Microcytosis and microcytic anaemia

GENERAL POINTS

Definition

- Anaemia is a pathological condition which results in the inability of all RBCs to transport sufficient oxygen to meet physiological requirements. It is defined by a haemoglobin level below the reference values (this limit varies according to sex and age).
- Microcytosis is defined by an MCV below the reference values (this limit varies according to age).
- Microcytic anaemia may be hypochromic or normochromic and, very rarely, hyperchromic (MCHC reference values: 32-35 g/dL).

INVESTIGATION

- **If Hb:**
  < 13 g/dL in males
  < 12 g/dL in females
  < 10.5 g/dL in pregnant women (second and third trimesters)
  < 13.5 g/dL at birth
  < 9 g/dL at 2 months
  Gradual increase up to puberty
- **With MCV:**
  < 80 fL in adults
  < 95 fL at birth
  < 70 fL from 6 months to 2 years
  Gradual increase up to puberty

NOTE: in the absence of hypochromia, haemoglobin levels may be estimated based on haematocrit (Hb ≈ 1/3 Ht).

Physiological variations

- Intra-individual biological variability (EFLM, 2019[1]): estimated at 3% for Hb, 1.7% for MCV and 0.9% for MCHC.
- Neonates:
  - neonatal polycythaemia (up to 23 g/dL) related to intra-uterine hypoxia. A slight increase in Hb levels due to haemoconcentration is observed in the first few days, followed by a gradual decrease falling to the lowest level at 2 months. Haemoglobin levels then increase up to puberty to reach adult levels (with Hb levels > 0.5 to 1 g/dL in boys owing to increased testosterone secretion);
  - Hb levels. Levels depend on the type of sample taken and are slightly higher in capillary samples, and significantly lower in cord blood samples;
  - physiological macrocytosis (up to 120 fL) notably related to the higher lipid content of the cell membrane. MCV remains elevated in the first 2 weeks, then gradually decreases to its lowest level at around 6 months, then gradually increases up to puberty to reach adult values. MCV is inversely proportional to the degree of prematurity and can be > 130 fL in certain extreme cases.
- Pregnancy: anaemia due to haemodilution which generally starts from the second trimester of pregnancy. This is related to an earlier and more extensive increase in plasma volume compared to the increase in corpuscular volume. Anaemia is more pronounced with increasing foetal weight and number.
- African populations: mean Hb levels are lower by 0.8 to 1 g/dL compared to that of Caucasian populations.

DIAGNOSTIC APPROACH

Eliminate artifactual causes

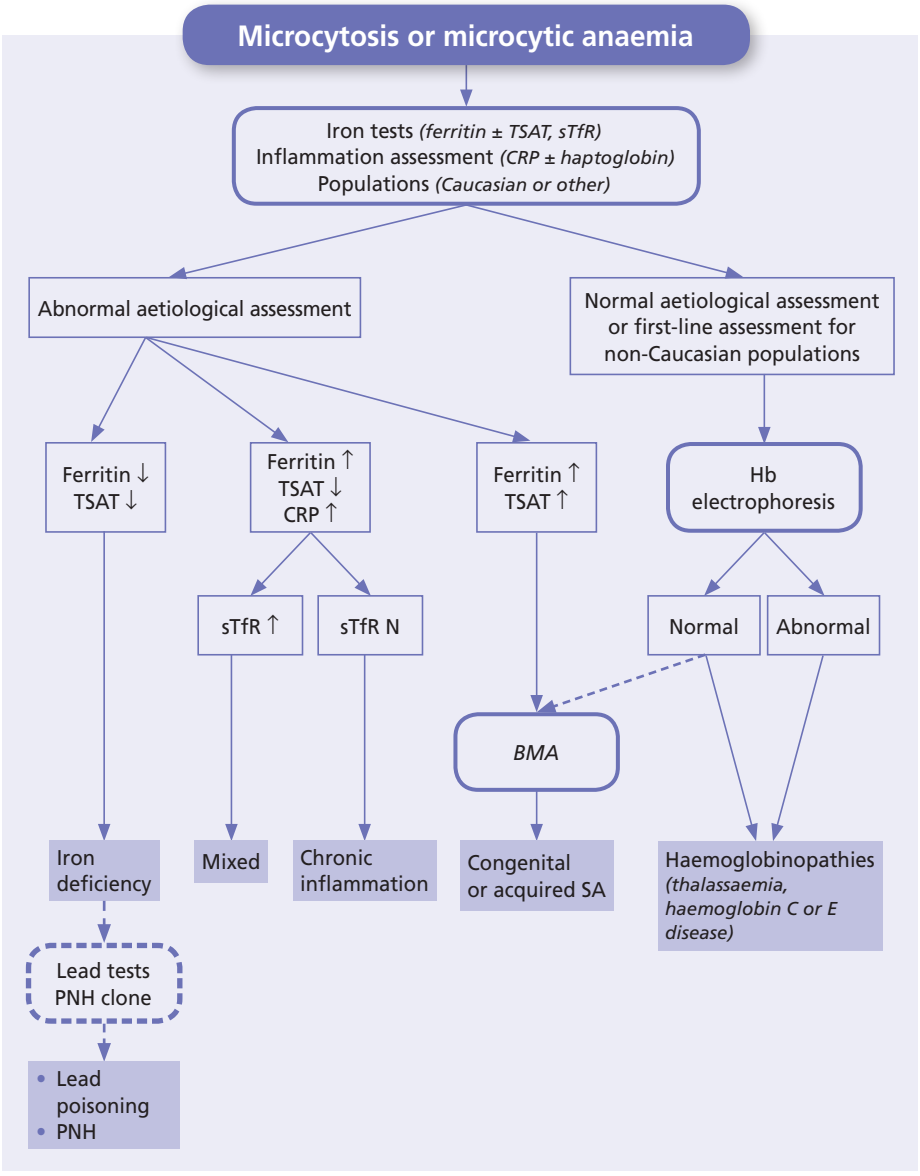
- Pseudoanaemia is a common artefact and is related to a pre-analytical problem in the majority of cases.

Causes	Procedure
Coagulated sample (e.g. difficulty taking the sample)	• Take a new sample
Diluted sample (e.g. patient administered an infusion)	• Take a new sample

NOTE: if blood gases are tested at the same time as CBC, Hb values may be compared in the event of suspicion of pseudoanaemia.

- Spurious microcytosis is rarely observed. MCV is a very stable parameter over time. A variation > 6 fL in less than 8 days (determined using the same analyser) should prompt investigation for a tube identification error or analytical interference.

Causes	Procedure
Plasma hypoosmolarity related to hyponatraemia (e.g. syndrome of inappropriate ADH secretion)	• New sample after correcting the cause of plasma hypoosmolarity


Microcytosis or microcytic anaemia
Iron tests (ferritin ± TSAT, sTfR)
Inflammation assessment (CRP ± haptoglobin)
Populations (Caucasian or other)
Abnormal aetiological assessment
Normal aetiological assessment or first-line assessment for non-Caucasian populations
Ferritin ↓ TSAT ↓
Ferritin ↑ TSAT ↓ CRP ↑
Ferritin ↑ TSAT ↑
Hb electrophoresis
sTfR ↑
sTfR N
Normal
Abnormal
BMA
Iron deficiency
Mixed
Chronic inflammation
Congenital or acquired SA
Haemoglobinopathies (thalassaemia, haemoglobin C or E disease)
Lead tests PNH clone
Lead poisoning
PNH

Decision-making algorithm

Adults

- Iron deficiency is the leading cause of microcytic anaemia. In the majority of cases, it is related to chronic bleeding. It is three times more common in females than in males, with an estimated incidence of 2-3% in the general population. This is related to menstruation (excessive iron loss), pregnancy (increased requirements) and more common dietary restrictions (insufficient iron intake).
- Haemoglobinopathies are a conventional cause of microcytosis in non-Caucasian populations.

Paediatric population

- Iron deficiency is also the most common aetiology. In the majority of cases, it is related to insufficient iron intake. Intolerance to cows' milk protein and repeated diarrhoea are also common causes.
- Inflammatory anaemia is rare (juvenile rheumatoid arthritis, lupus, Crohn's disease, neuroblastoma, Hodgkin's disease, etc.). Common ENT infections never give rise to this presentation.
- Lead poisoning is rare in developed countries and mainly concerns children living in old accommodation unfit for habitation. Lead poisoning is more common in sub-Saharan Africa, China, India, Pakistan, the Middle East, South America and certain Eastern European countries.

Elderly population

- Anaemia is the most common abnormal finding in the CBC. Its frequency significantly increases with age; approximately 5% at age 65, 15% at age 80 and 30% at age 90.
- Anaemia in the elderly should not be attributed to advanced age, and a pathological cause should always be investigated. After menopause, the incidence of iron-deficiency anaemia balances out between men and women, and remains a major cause of microcytic anaemia.
- Anaemia in the elderly is a multifactorial disorder in more than half of cases. The frequency of chronic inflammatory diseases, folate deficiency and CKD increases jointly with advancing age.

Guide to interpretation

Clinical presentation

Symptoms related to anaemia	• Microcytic anaemia usually develops gradually and is therefore well tolerated • Pallor and major asthenia are often the only symptoms
Orientation towards iron deficiency	• Symptoms affecting the skin and appendages (dry skin, dry, brittle hair, chipped nails, etc.) • Pica (irresistible desire to ingest non-food substances). The cause-effect relationship between the two is much debated
Orientation towards chronic inflammation	• Possible general symptoms in acute inflammation (fever, asthenia, sleep disorder) and chronic inflammation (intense asthenia and cachexia in extreme forms) • Presence or absence of identified source

Orientation towards Lead poisoning	• Gastrointestinal disorders (abdominal pain, constipation) • Neurological disorders (behavioural disorders, headache, antebrachial paralysis)
Orientation towards congenital SA	• Symptoms of iron overload (skin hyperpigmentation, heart and liver failure)

CBC findings

Severity of anaemia	• Inflammatory anaemia and Lead poisoning are moderate (rarely < 8 g/dL) • Iron-deficiency anaemia may be very severe (sometimes < 5 g/dL)
RBC and MCV correlation	• In iron deficiency and chronic inflammatory diseases, there is a correlation between the reduction in RBC count and MCV • In thalassaemia, a discrepancy is observed between the reduction in RBC count and MCV (the latter being more decreased)
RDW	• Often increased in iron deficiency (anisocytosis is generally the first abnormal finding evidenced) • Often normal in "minor" haemoglobinopathies and chronic inflammation • Dimorphism in congenital sideroblastic anaemia
Reticulocytes	• Always low in iron deficiency and chronic inflammation • Low or slightly raised in "minor" haemoglobinopathies and sideroblastic anaemia
% Microcytic RBC / % Hypochromic RBC	• Thalassaemic RBC tend to be more microcytic whereas iron-deficient RBC tend to be more hypochromic • A ratio > 6.4 allows 91.1% of microcytic patients to be correctly classed as thalassaemic (α-thalassaemia, β-thalassaemia or haemoglobin E disease)[2] • Chronic inflammatory diseases have a similar profile to iron deficiency • A "thalassaemia + iron deficiency" combination corresponds to an iron-deficiency profile • The performance of this test varies according to age and geographical origin
Thrombocytosis	• Common in iron deficiency and chronic inflammation • Generally absent in other causes

ADDITIONAL TESTS

➡ Blood smear

Initial prescription (adapted from the ISLH[3])

- Hb < 7 g/dL with MCV < 75 fL (adults), in an unknown patient and not in a bleeding context.
- RDW > 22%, in an unknown patient and not in an RBC transfusion context.

Main abnormal findings to be investigated

- Hypochromia:
  - pronounced hypochromia indicates iron deficiency. The severity of hypochromia and the hypochromic RBC count are correlated with the severity of iron deficiency. In extreme cases, the majority of RBC will be ring-shaped and may be associated with moderate poikilocytosis;
  - this is generally slight or absent in chronic inflammation and haemoglobinopathies (except for severe forms of thalassaemia);
  - a dimorphic RBC population (hypochromic + normochromic) is possible in iron deficiency in the process of developing or being corrected, and in congenital sideroblastic anaemia.
- Target cells:
  - small quantity (< 10%) in iron deficiency, chronic inflammatory diseases and heterozygous state for HbC;
  - numerous (> 10%) in the majority of other haemoglobinopathies. More than 50% of target cells are observed in case of homozygous state for HbC and HbE. These are combined with severe anisopoikilocytosis in thalassaemia major.
- Basophilic stippling:
  - absent in iron deficiency;
  - present in certain haemoglobinopathies (≈ reticulocytes) and secondary acquired sideroblastic anaemia.

➡ Iron tests

Initial prescription

- General population:
  - serum ferritin on a first-line basis;
  - transferrin saturation + CRP if iron deficiency is strongly suspected, and ferritin levels are normal or elevated.
- Pregnancy and infants < 5 years:
  - assay not performed on a first-line basis (as iron deficiency is the most common cause of anaemia in these 2 populations);
  - serum ferritin assay if iron supplementation fails + investigation for malabsorption, chronic bleeding and any other aetiology for anaemia.

- Other situations (cancer, IBD, liver disease, CKD, etc.):
  - serum ferritin + a marker for the amount of iron available for erythropoiesis (TSAT or percentage of hypochromic Red cells) + an inflammatory marker (e.g. CRP) are generally used;
  - soluble transferrin receptor (in the absence of hyperhaemolysis) or % HYPO (in the presence of concomitant hyperhaemolysis) is used when an inflammatory syndrome is observed;
  - ferritin + TSAT or % HYPO or reticulocyte Hb content is used in the event of CKD, with or without ESA therapy.

Interpretation of tests

- General overview.

	Iron deficiency	Inflammation	Mixed
Iron	↓	↓	↓
Ferritin	↓	↑	N or ↓
Transferrin	↑ then ↓	↓	variable
TSAT	↓	N or ↓	↓
sTfR	↑	N	↑
sTfR/log ferritin	↑	↓	N or ↑
% HYPO **CHr**	↑ ↓	N N	N or ↑ ↓

- Ferritin assay.

Threshold values proposed by the 2001 WHO/UNICEF/UNU report[3]		
Iron deficiency	Age < 5 years Age > 5 years	< 12 µg/L < 15 µg/L
Iron deficiency with infection	Age < 5 years Age > 5 years	< 30 µg/L /

NOTE: reduced serum ferritin is 100% specific to iron deficiency. Iron deficiency is very unlikely when serum ferritin is > 40 µg/L in the general population and > 70 µg/L in the context of inflammation or chronic hepatic failure. In patients with CKD, serum ferritin < 100 µg/L indicates true iron deficiency, serum ferritin > 100 µg/L with a pathological marker for iron availability indicates functional iron deficiency, and ferritin > 100 µg/L with a normal marker for iron availability indicates the absence of iron deficiency. The target value for patients on haemodialysis is higher, at 200 µg/L.

- Transferrin assay and transferrin saturation.

	Transferrin	Transferrin saturation
Reference values in adults	1.6-3.2 g/L	20-40%
Decreased	• Iron overload • Inflammation • Chronic liver failure • Undernutrition	• Iron deficiency • Pregnancy, contraception
Elevated	• Iron deficiency • Inflammation	• Iron overload

NOTE: in an inflammatory context, very low TSAT (typically < 10%) suggests concomitant iron deficiency. In patients with CKD, adequate iron reserves are defined by TSAT > 20%, as stipulated by most guidelines.

- Soluble transferrin receptor assay: this marker is not affected by the patient's inflammatory context (which is helpful in inflammatory syndrome or liver disease). It may be falsely elevated in the event of hyperhaemolysis (not recommended if thalassaemia, AIHA or vitamin B9/B12 deficiency, etc. is suspected). sTfR has greater sensitivity, but is less specific than serum ferritin, in an inflammatory context. Use of the "sTfR/log ferritin" index increases the sensitivity and specificity of ferritin and sTfR used separately.
- Percentage of hypochromic Red cells: a level > 6% suggests iron deficiency (marker for long-term iron-deficient erythropoiesis as RBC have a life span of 120 days). This marker is helpful in complex clinical situations, notably suspected iron deficiency in a patient with chronic inflammatory disease and haemolysis. It is included in certain guidelines in relation to chronic kidney disease.
- Reticulocyte haemoglobin content: a level < 29 pg suggests iron deficiency (marker for short-term iron-deficient erythropoiesis as reticulocytes have a life span of 1-2 days). This marker seems particularly beneficial for assessing response to replacement therapy at an earlier stage than the elevation of reticulocytes (the so-called reticulocyte crisis). It is also included in certain guidelines in relation to chronic kidney disease.

Impact on CBC

- Stages of iron deficiency : decreased reserves (ferritin ↓), followed by decreased serum iron with a compensatory increase in transferrin (TSAT ↓), followed by insufficient supply of iron to erythroblast precursors resulting in increased synthesis of membrane transferrin receptors (sTfR ↑), followed by a reduction in RBC size (MCV ↓) then Hb content (MCHC ↓) and, lastly, a quantitative reduction in erythropoiesis (Hb ↓). Gradual replacement of "healthy" RBC by "iron-deficient" RBC causes gradual anisocytosis (RDW ↑).
- Severity of abnormal values: there is a correlation between the reduction in Hb levels, RBC count and blood cell indices (MCV, MCHC). Hb levels can be very low (sometimes < 4 g/dL). Reticulocytes are always low. Concomitant thrombocytosis, or even thrombocytopenia, may sometimes be observed in very severe forms.

Inflammation assessment

Initial prescription

- General case: first-line assay of CRP.
- Suspected sepsis: First-line CRP + PCT.
- Suspected chronic inflammatory disease or inflammatory disease with "normal" CRP: assay of CRP + protein with a slow kinetic profile (fibrinogen, haptoglobin or orosomucoid).
- Erythrocyte sedimentation rate is a poor marker for inflammation (limited specificity and delayed kinetics) and therefore should no longer be used.

Interpretation of tests

- General overview.

	Acute (< 2-3 days)	Acute (> 2-3 days)	Chronic or regressive
CRP	↑ or ↑↑↑	↑ or ↑↑↑	N or ↑
Haptoglobin Orosomucoid Fibrinogen	N or ↑	↑ or ↑↑↑	↑ or ↑↑↑
CBC	N	Thrombocytosis	Normocytic or microcytic anaemia + thrombocytosis
Serum protein electrophoresis	↑ α1	↑ α1 and α2 ↓ albumin ± ↑ γ	↑ α1 and α2 ↓ albumin ± ↑ γ
ESR	N	↑ or ↑↑↑	↑ or ↑↑↑

NOTE: certain inflammatory diseases have a normal or subnormal CRP in the chronic phase (systemic lupus, primary Sjögren's syndrome, scleroderma). Fibrinogen and platelets may be reduced in the event of DIC. Acceleration of ESR has limited specificity for inflammation (also increases in the event of anaemia, hypergammaglobulinaemia, pregnancy, etc.). There is a correlation between haptoglobin and orosomucoid (hapto = 1.3 × oroso). In the event of conflicting results, haemolysis (↓ haptoglobin) and kidney disease (↑ orosomucoid) should be investigated as a priority.

- Utility of procalcitonin: this is a specific marker for bacterial infection which has a rapid kinetic profile (detectable from 3 hours and peak between 6 and 12 hours). The threshold for positivity is 0.2-0.5 ng/mL; there is a high risk of severe sepsis or septic shock for values > 2 ng/mL. False-negative results (early or local infection, effective antibiotic therapy) and false-positive results (neonates < 48 hours, first few days for multiple injuries and major burns) are observed.