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Morphology in the diagnosis of red cell disorders

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Abstract
Despite the advances in automated blood cell counting, the blood film retains a crucial role in the diagnosis of red cell disorders. It is particularly important in haemolytic anaemias and in the differential diagnosis of macrocytic anaemia. However, all cases of anaemia in which the diagnosis is not immediately obvious require a blood film. Blood film examination sometimes provides a definitive diagnosis but more often suggests a differential diagnosis that indicates which further tests are most appropriate. The blood film has the advantage of speed; this is clinically important in any severe anaemia but particularly in acute haemolytic anaemia, thrombotic thrombocytopenic purpura and megaloblastic anaemia. Polycythaemic as well as anaemic patients require blood film examination.

Modern automated blood counting instruments produce a great deal of data about characteristics of red cells. In addition to the red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) they may measure the red cell distribution width (RDW) and, less often, the haemoglobin distribution width (HDW). The RDW quantitates the range of sizes of individual red cells and thus correlates with anisocytosis. The HDW quantitates the range of haemoglobin concentrations of individual cells and thus correlates with anisochromasia. Some instruments can indicate that there are increased numbers of hypochromic or hyperchromic cells and can show if these are large or small. Some can indicate the presence of red cell fragments and many can detect the presence, and possibly the number, of nucleated red blood cells (NRBC). Most can now quantitate reticulocytes, although this will not necessarily be done on every sample. Most instruments provide histograms or red cell size; some also provide two-dimensional plots of size against haemoglobin concentration so that the laboratory scientist can recognize characteristic patterns. Nevertheless, despite the wealth of information available, there is still much to be learnt from careful examination of a blood film [1].

Blood film examination can validate automated instrument results and confirm or exclude factitious results. An increased MCV should always be confirmed by blood film examination; elevation may be factitious, as a result of the presence of a cold agglutinin. Red cell indices that appear improbable should also always lead to blood film examination. Factitious results leading to unlikely red cell indices can be the result of hyperlipidaemia or parenteral nutrition with lipid emulsions, turbidity as the result of a very high white cell count, the presence of a paraprotein, the presence of a cryoglobulin, accidental heating or freezing of a sample or continuing in vitro lysis in *Clostridum perfringens* septicaemia, ageing of a sample and contamination by subcutaneous fat [2]. The blood film generally indicates the nature of the problem responsible for the aberrant results. A platelet count that is low, high or otherwise unexpected should always be validated on a blood film. This will sometimes lead to detection of a red cell abnormality. A high platelet count may be the result of hyposplenism, easily detected on a blood film, or may be a factitious elevation resulting from the presence of numerous schistocytes or microspherocytes. A blood film will not only validate a low platelet count but may show red cell changes indicating the likely cause, e.g. liver disease, megaloblastic anaemia or a myelodysplastic syndrome.

In addition to validating and possibly explaining abnormalities shown in a full blood count, assessment of morphological features can also yield information that no automated instrument can yet give and can

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permit integration of morphological and numerical data in the light of the age, gender and clinical setting. Blood film examination is often useful in haemolytic anaemias, haemoglobinopathies and thalassaemias, microcytic anaemias, macrocytic anaemias and, less often, normocytic anaemias.

In the haemolytic anaemias, the blood film may provide a definitive diagnosis or a differential diagnosis. Certain conditions, e.g. hereditary elliptocytosis, South-East Asian ovalocytosis and hereditary pyropoikilocytosis, are so morphologically distinctive that the blood film permits an almost certain diagnosis. The film in South-East Asian ovalocytosis, for example, shows a unique combination of macro-ovalocytes, which appear to be almost double the size of the other cells, and stomatocytes, including stomatocytes with two stomas or Y-shaped or V-shaped stomas. Other blood film abnormalities are less distinctive but indicate a differential diagnosis. The distinction between spherocytes and irregularly contracted cells is very important since the diagnostic significance is quite different. Spherocytes most often indicate either hereditary spherocytosis or autoimmune haemolytic anaemia. However, in a neonate they may indicate alloimmune haemolytic anaemia due to transplacental passage of maternal antibodies and, in other circumstances, they may be indicative of a delayed haemolytic transfusion reaction or alloimmune haemolytic anaemia resulting from administration of anti-D or incompatible plasma. Microspherocytes, sometimes designated spheroschistocytes, have a somewhat different significance, indicating that red cell fragmentation has occurred. They may be present, together with other schistocytes, in microangiopathic haemolytic anaemias and mechanical haemolytic anaemias; in this context, the blood film is of critical importance in the speedy diagnosis of thrombotic thrombocytopenic purpura. In patients with severe burns microspherocytes are present together with microdiscocytes and budding red cells. In hereditary pyropoikilocytosis, microspherocytes are present, together with a striking range of other poikilocytes. Irregularly contracted cells resemble spherocytes in that they lack central pallor but can be distinguished from them by their irregular outline. They have a different significance from either spherocytes or microspherocytes. Irregularly contracted cells usually indicate either oxidant damage or instability of haemoglobin. They are characteristic of acute haemolysis in glucose-6-phosphate (G6PD) deficiency, following administration of oxidant drugs or chemicals (e.g. dapsone) to individuals with normal red cell enzymes and in certain haemoglobinopathies (see below). They are a feature of Zieve's syndrome (haemolytic anaemia associated with acute alcoholic fatty liver and hyperlipidaemia). In G6PD deficiency and following oxidant exposure irregularly contracted cells are often accompanied by keratocytes ('bite cells'), hemighost cells ('blister cells') and even ghost cells. Sometimes there are protrusions from the surface that, on a Heinz body preparation, are found to represent Heinz bodies. Detection of the characteristic features of oxidant-induced damage is very important in suggesting the diagnosis of G6PD deficiency since a G6PD assay is not always abnormal during acute haemolysis [3]. Rarely irregularly contracted cells are the result of release of copper from the damaged liver in the late stages of Wilson's disease. Other morphological clues to the presence and nature of a haemolytic anaemia include stomatocytosis, acanthocytosis, red cell agglutination, basophilic stippling or the presence of intracellular organisms. Stomatocytosis is usually the result of liver disease, often alcoholic liver disease. Less often it is the result of one of a range of rare inherited haemolytic anaemias. The importance of blood film examination is demonstrated by the recent recognition of a number of cases of phytosterolaemia as a result of the observation of stomatocytes in the blood film [4]; the authors suggested that all patients found to have hypercholesterolaemia should have a blood film examined in order that such cases should be recognized. Acanthocytes are characteristic of haemolytic anaemia resulting from liver failure and are also seen in a range of rare inherited haemolytic anaemias. Red cell agglutinates are characteristic of the presence of a cold agglutinin and of paroxysmal cold haemoglobinuria but are not usually seen in warm autoimmune haemolytic anaemia. Basophilic stippling is a rather non-specific abnormality but nevertheless can be an important clue to a diagnosis of lead poisoning or pyrimidine 5′ nucleotidase deficiency. Microorganisms relevant to haemolytic anaemia can be either parasites (in malaria or babesiosis) or bacteria (as in Oroya fever and rare cases of Whipple's disease). In some cases of haemolytic anaemia there are no distinctive features. Diagnostic possibilities then include non-spherocytic haemolytic anaemia, paroxysmal nocturnal haemoglobinuria and Wilson's disease.

The blood film in haemoglobinopathies and thalassaemias often shows target cells and sometimes irregularly contracted cells. In sickle cell disease there may be sickle cells, boat-shaped (or oat-shaped) cells, target cells and other features of hyposplenism, irregularly contracted cells, linear red cell fragments, distinctive sickle cell-haemoglobin C poikilocytes (when haemoglobin C is present) [5,6] and 'Napoleon hat cells' (when haemoglobin S-Oman is present) [7]. Sickle cell anaemia and sickle cell-haemoglobin C disease can usually be distinguished on a blood film, while awaiting more definitive tests, by consideration of the Hb and the morphological features [5,6]. Haemoglobin C disease has a very characteristic film with a mixture of target cells and irregularly contracted cells; sometimes the film of sickle cell-haemo-

globin C disease is similar but, in an emergency (e.g. pre-operatively), a sickle solubility tests permits the distinction to be made. Irregularly contracted cells are also characteristic of unstable haemoglobins and to a lesser extent haemoglobin E homozygosity and heterozygosity; they may be present in small numbers in β thalassaemia heterozygosity. A diagnosis of α^0 and β thalassaemia trait is more reliably suggested by the red cell indices than by blood film morphology; the blood film sometimes shows only microcytosis whilst in other patients there is also poikilocytosis, basophilic stippling and the presence of target cells. Among the α thalassaemias, basophilic stippling is particularly suggestive of haemoglobin Constant Spring. Haemoglobin H disease can usually be suspected from the blood film and the blood count, including the reticulocyte count. The blood film shows marked hypochromia and microcytosis and poikilocytosis is usually quite marked. The reticulocyte count is increased and, in contrast to α and β thalassaemia heterozygosity, the MCHC is usually clearly reduced.

The differential diagnosis in microcytic anaemia is sometimes aided by blood film examination. Useful features include the presence or absence of anisochromasia or a dimorphic blood film, the presence of basophilic stippling or Pappenheimer bodies and the presence of increased rouleaux or the features of hyposplenism. Anisochromasia is typical of untreated iron deficiency anaemia whereas a dimorphic blood film suggests either iron deficiency on treatment or a sideroblastic anaemia; congenital sideroblastic anaemia is usually associated with microcytosis whereas acquired sideroblastic anaemia is most often, but not always, associated with a mixture of hypochromic microcytic cells and normochromic macrocytic cells. Basophilic stippling suggests either thalassaemia trait or lead poisoning; the latter is quite rare in most countries but its recognition is very important to the patient. Iron deficiency anaemia and severe anaemia of chronic disease are not always distinguishable on the blood film but the presence of rouleaux, increased background staining or reactive changes in white cells favours the latter diagnosis. Hyposplenism in a patient with hypochromia and microcytosis suggests, in the absence of a history of splenectomy, that the patient has coeliac disease.

The blood film is often of value in the differential diagnosis of macrocytic anaemia. In megaloblastic anaemia anisocytosis, poikilocytosis, oval macrocytes and hypersegmented neutrophils are helpful features. In comparison, in liver disease or alcohol excess macrocytes are usually round, hypersegmented neutrophils are not present and there may be target cells or stomatocytes. If the patient has not had a splenectomy, features of hyposplenism in a patient with a macrocytic anaemia raise the possibility of megaloblastic anaemia as a result of coeliac disease; the blood film may be dramatically abnormal with very numerous Howell–Jolly bodies. When macrocytosis is the result of a myelodysplastic syndrome, the blood film may show a population of hypochromic microcytes and Pappenheimer bodies or there may be dysplastic features in other lineages; a high platelet count in a patient with macrocytic anaemia raises the possibility of the 5q-syndrome. Unexplained macrocytosis is sometimes a feature of multiple myeloma and in this case there is likely to be increased rouleaux formation and increased background staining to suggest the diagnosis. If macrocytosis is the result of a haemolytic anaemia there will be polychromasia and an increased reticulocyte count, with or without more specific features. Congenital dyserythropoietic anaemia is a rare cause of macrocytic anaemia, characterized by marked anisocytosis and poikilocytosis. A rather more common cause is the administration of drugs that lead to macrocytosis; the laboratory scientist or haematologist should be alert to the possibility that a patients may be taking antiretroviral drugs, such as zidovudine, without this fact being included in the clinical details given on the request form.

There are many causes of a normocytic normochromic anaemia, including renal failure and the early stages of both anaemia of chronic disease and iron deficiency. The blood film is often not very helpful but multiple myeloma can usually be suspected, because of the presence of rouleaux and background staining, and if there is a leucoerythroblastic film either idiopathic myelofibrosis or bone marrow infiltration should be suspected.

Blood film examination is relevant to patients with polycythaemia as well as patients with anaemia. The presence of an absolute increase in the basophil count (not reliably detected by automated counters) or giant platelets favours a diagnosis of polycythaemia vera rather than relative or secondary polycythaemia.

Conclusion

All cases of anaemia in which the diagnosis is not immediately obvious require a blood film. This is particularly important if the anaemia is severe and if rapid diagnosis is needed. The blood film should be interpreted in the light of the clinical history and the results of a blood count.

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