Hereditary spherocytosis

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Hereditary spherocytosis is a common inherited disorder that is characterised by anaemia, jaundice, and splenomegaly. It is reported worldwide and is the most common inherited anaemia in individuals of northern European ancestry. Clinical severity is variable with most patients having a well-compensated haemolytic anaemia. Some individuals are asymptomatic, whereas others have severe haemolytic anaemia requiring erythrocyte transfusion. The primary lesion in hereditary spherocytosis is loss of membrane surface area, leading to reduced deformability due to defects in the membrane proteins ankyrin, band 3, β spectrin, α spectrin, or protein 4.2. Many isolated mutations have been identified in the genes encoding these membrane proteins; common hereditary spherocytosis-associated mutations have not been identified. Abnormal spherocytes are trapped and destroyed in the spleen and this is the main cause of haemolysis in this disorder. Common complications are cholelithiasis, haemolytic episodes, and aplastic crises. Splenectomy is curative but should be undertaken only after careful assessment of the risks and benefits.

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Introduction

Hereditary spherocytosis refers to a group of heterogeneous inherited anaemias that are characterised by the presence of spherical-shaped erythrocytes (spherocytes) on the peripheral blood smear.[1–3] This disorder, including the very mild or subclinical forms, is the most common cause of inherited chronic haemolysis in northern Europe and North America where it affects about one person in 2000.[4–11] It has also been frequently described in other populations, notably in Japan.[12] Although the disease arises in all racial and ethnic groups, on the basis of clinical reports, it seems to be less common in African-American and southeast Asian people; however, comprehensive population survey data are unavailable for these populations.

Pathophysiological effects

The red blood cell or erythrocyte membrane is a dynamic and fluid structure with the strength and flexibility needed to survive 120 days in the circulation. Its lipid bilayer is composed mainly of phospholipids and cholesterol, with integral proteins embedded in the lipid bilayer that span the membrane and a membrane skeleton on the cytoplasmic side (figure 1).[1,13–16] The polar heads of the lipid bilayer are turned outward and their apolar fatty-acid chains face one another and form the inner core. The band-3 complex, is centred by a band-3 tetramer; band 3 can also exist as a dimer. Each band-3 monomer consists of a large transmembrane segment, with a long, branched polylactosaminoglycan attached on the non-cytoplasmic side, and a stalk-like cytoplasmic domain that anchors the ankyrin-1. The ankyrin-1 also binds to the C-terminal region of the β-spectrin chain and to protein 4.2. Glycophorin A exists as a dimer with several short, sialic-acid-containing glycans attached to it. The Rhesus (Rh) complex contains the Rh polypeptides, the Rh-associated glycoprotein (arranged as a heterotetramer), CD47, the Landsteiner-Wiener glycoprotein, and glycophorin B dimer. CD47 interacts with protein 4.2 and the Rh polypeptides to establish a contact with ankyrin-1. The Rh complex and the band-3 complex are thought to form a macrocomplex. In the junctional complex, protein 4.1R interacts with one end of several spectrin tetramers in the β-spectrin N-terminal, in a region containing actin short filaments and an array of actin-binding proteins—ie, dematin, tropomyosin, β-adducin, and tropomodulin. Outside the junctional complex, protein 4.1R interacts with transmembrane glycophorin C and p55. The α2β2 tetramer of spectrin forms a dense network, lining the inner surface of the lipid bilayer. The α-spectrin and β-chains are antiparallel with the head-to-head association of two dimers—ie, N-terminal region of α-spectrin chains with the C-terminal region of β-spectrin chains—at the self-association site to generate tetramers and higher-order oligomers (figure 1). The membrane and its skeleton provide the erythrocyte with deformability—ie, the ability to undergo substantial distortion without fragmentation or loss of integrity during microcirculation and the ability to withstand the shear stress of the arterial circulation.[1,13,17–19] Two factors are implicated in the pathophysiological effects of hereditary spherocytosis—an intrinsic red blood cell membrane defect and an intact spleen that selectively retains, damages, and removes the defective erythrocytes (figure 2).[1,20,21]

Search strategy and selection criteria

We mainly searched our personal database of references, which was prospectively built by monthly search of PubMed in the past 15–20 years, with the following key words and their combinations in all fields: "hereditary spherocytosis", "red cell membrane", "spectrin", "ankyrin", "band 3", "spherocytes", "splenectomy", "gallstones", or "cholecystectomy". We mainly selected studies from the past 10 years, but did not exclude commonly cited older publications. We only read the abstracts of articles published in languages other than English. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles and standard textbooks are occasionally cited to provide readers with more details and references than this Seminar has room for.

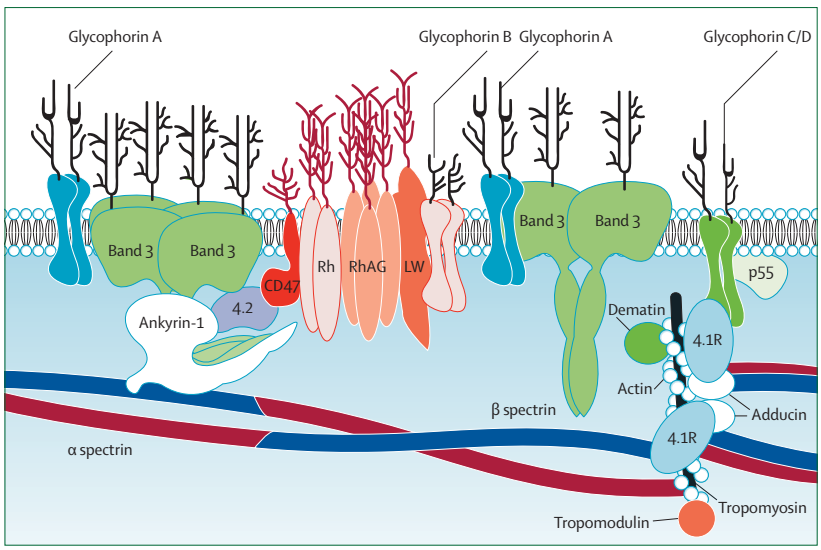

Figure 1: **A simplified cross-section of the red blood cell (erythrocyte) membrane**
The lipid bilayer forms the equator of the cross-section with its polar heads (small circles) turned outward. 4.1R=protein. 4.2=protein 4.2. Rh=Rhesus polypeptide. RhAG=Rh-associated glycoprotein. LW=Landsteiner-Wiener glycoprotein.

Although the main molecular defects in hereditary spherocytosis are heterogeneous, one common feature of the erythrocytes in this disorder is weakened vertical linkages between the membrane skeleton and the lipid bilayer with its integral proteins (table 1). Vertical linkages include spectrin, ankyrin-1, band-3, and protein-4.2 interactions; spectrin, protein-4.1R, glycophorin-C, and p55 interactions; Rh-complex and ankyrin-1 interactions; and other, as yet not fully defined lipid bilayer-membrane skeleton interactions (figure 1). When these interactions are compromised, loss of cohesion between bilayer and membrane skeleton occurs, leading to destabilisation of the lipid bilayer and release of skeleton-free lipid vesicles.[1,22] Two distinct pathways lead to reduced membrane surface area: (1) defects in spectrin, ankyrin, or protein 4.2 reduce the density of the membrane skeleton, destabilising the overlying lipid bilayer and releasing band-3-containing microvesicles; and (2) defects of band 3 lead to its deficiency and loss of the lipid-stabilising effect, which results in the loss of band-3-free microvesicles from the membrane. Both pathways result in reduced membrane surface area, a reduction in the ratio of surface area to volume, and the formation of spherocytes with reduced deformability that are selectively retained and damaged in the spleen (figure 2).[1,2,22]

Once trapped in the spleen, the abnormal erythrocytes undergo additional damage or splenic conditioning shown by further loss of surface area and an increase in cell density. Low pH, low concentrations of glucose and adenosine triphosphate, contact of erythrocytes with macrophages, and high local concentrations of oxidants contribute to conditioning. Some of these conditioned erythrocytes escape the hostile environment of the spleen, re-enter the systemic circulation, and are visable as the tail of cells on osmotic fragility tests (figure 2).[1] Splenic destruction of abnormal erythrocytes is the main cause of haemolysis in patients with hereditary spherocytosis.

Analysis of deformability profiles show that splenectomy is more beneficial for spectrin-deficient or ankyrin-deficient than for band 3-deficient red blood cells. Splenectomy prevents an early loss of immature cells in both types of deficiencies but it has an additional beneficial effect on mature spectrin-deficient or ankyrin-deficient cells, increasing their survival. Spectrin-deficient or ankyrin-deficient erythrocytes escape opsonisation by releasing band-3-containing vesicles. Hence, band-3 clusters needed for bivalent binding of low affinity, naturally occurring IgG antibodies might be retained in band-3-deficient erythrocytes with an excess of skeletal proteins but are released from spectrin-deficient or ankyrin-deficient cells in which vesicle budding is facilitated by an impaired skeleton.[23]

Causes

Membrane loss in hereditary spherocytosis is associated with defects in several membrane proteins. On the basis of densitometric quantification of membrane proteins separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis, this disease can be divided into subsets: (1) isolated deficiency of spectrin; (2) combined deficiency of spectrin and ankyrin; (3) deficiency of band-3 protein; (4) deficiency of protein 4.2; (5) deficiency of Rh complex; and (6) yet to be defined protein abnormalities (table 2).[20,24–26]

Mutations associated with isolated spectrin deficiency are defects of both α-spectrin and β-spectrin (*SPTA1* and *SPTB*, respectively) genes (online mendelian inheritance in man [OMIM] +182860 and +182870, respectively).[20,25] In general, hereditary spherocytosis caused by α-spectrin and β-spectrin mutations is associated with recessive and dominant inheritance, respectively. In healthy erythroid cells, production of α-spectrin chains is three-fold to four-fold greater than β-spectrin production.[27] Thus, a mutation of one β-spectrin allele is sufficient to cause spherocytosis whereas both α-spectrin alleles have to be affected for the disease to arise.

β-spectrin defects account for about 15–30% of cases of hereditary spherocytosis in northern European populations. Patients with β-spectrin deficiency typically have mild to moderately severe disease and do not need transfusion.[1,2,28] Blood smears from these individuals might contain prominent acanthocytes or spiculated spherocytes (figure 3). With rare exceptions, mutations of the β-spectrin gene are isolated and might be associated with monoallelic expression, suggesting that null mutations are common.[1,25,29,30] Several de-novo mutations of β spectrin have been described (table 2).[29–32]

α-spectrin defects account for about 5% of patients with hereditary spherocytosis and are only clinically

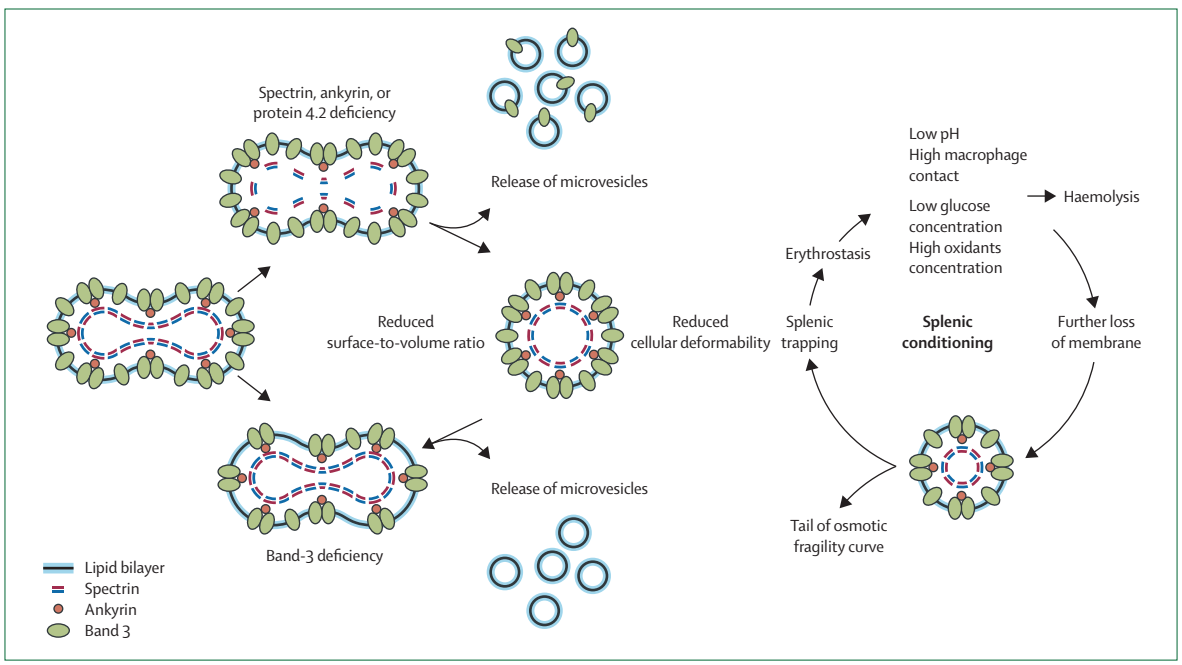

Figure 2: **Pathophysiological effects of hereditary spherocytosis**
Modified from Gallagher and colleagues[21] with permission.

apparent in the homozygous or compound heterozygous state. These patients have severe disease. Homozygous and compound heterozygous defects have been associated with null mutations and variants are associated with low-expression alleles.[33–36] For example, the α-*LEPRA* (low-expression allele Prague) allele produces about six-fold less of the correctly spliced α-spectrin transcript than does the healthy allele.[33] The presence of two null α-spectrin alleles is speculated to be lethal. Blood smears from patients with severe α-spectrin deficiency contain many microspherocytes, contracted erythrocytes, and abnormal poikilocytes (table 2).[24,33]

The biochemical phenotype of combined spectrin and ankyrin deficiency is the most common abnormality noted in about 40–65% of patients with hereditary spherocytosis in northern European populations,[36–38] but in only 5–10% of cases in Japan (table 2).[12] Patients with ankyrin defects have prominent spherocytosis without other morphological abnormalities (figure 3). Ankyrin mutations (OMIM +182900) cause both dominant and recessive disease that can range from clinically mild to severe.[1,20,25,38–41] About 15–20% of ankyrin-1 (*ANK1*) gene mutations reported are de novo;[25,32,38,42] recurrent mutations have been described.[43]

Mutations—eg, nucleotide –108T to C and –153G to A, and deletion of nucleotides –72/73 of the ankyrin promoter, leading to decreased ankyrin-1 expression have been identified in some patients.[44,45] A few patients with atypical hereditary spherocytosis associated with karyotypic abnormalities—ie, deletions or translocations of the ankyrin gene locus on chromosome 8p—have been described.[46,47] *ANK1* gene deletion might be part of a contiguous gene syndrome with manifestations of spherocytosis, mental retardation, typical faces, and hypogonadism.

Ankyrin-1 plays a pivotal role in the stabilisation of the membrane, providing the main membrane binding site for the spectrin-based membrane skeleton. Since it links β spectrin to band 3, ankyrin deficiency leads to a proportional reduction in spectrin assembly on the membrane despite normal spectrin synthesis.[48] The deficiency of one protein is strictly associated with the deficiency of the other and the extent of protein deficiency is related to the clinical severity. A high reticulocyte count might mask a reduction in ankyrin-1 in biochemical studies.[49]

Deficiency of band-3 protein arises in about 33% of patients, presenting with mild to moderate, dominantly inherited disease (table 2). Mushroom-shaped or pincered erythrocytes might be seen on peripheral blood smear (figure 3).[1,50] SDS-PAGE analysis shows a reduction in band 3 in about 20–30% of patients, as the wild-type allele in trans partly compensates for the mutated allele.[51] Erythrocyte membranes from these patients also have deficiency of protein 4.2.[52] A range of band-3 mutations (OMIM+109270) associated with hereditary spherocytosis have been reported; these mutations are spread out throughout the band-3 (*SLC4A1*) gene.[1,20,25,53–55]

Alleles that affect band-3 expression when inherited in trans to a band-3 mutation, aggravate band-3 deficiency and worsen clinical severity.[56–58] Severe disease has been reported in patients who are compound heterozygotes or homozygotes for band-3 defects.[59–62]

A subset of patients with band-3 protein deficiency, resulting from mutations in the region of the band-3 gene encoding the fifth outer loop of the transmembrane domain, have features of dehydrated hereditary stomatocytosis or cryohydrocytosis.[63] Thus, the clinical presentation ranging from band-3-related hereditary spherocytosis to dehydrated hereditary stomatocytosis and cryohydrocytosis is a continuum. Band-3 mutations sometimes also cause distal renal tubular acidosis with or without hereditary spherocytosis, the phenotype of southeast Asian ovalocytosis, and acanthocytosis. The pleiotropic clinical manifestations associated with band-3 mutations are remarkable in their diversity.[52]

Recessive hereditary spherocytosis due to homozygous mutations in protein 4.2 (*EPB42*) gene (OMIM *177070) is common in Japan,[12,64,65] but is rare in other populations (table 2).[24,66] On peripheral blood smear, ovalocytes and stomatocytes predominate with few spherocytes. Patients with heterozygous mutantions are asymptomatic. Membranes show an almost total absence of protein 4.2, resulting in a secondary reduction in CD47, a member of the Rh complex (figure 1).[61,67,68]

Rh deficiency syndrome identifies rare individuals who have either absent (Rh null) or moderately reduced (Rh mod) Rh antigen expression and mild to moderate haemolytic anaemia associated with the presence of stomatocytes and spherocytes on the peripheral blood film. Haemolytic anaemia is improved by splenectomy.[69,70] Rh proteins are part of a multiprotein complex that also includes Rh-associated glycoproteins (RhAG) and Landsteiner-Wiener glycoprotein, glycophorin B, and protein 4.2. The Rh-RhAG complex interacts with ankyrin

	Protein	Gene	Location	Exons	Aminoacids	Molecular weight×10^3 (gel/calculated)	Oligomeric state
SDS-PAGE band 1	α-spectrin	*SPTA1*	1q22-q23	52	2429	240/281	Heterodimer/tetramer
SDS-PAGE band 2	β- spectrin	*SPTB*	14q23-q24.1	32	2137	220/246	Heterodimer/tetramer
SDS-PAGE band 2.1	Ankyrin-1	*ANK1*	8p11.2	42	1880	210/206	Monomer
SDS-PAGE band 2.9	β-adducin	*ADD2*	2p13.3	16	726	97/80	Heterodimer/tetramer
SDS-PAGE band 3	Band 3 (AE1)	*SLC4A1*	17q21	20	911	90–100/102	Dimer/tetramer
SDS-PAGE band 4.1R	Protein 4.1	*EPB41*	1p33-p34.2	>23	588	80+78*/66	Monomer
SDS-PAGE band 4.2	Protein 4.2	*EPB42*	15q15-q21	13	691	2/77	Dimer/trimer
SDS-PAGE band 4.9†	Dematin	*EPB49*	8p21.1	15	383	48+52/43	Trimer
	p55	*MPP1*	Xq28	12	466	46+55/53	Dimer
SDS-PAGE band 5‡	β-actin	*ACTB*	7pter-q22	6	375	43/42	Oligomer
	Tropomodulin	*TMOD*	9q22	9	359	43/41	Monomer
SDS-PAGE band 6	G3PD	*GAPD*	12p13	9	335	35/36	Tetramer
SDS-PAGE band 7§	Stomatin	*STOM*	9q33.2	7	288	31/32	..
	Tropomyosin	*TPM3*	1q31	13	239	27+29/28	Heterodimer
SDS-PAGE band PAS-1¶	Glycophorin A	*GYPA*	4q31.21	7	131	36/14	Dimer
SDS-PAGE band PAS-2¶	Glycophorin C	*GYPC*	2q14-q21	4	128‖	32/14	..
SDS-PAGE band PAS-3¶	Glycophorin B	*GYPB*	4q31.21	5	72	20/8	Dimer
	Glycophorin D	*GYPD*	2q14 q21	4	107‖	23/11	..

AE1=anion exchange protein-1. G3PD=glyceraldehyde-3-phosphate dehydrogenase. PAS=periodic acid Schiff reagent. SDS-PAGE=sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Protein 4.1 is a doublet of 4.1a and 4.1b on SDS-PAGE gel. †Band contains both dematin and p55.; ‡Band contains β actin and tropomodulin. §Band contains stomatin and tropomyosin. ¶Glycophorins are visible only on PAS-stained gels. ‖Glycophorins C and D arise from alternative translation initiation sites.

Table 1: **Major human erythrocyte membrane proteins and gene defects**

	Patients with HS	Heredity	Prevalent mutations	Protein reduction	Disease severity	Peripheral blood smear
Ankyrin-1	USA and Europe 40–65%; Japan 5–10%	AD, AR, de novo	AD or de novo: null mutation; AR: mis-sense and promoter mutations	Spectrin and ankyrin-1 15–50%	Mild to moderate	Spherocytes
Band 3	20–35%	AD	Functionally null mutation	Band 3 15–35%	Mild to moderate	Spherocytes, occasional mushroom-shaped or pincered cells
α spectrin	<5%	AR	α-*LEPRA* allele and null mutation	α spectrin 50–75%	Severe	Spherocytes, contracted cells, and poikilocytes
β spectrin	15–30%	AD, de novo	Null mutation	β spectrin 15–40%	Mild to moderate	Spherocytes, 5–10% acanthocytes
Protein 4.2	USA and Europe <5%; Japan 45–50%	AR	Mis-sense (prevalence of 4.2 Nippon)	Protein 4.2 95–100%	Mild to moderate	Spherocytes, ovalostomatocytes

AD=autosomal dominant. AR=autosomal recessive. HD=hereditary spherocytosis. *LEPRA*=low-expression allele Prague.

Table 2: **Clinical and molecular characterisation of hereditary spherocytosis molecular defects**

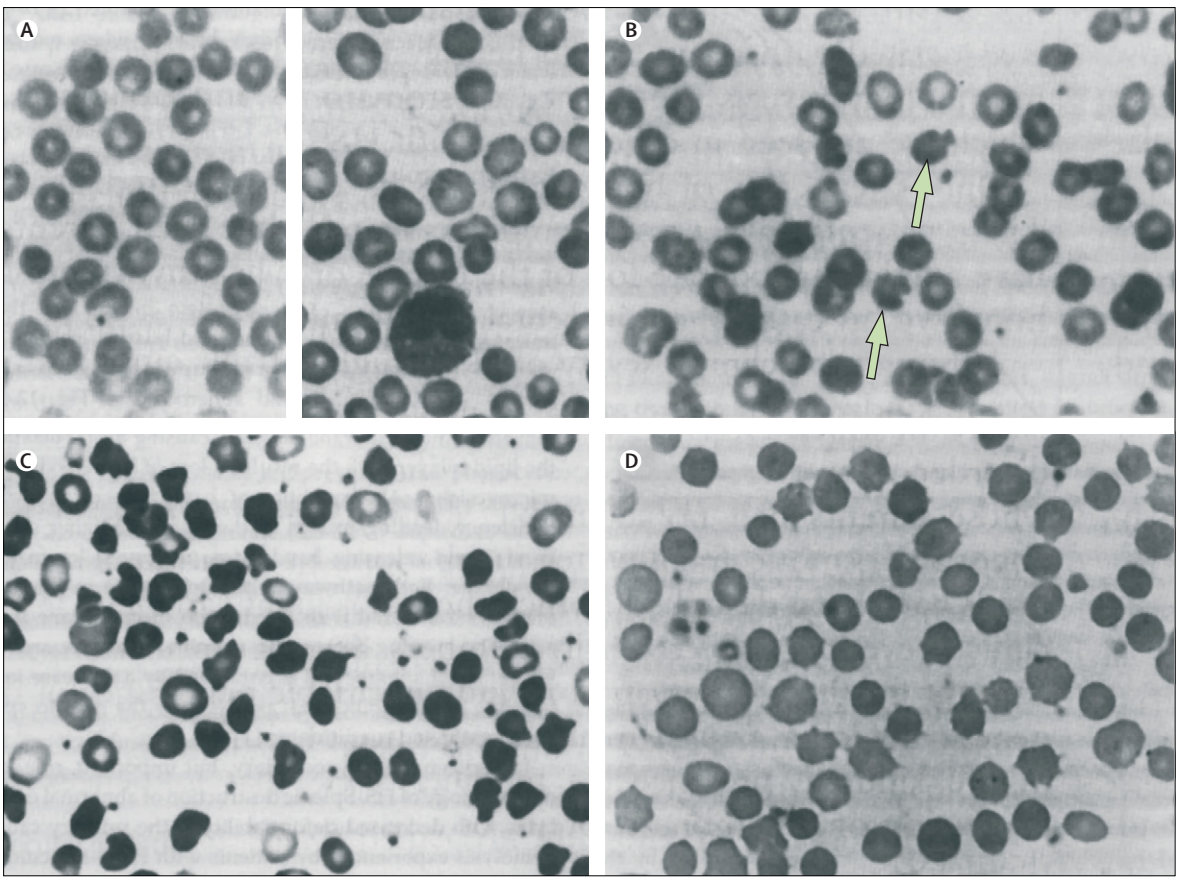

Figure 3: **Abnormal peripheral blood smears in hereditary spherocytosis (HS) due to different membrane defects**
(A) Two blood smears of typical moderately severe HS with a mild deficiency of red blood cell spectrin and ankyrin-1. Although many cells have spheroidal shape, some retain a central concavity. (B) HS with pincered red cells (arrows), as typically seen in HS associated with band-3 deficiency. Occasional spiculated red cells are also present. (C) Severe atypical HS due to severe, combined spectrin and ankyrin-1 deficiency. In addition to spherocytes, many cells have irregular contours. (D) HS with isolated spectrin deficiency due to a β-spectrin mutation. Some of the spherocytes have prominent surface projections resembling spheroacanthocytes. Reproduced from Gallagher and colleagues[3] with permission.

to link the membrane skeleton to the lipid bilayer.[71] Rh null erythrocytes have increased osmotic fragility, showing decreased membrane surface area and are dehydrated, as shown by reduced cell cation and water content and increased cell density, cellular features characteristic of spherocytosis associated with ankyrin, and band-3 and spectrin deficiencies.[69,70,72] The genetic basis of the Rh deficiency syndrome is heterogeneous. The amorph type is due to defects associated with the RH30 locus encoding the RhD and RhE polypeptides. The regulatory type of Rh null and Rh mod phenotypes results from suppressor or modifier mutations at the RH50 locus.[69,70,72,73]

Clinical features

The clinical manifestations of hereditary spherocytosis vary widely (panel 1). Manifestations of typical disease are haemolysis with anaemia, jaundice, reticulocytosis, gallstones, and splenomegaly, and spherocytes on peripheral blood smear (figure 3), increased erythrocyte osmotic fragility, and a positive family history of disease.[1–3,74] It is most commonly associated with dominant inheritance, although non-dominant and recessive inheritance has been described.[75] Initial assessment of a patient with suspected disease should include a family history and questions about history of anaemia, jaundice, gallstones, and splenectomy. Physical examination should seek signs such as scleral icterus, jaundice, and splenomegaly. After diagnosis of a patient with hereditary spherocytosis, family members should be examined. In general, affected individuals of the same family have similar degrees of haemolysis, but heterogeneous clinical patterns have also been reported in individual relatives due to additive effects of unequally expressed mutant alleles[56,57,60] or other modifier alleles, or due to co-inheritance of other haematological disorders. In particular, hereditary spherocytosis might be worsened or ameliorated by obstructive jaundice, β-thalassaemia and deficiencies of iron, vitamin B_{12}, folate, pyruvate kinase, or glucose-6-phosphate dehydrogenase.[1,76,77] One of the frequent associations in urban areas is heterozygosity for haemoglobin S or haemoglobin SC disease with

hereditary spherocytosis, which can result in a splenic infarct or acute splenic sequestration.[1,78]

Severity of hereditary spherocytosis is classified as mild, moderate, moderately severe, and severe according to a few common clinical laboratory variables, including haemoglobin and bilirubin concentrations, and reticulocyte count (table 3).[74]

About 20–30% of patients have mild disease with compensated haemolysis—ie, red blood cell production and destruction are balanced or nearly equivalent.[79] These patients are not anaemic and are usually asymptomatic with mild splenomegaly, slight reticulocytosis, and minimum spherocytosis, making diagnosis difficult in some cases. Osmotic fragility is often increased only after the blood is preincubated at 37°C for 24 h. Many of these individuals escape detection until adulthood when complications related to chronic haemolysis arise.[1] Haemolysis can be exacerbated by illnesses that cause splenomegaly, such as infectious mononucleosis, or by other factors, such as pregnancy or exercise.[1,80]

Because of the asymptomatic course in these patients, diagnosis of hereditary spherocytosis is often made during studies of the family or assessment of splenomegaly, gallstones, or anaemia from viral infection—eg, parvovirus B19 or influenza.[1,2,4]

About 60–70% of patients have moderate disease, which typically presents in childhood but can present at any age. In children, anaemia is the most frequent sign (50% of cases), followed by splenomegaly, jaundice, and a positive family history. The haemoglobin concentration is between 80–110 g/L and the proportion of reticulocytes is increased in most cases (table 3). The anaemia is usually asymptomatic except for fatigue or pallor, or both. Jaundice is seen occasionally in about half the patients, usually in association with viral infections. When present, it is acholuric and characterised by unconjugated indirect hyperbilirubinaemia and absence of bilirubinuria. The prevalence of palpable splenomegaly varies from about 50% in young children to 75–95% in older children and adults. Typically the spleen is moderately enlarged, but it can be massive. Although an association between spleen size and the severity of hereditary spherocytosis has not been established, it seems likely that one exists. Diagnosis is straightforward if spherocytosis, increased osmotic fragility, and a positive family history are present.[3]

A small group of patients, about 10%, have moderately severe disease, which is distinguished from moderate hereditary disease by low haemoglobin concentrations (table 3) and an intermittent need for transfusions. Additionally, reticulocytosis is greater (often >15%) and bilirubinaemia more pronounced (often >51 μmol/L) than in moderate disease.

About 3–5% of patients have severe hereditary disease with life-threatening anaemia, needing regular transfusions to maintain a haemoglobin concentration greater than 60 g/L. They almost always have non-dominant disease.[32,38,43] Most patients have isolated, severe spectrin deficiency, thought to be due to a defect in α spectrin, but a few have ankyrin or band-3 defects. In addition to spherocytes and microspherocytes, irregularly budding spherocytes or abnormal poikilocytes are sometimes seen

***Panel 1:* Major clinical and laboratory characteristics of hereditary spherocytosis**

- Anaemia*
- Splenomegaly*
- Jaundice*: from haemolysis* or biliary obstruction
- Haemolytic, aplastic, and megaloblastic crises
- Gallstones
- Rare manifestations: leg ulcers, gout, chronic dermatitis, extramedullary haemopoietic tumours, haematological malignant diseases, angioid streaks
- Neuromuscular disorders
- Cardiovascular disease
- Myopathy
- Spinocerebellar degeneration
- Reticulocytosis*
- Spherocytes*
- Increased mean corpuscular haemoglobin concentration and red blood cell distribution width*
- Increased proportion of hyperdense cells*
- Increased osmotic fragility (especially after incubation)*
- Negative direct antiglobulin test*
- Inheritance
- Dominant: about 75%
- Non-dominant: about 25% de novo or recessive
- Excellent response to splenectomy

*Typical signs of hereditary spherocytosis.

	Mild	Moderate	Moderately severe	Severe*
Haemoglobin (g/L)	Normal	>80	60–80	<60
Reticulocytes	<6%	>6%	>10%	>10%
Bilirubin (μmol/L)	17·1–34·2	>34·2	>34·2–51·3	>51·3
Peripheral smear	Some spherocytes	Spherocytes	Spherocytes	Microspherocytes and poikilocytosis
OF (fresh blood)	Normal or slightly increased	Increased	Increased	Increased
OF (incubated blood)	Increased	Increased	Increased	Increased
Splenectomy	Rarely†	If physical ability is decreased or in some cases†	Necessary (at >5 years)	Necessary (at >2–3 years)
Transfusions	0–1	0–2‡	>2	Regular
SDS-PAGE (protein deficiency)	Normal	Sp, Ank+Sp, band 3, protein 4.2	Sp, Ank+Sp, band 3	Sp, Ank+Sp, band 3
Heredity	AD	AD, de novo mutation	AD, de novo mutation	AR

AD=autosomal dominant. Ank=ankyrin-1. AR=autosomal recessive. OF=osmotic fragility. SDS-PAGE=sodium dodecyl sulphate polyacrylamide gel electrophoresis. Sp=spectrin. *Patients depend on regular transfusions. †Adults undergoing cholecystectomy or with pronounced jaundice. ‡Some patients need one or two transfusions during infancy.

***Table 3:* Classification of hereditary spherocytosis**

on peripheral blood smear (figure 3). Patients might develop iron overload with its attendant clinical complications, necessitating continuous chelation treatment. Without regular transfusions or splenectomy, or both, patients might have growth retardation, delayed sexual maturation, or extramedullary erythropoiesis with hepatosplenomegaly and bony changes—eg, thalassaemic faces.

The parents or other relatives of patients with recessive hereditary disease are carriers of an asymptomatic trait. They are clinically healthy and do not have anaemia, splenomegaly, hyperbilirubinaemia, or spherocytosis on peripheral blood smears.[74,75] Most relatives have subtle laboratory signs of hereditary disease, including slight reticulocytosis (1·5–3·0%) or slightly reduced haptoglobin values. The incubated osmotic fragility test is probably the most sensitive method for detection of carriers, especially by the 100% lysis point, which is greatly increased in carriers (mean sodium chloride 4·3 g/L [SD 0·5]) compared with controls (2·3 g/L [0·7]). The acidified glycerol lysis test might also be useful for the diagnosis of spherocytosis. About 1% of the population are estimated to be silent carriers.[5,6]

Unsplenectomised pregnant women with hereditary disease do not have clinically significant complications except for anaemia, which is aggravated by the plasma volume expansion that usually occurs in pregnancy and sometimes by increased haemolysis or megaloblastic crises. Transfusions are rarely necessary.[1,80]

In the neonate, hereditary disease often presents as jaundice in the first few days of life.[81,82] Kernicterus is a risk; thus, exchange transfusions are sometimes necessary, but in most patients the jaundice is controlled with phototherapy. Co-inheritance of Gilbert's syndrome, as detected by homozygosity for a TATA box polymorphism in the uridine-diphosphate glucuronyl-transferase 1A1 gene (*UGT1A1*), increases the frequency and severity of hyperbilirubinaemia in neonates with hereditary disease.[83,84] Hydrops fetalis associated with spectrin or band-3 defects is rare and has been reported in only four or five patients.[2,28,59,85]

Only 28–43% of neonates with hereditary disease are anaemic at birth (haemoglobin concentration <150 g/L) and severe anaemia is rare.[7,81,86] In most newborns, haemoglobin concentrations are within the normal range at birth, then decrease sharply during the first 3 weeks of life, leading to a transient, severe anaemia.[86] Up to three-quarters of these anaemic infants need blood transfusions. Most infants outgrow the need for transfusion by the end of their first year of life.[7,86] This transient anaemia is the result of erythropoiesis being slow as shown by a reticulocyte count that is low for the degree of anaemia. Regular subcutaneous administration of recombinant human erythropoietin to infants with hereditary spherocytosis was shown to be beneficial in reduction of the need for blood transfusions in an open-label study.[87] However, further study of the use of this hormone is needed in anaemic infants with hereditary spherocytosis before routine use can be recommended.

Close observation of infants with hereditary spherocytosis is necessary; haemoglobin concentrations and reticulocyte counts should be monitored at least once a month during the first 6 months to detect and treat late anaemia. The observation interval in the children with mild and moderate disease can be increased to 6–8 weeks after 6 months of life, and to 3–4 months in the second year of life. In childhood, each patient should have haemoglobin and bilirubin concentrations and reticulocyte counts checked every 6–12 months up to the age of 5 years, and roughly every year thereafter. After the neonatal stage, the criteria for red blood cell transfusion is a haemoglobin concentration of less than 50–60 g/L in the absence of other concomitant factors—eg, fever and viral illness. A yearly screen for parvovirus B19 serology could be done until a positive result for IgG is noted. This practice can be helpful for patients and parents since it will warn or reassure them of the risk of contracting this viral infection.

Complications

Gallbladder disease

Chronic haemolysis leads to the formation of bilirubinate gallstones, which are the most common complication of hereditary spherocytosis. Gallstones are noted in at least 5% of children less than 10 years of age;[7] the proportion increases to 40–50% in the second to fifth decades, with most stones arising between 10 and 30 years of age.[88] The co-inheritance of Gilbert's syndrome increases the risk of cholelithiasis by four-fold to five-fold.[89] Timely diagnosis and treatment will help prevent complications of biliary-tract disease, including biliary obstruction with pancreatitis, cholecystitis, and cholangitis. The best method for detection of gallstones is ultrasonography.[88] We recommend an abdominal ultrasound every third to fifth year in patients with hereditary spherocytosis, every year in those with both hereditary spherocytosis and Gilbert's syndrome, and before splenectomy. Once the spleen is removed, individuals with hereditary spherocytosis do not develop pigment stones.[90]

Haemolytic, aplastic, and megaloblastic crises

Patients with hereditary spherocytosis, like those with other haemolytic diseases, are at risk of increased crises.[1,3] Haemolytic crises are the most common, often triggered by viral illnesses and typically arise in childhood. Increased haemolysis is probably due to enlargement of the spleen during infections and activation of the reticuloendothelial system. Haemolytic crises are generally mild and are characterised by a transient increase in jaundice, splenomegaly, anaemia, and reticulocytosis; intervention is rarely necessary. Severe haemolytic crises are associated with substantial jaundice, anaemia, lethargy, abdominal pain, vomiting, and tender splenomegaly. For most patients, only supportive care is needed; red blood cell

transfusions are only needed if the haemoglobin concentrations are greatly reduced.

Aplastic crises following virally-induced bone marrow suppression are less common than haemolytic crises, but they can lead to severe anaemia, requiring treatment in hospital and transfusion, with serious complications, including congestive heart failure or even death. With rare exceptions, severe aplastic crises arise only once in life. The most common causative agent is parvovirus B19—the cause of erythema infectiosum (fifth disease)[91]—which confers life-long immunity. Parvovirus selectively infects erythropoietic progenitor cells and inhibits their growth. The characteristic laboratory finding is a low number of reticulocytes despite severe anaemia. The earliest laboratory sign is an increase in the serum iron concentration due to the loss of marrow erythroblasts and reduced haemoglobin synthesis. Bilirubin concentrations might decrease as the number of abnormal red blood cells that can be destroyed decreases. Parents should be advised to watch for pallor, extreme lassitude, and ashen conjunctivae after mild non-specific signs of infection such as fever, chills, vomiting, diarrhoea, abdominal pain, and myalgias. The characteristic rash of parvovirus infection in these patients is rare but when present is related to deposits of immune complexes. Aplastic crises usually last 10–14 days. Confirmation of

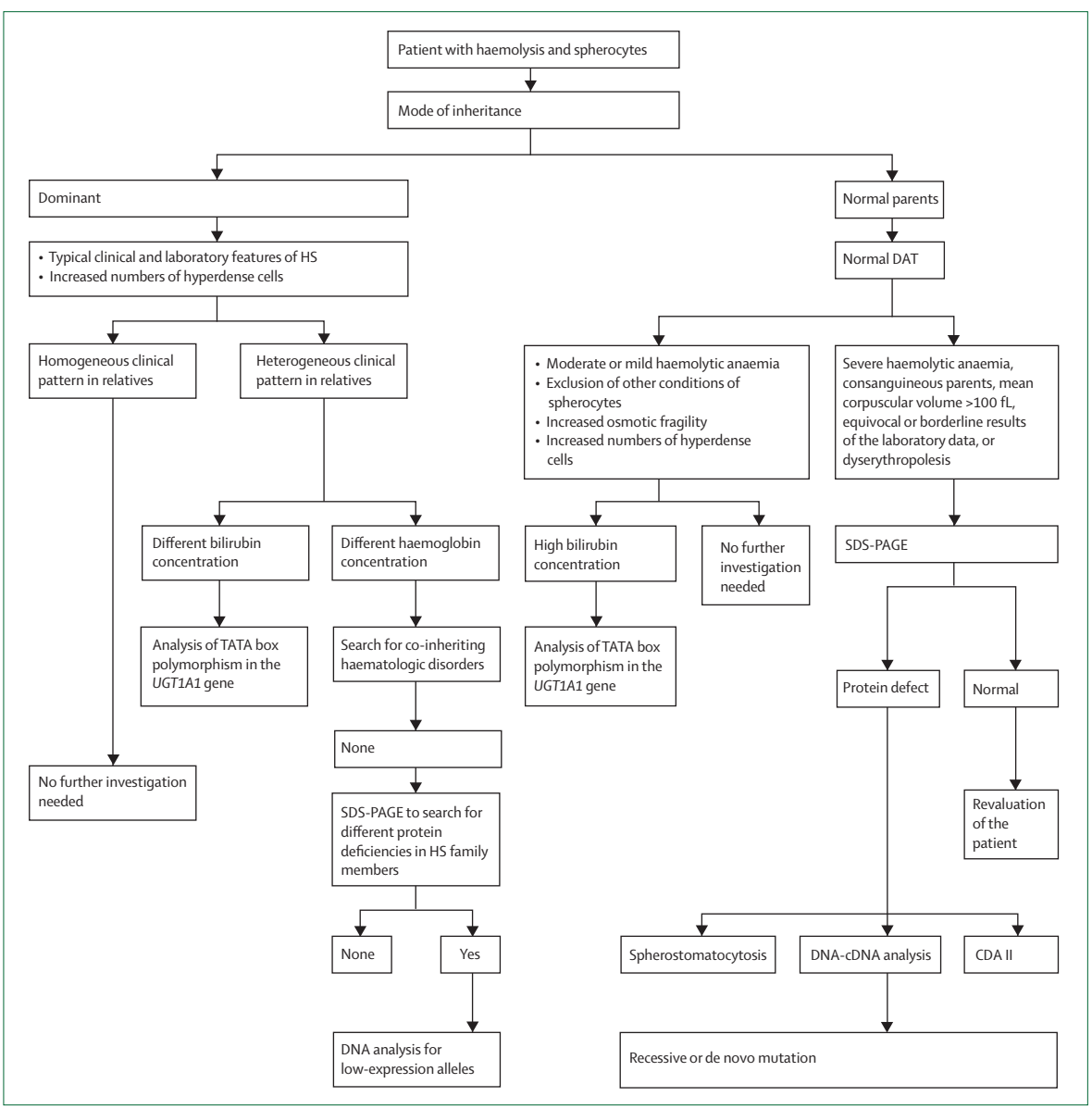

Figure 4: **Flow chart for the diagnosis of hereditary spherocytosis (HS)**
UGT1A1=uridine diphosphate glucoronsyl transferase 1A1. SDS-PAGE=sodium dodecyl sulphate polyacrylamide gel electrophoresis. DAT=direct antiglobulin test. CDA II=congenital dyserythropoietic anaemia type II.

the diagnosis by recording the raised parvovirus IgM titres is helpful to the doctors and patients. Intravenous immunoglobulin treatment is not recommended. Undiagnosed, asymptomatic patients with hereditary spherocytosis and compensated haemolysis might come to medical attention during an aplastic crisis.[92] Because parvovirus might infect several members of a family simultaneously, epidemics or outbreaks of hereditary spherocytosis have been reported. Importantly, for this reason, family members should be screened when erythroblastopenia arises in a patient with undiagnosed hereditary spherocytosis.

Megaloblastic crises caused by folate deficiency are very rare in developed countries where nutrition and prenatal care are good, but they can occur in patients with hereditary spherocytosis and increased folate demands—eg, pregnant women, growing children, or patients recovering from an aplastic crisis. To prevent folate deficiency, the recommendation is that unsplenectomised patients with hereditary spherocytosis and splenectomised patients with severe hereditary spherocytosis take folate 2–3 mg per day until the age of 5 years and 5 mg per day thereafter.

Rarely, patients with hereditary spherocytosis develop other complications (panel 1). In severe cases, growth failure, leg ulcers, or skeletal abnormalities resulting from bone marrow expansion can occur.[93] Extramedullary erythropoiesis might be associated with tumours, especially along the posterior thoracic or lumbar spine or in the hila of the kidneys.[1,94,95] These tumours often arise in non-splenectomised patients with mild disease and are the first manifestation of hereditary spherocytosis. Easily diagnosed by MRI, these masses stop growing and undergo fatty metamorphosis after splenectomy, but they do not shrink in size. Haematological malignant diseases—eg, multiple myeloma and leukaemia—have been reported in patients with hereditary spherocytosis, leading to the postulation that prolonged haemopoietic stress was a predisposing factor. However, cause and effect have not been proven.[96,97]

Diagnosis and laboratory features

Hereditary spherocytosis is usually diagnosed based on a combination of clinical and family histories, physical examination (for splenomegaly or jaundice), and laboratory data (full blood count, especially red blood cell indices and morphology, and reticulocyte count; figure 4).[1,3] Other causes of anaemia should be excluded, particularly autoimmune haemolytic anaemia, congenital dyserythropoietic anaemia type II, and hereditary stomatocytosis.

Interpretation of the direct antiglobulin test-negative autoimmune haemolytic anaemia can be difficult in an individual, particularly in an adult, with no family history. Flow cytometric assessment of red blood cell immunoglobulin density and the application of specific reagents might be helpful in the diagnosis of some cases of autoimmune haemolytic anaemia.[98] Moreover, the presence of reticulocytes with volume consistently lower and haemoglobin concentration consistently higher in hereditary spherocytosis than in autoimmune haemolytic anaemia could be useful in distinguishing spherocytes from these two diseases.[99]

Panel 2: Disorders with spherocytes on peripheral blood smear

- Hereditary spherocytosis
- Immune haemolytic anaemia
- Acute oxidant injury
- Microangiopathic and macroangiopathic haemolytic anaemias
- Haemolytic transfusion reactions
- Thermal injuries
- Liver disease
- Heinz body anaemias
- Hereditary pyropoikilocytosis
- Clostridial sepsis
- Zinc toxicity
- Poisoning with some snake, spider, and *Hymenoptera* venoms
- Severe hypophosphataemia
- Long blood storage (in vitro)
- ABO incompatibility (in neonates)
- Hypersplenism

The clinical picture of mild to moderate chronic anaemia, splenomegaly, jaundice, and gallstones in association with the presence of spherocytes on peripheral blood smear, and an increased osmotic fragility could result in the erroneous diagnosis of hereditary spherocytosis in some patients with congenital dyserythropoietic anaemia type II. This concern is particularly relevant for individuals with congenital dyserythropoietic anaemia type II and reticulocyte counts of 120×10^9 to 180×10^9 per L. Careful assessment of the ratio of reticulocyte count to haemoglobin concentration and the signs of iron overload, particularly in adult patients, can assist in the accurate diagnosis of disease.[100,101] However, diagnosis of congenital dyserythropoietic anaemia type II can be confirmed by the electrophoretic pattern of the red blood cell membrane showing a fast migrating band 3 or a bone-marrow smear examination showing an erythroid hyperplasia (with 10–40% of binucleate or multinucleate erythroblasts), or both.[101]

Hereditary stomatocytoses are very rare autosomal dominant haemolytic anaemias, often diagnosed as atypical hereditary spherocytosis. Macrocytosis is common in many but not all cases. The blood film might show a few spherocytes and stomatocytes. Target cells are seen in some disorders, especially dehydrated stomatocytosis. Osmotic fragility of red blood cells and mean corpuscular haemoglobin concentration can help to differentiate between the hydrocytosis and xerocytosis

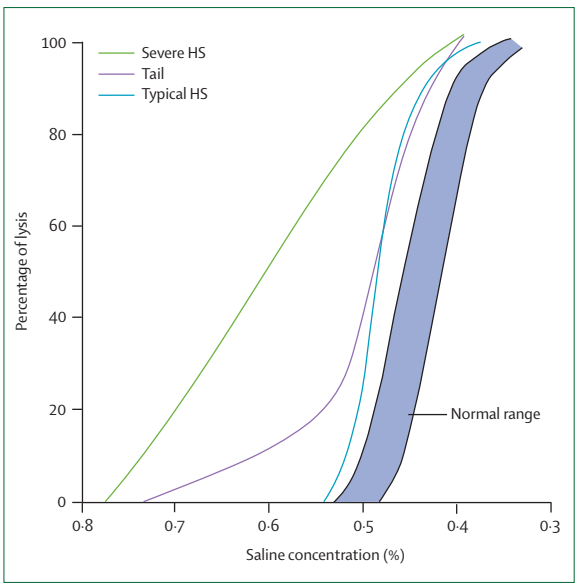

Figure 5: **Osmotic fragility curves in hereditary spherocytosis (HS)**
The shaded area is the normal range. Results representative of both typical and severe HS are shown. A tail, representing very fragile erythrocytes that have been conditioned by the spleen, is common in many patients with hereditary spherocytosis before splenectomy. Reprinted from Dacie with permission.[108]

variants. Moreover, the key test for diagnosis is osmotic gradient ektacytometry, available only in specialised laboratories. Differentiation of hereditary spherocytosis from hereditary stomatocytosis and related disorders is important because splenectomy is associated with a high risk of thromboembolic events in hereditary stomatocytosis.[102,103]

An incubated osmotic fragility test should be done after an initial laboratory investigation for diagnosis of hereditary spherocytosis that includes a complete blood count with peripheral smear, reticulocyte count, direct antiglobulin test, serum bilirubin, and a family history suggestive of hereditary spherocytosis. Rarely, additional, specialised tests are needed to confirm the diagnosis.

The diagnosis of hereditary spherocytosis is often more difficult in the neonatal period than later in life.[81] Splenomegaly is infrequent and at most the spleen tip is palpable; reticulocytosis is variable and usually not severe (only 35% of affected newborns have a reticulocyte count >10%). About 33% of neonates with hereditary spherocytosis do not have large numbers of spherocytes in their peripheral blood smears, whereas spherocytes are commonly seen in neonatal blood films in the absence of disease.[81] Because neonatal red blood cells are more osmotically resistant than are adult cells, the osmotic fragility test is less reliable for diagnosis of this disease. For these reasons, unless the need for diagnosis is urgent, tests should be postponed until the child is at least 6 months of age. If diagnostic tests are needed, appropriate osmotic fragility curves that have been developed for neonates should be used.

Several disorders can show spherocytes on peripheral blood smear (panel 2). Importantly, a smear from a patient with suspected spherocytosis should be of high quality, with the erythrocytes well separated and some cells with central pallor, because spherocytes are a common artifact on blood smears. Erythrocyte morphology is quite variable in hereditary spherocytosis. In patients with typical disease, spherocytes are obvious on blood smear. These cells are dense, round, and hyperchromic without the central pallor and have a reduced mean cell diameter. Infrequently, patients present with only a few spherocytes on peripheral smear or with many small, dense spherocytes, and abnormal erythrocyte morphology with anisocytosis and poikilocytosis. Specific morphological effects have been identified in association with some membrane protein defects in hereditary spherocytosis (figure 3).[12,30,51,53]

Most patients have a mild to moderate anaemia or no anaemia at all (compensated haemolysis). Neither the mean corpuscular volume nor the percentage of microcytic red cells has any diagnostic value. The mean corpuscular haemoglobin concentration is greater than 360 g/L in half of patients because of cellular dehydration.[104] Combination of the mean corpuscular haemoglobin concentration with the red blood cell distribution width can lead to a specificity of nearly 100%.[105] Some automated haematology analysers measure cell haemoglobin concentration of individual red blood cells, and increased numbers of dense dehydrated cells might identify all patients with hereditary spherocytosis without the need for additional laboratory tests, especially when one member of a family is already known to have the illness.[104–107] To rule out the diagnosis of disease in the presence of normal osmotic fragility, assessment of the state of cell hydration is essential. If mean corpuscular haemoglobin concentration or the percentage of hyperdense red blood cells is increased, a diagnosis of hereditary spherocytosis should be seriously considered despite normal osmotic fragility. These variables are less pronounced in splenectomised than in non-splenectomised patients with this disorder.

In the healthy erythrocyte, membrane redundancy gives the cell its characteristic discoid shape and provides it with abundant surface area relative to cell volume. In spherocytes, surface area relative to cell volume is reduced, resulting in the increased osmotic fragility noted in these cells. Osmotic fragility is tested by assessment of the extent of haemolysis of red blood cells suspended in increasingly hypotonic solutions of saline (figure 5). Healthy erythrocytes are able to increase their volume with increasing hypotonicity by swelling until they reach their critical haemolytic volume, after which lysis occurs. Spherocytes with reduced surface area reach their critical haemolytic volume at higher saline concentrations than do healthy erythrocytes and as such are osmotically more fragile. About 25% of individuals with hereditary spherocytosis will have a normal osmotic fragility of their freshly drawn red blood cells.[109] After

incubation at 37°C for 24 h, red blood cells from patients with hereditary spherocytosis lose membrane surface area more readily than do healthy cells. Incubated osmotic fragility test is thought to be the gold standard in the diagnosis of hereditary spherocytosis in a patient with direct antiglobulin test-negative spherocytic haemolytic anaemia, particularly one of northern European descent or someone with a positive family history of undiagnosed anaemia. When the spleen is present, a subpopulation of very fragile erythrocytes that have been conditioned by the spleen form the tail of the osmotic fragility curve that disappears after splenectomy (figures 2 and 5).

Osmotic fragility tests have a poor sensitivity because about 20% of mild cases of hereditary spherocytosis are missed.[110] It is unreliable in patients with small numbers of spherocytes, including those who have recently received a blood transfusion. The finding of increased osmotic fragility is not unique to hereditary spherocytosis and is also present in other disorders associated with spherocytosis—eg, autoimmune haemolytic anaemia. A normal osmotic fragility test result does not exclude the diagnosis of hereditary spherocytosis.[109] The test might be normal in the presence of iron deficiency, obstructive jaundice, and during the recovery phase of an aplastic crisis when the reticulocyte count is increased.[110]

A rapid flow cytometric analysis of eosin-5-maleimide bound to erythrocytes has been introduced as a screening test for the diagnosis of hereditary spherocytosis and for the study of membrane protein deficiency.[111,112] This test holds great clinical promise for standardised testing for hereditary spherocytosis.

The best method for showing reduced membrane surface area of spherocytes is osmotic gradient ektacytometry, available only in specialised laboratories.[104,113]

Quantification of the amount of spectrin or ankyrin-1, or both, band-3 protein, or protein 4.2 in the erythrocyte membrane can support the diagnosis of disease in atypical cases. However, these measurements are difficult to implement because small variations from normal should be accurately measured since membranes from some patients might have only a 10–15% reduction in the affected protein. The simplest method is SDS-PAGE of red blood cell membranes, but this technique reveals abnormalities in only 70–80% of patients.[37] Amounts of spectrin and ankyrin-1 are best assessed by RIA or EIA.[24,36,114] However, these methods are available only in a few specialised laboratories.

Identification of a membrane protein deficiency facilitates identification of the underlying genetic defect. PCR-based mutation screening techniques have been applied to the detection of hereditary spherocytosis-associated mutations.[38,40] Because most of the proteins that cause this disease are large and contain many exons, this approach is difficult. Identification of the molecular defect is, however, useful—eg, in undiagnosed, transfusion-dependent patients.[35,85] Alternatively, candidate genes can be identified by comparison of genomic DNA and reticulocyte mRNA (as cDNA) for reduced expression of one allele (figure 4).[31,32,38–40,42] From a clinical perspective, a biochemical and molecular analysis might be useful only in selected patients. Patients with an appropriate clinical history in the family usually do not request further investigations. However, those with dominantly inherited hereditary spherocytosis with relatives showing a wide clinical heterogeneity might be assessed for co-inheritance of another erythrocytic defect (thalassaemic trait, sickle haemoglobin, or pyruvate kinase or glucose-6-phosphate dehydrogenase deficiency) or for low-expression alleles occurring in trans to the hereditary spherocytosis allele. SDS-PAGE of membrane proteins should be done in these patients with low-expression alleles.

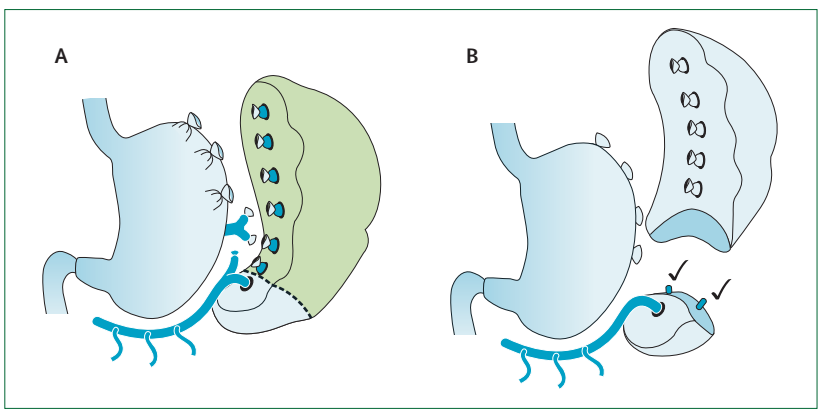

Figure 6: **Surgical technique used for partial (about 80%) splenectomy**
(A) All vascular pedicles supplying the spleen are divided except those arising from the left gastroepiploic vessels. (B) The upper pole of the spleen is removed at the boundary between the well perfused and poorly perfused tissue. Reproduced from Tchernia and colleagues[140] with permission from the American Society of Hematology.

Some characterisation of the underlying mutation might be useful in families with unclear heritage and severe or atypical disease. Candidate genes can be identified by SDS-PAGE. Alternatively, silent polymorphisms offer a rational approach to the study of disease. Frameshift or non-sense mutations are most common in hereditary spherocytosis, and no mutant RNA (or protein) is present in reticulocytes. A finding that one ankyrin-1, β-spectrin, α-spectrin, or band-3 gene is not expressed by comparison of the frequent polymorphisms in genomic DNA and RNA/cDNA (loss of heterozygosity) is proof that a null mutation exists. This method also has been used for identification of candidate genes for de-novo mutations in patients with this disease.[41] All the exons in the candidate genes can then be screened but it is time-consuming and very expensive.

Other laboratory manifestations in hereditary spherocytosis are markers of persisting haemolysis. Reticulocytosis and increased indirect bilirubin, lactate dehydrogenase, and urinary and fecal urobilinogen, and reduced haptoglobin indicate an increased erythrocyte production or destruction.

Treatment and outcome

Splenectomy

Splenic sequestration is the main determinant of erythrocyte survival in patients with hereditary spherocytosis. Thus, splenectomy cures almost all patients with this disorder, eliminating the anaemia and hyperbilirubinaemia and reducing the reticulocyte count to nearly normal.[3,23,75] After splenectomy, spherocytosis and altered osmotic fragility persist, but the tail of the osmotic fragility curve disappears. Although patients with very severe disease are not completely cured by splenectomy, their clinical improvement after splenectomy is striking. The rare splenectomy failure is usually associated with an accessory spleen.[115]

Early complications of splenectomy include local infection, bleeding, and pancreatitis. The serious long-term complication is overwhelming postsplenectomy infection with encapsulated bacteria, mostly *Streptococcus pneumoniae*, and, in some geographic regions (America and Africa), parasitic infection.[116] A postsplenectomy mortality rate of 0·05–0·30 per 100 person-years of follow-up has been reported.[117,118] Immunisation against pneumococcus, *Haemophilus influenzae* type b, and meningococcus, the use of prophylactic penicillin after splenectomy, or the promotion of early antibiotic treatment for febrile illnesses after splenectomy, or both, have reduced but not eliminated postsplenectomy infection.[119–125]

Because the risk of postsplenectomy infection is very high in infancy and early childhood, splenectomy should be delayed until 6–9 years of age if possible, but should not be done before 3 years of age, even if chronic transfusion is needed in the interim. Further delay might be harmful because the risk of cholelithiasis increases greatly in children after 10 years of age.

Indications for splenectomy and cholecystectomy

Risks and benefits should be assessed carefully before splenectomy is done. A multitude of factors affect the decision to do splenectomy, including the risk of postsplenectomy infection, the emergence of penicillin-resistant pneumococci, access to medical care, and the increased risk of ischaemic heart disease, cerebral stroke, pulmonary hypertension, and thrombosis later in life.[126–130] Hereditary spherocytosis management guidelines recognise these important considerations and recommend detailed discussion between health-care providers, the patient, and family when splenectomy is considered.[131]

After consideration of the risks and benefits, a reasonable approach would be to splenectomise all patients with moderately severe and severe hereditary spherocytosis and all those who have symptomatic haemolytic anaemia, growth retardation, skeletal changes, leg ulcers, or extramedullary haemopoietic tumours. Other candidates for splenectomy are older patients with hereditary spherocytosis who have vascular compromise of the vital organs or, for cosmetic reasons, adult patients with pronounced visible jaundice due to the combination of mild to moderate hereditary spherocytosis and Gilbert's syndrome (table 3).[2,89] Whether patients with moderate disease and compensated, asymptomatic anaemia should be splenectomised remains controversial. Patients with mild disease and compensated haemolysis can be followed up and referred for splenectomy if clinically suggested. However, the indication for splenectomy should be considered carefully because the mean haemoglobin values in splenectomised patients are higher than those in healthy individuals[104] and splenectomy could predispose to late adverse thrombotic events.[126]

Treatment of patients with mild to moderate hereditary spherocytosis and gallstones is also debatable, especially since new treatments for cholelithiasis, including laparoscopic cholecystectomy, endoscopic sphincterotomy, cholecystostomy (simple removal of stones without the gallbladder) and extracorporeal cholecystolithotripsy, reduce the risk of this complication.[132,133] If splenectomy is done in a patient with symptomatic gallstones, cholecystectomy or cholecystostomy should be done concomitantly.[134] Prophylactic cholecystectomy at the time of splenectomy is not suggested for patients without cholelithiasis.[135,136]

When splenectomy is warranted, laparoscopic splenectomy is the method of choice.[137–139] Although laparoscopic splenectomy needs a longer operative time, it results in less postoperative discomfort, a quicker recovery time, shorter hospital stay, decreased costs, and smaller scars than does open splenectomy. Even huge spleens (>500 g) can be removed laparoscopically. Near-total splenectomy has been advocated for infants and young children with substantial anaemia associated with hereditary spherocytosis (figure 6).[140–144] It is usually an open operation, but a laparoscopic procedure has been reported.[145,146] The goal is to allow for palliation of haemolysis and anaemia while maintaining residual splenic immune function. Splenic regrowth necessitating reoperation is a concern. The procedure seems to reduce the severity of the disease by about one grade and thus can be recommended for children with severe hereditary spherocytosis between 3 and 5 years of age. A second total splenectomy might be done after age 5–6 years if the results are not completely satisfactory. More experience and a long follow-up are needed to assess effectiveness of near-total splenectomy

Before splenectomy, patients should be immunised against *S pneumoniae*, *H influenzae* type b, and *Neisseria meningitidis*, preferably several weeks preoperatively. The use and duration of prophylactic antibiotics after splenectomy is controversial, especially since the worldwide emergence of penicillin-resistant pneumococci. Scientific justification for any specific regimen does not exist—eg, first 3–5 years after surgery versus lifelong, and expert opinions differ widely on the use of different regimens.[147]

Conclusion and future directions

Challenges for the future are: (1) development of accurate, sensitive, and specific diagnostic laboratory tests for hereditary spherocytosis; (2) identification of the molecular basis of disease, especially for the 10–15% of patients for whom the genetic defects have yet to be defined; and (3) establishment of criteria to assess appropriate indications for splenectomy, timing of splenectomy in children with severe disease, and the long-term outcome of near-total splenectomy.[147]

Conflict of interest statement

We declare that we have no conflict of interest.

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