Diagnosis and management of congenital haemolytic anaemia

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Congenital haemolytic anaemias are hereditary conditions resulting from an increase in the rate of red cell destruction.[1] Four main types of red cell abnormality can cause haemolysis:

- cytoskeletal membrane disorders (eg hereditary spherocytosis (HS), hereditary elliptocytosis (HE), hereditary pyropoikilocytosis (HPP))
- disorders of membrane cation transport (eg hereditary stomatocytosis)
- enzyme deficiencies (eg dehydrogenase (G6PD) deficiency, pyruvate kinase (PK) deficiency)
- disorders of haemoglobin synthesis (eg sickle cell anaemia, unstable haemoglobins).

Congenital haemolytic anaemia can also be a feature of more generalised metabolic problems not directly involving the red cells such as lipid abnormalities (eg abetalipoproteinaemia, sitosterolaemia) and Wilson's disease. Although most thalassaemia syndromes involve an element of haemolysis, the predominant defect is ineffective erythropoiesis and the former will not be considered further in this article.

General approach to diagnosis

It is first necessary to decide whether there is any evidence of haemolysis, which typically is suggested by anaemia, reticulocytosis, high serum lactate dehydrogenase levels, unconjugated hyperbilirubinaemia and low serum haptoglobin levels. A blood film should be reviewed; if this shows marked fragmentation, the possibility of angiopathic haemolysis, such as disseminated intravascular haemolysis or haemolytic uraemic syndrome, should be considered. If haemolysis is present, the direct antiglobulin test will identify the vast majority of cases of autoimmune haemolysis; if it is negative, congenital red cell abnormalities should be considered.

Cytoskeletal membrane disorders

The group of hereditary cytoskeletal membrane disorders arise from mutations of genes controlling the proteins of the membrane and their interaction which results in change to the shape of the red cells.[2] The inheritance pattern is typically autosomal dominant.

Hereditary spherocytosis

Probably the commonest cause of congenital haemolytic anaemia in northern Europeans is HS. It is less well characterised in other populations, although it certainly occurs in people of African and Asian origin. It is characterised by the spherical shape of the affected red cells. The pattern of inheritance is autosomal dominant in 75% of cases; in the others the parents are apparently normal. HS can result from mutations in seven different genes, but mutations in the genes coding for ankyrin, band 3 or β-pectrin are most common in the autosomal dominant form.[3]

Clinically, HS commonly presents as anaemia of variable severity, jaundice, which is often present neonatally, and palpable splenomegaly (the main site of red cell destruction).[4]

Diagnosis. Diagnosis of HS is based on:

- an appropriate family history
- spherocytes on the blood film
- mean cell haemoglobin concentration which is increased in approximately 50% of patients
- increased osmotic fragility.

Binding of the fluorescent dye EMA, as measured by flow cytometry, is reduced in most cases of HS and can be used as a confirmatory test in atypical cases with no family history, particularly to confirm the diagnosis prior to splenectomy.[5]

Treatment. Many patients require no specific treatment, although folic acid is sometimes used because of the increased rate of erythropoiesis. Splenectomy significantly increases the haemoglobin and

Table 1. Useful initial investigations for possible congenital haemolytic anaemia.

Test	Possible result
Full blood count	Anaemia
Blood film	Polychromasia, spherocytes, anisopoikilocytosis, stomatocytosis, sickle cells
Reticulocyte count	Increased
Liver function tests	Unconjugated hyperbilirubinaemia
Serum haptoglobin	Decreased
Lactate dehydrogenase	Increased
Coombs' test (direct autoantibody test)	Negative in congenital haemolysis
Osmotic fragility tests	Increased in presence of spherocytes
Red cell G6PD and pyruvate kinase assays	Low levels in deficiency states
Haemoglobin analysis	Detects sickle and most other abnormal haemoglobins

G6PD = glucose-6-phosphate dehydrogenase.

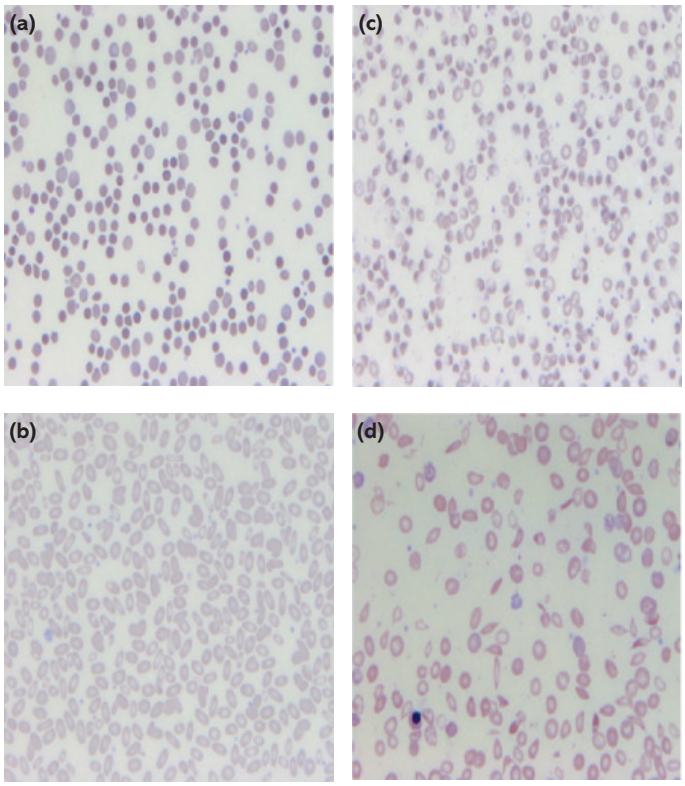

Fig 1. Blood films: (a) hereditary spherocytosis, (b) hereditary elliptocytosis, (c) glucose-6-phosphate dehydrogenase deficiency, (d) sickle cell anaemia.

reduces haemolysis in most patients with HS. It is indicated if the patient requires regular or frequent blood transfusions or has significant symptoms such as failure to thrive or disabling fatigue. Symptomatic gallstones may require a cholecystectomy, and splenectomy is usually performed at the same time to reduce the risk of further cholelithiasis.[6]

Hereditary elliptocytosis and hereditary pyropoikilocytosis

HE is typically an autosomal dominant condition characterised by the elliptical shape of the red blood cells. It is usually asymptomatic, causing minimal haemolysis. Occasionally it can cause severe haemolysis, which is often more apparent in the newborn period. Homozygosity for mutations causing elliptocytosis typically results in HPP, a severe haemolytic anaemia with bizarrely shaped red cells.[7] Treatment includes folic acid supplementation. In homozygous HE or HPP, severe haemolytic anaemia may develop and splenectomy may be beneficial.

Disorders of membrane cation transport

Disorders of membrane cation transport are characterised by red cells with a mouth-like slit rather than the normal central circular area of pallor. They typically involve increased leak of sodium and potassium across the red cell membrane, resulting in an altered state of red cell hydration and haemolysis. Diagnosis is made on the basis of clinical suspicion and measurement of red cation transport or ektacytometry.[2,8]

Hereditary stomatocytosis

Dehydrated hereditary stomatocytosis. This is also known as hereditary xerocytosis or cryohydrocytosis. The red blood cells have a membrane abnormality, with increased permeability to cations with a greater efflux of K^+ than of Na^+. This causes red cell dehydration and consequently results in osmotically resistant xerocytes.[9] The clinical presentation ranges from mild to moderate haemolytic anaemia, jaundice, splenomegaly and cholelithiasis. Iron overload may develop later in life. The leak of K^+ from the erythrocytes can cause marked pseudohyperkalaemia, and the condition is also linked to spontaneously resolving fetal and neonatal ascites. Splenectomy is not beneficial and has been associated with increased risk of thrombosis.[2]

Overhydrated hereditary stomatocytosis. This disorder is also known as hydrocytosis. The primary defect is due to increased Na^+ and K^+ permeability. The influx of Na^+ is greater than the loss of K^+, resulting in a net influx of water, overhydration and swelling. The resulting increased osmotic fragility and reduced deformability of erythrocytes leads to haemolysis,[10] which is usually mild and does not require intervention.

Red cell enzyme defects

Glucose-6-phosphate dehydrogenase deficiency

The commonest inherited enzyme deficiency is G6DP, affecting 400 million people worldwide. The inheritance is

Table 2. Agents likely to cause haemolytic anaemia in glucose-6-phosphodiesterase deficiency.

Agent	
Antibiotics	Sulphonamides, dapsone, co-trimoxazole, septrin, quinolones, nalidixic acid
Analgesics	Phenacetin
Antimalarials	Maloprim, primaquine, pentaquine
Other drugs	Rasburicase, flutamide, methylene blue
Acidosis	Infections, fever, diabetic ketoacidosis
Food	Fava beans
Other	Naphthalene (mothballs)

X-linked, typically resulting in affected males. However, it is not uncommon to see females with acute haemolysis due to G6PD deficiency because of either homozygosity or skewed inactivation of the X chromosome. The condition is most prevalent in West Africa, the Mediterranean, the Middle East and South-East Asia, and there is some evidence that it gives protection against malaria and its complications.[2] Typically, patients are well, with no evidence of haemolysis unless exposed to certain drugs or fava beans which cause acute intravascular haemolysis. This may present as jaundice, fever, backache and pallor with dark urine due to haemoglobinuria.[11]

G6PD deficiency is also an important cause of prolonged or severe neonatal jaundice, particularly when co-inherited with Gilbert's syndrome.[2] Less commonly, severe deficiency can result in chronic haemolysis.

Diagnosis rests upon the typical history, with blood tests showing intravascular haemolysis. A blood film should show contracted and fragmented cells, with hemighosts in which the haemoglobin occupies one half of the cell. The diagnosis is confirmed by measurement of G6PD activity in red cells.

Management depends on the symptoms. It is important to stop the offending drug and treat any infection. A blood transfusion may be needed. Renal function should be supported as intravascular haemolysis can result in renal impairment. Neonatal jaundice may require phototherapy and exchange transfusion.[12]

Pyruvate kinase deficiency

PK deficiency is the commonest enzymopathy, causing chronic, non-spherocytic haemolytic anaemia. The exact prevalence is not known, but there are probably 200–300 patients in the UK with the condition. The inheritance pattern is autosomal recessive. PK catalyses the final step in the glycolytic pathway leading to the accumulation of 2,3-diphosphoglycerate, which causes low oxygen affinity and results in the anaemia being typically well tolerated.[13]

The clinical severity varies from asymptomatic anaemia to a transfusion-dependent condition. Jaundice and gallstones may be present, and there is often progressive iron overload from increased iron absorption and blood transfusion. A blood film reveals poikilocytosis and distorted 'prickle' cells. The diagnosis is made by direct enzyme assay, although significant deficiency may be missed by standard assays which are not sensitive to subtle variations in enzyme kinetics. Assays at low substrate concentration and different temperatures may be helpful, with DNA analysis of the PK-LR gene.[2,14]

Management depends on the severity. Intermittent or regular blood transfusions may be necessary. Splenectomy leads to a small increase in haemoglobin in most cases. Patients should be monitored regularly for iron accumulation.

Rarer red cell enzymopathies

There are more than 15 other red cell enzymes whose deficiency has been identified as causing haemolytic anaemia. These are all rare and many involve only a handful of cases worldwide. Sometimes red cell enzymopathies can present as part of a more generalised metabolic problem, often associated with neuromuscular problems. All can be diagnosed by specific enzyme assay, although studies suggest that once G6PD and PK deficiency have been excluded, about 70% of cases of congenital non-spherocytic haemolytic anaemia remain undiagnosed despite extensive enzymopathy testing.

Disorders of haemoglobin synthesis

Sickle cell disease

Disorders of haemoglobin synthesis are a group of conditions characterised by the presence of sickle haemoglobin, intermittent vaso-occlusion and haemolysis. The commonest and most severe form is sickle cell anaemia (HbSS), but HbS can be co-inherited with a number of other β-globin mutations to form sickle cell disease; the most common of these is

Table 3. Rare red blood cell (RBC) enzymopathies. Reproduced with permission of Blackwell Publishing.[2]

RBC enzymopathy	Inheritance	Characteristic features
Pyrimidine 5' nucleotidase deficiency	Autosomal recessive	Basophilic stippling Mild haemolysis. Possible association with learning difficulties
Hexokinase deficiency	Autosomal recessive	Typically mild haemolysis
Triose phosphate isomerase deficiency	Autosomal recessive	Variable haemolysis with progressive neurological deterioration and early death
Phosphofructokinase deficiency	Autosomal recessive	Mild haemolysis Pain on exercise and fatigue Myoglobinuria Typically fatal
Aldolase deficiency	Autosomal recessive	Haemolysis Myopathy Mental retardation
Phosphoglycerate kinase deficiency	X-linked recessive	Haemolysis Muscle pain Weakness Aphasia Myoglobinuria
Glutathione synthetase deficiency	Autosomal recessive	Neurological defects 5-oxoprolinuria

Key Points

- Congenital haemolytic anaemia is typically suggested by anaemia, reticulocytosis, high serum lactate dehydrogenase levels, unconjugated hyperbilirubinaemia, low haptoglobin levels and a negative direct antiglobulin test
- Hereditary spherocytosis is the commonest cause of congenital haemolytic anaemia in northern Europeans
- Glucose-6-phosphate dehydrogenase deficiency is X-linked. It is more prevalent in West Africa, the Mediterranean, the Middle East and South-East Asia
- The two main pathological processes in sickle cell disease are vaso-occlusion and haemolysis, both related to polymerisation of the deoxygenated haemoglobin molecule containing the mutated β-globin chain
- Unstable haemoglobins are typically due to point mutations causing amino acid substitution in the α- or β-globin chains, the commonest probably being Haemoglobin Köln

KEY WORDS: congenital haemolytic anaemia, glucose-6-dehydrogenase deficiency, hereditary spherocytosis, sickle cell disease, unstable haemoglobins

HbSC. The haemoglobin S mutation is the result of valine substituting for glutamic acid at position 6 in the β-globin chain. Sickle cell disease is a strikingly variable condition; many of the contributing factors are unknown, although higher fetal haemoglobin levels have been linked to less severe clinical problems.[15]

Both the two main pathological processes in sickle cell disease, vaso-occlusion and haemolysis, are related to the polymerisation of the deoxygenated haemoglobin molecule containing the mutated β-globin chain. The ischaemic damage caused by vaso-occlusion has been appreciated for many years, but it has recently emerged that haemolysis may also contribute to some of the complications such as pulmonary hypertension, leg ulcers and priapism. Haemolysis causes release of free haemoglobin; this binds rapidly to nitric oxide (NO), resulting in functional NO deficiency. This process is best studied in sickle cell disease, but is likely to contribute to the increased risk of pulmonary hypertension found in other conditions causing chronic haemolysis, such as pyruvate kinase and hereditary spherocytosis.

Sickle cell disease has a wide range of clinical manifestations, including acute and chronic pain, increased risk of certain infections due to hyposplenism, avascular bone necrosis, nocturia or enuresis, cholelithiasis, acute and chronic pulmonary damage, and retinopathy. It is the commonest cause of stroke in childhood, affecting about 6% of children by the age of 10 years.

Management. This includes primary prevention of infection with pneumococcal vaccines and penicillin, with specific treatment of the complications encountered. There is evidence that children at high risk of stroke can be identified using transcranial Doppler scanning and that regular blood transfusions can reduce the risk of stroke by 90%. Hydroxyurea reduces the frequency of acute pain and acute chest syndrome. Allogeneic bone marrow transplantation is potentially indicated in children requiring regular blood transfusions.[2,16]

Unstable haemoglobins

Unstable haemoglobins are typically due to point mutations causing amino acid substitutions in the α- or β-globin chains. These substitutions weaken non-covalent bonds, allowing the haemoglobin to denature and precipitate as insoluble globins which may attach to the cell membrane forming Heinz bodies.[17] Patients may present in various ways: congenital non-spherocytic haemolytic anaemia, splenomegaly, pigmented gallstones, Heinz body haemolytic anaemia with sensitivity to oxidant drugs, a thalassemia-like peripheral blood picture with hypochromic red cells, increased formation of methaemoglobin or severe neonatal jaundice.[2]

Diagnosis. Diagnosis of unstable haemoglobins may be suggested by tests showing decreased haemoglobin stability, such as the isopropanol or heat stability tests. Staining with brilliant cresyl blue may show typical Heinz bodies in the red cells. Haemoglobin electrophoresis may detect the mutant haemoglobin, but if it is very unstable it may not be detectable in the blood. DNA analysis of the globin genes will show the mutation causing the abnormal haemoglobin. Unstable haemoglobins are rare and occur sporadically; the commonest is probably Haemoglobin Köln (β98,Val→Met). Haemolysis is variable and can result in transfusion dependence.

Management. The response to splenectomy is typically good, although only limited experience exists for any particular variant. Infection and oxidative drugs may precipitate acute haemolysis. Gallstones may require a cholecystectomy. Folic acid is usually given.[17]

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Von Willebrand disease

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Von Willebrand disease (vWD) is the most common of the inherited bleeding disorders, with a prevalence of symptomatic vWD around 125 per million.[1] It is caused by mutations affecting the von Willebrand factor (vWF) gene which result in either quantitative or qualitative abnormalities of vWF.

Patients with vWD have a mucocutaneous pattern of bleeding, with easy bruising associated with trauma and may report spontaneous bruising. Epistaxes and menorrhagia are common features. Prolonged bleeding following haemostatic challenges, including minor cuts, dental extractions and surgical procedures, is also typical.

vWF is synthesised predominantly in blood vessel endothelial cells but is also produced by bone marrow megakaryocytes and incorporated into platelet alpha granules.[2] The vWF gene is located on chromosome 12. The primary gene product is a protein with a molecular weight of approximately 250 kDa. Within cells of synthesis the vWF protein undergoes polymerisation, forming a range of multimers up to 80 protein units in size. vWF multimers are secreted from blood vessel endothelial cells directly into the circulation or are retained in the cells in specialised storage granules called Weibel-Palade bodies from which they are released as a response to vascular injury.

The main function of vWF is to facilitate platelet binding to blood vessel subendothelium at sites of vascular damage in high shear flow environments such as small arteries and the microvasculature (Fig 1).[3] Once platelets are anchored in place, vWF enables aggregation of platelets recruited to the site of injury culminating in the formation of a haemostatically effective platelet plug. The high molecular weight multimer forms of vWF are required for these platelet interactions.

vWF also functions as the carrier protein for clotting factor VIII (FVIII), protecting it from proteolytic degradation in the circulation and transporting it to sites of vascular injury to enable it to carry out its crucial role in coagulation. Qualitative and quantitative defects in vWF result in impairment of the haemo-

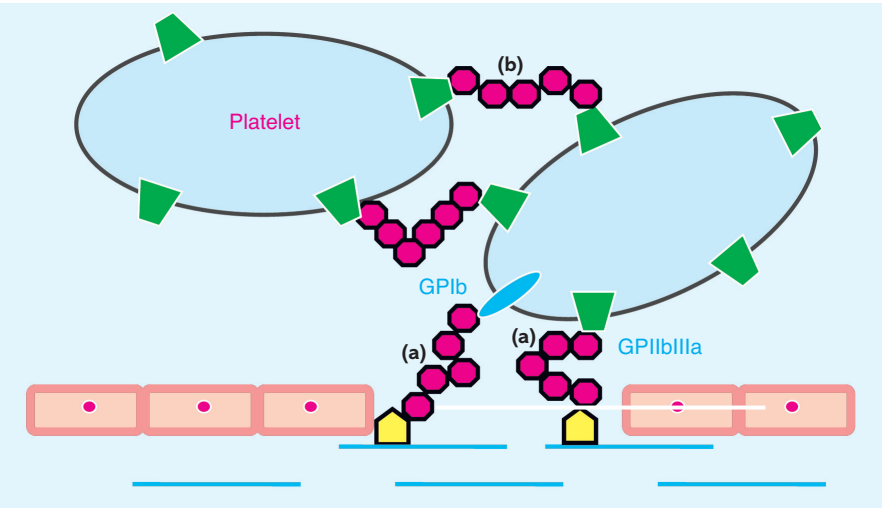

Fig 1. Role of von Willebrand factor (vWF) in haemostasis: (a) binding of vWF multimers to subendothelial collagen with capture of platelets, initially by binding to the glycoprotein (GP) Ib receptor and then to the GPIIbIIIa receptor; (b) aggregation of platelets by interbridging of vWF multimers between GPIIbIIIa receptors. Each vWF molecule can bind a factor VIII molecule (not shown) which is released at the site of haemostasis activation by the action of thrombin.