IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Advances In Laboratory Diagnosis Of Anemia: Current Approaches And Future Perspectives

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Abstract

Anemia is a common blood condition found all over the world and is a significant public health problem, affecting people of all ages. Correct diagnosis in the laboratory is crucial to understand the root cause, the level of the disease, the right treatment and surveillance of the treatment response. The traditional tests used for the evaluation of anemia remain the same: complete blood count (CBC), peripheral blood smear examination, reticulocyte count, iron studies, vitamin B12 and folate level determination, hemolysis markers and a bone marrow examination. Some of these procedures, however, may be not be useful in complex, inherited and atypical cases. The use of flow cytometry, high-performance liquid chromatography (HPLC), capillary electrophoresis, molecular diagnostic techniques, cytogenetics, mass spectrometry, and point of care testing (POCT) have improved the ability of laboratory medicine to make diagnoses.Recent developments in laboratory medicine have improved diagnostic accuracy by using flow cytometry, high performance liquid chromatography (HPLC), capillary electrophoresis, molecular diagnostic techniques, cytogenetics, mass spectrometry and point of care testing (POCT). Recent advances in the study of iron metabolism and erythropoiesis have introduced new emerging biomarkers including hepcidin, soluble transferrin receptor (sTfR), reticulocyte hemoglobin content (Ret-He), erythroferrone and growth differentiation factor-15 (GDF-15). The incorporation of AI, machine learning, digital pathology, and automated hematology platforms has further enhanced diagnostic precision, allowing for quicker data analysis and standardizing interpretation. The focus of future developments will be on multi-omics approaches, single-cell sequencing, wearable diagnostic devices, nanotechnology-based biosensors, lab-on-a-chip technologies and AI-driven precision diagnostics, all of which will help in personalized management of anemia. Combining traditional lab examinations with molecular testing and digital innovations will make a diagnostic process more efficient, better clinical decisions and help achieve better patient outcomes.

Keywords: Anemia; Laboratory diagnosis; Hematology; Biomarkers; Flow cytometry; Molecular diagnostics; Artificial intelligence.

1. Introduction

Anemia is a common hematological disease in all age groups which is one of the most common diseases around the world. World Health Organization (WHO) defines anaemia as a reduction in the oxygen-carrying capacity of the red blood cells (RBC) due to a below normal concentration of haemoglobin (Hb) in the blood, which depends on age, sex, physiological status and altitude. An estimated 25% of the world's population suffers from anaemia, and preschool children, pregnant women, women of reproductive age and older adults have the highest prevalence [1]. Although iron deficiency is the most common cause of anemia, other nutritional deficiencies, chronic inflammatory diseases, infectious disorders, inherited hemoglobinopathies, malignancies and bone marrow disorders also play an important role in the worldwide burden of anemia. In addition to its clinical symptoms, anemia can negatively affect physical performance, cognitive growth, work productivity, pregnancy outcomes, susceptibility to infection, hospital stay, morbidity and mortality. As a result, anemia is a major socio-economic problem, especially in low and middle income countries where nutritional deficiencies and poor access to healthcare services are prevalent [2]. Anemia is a heterogeneous group of diseases with multiple causes and mechanisms. Anemia can be distinguished from a morphological point of view on the basis of the mean corpuscular volume (MCV), which classifies it as microcytic, normocytic or macrocytic anemia. Microcytic anemia is most commonly seen with iron deficiency, thalassemia, and anemia of chronic disease while normocytic anemia is often seen with acute blood loss, chronic kidney disease, hemolytic disorders, and bone marrow suppression [3]. Macrocytic anemia is typically caused by vitamin B12 or folate deficiency, liver disease, alcoholism, hypothyroidism or myelodysplastic syndrome. From a functional perspective, anemia can also be classified as hemolytic anemia (augmented erythrocyte destruction), acute or chronic blood loss, impaired erythropoiesis or mixed anemia (where multiple mechanisms of anemia occur at the same time). As several etiological factors can be present in a single patient, diagnosis may not always be possible by just relying on clinical manifestations and may require a systematic laboratory-based evaluation.

Table 1. Morphological Classification of Anemia Based on Mean Corpuscular Volume (MCV).

Type of Anemia	MCV (fL)	Common Causes	Typical Laboratory Findings	Peripheral Blood Smear
Microcytic	<80	Iron deficiency anemia, Thalassemia, Sideroblastic anemia, Anemia of chronic disease (late stage)	↓ Hb, ↓ MCV, ↓ MCH, ↓ Ferritin (IDA), ↑ TIBC	Microcytes, hypochromia, anisopoikilocytosis [4]
Normocytic	80–100	Acute blood loss, Hemolytic anemia, Chronic kidney disease, Aplastic anemia, Early anemia of chronic disease	↓ Hb, Normal MCV, Variable reticulocyte count	Normocytic RBCs, occasional polychromasia
Macrocytic	>100	Vitamin B12 deficiency, Folate deficiency, Liver disease, Alcoholism, Myelodysplastic syndrome	↑ MCV, ↓ Hb, Hypersegmented neutrophils	Macro-ovalocytes, hypersegmented neutrophils [5]
Dimorphic (Mixed)	Variable	Combined iron and vitamin B12/folate deficiency, Post-transfusion states	Increased RDW, mixed RBC indices	Mixed microcytic and macrocytic RBCs

Anemia often has a vague clinical presentation, including fatigue, weakness, dizziness, pallor, dyspnea, and decreased exercise tolerance, which are shared by many illnesses. So laboratory testing is the most fundamental step in the accurate diagnosis, classification and treatment. Standard diagnostics always start with complete blood count (CBC) which gives important hematological indices, such as hemoglobin concentration, hematocrit, red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW). Further evaluation of erythrocyte morphology is done by peripheral blood smear examination for identification of characteristic abnormalities helpful in differential diagnosis [6]. Other tests, including the reticulocyte count, serum ferritin, serum iron, total iron binding capacity, transferrin saturation, vitamin B12 and folate levels, lactate dehydrogenase, bilirubin, haptoglobin and direct antiglulin tests are helpful in evaluating erythropoietic activity, nutritional status and hemolysis. The field of laboratory medicine has experienced significant advances over the past few years with the introduction of new technologies such as flow cytometry, high performance liquid chromatography (HPLC), capillary electrophoresis, molecular genetic testing, next generation sequencing (NGS), and the Digital Hematology Platform. In addition, novel biomarkers such as hepcidin, soluble transferrin receptor (sTfR), reticulocyte hemoglobin content (Ret-He), erythroferrone and growth differentiation factor-15 (GDF-15), in addition to artificial intelligence (AI)-assisted diagnostic algorithms, are improving diagnostic accuracy and enable earlier disease diagnosis and personalized clinical decision making. In the last few years, new laboratory technologies to revolutionized the diagnosis of anemia have been developed, such as an early diagnosis, accurate etiological classification and improved monitoring of treatment. Advanced diagnostic techniques, however, are still significant challenges, particularly in resource-limited settings, due to their limited accessibility and cost, as well as lack of standardization and implementation. Thus, it is important to have a new synthesis of all the current laboratory techniques. This review should include all conventional laboratory investigations and new diagnostic

tools currently employed in the evaluation of anemia, critically discuss new biomarkers and molecular-based diagnostic strategies, and explore the future perspective of using artificial intelligence in hematological diagnostics that could improve the accuracy of diagnosis of anemia, optimize patient management, and contribute to precision medicine for anemia [7].

2. Pathophysiology of Anemia

Anemia occurs due to inadequate production of red blood cells (RBCs) to meet the body's physiological needs or to the loss or destruction of RBCs from the circulation at a rate that is greater than the rate at which the bone marrow can replace them. In a normal state, erythropoiesis is a well-regulated process which maintains sufficient hemoglobin levels and oxygen delivery to tissues. Multipotent hematopoietic stem cells develop into mature erythrocytes through a series of well-defined developmental stages, including proerythroblasts, basophilic erythroblasts, polychromatic erythroblasts, orthochromatic erythroblasts, reticulocytes and mature erythrocytes, occurring primarily in the bone marrow [8]. Erythropoietin (EPO) is a glycoprotein hormone produced by the kidneys, in response to tissue hypoxia, which primarily regulates this process. Properly functioning erythropoiesis also requires sufficient amounts of essential nutrients, including iron, vitamin B12, folate, amino acids and trace elements, and a healthy bone marrow microenvironment. Mature erythrocytes remain in the circulation for about 120 days after which they are eaten by macrophages in the spleen and liver and iron is recycled for the production of new hemoglobin molecules. Anemia may be divided into three main mechanisms: blood loss, decreased production of RBCs, and increased destruction of RBCs. Blood loss may be acute, traumatic, surgical or due to gastrointestinal bleeding, or chronic, as in peptic ulcer disease, colorectal malignancy, heavy menstruation or parasitic infections. Iron deficiency anemia is the result of chronic blood loss which slowly reduces the iron stores [9]. decreased production of RBCs is frequently due to inadequate bone marrow function, decreased production of EPO in chronic kidney disease, nutrient deficiencies, infiltration of bone marrow by malignant cells, aplastic anemia, or suppression from chemotherapy and chronic diseases. The deficiency of iron, vitamin B12 or folate has a known effect on DNA replication and hemoglobin synthesis during maturation of the erythroid cell, resulting in characteristic microcytic or macrocytic anemia. Hemolysis can increase the destruction of erythrocytes and can be caused by inherited or acquired diseases, thereby shortening the RBC life span. The hereditary diseases include sickle cell disease, thalassemia, hereditary spherocytosis, glucose-6-phosphate dehydrogenase (G6PD) deficiency, pyruvate kinase deficiency, while the acquired diseases include, autoimmune hemolytic anemia, infections, mechanical hemolysis, microangiopathic disorders and drug-induced hemolysis [10]. Any of the following lab values are commonly elevated in cases of accelerated hemolysis: lactate dehydrogenase (LDH), indirect bilirubin, reticulocytosis, and a low serum haptoglobin. Another significant mechanism associated with anemia is chronic inflammation. Hepcidin, the master regulator of systemic iron homeostasis, is synthesized in the liver by inflammatory cytokines, especially interleukin-6 (IL-6). Iron absorption in the intestine is reduced by hepcidin and the release of iron from macrophages is reduced by the degradation of the iron exporter ferroportin, leading to functional iron deficiency despite sufficient iron stores. At the same time, inflammatory mediators inhibit the production of erythropoietin, and inhibit proliferation of erythroid progenitor cells, thus helping to cause anemia of chronic disease. Anemia is caused by genetic abnormalities also by mutations in hemoglobin synthesis, membrane integrity, enzyme function or maturation of the erythrocytes. The use of molecular genetics has led to the identification of many mutations that are responsible for inherited anemias and this has led to refinement of diagnosis and development of targeted therapy. An appreciation of these multiple pathophysiological pathways is important to determine which laboratory tests to use, to perform an accurate etiological diagnosis and to inform individualized patient care [11].

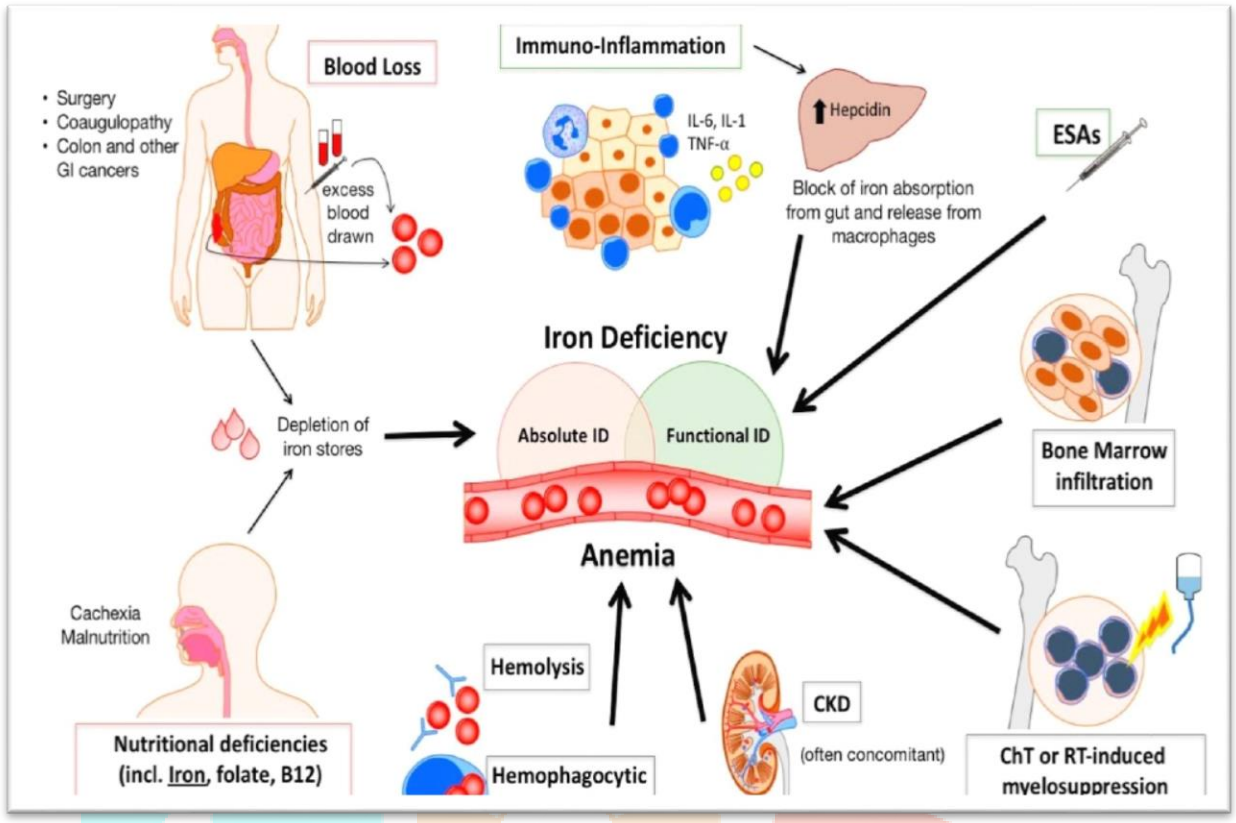

Figure 1 : Overview of the major pathophysiological mechanisms leading to anemia.

3. Conventional Laboratory Diagnosis of Anemia

The laboratory approach to the evaluation of anemia is systematic and based on a thorough history and examination to identify the cause of the anemia, its severity, and the proper treatment. Laboratory tests are still the foundation of the diagnosis of anemias and are available in the majority of medical practices. These investigations are useful for information about the number of erythrocytes, their shape and size, their production, nutritional status, hemolysis or bone marrow dysfunction. By combining several lab values, physicians may be able to distinguish between different types of anemia and, if needed, perform more specific testing [12].

3.1 Complete Blood Count (CBC)

The first and most important laboratory test performed in patients with suspected anemia will be the complete blood count (CBC). The automated hematology cells give a quantitative analysis of hemoglobin (Hb), hematocrit (HCT), red blood cell (RBC) count and erythrocyte indices, which give an immediate picture of the hematological status. The main test for diagnosis of anaemia is to measure the haemoglobin concentration which is a measure of the oxygen carrying capacity of the circulating blood. Hematocrit is the percentage of blood volume that is made up of erythrocytes and is generally proportional to hemoglobin concentration. The absolute number of circulating erythrocytes is given by the RBC count, which is useful in differentiating the various hematological disorders. The other blood indices of an anemia are also known as red blood cell indices. The red blood cell indices such as MCV may help to narrow the diagnosis of anemia to microcytic (<80 fL), normocytic (80–100 fL), or macrocytic (>100 fL). Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin in an individual red blood cell, while mean corpuscular hemoglobin concentration (MCHC) is the average amount of hemoglobin in each RBC cell, and helps to determine if a cell is hypochromic or hyperchromic. RCDW provides a good index of the variation in red cell size (anisocytosis) and is especially useful to differentiate iron deficiency anemia, which tends to have an elevated RDW, from thalassemia trait, where the RDW may not be elevated. The CBC is the first-line test in clinical

hematology that, when taken together, is essential to understanding the severity of anemia, morphology of the anemia, and possible causes [13].

3.2 Peripheral Blood Smear

The peripheral blood smear is used to supplement the automated hematology data and is a direct examination of the erythrocytes. With adequate staining using Wright-Giemsa stain it is possible to evaluate the size, shape, color, inclusion and distribution of red blood cells. Typical morphological anomalies are often helpful for diagnosis. Macrocytes indicate vitamin B12 or folate deficiency, liver disease or myelodysplastic syndromes, while microcytes are seen in iron deficiency anemia or thalassemia. The hypochromia is due to decreased intracellular hemoglobin production and is seen frequently in iron deficiency. Spherocytes are small, dense, erythrocytes with no central pallor and are indicative of hereditary spherocytosis or autoimmune hemolytic anemia. Target cells are frequently seen in thalassemia, liver disease and some hemoglobinopathies. Schistocytes are signs of microangiopathic hemolytic anemia, disseminated intravascular coagulation and mechanical hemolysis. Pathognomonic for sickle cell disease and related hemoglobinopathies are the sickle cells. Other clues to differential diagnosis obtained from peripheral smear include rouleaux formation, nucleated red blood cells, Howell–Jolly bodies, basophilic stippling, and polychromasia. For these reasons, blood smear studies are a vital part of automated hematology testing and often guide further confirmatory studies [14].

3.3 Reticulocyte Count

Reticulocytes are young erythrocytes which contain a network of remnants of the cell membrane and are newly released from the bone marrow into the peripheral blood. The amount of these can be used as an indirect indicator of bone marrow erythropoietic activity and regenerative capacity. The modern automated analyzers measure the number of reticulocytes by fluorescent dyes that can bind the remaining ribonucleic acid (RNA) in immature erythrocytes. In some cases a corrected reticulocyte count or a reticulocyte production index (RPI) will be performed to take into consideration the severity of the anaemia and to get an accurate picture of the marrow's response. Elevated reticulocytes may be seen after acute blood loss, hemolytic anemias, or after correcting nutritional deficiencies. In contrast, a low reticulocyte level can indicate that the bone marrow is not responding to the red blood cell loss, as occurs in aplastic anemia, bone marrow infiltration, chronic kidney disease, nutritional deficiencies, and ineffective erythropoiesis. Thus, reticulocyte evaluation is important in the differentiation of hypoproliferative from regenerative anemias [15].

3.4 Iron Studies

Iron studies are very useful in diagnosis of iron metabolism disorders and in distinguishing iron deficiency anemia from anemia of chronic disease and other microcytic anemia. Serum iron is the level of iron responsible for iron transport and is mostly bound to transferrin, but shows a large biological variation. Ferritin is a more accurate measurement of the total body iron stores which is usually low in iron deficiency anemia. Ferritin is an acute phase reactant, however, so inflammatory diseases can raise ferritin levels in the presence of iron deficiency. Total iron-binding capacity (TIBC) is the amount of iron that transferrin can bind to, and unsaturated iron-binding capacity (UIBC) is the amount of iron that transferrin can still bind. Transferrin saturation – obtained from serum iron and TIBC – indicates the percentage of transferrin bound to iron, and is markedly low in iron deficiency anemia. Ferritin, serum iron, TIBC, UIBC and transferrin saturation are used together to greatly improve diagnostic accuracy and to help differentiate absolute iron deficiency from functional iron deficiency (associated with chronic inflammation) [16].

3.5 Vitamin B12 and Folate

Vitamin B12 and folate status should be measured in all patients with macrocytic anemia. Pernicious anemia, gastric surgery, malabsorption syndromes and prolonged folate deficiency, or dietary deficiency, are common causes of vitamin B12 deficiency, while folate deficiency occurs with inadequate dietary intake, alcoholism, pregnancy and malabsorption syndromes. Both defects are associated with failure of DNA synthesis and result in suboptimal erythropoiesis and in the marrow, in both cases, in characteristic megaloblastic changes. Lab results generally show macrocytosis, hypersegmented neutrophils, increased mean corpuscular volume and decreased concentrations of serum vitamins. Other biomarkers, such as methylmalonic acid and homocysteine, can help with the diagnosis, especially when the B12 level is in the "borderline" range. Vitamin B12 deficiency can cause irreversible neurological damage even if it is corrected after the onset of anemia and early identification is essential [17].

3.6 Hemolysis Markers

Biochemical markers of accelerated erythrocyte destruction are measured in the lab when hemolytic anemia is suspected. Lactate dehydrogenase (LDH) is released from damaged erythrocytes and is usually raised in both intravascular (in the blood stream) and extravascular (in tissues) hemolysis. An elevated unconjugated (indirect) bilirubin level is caused by the rapid destruction of red blood cells leading to the loss of hemoglobin. During intravascular hemolysis, free hemoglobin is released into plasma, which causes the decrease in serum haptoglobin because free hemoglobin is rapidly removed. The direct antiglobulin (Coombs) test is a fundamental immunohematological laboratory test to identify the presence of immunoglobulin G (IgG) and complement on the surface of erythrocytes. A positive test will confirm the diagnosis of autoimmune hemolytic anemia, while a negative test will indicate that non-immune mechanisms of hemolysis are at play. These laboratory markers are also helpful in making the diagnosis and classification of hemolytic disorders when used in combination [18].

3.7 Bone Marrow Examination

Bone marrow aspiration and trephine biopsy are reserved for patients in whom the cause of anemia remains unclear after basic laboratory workup and/or primary bone marrow diseases are suspected. They offer direct evaluation of marrow cellularity, maturation of the erythroid series, iron stores, fibrosis, dysplasia, malignant infiltration and hematopoietic activity. Bone marrow examination is especially indicated in cases of aplastic anemia, myelodysplastic syndromes, leukemia, lymphoma, plasma cell dyscrasias, unexplained pancytopenia, sideroblastic anemia and suspected metastatic malignancy. The protocol also allows for additional tests such as cytogenetic, flow cytometric, immunophenotypic, microbiological, and molecular tests, improving diagnosis. Although it has excellent diagnostic potential, bone marrow examination is invasive, relatively costly and requires specialized training. It can be painful, bleed and can cause patients to be uncomfortable, which are potential complications, and which is why it is not routinely used as a first line investigation. Therefore, bone marrow examination is usually performed in limited clinical situations when other less invasive laboratory tests have failed to reach a definite diagnosis and/or more advanced forms of hematological disease are likely to be present [19].

Table 2. Characteristic Laboratory Findings in Common Types of Anemia.

Laboratory Parameter	Iron Deficiency Anemia	Anemia of Chronic Disease	Megaloblastic Anemia	Hemolytic Anemia	Thalassemia	References
Hemoglobin	↓	↓	↓	↓	↓	[20]
MCV	↓	Normal/↓	↑	Normal	↓	[21]
RDW	↑	Normal/↑	↑	↑	Normal/slightly ↑	[22]
Reticulocyte Count	↓	Normal/↓	↓	↑	Normal/↑	[23]
Serum Ferritin	↓	Normal/↑	Normal	Normal/↑	Normal/↑	[24]
Serum Iron	↓	↓	Normal	Normal	Normal	[25]
TIBC	↑	↓/Normal	Normal	Normal	Normal	[26]
Transferrin Saturation	↓	↓	Normal	Normal	Normal	[27]
Vitamin B12	Normal	Normal	↓	Normal	Normal	[28]
Folate	Normal	Normal	↓ (if folate deficiency)	Normal	Normal	[29]
LDH	Normal	Normal	↑	↑↑	Slightly ↑	[30]
Bilirubin	Normal	Normal	Mild ↑	↑	Mild ↑	[31]
Haptoglobin	Normal	Normal	Normal	↓	Normal	[32]
Peripheral Blood Smear	Microcytic hypochromic RBCs	Normocytic/mild microcytosis	Macro-ovalocytes, hypersegmented neutrophils	Spherocytes/schistocytes	Target cells, microcytes	

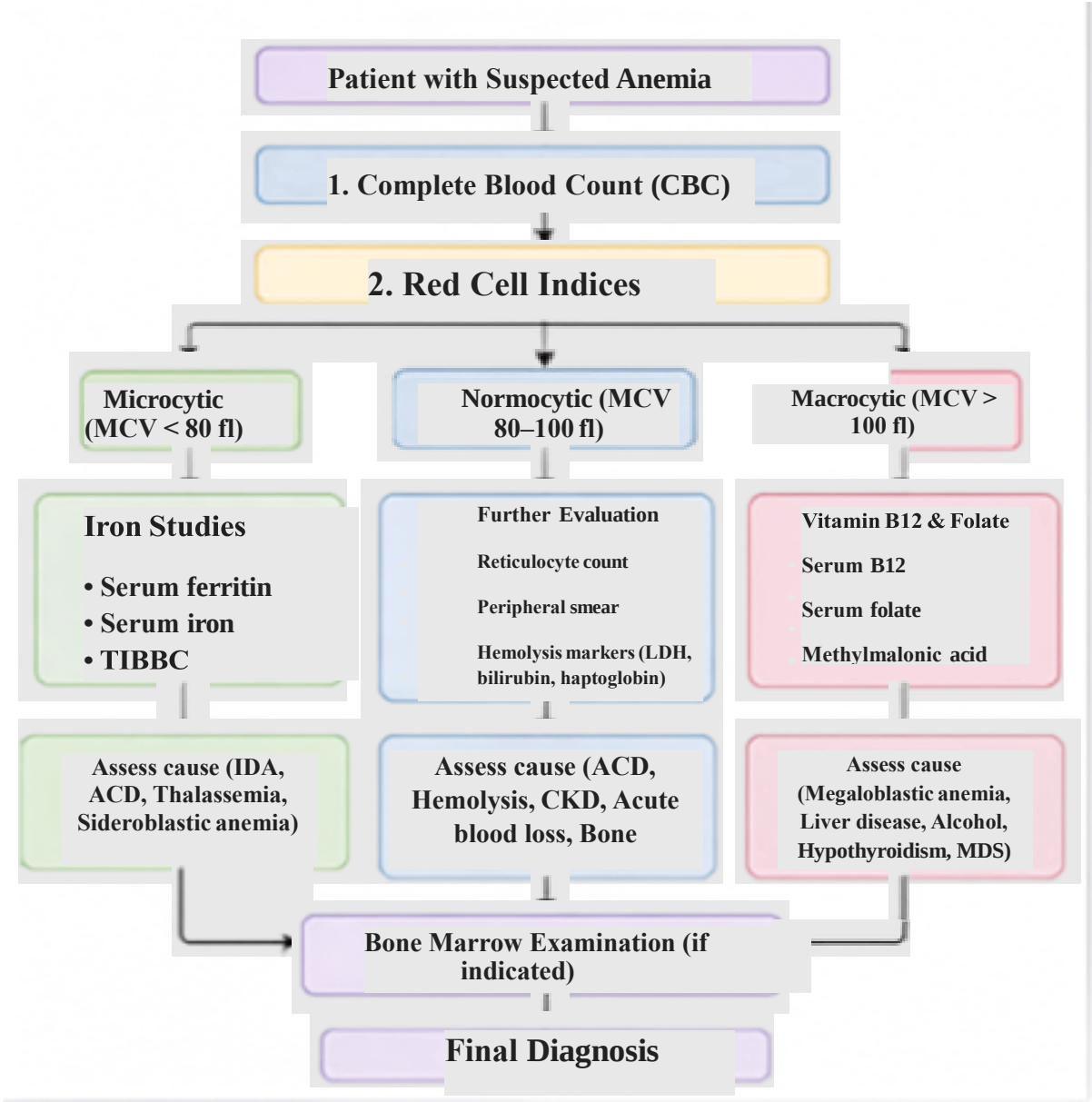

Figure 2. Routine laboratory diagnostic workflow for the evaluation of anemia.

4. Advanced Laboratory Techniques

Over the past few years, the diagnostic accuracy, sensitivity and specificity of evaluating anemia have greatly improved in the laboratory. Conventional laboratory investigation is the primary tool for the diagnosis of hematological disorders, but advanced diagnostic technologies can provide precise characterization of the hematological disorders,-help in early diagnosis of the disease, identification of underlying genetic abnormalities and can assist in the therapeutic decision making individually. These techniques are especially useful in atypical, complex and inherited anemias in which traditional investigations are unable to pinpoint a diagnosis. Modern hematology has been revolutionized by the incorporation of cutting-edge laboratory techniques, which have greatly increased the efficiency of the diagnostic process and improved patients' outcomes [33].

4.1 Flow Cytometry

Flow cytometry is a high throughput analysis method that uses laser technology to analyze the physical and immunophenotypic properties of cells in a fluid stream one by one. It allows fast assessment of cell size, granularity, fluorescence intensity and surface and intracellular antigen expression using fluorochrome-labeled monoclonal antibodies. Flow cytometry has become an essential tool in haematology for the diagnosis of haematological malignancies, the assessment of bone marrow disease and the characterization of abnormal erythrocyte populations. It is one of the most well known uses in the diagnosis of anemia, where it is used to detect the absence of

glycosylphosphatidylinositol (GPI)-anchored proteins including CD55 and CD59 on erythrocytes and granulocytes in the case of paroxysmal nocturnal hemoglobinuria (PNH). Flow cytometry is also valuable in the diagnosis of leukemia-associated anemia for immunophenotyping abnormal leukocyte populations and for determining extent of bone marrow involvement. In addition, automated flow cytometric reticulocyte analysis is useful to accurately count reticulocytes, and to measure the immature reticulocyte fraction (IRF) and evaluate bone marrow regenerative activity. Flow cytometry has advantages in precision, turnaround time, being objective, and it is more reproducible than manual microscopy [34].

4.2 High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is considered the reference laboratory technique for the qualitative and quantitative analysis of hemoglobin fractions. It resolves the various types of hemoglobin by retention time in a chromatographic column and allows the correct identification of both normal hemoglobin and abnormal hemoglobin species. HPLC is widely used in the diagnosis of thalassemia as well as other hemoglobinopathies such as sickle cell disease, hemoglobin C disease, hemoglobin E disease and compound heterozygous disorders. The determination of HbA, HbA_2, HbF and abnormal hemoglobin variants can be helpful in diagnosis of β-thalassemia trait, β-thalassemia major, and other inherited hemoglobinopathy disorders. The method is automated, highly reproducible and can analyze several samples within a reasonably short time frame, thus enabling large-scale population screening and newborn screening programs. Although this has good analytical capability, some rare hemoglobin variants may have similar chromatograms and require molecular testing for confirmation [35].

4.3 Capillary Electrophoresis

Another analytical platform that has developed in recent years is capillary electrophoresis (CE) which has proven to be a useful tool in hemoglobin variant analysis. This is based on the use of a narrow silica capillary column and high voltage for separation of hemoglobin molecules based on electrophoretic mobility. Capillary electrophoresis provides increased automation, resolution, analytical sensitivity and analysis time over traditional electrophoresis methods. HPLC or capillary electrophoresis can be used for the diagnosis of haemoglobinopathy screening with excellent results, but HPLC has certain advantages over capillary electrophoresis and vice versa. HPLC is a standard reference method in many clinical laboratories used to give very accurate quantitative analysis of hemoglobins produced. However, in other instances, certain hemoglobin variants may be more effectively separated by capillary electrophoresis (CE) that can result in reduced diagnostic confusion, whereas HPLC may not. These techniques are often used in combination and in clinical practice some form of confirmatory molecular testing may be needed if there is discordant or inconclusive results [36].

4.4 Molecular Diagnostics

Since the advent of molecular diagnostic techniques which allow direct detection of disease-causing genetic mutations, the investigation of inherited anemias has undergone a revolution. One of the most common molecular techniques is the polymerase chain reaction (PCR) which is still used to detect specific pathogenic variants of hemoglobinopathies and congenital anemia syndromes. This is further extended by Quantitative PCR (qPCR) which quantifies the number of genes (deletions and duplications) and the level of expression (quantitative abnormalities). Multiplex PCR is a diagnostic technique that amplifies multiple targets of the genetic material in one reaction, enhancing diagnostics both in terms of efficiency and cost and decreasing turnaround time. The field of hematological diagnosis was revolutionized more recently by the technique of next-generation sequencing (NGS), which allows the analysis of many genes or the whole exome simultaneously. For cases of unexplained congenital anemia and genetically heterogeneous disorders, NGS is proving to be a wonderful tool to

identify new pathogenic variants, as well as having high sensitivity for identifying known pathogenic variants. Such molecular tests have wide clinical applications for the diagnosis of thalassemia, sickle cell disease, congenital dyserythropoietic anemia, Diamond–Blackfan anemia, hereditary spherocytosis, enzyme deficiency (e.g., glucose-6-phosphate dehydrogenase deficiency) and many rare inherited bone marrow failure syndromes. Molecular testing can also help in the genetic counselling, pre-natal diagnosis, carrier detection and devising therapy [37].

4.5 Cytogenetics

Cytogenetic analysis is important for giving information about chromosomal abnormalities of acquired bone marrow disorders that result in anemia. Traditional karyotyping can be used to identify both numerical and structural chromosomal abnormalities, while fluorescence in-situ hybridization (FISH) is more sensitive in the detection of certain gene abnormalities and structural chromosomal abnormalities. Cytogenetic testing is particularly important for the diagnosis and prognosis of myelodysplastic syndromes (MDS), aplastic anemia, and acute leukemias and other conditions affecting the bone marrow where erythropoiesis is not occurring. Genetic abnormalities such as deletion 5q, monosomy 7, trisomy 8 and/or complex karyotype help classify the disease, help predict prognosis, select therapy and follow up of the disease. Thus, cytogenetic testing is now an essential part of today's hematopathology practice [38].

4.6 Mass Spectrometry

A new analytical technique that is becoming used in hematology is mass spectrometry. This technique can measure the mass to charge ratio of bio-molecules with high accuracy, crucial for the accurate identification of proteins, peptides, metabolites and hemoglobin variants. It has been demonstrated that the use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) and liquid chromatography–mass spectrometry (LC–MS) is important for detecting rare hemoglobin variants which could be difficult to analyze by traditional methods. Besides hemoglobin analysis, mass spectrometry can help in the discovery of biomarkers, metabolomic profiling and in the quantitative analysis of iron metabolism-related proteins. Although currently restricted to certain applications with significant equipment expenses, the technology is becoming increasingly developed and is expected to be increasingly applied in precision hematology and in translational studies [39].

4.7 Point-of-Care Testing (POCT)

The use of point of care testing (POCT) is gaining significance as rapid assays for assessment of haematology in non-centralized laboratories. The portable hemoglobin analyzers and handheld diagnostic devices give instant results from a small amount of blood collected by a finger prick. These systems have many applications in the emergency room, outpatient clinics, rural health care facilities, maternal health, transfusion centers, and at resource-limited facilities. The greatest advantage of POCT is the low sample volume required, rapid turnaround time, increased accessibility, simpler equipment and infrastructure needs, and the ability for timely clinical decision making. There are however a number of challenges, such as analytical accuracy, operator-dependent errors, quality control, device calibration, environmental influences and the lack of availability of comprehensive hematological parameters. Thus, abnormal POCT results usually need to be corroborated by standardised laboratory tests [40].

4.8 Digital Hematology Platforms

The digital hematology platforms are one of the most rapidly evolving segments of laboratory medicine, combining automated microscopy, artificial intelligence (AI) and machine learning with digital image analysis to enhance hematological diagnosis. The automated digital morphology systems capture high-resolution images of peripheral blood smears and uses sophisticated algorithms with very little human interaction to classify erythrocytes, leukocytes and platelets. Benefits of digital microscopy include increasing laboratory workflow efficiency, decreasing observer variability, more

standardization, remote consultation, and efficient quality assurance. Abnormal erythrocyte morphology such as microcyte, macrocyte, schistocyte, target cell, spherocyte and sickle cell can be accurately identified with AI-assisted image analysis to help identify specific anemia subtypes early on. Seamless data management and clinical reporting through the integration with laboratory information systems. Despite this, a large investment, validation in a variety of patient cohorts, algorithm standardization, and proper regulation are needed for digital hematology platforms to be widely used.

Table 3. Comparison of Conventional and Advanced Laboratory Techniques Used in Anemia Diagnosis.

Parameter	Conventional Techniques	Advanced Techniques
Examples	CBC, Peripheral blood smear, Iron studies, Bone marrow examination	Flow cytometry, HPLC, Capillary electrophoresis, PCR, NGS, Mass spectrometry [41]
Primary Purpose	Initial screening and anemia classification	Identification of specific etiologies and genetic disorders
Diagnostic Accuracy	Moderate to High	High to Very High [42]
Sensitivity	Moderate	High
Specificity	Moderate	High
Turnaround Time	Minutes to hours	Hours to several days [43]
Cost	Low	Moderate to Very High
Technical Expertise	Basic laboratory training	Specialized personnel required [44]
Infrastructure Requirement	Standard clinical laboratory	Advanced molecular and specialized laboratory
Detection of Genetic Disorders	Limited	Excellent [45]
Role in Personalized Medicine	Limited	Extensive
Clinical Application	Routine diagnosis and monitoring	Complex, inherited, refractory, and unexplained anemias [46]
Major Limitation	Limited specificity for complex disorders	High cost and limited availability

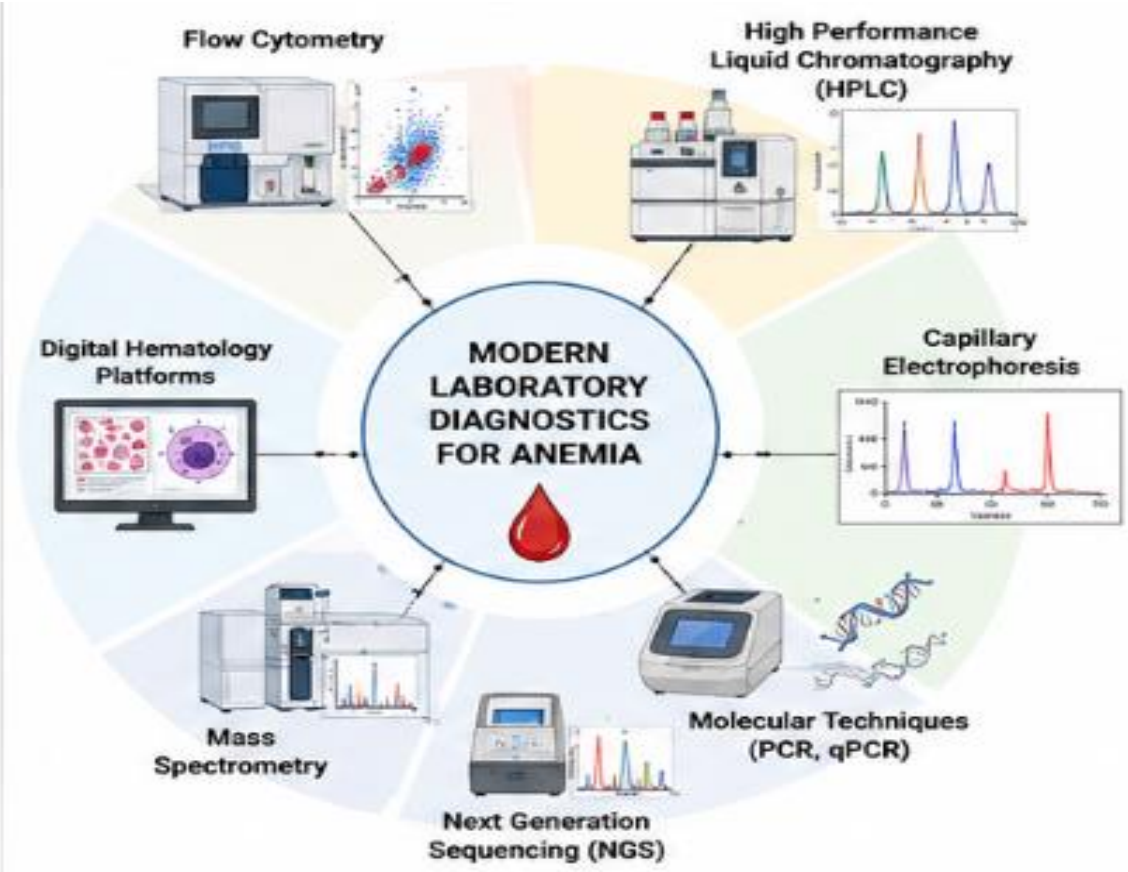

Figure 3. Advanced laboratory techniques currently used for the diagnosis of anemia

5. Emerging Biomarkers in Anemia Diagnosis

The discovery of new markers has significantly improved the laboratory diagnosis of anaemia by allowing a more accurate assessment of iron metabolism, erythropoietic activity, and disease mechanisms. Unlike traditional laboratory tests, which may not be specific enough to distinguish the various types of anemia, new biomarkers offer clues to iron homeostasis, bone marrow reactions and inflammatory control. These biomarkers are especially helpful in the diagnosis of iron deficiency anemia (IDA) vs anemia of chronic disease (ACD), for the detection of functional iron-deficiency (FID) and for the monitoring of the therapeutic response as well as to aid in individual patient management. Several biomarkers have proven powerful clinical utility, but remain limited in use because of the availability of the assays, cost and standardization.

5.1 Hepcidin

Hepcidin is a peptide hormone that is mainly produced by hepatocytes and is considered as the master regulator of systemic iron homeostasis. It binds to the iron exporter ferroportin, which results in its internalization and degradation, and regulates the transport of iron into the intestine and the release of iron from macrophages and hepatocytes. Increased hepcidin levels lower the amount of iron in the plasma, while decreased levels increase intestinal iron absorption and mobilization of iron stores, all of which lead to functional iron deficiency. The measurement of hepcidin in the serum has become a potential biomarker to distinguish iron deficiency anemia and anemia of chronic disease. Iron deficiency almost always is characterized by low hepcidin levels, while inflammatory conditions, chronic infections, auto-immune disorders and chronic kidney disease are associated with elevated hepcidin levels, mainly through the action of interleukin-6 (IL-6). As a result, the measurement of hepcidin could be useful in predicting the response of patients to oral iron therapy and those patients who might benefit from iron supplementation via IV [47].

5.2 Soluble Transferrin Receptor (sTfR)

Soluble transferrin receptor (sTfR) is a proteolytic product of the membrane-bound transferrin receptor (TfR) on the surface of developing erythroid cells. The serum sTfR concentration is a measure of cellular iron requirement, erythropoietic activity, and is relatively non affected by inflammatory conditions. It is important to note that sTfR will not be lowered during inflammatory conditions, unlike serum ferritin; this would make sTfR useful to differentiate iron deficiency anemia from anemia of chronic disease. In patients with mixed etiologies or chronic inflammatory diseases, the ratio of soluble transferrin receptor to logarithmic ferritin (sTfR/log ferritin index) further enhances the diagnostic accuracy. There is growing evidence that sTfR could be added to the full body iron assessment strategies, particularly in patients with chronic kidney disease (CKD), inflammatory bowel disease (IBD), and malignancies [48].

5.3 Reticulocyte Hemoglobin Content (CHr/Ret-He)

RHc or Ret-He (cellular hemoglobin in reticulocytes or reticulocyte hemoglobin equivalent) is the hemoglobin content of newly formed reticulocytes. The number of reticulocytes in the blood stream is a measure of the availability of the iron for erythropoiesis since they take only 1 to 2 days to mature into erythrocytes. Ret-He has become an increasingly popular early and sensitive indicator of IRAN, and can be detected earlier than the levels of conventional biochemical markers become abnormal. It is especially effective in patients using erythropoiesis-stimulating agents, those on hemodialysis, children and pregnant women. In addition, repeated Ret-He measurements offer a useful tool for monitoring treatment response to iron therapy and efficacy of therapy in a short time [49].

5.4 Immature Reticulocyte Fraction

Maturing reticulocytes are a part of the reticulocyte population that has the highest levels of remaining RNA and are referred to as the immature reticulocyte fraction (IRF). Fluorescence-based flow cytometric methods are used by automated hematology analyzers to measure IRF. IRF also acts as an early marker of bone marrow regeneration, and will rise several days before the absolute reticulocyte count does. Increased IRF is seen in recovery from an acute blood loss, hemolytic anemia, successful correction of a nutritional deficiency, bone marrow transplantation or chemotherapy-induced marrow suppression. Therefore, IRF can be used to monitor the recovery of bone marrow and the effectiveness of therapeutic measures [50].

5.5 Erythroferrone

Increased erythropoietic activity results in the production of the hormone, erythroferrone, predominantly by erythroblasts stimulated by erythropoietin. It inhibits hepcidin production in the liver, which increases iron uptake in the intestine and mobilizes the iron from stores for hemoglobin production. Increased erythroferrone levels have been found in diseases of poor erythropoiesis, such as beta-thalassemia, congenital dyserythropoietic anaemias and inherited erythroid disorders. Its use in routine clinical practice is still limited, but erythroferrone is an attractive biomarker for the comprehension of the erythropoietic regulation and may be part of new precision medicine strategies for anemia treatment [51].

5.6 Growth Differentiation Factor-15 (GDF-15)

Growth differentiation factor-15 (GDF-15) is a cytokine of the transforming growth factor-β (TGF-β) superfamily that is induced by stress. GDF-15 is increased by ineffective erythropoiesis, tissue injury, inflammation and oxidative stress. Increased levels of GDF-15 have been found in β-thalassemia, myelodysplastic syndromes, congenital sideroblastic anemia, and some cancers. GDF-15 also acts to inhibit hepcidin production, thus allowing more iron to be released into circulation even when body iron stores are high. GDF-15 is not disease-specific, but could be used alongside other biomarkers to assess disorders related to ineffective erythropoiesis and iron overload [52].

Clinical Applications

The introduction of novel biomarkers has broadened the scope of the laboratory haematology diagnostic armamentarium, allowing earlier detection of iron deficiency, classification of anaemias, evaluation of erythropoietic activity and monitoring of therapeutic response. They are especially useful in complex clinical conditions like chronic inflammatory diseases, chronic kidney disease, malignancies, pregnancy, paediatric anemia and hereditary hematological disorders. The use of these biomarkers together with routine laboratory testing enhances the precision of diagnosis and contributes to personalized care of patients [53].

Advantages

Emerging biomarkers have shown to be more sensitive than the traditional laboratory parameters and less affected by inflammatory conditions, and can also provide a dynamic evaluation of iron metabolism and erythropoiesis. They help to make a more accurate distinction between absolute and functional iron-deficiency, allow for more timely therapeutic intervention, help to monitor response to therapy and can be used in precision medicine approaches. Multiple biomarker integration is also useful for diagnosis in patients with mixed and/or multifactorial anemia.

Limitations

While these biomarkers hold great clinical potential, there are some limitations that preclude their routine use. No standardized reference ranges or assays are yet in existence, and there is some variation among laboratories. Numerous assays demand particular equipment, which makes diagnostics more expensive and impractical in resource-limited settings. Moreover, a number of biomarkers, especially erythroferrone and GDF-15, need to be further clinically validated at a large scale before being integrated into international diagnostic guidelines. At present, these biological markers should be used in addition to the typical blood parameters and the clinical situation of the patient.

Table 4. Comparison of Emerging Biomarkers for the Laboratory Diagnosis of Anemia

Biomarker	Primary Function	Clinical Utility	Major Advantages	Major Limitations
Hepcidin	Regulates systemic iron homeostasis	Differentiates iron deficiency anemia from anemia of chronic disease; predicts response to iron therapy	Direct indicator of iron regulation; useful in inflammatory anemia	Limited assay standardization; affected by inflammation and circadian variation [54]
Soluble Transferrin Receptor (sTfR)	Reflects cellular iron demand and erythropoietic activity	Detects iron deficiency, especially in inflammatory conditions	Minimally influenced by inflammation; high diagnostic accuracy	Higher cost; limited availability in routine laboratories
Reticulocyte Hemoglobin Content (CHr/Ret-He)	Assesses recent iron incorporation into reticulocytes	Early detection of iron-restricted erythropoiesis; monitoring iron therapy	Detects iron deficiency before ferritin changes; rapid response to treatment	Requires modern automated hematology analyzers

Immature Reticulocyte Fraction (IRF)	Indicates early bone marrow regenerative activity	Monitors marrow recovery and treatment response	Sensitive marker of erythropoietic recovery	Instrument-dependent; limited standardized cut-off values [55]
Erythroferrone	Suppresses hepcidin during increased erythropoiesis	Evaluates ineffective erythropoiesis and inherited anemias	Provides insight into erythropoietic regulation	Primarily research use; limited clinical validation
Growth Differentiation Factor-15 (GDF-15)	Regulates iron metabolism and stress response	Supports diagnosis of ineffective erythropoiesis and iron overload disorders	Useful adjunct in thalassemia and myelodysplastic syndromes	Poor disease specificity; not recommended as a standalone diagnostic marker

6. Artificial Intelligence and Digital Innovations

Artificial intelligence (AI) is a revolutionary technology in laboratory medicine that holds promise to make new contributions to the diagnosis, classification, and management of anemia. AI can use computational algorithms to analyze hematological parameters and improve the accuracy of diagnosis, minimizing human errors, and speeding clinical decision-making. The introduction of recent developments in machine learning (ML), deep learning (DL), digital pathology and clinical decision support systems (CDSS) has led to the automation of the analysis of laboratory parameters and microscopic images, helping to detect hematological abnormalities earlier, thus enabling personalised care. These innovations are complimentary to the standard laboratory-based techniques as they facilitate objective, reproducible and high throughput analyses which help precision hematology [56].

6.1 AI-Assisted Complete Blood Count (CBC) Interpretation

Anemia is the most common indication for a complete blood count (CBC) test, which provides multiple parameters from the blood and thus must be interpreted as a whole. AI assisted CBC analysis uses computational algorithm to assess hemoglobin, hematocrit, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), reticulocyte indices, and other hematological parameters simultaneously. AI systems can identify patterns in multidimensional data sets, categorize anemia as microcytic, normocytic, or macrocytic, and provide a list of potential causes such as iron deficiency anemia, megaloblastic anemia, anemia of chronic disease, hemolytic anemia, and thalassemia. Automated interpretation helps reduce inter-observer variability, aids in reporting, and helps the clinician prioritize appropriate confirmatory investigations [57].

6.2 Machine Learning Models

One area of AI is machine learning, which uses algorithms to analyze past data to see patterns for diagnosis and to create models to predict future conditions. Several supervised learning models have been applied to the successful classification of the various types of anemia using routinely available laboratory parameters like decision tree, random forest, support vector machine, and gradient boosting algorithms. Supervised Learning techniques also can be applied to discover underlying association in huge and complex hematological data sets for patient stratification and disease phenotyping. Combining laboratory data, demographic data, clinical history, nutritional status, inflammatory markers, and genetic data can enhance diagnostic accuracy, which can be achieved through machine learning models. These models have also been used to predict treatment response, to determine which

patients would be at risk of severe anemia, to estimate transfusion requirements, and to aid in population-based screening programs, as well as disease classification. These algorithms can be refined as more data becomes available over time, leading to better predictions.

6.3 Deep Learning for Blood Smear Analysis

The field of automated microscopic image analysis has been transformed by deep learning, especially CNNs. Deep learning algorithms can be applied to digital images of peripheral blood smears to detect subtle peripheral blood morphological abnormalities with high sensitivity and specificity. Automated systems are able to accurately identify and classify abnormalities of the erythrocytes such as microcytes, macrocytes, hypochromic cells, target cells, schistocytes, spherocytes, sickle cells, elliptocytes, and nucleated red blood cells. Deep learning can greatly speed up the time it takes to interpret images, enhance consistency, and minimize observer-dependent variability when compared to traditional manual microscopy. The systems are also able to automate the quantification of abnormal cell populations and enable early detection of rare blood disorders. Deep learning can be applied to digital microscopy platforms, allowing for the processing of high volumes of samples in a short period, boosting laboratory efficiency and quality assurance [58].

6.4 Digital Pathology

The new digital pathology solutions integrate whole slide imaging, cloud storage, image analysis software and artificial intelligence to provide a complete digital workflow for hematological diagnosis. The high resolution scanners turn the old glass slides into digital image information that can be analyzed remotely by the hematopathologist or processed automatically by a powerful image recognition algorithm. Digital pathology contributes to the standardization of diagnosis, allows telehematology services and enables multidisciplinary consultation between geographically dispersed healthcare centers. Automated image analysis can produce more accurate cell count, cell morphology classification and identification of abnormalities in erythrocytes and provide permanent digital images for education, QA and research. In addition, the system can be integrated with laboratory information systems (LIS) and electronic health records (EHRs), allowing for easy data transfer and full patient care [59].

6.5 Clinical Decision Support Systems

Clinical decision support systems (CDSS) are AI applications that integrate laboratory results, patient demographics, clinical history, imaging results and molecular diagnostic data to make clinical decisions with evidence-based recommendations for diagnosis. Such systems can assist the healthcare expert in making a differential diagnosis, recommending additional laboratory tests to assist the diagnosis, suggesting appropriate clinical guidelines, and assisting in the personalisation of treatment. By analysing patient data over time, CDSS can automatically interpret complex lab profiles, differentiate between many possible causes of anaemia, suggest iron studies or molecular testing when appropriate and monitor the therapeutic response over time. The use of AI-driven decision support can help to minimize delay in diagnosis, improve conformity with clinical guidelines, optimize resource use and boost patient results in personalized medicine applications [60].

Advantages

AI and digital innovations provide some key benefits when it comes to the diagnosis of anemia. Automated data analysis offers increased diagnostic accuracy, observer independence and consistency, and reproducibility. High-throughput processing not only dramatically cuts laboratory turnaround time, but also improves workflow efficiency. AI algorithms can integrate a range of clinical, laboratory, imaging and genomic information, providing a full picture for patients and assistance in precision medicine. Digital pathology allows for remote consultations, consistent reporting, ongoing quality control, and education training. In addition, predictive analytics produced by the machine

learning algorithm allows for the detection of the disease sooner, risk stratification, optimization of treatment, and better long-term patient monitoring.

Current Limitations

Despite the advances shown, there are still a number of challenges that need to be overcome before AI-based hematology systems can be widely adopted in clinical practice. These biases and lack of generalizability can occur because machine learning models rely heavily on the quantity, diversity, and quality of the data they are trained with, which can differ from other populations. There are several challenges to overcome, such as standardized validation protocols, interoperability issues between health information systems, and regulatory requirements. Moreover, there is a need for major investments in digital infrastructure, computer hardware, software and software support, as well as staff training. However, there are ethical issues to consider, such as patient privacy, data security, algorithm transparency, and clinician accountability, that need to be addressed before the widespread adoption. While AI can be a useful adjunct to laboratory diagnostics, it cannot replace clinical experience and conventional laboratory testing at the present time.

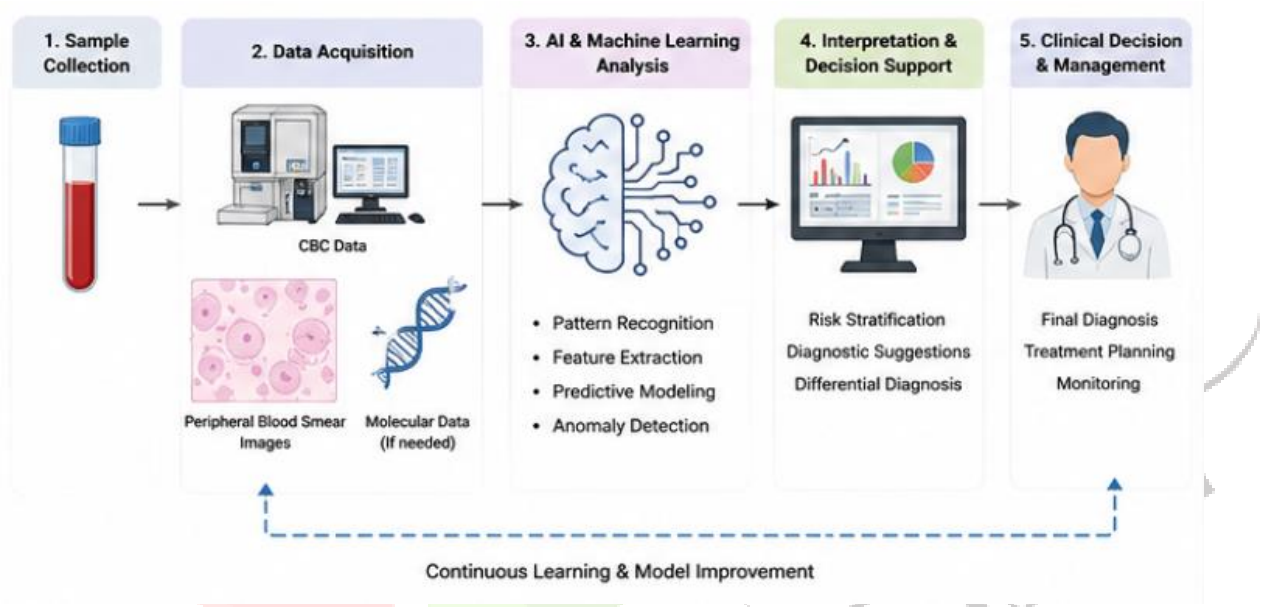

Figure 4: AI-integrated workflow for anemia diagnosis

7. Diagnostic Algorithms for Different Types of Anemia

Diagnostic algorithm, it is a system that is designed to detect the causes of anemia systematically and accurately select the correct treatment against it. Laboratory tests may be done in a step-wise fashion, as anemia is a symptom of many disease states. A complete blood count (CBC) and red blood cell indices are usually the first tests conducted, followed by a more in-depth biochemical, hematological and molecular diagnostics based on the results of those tests. The implementation of these advanced diagnostic techniques in conjunction with conventional lab tests enhances diagnostic accuracy, reduces unnecessary investigations and enables timely therapeutic intervention.

7.1 Iron Deficiency Anemia: Diagnostic Pathway

The most common type of anemia worldwide is iron deficiency anemia (IDA) which is mainly diagnosed by assessing iron metabolism. A CBC is first ordered to show microcytic hypochromic anemia due to decreased hemoglobin concentration and reduction in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and increased red cell distribution width (RDW). Microcytosis, hypochromia, anisocytosis and poikilocytosis are seen in blood smear. Further iron studies are necessary to confirm the diagnosis. Low serum ferritin is the best markers of iron deficiency and is typically associated with low iron and high total iron-binding capacity (TIBC), low transferrin saturation, and high unsaturated iron-binding capacity (UIBC). However, other biomarkers

like soluble transferrin receptor (sTfR), reticulocyte hemoglobin equivalent (Ret-He) and hepcidin can be useful to enhance diagnostic accuracy in cases where inflammatory disorders are present. In the event that iron deficiency is confirmed, further investigation should be undertaken to determine the cause of the iron deficiency, which may include chronic gastrointestinal bleeding, excessive menstrual blood loss, malabsorption syndromes, nutritional deficiency, or malignancy [61].

7.2 Anemia of Chronic Disease: Diagnostic Algorithm

This type of anemia is called anemia of chronic disease (ACD) or anemia of inflammation and is often seen in patients with chronic infections, autoimmune diseases, malignancies, and chronic kidney disease. CBC results typically reveal normocytic normochromic anemia; with longer disease a mild microcytosis may be present. Laboratory tests include blood tests for inflammatory markers, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), and blood tests for iron studies. Ferritin level is usually normal or high because it is an acute phase reactant and iron level and transferrin saturation are low. The TIBC is usually low or normal which rules out iron deficiency anemia. Serum hepcidin and soluble transferrin receptor (sTfR) can also help distinguish functional iron deficiency from absolute iron deficiency. Renal function and serum erythropoietin levels should be evaluated in patients with chronic kidney disease. Underlying inflammatory diagnosis and management continue to be the main concerns [62].

7.3 Megaloblastic Anemia: Diagnostic Algorithm

The major cause of megaloblastic anemia is defect in DNA synthesis due to vitamin B12 or folate deficiency. The first step in laboratory evaluation is to find that the patient has macrocytic anemia, which will be seen as an elevated MCV and low hemoglobin level. Peripheral blood smears usually show macro-ovalocytes, anisopoikilocytosis and hypersegmented neutrophils. To detect nutritional deficiencies, the levels of vitamin B12 and folate in serum should be measured. Methylmalonic acid (MMA) and homocysteine allows for better diagnostic sensitivity, especially early B12 deficiency, when there is an equivocal result. Further tests include: blood tests for intrinsic factor antibodies, parietal cell antibodies, gastrointestinal blood tests for malabsorption syndromes, liver function tests, thyroid tests. Bone marrow examination is not performed in routine cases, but is used in atypical cases when there is a suspicion that the myelodysplastic syndromes or other marrow conditions may be present.

7.4 Hemolytic Anemia: Diagnostic Approach

To confirm the diagnosis of hemolytic anemia, the destruction of the erythrocytes must be accelerated and the mechanism of the destruction must be evaluated. Laboratory investigation will include CBC, reticulocyte count and peripheral blood smear. Reticulocytosis is a compensatory reaction of the bone marrow, and typical morphology of the erythrocytes may suggest the specific etiology. Biochemical markers of hemolysis include an increase in lactate dehydrogenase (LDH); an increase in unconjugated bilirubin; a decrease in serum haptoglobin; and an increase in plasma free hemoglobin. The direct antiglobulin (Coombs) test helps to distinguish immune-mediated hemolysis from non-immune causes. Depending on clinical presentation, additional testing may be indicated such as glucose-6-phosphatase dehydrogenase enzyme assay, osmotic fragility testing, hemoglobin electrophoresis, flow cytometry (paroxysmal nocturnal hemoglobinuria) and molecular genetic testing [63].

7.5 Hemoglobinopathies: Laboratory Workflow

Laboratory evaluation for hemoglobinopathies is sequential laboratory investigations including hematological, biochemical and molecular. The preliminary laboratory tests include CBC which often shows microcytic anemia and relatively normal or elevated red blood cell count in thalassemia carriers. Anisopoikilocytosis, target cells, nucleated erythrocytes and basophilic stippling can be observed in the peripheral blood smear. HbA, HbA_2, HbF and abnormal hemoglobin variants such as HbS, HbC and HbE can be measured using hemoglobin analysis by high performance liquid chromatography (HPLC) or capillary electrophoresis for the purpose of confirming. Molecular diagnostic assays can also detect disease causing mutations in genes, including polymerase chain reaction (PCR), Sanger sequencing and next generation sequencing (NGS) which can be used in complex or indeterminate cases. Such methods may also be applied to carrier screening, prenatal diagnosis and genetic counseling and family studies. The combination of conventional laboratory tests and state-of-the-art molecular testing of the genes that cause inherited hemoglobin disorders results in maximum diagnostic certainty and the best management of the patient. Algorithms used in the laboratory to diagnose anemia, in general, are structured and are a rational, economical approach. First, simple investigations and then more complex biochemical and molecular methods allows for the correct diagnosis of the causative agent and prompt treatment, avoiding unnecessary tests. Future algorithms will be more precise, standardized and personalized with the rising use of emerging biomarkers [64].

8. Comparative Evaluation of Current Diagnostic Modalities

Laboratory diagnosis of anemia is based upon the use of a number of standard and specialized diagnostic methods, each of which offers additional information on the morphology of the erythrocytes, the iron metabolism, the function of the bone marrow, and genetic abnormalities. The etiology of all anemias can't be determined by one laboratory test; a comprehensive approach is necessary. The complete blood count (CBC), peripheral blood smear, and iron profile are the primary tests performed, and they continue to be the most readily available, rapid, and inexpensive tests for initial evaluation. These tests are very useful to screen patients, determine the severity of anemia and direct further investigations. Advanced laboratory techniques offer significantly higher diagnostic specificity and are of great value in complicated, inherited, and unexplained cases of anemia. Flow cytometry is useful for precise immunophenotypic diagnosis of the hematological disorders and is essential for the diagnosis of paroxysmal nocturnal hemoglobinuria (PNH) and to assess leukemia associated anemia. The gold standard for quantifying hemoglobin fractions is now high-performance liquid chromatography (HPLC) and is the method used to diagnose thalassemia and other hemoglobinopathies. The molecular diagnostic techniques used, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), enable direct identification of pathogenic genetic changes associated with inherited anemias, which helps to make an accurate diagnosis, screen for carriers, provide genetic counselling and individual treatment planning. The choice of diagnostic modality is dependent on a number of factors such as suspected etiology, clinical presentation, laboratory infrastructure, availability of specialized equipment, cost, and turnaround time. Advanced molecular technologies provide high precision diagnosis, but are not widely used due to their increased cost, technical expertise requirements and limited availability in many healthcare settings. Therefore, the most efficient and cost effective approach would be to have a tiered diagnostic testing protocol starting with routine hematological testing and moving to specialized testing if clinically indicated. This streamlined process provides optimal diagnostic accuracy and reduces over-investigation and healthcare costs [65].

Table 5. Comparative Evaluation of Current Laboratory Diagnostic Modalities for Anemia [66].

Diagnostic Method	Sensitivity	Specificity	Relative Cost	Turnaround Time	Primary Clinical Utility
Complete Blood Count (CBC)	High	Moderate	Low	10–30 minutes	Initial screening, anemia classification, assessment of severity
Peripheral Blood Smear	Moderate–High	Moderate	Low	30–60 minutes	Morphological evaluation of erythrocytes and differential diagnosis
Iron Profile (Ferritin, Serum Iron, TIBC, TSAT)	High	High	Low–Moderate	2–6 hours	Diagnosis of iron deficiency and anemia of chronic disease
Flow Cytometry	Very High	Very High	High	4–24 hours	Diagnosis of PNH, leukemia-associated anemia, reticulocyte analysis
High-Performance Liquid Chromatography (HPLC)	Very High	Very High	Moderate–High	4–24 hours	Detection and quantification of hemoglobin variants and thalassemia
Polymerase Chain Reaction (PCR)	Very High	Very High	High	1–2 days	Identification of known genetic mutations in inherited anemias
Next-Generation Sequencing (NGS)	Excellent	Excellent	Very High	3–14 days	Comprehensive genomic analysis of congenital and unexplained anemias

9. Challenges and Limitations

Although diagnostic advances have been made, there remain a number of obstacles to the general adoption of more sophisticated technologies related to anemia diagnosis. Feasibility is one of the key challenges as access to diagnostic facilities, including specialized laboratories, molecular diagnostic platforms, and trained healthcare practitioners is limited, especially in LMICs. Traditional laboratory tests, including the complete blood count and peripheral blood smear, continue to be the mainstay of diagnosis in many resource-limited environments, and are often not sensitive enough or specific enough to diagnose complex or inherited anemias. The use of advanced diagnostics equipment, such as flow cytometers, high performance liquid chromatography (HPLC) systems, next generation

sequencing (NGS) platforms and mass spectrometry is also limited due to the high cost of this equipment. Besides the equipment costs, maintenance costs, availability of the reagents, quality assurance programs etc., add to the financial burden on health care institutions. Besides, many of these technologies demand highly trained laboratory personnel for sample preparation, instrument operation, data interpretation and quality control, in addition to staffing needs. One of the other limitations is the absence of standardized laboratory practices and agreed reference values for some of the emerging markers such as hepcidin, erythroferrone and growth differentiation factor-15 (GDF-15). The diverse methods of assay, calibration, and analytical platforms may result in different results from one lab to another. In addition, there is variation in the interpretation of laboratory results based on patient factors, concurrent inflammatory diseases, nutritional status and genetic background; therefore, thorough clinical correlation is essential. Novel biomarkers have shown great promise for diagnostics but are not widely commercially available nor are they routinely used for clinical testing; they also have relatively high testing costs. International standardization, increased accessibility, staff training and cost-effective diagnostic approaches will be necessary to overcome these barriers for the achievement of equitable and accurate diagnosis of anaemia globally.

10. Future Perspectives

The advent of laboratory medicine, molecular biology and computer technology are likely to contribute substantially to the diagnosis and management of anemia. The combination of several omics technologies (genomics, transcriptomics, proteomics, metabolomics and epigenomics) will enhance the understanding of disease mechanisms, the identification of new biomarkers and disease classification. High resolution analysis of cellular and molecular alterations is a new possibility to study erythropoiesis, bone marrow disorders and congenital anemias with emerging technologies, like liquid biopsy and single-cell sequencing. Meanwhile, AI-powered precision diagnostics will be expected to increase the accuracy of automated interpretation of lab results, aid in the prediction of disease and guide personalised treatment. Wearable hemoglobin monitoring devices, nanotechnology based biosensors and lab-on-a-chip systems can offer rapid, minimally invasive and point-of-care testing, especially in resource poor settings. In addition, the expansion of telehematology will allow for remote consultation and digital laboratory reporting, which will enhance specialist care. Overall, these technological advances are predicted to expedite the shift to personalized medicine, with the goal of earlier diagnosis, better therapeutic monitoring, and more patient-centered treatment of anemia.

11. Conclusion

Anemia continues to be a major health problem around the world and should be correctly diagnosed in the laboratory for clinical management. Conventional laboratory tests such as CBC, peripheral blood smear, iron studies, Vitamin B12 levels, folate levels, peripheral blood markers of hemolysis and bone marrow examination continue to be useful for diagnostic evaluation. Recent developments, such as the use of flow cytometry, HPLC, molecular diagnostics, next generation sequencing, new biomarkers and artificial intelligence, have greatly improved diagnostic accuracy and etiological classification. However, there are some challenges to be overcome, including cost, access, standardization and expertise, that could hinder the widespread use of this, particularly in low-resource settings. The integration of molecular diagnostics, AI, multi-omics technologies and portable diagnostics platforms into the future is likely to facilitate the diagnosis of anemia in a more rapid, accurate and personalized manner. Further studies and harmonization at the global level will be required to achieve optimal patient outcomes and to reduce the burden of anemia globally.

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