

Anaemia: Approach to diagnosis

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Anaemia is defined as a condition in which the number of red cells or their oxygen-carrying capacity is insufficient to meet physiological needs. It is the most common disorder globally and one of the conditions that general practitioners most frequently encounter. In the World Health Organization global database, anaemia is estimated to affect 1.6 billion people. As anaemia manifests in a wide range of conditions, it is important to embrace a structured diagnostic approach. The recommended approach set out in this article incorporates clinical and pathophysiological considerations, red cell characteristics, and bone marrow activity. In this issue of CME, the first of two parts on anaemia, the causes of anaemia related specifically to decreased red cell production are discussed.

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Red cells are produced in the bone marrow (BM) from myeloid progenitor cells, where their production requires a permissive BM micro-environment and adequate substrate (including iron, vitamin B₁₂ and folate) for Hb, protein and DNA synthesis. Erythropoiesis is controlled by erythropoietin (EPO), which is synthesised by peritubular fibroblasts in the renal cortex in response to reduced oxygen tension.

According to the World Health Organization (WHO), anaemia is defined as 'a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs.' It is the most common disorder globally and one of the conditions that general practitioners most frequently encounter. Anaemia is rarely an isolated disease and is most often a sign of an acquired or inherited disorder.^[1]

According to the WHO global database,^[2] anaemia is estimated to affect 1.6 billion people. The highest prevalence is found in preschool-age children (47.4%), followed by pregnant females (41.8%), non-pregnant females (30.2%), school-age children (25.4%), and males (12.7%).^[2]

When investigating patients with anaemia, two fundamental questions have to be considered: (i) What is the cause of the anaemia?; and (ii) What is the urgency for correcting the anaemia, i.e. is a blood transfusion or other urgent intervention indicated?

Classification

Anaemia may be classified as follows:

Based on red cell characteristics (red cell size, chromia and morphology):

- hypochromic microcytic
- macrocytic normochromic
- normochromic normocytic
- morphology:
 - leuco-erythroblastic
 - micro-/macroangiopathic.

Based on underlying mechanism:

- decreased BM production/output:
 - BM aplasia/infiltrate

- ineffective haematopoiesis, e.g. megaloblastic anaemia (MA), myelodysplastic syndromes (MDSs), HIV
- substrate deficiency
- EPO insufficiency
- peripheral loss/destruction:
 - bleeding
 - sequestration
 - haemolysis.

In this issue of CME, the focus is on anaemia resulting from decreased BM output. Anaemia caused by peripheral loss will be discussed in the next issue.

Diagnostic approach to a patient with suspected anaemia

As anaemia may manifest in a wide range of disorders, a streamlined diagnostic approach is vital (Fig. 1). The approach outlined here is based on the abovementioned classification, clinical scenario and full blood count (FBC) findings.^[3]

Patients generally present with symptoms of anaemia, i.e. increased tiredness/fatigue, dyspnoea and decreased effort tolerance. The severity of symptoms depends on the degree of anaemia and rate of Hb decrease. Therefore, at a given Hb level, anaemia from acute blood loss is likely to manifest more severely than anaemia of insidious onset (weeks to months). Symptoms during early childhood should remind one of possible inherited forms of anaemia, e.g. thalassaemia.

History

A detailed history is of paramount importance and often eliminates much of the speculation during investigation. This should include:

- interrogation of the presenting complaint and duration of the problem
- transfusion history
- dietary history, including pica (craving for unusual food items, generally associated with iron deficiency)
- travel history (to endemic malarial or other infectious areas)
- change in bowel habits
- bleeding (e.g. gastrointestinal and genito-urinary)

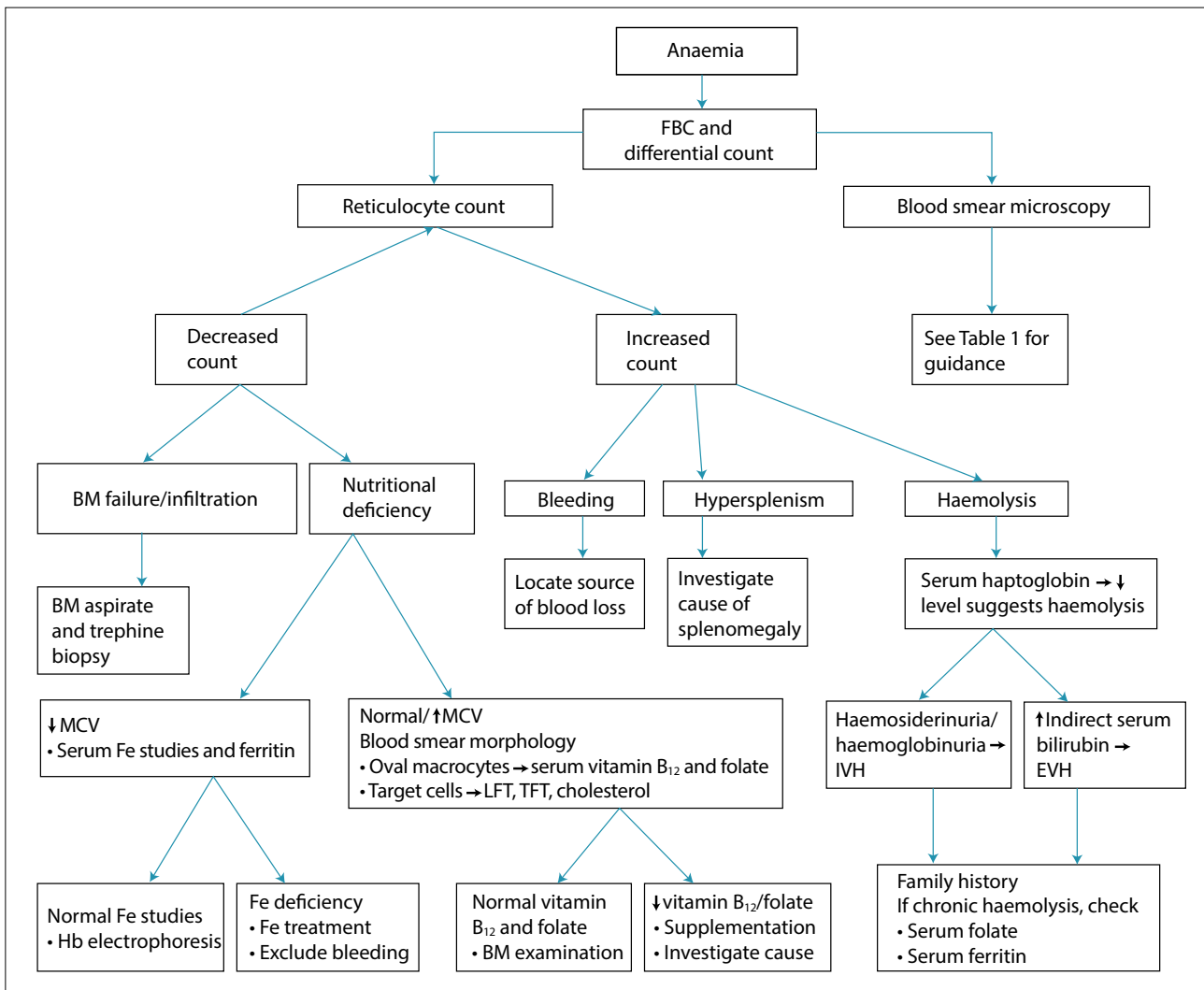


Fig. 1. Algorithm depicting diagnostic approach to anaemia. (TFT = thyroid function test; LFT = liver function test; MCV = mean cell volume; IVH = intravascular haemolysis; EVH = extravascular haemolysis; FBC = full blood count; Fe = iron.)

- drug history (e.g. anticoagulants, antiplatelet agents, renotoxic agents, anticonvulsants)
- chronic disease (e.g. HIV, tuberculosis (TB))
- surgery (e.g. gastrectomy, small-bowel surgery)
- current or recent pregnancy
- family history (particularly in children).

Clinical examination

A wide range of signs and symptoms may be apparent. Systematic examination directs further investigation and may reveal the possible cause.

- Skin and mucous membrane:
 - pallor is the cardinal clinical sign for anaemia, which should be confirmed by measuring the Hb level
 - angular stomatitis
 - glossitis in nutritional deficiencies
 - koilonychia (spoon-shaped nails) in iron deficiency
 - premature greying, which often accompanies MA

- scleral icterus, which indicates possible haemolysis or ineffective erythropoiesis.
- Neuromuscular:
 - muscle weakness
 - headache, lack of concentration, drowsiness, tinnitus
 - paraesthesias, peripheral neuropathy, ataxia and loss of vibration sense, and proprioception in pernicious anaemia.
- Cardiovascular:
 - hyperdynamic circulation with haemic ‘flow’ murmurs
 - cardiac failure.
- Clues for infection, malignancy (e.g. lymphoma, leukaemia, metastatic carcinoma):
 - hepatosplenomegaly
 - lymphadenopathy
 - bleeding manifestations (petechiae, purpura, ecchymosis), BM failure.

Laboratory testing

An FBC, differential and reticulocyte counts together with microscopic blood smear

examination should be the starting point of investigations. These confirm the clinical suspicion of anaemia and direct further investigation. Local laboratory/population reference ranges that are age and gender specific should be used. The normal reference ranges for Hb in adults in the Witwatersrand area, Johannesburg, South Africa (SA) are as follows:^[4]

- male: 13.8 - 17.9 g/dL (anaemia <13 g/dL)
- female: 12.4 - 15.5 g/dL (anaemia <12 g/dL; pregnancy <11 g/dL).

A reticulocyte count gives an indication of the BM status, i.e. decreased activity v. appropriate response to the anaemia. A reticulocyte production index (RPI) provides a more accurate representation of marrow activity than an isolated reticulocyte count, as it corrects for the degree of anaemia and presence of immature reticulocytes in the peripheral blood. The RPI is calculated as follows:

Table 1. Red cell morphological characteristics

Morphological observation	Significance	Further tests indicated and expected result
Oval macrocytes, teardrops, basophilic stippling, right shift	MA	Serum vitamin B ₁₂ and folate levels
Hypochromia Microcytosis	Iron deficiency anaemia (also see pencil cells) Chronic disorder; thalassaemia trait	Serum ferritin, transferrin and iron levels Hb electrophoresis/HPLC
Sickle cells	Seen in sickle cell disease	Hb electrophoresis to confirm presence of Hb S, and quantitate Hb F level
Spherocytes	Noted in hereditary spherocytosis, warm AIHA	Coombs test: positive for IgG in warm AIHA Red cell membrane analysis: selected membrane protein abnormalities
Elliptocytes/ovalocytes	Hereditary elliptocytosis/ovalocytosis	Red cell membrane analysis: selected membrane protein abnormalities
Autoagglutination	Cold AIHA	Coombs test: positive for C3d in cold AIHA
Red cell fragmentation	If platelets decreased → microangiopathic haemolysis If platelets normal → macroangiopathic haemolysis	Microangiopathic haemolysis: DIC screen → consumptive coagulopathy U&E: marked renal dysfunction in HUS Altered neurological status: suspect TTP
Malaria	Life-threatening infection	Identify species Monitor FBC and parasite count while on treatment

AIHA = auto-immune haemolytic anaemia; U&E = urea and electrolytes; DIC = disseminated intravascular coagulation; HUS = haemolytic uraemic syndrome; HPLC = high-performance liquid chromatography; TTP = thrombotic thrombocytopenic purpura.

- % reticulocytes × patient haematocrit/45 ÷ reticulocyte maturation time (days) in peripheral blood

The reticulocyte maturation time is calculated as follows:

- haematocrit >40% = 1 day, 30 - 40% = 1.5 days, 20 - 30% = 2 days, <20% = 2.5 days

A decreased RPI signifies a suboptimal BM response for correction of the anaemia. Clues for further investigation are derived from red cell indices (Fig. 1) and peripheral blood smear microscopy (Table 1).

BM examination is appropriate if pathology is suspected, e.g. BM infiltration, BM failure, and myelodysplasia. BM aspirate smears permit morphological assessment in cellular detail, but the architecture is disrupted, whereas in trephine biopsy sections the architecture is preserved, which makes it possible to detect pathology, such as granulomata and fibrosis.

More specialised tests are performed in individuals suspected of having rarer conditions that cause anaemia.

Important causes of anaemia due to inadequate BM output

Causes of red cell production failure include EPO deficiency (usually secondary to chronic kidney disease), substrate deficiency, ineffective erythropoiesis, BM aplasia/hypoplasia and BM displacement (malignancy/fibrosis), all of which are characterised by a low RPI.

BM aplasia/hypoplasia (primary BM failure)

BM failure may be inherited or acquired. In SA, the majority of patients have acquired idiopathic aplastic anaemia (AA). Inherited BM failure syndromes generally manifest in childhood and represent <10% of patients with AA.

Patients may present with one or more of the following: (i) symptoms of anaemia; (ii) infection (due to leucopenia/neutropenia); and (iii) mucocutaneous bleeding (due to thrombocytopenia). The FBC shows a pancytopenia of varying degrees (depending on the severity), reticulocytopenia with a low RPI, and reversal of the normal neutrophil:lymphocyte ratio. The diagnosis is confirmed on microscopic BM examination.

Numerous secondary causes and associations have been identified in AA, but in 60 - 70% of cases the cause remains unknown. It has been proposed that a triggering event, such as a virus or drug, leads to an immune response and to inappropriate expansion of cytotoxic T cells that destroy haematopoietic cells. Causes include viruses (e.g. hepatitis virus, HIV, Epstein-Barr virus, cytomegalovirus); autoimmune disorders (e.g. systemic lupus erythematosus, eosinophilic fasciitis, Sjögren's syndrome); drugs (a wide range, including sulfonamides, anticonvulsants, antimalarials; chemicals (e.g. benzene); and environmental factors (e.g. agricultural pesticides and radiation). The idiopathic variety is the largest group. Paroxysmal nocturnal haemoglobinuria (PNH), caused by expansion of a clone with mutation of the glycosylphosphatidylinositol gene, manifests with the clinical triad of haemolysis, thrombosis and BM failure. PNH clones can be detected on flow cytometry.

Some inherited disorders are characterised by BM hypoplasia/aplasia (affecting one or more haematopoietic cell lines), and are usually accompanied by one or more somatic abnormalities, i.e. part of a clinical syndrome. Inherited BM failure syndromes may be present at birth or develop later in childhood. Of these, the syndromes that affect all three cell lines include Fanconi anaemia, Schwachman-Diamond syndrome and dyskeratosis congenita. Further discussion of these syndromes is beyond the scope of this edition of CME.

Pure red cell aplasia is characterised by an isolated, often severe anaemia with a marked reticulocytopenia. It may be congenital

(Diamond Blackfan syndrome) or acquired (including viral infections, lymphoproliferative disorders, autoimmune disorders or drugs). In SA, parvovirus B19 infection in HIV-positive individuals is the most common cause of pure red cell aplasia. BM examination reveals the absence or maturation block of erythroid progenitors.

Patients with BM failure should be referred to a specialist haematology/oncology unit for further work-up and management.

BM displacement by malignancy or fibrosis

The presence of a leuco-erythroblastic reaction and teardrop poikilocytosis on peripheral blood smear microscopy should lead one to suspect possible BM infiltration.

Leukaemia and lymphoma can be further characterised on flow cytometry, cytogenetic analysis and polymerase chain reaction testing on a bone marrow aspirate sample. Immunohistochemical stains are also available to identify subtypes and cell types on BM trephine biopsy sections.

Ineffective haematopoiesis

In ineffective haematopoiesis, the BM activity ranges from normal to increased, but cells die before or shortly after release into the circulation. Common causes for ineffective haematopoiesis include MA (discussed below), drugs that interrupt DNA synthesis or folate metabolism (e.g. methotrexate, trimethoprim, phenytoin), HIV infection, and the MDSs. The MDSs are clonal haematopoietic disorders characterised by peripheral blood cytopenias and dysplastic haematopoiesis, with a predisposition towards developing acute leukaemia.

When suspected, MA should be excluded by means of serum vitamin B₁₂ and folate levels (performed prior to blood transfusion), as should HIV and other chronic infections. Where no other cause for ineffective haematopoiesis is found, further work-up for MDSs may be necessary (including BM examination with cytogenetic analysis), particularly in older patients.

Substrate deficiency

Iron deficiency is the most common cause of anaemia, occurring in an estimated 15% of the world's population.^[5] The causes of iron deficiency can be broadly classified as inadequate iron intake (e.g. nutritional deficiency, iron malabsorption) or excessive iron loss (mostly due to bleeding). It is most common in young children and women of childbearing age because of the increased iron demands related to rapid growth and menstrual blood losses/pregnancy, respectively.

Iron deficiency causes a microcytic and/or hypochromic anaemia. Serum iron studies show low serum ferritin and raised transferrin (TF) levels, and low TF percentage saturation. The differential diagnosis for microcytic anaemia includes anaemia of chronic disorder (ACD), thalassaemia trait and sideroblastic anaemia. The frequency of these differential diagnoses depends on the demographics of the population served, with thalassaemia trait being common in patients of Indian or Mediterranean ancestry, and ACD occurring frequently in patients with chronic infection (e.g. HIV or TB), malignancy or autoimmune disorders. ACD is caused by a combination of functional iron deficiency (due to reticulo-endothelial iron blockade), and EPO deficiency or resistance, and is characterised on iron studies by raised ferritin and low TF levels with low or normal TF percentage saturation. The anaemia is usually normocytic and normochromic, but can be microcytic in approximately one-third of cases due to long-standing iron restriction at the macrophage level.

Occasionally, the distinction between iron deficiency anaemia (IDA) and ACD can be blurred, as ferritin is an acute-phase protein and may be falsely normal or raised in patients with iron deficiency in the presence of acute inflammation. In this setting, the gold standard for discriminating IDA from ACD is evaluating BM iron stores microscopically. Measuring the soluble TF receptor (STFR) levels and calculating the STFR index (STFR:log ferritin ratio) is also of use in this regard, where a ratio >2 suggests iron deficiency with coexisting ACD.^[6] Unfortunately, STFR testing is not universally available in SA laboratories. Of note is that serum iron levels are of minimal use in assessing iron stores, reflecting recent iron intake as opposed to overall iron status. IDA should therefore not be diagnosed solely on the basis of a low serum iron level.

To establish if someone has iron deficiency, one has to include a thorough dietary history, with emphasis on the frequency of red meat consumption, excessive dietary phytates (present in grains and legumes) or tannins (present in tea), which inhibit iron absorption, and pica. In the absence of an obvious nutritional cause, a source of bleeding should be sought (e.g. urogenital, gastrointestinal), particularly in men and postmenopausal women.

Oral iron supplementation is the mainstay of therapy for IDA, but intravenous iron is occasionally necessary when more rapid correction of the Hb level is desired, if iron malabsorption is the main cause for the IDA, or if oral iron is not tolerated. A blood transfusion is indicated for severe, symptomatic anaemia, especially in patients who are bleeding. Treatment of IDA should aim at restoring the Hb to normal, followed by a further 4 - 6 months of therapy to replenish iron stores (Table 2). Iron should ideally be given between meals together with vitamin C to maximise absorption, but it can be administered with food or at reduced doses in patients who experience excessive abdominal side-effects (e.g. abdominal discomfort, nausea or constipation). For patients who are refractory to oral iron therapy, iron malabsorption, ongoing blood loss and non-compliance should be considered.

The treatment of ACD is centred on management of the underlying cause. There may be a role for EPO supplementation, particularly in patients with underlying malignancy^[7] and HIV.^[8] Intravenous iron may additionally benefit patients with functional iron deficiency.^[9]

MA is the other major nutritional anaemia caused by vitamin B₁₂ or folate deficiency. As both are required for purine biosynthesis, a deficiency of either nutrient hampers normal DNA synthesis, which results in ineffective haematopoiesis.

Vitamin B₁₂ deficiency is the most frequent cause of MA seen in general practice, the most common cause being pernicious

Table 2. Suggested therapeutic protocols for nutritional anaemia

Micronutrient deficiency	Suggested therapeutic protocol
Iron	Ferrous sulphate 200 mg orally 3 times daily between meals together with vitamin C for 4 - 6 months
Folate	5 mg orally daily for 2 - 4 months (or while the underlying risk factor persists)
Vitamin B ₁₂	1 000 µg daily intramuscularly for 5 - 7 days, followed by 1 000 µg intramuscularly weekly for a month, and then 1 000 µg intramuscularly every 2 months for life

Table 3. Causes of vitamin B₁₂ and folate deficiencies

Vitamin B ₁₂ deficiency	Folate deficiency
Dietary deficiency	Dietary deficiency
Ovolactovegetarians	Poor diet (e.g. alcoholics, elderly)
Malabsorption	Malabsorption
Pernicious anaemia	Inflammatory bowel disease
Ileal pathology	Coeliac disease
Crohn's disease/ulcerative colitis	Short-bowel syndrome
Infection, e.g. HIV, TB	Impaired folate metabolism*
Infiltration, e.g. lymphoma surgery	Drugs (e.g. methotrexate, anticonvulsants, antimalarials)
Gastrectomy, ileal resection	Alcoholism
Diphyllobothrium latum (fish tapeworm)	Hypothyroidism
Blind loop syndrome	Excessive folate demand
Drugs (e.g. proton pump inhibitors, metformin)	Chronic haemolytic anaemia
	Pregnancy and lactation (particularly if the diet is poor)

*Serum folate levels may be normal.

anaemia. The latter leads to impaired vitamin B₁₂ absorption owing to immune-mediated destruction of the gastric parietal cells and consequent intrinsic factor deficiency. In contrast, dietary deficiency and the postpartum phase most commonly cause folate depletion. Dietary deficiency is currently relatively uncommon in SA owing to mandatory fortification of maize meal and wheat flour. Other causes of vitamin B₁₂ and folate deficiency are summarised in Table 3.

MA is diagnosed by demonstrating low serum levels of vitamin B₁₂ or folate, along with characteristic peripheral blood smear morphology (including macro-ovalocytosis, teardrops, varying numbers of red cell fragments and basophilic stippling, and hypersegmentation (right shift) of neutrophils). Importantly, MA is not universally macrocytic, and cannot be excluded solely owing to the presence of a normal or even a low mean cell volume. When MA is strongly suspected, a trial of vitamin B₁₂ and folate supplementation is advisable, particularly if levels are borderline low.

When vitamin B₁₂ deficiency is diagnosed, further investigation for pernicious anaemia is warranted. Intrinsic-factor antibodies are positive in 50 - 70% of cases.^[10] In patients with pernicious anaemia, other organ-specific auto-immune disorders should be excluded (e.g. Addison's disease and auto-immune thyroiditis). Vitamin B₁₂ deficiency is treated with lifelong parenteral vitamin B₁₂ replacement therapy, while folate depletion is treated with oral folate supplementation (Table 3). Importantly, folate administration in patients with MA secondary to vitamin B₁₂ deficiency may partially correct the anaemia, but does not address the effects of vitamin B₁₂ deficiency on the central nervous system. Owing to the risk of neurological complications (such as subacute combined degeneration of the spinal cord) in patients with vitamin B₁₂ deficiency, MA should not be presumptively treated with folate alone, as this may

accelerate irreversible neurological damage. Although the mainstay of treatment in vitamin B₁₂ deficiency is supplementation with vitamin B₁₂, patients with very severe/life-threatening anaemia may benefit from a blood transfusion. Crucially, these patients should be transfused conservatively and cautiously (no more than 1 or 2 units of blood should be transfused, each unit running over ~2 - 4 hours, followed by 20 mg of intravenous furosemide (Lasix) after each unit), as aggressive transfusion practices are associated with a risk of sudden death in this setting.

Conclusion

There are numerous and varied causes of anaemia. Given the vastness of the topic, a thorough history, physical examination and systematic laboratory investigation are key elements towards determining the cause of the condition.

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