1. Guidelines for Investigating Anaemia

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1.1 Guidelines to Investigate Non Haemolytic Anaemia

The following guidelines are mainly based on laboratory investigations, keeping the relevant clinical features and laboratory quality assessment in mind.

Hb% is the sole parameter to decide whether the patient has anemia or not.

Therefore the method used to estimate this has to have good specificity and reproducibility.

Cyanmethaemoglobin method with spectrophotometric estimation has stood the test of time to be the best. It is also relatively a low cost test and does not need much expertise to perform.

A haemoglobin value of

11.5 g/dl for adults.
11.0 g/dl for pregnant female
10.5 g/dl for children between 1-2 yrs.

for practical purposes, may be considered as lower normal limits, and any value less than this needs to be considered as anemia. At birth, the normal minimum value of Hb is 12.5 g/dl and at 1 year 10.5g/dl ,as a result of the physiological changes taking place in the infancy. It rises gradually to normal adult range by puberty.

Therefore age related normal ranges of haemoglobin value need to be available especially in Paediatric practice. Age and sex related reference ranges should be kept available for clinical and laboratory staff to refer often.

Anaemia is subdivided into hypochromic microcytic, macrocytic and normochromic normocytic types for investigational purposes. This **morphological** typing often help to arrive at an aetiological diagnosis. The following routine investigations give further diagnostic information.

- Full Blood Count (FBC)
- Blood picture
- Reticulocyte count

The FBC is a deceptively simple test to perform and interpret, with the help of the haematology auto analyzer. The haematological indices like MCV, MCH, RBC, RDW which are readily produced by the analysers make the subclassification of anaemiua easy. The less resourced labs can still get adequate information by doing a PCV, a manual WBC count, a differential count and when necessary a manual red cell count. The manual methods also have a place as a tool in checking the quality of the auto analyzers.

A well prepared and well stained blood film is an asset for the Pathologist to produce a quality **Blood picture** report. It should show relevant red cell, white cell and platelet changes, which are important to identify the cause of

anemia . Preparation of a good blood film is the work of adapting the correct methods of slide cleaning, preparation of the stain and following the right steps of the staining procedure . However, MCV may be normal in mixed deficiency anaemia or in the presence of large numbers of polychromatic cells. In these situations, a blood picture will be most useful.

Reticulocyte count is performed manually, or using advanced auto analyzers. The manual reticulocyte count is the preferred method as it is cheaper and more specific. The relative reticulocyte count expressed as a percentage may be misleading. Therefore the absolute reticulocyte count needs to be calculated using the red cell count which can be obtained from an analyzer. The corrected reticulocyte count, an estimate of the true reticulocyte count corrected for anemia, can be obtained by either of the formulae given below

$$\frac{PCV}{0.45} \text{ or } \frac{Hb}{15}$$

1.1.1 Hypochromic Microcytic Anaemia (MCV<76, MCH<27)

Common causes of hypochromic microcytic anaemia are,

- iron deficiency anaemia
- thalassaemia trait
- long standing anaemia of chronic disease
- sideroblastic anaemia

Before iron deficiency anaemia develops a patient can be in a state of complete depletion of iron stores with normal Hb and MCV. Often MCH appears to be the first parameter to show a low value when a patient develops iron deficiency anaemia. Iron studies (serum ferritin, serum iron, total iron binding capacity) should be performed, if the cause for anaemia is not clinically obvious like in haemorrhoids, menorrhagia, nutritional deficiency etc.
The following table provides information on how to identify the cause of a hypochromic microcytic anemia by iron studies.

Disorder	Serum ferritin	TIBC	Serum iron	Bone marrow iron
Iron deficiency	↓	↑	↓	↓
Thalassaemia trait and minor haemoglobinopathies	N or ↑	N	N or ↑	N or ↑
Anaemia of chronic disorder	N or ↑	↓	↓	N or ↑
Iron deficiency with inflammation	↑	↑	Nor ↑	↓

Serum ferritin and serum iron levels do not truly reflect iron status in certain situations.
Serum ferritin may give a falsely high value when there is a coexisting infection or inflammatory disorder,during

febrile illnesses, in acute or chronic liver disease, and in acute leukaemias.

Serum iron can be falsely high after a blood transfusion, and while on iron therapy, and it can be low in chronic renal failure with or without dialysis

Serum soluble transferrin receptor level helps in differentiating iron deficiency anaemia from anaemia of chronic disorder. A high value (normal 2.5-8.5 mg/l) is seen in early iron deficiency. Increased red cell protoporphyrin level is a stable measure of iron deficiency over previous few weeks, provided sideroblastic anaemia and Lead poisoning is excluded. (Grade Y)

Hypochromic microcytic anemia with normal or increased iron stores warrant few more investigations for a final diagnosis. Elevated RBC, normal MCHC along with the investigations of a haemoglobinapthy should identify the type of thalassaemia or haemoglobinopathy trait.

Bone marrow morphology is sufficient for the diagnosis of sideroblastic anaemia but need more information to find the cause.

Genetic studies to identify the specific mutation can confirm the diagnosis and type further in the above disorders. (Grade Z)

1.1.2 Macrocrocytic Anaemia (MCV>96fl, MCH 27-32pg)

Causes for macrocytosis could be physiological or pathological.

Physiological macrocytosis is seen in the neonatal period and in pregnancy.

Pathological causes can be broadly divided as megaloblastic macrocytosis and normoblastic macrocytosis.

Megaloblastic erythropoiesis is characterized by the presence of oval macrocytes while non megaloblastic erythropoiesis has round macrocytes.

A detailed clinical history and examination is useful in identifying the cause of macrocytosis.

The history and examination should include – alcohol intake, diet, drugs, malabsorptive states, features of hypothyroidism, presence of other autoimmune disorders, presence of liver disease, smoking, presence of chronic lung disease etc.

The full blood count, blood picture and the retic count often are sufficient to differentiate megaloblastic from non megaloblastic macrocytosis .

Certain features like oval macrocytes, tear drop cells, fragmented cells, pancytopenia and hypersegmented neutrophils in the blood film,favour the diagnosis of megaloblastosis, which should be confirmed with a bone marrow biopsy prior to starting specific replacement

therapy. The other important dignostic step is to differentiateB_{12} and Folate dependent megaloblastosis from B_{12} and Folate independent megaloblastosis. B_{12} dependent causes broadly include

* nutritional deficiency of B_{12} or Folate
* non availability of the absorption area in the intestine
* utilization of B_{12} by abnormal bacterial flora or parasites
* Food cobalamine deficiency

Schilling's test with and without food , gastric and intestinal biopsy, intrinsic factor antibody level etc. are performed according to the diagnosis suspected.

Presence of B_{12} or Folate deficiency in these situations is confirmed by serum B_{12} and Folate levels, keeping in mind the following diagnostic pitfalls.

- Serum or red cell Folate can be low in about 60 % of B_{12} deficient patients and serum B_{12} can be low in Folate deficient patients, in normal pregnancy, in liver disease, inpatients on antiepileptic drugs,and in multiple myeloma.
- Mild red cell changes in the blood film and in the indices can occur before B_{12} and Folate levels drop.
  Serum folate level drops before red cell folate but red cell folate is normally performed as it is more reliable.
- Both Homocystine and Methyl malonic acid are almost always elevated in B_{12} deficiency and only homocystine is elevated in Folate deficiency

However in the present setup a theraputic trial with B_{12} and Folate may be all that is needed for B_{12} and Folate dependent megaloblastosis.

But if B_{12} and folate independent causes like
TC II deficiency
Homocystine urea
Or defects in the pathway of DNA metabolism
is the cause, the following steps need to be considered.

The B_{12} and folate level can be normal or increased in these disorders. Serum holo TC II level is the way to confirm TC II deficiency.

Serum B_{12} is elevated without related abnormalities in the following disorders

Myeloproliferative disorders
TC II deficiency

Normoblastic macrocytosis has some identified causes. The common causes are

* Hypothyroidism
* Obstructive airways disease
* Alcoholism
* Liver disease
* Fanconi's anaemia
* Aplastic anaemia

1.1.3 Normochromic Normocytic Anaemia

In addition to the normochromic and normocytic red cell picture, the red cell count, reticulocyte count, MCV and the MCH are vital parameters in this entity.

Reduced RBC and reticulocyte count indicates poor red cell production which can be due to

- anemia of chronic disease
- acute systemic illness
- malignancy.

A high reticulocyte count in the presence of normocytic normochromic anaemia may be seen in acute blood loss even an iatrogenic one.

Normal or high serum ferritin level with low serum iron and TIBC, normal percentage saturation is the pattern in an uncomplicated anaemia of chronic disease.

The investigations focused accordingly should include :

- ESR
- CRP
- liver profile
- renal profile
- LDH
- tumour markers
- autoimmune profile .

1.2 Guidelines to Investigate Haemolytic Anaemias

1.2.1 Introduction

Haemolysis means that the rate of destruction of red cells is increased above normal. The bone marrow can increase the production of red cells by six fold, partly from increased cellularity of existing haemopoetic cells and partly by expansion of the marrow in long bones and other sites of fatty marrow. When the rate of haemolysis exceeds the rate of production, the result is anaemia.

1.2.2 First line investigations:

- Full Blood Count with indices
- Reticulocyte count/ Absolute retic count/ Retic index
- Serum bilirubin
- Urine urobilinogen.
- Serum haptoglobin/ haemopexin
- Urine Haemoglobin & Haemosiderin
- Blood picture

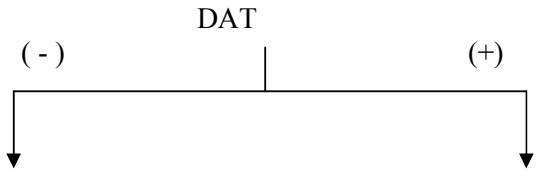
1.2.3 Second line investigations:

Depending on blood picture findings, the second line tests are decided.

Blood picture findings could be;

- **Spherocytes**
- **Elliptocytes**
- **Fragmented cells**
- **Agglutination**
- **Non- specific.**
- **Haemoglobinopathy**

When the blood picture shows **Spherocytes**



- Osmotic Fragility test
- Glycerol Lysis test
- Cryohaemolysis .
- Cell membrane protein electrophoresis
- Family Screening

Investigate for Autoimmune Haemolytic anaemia and other causes

When the blood picture shows **Elliptocytes**

- Membrane studies
- Family screening

When the blood picture shows **Fragmented cells** - according to morphology

- Glucose-6 Phosphate Dehydraganase deficiency
    - Brewer's test (When retic count is normal)
    - Florescent screening test for G6PD
    - Heinz bodies
- Pyruvate Kinase deficiency
    - Pyruvate kinase assasy
- Microangiopathic anaemias
    - Coagulation screening
    - D-Dimers/FDP
    - Renal profile
    - Liver profile
- Drug induced & other acquired causes
- Other causes
    - Infections
    - Physical/ Chemical/mechanical damage to redcells.

When the blood picture shows **Agglutination**

- Direct coombs test
- Mono-specific test
- Cold agglutinin titre
- Look for aetiology
- Bone marrow if indicated

When the blood picture is a **Non- specific blood picture**

DAT $+^{ve}$ ⟶ Doneth - Landsteiner test

DAT- ^{ve} ⟶ HAM's
Flowcytometry for CD markers
Bone marrow
Urine for Haemosiderin

When the blood picture shows **Haemoglobinopathy** features

- Haemoglobin electroporesis :acid and alkaline
- Sickling test
- HPLC
- Isopropanyl test.

- Heat stability test.
- Heinz bodies elastration
- Acid elusion test.
- Alkaline denaturation test and Hb F estimation
- Quantitation of haemoglobin variants.
- Isoelectric focusing

1.2.4 References

1. Victor A, Catovsky D,Edward GD, Tuddenham,. *Postgraduate Haematology*, ,5th ed.2005.Blackwell Publishing

2. Guidelines for the Diagnosis and Management of Hereditary Spherocytosis - *British Journal of Haematology 2004; 126:455-474*

3. Guidelines for the management of iron deficiency anaemia - British Society of Gastroenterology - May 2005