Anaemia: A Diagnostic Approach

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Anemia is operationally defined as a reduction in one or more of the major RBC measurements:

- Hemoglobin concentration
- Hematocrit
- RBC count

Concentration measures: affected by plasma volumes

Most accurately measured by obtaining a RBC mass via isotopic dilution methods.
Erythrocyte Development

- IL-3 SCF EPO
- BFU-E
- CFU-GEMM
- CD33 CD34
- Proerythroblast
- EPO
- Gly A
- Erythrocyte
- Reticulocyte
- Gly A
- EPO
- Erythroblast
Causes

- Blood loss
- Increased RBC destruction (haemolysis)
- Decreased production of RBC
Initial Approach:

- Laboratory blood values
  - Hb (12-16mg/dl)
  - WCC (4-11 x 10⁹/l)
  - Platelets (140-440 x 10⁹/l)
  - MCV (76-96 fl)
  - MCH (27-32 pg)
- WCC differential
Two main approaches that are not mutually exclusive:

1. Biologic or kinetic approach.
2. Morphology.
The reticulocyte count (kinetic approach)

- Increased reticulocytes (greater than 2-3% or 100,000/mm$^3$ total) are seen in blood loss and hemolytic processes.

- Up to 25% of hemolytic anemias will present with a normal reticulocyte count due to immune destruction of red cell precursors.

- Retic counts are most helpful if extremely low (<0.1%) or greater than 3% (100,000/mm$^3$ total).

- Reticulocyte count must be adjusted for the patient's hematocrit.
Morphological Approach
(big versus little)

First, measure the size of the RBCs:

• Use of volume-sensitive automated blood cell counters, such as the Coulter counter.
• By calculation from an independently-measured red blood cell count and hematocrit:
  \[
  \text{MCV (femtoliters)} = 10 \times \text{HCT (percent)} \div \text{RBC (millions/µL)}
  \]

• Macrocytic (MCV>100)
• Normocytic
• Microcytic (MCV<83)
Blood loss:

Blood loss may be caused by:

- trauma
- gynaecologic - menorrhagia
- gastrointestinal bleeding
- haematuria
- bleeding diatheses
Increased destruction can occur in:

- **hereditary defects:**
  - haemoglobinopathies - sickle, thalassaemia
  - membrane defects - spherocytosis
  - enzyme problems - G6PD deficiency

- **acquired problems**
  - haemolytic anaemias - immune and autoimmune

- **burns**
Reduced proliferation

• iron deficiency
• anaemia of chronic disease
• renal failure - reduced erythropoietin production
• bone marrow disease:
  • aplastic anaemia
  • infiltrative disorders - leukaemia, myelofibrosis, cancer
• pure red cell aplasia

Defective maturation of red cell precursors - increased erythropoietin, marrow hyperplasia but ineffective erythropoiesis due to

• defective nuclear maturation - megaloblastic anaemia
• defective cytoplasmic maturation:
Haemolytic anaemias
Haemolytic anaemia

- Normal red cell life span 100-120 days
- A haemolytic anaemia occurs if that life span is shortened
- Predominant site of RBC destruction is the red pulp of the spleen.
- Sustained reticulocytosis and indirect bilirubinaemia
Clinical features of haemolytic anaemias

- Symptoms related to anaemia (i.e. breathlessness-fatigue)
- Symptoms of the underlying disorder
- Jaundice
- Increased incidence of pigmented gallstones
- Splenomegaly
- Changes in urine colour
Confirm haemolysis is occurring:

- Check FBC
- Check blood film
- Increased reticulocytes
- Increased LDH
- Increased serum bilirubin
- Haptoglobin levels
Further diagnosis

- Warm and cold agglutination essays, Hb electrophoresis, Combs test, etc

- Haematologic consultation should be obtained in virtually all patients with new
Inherited haemolytic anaemias

• Red cell membrane defects

• Disorders of red cell metabolism

• Abnormal haemoglobins
classic spherocytes with loss of central pallor in the erythrocytes.
Acquired Haemolytic anaemia

- Immune
  - Autoimmune
    - Idiopathic
    - Secondary
  - Alloimmune
- Non Immune
- Drug induced
Autoimmune haemolytic anaemia

- **Warm Antibody**
  - Idiopathic
  - Secondary
    - CLL
    - Connective tissue disorders
    - Lymphomas
    - Drugs eg Methyldopa

- **Cold Antibody**
  - Idiopathic
  - Secondary
    - Mycoplasma
    - Infectious mononucleosis
    - Lymphoma
    - Paroxysmal cold haemoglobinuria
Alloimmune Haemolytic Anaemia

- Haemolytic transfusion reactions eg ABO mismatch

- Haemolytic disease of the newborn eg Rhesus incompatibility
Non immune haemolytic anaemia

- Red cell fragmentation
  - DIC,
  - Cardiac valves
  - HUS, TTP
  - March haemoglobinuria

- Infections
  - Malaria, Clostridium
  - Drugs, chemicals
  - Venoms
  - Burns

- Chemical/ Physical
Common Causes of Hemolytic Anemia in the Adult

Extravascular destruction of red blood cells

**Intrinsic red blood cell defects**
- Enzyme deficiencies (e.g., G6PD or pyruvate kinase deficiencies)
- Hemoglobinopathies (e.g., sickle cell disease, thalassemias, unstable hemoglobins)
- Membrane defects (e.g., hereditary spherocytosis, elliptocytosis)

**Extrinsic red blood cell defects**
- Liver disease
- Hypersplenism
- Infections (e.g., bartonella, babesia, malaria)
- Oxidant agents (e.g., dapsone, nitrites, aniline dyes)
- Other agents (e.g., lead, snake and spider bites)
- Microangiopathic (e.g., DIC, TTP-HUS)
- Autoimmune hemolytic anemia (warm- or cold-reacting, drugs)
- Intravenous immune globulin infusion
- Large granular lymphocyte leukemia

Intravascular destruction of red blood cells

- Microangiopathy (e.g., aortic stenosis, prosthetic valve leak)
- Transfusion reactions (e.g., ABO incompatibility)
- Infection (e.g., clostridial sepsis, severe malaria)
- Paroxysmal cold hemoglobinuria
- Paroxysmal nocturnal hemoglobinuria
- Following intravenous infusion of Rho(D) immune globulin
- Following intravenous infusion with hypotonic solutions
- Snake bites
• Diminished production of RBCs is suggested in all patients without evidence of either blood loss or haemolysis
Microcytic anaemias

Anaemia of Chronic Disease
Anaemia of chronic disease

- Causes
  - Chronic infection eg abscess, TB
  - Rheumatoid arthritis
  - Ulcerative colitis
  - Malignancy
  - Chronic renal failure
Anaemia of chronic disease

• Mechanisms
  • Shortened red cell survival
  • Impaired marrow response to anaemia
  • Impaired erythropoietin production
  • Defective iron transport
  • Hepcidin??

• Laboratory findings
Underproduction

**Normocytic**
- Anemia of chronic disease
- Mixed deficiencies
- Renal failure

**Microcytic**
- Iron deficiency
- Thal. trait
- Anemia of chronic disease (30-40%)
- Sideroblastic anemias
Iron Deficiency Anemia vs. Inflammatory Block

- Smear:
  - hypochromic and microcytic (low MCV) RBCs, usually not seen unless Hct < 30%

\[ \downarrow \text{Ferritin: a measure of total body iron stores, but also an acute phase reactant} \]

- \(<15 \mu g/l = \text{Fe deficiency,} >150 \mu g/l = \text{Not Fe deficiency}\)
- 15-150 \(\mu g/l = ?\)
Iron Deficiency Anemia vs. Inflammatory Block

- Low Iron Saturation (Fe/TIBC ratio)
  - ↓ Fe (not reliable)
  - ↑ TIBC
  - Fe/TIBC (% saturation) <15%
- BM bx: absent Fe stores
- Therapeutic Trial of Oral Iron
Causes of iron deficiency anaemia

1. Inadequate intake
   - Dietary iron
   - Iron supplements

2. Failure of absorption
   - Gastrointestinal symptoms
   - Hx of Coeliac disease
   - Overt blood loss from bowel or change of bowel habit
   - Menorrhagia

3. Increased blood loss
   - Pregnancy
Main causes are

• Blood loss from anywhere
  • gut / PV / PU / respiratory tract etc
• Increased demand
  • pregnancy / growth
• reduced intake
  • diet / malabsorption
What further investigations should be

• Serum ferritin +/- serum iron
• B12 / folate
• FOBs
• Bioprofile
• +/- Coeliac screen
• +/- Gastroscopy and/or colonoscopy
What further investigations should be

If indicated:

- Coagulation screen
- Haemoglobin electrophoresis
Hypochromic Anaemia

- Ferritin
  - Reduced
  - Normal
  - Increased

- Iron deficiency
- Haemoglobinopathy
- Sideroblastic Anaemia
- Other causes of high ferritin

Anaemia of Chronic disorders
Iron deficiency anaemia
Hb < normal limits
MCV < 76 fl
Ferritin < 15 μg/dl

Choose A or B or run both concurrently

A
- Gastroscopy and small bowel biopsy
  - Gluten free diet
    - Coeliac
    - Surgery or palliative care
      - Gastric Cancer
      - Treat other pathology
        - Other pathology
          - Normal
            - Has path B been done?
              - No → B
              - Yes → Iron replacement
                - Has path A been done?
                  - No → See section 4.3
                  - Yes → See section 3.4

B
- Colonoscopy or barium enema (sigmoidoscopy if indicated)
  - Colon Cancer
    - Other pathology
      - Surgery or palliative care
        - Other pathology
          - Normal
            - Has path A been done?
              - No → B
              - Yes → Iron replacement
                - Has path A been done?
                  - No → See section 3.4
                  - Yes → See section 4.3
Macrocytic anaemias
Causes of macrocytosis

- Megaloblastic anaemia
  - Raised MVC with megaloblastic changes in the bone marrow
- Vitamin B12 deficiency
- Folate deficiency
- Myelodysplasia
- Liver disease
- Alcohol excess
- Hypothyroidism
- Aplastic anaemia
- Cytotoxic drugs
Megaloblastic Anaemia

A characteristic cell morphology caused by impaired DNA synthesis
Typical peripheral blood
How do B12 + Folate cause anaemia?

- DNA consists of purine/pyrimidine bases - Folate is required for their synthesis.
- B12 is essential for cell folate generation.
What further investigations should be performed?

- Blood film
- B12, Folate, Ferritin
- Liver function tests
- Thyroid function tests
- Reticulocyte count
- Coeliac screen
- Intrinsic factor and parietal cell antibodies
- Bone marrow – only if above normal
Vitamin B12

- Sources – liver/meat/fish and dairy products
- Loads in most diets compared to needs - but only from animal sources
- Daily intake 3-30 microgram
- Adult daily requirement 1-2 microgram
- Body stores 3-5 mg in the liver (2-4 yr supply)
Vitamin B12 absorption

- B12 attaches to intrinsic factor (IF) in the stomach
- IF – a glycoprotein secreted by the parietal cells
- B12/IF passes to the terminal ileum where absorption takes place
Causes of B12 deficiency

- Strict vegetarianism
- Malabsorption
  - Pernicious anaemia
  - Gastrectomy
- Coeliac disease
- Disease involving the terminal ileum
Folate

- Dietary sources - eggs, green vegetables, liver, nuts
- Absorbed in the jejunum
- Daily intake 600-700 microgram
- Daily requirement 100 microgram

Causes of Folate Deficiency

- Dietary – infancy and old age
- Malabsorption – coeliac disease
Pernicious anaemia

- Autoimmune disease
- Gastric atrophy
- Anti parietal antibodies 90%
- Anti intrinsic factor antibodies 70%
- Often associated with other autoimmune disorders

Clinical Features

- Related to anaemia
Pernicious Anaemia

- Diagnosis
  - B12 levels
  - IF/Parietal cell antibodies
  - Bone marrow
  - Schilling test
    - Saturate B12 stores IM
    - Give PO radiolabelled B12
SACDC

- Any cause of severe B12 deficiency
- Anaemia not an absolute requirement
- Demyelination of dorsal + lateral columns
- Peripheral nerve damage
- Presents as:
  - Peripheral neuropathy / Paraesthesiae
  - Numbness and distal weakness
  - Unsteady walking
  - Dementia
Final comments regarding diagnostic approaches

• Methylmalonate
• Homocysteine
• Bone marrow aspirates and biopsy
References

- For excellent patient education resources, visit eMedicine's [Blood and Lymphatic System Center](http://emedicine.com). Also, see eMedicine's patient education article [Anemia](http://emedicine.com).


- Stamatoyannopoulos G, Majerus PW, Perimutter RM: The