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Anemia: Challenges in Diagnosis and Discovery-An Updated Review

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Abstract:

Background: Anemia is a significant global health issue, characterized by a decrease in red blood cell count or hemoglobin concentration, leading to symptoms like fatigue and breathlessness. It is classified based on the underlying cause, severity, and morphology, with common causes including micronutrient deficiencies, infections, and genetic disorders. The World Health Organization (WHO) provides guidelines for anemia diagnosis and treatment, but challenges persist in its accurate diagnosis, particularly in low-resource settings. This paper focuses on the methodologies and challenges in anemia diagnosis, particularly hemoglobin (Hb) measurement, and explores other contributing factors like iron deficiency and genetic causes.

Aim: The aim of this review is to examine the methods, equipment, and quality control aspects in Hb measurement for anemia diagnosis, as well as to identify the challenges in addressing the underlying causes of anemia.

Methods: This paper reviews the different Hb measurement techniques, including spectrophotometric methods like the cyanmethemoglobin method, as well as alternative methods such as the hemoglobin color scale. It also addresses diagnostic challenges associated with sample handling, quality control, and the role of other factors in anemia diagnosis.

Results: The review highlights that while Hb measurement is the most common method for diagnosing anemia, it cannot pinpoint the underlying cause. Diagnostic methods vary in accuracy, and there are challenges in handling samples and ensuring precision in low-resource settings. Additional tests for micronutrient levels and genetic analysis are essential for accurate diagnosis.

Conclusion: Accurate anemia diagnosis requires effective Hb measurement and a comprehensive understanding of its underlying causes. Improved diagnostic strategies and better implementation of WHO guidelines are necessary to reduce anemia prevalence and guide effective interventions.

Keywords: Anemia, Hemoglobin measurement, Diagnosis, Micronutrient deficiency, Genetic causes, WHO guidelines.

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Introduction:

This paper has been developed as a contributing document for the Comprehensive Framework for Integrated Action on the Prevention, Diagnosis, and Management of Anemia, led by the World Health Organization (WHO). It represents one of five papers designed to offer insights into the diagnosis of anemia and its underlying causes with acceptable accuracy and precision for both individuals and populations, aiming to prioritize actions that reduce the prevalence of anemia. The remaining four papers address the objectives of the framework, including the determinants of anemia, preventive and therapeutic interventions, and the integrated management of anemia across the life course [1-4]. The objective of this paper is to examine the methods, equipment, and aspects related to sample handling and quality control in hemoglobin (Hb) measurement for anemia diagnosis. Given that Hb measurement alone cannot determine the cause of anemia, this paper also addresses other factors such as iron deficiency, infections, and genetic-related causes, while identifying key gaps and challenges in generating effective strategies for anemia prevention and control.

Definition and Classification of Anemia

Anemia is characterized by a decrease in the number of red blood cells (RBCs) or the hemoglobin concentration within them, below normal levels [5]. Clinically, anemia may present symptoms such as fatigue, palpitations, headache, and shortness of breath, along with signs such as conjunctival and palmar pallor. Although these signs are not highly sensitive and only moderately specific for diagnosing anemia, they remain useful when laboratory assessments are limited [6]. In clinical practice and public health, Hb concentration is the most commonly used indicator to diagnose anemia. Hb concentration is influenced by several factors including age, sex, pregnancy, health status, and genetic and environmental factors [9, 10]. For example, in newborns, normal Hb levels range from 170 to 210 g/L, which decrease over the first few months of life to around 100 g/L for infants aged 6-9 months [11]. These values then increase throughout childhood, reaching approximately 110 g/L up to 59 months, 115 g/L up to 11 years, and 120 g/L for adult females and 130 g/L for adult males [12-15]. Sex differences in Hb levels become apparent during puberty, as menstruation negatively affects iron stores in females, while testosterone enhances erythropoiesis in males [16, 17]. In pregnancy, blood volume expansion leads to dilution of Hb concentration during the first and second trimesters, with a gradual rise in the third trimester [4, 18-20]. Increased altitude and smoking can elevate Hb concentrations as a physiological adaptation to hypoxia, necessitating more Hb to compensate for reduced oxygen saturation, which stimulates erythropoietin production. WHO provides guidelines for adjusting Hb concentrations based on these factors to improve the accuracy of anemia prevalence estimates in affected populations.

Some studies suggest that race is a biological factor influencing Hb distribution, which could contribute to differences in anemia prevalence [21-24]. However, other studies argue against using race as a biological factor, highlighting that race is a social construct and that inequitable social determinants of health may explain these variations [25, 26]. Genetic studies have revealed that certain genetic variants are

linked to alterations in iron status, indicating that genetic ancestry may influence global variations in Hb concentrations and anemia prevalence [22].

However, genetic ancestry should not be equated with race, as race remains a social construct [27]. Consequently, some experts have called for the removal of race-based guidance in hematology, as phenotypic or self-identified race in clinical decisions may perpetuate existing racial health disparities [28]. WHO reports indicate that black individuals generally have lower Hb concentrations than white individuals (approximately 10 g/L), and these differences persist across age groups, regardless of health, nutrition, or socioeconomic factors [10]. However, other WHO documents do not consider race or genetic ancestry as factors [29]. Anemia can be classified by its underlying causes, such as increased red blood cell loss or reduced production. It may also be categorized as microcytic (e.g., iron deficiency or thalassemia), normocytic (e.g., inflammation), or macrocytic (e.g., vitamin B12/folate deficiency, liver disease, myelodysplasia, or hypothyroidism) [10]. Morphological characterization is typically used to support diagnosis and inform treatment, particularly in conjunction with laboratory results and clinical evaluation. However, this method is limited in determining the etiology of anemia. Anemia can also be classified based on its severity, either at the individual level or as a public health burden. In both cases, anemia is categorized as mild, moderate, or severe. The classification of anemia as a public health issue is outlined in the following:

Classification of Public Health Significance of Anemia Based on Prevalence Estimated from Hemoglobin Levels [29]:

- **Severe**: Prevalence of 40% or higher
- **Moderate**: Prevalence between 20.0% and 39.9%
- **Mild**: Prevalence between 5.0% and 19.9%
- **Normal**: Prevalence of 4.9% or lower

Hemoglobin Cutoffs To Define Anemia

The hemoglobin (Hb) thresholds used by the World Health Organization (WHO) to define anemia at the individual level were initially established in 1968, following technical meetings of an expert group consisting of clinical and public health professionals who reviewed the evidence available at the time. The evidence primarily consisted of data from five studies involving predominantly Caucasian populations in Europe and North America [29]. These cutoff values were slightly modified in 2001 and have since remained the official WHO thresholds [29]. The current guidelines set specific Hb concentration ranges for diagnosing anemia across different age and population groups. For instance, in children aged 6-59 months, Hb values of 110 g/L or higher are considered normal, values ranging from 100-109 g/L are classified as mild anemia, 70-99 g/L as moderate anemia, and values below 70 g/L as severe anemia. Similarly, for children aged 5-11 years, normal Hb levels are defined as 115 g/L or higher, with mild anemia ranging from 110-114 g/L, moderate anemia from 80-109 g/L, and severe anemia below 80 g/L. For other groups, such as nonpregnant women aged 15 years and older, a normal Hb level is 120 g/L or higher, with mild anemia defined between 110-119 g/L, moderate anemia between 80-109 g/L, and severe anemia below 80 g/L. Pregnant women have slightly different thresholds, with normal Hb levels defined as 110 g/L or higher, mild anemia from 100-109 g/L, moderate anemia from 70-99 g/L, and severe anemia below 70 g/L. For men aged 15 years and older, the normal Hb level is 130 g/L or higher, with mild anemia between 110-129 g/L, moderate anemia between 80-109 g/L, and severe anemia below 80 g/L. WHO is currently updating its guidelines for the use and interpretation of Hb concentrations for both individual and population-level diagnoses of anemia. This process adheres to the established WHO guideline development procedures and aims to produce an updated guideline for clinical and public health applications. Any revisions to these cutoffs will be crucial, not only for accurately identifying individuals with anemia but also for determining public health challenges within populations, facilitating interventions, and evaluating the performance and comparability of existing Hb measurement tests.

Causes Of Anemia

The World Health Organization (WHO) has outlined a range of determinants contributing to anemia, categorized into biological, infection- and inflammation-related, genetic, and social, behavioral, and environmental factors [10]. Among the biological causes, micronutrient deficiencies, particularly iron deficiency, are the most prevalent. Iron deficiency can either be absolute, where the body's iron stores are insufficient to meet iron requirements, or functional, where iron is available but its utilization is impaired due to reduced mobilization and absorption aimed at limiting its access to pathogenic organisms. Both forms of iron deficiency can coexist within individuals or populations [32]. Other less common micronutrient deficiencies contributing to anemia include those of vitamins A, B2, B6, B9, B12, C, D, and E, as well as copper and zinc. Such deficiencies may arise when the intake of micronutrients is insufficient to meet bodily demands over time, particularly in situations involving low consumption, poor bioavailability, excessive inhibitors or inadequate enhancers of absorption, increased physiological demands (e.g., during rapid growth phases such as infancy, adolescence, or pregnancy), and/or elevated losses [33].

In addition to nutritional factors, non-nutritional causes of anemia include inflammation induced by infections such as tuberculosis, malaria, and HIV, as well as noninfectious causes like cancer, organ failure, and autoimmune diseases. Parasites such as hookworm and schistosomiasis also contribute to anemia through blood loss. Genetic disorders such as thalassemia, sickle cell disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and red blood cell membrane disorders are further contributors to anemia. The upstream risk factors, which are often environmental or related to support systems, include poor sanitation, unsafe drinking water, inadequate personal hygiene, economic disparities, political instability, limited institutional capacity and resources, and unfavorable climatic or environmental conditions [10, 34]. Other upstream factors that significantly impact anemia, particularly in women, include poverty, obesity, low educational levels, poor household wealth, cultural norms, lack of empowerment, rural residency, insufficient healthcare access, inadequate nutrition knowledge, improper health policies, limited access to healthcare services, and insufficient maternal and childcare practices. Additionally, the vulnerability of women and children, which may be influenced by early childbirth, high parity, and short birth spacing, further exacerbates the risks of anemia [33].

Diagnosing Anemia

The availability of accurate, reliable, acceptable, and cost-effective diagnostic tools for anemia and its primary determinants is crucial for understanding the prevalence and distribution of the condition and for formulating appropriate interventions aimed at prevention and treatment [2][3][4]. Anemia is most frequently diagnosed by measuring the hemoglobin (Hb) concentration in blood. However, other approaches can be utilized, including assessing hematocrit (packed cell volume), and more specifically, red blood cell (RBC) parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and reticulocyte count. Additional diagnostic indicators include blood film examination, Hb electrophoresis (or high-performance liquid chromatography), micronutrient biomarker measurements, the Hb color scale, and clinical manifestations [35][36]. The selection of diagnostic markers varies, as each tool identifies different individuals with anemia due to their varying measurement of metabolites or biological processes.

Measuring Hb

Ideally, Hb measurement should be conducted in well-equipped clinical laboratories. Hb determination methods primarily rely on the spectrophotometric properties of Hb or its derivatives, such as cyanmethemoglobin, considered the gold standard for Hb estimation [9][37][38]. Numerous alternative methods are available, relying on the color of Hb, such as the hemoglobin color scale, the Sahli method, the Lovibond–Drabkin technique, and the Tallqvist technique. Additionally, specific gravity-based methods, which compare blood samples to a copper sulfate solution, exist. Each method operates on distinct principles and offers unique advantages and limitations. The most frequently used Hb measurement

techniques are summarized as follows: these methods, excluding noninvasive ones, can utilize arterial, venous, or capillary blood samples.

The cyanmethemoglobin method is the gold standard for Hb determination, being inexpensive, stable, and internationally recognized with a reference standard calibrator. However, potassium cyanide used in this method is toxic in high concentrations, complicating its disposal. In this process, blood is mixed with Drabkin's solution—a combination of potassium cyanide, potassium ferricyanide, and potassium dihydrogen phosphate. Erythrocytes are lysed, and Hb is released. Potassium ferricyanide then converts Hb to methemoglobin, which reacts with potassium cyanide to form cyanmethemoglobin. The absorbance at 540 nm is measured spectrophotometrically, and the Hb concentration is calculated by comparison with a standard cyanmethemoglobin solution. This technique quantifies most Hb derivatives, including oxyhemoglobin, methemoglobin, and carboxyhemoglobin (excluding sulfhemoglobin) [39]. Automated hematology analyzers often utilize the cyanmethemoglobin method or generate other Hb derivatives, determining their absorbance at wavelengths specific to the Hb derivative. Some analyzers, employing sodium lauryl sulfate (SLS) instead of cyanide reagents, offer less stable solutions, although these analyzers maintain high precision, robust quality control, and calibration using commutable reference standards, making them preferred for Hb measurement.

The alkaline hematin method involves the use of cetrimide, which lyses erythrocytes and precipitates Hb. The resulting conjugate with boronic acid forms a red precipitate that is measured at 540 nm [40]. The WHO Hemoglobin Color Scale, used to estimate Hb concentration from a drop of blood, consists of a card with six shades of red that correspond to various Hb levels (40, 60, 80, 100, 120, and 140 g/L). This method is more precise than clinical examinations of the conjunctiva or nail beds and is cost-effective [41][42], but should only be employed when more accurate devices are unavailable. Variants of color scale methods include the Lovibond–Drabkin, Tallqvist, and Sahli methods. The Lovibond–Drabkin technique measures cyanmethemoglobin, comparing the color of blood to a standard color disc [43]. The Tallqvist method requires placing a blood drop on blotting paper and interpreting the Hb concentration by comparing the color to standards on the paper [37]. Sahli's method involves hydrochloric acid converting Hb to acid hematin, which is then diluted until the color matches the comparator block. This method, which is inexpensive and does not require electricity, uses only 20 μL of blood, and results are obtained within three minutes of sample addition [44].

The copper sulfate method, based on blood's specific gravity, involves placing a blood droplet into a copper sulfate solution with a specific gravity equivalent to that of blood containing known Hb concentration. The method determines Hb concentration by comparing the sample's specific gravity to a copper solution of specific gravity 1.053, which corresponds to an Hb level of 125 g/L. This method was once used for blood donor screening. While nonspectrophotometric methods are increasingly infrequent, advances in technology have led to the development of noninvasive devices for detecting Hb concentrations. These technologies, which rely on pulse oximetry or white light to capture transmission data from tissue capillaries, are still in the experimental phase, and their performance in clinical and population settings requires further validation and research [45][46]. Noninvasive transcutaneous pulse co-oximetry devices have been developed to measure total Hb and its components (oxy-, carboxy-, and methemoglobin). One such device, from Masimo Corp, provides continuous (Radical-7™) and intermittent (Pronto-7™) measurements, utilizing distinct algorithms. The Radical-7 additionally estimates carboxy- and methemoglobin levels. Another innovative technology, occlusion spectroscopy, involves a ring-shaped sensor attached to the finger. This sensor temporarily stops blood flow, producing an optical signal that provides a high signal-to-noise ratio for estimating Hb concentration.

Equipment for Hemoglobin Determination

Automated hemoglobin (Hb) analyzers are extensively utilized for examining blood composition, cell morphology, hematocrit, and Hb concentrations. These devices offer superior accuracy and precision, delivering results in a fraction of the time when compared to traditional manual methods [11]. In addition to determining Hb levels, automated hematology analyzers can assess red blood cell (RBC) size and count,

as well as quantify other blood cells, such as white blood cells and platelets, thereby generating a complete blood count (CBC) that aids in diagnosing the etiological causes of anemia, along with various diseases and genetic conditions. However, the initial investment, ongoing maintenance costs, and the necessity for trained laboratory personnel to operate such equipment can restrict its widespread use, particularly in field research settings.

In contrast, for field studies, emergency situations, or environments with limited resources, portable analyzers, often referred to as point-of-care (POC) devices, such as hemoglobinometers or field photometers, are commonly employed. These devices are compact, user-friendly, and relatively cost-effective, requiring only a small sample of capillary or venous blood. They are also advantageous in that they do not need refrigeration or electricity and provide immediate digital readings of Hb levels. The HemoCue® device is widely used in field settings for Hb measurements. It generates an immediate numerical Hb value from various blood types, including venous, capillary, or arterial. Blood is placed into a cuvette, which undergoes a chemical reaction converting Hb to azide-Hb. The concentration is then measured using absorption photometry at two wavelengths (570 nm and 880 nm). This device, first introduced in the mid-1980s, was enhanced in 2002 with the Hb 201+ system, and subsequent versions, including the HemoCue 301 and 801 systems developed after 2008, enabled Hb detection in the same blood samples without requiring the chemical conversion.

The HemoCue 301 operates by quantifying the absorbance of both oxygenated and deoxygenated Hb, while compensating for turbidity at a wavelength of 880 nm. Hb concentration is estimated by measuring the absorbance of whole blood at the isosbestic points for Hb/HbO2, specifically at 506 nm and 880 nm, to account for turbidity. This system requires just 10 μL of blood and delivers results in approximately 3 seconds. It functions within a temperature range of 10 to 40°C, measuring Hb concentrations from 0 to 25.6 g/dL. The HemoCue Hb 801, a more recent model, operates similarly but delivers results in less than one second. It is particularly useful for rapid diagnosis at the patient's bedside (https://www.hemocue.us/wp-content/uploads/2021/02/MMUS-01244-Hb-301-Product-Profile.pdf).

The precision of these POC analyzers, when using venous blood samples from the same individual, exhibits a wider confidence interval (95% CI width of 5 g/L) compared to automated analyzers, which tend to have a narrower CI (95% CI width of <3 g/L). This discrepancy can be attributed to factors such as the smaller blood volume (10 μL versus 200 μL) and potential errors in loading the microcuvettes. However, when using capillary blood samples, especially those obtained via finger pricks, the variation in Hb readings increases significantly, with a CI width exceeding 12 g/L, even with experienced personnel. This issue has been acknowledged in other studies [47, 48, 49]. Furthermore, a systematic bias of up to 4 g/L has been identified across different devices, irrespective of the model, which introduces significant errors in Hb measurements, both in individual and population assessments. To address this, the USAID-Advancing Nutrition project is conducting an intercountry study [50] aimed at refining procedures to enhance Hb measurement accuracy using field photometers.

Extensive research has been conducted to assess the performance of HemoCue devices. Studies have consistently shown that the Hb-301 system produces higher Hb readings than the Hb-201+, [51], and when comparing capillary blood samples analyzed by HemoCue devices with venous blood samples tested by automated hematology analyzers or cyanmethemoglobin reference methods [38, 52-54]. Discrepancies between study results can be attributed to several factors, including measurement errors, the blood sampling site (capillary versus venous blood), analytical setting (laboratory versus field), and population characteristics (e.g., healthy adults, children, pregnant women, or patients with illnesses). The World Health Organization (WHO) recommends the use of automated hematology analyzers or hemoglobinometers for diagnosing anemia in pregnant women, especially in settings where a full blood count is not available. Screening using hemoglobinometers is preferred over the hemoglobin color scale, as research suggests the latter is less effective in detecting severe anemia in pregnant women, and the consequences of missing severe anemia are more critical than failing to detect mild or moderate anemia [33, 55].

Another reagent-free device, the DiaSpect, measures Hb using broad-spectrum photometry. The DiaSpect technology employs a white light-emitting diode (LED) to illuminate the blood sample, with an optical sensor detecting the absorbance across a broad wavelength range. This results in a more specific measurement with reduced sensitivity to interference [www.ekfdiagnostics.com]. A 2021 study by Young et al. [46] evaluated the performance of noninvasive devices (smartphone applications and the Masimo Pronto) alongside POC analyzers like the HemoCue Hb-301 and Hb-801, comparing them to a gold-standard hematology analyzer using venous blood. Noninvasive devices showed poor correlation with reference Hb, while the HemoCue system demonstrated a stronger correlation. The authors concluded that the diagnostic accuracy of the HemoCue devices was comparable to the reference method, whereas noninvasive devices, though highly acceptable to users, exhibited considerable biases. Given the need for reliable, reproducible, and affordable equipment for rapid Hb quantification in both field conditions and clinical practice, particularly for simultaneous detection of additional markers such as inflammation, malaria, iron, or other nutritional deficiencies, further research and development in this area are crucial.

Preanalytical or Sample-Related Aspects of Hb Measurement

Hemoglobin (Hb) can be quantified using venous, arterial, or capillary blood samples. In controlled environments, such as clinical laboratories or hospitals, automated hematology analyzers are typically employed for this purpose, as they provide high accuracy and reliability. However, these devices are expensive and not suitable for use in field settings. In most cases, the measurement of Hb concentration in venous blood using the methemoglobin method, either manually or with automated analyzers, is the most common approach for estimating Hb levels and diagnosing anemia [29]. In contrast, in field or remote settings, various factors may affect the accuracy and reliability of Hb measurements. These factors are particularly critical in population-based Hb assessments and anemia surveys, which are often conducted in areas with limited laboratory infrastructure. Environmental influences, such as extreme temperatures, high humidity, or geographical challenges, can impact the precision of Hb determination. Furthermore, logistical challenges in transporting samples, particularly ensuring the integrity of cold chain protocols, can further complicate the process. Social and cultural beliefs regarding blood sampling can also influence the collection of samples, irrespective of whether the measurements are performed in clinical or field settings. These cultural considerations must be taken into account to ensure compliance and reliable data collection.

An analysis of 11 studies, involving diverse populations (children, men, non-pregnant women, and pregnant women) from seven countries (Cambodia, India, The Gambia, Ghana, Laos, Rwanda, and the United States), investigated variables such as the blood sampling site (capillary versus venous), the equipment used (HemoCue versus automated hematology analyzers), and the specific models of the HemoCue device (201+ versus 301). The study revealed significant variability in Hb concentrations when comparing capillary and venous blood samples and when using different devices (HemoCue Hb 201+ or Hb 301 versus automated analyzers) [56]. Substantial biases and imprecision have been reported when comparing capillary and venous blood samples for Hb measurement. This underscores the need for standardized and harmonized methodologies, particularly for field-based population surveys, where capillary blood samples are often utilized for anemia prevalence assessments [57]. Alternatively, using venipuncture as the preferred blood source could help reduce these biases. Studies have consistently found that capillary Hb concentrations were significantly higher than venous Hb concentrations when measured using the same detection devices, such as HemoCue, or when comparing HemoCue results with those obtained from automated hematology analyzers [58].

A study comparing Hb distributions across different population-based surveys, which were matched by country and time, analyzed data from 17,719 children (aged 6–59 months) and 21,594 non-pregnant women (aged 15–49 years). These surveys measured Hb concentrations using capillary (DHS) or venous (BRINDA) blood, with significant differences observed in anemia estimates between the two methods. Three out of the four countries studied showed substantial variations in anemia prevalence estimates, with discrepancies ranging from 1 to 31 percentage points. The BRINDA surveys consistently reported lower anemia estimates compared to DHS, both for children and women [59]. The USAID

Advancing Nutrition project, mentioned previously, is investigating the most effective procedures for determining Hb concentrations in population surveys. This project involves a comparison of three HemoCue device models (201+, 301, and 801) against a certified automatic analyzer, using venous blood samples from women of reproductive age and children under 5. It also aims to evaluate the performance of these devices when using pooled capillary blood or capillary blood drops (third drop) compared to venous blood samples [50].

Quality Control of Hb Determinations

To ensure the reliability of Hb measurements, quality control procedures must be established across the preanalytical, analytical, and postanalytical phases. Key aspects to address include patient preparation, personnel training, adherence to international calibration standards, equipment calibration or validation methods, blood collection techniques, and the monitoring of data collection. It is also critical to ensure accurate result recording, regular cleaning and maintenance of equipment, and reproducibility of results, all in accordance with international laboratory quality control standards [9, 54, 60].

In conclusion, the optimal method for Hb determination is the use of venous blood samples, analyzed with automated hematology analyzers, accompanied by robust quality control measures. Although these practices are recommended, practical constraints, such as logistics and cost, may limit their implementation in field-based population surveys, potentially resulting in smaller sample sizes than those typically employed in such studies. When these optimal practices are not feasible, the use of an approved point-of-care (POC) device, calibrated to international standards, could be considered as an alternative, provided that venous blood is used. However, if capillary blood is required, using pooled drops from a single capillary blood draw may reduce variability and yield results more consistent with those obtained from venous blood. There is a need for more detailed methodologies to improve the collection of pooled capillary blood samples. In all instances, the blood source, method of sample collection, and the specific Hb determination method used should be clearly stated in any report on anemia, whether at the individual or population level. Additionally, the report should detail the quality control and assurance measures implemented. It is important to recognize that data derived from different blood sources or methods may not be directly comparable at the individual or population level.

Diagnosing the Underlying Causes of Anemia

Diagnosing Iron-Related Causes

While measuring hemoglobin (Hb) levels is essential for diagnosing anemia, Hb concentration alone is insufficient for determining its underlying cause. For instance, in cases of iron deficiency anemia, Hb levels do not provide an accurate indication of iron status. To assess iron deficiency, additional tests are required, such as measuring serum ferritin or soluble transferrin receptor (sTfR), which are the most commonly used markers. Other indicators include total iron-binding capacity, transferrin saturation, zinc protoporphyrin concentration, reticulocyte Hb, erythrocyte protoporphyrin concentration, or, in some instances, bone marrow biopsy. However, the use of these biomarkers in population-level or field studies may be impractical due to their associated costs, equipment requirements, maintenance, and the need for specialized training and laboratory staff [34].

Ferritin concentration serves as an indicator of iron stores but may be misleading in cases of concurrent infection or inflammation. Although ferritin decreases in iron deficiency, it can also increase in response to inflammation, making its interpretation in such contexts complex. To address this, the presence of infection or inflammation must be identified, often through acute-phase proteins such as C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP). These proteins help differentiate between inflammation and iron deficiency, as they elevate for different durations compared to ferritin. The World Health Organization (WHO) updated ferritin concentration cutoffs in 2020 to refine the definition of iron deficiency, incorporating adjustments for infection and inflammation [61]. For monitoring the effects of interventions at the population level, WHO recommends the use of both serum ferritin and Hb, in conjunction with indicators of inflammation, to assess iron status [62]. Serum sTfR concentration provides

a semi-quantitative assessment of iron deficiency, with the advantage of being less influenced by inflammation than ferritin. However, sTfR concentrations can increase in cases of accelerated erythropoiesis, as seen in hemolysis or ineffective erythropoiesis. The sTfR:SF ratio can offer valuable insights into iron status, distinguishing between individuals with iron deficiency, normal iron balance, and elevated iron stores [63].

In 2011, Lynch [64] introduced additional iron status indicators, including serum iron, transferrin saturation, and red blood cell (RBC) zinc protoporphyrin (RBC ZPP), which reflect the adequacy of iron for RBC production. In iron deficiency, serum iron and transferrin saturation decrease, while RBC ZPP levels rise. However, elevated RBC ZPP can also occur in inflammatory conditions and lead exposure. In clinical practice, a complete blood count (CBC) is typically performed using an automated hematology analyzer, which measures Hb and hematocrit, along with RBC indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), as well as total and differential white blood cell counts, platelet counts, and the reticulocyte index. The CBC is a valuable tool for diagnosing anemia, infections, and certain cancers. The reticulocyte count provides an estimate of RBC output from the bone marrow, and a blood smear can help assess RBC morphology. Other diagnostic tests may include bone marrow biopsy, hemoglobin electrophoresis for hemoglobin variants (e.g., HbS), beta-globin gene disorders (e.g., thalassemia), serum creatinine, erythropoietin levels, liver function tests, coagulation profiles, or hemolysis assessments [65, 66]. In iron deficiency anemia, the MCV, MCH, and MCHC are typically reduced, while RBC distribution width is increased. Reticulocyte Hb concentration, which measures iron availability to RBCs recently released from the bone marrow, is widely available on modern automated analyzers and is reduced in cases of iron deficiency [15].

To enhance the accuracy of iron deficiency estimates in population studies, combinations of these indicators may be necessary. However, the high cost of many of these tests may limit their feasibility for widespread use [64, 67]. The measurement of hepcidin concentration is emerging as a promising method for distinguishing between absolute and functional iron deficiency. Low hepcidin levels indicate a physiological need for iron and predict a positive response to iron supplementation, enabling personalized treatment plans. Mass spectrometry and immunochemistry-based methods have been developed to quantify hepcidin, although results may vary significantly across different measurement techniques. For hepcidin testing to be effective in clinical practice and research, comparability and analytical reliability are essential. The use of calibration materials that are commutable with human plasma or serum will facilitate standardization and enable routine hepcidin testing. Recent advancements, such as the development of a two-level secondary reference material for hepcidin assays, hold promise for global standardization of these tests [68].

Diagnosing Infectious Causes

The diagnosis of anemia of inflammation is characterized by normocytic and normochromic anemia, meaning a normal mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). This condition is typically seen in patients who exhibit signs of systemic inflammation, such as an elevated erythrocyte sedimentation rate or C-reactive protein (CRP) levels, alongside evidence of iron restriction that is not attributable to systemic iron deficiency. Specifically, low transferrin saturation coupled with elevated serum ferritin levels suggests anemia of inflammation. A significant challenge in diagnosing the underlying cause lies in the frequent coexistence of absolute iron deficiency and anemia of inflammation. This is especially prevalent in patients suffering from blood loss due to underlying diseases, as well as in those with iron deficiency arising from malnutrition, prolonged inflammation, or increased iron demands in growing children or pregnant women [69, 70]. The complexity of iron metabolism and its impact on various biomarkers often leads to misdiagnosis. Commonly, anemia of chronic disease is mistaken for iron deficiency, as laboratory features may include low serum iron, normal iron binding capacity, and normal or high ferritin, complicating the accurate interpretation of iron indices.

Malaria remains one of the leading global causes of anemia. In 2020, an estimated 241 million malaria cases occurred worldwide, with 95% of these in the World Health Organization (WHO) African

Region [71]. Iron deficiency is highly prevalent in malaria-endemic regions, and the relationship between iron deficiency and malaria is intricate. The WHO currently recommends administering iron supplements alongside public health measures for malaria prevention, diagnosis, and treatment [75]. Malaria can be diagnosed through various techniques, such as microscopic examination of stained blood smears, rapid diagnostic tests, or molecular methods like polymerase chain reaction (PCR). Additionally, emerging diagnostic methods include loop-mediated isothermal amplification, nucleic acid sequence-based amplification, and saliva-based tests for Plasmodium protein detection. Rapid diagnostic tests are particularly advantageous due to their ease of use, speed, and low cost. However, tests detecting histidine-rich protein 2 (HRP2) may remain positive for extended periods due to antigen persistence, and the recent spread of pfhrp2/3 gene deletions raises the risk of false-negative results. Consequently, the development of point-of-care (POC) devices that can simultaneously diagnose malaria, anemia, and potentially iron deficiency is of considerable interest for large-scale population studies [76].

Diagnosing Genetic Causes

Genetic variation plays a pivotal role in determining hemoglobin (Hb) concentrations and the risk of anemia. The most prominent and widely occurring genetic variants affecting Hb are found within the HBA1, HBA2, and HBB genes, which are primary contributors to anemia globally [77]. Inherited hemoglobin disorders, particularly thalassemia and, to a lesser extent, sickle cell trait, represent some of the leading causes of anemia worldwide. Approximately 5% of the global population is estimated to carry a significant hemoglobin variant, with the highest prevalence observed in Africa (18%) and Asia (7%). These genetic disorders, including sickle cell anemia and various forms of thalassemia, continue to increase in prevalence, particularly in regions where the incidence of these conditions is rising. Annually, around 330,000 children are born with serious inherited hemoglobin disorders, with over 80% of these births occurring in low- and middle-income countries [10, 78, 79].

The diagnostic process for thalassemia involves a complete blood count (CBC), reticulocyte count, and hemoglobin electrophoresis or related methods for diagnosing beta thalassemia or sickle cell disease. Genetic testing may also be employed for identifying alpha thalassemia. To diagnose sickle cell anemia or detect the sickle cell trait, it is essential to assess the presence and relative concentration of Hb S in a blood sample, typically through hemoglobin electrophoresis, or by identifying mutations in the hemoglobin genes. Point-of-care analyzers are available that can concurrently diagnose anemia and sickle cell disease/trait [80, 81]. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked enzyme deficiency that affects over 400 million people globally, with a significant prevalence in malaria-endemic areas. Although many individuals remain asymptomatic, this deficiency can lead to neonatal jaundice, chronic congenital hemolytic anemia, and acute hemolytic anemia. The latter is triggered by oxidative damage in red blood cells (RBCs), which may occur due to the ingestion of fava beans (known as “favism”), certain medications, or infections. The diagnosis is typically made through phenotypic tests that assess G6PD enzyme activity in blood samples. A diagnosis is often suspected in males from regions such as Africa, Asia, the Middle East, and the Mediterranean who have a family history of G6PD deficiency, or in cases where there is sudden onset of intravascular hemolysis and characteristic morphological changes in RBCs, such as the presence of “bite” or “blister” cells.

In summary, the diagnosis of anemia begins with the measurement of hemoglobin concentration. Subsequently, additional hematological indices can help pinpoint the underlying causes and guide further diagnostic efforts. These may include tests for renal or hepatic disease, cancer, parasitic infections (including malaria), HIV, tuberculosis, malabsorptive conditions, micronutrient deficiencies, and genetic disorders. From a population-level perspective, understanding factors such as socioeconomic conditions, the prevalence of parasitic infections (including malaria), genetic hemoglobin variants, nutritional status, rates of wasting and stunting, infant mortality, food consumption patterns, environmental pollution, and water and sanitation conditions can help identify the root causes of anemia. Addressing these conditions from a public health standpoint involves diagnosing and managing iron deficiency, malaria, and genetic variations that affect Hb concentrations, all of which are crucial for mitigating the burden of anemia.

Conclusion:

Anemia remains a pressing global health challenge, significantly affecting populations worldwide, particularly vulnerable groups such as pregnant women, children, and those in low-income settings. The accurate diagnosis and understanding of anemia's underlying causes are critical for effective prevention and treatment strategies. This paper examined the methods used in hemoglobin (Hb) measurement, the gold standard for diagnosing anemia, while highlighting the limitations and challenges in the accurate diagnosis of anemia, particularly in resource-limited settings. Hemoglobin measurement alone, while central to anemia diagnosis, cannot identify the specific cause of anemia. Other contributing factors, including iron deficiency, infections, and genetic disorders, must also be considered. For instance, iron deficiency anemia, the most common form, is influenced by nutritional intake, environmental factors, and underlying health conditions. Infections such as malaria, tuberculosis, and HIV, along with parasitic infestations like hookworm and schistosomiasis, also contribute significantly to anemia, particularly in tropical regions. Additionally, genetic disorders such as thalassemia and sickle cell disease play a critical role in the global prevalence of anemia. The review underscored the need for accurate, cost-effective diagnostic tools that go beyond simple Hb measurements. Methods like the cyanmethemoglobin test and alternatives such as the hemoglobin color scale and automated analyzers each have their strengths and limitations. In addition, a more comprehensive diagnostic approach, integrating other tests for micronutrients and genetic markers, is necessary to provide a holistic understanding of anemia's etiology. To address these challenges, WHO's guidelines on anemia diagnosis must be continuously updated and implemented rigorously. By refining diagnostic methods and incorporating additional factors, public health systems can better identify and manage anemia, ultimately reducing its global burden. Moreover, improving access to healthcare, education, and proper nutrition is crucial in the prevention and control of anemia. In conclusion, combating anemia requires not only accurate diagnostics but also comprehensive interventions that address its multifaceted causes, from micronutrient deficiencies to genetic factors and environmental influences.

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الملخص:

الخلفية: فقر الدم هو مشكلة صحية عالمية هامة، يتميز بانخفاض عدد خلايا الدم الحمراء أو تركيز الهيموغلوبين، مما يؤدي إلى أعراض مثل التعب وضيق التنفس. يُصنف فقر الدم استنادًا إلى السبب الكامن وراءه، وشدته، وتكوينه، وتشمل الأسباب الشائعة نقص المغذيات الدقيقة، العدوى، والاضطرابات الوراثية. تقدم منظمة الصحة العالمية (WHO) إرشادات لتشخيص فقر الدم وعلاجه، لكن التحديات لا تزال قائمة في التشخيص الدقيق، خصوصًا في المناطق ذات الموارد المحدودة. تركز هذه الورقة على الأساليب والتحديات في تشخيص فقر الدم، خصوصًا قياس الهيموغلوبين(Hb) ، وتستكشف العوامل المساهمة الأخرى مثل نقص الحديد والأسباب الوراثية.

الهدف: الهدف من هذه المراجعة هو دراسة الأساليب، والأدوات، وجوانب مراقبة الجودة في قياس الهيموغلوبين لتشخيص فقر الدم، بالإضافة إلى تحديد التحديات في معالجة الأسباب الكامنة وراء فقر الدم.

الأساليب: تستعرض هذه الورقة تقنيات قياس الهيموغلوبين المختلفة، بما في ذلك الطرق الطيفية مثل طريقة السيانميتهيموغلوبين، بالإضافة إلى الطرق البديلة مثل مقياس لون الهيموغلوبين. كما تتناول التحديات التشخيصية المرتبطة بمعالجة العينات، ومراقبة الجودة، ودور العوامل الأخرى في تشخيص فقر الدم.

النتائج: تشير المراجعة إلى أنه على الرغم من أن قياس الهيموغلوبين هو الطريقة الأكثر شيوعًا لتشخيص فقر الدم، إلا أنه لا يمكنه تحديد السبب الكامن. تختلف طرق التشخيص في دقتها، وهناك تحديات في معالجة العينات وضمان الدقة في الأماكن ذات الموارد المحدودة. كما أن الفحوصات الإضافية لمستويات المغذيات الدقيقة والتحليل الوراثي ضرورية لتشخيص دقيق .

الخلاصة: يتطلب تشخيص فقر الدم الدقيق قياسًا فعالًا للهيموغلوبين وفهمًا شاملاً لأسبابه الكامنة. هناك حاجة إلى تحسين الاستراتيجيات التشخيصية وتنفيذ أفضل لإرشادات منظمة الصحة العالمية لتقليل انتشار فقر الدم وتوجيه التدخلات الفعالة.

الكلمات المفتاحية: فقر الدم، قياس الهيموغلوبين، التشخيص، نقص المغذيات الدقيقة، الأسباب الوراثية، إرشادات منظمة الصحة العالمية.