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Additional documents:
1. Blood cell development (pdf)
2. Diagnosis from blood smear (pdf)

Introduction

This is the first of a set of documents and films that cover commonly encountered diagnoses and will also serve as an introduction to blood film morphology. The main aim is to ensure that all scientists are at a basic level of knowledge before film training starts. There are questions to be completed at the end of each section as revision. If you have any questions while you are working through any of the sections, you can email me at vlowe@clinpath.com .

Section 1 outlines some of the most commonly encountered diagnoses.

What is a hypersegmented neutrophil?

Hypersegmented neutrophils may be over-estimated as a film with 5-lobed neutrophils will stand out among other films that (for hospital patients) are more likely to have a left shift.

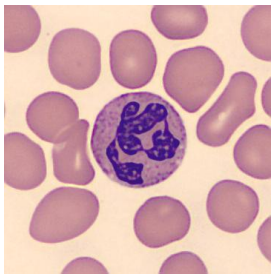
- A neutrophil must have at least 6 lobes to be considered hypersegmented.
- Other granulocytes may also show hypersegmentation, eg 3 lobes in an eosinophil, but this is much rarer.

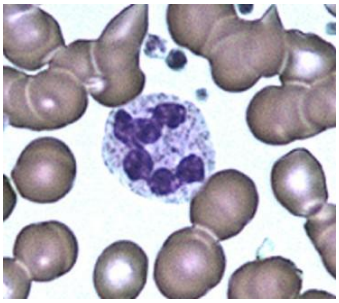
Hypersegmented neutrophils show:

- increased size
- 6 or more lobes
    - in normal patients no more than 3% of neutrophils have more than five lobes
    - in megaloblastic anaemia the average lobe count is increased and neutrophils with 6, 7, or 8 lobes may be seen

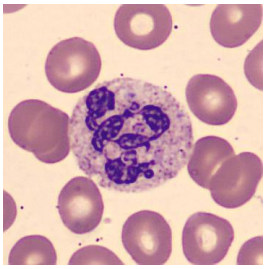
Hypersegmentation is often indicative of reduced DNA synthesis

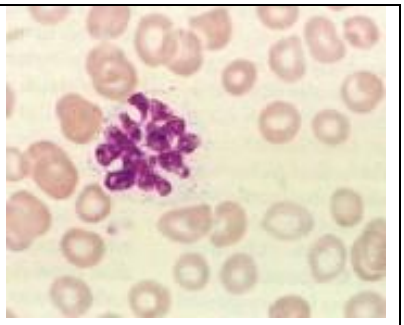
- commonly associated with:
    - vitamin B12 deficiency
    - folate deficiency
    - anti-metabolite therapy
    - alcoholism
- occasional hypersegmented neutrophils may be seen in:
        - blood loss
        - iron deficiency



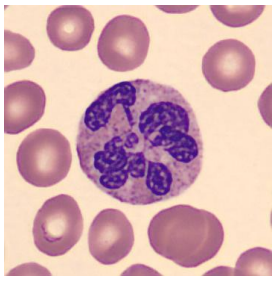


HYPERSEGMENTED NEUTROPHILS





MACROPOLYCYTES



What is a macropolycyte?

Macropolycytes are:

- (usually) neutrophils
- approximately double normal size with
    - proportionate increase in the size of the nucleus
    - increase in nuclear lobulation
    - increased number of nuclear drumsticks (in women)
- usually tetraploid
    - represent a missed cell division

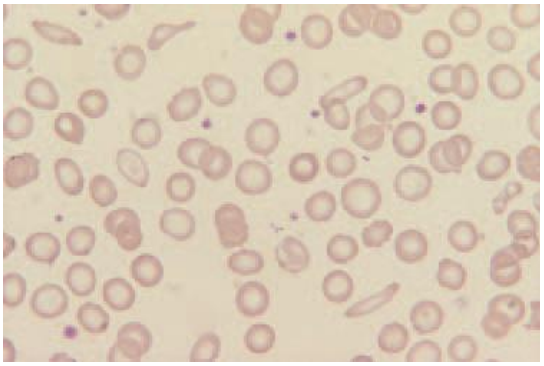
Macropolycytes can occasionally be found in normal, healthy individuals but are more common in:

- reactive conditions (including infections)
- nutritional deficiencies
    - may show additional cytological abnormalities including a more open chromatin pattern
- growth factor administration
- HIV
- myelodysplastic syndromes (MDS)
- myelodysplasia-related acute myeloid leukaemia (AML)
- myeloproliferative neoplasms
- chemotherapy

Nutritional deficiencies

Iron deficiency anaemia

Iron deficiency is the most common nutritional deficiency in the world (based on WHO figures).



The blood film of a patient with iron deficiency anaemia showing anisocytosis, poikilocytosis (including elliptocytes), hypochromia and microcytosis.

Blood film and count
initial normocytic normochromic anaemia with anisocytosis followed by development of:

- poikilocytosis
- elliptocytes
- pencil cells
- target cells (occasional)
- anisohypochromasia
    - not all cells are hypochromic in mild iron deficiency
- microcytosis and fall in MCH
- basophilic stippling is rare
- increased RDW
- reticulocyte percentage may be normal or elevated, absolute reticulocyte count is normal or reduced
- increased platelet count is common and may be due to:
    - iron deficiency itself
    - blood loss
    - underlying malignant disease
- in severe iron deficiency leucopenia and thrombocytopenia occur in up to 10% of patients
- occasional hypersegmented neutrophils are sometimes present and are not necessarily indicative of coexisting vitamin B12 or folate deficiency

Further tests
Iron studies:

- low serum ferritin is diagnostic
- serum ferritin is raised in inflammation and may mask iron deficiency

Definitive test (although rarely performed) is the demonstration of absent bone marrow iron.

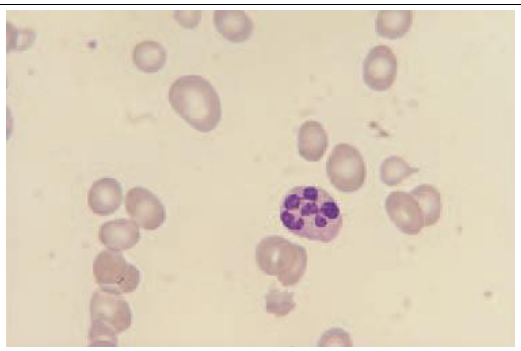
B12 and folate deficiency

Megaloblastic anaemia shows:

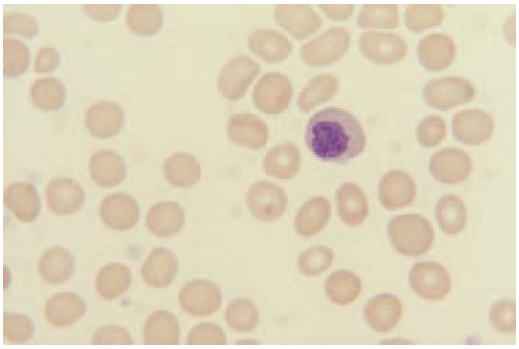
- dyserythropoiesis
- increased size of erythroid precursors
- asynchronous maturation of nucleus and cytoplasm so that cytoplasmic maturation is in advance of nuclear maturation

B12/folate deficiency clinical features include:

- glossitis
- mild splenomegaly
- mild jaundice resulting from ineffective haemopoiesis
- in B12 deficiency there may also be:
  - optic atrophy
  - dementia
  - peripheral neuropathy
  - subacute combined degeneration of the spinal cord (causing spastic paraparesis and reduced proprioception)



The blood film of an elderly woman with both malabsorption of vitamin B12 and dietary deficiency of folic acid showing marked anisocytosis, macrocytosis, several oval macrocytes, a teardrop poikilocyte and a hypersegmented neutrophil.



The blood film of a patient with pernicious anaemia showing macrocytosis and a circulating megaloblast.

Blood film and count

Characteristic blood film features include:

- anaemia
- reduced Hb, haematocrit and red cell count in parallel with the increase in the MCV and MCH
- normal MCHC
- RDW increases (precedes the rise in the MCV)
- anisocytosis
- poikilocytosis
- oval macrocytes
- teardrop cells
- neutrophil hypersegmentation (when hypersegmentation is absent the chromatin pattern is more open than normal)
- occasional hypersegmented eosinophils may be present
- macropolycytes (occasional)
- basophilic stippling (sometimes)

As anaemia becomes more severe there is:

- increasing anisocytosis and marked poikilocytosis
- appearance of microcytes and fragments
- possible hypochromic microcytes and hypochromic fragments (resulting from dyserythropoiesis rather than coexisting iron deficiency)
- small number of Howell–Jolly bodies
- occasional circulating megaloblasts and granulocyte precursors may appear
- platelet count falls
- possible moderate neutropenia and mild lymphopenia
- no polychromasia despite severe anaemia as the reticulocyte count is low

When megaloblastic anaemia develops acutely there may be:

- a sudden failure of bone marrow output of cells resulting in pancytopenia with a normal MCV
- few or no macrocytes or hypersegmented neutrophils
- polychromasia is absent as the reticulocyte count is very low
- this 'megaloblastic arrest' can occur in acutely ill patients, often in association with pregnancy, surgery or sepsis.

Megaloblastic anaemia resulting from folic acid antagonists, eg methotrexate, is indistinguishable from that due to vitamin B12 or folate deficiency.

When iron deficiency coexists with deficiency of either vitamin B12 or folic acid:

- blood film features are variable
- possible hypochromic microcytes in addition to macrocytes
- features of iron deficiency may be predominant with only the presence of hypersegmented neutrophils suggesting a possible double deficiency. (However, hypersegmented neutrophils may be seen in uncomplicated iron deficiency and for other reasons.)
- iron deficiency is sometimes unmasked when vitamin B12 or folic acid deficiency is treated

When B12 and folate deficiency are treated recovery proceeds as follows:

- lag phase of a few days
- white cell and platelet counts rise
- polychromatic macrocytes are produced
- Hb rises
- in pancytopenia there may be a rebound thrombocytosis, often associated with left shift or a leucoerythroblastic blood film
- hypersegmented neutrophils persist in the peripheral blood for 5–7 days, or longer in those who were cytopenic.

Further tests

- B12 and folate assays
    - serum vitamin B12 concentration is reduced in about 97% of patients with clinical evidence of vitamin B12 deficiency
    - red cell folate assay is more specific for significant tissue deficiency of folate
- pernicious anaemia is the most common cause of vitamin B12 deficiency, and can be diagnosed by:
    - tests for intrinsic factor
    - parietal cell antibodies are also usually present in pernicious anaemia
    - the Schilling test can determine whether there is a gastric or intestinal cause of vitamin B12 malabsorption
- coeliac disease may cause folic acid deficiency or, less often, vitamin B12 deficiency:
    - antibody to endomysium is the most useful serological test
    - IgA anti-endomysial antibodies in patients with coexisting IgA deficiency (increased in frequency in patients with coeliac disease) may be negative
    - definitive test for coeliac disease is a small bowel biopsy

Renal disease

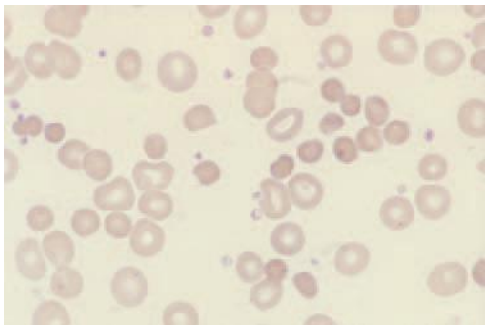
- Red cell survival is usually reduced in acute renal failure.
  - features of a ***mild*** microangiopathic haemolytic anaemia ***may*** be present, including a small numbers of fragments
  - if there are significant numbers of fragments, consider other causes for the red cell changes
- Anaemia of chronic renal failure:
  - exacerbated by the reduced red cell survival
  - primary deficiency of erythropoietin is the main cause of anaemia
    - kidneys are responsible for approximately 90% of erythropoietin production
  - level of anaemia is directly related to the level of residual renal function

Liver disease

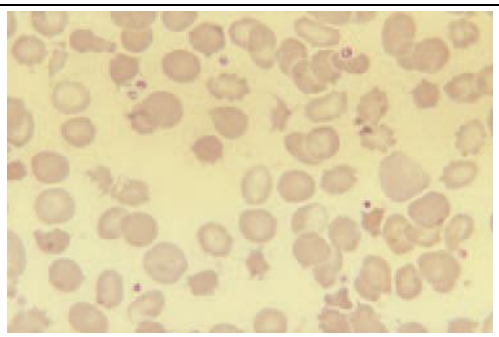
Liver disease may be associated with:

- Zieve's syndrome:
  - acute alcoholic liver disease associated with hyperlipidaemia and acute haemolysis
  - abnormal cells may include spherocytes
  - irregularly contracted cells are usually more common
- Spur cell haemolytic anaemia:
  - characterized by numerous acanthocytes
  - seen in liver failure of any aetiology

More commonly a mild anaemia and mild thrombocytopenia are seen.



The blood film of a patient with Zieve's syndrome as a complication of acute alcoholic liver disease showing irregularly contracted cells and polychromatic macrocytes.



The blood film of a patient with terminal liver disease of unknown aetiology showing numerous acanthocytes ('spur cell' haemolytic anaemia).

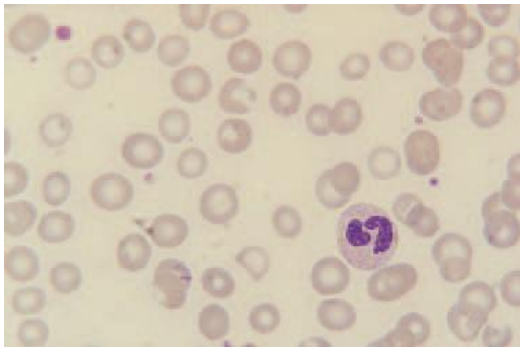
Anaemia of chronic disease

'Anaemia of chronic disease' describes anaemia that results from:

- chronic infection or inflammation
- malignant disease

is characterized by:

- low serum iron
- adequate bone marrow iron stores
- defective incorporation of iron into haemoglobin
- reduced erythropoietin production in response to anaemia
- some shortening of red cell survival



The blood film of a patient with the anaemia of chronic disease consequent on a lymphoma, showing mild anisocytosis, poikilocytosis and hypochromia.

Blood film and count

Mild anaemia of chronic disease:

- normocytic and normochromic

Severe anaemia of chronic disease:

- hypochromia and microcytosis develop
- microcytosis may be as marked as iron deficiency
- RDW is variable
- absolute reticulocyte count is reduced
- features of chronic inflammation may be present:
    - neutrophilia
    - thrombocytosis
    - increased rouleaux formation
    - increased background staining
    - raised ESR and CRP

Further tests

- Iron studies in anaemia of chronic disease:
    - serum iron and serum transferrin are reduced
    - serum ferritin is increased
- The combination of iron deficiency and anaemia of chronic disease cannot be diagnosed by the blood film and biochemical tests.
- Bone marrow biopsy can confirm iron deficiency, but is rarely used.

Hyposplenism

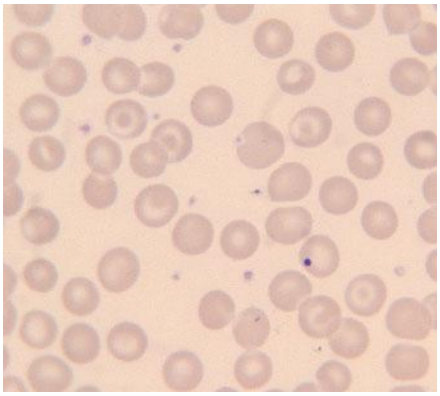
Reduced or absent splenic function is referred to as hyposplenism. It can result from:

- congenital absence of the spleen
- surgical removal
- loss of splenic function, eg. splenic atrophy or infarction

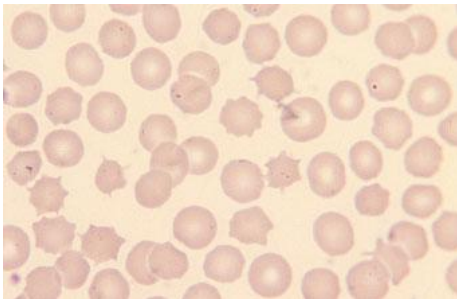
Mild hyposplenism is normal in the neonatal period, particularly in premature neonates.

Many conditions can be associated with hyposplenism:

- Ageing
- Haematological disorder
  - Sickle cell disease
  - Thrombocythaemia
  - Myelofibrosis
  - Malaria
  - Lymphomas
- Circulatory
  - Splenic arterial/venous thrombosis
- Autoimmune disease
  - Systemic lupus erythematosus
  - Rheumatoid arthritis
- Gastrointestinal (? immune basis)
  - Gluten-induced enteropathy
  - Dermatitis herpetiformis
  - Crohn's disease
  - Ulcerative colitis
- Infiltrations
  - Lymphomas
  - Sézary syndrome
  - Secondary carcinomas, especially breast
  - Cysts, e.g. hydatid
- Nephrotic syndrome
- Drugs
  - Methyldopa
  - Intravenous gammaglobulin
  - Corticosteroids
- Irradiation
- Splenectomy and splenic embolisation



A cell containing a Howell–Jolly body in a patient who has had a splenectomy. There are also several target cells.



Spherocytes, spheroacanthocytes and red cells containing Pappenheimer bodies following splenectomy for hereditary spherocytosis.

Blood film and count

When splenectomy is carried out in a patient with a haematological abnormality, post-splenectomy features are often modified by the effects of the underlying disease.

Red cell changes:

The blood film shows:

- target cells
- Howell–Jolly bodies

- acanthocytes (sometimes)
- occasional spherocytes
- occasional Pappenheimer bodies (siderotic granules)
- increase in the number of reticulocytes
- occasional nucleated red cells

White cell changes:

After splenectomy there is:

- rise in the total leucocyte count
- neutrophilia in the immediate postoperative period
- later there is often an increase in both lymphocytes and monocytes
    - persistent postsplenectomy lymphocytosis can occasionally mask early chronic lymphocytic leukaemia
    - progressive rise in lymphocyte count requires review by a Haematologist
- slight increase in eosinophils and basophils occasionally reported
- greater leucocytosis in response to infection
    - often marked left shift with myelocytes and occasionally more primitive cells

Platelet changes:

- thrombocytosis is often seen post splenectomy
    - may fall to normal or near normal values over the following 1–2 months
- large and pleomorphic platelets may persist

Immunological effects:

- Spleen is important in immunoglobin synthesis:
    - fall in the IgM post splenectomy
    - IgG levels do not change
    - IgA and IgE increase.
- Splenectomy in adults without complicating disease is not usually associated with a substantially increased incidence of infection.

Further tests

- CT scan or other imaging technique to confirm unexpected hyposplenism.

- Lymphocyte surface markers may be worthwhile to exclude a lymphoproliferative disorder where there is a persistent and/or progressive lymphocytosis.

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