The blood film as a diagnostic tool

Introduction

In the general haematology laboratory the patient's samples are usually analysed on an automated machine which will produce the full blood count result, together with a digital plot of the blood cells depending on the cell's volume and density of staining. An abnormal full blood count is a common finding with many different conditions. However, the result may give little information about the actual cause of the abnormality, and therefore further investigations such as a blood film are necessary.

A blood film or smear is a way of visualizing the morphological features present in the cells of a patient's blood sample and gives a huge amount of additional information. The haematology laboratory will produce a blood film in response to certain abnormal blood findings, including raised or decreased counts cell counts (*Table 1*), or a 'flag' of abnormal cell populations detected in the cell scatter-plots graphics. On average between 2 and 10% of full blood counts may need a blood film (Galloway and Osgerby, 2006). If felt appropriate a blood film can also be specifically requested by general medical doctors, and liaison with the haematology department can help with the diagnosis of many different conditions.

A blood film is made by placing a drop of blood on a glass slide and spreading it thinly before allowing it to dry. It is then stained with a dye such as Romanowsky or Giemsa. The slide is mounted with a cover slip and visualized with light microscopy at between x10 and x100 magnification. The low magnification is used to look at the general distribution and proportions of blood cells, while higher magnification is used to look at the actual morphological characteristics of the individual cells.

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Red cell disorders

Red cell disorders can be characterized as those with too many red cells, too few red cells, or disorders of red cell structure and function. Anaemia is a common finding on a full blood count, and initially the mean corpuscular volume and haemoglobin results should be reviewed. The mean corpuscular volume refers to the size of the red cells and the mean corpuscular haemoglobin refers to the average mass of haemoglobin per red blood cell in a sample of blood. Anaemic conditions can give a variety of results which can point to a potential diagnosis (*Table 2*).

The blood film should then be viewed to further help with the diagnosis of the anaemia, and can be used to confirm the size, shape and density of the red cells. For example a film of iron deficiency will shown small, pale cells, and may also show 'target' red cells and the elongated 'pencil cells'. Vitamin B_{12} or folate deficiency films may show large red cells and hypersegmented neutrophils. Reticulocytes are seen as larger, blue-tinged polychromatic cells and are seen in increased numbers in conditions such as haemolytic anaemia. Nucleated red cells are not normally found in the peripheral blood but can be seen in patients who have marrow infiltration or have previously had a splenectomy. Some examples of the various red cell structural variations are seen in *Figure 1*.

Polycythaemia is a condition where the haemoglobin level is increased. This can be classified as true polycythaemia where there is an actual increase in the red cell population, or pseudo-polycythaemia where there is a relatively normal number of red cells with a reduced circulating plasma volume as a result of dehydration or diuretic therapy. True polycythaemia can be sub-classified as primary proliferative polycythaemia (polycythaemia rubra vera), or secondary to hypoxia or increased erythropoietin production.

The blood film can be used to visually confirm the increased red cell number, or the presence of any primitive myeloid precursors that may suggest a primary myeloproliferative disorder. The blood film should be used in conjunction with diag-

Table 1. Suggested indications for a blood film review

Haemoglobin < 7.0 or > 2.0 g/dl above reference range for age or sex
Reticulocytes > 0.1%
Nucleated red blood cells detected
White blood cell < 4.0 or > 30x10^9/litre
Neutrophils < 1.0 or > 20x10^9/litre
Lymphocytes > 5.0x10^9/litre
Monocytes > 1.5x10^9/litre
Eosinophils > 2.0x10^9/litre
Basophils > 0.5x10^9/litre
Platelets < 100 or > 1000x10^9/litre
Mean corpuscular volume < 75 or > 105 fl
Automated analyser detects abnormal cell population
No white blood cell differential given
Film requested by clinician
Infective disease (malaria, leishmaniasis, trypanosomiasis)

Adapted from Barnes et al (2005)

Table 2. Classification of anaemia according to the full blood count analysis parameters

Microcytic anaemia (low mean corpuscular volume)	Iron deficiency
	Anaemia of chronic disease
	Thalassaemia
	Sickle cell disease
Normocytic anaemia (normal mean corpuscular volume)	Anaemia of chronic disease
	Mixed iron, vitamin B_{12} or folate deficiency
	All other causes
Macrocytic anaemia (raised mean corpuscular volume)	Vitamin B_{12} or folate deficiency
	Alcoholism
	Liver disease
	Myelodysplasia
	Hypothyroidism
	Drugs, e.g. hydroxycarbamide
	Pregnancy
	Reticulocytosis

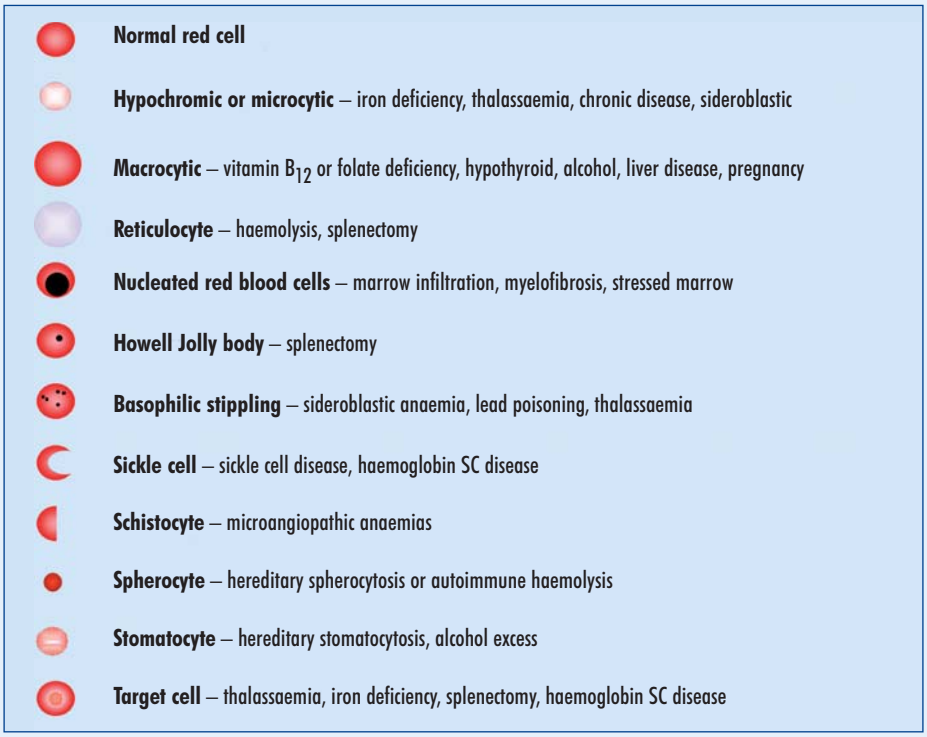


Figure 1. Red blood cell morphology.

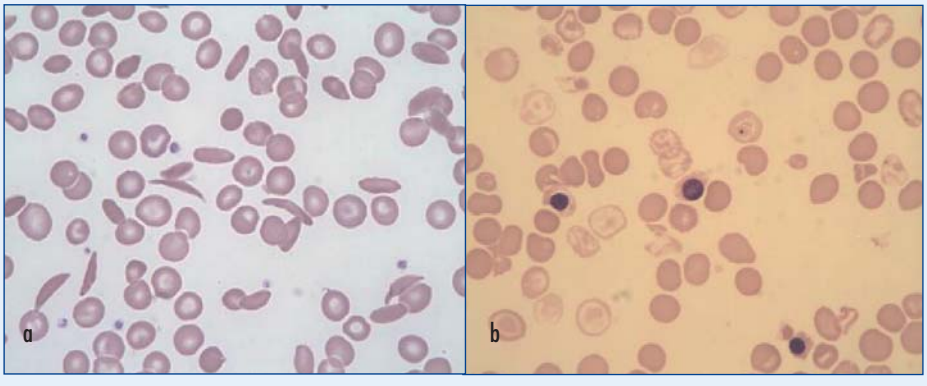
nostic tests such as the JAK2 molecular mutation and the red cell mass-plasma volume investigation (McMullin et al, 2007).

Many other conditions can affect the actual structure of the red cells. Congenital red cell cytoskeleton defects include hereditary spherocytosis, eliptocytosis and stomatocytosis, and the morphological features of the red cells are the main initial screening tool to obtain the diagnosis. Spherocytic red cells are smaller, spherical and denser than normal red cells. Eliptocytes are oval in appearance, while stomatocytes contain a central bar-like central pallor rather than the usual circular appearance.

The haemoglobinopathies are extremely common worldwide and the blood film gives a variety of diagnostic appearances. α or β thalassaemia trait may just show small and pale, but otherwise normal red cells, while the major thalassaemias can show many morphological abnormalities including target cells, hypochromic cells, reticulocytes and misshapen red cells. A blood film can also be used to diagnose sickle cell disease, where the red cells may show a crescentic shape (*Figure 2*).

Another group of conditions where the red cell morphology is essential is the microangiopathic haemolytic anaemias (*Table 3*), caused by non-immune haemolysis. Red cell fragments or schistocytes may be seen, together with other features such as thrombocytopaenia. This appearance of the blood film should warn the physician about the potential presence of a haematological emergency.

Figure 2. Red cell disorders. a. Sickle cell disease showing multiple sickle cells. b. Thalassaemia major, showing hypochromic cells, nucleated cells and target cells.



White cell disorders

There are five main types of white cell that are seen in a normal blood film (*Figure 3*) and these can be affected by many different conditions.

Neutropaenia can lead to a decrease in the total white blood cell count and can be caused by a decrease in production of cells such as in bone marrow infiltration conditions. Alternatively there can be increased destruction of neutrophils, for example as a result of autoimmune neutropaenia. A blood film in a neutropaenic patient is essential to rule out significant causes such as leukaemia, where primitive white cells may be seen in the blood. Lymphocytopaenia is less common. It can be congenital such as in severe combined immunodeficiency or acquired as in human immunodeficiency virus infection.

An increase in the number of white cells may be a reaction to an infective or inflammatory condition; alternatively it may indicate a significant primary haematological disorder. Viral infections can cause a lymphocytosis, while infectious mononucleosis can cause a variety of atypical mononuclear white cells. Bacterial infections can cause a neutrophilia (or a neutropaenia) and may show additional features on a blood film such as toxic granulation or vacuolation of the white cells. Very severe infections can lead to a 'leukaemoid' blood film appearance because of the presence of more primitive white cells in the blood, mimicking the appearance of leukaemia.

Table 3. Microangiopathic haemolytic anaemias

Disseminated intravascular coagulation
Thrombotic thrombocytopaenic purpura
Haemolytic uraemic syndrome
Pre-eclampsia
Acute fatty liver of pregnancy
HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome
Antiphospholipid syndrome

A malignant expansion of a white blood cell clone in the marrow can lead to the presence of leukaemic cells in the peripheral blood. Acute leukaemias can be myeloid or lymphoid in origin, and leukaemic blast cells may be visible in the blood, with a reduction in normal blood white cells. Blastic cells are often larger than normal white cells with abnormal granulation in the cytoplasm and nucleoli visible in the nucleus.

It can occasionally be difficult to differentiate myeloblasts from lymphoblasts, although the presence of fine needle-like 'auer rods' in the cytoplasm indicates a myeloid origin. In chronic lymphocytic leukaemia there may be an increase in small, mature lymphoid cells and smear cells (fragile lymphocytes burst in the process of making the blood film). In chronic myeloid leukaemia the blood film demonstrates abnormal eosinophils and basophils, plus primitive bone marrow white cells: blasts, promyelocytes, myelocytes and metamyelocytes not found in normal blood films (*Figure 4*).

Occasionally cases of lymphoma can develop a leukaemic phase, i.e. lymphoma cells present in the peripheral blood. The B cell disorder Burkitt's lymphoma has very blue, vacuolated lymphoid cells, while hairy cell leukaemia cells tend to be small with villus cytoplasmic projections. Certain T cell disorders can also affect the blood with a variety of different white cell appearances, such as cells with a ceribriform nuclear pattern in Sezary syndrome, or a 'clover leaf' appearance in adult T-cell leukaemia lymphoma.

Platelet disorders

An increase in platelet numbers can be seen as a response to infection or inflammation in the body. Acute haemorrhage or iron deficiency can also lead to a thrombocytosis. A blood film has a limited value in distinguishing reactive from malignant thrombocytosis, but certain features may help point towards a primary marrow disorder. In myeloproliferative disorders such as essential thrombocythaemia there is a thrombocytosis with platelet anisocytosis (a variety of size platelets). In comparison myelofibrosis may have the additional features of 'tear drop' shaped and nucleated red cells, plus primitive white cells on the blood film.

Thrombocytopaenia can be the result of a decreased production of platelets, such as caused by marrow infiltration. Increased destruction can be caused by immune thrombocytopaenia, the microangiopathic haemolytic anaemias, or can be drug induced such as heparin-induced thrombocytopaenia, and a film is essential. A falsely low platelet count can also occasionally be detected on the full blood count as a result of the curious condition of platelet clumping. A patient may have a normal true platelet count but when the blood sample is collected in an EDTA bottle the platelets stick together and give a falsely low reading (*Figure 5*). This condition is not clinically significant and may be suggested on the automated full blood count analyser, but is easily identified on the blood film. A more accurate platelet count can be acquired if the sample is collected in a citrate bottle.

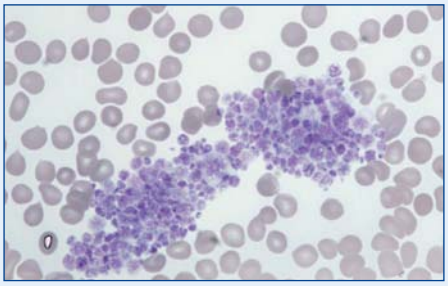


Figure 5. Platelet clumping.

Pancytopaenia

A blood film is essential if a patient develops a pancytopaenia as many of the conditions are clinically highly significant. Chemotherapy, vitamin B_{12} or folate deficiency can cause a decrease in production of the cell lines, while in myelodysplasia there is a dysregulated cell production and abnormalities in apoptotic cell death.

The neutrophils may have an abnormal nuclear hour-glass or circular appearance, or there may be abnormal cytoplasmic granulation. Alternatively marrow infiltration can occur as a result of leukaemia, lymphoma or metastatic cancer such as breast or prostate carcinoma. There may be a leuco-erythroblastic film where primitive white and nucleated erythroid cells are seen. The malignant cells may also be seen in the peripheral blood, while marrow infiltration with reticulin or collagen can lead to myelofibrosis. All of these conditions can demonstrate significant features on the blood film to indicate a potential diagnosis. A subsequent bone marrow examination is often essential for confirmation of the cause.

Tropical diseases

A blood film can also help with diagnosis of various tropical diseases. A patient with malaria may show the typical 'signet ring' parasites present in the red blood cells.

Figure 3. Normal peripheral blood white cells.

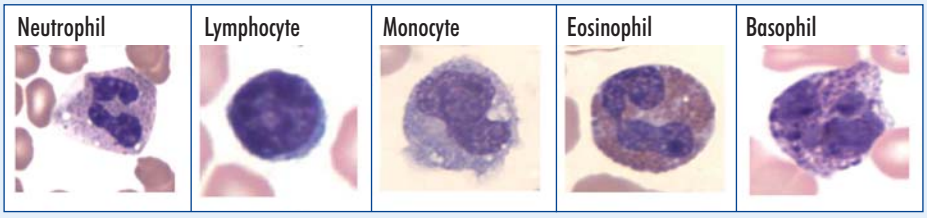


Figure 4. Examples of leukaemia. a. Acute myeloid leukaemia showing large blastic cells with sizeable nuclei and prominent nucleolus. b. Chronic myeloid leukaemia with all stages of myeloid cells present: blast, promyelocyte, myelocyte, metamyelocyte and neutrophil.

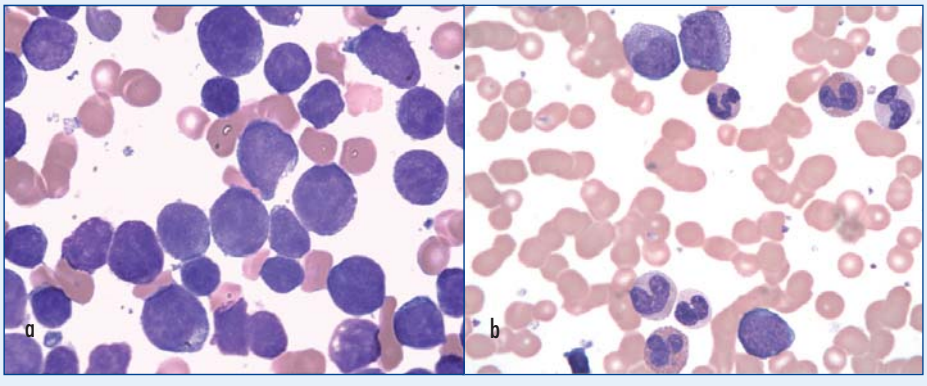


***Figure 6. Tropical diseases. a. Malaria, caused by* Plasmodium falciparum. *b. Microfilariasis, caused by* Loa loa.**

The thick blood film is used to diagnose the presence of malaria, while a thin film is used to look at the morphological features and the presence of trophozoites or gametocytes to subclassify the different types of malaria. Alternatively the worms of the microfilariasis conditions can be seen in the blood under a Giemsa stain (*Figure 6*), or the motile trypanosomes of trypanosomiasis.

Conclusions

A blood film is an essential investigation to help determine the cause of many blood count abnormalities. Close liaison with the haematology laboratory and medical staff can give a rapid service which can aid in the diagnosis of many medical conditions. BJHM

Conflict of interest: none.

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KEY POINTS

- Many haematological and general medical conditions lead to abnormalities in the full blood count.
- The blood film is an essential tool to help with the diagnosis of these conditions.
- The cell numbers and characteristics can be seen by morphology.
- The information given can often lead to a diagnosis, or may suggest secondary investigations.