Occasional articles

Current problems in haematology 2:
Hereditary spherocytosis

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Introduction

In Northern Europeans, hereditary spherocytosis is the most common of the hereditary haemolytic anaemias, with an incidence of 1 in 5000. Important recent advances in this disease have concentrated mainly on the molecular basis of aetiology. This brief review considers the influence that this molecular knowledge is having on current trends in diagnosis and management.

Clinical features

The principal clinical manifestations of hereditary spherocytosis—namely, extra-vascular haemolysis coupled with splenomegaly—have been well documented.¹ A common feature (ranging from 10–55%) is premature cholelithiasis, those most severely affected develop pigment gall stones in the first decade of life; in some cases this is the presenting feature. Other well recognised complications include aplastic crisis secondary to viral infection (usually parvovirus), haemolytic crises, folate deficiency and, rarely, chronic leg ulcers.

It has long been appreciated that hereditary spherocytosis exhibits considerable phenotypic heterogeneity. Most patients (66%) present in childhood with mild to moderate anaemia. At one end of a broad spectrum are those who present in infancy, often with severe neonatal jaundice and subsequent transfusion dependent anaemia. At the other

extreme, a subset (about 25%) of patients are asymptomatic, their compensated haemolytic state diagnosed incidentally often as late as the fourth–seventh decades of life.

Mode of inheritance is variable. Early descriptions cite autosomal dominant as the inheritance pattern. Although this is the most common (75% of cases), it is not the sole mode of inheritance and it is thought that a major proportion of the remaining 25% represent *de novo* mutations. A very small group of patients exhibiting autosomal recessive inheritance have more recently been identified.

As a general rule, those patients homozygous for the autosomal recessive genotype have a severe clinical course. The autosomal dominant disease produces a wide clinical spectrum. It should be appreciated, however, that the link between severity and inheritance is complex (see pathogenesis below), and there is a degree of overlap between these two broad groups.

Pathogenesis**MOLECULAR PATHOLOGY**

In the recent past the application of modern techniques including electron microscopy, protein binding studies, and protein analysis has led to a greater understanding of the structure of the erythrocyte membrane and its related cytoskeleton. Furthermore, it has

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Schematic representation of the red cell membrane and cytoskeleton showing sites of principal defects known in hereditary spherocytosis. HS (Sp+) = spectrin deficiency; HS (Ank+) = ankyrin deficiency; HS (Sp-4.1) = abnormal spectrin/protein 4.1 binding.

become clear that the functional abnormality in hereditary spherocytosis results from quantitative and qualitative defects in the one or more structural proteins of the cytoskeleton. To describe these abnormalities it is first necessary to review briefly current knowledge of the cytoskeleton structure.²⁻⁴

NORMAL ERYTHROCYTE MEMBRANE

The erythrocyte membrane consists of a lipid bilayer traversed by integral proteins. When red cell membranes are treated with a non-ionic detergent a second group of proteins are identified. These proteins form an inter-connecting lattice which lies just beneath the lipid bilayer to which it is attached via the integral proteins. This protein meshwork constitutes the cytoskeleton, which forms a supporting structure for the lipid bilayer and probably has a principal role in maintaining the shape and deformability of the mature red cell.

The four major proteins in the cytoskeleton are spectrin, actin, ankyrin and protein 4.1 (figure). Spectrin is by mass the most abundant of these. It is a filamentous molecule consisting of an α and β subunit, which are twisted together in anti-parallel along their length to form a heterodimer. These subunits are further divided into domains (α I-V and β I-IV, respectively) which are responsible for specific functions such as binding to other components of the skeleton. Heterodimers are joined at their head end (at the α I and β I domains) to form tetramers and higher oligomers. It is this oligomeric spectrin which forms a two-dimensional framework when its tail ends are connected by junctional complexes of actin (a globular protein), the binding of which is facilitated by protein 4.1.

This skeleton is attached to the inner surface of the membrane via two linkages. Ankyrin is attached to the β unit of tetrameric spectrin between the junctional complexes, binding the structure to an integral protein (protein 3). A second attachment is provided by protein 4.1 which is tethered to glycoporphins in addition to the anion transporter. For a more detailed account of this structure the reader is referred to the excellent review by Zail.⁴

Other proteins identified in the cytoskeleton are, for example, tropomyosin and proteins 4.2 and 4.9—their structural and functional roles in skeletal function have yet to be fully defined.

MEMBRANE ABNORMALITIES IN HEREDITARY SPHEROCYTOSIS

Several abnormalities of the cytoskeleton have been described in hereditary spherocytosis.

Spectrin deficiency is perhaps the best characterised. It is associated with both autosomal dominant and autosomal recessive hereditary spherocytosis. In autosomal dominant disease spectrin deficiency is variable, with spectrin concentrations ranging from 60–85% of normal. In contrast, patients with recessive hereditary spherocytosis tend to have more severe spectrin deficiency, with 30–74% of normal levels. Irrespective of the mode of inheritance the degree of spectrin deficiency is inversely related to clinical severity and is

predictive of the response to splenectomy.⁵ Recessive patients with less than 50% spectrin, for example, have transfusion dependent anaemia which usually fails to correct completely with splenectomy.

The mechanism of this observed spectrin deficiency is unclear, but theories revolve around the concept of diminished spectrin production or synthesis of dysfunctional mutant spectrin. Some of these hypotheses have received support from studies in animal models as well as in man.²⁻⁴ For example, spectrin deficient mouse mutants have been studied in detail. These animals have complete or partial deficiency of either the α or β subunit depending on the mutation for which they are homozygous. Autosomal recessive hereditary spherocytosis in man may be due to inheritance of a polymorphism of the α subunit DNA. This is supported by the finding that in some people a structural variant of the α subunit exists, the mutation lying in the α II domain. Conversely, in the dominant form, an abnormality of the α subunit has been excluded by investigation with an α cDNA probe. The defect in dominant hereditary spherocytosis is, therefore, likely to involve the β spectrin subunit, although the precise mechanism remains unclear.

Quantitative defects in other cytoskeleton proteins have also been identified. Complete absence of ankyrin has been noted in some affected mice with concomitant spectrin deficiency, and in two patients reduction of ankyrin to 50% of normal has been described. *Functional abnormalities* of the cytoskeleton components exacerbate protein deficiency in a subgroup of patients. In particular, defective binding of β spectrin to protein 4.1 is associated with dominant hereditary spherocytosis, and a structural abnormality of the β IV domain (the putative protein 4.1 binding site) has been shown in some of these cases.⁶

GENETICS

The variation in the clinical picture is a reflection of pronounced heterogeneity in the genetic basis of each of the cytoskeleton abnormalities outlined above. This is a rapidly expanding area of our knowledge concerning not only hereditary spherocytosis but also the other hereditary erythrocyte membrane disorders. Extensive work has been done in murine models of spherocytosis. More recent work in man has exposed the chromosomal locations for some proteins, such as α spectrin (1q22-q25), β spectrin (14q23-24.2), and protein 4.1 (1p32-1pter). Evidence for localisation of the ankyrin gene on chromosome 8, based on clinical data from a pair of siblings with dysmorphic features, hereditary spherocytosis, and deletion of the short arm of chromosome 8, has been corroborated and the exact locus determined (8p11-21.1). More recently, the cDNA for ankyrin has been cloned and sequenced.

By mapping “candidate genes” a greater understanding of the genetic defects responsible for—for example, spectrin or ankyrin deficiency—has been achieved. Recently many mutations in several different genes have been

postulated or shown, and these have been succinctly reviewed by Davies and Lux.⁷

PATHOPHYSIOLOGY

The mechanism by which these biochemical abnormalities result in premature red cell destruction is poorly understood. It has been suggested that changes in the cytoskeleton leave areas of the lipid bilayer unsupported. Small expanses of cell membrane are thus lost, reducing the cells surface area and impairing its flexibility. This theory is supported by the finding that, *in vitro*, incubated erythrocytes of patients with hereditary spherocytosis shed portions of membrane as microvesicles until they assume a spherical shape. Clearly, a prime determinant of haemolysis in these patients is splenic destruction. Proposed mechanisms include physical entrapment in splenic vessels and ingestion by cells of the mononuclear phagocyte system. Both these theories are confirmed by morphological studies of diseased spleens with light and electron microscopy; these show red cell trapping in splenic cords and, to a lesser extent, phagocytosis by cordal macrophages. Further theories rely on the concept that there is an unfavourable environment in the spleen which damages erythrocytes passing through it. Low pH or glucose deficiency, or both, may affect cell metabolism, while high local concentration of toxic free radicals produced by adjacent phagocytes could directly affect the cytoskeleton. The latter explanation is supported by the interesting finding that in cases of hereditary spherocytosis with defective spectrin-protein 4.1 binding, spectrin is abnormally sensitive to oxidation.⁶

Irrespective of these clearly complex cellular events, and certainly others yet to be described, the net result is extravascular haemolysis which is largely corrected by splenectomy.

Diagnosis

Investigation of patients with suspected hereditary haemolytic anaemias can be divided into (a) baseline tests to establish the diagnosis and differentiate it from other causes of haemolysis; and (b) more sophisticated assessment of the genetic and molecular defect.

Elucidation of a family pedigree combined with a full blood count, film, Coomb's test and osmotic fragility tests for the patient and first degree relatives comprise the essential initial investigation. Mild to moderate anaemia is usual, with normal or slightly reduced mean red cell volume, and a raised mean red cell haemoglobin concentration and reticulocyte count. The emphasised heterogeneity of this condition also applies to the blood film appearances. These range from mild spherocytosis, through predominance of microspherocytes with the additional presence of acanthocytes, to grossly abnormal morphology including microspherocytes, acanthocytes, and poikilocytes.

An osmotic fragility test remains an essential diagnostic manoeuvre despite its numerous disadvantages. The main problem is poor sensitivity, particularly in patients with

relatively mild defects in which group it is reported by some that as many as 25% of cases may be missed despite preincubation. It must also be appreciated that specificity is sub-optimal as increased fragility can be found in immune mediated and other haemolytic conditions.

The acidified glycerol lysis test has provided an alternative method for overcoming these problems. This test is based on the measurement of rate rather than the extent of haemolysis. Drawbacks remain, not only in the form of incomplete specificity (positive in pregnancy and renal failure) but in a degree of interoperator variability in sensitivity. Autohaemolysis, a third laboratory test of haemolysis also suffers from the disadvantage of poor specificity. Although its popularity is waning, this test can be a useful screening exercise in the investigation of possible congenital haemolytic states, serving mainly to help exclude extrinsic red cell causes. A positive autohaemolysis test usually points to an intrinsic red cell abnormality while a negative test is generally unhelpful.

Unfortunately, there is not a single unequivocal test for hereditary spherocytosis suitable for routine use, but the initial investigations outlined above plus osmotic fragility and autohaemolysis data are sufficient to confirm the diagnosis in most cases with a good supporting clinical history.

In selected patients where the diagnosis is in doubt, additional evaluation can now be carried out using the specialised techniques described above. Analysis of red cell spectrin content, either by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) or, more accurately, by radioimmunoassay of whole red cell lysates, may be particularly useful in this respect (as spectrin concentrations have been found to be normal in patients with immune haemolytic anaemias). It should also be possible to predict disease severity and response to splenectomy from the degree of spectrin deficiency.

At present more elaborate assessment of the precise genetic and molecular defects, such as SDS-PAGE quantification of other cytoskeleton proteins, cytogenetic analysis, linkage studies and gene mapping, is largely of academic interest. Increasing the number of affected families investigated in this manner, however, could ultimately lead to clinical application of these techniques.

Management

The management of patients with hereditary spherocytosis is one aspect of the disease which has not seen significant change for some considerable time. Transfusion support, splenectomy and cholecystectomy remain the major treatment options, although clearly the phenotypic severity will dictate how these management options interact.

At the extremes of the spectrum the course of action is well defined. Severely affected patients present early, their anaemias demanding regular transfusional support. These are the patients in whom early splenectomy, while

usually failing to correct fully the clinical defect, will produce symptomatic relief and reduce (indeed usually negate) the requirement for transfusion, with obvious implications for future iron overload. Likewise, few clinicians would disagree that the elderly, asymptomatic patient with mild anaemia should be spared splenectomy.

Between these two distinct groups, however, lie a subset of patients in whom the role of splenectomy is more controversial. Until relatively recently the consensus was that splenectomy was indicated in all but the mildest cases of hereditary spherocytosis. In the patients with autosomal dominant hereditary spherocytosis and mild anaemia splenectomy effects a cure with a postoperative rise in haemoglobin and haematocrit to almost normal concentrations. Splenectomy also removes the risk of severe haemolytic crises, usually precipitated by infection, and this is the principal argument cited by the proponents of the procedure. Reviews on the use of splenectomy in haemolytic disease have claimed low morbidity and no mortality with excellent symptomatic relief in patients with hereditary spherocytosis.¹⁸

Presently, however, the vogue seems to be towards avoidance of splenectomy, the main anxiety lying in the incidence of subsequent sepsis. In terms of paediatric practice this anxiety has been reduced by delaying splenectomy, where possible until after 6 years of age. Sepsis is also minimised by the administration of polyvalent pneumococcal vaccine and prophylactic benzyl penicillin. Despite these approaches there is a disturbing incidence of late sepsis after splenectomy. Evans found 25 reported cases of late (older than 10 years) sepsis, five of which occurred in patients with hereditary spherocytosis.⁹ To heighten concern at these figures, three previously healthy young patients with hereditary spherocytosis (aged 17–30 years) died of overwhelming pneumococcal sepsis.

The final decision as to whether splenectomy is appropriate in these intermediate patients rests with the clinician and is based on age, symptoms, and perhaps likelihood of compliance with antibiotic prophylaxis. Having elected to proceed with splenectomy, a careful search must be made at the time of operation to remove any splenunculi, if relapse is to be avoided.

The indications for cholecystectomy in hereditary spherocytosis are rather more well defined. The main group of patients who will benefit are those who have symptomatic cholelithiasis. Before elective splenectomy, however, care must be taken to find any gall stones with abdominal ultrasonography. If found to contain stones, the gall bladder should be removed at the time of operation following a preoperative cholangiogram. Despite a low

incidence of cholelithiasis before the age of 10 years, intraoperative examination of the gall bladder is prudent in children who are having their spleens removed.

All patients with haemolysis are at risk of folate deficiency, therefore concentrations should be monitored and supplements prescribed when indicated. Clinicians must also be alert to the possibility of infection which, whether responsible for haemolytic or aplastic crises in those who have not had their spleens removed, or overwhelming sepsis in those who have, requires prompt appropriate action.

Finally, as hereditary spherocytosis is a genetic disorder counselling patients of child-bearing age and the parents of those presenting in childhood is essential. This aspect will be highlighted in the future as advances in molecular biology ultimately make prenatal diagnosis available at least to those at risk of having a severely affected child. Although the severity of the disease is often insufficient to suggest prenatal diagnosis, the application of DNA technology enables diagnosis as early as the tenth week of pregnancy. In the case of hereditary spherocytosis this state is fast approaching as the molecular and genetic basis is further defined. The lack of specificity of the phenotypic tests precludes diagnosis on cord blood at 18–20 weeks gestation as is now being used for the red cell enzymopathies.

Summary

Hereditary spherocytosis is a relatively common haematological disorder and will be encountered by all haematologists. The abundance of new information, dealing principally with molecular and genetic aspects of pathophysiology, is beginning to have implications for its investigation and management. While these advances have not yet exerted a large influence at therapeutic level, the promise of such advents as prenatal diagnosis make this an exciting field to watch.

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