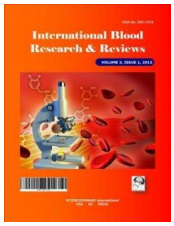

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Approach to Anaemia Diagnosis in Developing Countries: Focus on Aetiology and Laboratory Work-Up

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Introduction: Anaemia is a significant public health problem in developing countries. Anaemia is never normal. The etiology of the anaemia should always be sought. Diagnosis of its cause and early treatment is crucial to improving the quality of life among affected persons. There is a need to provide practicing physicians with a good theoretical framework and a practical algorithm for arriving accurately at anaemia diagnosis.
Objective: This article seeks to collect, collate and concisely review anaemias with emphasis on the prevalent aetiologies and laboratory diagnosis in developing countries
Results: The etiology of anaemia in developing countries is myriad and requires accurate diagnosis. Nutritional (substrate) deficiencies and chronic diseases account for a significant proportion of acquired anaemias. The predominating inherited causes include haemoglobinopathies, red cell enzymopathies and membranopathies. A systematic approach will help the physician paddle through the large list of differentials, to cone down on precise diagnosis. Relevant clinical history, physical examination and baseline investigations are imperative. Further

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evaluations should be conducted in unresolved cases using suggested practical algorithms such as the morphologic and/or kinetic approach.
Conclusion: Baseline investigations including full blood count, reticulocyte count and peripheral blood film should be requested on patients presenting with anaemia. Relevant authorities should ensure availability of these basic tests in all health facilities. Consultations with hematology unit should be engaged when necessary.

Keywords: Anaemia; diagnosis; laboratory work-up; anaemia work-up; developing countries.

1. INTRODUCTION

The term, 'anaemia' is derived from two ancient Greek words, 'an' meaning 'without' and 'haima' meaning 'blood'. Literally, anaemia means bloodlessness or low blood level. Technically, anaemia describes a condition in which an individual's hemoglobin level (or hematocrit) falls two standard deviations below the average mean of normal for individuals of same age, sex and altitude [1,2]. The functional consequence of anaemia is decreased oxygen carrying capacity of the blood and general tissue hypoxia. Anaemia itself, is not a diagnosis, but rather a feature of an underlying disease.

The causes of anemia may be categorized in terms of patient's red cell appearance or size (cytometric or morphologic classification), the underlying patho-physiologic mechanism (aetiologic or erythrokinetic or biologic classification), marrow responsiveness or based on its biochemical or molecular basis [3]. Based on red cell morphology, anaemia may be described as microcytic-hypochromic, normocytic-normochromic or normochromic-macrocytic. From the kinetic stand-point, anaemias are grouped as anaemia of blood loss (haemorhage), haemolytic anaemia or anaemia of bone marrow failure. Based on marrow response to anaemia, anaemia may be grouped as hypo-regenerative (reticulocyte count < 50,000/ul), normo-regenerative (reticulocyte count between 50,000 and 100,000/ul), hyper-regenerative (> 100,000/ul) [4]. Normo-regenerative anaemia may often be difficult to diagnose and are often due to multiple aetiologies [4].

Undoubtedly, anaemia is the most common haematology laboratory feature among patients [4,5]. More than 90% of patients with primary haemopathies present with anaemia [5]. Anaemia is a feature of many topical diseases including Human immunodeficiency virus/Acquired Immunodeficiency syndrome (HIV/AIDS), malaria, and tuberculosis, parasitic infections such as schistosomiasis and hookworm infestations.

Making early and accurate diagnosis of tropical diseases, as well as prompt treatment decisions is related to the physician's ability to properly investigate anaemia. It is imperative for physicians, especially those in developing countries where the burden of anaemia is highest [4], to be equipped with requisite knowledge on how to investigate anaemia. Therefore, the objective of this article is to provide a general overview of anaemia, its causes and laboratory evaluation especially as it relates to developing countries. Relevant standard texts as well as journal articles on major databases including google scholar and pubmed were accessed, collated and summarized as appropriate sections.

2. ANAEMIA DEFINITIONS, DETERMINATION AND EPIDEMIOLOGY

The diagnosis of anaemia is established by low haemoglobin levels, haematocrit or reduced number of circulating red cells. In clinical practice, often times, anaemia is defined as blood haemoglobin concentration or haematocrit below established cut-offs. The lower cut-offs for definition of anaemia differs from individual to individual based on age, sex, geographical location, pregnancy status, smoking, altitude and ethnicity [6]. As such, there is need for clear definitions and reference intervals. According to the World Health Organisation (WHO), for non-smoking, non-African extraction individuals living at an altitude below 1000 meters, anaemia categories are presented in Table 1 [6-10].

According to Centre for Disease control and Prevention (CDC), anaemia in pregnancy is defined by Hb less than 11 g/dl in the first and third trimesters, and 10.5 g/dl in the second trimester [11]. Some authorities consider very severe anaemia to be Hb value less than 4 g/dl

Table 1. Who anaemia categories (haemoglobin cut-offs in g/dl)

Age groups:
Adult males above 15 years: Less than 13 g/dl
Non-Pregnant females above 15 years: Less than 12 g/dl
Teens aged 12 to 14.99 years: Less than 12 g/dl
Children aged 5 to 11.99 years: Less than 11.5 g/dl
Children aged 6 months to 4.99 years: Less than 11 g/dl
Pregnant women: Less than 11 g/dl
Severity of anaemia
Mild (10 to 10.9 g/dl)
Moderate (7 to 9.9 g/dl)
Severe (less than 7 g/dl)

while hyperanemia is haematocrit value less than 10% [12,13]. In individuals of African extraction, a cut-off of about 1 g/dl lower is recommended [6]. Even when haemoglobin value is not below the normal reference point, anaemia may be considered when haemoglobin level has significantly decreased below the individual's steady state value. Low borderline haemoglobin values may be seen in evolving disease conditions where there may still be adequate compensation or marrow reserves. Pseudoanaemia, otherwise referred to as relative or spurious anaemia is caused by expanded plasma volume due to haemodilution as in dilutional anaemia of pregnancy or a falsely reduced red cell mass due to redistribution as in splenomegaly.

The primary method for anaemia determination is haemoglobin testing. There are various methods for haemoglobin testing. The most accurate method is the colorimetric haemoglobinometry using cyanmethaemoglobin method [14]. Other less reliable methods include use of visual scales or haemoglobinometers. In developing countries, manual estimation of haematocrit (or packed cell volume) is commonly done using the micro-haematocrit (erythrocyte centrifugation) method. It is shown to be reliable for routine clinical purposes. A positive linear correlation exists between haematocrit and haemoglobin concentration. This has been validated with a correlation of 'Haematocrit = 2.62 x (haemoglobin level) + 3.67', coefficient of 0.98 and probability value less than 0.001 [15]. As a rule of thumb, packed red cell volume (PCV) is equivalent to 3 times the haemoglobin concentration levels.

An estimated two billion (more than one-third) of the world population are affected by anaemia [8, 16]. Its burden is higher in developing countries. Women of child bearing age and children bear the highest burden of anaemia [8,16,17]. WHO estimates that over 30% of all women and about 52.8 to 61.3% of pregnant women in developing nations are anaemic [8,16]. Similarly, among hospital patients, a high burden of anaemia has been reported [18-20]. In a recent retrospective study in Nigeria, the prevalence of anaemia among patients and clients receiving care in a tertiary hospital was observed to be 27.3% [21].

3. AETIOGENESIS OF ANAEMIA

All forms of anaemia may be categorized as haemolytic, hypoproliferative (aregenerative) or haemorhagic. Haemolytic anaemia results from accelerated central or peripheral destruction of red cells. Hypoproliferative anaemia, otherwise called anaemia of bone marrow failure, results from decreased central production of red cells and/or its defective release from the bone marrow. Anaemia of blood loss or haemorhage results from a breach in the integrity of blood vessels in the body, resulting in acute or chronic shortage of red cells/red cell mass. Hypoproliferative causes are either strictly production defects or maturation defects (otherwise called ineffective erythropoiesis). In terms of marrow reticulocyte response to anaemia, a list of its possible differentials is exemplified in Fig. 1.

Haemolysis describes a pathological state in which red cell survival is shortened below its normal interval [22,23]. Red cell life span is normally about 100 to 120 days in vivo. Haemolysis may be acute or chronic (depending on the rapidity of onset), inherited or acquired, immune or non-immune, intrinsic or extrinsic. Intrinsic causes are due to intracorpuscular defects in the red cells and they include red cell membranopathies and cytoplasmic defects. Examples of red cell membranopathies are hereditary spherocytosis, elliptocytosis, south-

east Asian ovalocytosis, pyropoikilocytosis and others. Red cell cytoplasmic defects may further be grouped as haemoglobinopathies and enzymopathies. Two major broad forms of haemoglobinopathies include sickle cell disease and thalassemia. Enzymopathies include Glucose 6 Phosphate dehydrogenase (G6PD) deficiency, pyruvake kinase (PK) deficiency and others [24]. G6PD deficiency affects about 4 to 26% of Nigerians and 20 - 26% of the Nigerian male population [25-26]. In other developing African nations including Ghana, Kenya, Burkina Faso, Tanzania and Mali, frequency of G6PD deficiency ranges between 5 – 23.8% [27]. In India, prevalence of G6PD deficiency ranges from complete absence to as high as 27%, with average of about 10% [28]. On the other hand, extracorpuscular defects include immune and non-immune causes. Immune mediated haemolysis may be allo-immune, auto-immune or drug-induced in origin. In about 30 to 50% of cases, the cause of immune haemolysis is primary idiopathic, while others occur secondary to underlying diseases [22,29,30]. These secondary causes of immune haemolysis include neoplasms such as haematological malignancies and solid tumors, infections especially viral, connective tissue diseases or drugs [29]. Non-immune haemolysis may be due to exposure to toxins, infections, microangiopathy, as well as paroxysmal nocturnal haemoglobinuria (PNH). Toxaemias such as uraemia, snake and spider venoms are associated with reduced red cell lifespan [31]. Infections by microbes such as *Plasmodium falciparum, Babesia microti, Clostridium perfringens, Barthonella* species are known to cause direct lysis of red cells [22,23,32]. Malaria is a significant cause of anaemia in tropical Africa and other endemic areas, especially among under 5 children and pregnant women [33-38]. Micro-angiopathy may be caused by mucinous adenocarcinomas, haemolytic uraemic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP), malignant hypertension, connective tissue diseases, burns, vasculitis, disseminated intravascular coagulopathy (DIC) [9,22]. It should be noted that HUS/TTP and DIC are thrombotic microangiopathies (TMA). Anaemia in TMA results from red cell lysis caused by the abnormal fibrin strand meshwork deposited within the microvasculature. DIC is a thrombo-haemorhagic complication which occur secondary to several underlying disease processes. DIC is characterized by systemic (widespread) activation of coagulation system, resulting in obstruction of blood supply to vital organs and overconsumption of coagulation proteins and platelets [39]. Known causes of DIC includes severe sepsis, acute haemolytic transfusion reaction, any form of shock, severe head injury or trauma, massive blood transfusion, vascular malformations, severe pancreatitis, obstetric complications such as intrauterine fetal demise, placental abruption, amniotic fluid embolism and others [39,40]. PNH is an acquired, progressive membrane defect that results from complement mediated lysis of red cells due to a defect in the PIG-A gene which anchors cyto-protective, complement regulating membrane surface proteins such as CD55 (decay accelerating factor, DAF), C8 binding protein (HRP) and most importantly, CD59 (membrane inhibitor of reactive lysis, MIRL) [41,42]. Most extracorpuscular defects are acquired, while most intracorpuscular defects are inherited except PNH.

Anaemia of bone marrow failure is a very significant cause of anaemia worldwide especially in developing countries largely due to nutritional deficiency anaemia. Marrow underproduction of red cells may occur at the stem cell, progenitor cell, or precursor cell pools. Known causes of marrow failure includes nutritional deficiencies, aplastic anaemia, sideroblastic anaemia, anaemia of chronic inflammation, myelophthisic anaemia, anaemia of renal failure, endocrine causes and congenital dyserythropoietic anaemias [9]. The highest burden of hypo-proliferative anaemia worldwide is caused by nutritional (substrate) deficiency (predominantly iron deficiency), closely followed by anaemia of chronic diseases [4,43]. Despite being the most abundant element/metal in the earth's crust, WHO estimates that approximately half (50%) of all cases of anaemia worldwide can be attributed to iron deficiency [43,44]. Micronutrients are necessary erythropoietic precursors required for normal haemopoiesis. They include vitamins B1, B2, B3, B6 (pyridoxine), B9 (folate), B12 (cobalamin) and trace metals such as iron, cobalt and copper [45]. Aplastic anaemia may be an inherited syndrome as in Fanconi'sanaemia, dyskeratosis congenital (DKC) or Schwachman diamond syndrome. In acquired aplastic anaemia, 60 to 70% (two-thirds) of cases are idiopathic, others are secondary to viral infections, irradiation, provocative drugs, pregnancy and graft versus host disease (GvHD) [46]. Anaemia of chronic inflammation, also known as anaemia of chronic disease, occurs in chronic inflammatory states such as autoimmune diseases, malignancies and

chronic infections such as tuberculosis, HIV/AIDS and others [47]. Malignancies involving midline and paired organs such as breasts, lungs, prostate, and cervix have predilections for bone marrow involvement [48,49]. Bone marrow suppression resulting from marrow infiltration by ectopic tumor cells is termed myelophthisic anaemia. Hormones such as thyroxine and androgen are also important drivers of erythropoiesis. As such, endocrinopathies including addison's disease, hypothyroidism and hypogonadotrophic states are associated with hypoproliferative anaemia.

Please note that some forms of anaemia have multiple aetiologies. For instance, anaemia of chronic renal failure is associated with bone marrow underproduction owing to reduced erythropoietin drive alongside nutritional deficiencies (vitamin K deficiency), decreased red cell survival as well as haemorhage which may be precipitated by uraemic coagulopathy, gastritis and platelet dysfunction. Additionally, haemodialysis contributes to iron and folate deficiency, blood loss and ex-vivo red cell destruction [50].

4. PATHOPHYSIOLOGY AND CLINICAL ASPECTS OF ANAEMIA

Generally, symptomatology of anaemia among patients depends on its speed of onset, its clinical severity, patient's cardio-vascular reserve and presence of co-morbidities [9]. Clinical features of anaemia may be specific or non-specific. Non-specific features include hypoxia related effects of anaemia on organ systems most especially the brain, heart and muscles. Hypoxic effects of anaemia include physical fatigue, general malaise, dimness of vision, fainting spells, dizziness, tinnitus, palpitations, angina of efforts (if pre-existing heart disease), amenorhoea (in women), exercise intolerance, dyspnoea and paraesthesia [31]. Also, there will be pallor of mucous membrane surfaces of the eyes/conjunctiva, oral cavity, palms, sole of the feet, or the entire skin in newborns. However, clinical pallor may not be evident until haemoglobin levels less than 9 g/dl [51,52]. Specific features are related to the underling aetiology such as koilonychia in iron deficiency anaemia and atrophic painful glossitis in megaloblastic anaemia. Normally, a clinician will suspect anaemia based on one or more of the above listed symptoms and sign.

In anaemic conditions, host compensatory mechanisms are activated. These physiological adaptations include a hyperdynamic circulation, erythroid hyperactivity, increased 2, 3 DPG production, redistribution of blood flow from the peripheral (skin) to vital organs such as brain, heart and muscles [9,53]. Erythroid activity may be increased as much as 5 – 7 times normal. Decreased viscosity of anaemia blood and high levels of 2, 3 DPG helps to improve tissue perfusion by oxygen [53]. However, if hypoxia of vital organs persists for too long, compensatory mechanisms are lost. Cardiac decompensation culminates in anaemic heart failure. Features of anaemic heart failure include tachycardia, tachypnoea, dyspnoea, tender hepato-splenomegaly and bilateral pedal oedema [9]. If prompt appropriate intervention is not rendered, death ensues.

5. ANAEMIA WORK-UP

Laboratory evaluation of anaemia is crucial to diagnostic formulations in patient care. In clinical practice, accurate diagnosis relies on a tripod of clinical history, physical examination and laboratory investigations. Clinical history should include socio-demographic data noting age, sex, occupation, geographical location and ethnicity as it may have a bearing on the cause of anaemia. For instance, iron deficiency anaemia is more likely to be related to growth and development in children while chronic blood loss is a more likely cause in elderly persons. History suggestive of haemorhage or haemolysis, social history, family history, drug intake and nutritional history and other relevant details should also be elicited. A general physical examination and systemic examination especially cardiovascular and neurological systems are important. Physical signs such as jaundice and chronic leg ulcers may suggest a congenital haemolytic anaemia.

Anaemia may be multi-factorial in origin (anaemia of mixed origin). Sometimes, its cause may be obvious from clinical history alone, as in straight-forward acute blood loss. However, many cases of anaemia require more detailed history, physical examination and laboratory investigations to elucidate its cause(s) [5]. Laboratory investigations will often be required to establish or exclude possible differentials. Such investigation profiles engaged in the diagnosis and treatment of anaemia are termed anaemic work-up. Anaemia work-up investigations are intended to define anaemia, to establish its causes and to monitor response to treatment.

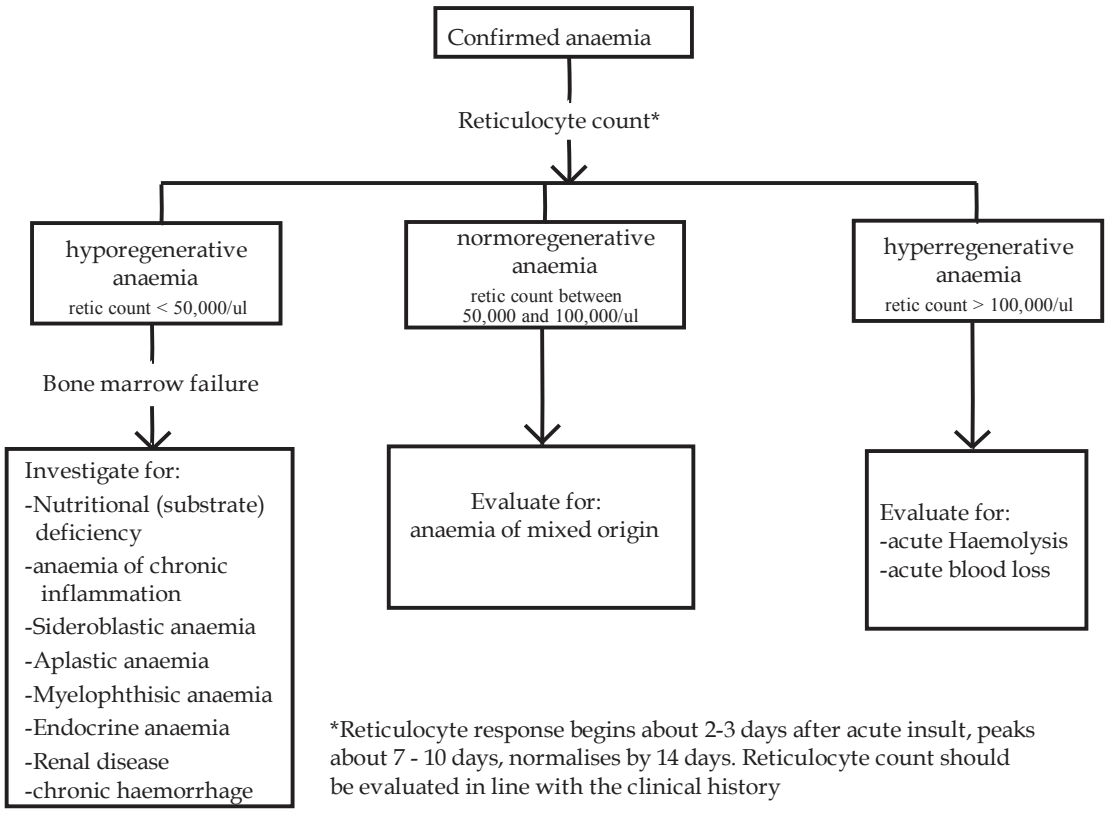

Fig. 1. Aetiologic classification of anaemia

Laboratory investigation of anaemia should never be in isolation, rather it should be directed by a patient's clinical history and physical examination findings. For instance, it would be clinically absurd to request bone marrow aspiration (BMA) plus biopsy or immunophenotyping/flow cytometry or even haematinic assays at first clinical interview with a paediatric patient presenting to you with a history of delayed growth, chronic anaemia/pallor, jaundice and recurrent bone pains. Such a patient would rather benefit from a full blood count (FBC), reticulocyte count, peripheral blood film (PBF), haemoglobin electrophoresis, high performance liquid chromatography (HPLC), biochemical markers of haemolysis, as the history points toward sickle cell disease as a top differential.

As a baseline, initial laboratory work-up when investigating anaemia should include full blood count (including red cell indices), reticulocyte count and peripheral blood film [54-56]. Arguably, reticulocyte count is the single most important laboratory investigation in any case of anaemia. Reticulocytes are the youngest anucleate red cells in the peripheral circulation and they take about 1- 2 days for maturation in the periphery [54]. Normally, in anaemic conditions, reticulocyte production is increased in order to compensate for the decreased red cell mass. Normal reticulocyte count in adults is 0.5-1.5% and 2-5% in newborns [23,45]. Increased reticulocyte count is a sign of adequate marrow regeneration in response to anaemia following haemolysis or haemorrhage or haematinic therapy. Poor reticulocyte response suggests a hypoproliferate anaemia or bone marrow failure [57]. Reticulocyte count may be reported as percentage or absolute counts or corrected for the degree of anaemia. Evaluation of anaemia using reticulocyte count is more reliable through the use of indices such as the reticulocyte production index (RPI). In severe anaemia, the rate of red cell release from the marrow exceeds the normal rate and this gives a false impression of increased production. To correct for this phenomenon (where release exceeds production), RPI is more sensitive and is used for shift correction. Generally, normal RPI is 1. RPI > 3 suggests anaemia with adequate regeneration. RPI < 2 suggests anaemia with inadequate regeneration [58]. A full blood count (FBC) is

important because anaemia is not always isolated. Bi- or pancytopenia may suggest underlying diseases causing central suppression of haemopoiesis such as malignancies, nutritional deficiencies or HIV/AIDS. It is therefore important to request a FBC rather than just a PCV (haematocrit). Red cell indices such as mean corpuscular volume(MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration(MCHC) are a part of full blood count.They are useful in categorizing anaemia as either normocytic, microcytic or macrocytic (Fig. 2). Major differentials of microcytic hypochromic anaemia are iron deficiency anaemia, thalassemias, sideroblastic anaemia and occasionally, anaemia of chronic diseases [9,59].

Normocytic anaemia may be seen in combined (mixed) nutritional deficiency states, acute blood loss, anaemia of chronic diseases and anaemias due to endocrine dysfunction [60]. Macrocytic anaemias may be megaloblastic or non-megaloblastic [61,62]. Megaloblastic causes include folate and B12 deficiency, as well as acute nitrous oxide poisoning. Whereas, non-megaloblastic macrocytosis is observed in alcoholism, neonates, myeloma, hypothyroidism, liver disease, pregnancy, aplastic anaemia and myelodysplastic syndrome [61,62]. Clinical indications for a peripheral blood film (PBF) request are myriad and they include cases listed in Table 2 [63,64].

PBF should be reported by a haemato-morphologist. This requires that adequate clinical details should be provided by the requesting clinician, in order to facilitate holistic review of patient's blood smear by the haemotologist.

Further laboratory evaluations would include specific investigations to pin down the exact cause of the anaemia. Further anaemia work-ups include other haematological, biochemical, microbiology or histo-pathological tests. They include C-reactive protein/Erythrocyte Sedimentation Rate (CRP/ESR), renal function test (E/U/Cr), liver function test (LFT), anti-globulin test, haemoglobin electrophoresis, G6PD assay, stool for occult blood, flow cytometry for CD55/59, bone marrow cytology and histology, lymph node histology, haematinic assays, prussian blue reaction, cytogenetic studies, erythropoietin assay, serum hepcidin levels, hormone assays (serum androgen and thyroxine levels), GIT studies and so on [5,9,55,65,66]. Erythrocyte sedimentation rate (ESR) is raised in chronic inflammation. ESR is rather an unspecific marker. However, a very high ESR (above 100 mm in 1 hour) may be seen in multiple myeloma and other plasma cell dyscrasia, as well as polymyalgia rheumatica and tuberculosis. Normal ESR level (Westergren method) is less than 15 mm in one hour in adult males and less than 20 mm in one hour in adult females. ESR may be slightly reduced in sickle cell disease and slightly increased in the elderly. On the other hand, C-reactive protein (CRP) gives a more sensitive and accurate reflection of acute inflammation. Normal adult CRP levels is about 1 to 5 mg/l. Deranged renal function and liver function tests may point to anaemia of chronic renal failure and liver disease respectively. Direct and indirect antiglobulin tests, also called Coomb's test were designed by Coombs and colleagues in 1945 for detection of non-agglutinating antibodies in the serum. Coombs test are useful in investigating immune related causes such as auto-immune anaemias and haemolytic transfusion reactions [67]. Haemoglobin electrophoresis and HPLC are highly informative in diagnosis or exclusion of abnormal haemoglobin variants such as sickle cell disease and thalassemia [56,68,69]. G6PD assay should be requested in suspected cases of haemolytic anaemia especially in males. Patients with history of chronic cough, chronic diarrhoea and significant weight loss, as well as individuals with history of sexually risky behaviors should be referred for retroviral screening (RVS) following proper counseling and consent. Stool test for occult blood is unspecific and may be associated with false-positives depending on the individual's diet or use of iron containing tablets. However, true-positive result suggests some sort of GI bleeding, maybe peptic ulcer disease or gastro-intestinal malignancy. Gastro-intestinal endoscopies and CT/MRI may be required for further evaluation. Lymph node biopsy histology is indicated in suspected lymphomas or metastatic lymphadenopathy. Bone marrow aspiration and biopsy is an invasive procedure, as such patients should be carefully selected, educated and consenting. Not all cases of anaemia will require bone marrow examinations. BMA and biopsy is indicated in cases of unexplained anaemia or leukocytosis despite peripheral blood analysis, unexplained lymphadenopathy or splenomegaly, suspected acute leukaemias, megaloblastic anaemia, advanced lymphomas or non-haematologic malignancies with marrow involvement and so on [55,70]. Marrow blood is also useful for microbiological cultures, cytogenetic studies and Perl's staining (Prussian blue reaction) for

marrow iron stores. Haematinic assays include serum iron levels, total iron binding capacity (TIBC), percentage transferrin saturation (TSAT), serum ferritin levels, serum transferrin receptor levels, serum folate and red cell folate levels as well as serum cobalamin levels. These assays, with their respective limits and pitfalls, are engaged in assessing iron, folate or cobalamin deficiency anaemias. In suspected haemolytic anaemia, relevant biochemical tests for haemolysis includes serum bilirubin levels, serum enzymes including aspartate transaminase and lactate dehydrogenase, haptoglobin and haemopexin levels [60]. Haptoglobin is more sensitive and specific than indirect bilirubin assay. A combination of raised lactate dehydrogenase (LDH) levels alongside reduced serum haptoglobin is 90% specific for diagnosing haemolysis. Normal serum LDH coupled with serum haptoglobin level above 25 mg/dl is 92% sensitive to exclude haemolysis [71]. Haptoglobin level (in the absence of liver cirrhosis) below 28mg/d is 92% sensitive and 98% specific for predicting haemolysis [72]. A practical schema for evaluation of haemolytic anaemia is provided in Fig. 3. Examination of the peripheral blood is usually sufficient in diagnosis of haemolytic anaemia. However, most cases of reticulocytopenic anaemia or pancytopenic anaemia resulting from marrow failure will require bone marrow examination for adequate diagnosis.

In steady state, normal non-anaemic serum erythropoietin (EPO) level is about 5 to 20 milliunits/ml [73]. Low EPO levels suggest renal disease. In response to anaemia in absence of renal damage, endogenous EPO levels increase exponentially in order to compensate and promote production of red cells. However, significant anaemia especially in HIV/AIDS with serum endogenous EPO levels below 500 mu/ml may be corrected with exogenous (recombinant) EPO treatment [74].

Detection of rheumatoid factor and anti-nuclear antibodies are relevant in investigation of auto-immune diseases/connective tissue diseases. Radiological studies such as abdominal X-rays, abdominal ultrasonography and CT scan may be used to delineate occult intra-abdominal malignancies. Work-ups such as blood culture and other microbiological cultures, blood film for malaria parasite, stool for ova and parasite, retroviral screening, urine m/c/s, lumber puncture (CSF) analysis are relevant to evaluating infection/infestation associated anaemia. Advanced techniques including cytogenetics and nucleic acid amplication protocols such as PCR are required for evaluation of primary haemopathies especially haematological malignancy.

This list of anaemia work-up investigations is by no means exhaustive. However, anaemic work-ups should be targeted towards confirming or refuting its likely aetiology in a systemic unprejudiced manner, considering the high cost of laboratory tests. A physicians ability to select the most appropriate and relevant investigations in anaemia work-up depends on the depth of his knowledge database and clinical experience. A good physician is one who knows his limits. Consultations should be sought or referrals made to appropriate specialist teams where and when necessary.

6. GENERAL APPROACH TO ANAEMIA TREATMENT

General approach to treating anaemia include methods for promotion of red cell production in hypoproliferative anaemia such nutritional supplements in nutritional anaemia, limitation of red cell destruction in haemolytic anaemia such as immunosuppressive therapy in auto-immune haemolytic anaemia and arrest of bleeding in anaemia of blood loss. Severe symptomatic anaemia requires replacement with red cell concentrate (packed red cells). Specialist blood component should be prescribed as the situation warrants. Haematology consultations should be sought when in doubt.

Blood transfusion is not totally innocuous and should not be undertaken lightly. There must be a clear indication for every transfusion event. Transfusion should be withheld except the anaemia is severe (haemoglobin less than 7 g/dl), symptomatic at any level, evidence for continuing blood loss or in pre-operative/procedure/surgical preparations. In disease conditions associated with lower affinity haemoglobins such as sickle cell disease, transfusions should be withheld except in occasions of very severe anaemia (haemoglobin level less than 4 g/dl) or acute worsening anaemia. While symptomatic relief with blood transfusion is offered, the cause of the anaemia should be pursued. Erythropoiesis stimulating agents such as erythropoietin and haematinics should be administered where indicated. Tactful skills (bloodless surgeries) should be engaged at surgeries. Definitive treatment should be directed at the underlying disease.

Table 2. Indications for peripheral blood film

Unexplained peripheral blood cytopenias	Anaemia Leucopenia Thrombocytopenia
Unexplained high leucocyte counts	
Jaundice or haemolysis	
Features suggestive of an inherited haemolytic anaemia	
Suspected leukaemias or lymphomas	
Liver or renal failures	
Severe bacterial sepsis and parasitic infections	
Advanced cancers with possible bone marrow involvement	
Cases of nutritional anaemias	
Others	

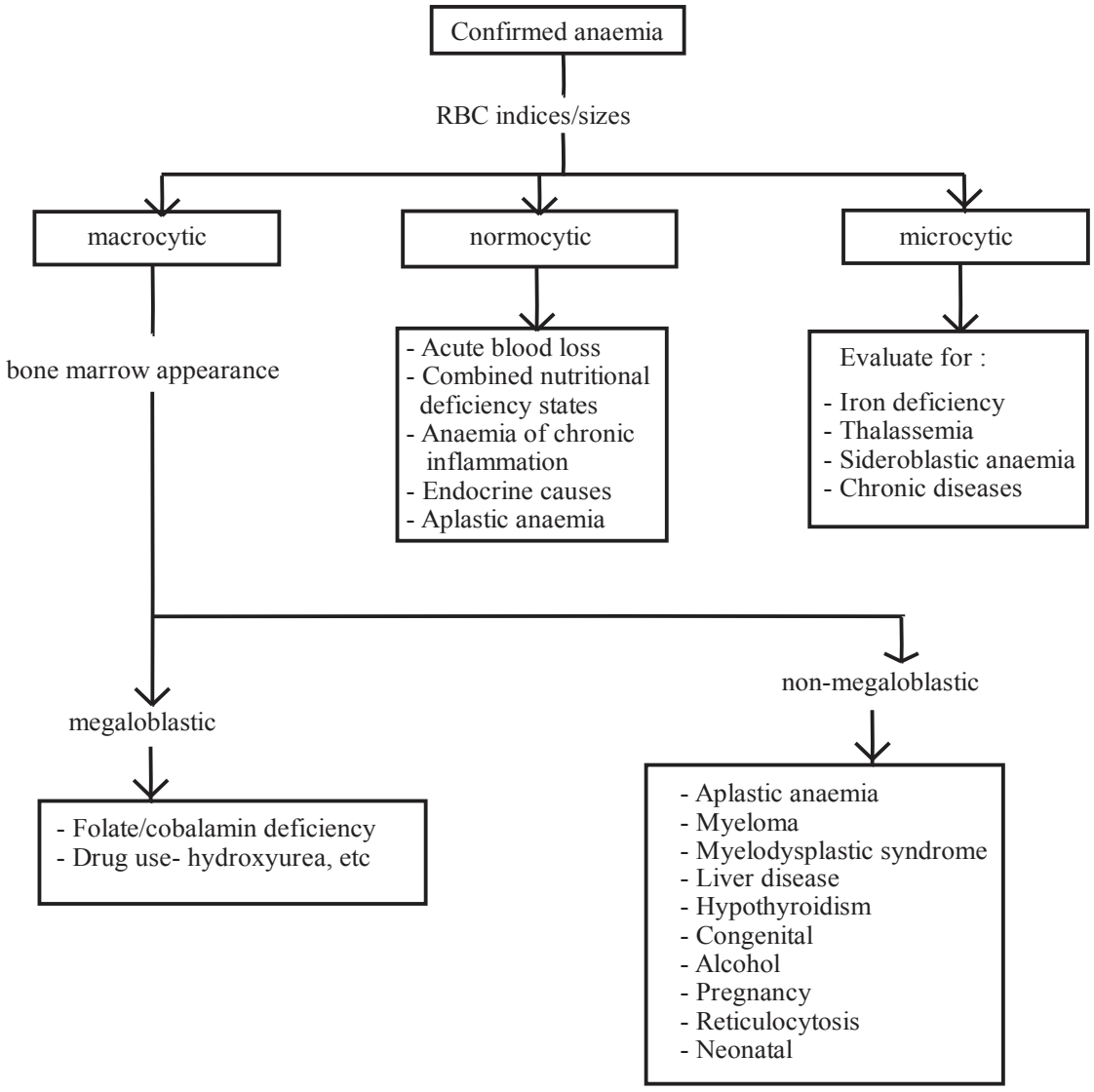

Fig. 2. Diagnostic algorithm for anaemia evalution

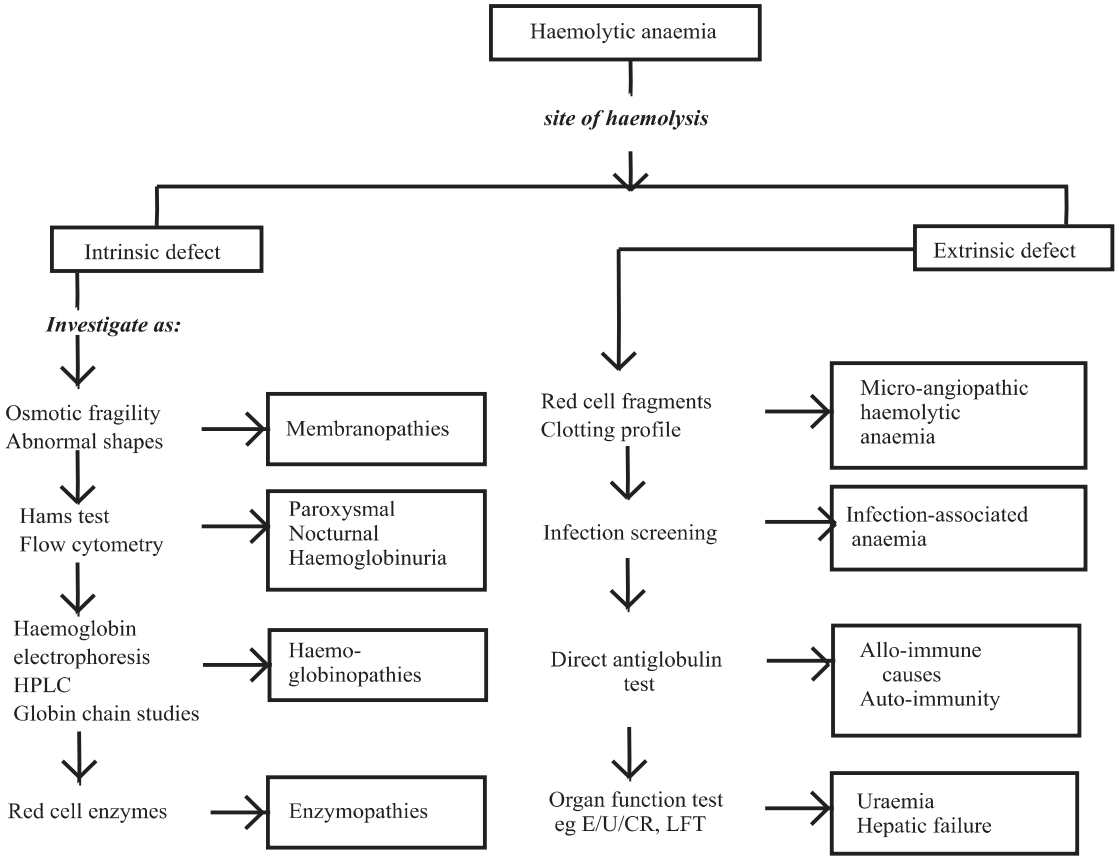

Fig. 3. Evaluation of haemolytic anaemia

7. CONCLUSION

Laboratory investigations are not substitutes for good clinical skills in patient interview and physical examination. Physicians should treat patients, not laboratory tests. However, in appropriate situations, relevant laboratory investigations that would facilitate patient care should be requested.

The author also recommends that FBC, reticulocyte count and PBF should be requested on any patients presenting with anaemia as initial baseline investigations. Relevant authorities and stakeholders should ensure that these tests are routinely available in all health facilities. This therefore calls for improvements in diagnostic services especially in developing nations. Automation of medical laboratories is highly desirable as it reduces processing time and manpower needs and improves diagnostic accuracy. Though more expensive to install and operate, automation is a more suitable alternative for laboratories with high sample volume.

Blood transfusion is never a quick-fix unless the cause of anaemia is found and treated. As such, the cause of anaemia should always be sought by the attending physicians at all levels of health-care. Prompt actions should be taken as anaemia may portray life threatening causes such as malignancies. Difficult cases should be referred to a haematologist and other appropriate specialists.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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