Current Practice

Clinical and laboratory evaluation of childhood anaemia

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Introduction

Anaemia is a common public health problem in the world. It is estimated that over 1.9 billion people across the globe suffer from anaemia[1]. The highest prevalence is reported in South Asian and the Sub-Saharan African regions. Children, those who are aged between 6 months to 5 years in particular, are at increased risk of developing anaemia[2]. The prevalence of childhood anaemia ranges between 20-60% in most developing countries[3]. The National Nutrition and Micronutrient Survey (2012) reported that the prevalence of anaemia among children in Sri Lanka as 15.1%[4].

Despite being one of the most studied clinical problems, the definition of anaemia is still somewhat arbitrary. This is primarily due to the wide variation in normal levels of haemoglobin seen in different age groups, sexes, ethnic groups and physiological states[5]. Haemoglobin concentration is highest in newborns (14.5 - 22.5 g/dl) which drops rapidly over the first two months to reach a nadir at 9-10 g/dl[6]. This reduction, which is known as physiological anaemia of infancy, does not require treatment. Thereafter, haemoglobin gradually rises and stabilises above 11.0 g/dl by the age of 6 months. Hence, haemoglobin level below 11.0 g/dl in a child between 6 months to 5 years is considered as anaemia[7].

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Aetiology of anaemia

The aetiology of anaemia in children is diverse. The simplest classification based on the mechanism of causation, divides these causes into two main groups; i.e. decreased production of red blood cells (RBC) or increased loss of RBC (haemolysis or haemorrhage) (Figure 1). Decreased production of RBC can be due to nutritional deficiencies (iron, folate and vitamin B_{12}) or failure or infiltration of the bone marrow (Box 1). Haemolytic anaemias are sub-classified into intrinsic defects in the RBC (membranopathies, enzymopathies and haemoglobinopathies) and extrinsic factors that include immune-mediated haemolysis, drugs, toxins and mechanical destruction. Careful evaluation of the history and examination is essential to narrow down the differential diagnosis which should be followed by stepwise investigations to arrive at the exact diagnosis[8]. The subsequent sections of this paper present a practical approach to all paediatricians to identify the specific aetiology causing anaemia with the least number of investigations.

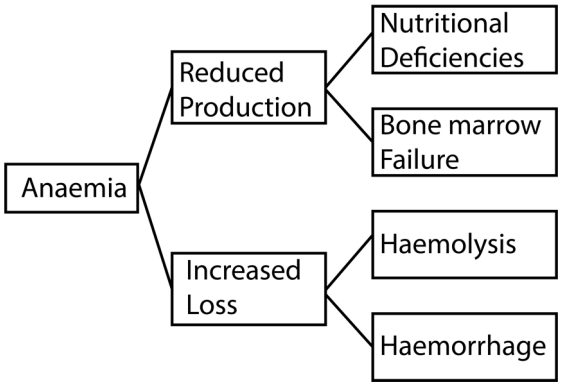


Figure 1: ***Classification of mechanisms causing anaemia***

Box 1: Aetiology of childhood anaemia

Anaemia due to reduced production of red blood cells

Nutritional deficiencies
- Iron deficiency
- Folic acid deficiency
- Vitamin B_{12} deficiency

Bone marrow failure or infiltration
- Pure red cell aplasia (e.g. Diamond Blackfan Anaemia, Transient erythroblastopenia of childhood, Red cell aplasia associated with parvovirus B19 infection)
- Aplastic anaemia (e.g. Fanconi anaemia, Acquired aplastic anaemia)
- Haematological malignancies (e.g. Acute leukaemia, Lymphoma)
- Anaemia of chronic diseases

Anaemia due to increased loss of red blood cells

Haemolytic anaemia
- Membranopathies
  - Hereditary spherocytosis
  - Hereditary elliptocytosis
- Enzymopathies
  - Glucose 6-phosphate dehydrogenase (G6PD) deficiency
  - Pyruvate kinase deficiency
- Haemoglobin disorders
  - Thalassaemia (eg: β-thalassaemia, α-thalassaemia, haemoglobin E disease)
  - Sickle cell disease
  - Other haemoglobinopathies
- Extracellular factors
  - Auto immune haemolytic anaemia
  - Haemolytic uremic syndrome
  - Thrombotic thrombocytopenic purpura
  - Disseminated intravascular coagulation
  - Drugs and toxins
  - Haemangiomas

Haemorrhage

Clinical evaluation – History

A vast majority of children with anaemia are asymptomatic and are diagnosed during physical examination or investigations performed for different reasons. A detailed history focusing on the following points is important to identify the aetiology of anaemia;

- ***Symptoms of anaemia:*** Lethargy, irritability, poor feeding and exercise intolerance are common symptoms of anaemia. These symptoms are present only in patients with severe or rapid onset anaemia.
- ***Onset and duration of anaemia:*** This provides useful information to identify possible causes. Most pathologies causing anaemia are of insidious onset. However, acute-onset anaemia could be seen in glucose 6-phosphate dehydrogenase (G6PD) deficiency, bone marrow infiltration, such as in acute leukaemia, auto immune haemolytic anaemia and microangiopathic haemolytic anaemia (haemolytic uremic syndrome, thrombotic thrombocytopenic purpura and disseminated intravascular coagulation).
- ***Bleeding manifestations***: Both revealed and concealed (especially in the gastrointestinal tract) bleeding could lead to anaemia. Alternatively, bleeding could be a symptom of thrombocytopenia which is associated with anaemia in bone marrow pathologies.
- ***Urine colour:*** Dark cola-coloured urine suggests haemoglobinuria due to intravascular haemolysis in haemolytic anaemias. Dark coloured urine is also seen in haemolytic anaemia due to high levels of urobilin in urine.
- ***Past history:*** Review of previous haemoglobin values and red cell indices provide useful information on the chronicity of anaemia**.** Instead, underlying medical conditions or

chronic diseases may indicate anaemia of chronic disease.

- ***Birth history:*** Prematurity is associated with anaemia of prematurity**.** Neonatal hyperbilirubinemia is suggestive of a haemolytic disease like hereditary spherocytosis, G6PD deficiency or pyruvate kinase deficiency.
- ***Developmental history:*** Fanconi anaemia and Diamond Blackfan anaemia can be associated with developmental delay.
- ***Dietary history****:* Nutritional deficiency of iron, folate and vitamin B_{12} are common causes for anaemia in children.
- ***Drug history:*** Antibiotics, antimalarials and analgesics can precipitate haemolysis in G6PD deficiency.
- ***Family history:*** Family history of anaemia, gall stones or splenomegaly suggest hereditary haemolytic diseases like hereditary spherocytosis or thalassaemia.
- ***Social history:*** Recent travel history to tropical African regions or India points towards infective causes of anaemia (e.g. malaria).

Clinical evaluation – Examination

The physical examination of children suspected of having anaemia should focus on the following;

- ***General appearance:*** Irritability, excessive crying and listlessness are general examination findings of anaemia.
- ***Pallor:*** Conjunctival and skin pallor provides a rough assessment of the degree of anaemia.
- ***Jaundice:*** Jaundice is suggestive of a haemolytic process.
- ***Growth parameters:*** Growth can get affected in severe anaemia. Conversely, some causes of anaemia (e.g. Fanconi anaemia) are associated with short stature.
- ***Facial appearance:*** Features of bone marrow expansion like frontal bossing, maxillary hyperplasia and dental malocclusion suggests thalassaemia major. However, these manifestations are rare except in extreme cases.
- ***Skin manifestations:*** Hyperpigmentation and café-au-lait patches are associated with Fanconi anaemia. Petechiae and purpura are suggestive of mucocutaneous bleeding due to thrombocytopenia or bone marrow infiltration. The skin due to nutritional deficiency can be coarse and rough to the touch.
- ***Thumb abnormalities:*** Thumb abnormalities are associated with Fanconi anaemia.
- ***Nutritional deficiencies:*** The presence of angular stomatitis and glossitis suggest iron deficiency. Koilonychia, is also seen with severe iron deficiency.
- ***Hepatomegaly:*** Hepatomegaly is suggestive of thalassaemia or haematological malignancies.
- ***Splenomegaly:*** Splenomegaly suggests thalassaemia, haematological malignancies or conditions associated with extravascular haemolysis (e.g. hereditary spherocytosis and elliptocytosis).
- ***Lymphadenopathy:*** Generalised lymphadenopathy is suggestive of haematological malignancies.

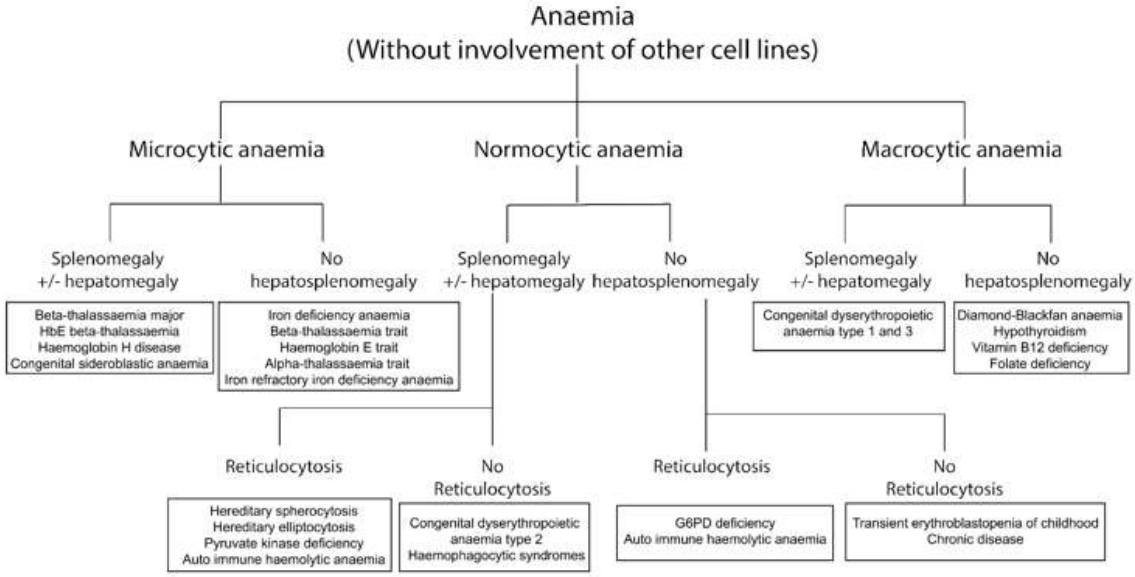
Initial investigation – Full blood count

Using a full blood count report from an automated analyser, the differential diagnosis of anaemia can be narrowed down substantially. Very useful information which are outlined below can be gathered from a full blood count.

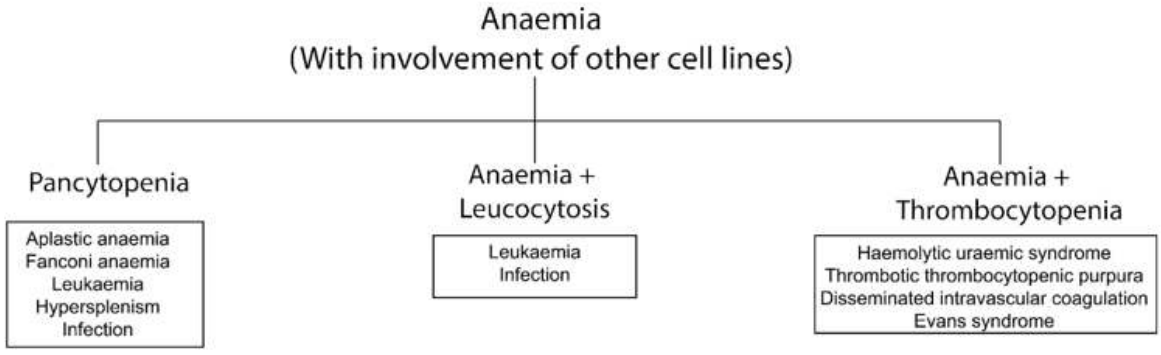
- ***Haemoglobin:*** The haemoglobin level is essential to confirm anaemia and define its severity. Anaemia is diagnosed when the haemoglobin is <11.0g/dl in children between 6 months to 5 years and <11.5g/dl in children between 5-12 years. Severe anaemia is defined as haemoglobin <7.0g/dL.
- ***White blood cell count:*** Leukopenia suggests bone marrow failure or hypersplenism and leucocytosis supports a diagnosis of haematological malignancy. However, normal white blood cell count does not exclude leukaemia.
- ***Platelet count:*** Thrombocytopenia is associated with microangiopathic haemolytic anaemia. Also, it may be an indicator of pancytopenia or bone marrow infiltration.
- ***Mean corpuscular volume (MCV):*** Normal range of MCV varies with age. However, in children the normal range is approximately 75-95fL. Anaemia is classified based on the MCV into microcytic (MCV<75fL), normocytic (MCV 75-95fL) and macrocytic (MCV>95fL) anaemia.
- ***Mean corpuscular haemoglobin (MCH):*** Normal range is between 27-32pg. A low MCH indicates decreased haemoglobin content per cell and is typically seen in iron deficiency and thalassaemia.
- ***Mean corpuscular haemoglobin concentration (MCHC):*** Low MCHC is typical of iron deficiency and high MCHC values reflect spherocytosis.
- ***Red cell distribution width (RDW):*** This is a measure of the variation in RBC size. A high RDW implies a large variation in RBC size and a low RDW implies a homogeneous population of RBCs. High RDW is characteristic of iron deficiency anaemia.
- ***Reticulocyte count and percentage:*** Normal reticulocyte percentage is 1-2%. Reticulocytosis (>2%) suggests haemolytic

anaemia while reticulocytopenia is suggestive of red cell aplasia or aplastic anaemia.

Based on the clinical findings and results of full blood count, differential diagnosis of anaemia in children can be narrowed down to a considerable extent. Flow-chart 1 presents a rational guide to arrive at a diagnosis when anaemia is isolated without involvement of other cell lines (white blood cells and platelets). Flow-chart 2 provides the differential diagnosis when the anaemia is associated with abnormalities in other cell lines. Further confirmatory investigations should be planned based on the likely diagnosis according to these flow-charts.



Flowchart 1: ***A rational approach to narrow down the differential diagnosis in a child presenting with anaemia without abnormalities in the white blood cells and platelets***



Flowchart 2: ***An approach to identifying the aetiology in children who present with anaemia with abnormalities in white blood cells or platelets***

Investigations of microcytic anaemia

The two most important investigations that help to differentiate common causes of microcytic anaemia are serum ferritin and haemoglobin subtype quantification by high performance liquid chromatography (HPLC) or capillary electrophoresis (CE).

- ***Serum ferritin:*** Serum ferritin is the most widely used investigation to diagnose iron deficiency. Although the reference range may vary from laboratory to laboratory, a serum ferritin value less than 15ng/dL in a child is confirmatory of iron deficiency anaemia. However, serum ferritin is an acute phase protein thus, could erroneously be elevated in the presence of inflammation leading to false negative results.
- ***Serum iron profile:*** This includes, serum iron, total iron binding capacity and percentage saturation of transferrin. Serum iron profile is less affected by inflammation therefore, is suitable in a child with infection or inflammation. In iron deficiency, serum iron is

low, total iron binding capacity is increased and transferrin saturation is <16%.

- ***Haemoglobin subtype quantification (HPLC or CE):*** Haemoglobin HPLC or CE is useful in diagnosing β-haemoglobinopathies (Table 1)[9]. In instances where interpretation is difficult, family screening with HPLC/CE studies of parents and siblings are helpful.

Table 1: Quantification of haemoglobin subtypes in haemoglobinopathies

Subtype	HbA	HbA2	HbF	Other
Normal	>90%	<3.4%	<2%	-
β-thalassaemia trait	>90%	3.5%-7.0%	1-3%	-
Hb E trait	50-80%	<3.4%	1-3%	Hb E: 20%-50%
Hb S trait	50-80%	<7.0%	1-3%	Hb S: 20%-50%
β-thalassaemia major	<10%	<3.4%	>70%	-
Hb E β-thalassaemia	<10%	<3.4%	30%-70%	Hb E: 30%-50%
Hb S β-thalassaemia	<30%	<3.4%	<20%	Hb S: 50%-80%
α-thalassaemia	>90%	<3.4%	<2%	-

Normal serum ferritin or iron profile and normal haemoglobin subtype quantification excludes both iron deficiency and β-haemoglobinopathy in a child with microcytic anaemia. Among such children, a large majority have α-thalassaemia trait. A recent island wide survey revealed that approximately 8% of Sri Lankan population has α-thalassaemia trait[10]. Similarly, iron deficiency can coexist with α- or β-thalassaemia[11]. Therefore, considering the importance of prevention of β-thalassaemia, it is important to investigate for β-thalassaemia trait even if a diagnosis of iron deficiency is made in a child with microcytic anaemia[12,13].

- ***α-globin gene molecular studies:*** α-thalassaemia trait could only be diagnosed by molecular studies. Currently, seven common deletional α-thalassaemia mutations ($\alpha^{-3.7}$, $\alpha^{-4.2}$, $--^{MED}$, $--^{SEA}$, $--^{THAI}$, $--^{FIL}$ and $--^{20.5}$) can be identified by multiplexed polymerase chain reaction method in Sri Lanka.
- ***Haemoglobin H inclusion bodies:*** The peripheral blood film will be positive for Haemoglobin H inclusion bodies in patients with haemoglobin H disease. This low-cost test is available in haematology laboratories in most teaching hospitals.
- ***Bone marrow aspiration and biopsy:*** Bone marrow aspiration and biopsy is rarely required in the investigation of a hypochromic microcytic anaemia. The only indication is in a child with microcytic anaemia and hepatosplenomegaly in whom thalassaemia is conclusively excluded. These children could have rare congenital sideroblastic anaemia which is diagnosed by the presence of ring sideroblasts in the bone marrow.
- ***Genetic studies to identify mutations causing iron refractory iron deficiency anaemia:*** Iron refractory iron deficiency anaemia is suspected in children who have identical clinical and laboratory characteristics of iron deficiency anaemia (microcytic anaemia and low serum ferritin) but do not respond to oral iron. This rare entity is due to a variety of genetic mutations of which mutations in the *TMPRSS6* gene are well characterised. These mutations can be identified by DNA sequencing however, facilities are not available in Sri Lanka.

Investigations of macrocytic anaemia

The following investigations are helpful to arrive at a diagnosis in a child with macrocytic anaemia.

- ***Blood picture:*** Presence of oval macrocytes and hyper segmented neutrophils suggest the possibility of megaloblastic anaemia due to folate or vitamin B_{12} deficiency.
- ***Bone marrow aspiration and biopsy:*** In megaloblastic anaemia the bone marrow is hypercellular, with erythroid hyperplasia and giant metamyelocytes. Markedly reduced or virtually absent erythrocyte precursors in the marrow suggests pure red cell aplasia (e.g. Diamond Blackfan anaemia). Bone marrow erythroid hyperplasia, megaloblastosis and binucleated and multinucleated polychromatophilic erythroblasts are indicative of congenital dyserythropoietic anaemia.
- ***Serum folic acid / RBC folate levels:*** These will be low in the presence of folate deficiency.
- ***Serum vitamin B_{12} levels:*** Serum vitamin B_{12} will be low in megaloblastic anaemia due to Vitamin B_{12} deficiency. Red cell folate levels are also reduced in the presence of vitamin B_{12} deficiency.
- ***Thyroid function test:*** This is important to exclude hypothyroidism as a cause for macrocytic anaemia.
- ***Erythrocyte adenosine deaminase (ADA) activity:*** ADA is increased in most patients with Diamond Blackfan anaemia.

Investigations of normocytic anaemia

Normocytic anaemia is most commonly due to haemolytic anaemia or bone marrow failure. It may or may not be associated with involvement of other cell lines. Haemolysis could be either extravascular (occurring within phagocytic macrophages in the spleen, liver or lymph nodes) or intravascular (occurring within blood vessels). Hereditary spherocytosis, hereditary elliptocytosis, pyruvate kinase deficiency and warm type autoimmune haemolytic anaemia result in extravascular haemolysis while G6PD deficiency, cold type auto immune haemolytic anaemia and microangiopathic haemolytic anaemia predominantly cause intravascular haemolysis. Following investigations aid to arrive at the diagnosis of normocytic anaemia which is not associated with abnormalities in white blood cells or platelets.

- ***Reticulocyte count:*** Reticulocytosis (reticulocyte count >2%) is indicative of haemolytic anaemia while reticulocytopenia is an indicator of bone marrow aplasia.
- ***Blood picture:*** Blood picture is possibly the most useful investigation to find the cause in a child with normocytic anaemia. Most haemolytic anaemias have characteristic blood picture features;
    1. Microspherocytes – hereditary spherocytosis or autoimmune haemolytic anaemia
    2. Elliptocytes – hereditary elliptocytosis
    3. Bite cells, and blister cells – G6PD deficiency
    4. Fragmented RBC (schistocytes and helmet cells) – microangiopathic haemolytic anaemia

  Additionally, blood picture will be useful in evaluating abnormalities in white blood cells (e.g. blast cells in acute leukaemia) and platelets (e.g. thrombocytopenia in microangiopathic haemolytic anaemia and large platelets in Evans syndrome) that points towards a diagnosis in normocytic anaemia.
- ***Serum bilirubin (total and direct):*** Indirect hyperbilirubinemia is suggestive of a haemolytic process.
- ***Serum lactate dehydrogenase:*** Elevated lactate dehydrogenase suggests haemolytic disease.
- ***Haemoglobinuria:*** Presence of haemoglobin in urine and cola coloured urine is an indicator of intravascular haemolysis.
- ***Direct agglutination test:*** Positive direct agglutination test (Direct Coombs test) is suggestive of immune mediated haemolysis and autoimmune haemolytic anaemia.
- ***Osmotic fragility test:*** Osmotic fragility is increased in the presence of spherocytes in hereditary spherocytosis. However, this test is not specific for hereditary spherocytosis. It may be positive in the presence of auto immune haemolysis as well in the presence of micro spherocytes. Auto haemolysis test and glycerol lysis test though more specific for spherocytosis offer no advantage over the osmotic fragility test.
- ***G6PD enzyme activity:*** Decreased G6PD enzyme activity in RBC is confirmatory of G6PD deficiency. However, this test can be falsely negative during a haemolytic crisis of G6PD deficiency. To confirm G6PD deficiency, G6PD enzyme levels or assay should be repeated approximately 3 to 4 months following the episode of acute haemolysis. A reticulocyte count should be performed prior to the G6PD assay to confirm that the child is not actively haemolysing RBCs.

Normocytic anaemia without reticulocytosis is due to transient erythroblastopenia of childhood (TEC), anaemia of chronic disease or rarely congenital dyserythropoietic anaemia (CDA) type 2. TEC should be suspected in an otherwise healthy child with normocytic anaemia and reticulocytopenia. In these children, extensive investigations are not necessary, provided that the blood picture does not suggest RBC disorder or leukaemia.

When anaemia is associated with reductions in other cell lines, aplastic anaemia, hypersplenism or leukaemia should be suspected. In such instances urgent bone marrow aspiration and biopsy is indicated.

- ***Bone marrow biopsy:*** Bone marrow aspiration and biopsy is helpful to examine the erythropoiesis, granulopoiesis and thrombopoiesis as well as the entire bony trabecular architecture. Special tests, including cytogenetics, karyotyping, and stains are used to arrive at a diagnosis, depending on the underlying condition. In aplastic anaemia the bone marrow is hypocellular and the marrow space is replaced by fat cells. Inherited marrow failure syndromes (e.g. Fanconi anaemia, Schwachmann Diamond Syndrome) often show dysplastic features such as hypo-nucleated small megakaryocytes, multinucleated red cells and hypo-lobulated and hypo-granular myeloid cells. Leukaemia results in replacement of the cellular component of the marrow by immature or undifferentiated cells.

Conclusion

Evaluating a child with anaemia includes a careful history, examination, and well-planned investigations. Most diagnoses can be easily made. However, in some instances (e.g. congenital dyserythropoietic anaemia, iron refractory iron

deficiency anaemia and haemolytic anaemia due to rare red cell enzyme deficiencies) the diagnosis is difficult and time consuming. Nonetheless, most of these unidentified causes of anaemia may be diagnosed using molecular genetic tests in the near future.

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