic anemia. An osmotic fragility test may help evaluate increased RBC fragility in suspected hereditary spherocytosis. Parvovirus B19 serology or DNA testing may be appropriate when transient red cell aplasia is suspected, particularly in immunocompromised patients. Blood lead concentration should also be assessed when lead exposure is suspected, especially in children, because lead toxicity interferes with normal hemoglobin synthesis. Imaging and invasive investigations are reserved for selected clinical situations. Abdominal ultrasonography can assess spleen size and identify splenomegaly associated with hemolytic anemia or hypersplenism. Computed tomography of the abdomen and pelvis may help locate potential sources of chronic or unexplained gastrointestinal bleeding. Capsule endoscopy provides visualization of the small intestine and can identify occult bleeding sources that may not be detected through conventional gastrointestinal investigations. Bone marrow aspiration and biopsy may be required when laboratory findings suggest impaired marrow function, marrow infiltration, fibrosis, or unexplained abnormalities in blood cell production. These procedures should be performed following appropriate hematological consultation. Overall, anemia evaluation should follow a stepwise approach rather than relying on a single laboratory measurement. CBC and RBC indices provide the initial classification, while reticulocyte count, iron studies, peripheral smear, and targeted biochemical investigations help establish the underlying mechanism. Additional immunological, genetic, imaging, and bone marrow investigations should be selected according to the clinical findings. This systematic approach supports accurate diagnosis, appropriate treatment selection, and identification of potentially serious underlying disorders [22][23][24].

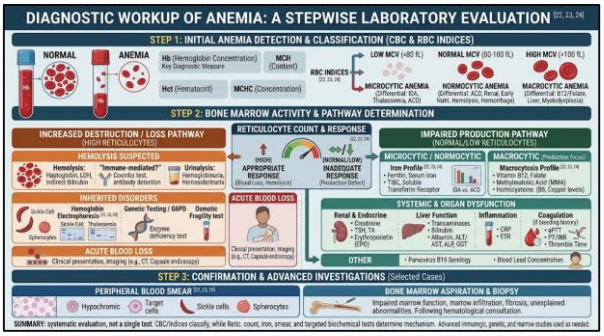


Figure-4: Diagnostic Tests for anemia screening.

Results, Reporting, and Critical Findings

The interpretation and reporting of laboratory findings in patients with anemia require integration of clinical history, physical examination, and hematological investigations. A detailed patient assessment helps identify potential causes and determine the most appropriate diagnostic pathway. Information regarding dietary habits, medication use, previous medical conditions, family history, recent infections, and possible bleeding episodes can provide important clues. Physical examination may reveal pallor, jaundice, tachycardia, splenomegaly, or other findings that support specific etiological diagnoses. When clear clinical indicators are present, investigations can be directed toward the most likely cause, allowing earlier diagnosis and treatment. However, when the underlying etiology remains uncertain, a broader diagnostic evaluation is necessary to systematically exclude potential causes. The differential diagnosis of anemia includes several major categories. These comprise chronic diseases and malignancies, nutritional deficiencies, traumatic injuries, inherited genetic disorders, infections, autoimmune conditions, and exposure to medications or chemicals. Each category may affect RBC production, survival, or circulating volume through different mechanisms. Therefore, laboratory results should be interpreted in the context of the patient's clinical condition rather than considered independently. An important initial step is determining whether the patient has active bleeding and estimating its severity. This assessment is essential because acute blood loss may require immediate intervention before completing the full diagnostic evaluation. The clinician should assess vital signs, hemodynamic stability, evidence of external bleeding, and possible internal hemorrhage. In trauma patients, the Advanced Trauma Life Support (ATLS) framework provides a standardized approach to classifying hemorrhage according to the estimated percentage of circulating blood volume lost. This classification supports decisions regarding fluid resuscitation, blood transfusion, and further emergency management [24].

Outside traumatic injuries, the gastrointestinal, gynecological, and genitourinary systems represent the most common sources of clinically significant bleeding [25]. Gastrointestinal blood loss may occur through visible bleeding or occult hemorrhage that develops gradually. Gynecological bleeding, particularly heavy menstrual bleeding, can contribute to chronic iron depletion. Genitourinary bleeding may also result in progressive RBC loss. Additional sources include occult hemorrhage within the chest, abdomen, or pelvis, particularly in patients receiving anticoagulant therapy. Hematoma formation may provide further evidence of internal bleeding. Hemodynamic stability remains the primary consideration when determining the urgency of treatment. Management should prioritize restoration of circulating blood volume, control of active bleeding, and treatment of the underlying cause [26]. When acute hemorrhage is unlikely, laboratory findings should be used to classify anemia according to RBC size, hemoglobin content, and cellular morphology. Based on mean corpuscular volume (MCV), anemia is categorized as microcytic, normocytic, or macrocytic. Mean corpuscular hemoglobin concentration (MCHC) helps distinguish hypochromic from normochromic anemia. Peripheral blood smear examination provides additional morphological information and may reveal abnormalities in RBC shape, size, and distribution. These findings can suggest specific disorders, including inherited hemoglobin abnormalities and other hematological conditions. Hemoglobin electrophoresis provides further diagnostic information by identifying abnormal hemoglobin fractions and supporting the diagnosis of conditions such as sickle cell disease and thalassemia [27,28].

Two fundamental questions should guide the interpretation of the initial laboratory results. The first is whether the bone marrow is producing sufficient RBCs to compensate for anemia. The reticulocyte count and reticulocyte percentage provide an estimate of bone marrow activity. An increased reticulocyte response generally suggests that the marrow is compensating for peripheral RBC loss or destruction. Conversely, a reduced or inadequate response indicates impaired RBC production. Although these measurements are not entirely specific, they are important tools for distinguishing between production-related and regenerative forms of anemia. The second question is whether hemolysis is contributing to the condition. Laboratory assessment commonly includes serum lactate dehydrogenase (LDH), haptoglobin, and indirect bilirubin. Increased LDH and indirect bilirubin, together with reduced haptoglobin, support the presence of increased RBC destruction. However, these parameters are individually nonspecific and should be interpreted collectively alongside the clinical presentation, reticulocyte count, and peripheral blood smear. Further investigations should follow the morphological classification of anemia. In microcytic anemia, iron studies are particularly

important for identifying iron deficiency and evaluating disturbances in iron metabolism. Macrocytic anemia requires assessment of vitamin B12 and folate concentrations, with methylmalonic acid and homocysteine measurements providing additional diagnostic information when indicated. Thyroid dysfunction should also be considered in selected patients. Normocytic anemia requires evaluation for blood loss, hemolysis, chronic disease, renal impairment, and reduced bone marrow production. This stepwise approach improves diagnostic efficiency and reduces unnecessary investigations.

Red Blood Cell Transfusion Guidelines

RBC transfusion is an important therapeutic intervention in selected patients with anemia, but the decision should depend on the patient's clinical condition rather than hemoglobin concentration alone. According to the American Academy of Family Physicians (AAFP) position statement issued on March 15, 2011, transfusion indications include acute sickle cell crisis, acute blood loss exceeding 1,500 mL or approximately 30% of circulating blood volume, and symptomatic anemia that significantly impairs the patient's ability to function without correction. The AAFP statement also discussed evidence from an updated Cochrane review supporting a restrictive transfusion strategy in patients with noncardiac disorders. This approach recommended a hemoglobin transfusion threshold of approximately 7 g/dL rather than the previously used threshold of 10 g/dL. The reported analysis demonstrated a 54% relative reduction in the number of blood units transfused and an associated reduction in 30-day mortality following adoption of the restrictive approach [29,30]. Nevertheless, transfusion decisions require consideration of symptoms, hemodynamic status, ongoing blood loss, comorbidities, and the underlying cause of anemia. Patients with active hemorrhage or clinical instability may require urgent transfusion regardless of whether their measured hemoglobin concentration has reached a predefined threshold. Conversely, stable patients with chronic anemia may tolerate lower hemoglobin concentrations and benefit from treatment directed at the underlying cause. Appropriate interpretation and reporting of laboratory findings therefore remain essential for identifying critical abnormalities, guiding transfusion decisions, and ensuring timely clinical management [29,30].

Quality Control and Laboratory Safety

Quality assurance (QA) is a structured and systematic process designed to ensure that laboratory procedures consistently produce accurate, reliable, and reproducible results. It represents an essential component of laboratory practice, particularly in anemia screening, where diagnostic decisions depend on the accuracy of hematological and biochemical measurements. Quality assurance covers all stages of the laboratory testing process, including preanalytical, analytical, and postanalytical phases. Each phase can introduce errors that may affect test results and subsequent clinical decisions. Therefore, implementing comprehensive quality management procedures is necessary to maintain laboratory performance and support accurate anemia diagnosis [31]. The preanalytical phase includes activities performed before sample analysis, such as patient identification, specimen collection, labeling, transportation, and storage. Errors during this stage may result from incorrect patient identification, inappropriate blood collection techniques, insufficient sample volume, contamination, hemolysis, or unsuitable storage conditions. These problems can affect the reliability of tests such as the complete blood count (CBC), serum iron, and ferritin measurements. Standardized specimen collection procedures, appropriate anticoagulant selection, accurate labeling, and proper sample handling are therefore essential. Laboratory personnel should also verify specimen suitability before accepting samples for analysis.

The analytical phase involves performing laboratory tests using validated methods, calibrated instruments, and appropriate reagents. Internal quality control (IQC) is a major component of this phase and is used to monitor analytical performance before patient results are released. In anemia screening, IQC is particularly important for tests including CBC, serum ferritin, serum iron, total iron-binding capacity (TIBC), transferrin saturation, and vitamin B12 and folate measurements [32,33]. Regular quality control procedures help identify analytical errors, instrument malfunctions, reagent deterioration, and variations that could compromise diagnostic accuracy. IQC generally involves daily testing of multilevel control materials representing different concentrations or ranges of analytes. These controls allow laboratory personnel to assess whether instruments and analytical methods are performing within predefined acceptable limits. Instrument calibration, reagent stability verification, maintenance schedules, and systematic documentation are also essential components of this process. Statistical methods are used to monitor control results and identify analytical variations over time. Levey–Jennings charts provide a graphical representation of quality control measurements in relation to established mean values and standard deviations. They help identify random and systematic errors and indicate when corrective action may be necessary. Results outside acceptable control limits should be investigated before patient results are reported. Appropriate corrective measures may include instrument recalibration, reagent replacement, equipment maintenance, and repetition of control measurements [34].

External quality assurance (EQA) complements IQC by providing an independent assessment of laboratory performance. EQA

programs distribute standardized and blinded specimens to participating laboratories. Each laboratory analyzes these specimens using its routine procedures and submits the results for comparison with assigned target values or the performance of other participating laboratories. This process helps identify analytical bias, methodological differences, and systematic discrepancies that may not be detected through routine internal monitoring. When significant deviations occur, laboratories should investigate their causes and implement corrective measures to improve analytical performance [35,36]. Participation in both IQC and EQA programs is essential for maintaining consistent laboratory standards. While IQC provides continuous monitoring of routine analytical performance, EQA offers an opportunity to assess the accuracy and comparability of results across different laboratories. Together, these procedures strengthen confidence in anemia-related investigations and support the reliability of diagnostic decisions. Maintaining accurate laboratory records, reviewing quality indicators, documenting corrective actions, and providing continuous staff training further contribute to effective quality management. These activities also help laboratories maintain compliance with recognized quality standards and improve patient care through reliable diagnostic reporting [35,36].

Laboratory Safety

Maintaining a safe laboratory environment is equally important, particularly in facilities performing hematological investigations and anemia screening. Laboratory safety procedures aim to protect healthcare personnel, patients, visitors, and the surrounding environment from biological, chemical, and physical hazards. Blood specimens may contain infectious agents, including blood-borne pathogens. Therefore, all human blood samples should be handled according to established biosafety precautions, regardless of the patient's known infection status [37]. The use of appropriate personal protective equipment (PPE) is a fundamental safety requirement. Laboratory personnel should wear suitable laboratory coats, gloves, and eye or face protection when procedures may generate splashes or aerosols. Hand hygiene should be performed before and after specimen handling and after glove removal. Eating, drinking, and storing personal food items in laboratory work areas should be prohibited. Work surfaces and equipment should also undergo regular cleaning and disinfection using appropriate procedures. Safe specimen collection is particularly important during anemia screening. Sterile, age-appropriate lancets should be used for capillary blood sampling, and single-use collection devices should never be reused. Sharps must be discarded immediately into designated puncture-resistant containers. Blood-contaminated materials, including collection tubes, gloves, and disposable laboratory supplies, should be segregated and discarded according to institutional biomedical waste procedures. Personnel should receive appropriate training in specimen handling, spill management, exposure prevention, and emergency response.

Chemical safety must also be considered when handling laboratory reagents, stains, and cleaning agents. Reagents should be stored and used according to their safety data sheets, which provide information about potential hazards, required protective measures, and appropriate disposal procedures. Laboratories should maintain adequate ventilation and ensure that emergency equipment, including eyewash stations and safety showers where required, remains accessible and functional. Regular inspection of laboratory equipment and safety systems helps identify potential hazards before they result in accidents. Laboratories should establish written safety policies and provide continuous training to ensure that personnel understand their responsibilities. Compliance with recognized standards, including International Organization for Standardization (ISO) 15190 and applicable Occupational Safety and Health Administration (OSHA) requirements, supports the implementation of appropriate laboratory safety and risk-management practices [38].

Conclusion:

Anemia is a multifactorial hematological condition that requires systematic screening and diagnostic evaluation. Its development involves impaired red blood cell production, increased cellular destruction, or blood loss, with nutritional deficiencies, chronic diseases, infections, and inherited disorders representing important causes. Complete blood count remains the primary screening investigation, while reticulocyte count, peripheral blood smear, iron studies, and targeted biochemical tests help establish the underlying etiology. Additional investigations should be selected according to clinical findings and initial laboratory results. Accurate diagnosis also depends on appropriate interpretation of critical findings, assessment of transfusion requirements, and recognition of potentially serious underlying conditions. Furthermore, internal and external quality control programs, standardized laboratory procedures, and adherence to safety requirements are essential for maintaining reliable results. Integrating these elements into routine laboratory practice supports early diagnosis, guides treatment decisions, and improves patient care.

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