Introduction

Antonie van Leeuwenhoek,1 a brilliant Dutch microscopist, who observed and described the red cell, was the first to publish, in Philosophical Transactions of the Royal Society in 1675, a remarkable description of the unique features of human red blood cells (RBC). The non-nucleated erythrocyte is unique among human cells in which the plasma membrane, it is only a structural component, and accounts for all its diverse antigenic, transport, and mechanical characteristics.

The erythrocyte membrane2 is the most thoroughly studied biologic membrane. Although it constitutes only about 1% of the total weight of the red cell, the membrane plays a pivotal role in the maintenance of erythrocyte integrity in different ways. It responds to erythropoietin during erythropoiesis and imports the iron required by the cell for hemoglobin (Hb) synthesis. It retains vital compounds as organic phosphates and removes metabolic waste. The erythrocyte membrane sequesters the reductants required to prevent corrosion by oxygen. It helps to regulate erythrocyte metabolism by selectively and reversibly binding and inactivating glycolytic enzymes. It exchanges chloride and bicarbonate ions and maintains an adequate pH. The membrane maintains a slippery exterior so that erythrocytes do not adhere to endothelial cells or aggregate and occlude the microcirculation, furthermore it provides strength and flexibility while enduring to circulatory stresses during its 120 days life span. The erythrocyte membrane is composed of mobile, asymmetrically distributed proteins and lipids that interact in a variety of ways. In this review are discussed the two of the most common abnormalities of red cell shape, hereditary spherocytosis, elliptocytosis, and miscellaneous disorders that affect the erythrocyte membrane.

Hereditary spherocytosis

Hereditary spherocytosis (HS) is the most common congenital hemolytic anemia in Caucasians, with an estimated prevalence ranging from 1:2000 to 1:5000. Approximately 75% of cases display an autosomal dominant pattern of inheritance, the remaining comprise recessive forms and de novo mutations. It was first recognized more than 100 years ago by two Belgian physicians, Vanlair and Masius who gave the description of a woman who suffered from anemia, jaundice, splenomegaly and recurrent abdominal pain. Later a case report of Minkowski in 1900 and the classic description of osmotic fragility and reticulocytosis by Chauffard stated the diagnosis.
Four abnormalities in red cell membrane proteins have been identified and include: i) spectrin deficiency alone; ii) combined spectrin and ankyrin deficiency; iii) band 3 deficiency; and iv) protein 4.2 defects. Spectrin deficiency is the most common defect.

**Spectrin deficiency**

Mutations of α-spectrin are associated with recessive forms of HS, whereas mutations of β-spectrin occur in families with autosomal dominant forms of HS. The synthesis of α-spectrin is 3-fold greater than that of β-spectrin. The excess α chains are normally degraded. Heterozygotes for α-spectrin defects produce sufficient normal α-spectrin to balance normal β-spectrin production. Defects of β-spectrin are more likely to be expressed in the heterozygous state because synthesis of β-spectrin is the rate-limiting factor. Red cell membranes isolated from individuals with autosomal recessive HS have only 40-50% of the normal amount of spectrin (relative to band protein 3), whereas red cell spectrin levels range from 60-80% of the normal amount in the autosomal dominant form of HS.

Identification of an α-spectrin mutation involves a point mutation at codon (969), resulting in an amino acid substitution (alanine/aspartic acid) at the corresponding site of α-spectrin in 50% of patients with severe recessive HS. Mutations involving the α-spectrin β-spectrin gene also occur, each resulting in spectrin deficiency. The first identified point mutation leads to a defective binding of spectrin to protein 4.1. Several other β-spectrin mutations have been identified. Some of these mutations result in impaired β-spectrin synthesis. Others produce unstable β-spectrins or abnormal β-spectrins that do not bind to ankyrin and undergo proteolytic degradation.

**Ankyrin defects**

HS is described in patients with translocation of chromosome 8 or deletion of the short arm of chromosome 8 where the ankyrin gene is located, and patients with HS and deletion of chromosome 8 are shown to have a decrease in red cell ankyrin content. Ankyrin is the principal binding site for spectrin on the red cell membrane. Studies of cytoskeletal protein assembly in reticulocytes indicate that ankyrin deficiency leads to decreased incorporation of spectrin. In HS caused by ankyrin deficiency, a proportional decrease in spectrin content occurs, although spectrin synthesis is normal. Of particular interest, 75-80% of patients with autosomal dominant HS have combined spectrin and ankyrin deficiency and the 2 proteins are diminished equally.

**Band 3 deficiency**

Band 3 deficiency has been recognized in 10-20% of patients with mild-to-moderate autosomal dominant HS. These patients also have a proportionate decrease in protein 4.2 content on the membrane. In some people with HS who are deficient in band 3, the deficiency is considerably greater in older RBCs. This suggests that band 3 protein is unstable.

**Protein 4.2 (pallidin) deficiency**

Hereditary hemolytic anemia has been described in patients with a complete deficiency of protein 4.2. Spherocytes, elliptocytes, or spherovolocytocytes have characterized RBC morphology. Deficiency of protein 4.2 in HS is relatively common in Japan. One that appears to be common in the Japanese population (protein 4.2 Nippon) is associated in the homozygous state with a red cell morphology described as spherocytic, ovalocytic, and elliptocytic. Another mutant protein 4.2 (protein 4.2 Lisboa) is caused by a deletion that results in a complete absence of protein 4.2. This is associated with a typical HS phenotype.

**Pathophysiology and mechanisms of membrane surface loss**

There are two hypotheses concerning the mechanism of membrane loss. In hypothesis 1 it is assumed that the membrane (the lipid bilayer and integral membrane proteins) is directly stabilized by interactions with spectrin or other elements of the membrane skeleton. Spectrin-deficient areas, lacking support, bud off, leading spherocytosis. In hypothesis 2 it is assumed that the membrane is stabilized by interactions of band 3 with neighboring lipids. The influence of band 3 extends into the lipid milieu because the first layer of immobilized lipids are missing. Spectrin/ankyrin deficiency allows band 3, owing to a targeted gene disruption, has marked membrane vesiculation and spherocytosis despite a nearly intact membrane skeleton.

**Clinical presentation**

The clinical presentation of hereditary spherocytosis is primarily dependent on the severity of hemolysis. Prolonged or excessive hyperbilirubinemia in the neonatal period may be an early manifestation of severe disease. In older children and young adults, symptoms of anemia, intermittent jaundice, or splenomegaly may lead to evaluation and diagnosis. Hereditary spherocytosis resulting from autosomal dominant HS is the most common form. The clinical presentation of HS caused by α-spectrin and β-spectrin mutations are quite similar to those of autosomal dominant HS. Patients with α-spectrin mutations typically have normal red cell mass, whereas patients with β-spectrin mutations may have a slightly decreased red blood cell mass. In both cases, the degree of anemia is variable, and the degree of spherocytosis is not a reliable indicator of the severity of the mutation. The clinical features of HS caused by ankyrin mutations are more variable than those of HS caused by spectrin mutations. The degree of anemia is variable, as are the degree of spherocytosis and the red cell mass. In some cases, the hemolytic anemia is mild, and in others it is severe. In general, patients with HS caused by ankyrin mutations have a more severe clinical course than those with HS caused by spectrin mutations. The clinical features of HS caused by protein 4.2 mutations are similar to those of HS caused by spectrin mutations. Patients with protein 4.2 mutations typically have normal red cell mass, and the degree of anemia is variable. The degree of spherocytosis is not a reliable indicator of the severity of the mutation. In general, patients with HS caused by protein 4.2 mutations have a more severe clinical course than those with HS caused by spectrin mutations.
mal dominant inheritance is commonly associated with a family history of anemia, jaundice, splenectomy, cholelithiasis, and cholecystectomy. A negative family history is to be expected in patients in whom HS results from spontaneous mutation or autosomal recessive inheritance. In those kindred with autosomal recessive inheritance, the presumed homozygotes have marked spherocytosis and severe hemolysis. Patients with interstitial gene deletions in chromosome 8p11.1-8p21.20 that include ankyrin and neighboring genes have psychomotor retardation and hypogonadism. The observation that erythrocyte ankyrin and erythrocyte β-spectrin are expressed in muscle, brain, and spinal cord, raises the hypothesis that patients with HS also suffer from defects of these proteins.

Laboratory features

Peripheral blood smear

Spherocytes are the hallmark of HS. They are dense, round, and hyperchromic, lack pallor and have a decreased mean diameter. In most HS patients spherocytes and microspherocytes are the only abnormal cells on the peripheral smear.22,23 Acanthocytes or hyperchromic echinocytes are present in patients with β-spectrin defects. Acanthopikilocytes and ovalostomatocytes have been observed in some patients with protein 4.1 deficiency.

Red cell indices

Most patients have mild to moderate anemia (Hb: 9-12 g/dL), or no anemia at all. In the most severe cases the hemoglobin may drop to levels as low as 4-5 g/dL. The mean corpuscular hematocrit concentration of HS red cells is increased owing to relative cellular dehydration.

Osmotic fragility test

Osmotic fragility testing24 is performing by suspending red cells in increasingly hypotonic buffered NaCl. In hypotonic solutions normal erythrocytes are able to increase their volume by swelling until they become spherical and burst, releasing hemoglobin into the supernatant. Cells that begin with a decreased surface/volume ratio, like spherocytes or stomatocytes, reach the spherical limit at a higher NaCl concentration than normal cells and termed osmotically fragile. After incubation at 37°C for 24 h, HS red cells lose the membrane area more rapidly than normal because their membranes are leaky and unstable. This incubation accentuates the defect in HS erythrocytes and brings out the defect on osmotic fragility, which is the test routinely available for diagnosing HS. When spleen is present, a subpopulation of very fragile erythrocytes that have been conditioned by the spleen, form the tail of the osmotic fragility curve. This tail disappears after splenectomy.

Acidified glycerol lysis test and Pink test

The glycerol lysis test, in which glycerol is employed to retard the osmotic swelling of red cells, was developed as an alternative to the osmotic fragility test. It is based on the rate rather than on the extend of hemolysis. An acidified version, the acidified glycerol lysis test (AGLT) is more accurate if the samples are preincubated at room temperature for 24 h. The half time for AGLT lysis is more than 30 min for normal samples, and less than 5 min for HS samples. Another adaptation for glycerol test called the Pink test is more reproducible but requires 1 day preincubation.24

Hypertonic cryohemolysis test

It is based on the fact that HS red cells are particularly sensitive to cooling at 0°C in hypertonic solutions. The test is independent of the surface/volume ratio which is an advantage compared to other tests.25

Autohemolysis test

The autohemolysis of erythrocytes after 48 h at 37°C is normal. In HS autohemolysis of spherocytes is increased by 15-45% in the absence of glucose, and reduces by the addition of glucose whereas in acquired disorders such as immune-mediated anemia, the degree of autohemolysis is not reduced.26

Membrane protein analysis

These useful tests include structural and functional studies of erythrocyte membrane protein such as protein quantitation, limited tryptic digestion of spectrin, membrane protein synthesis and assembly or ion transport studies.27,28

Determination of the molecular defect

Various mutation detection screening techniques have been employed, the most popular among them is the single stranded conformation polymorphism analysis. Mutation detection can be performed using direct nucleotide sequence analysis of polymerase chain reaction amplified genomic DNA or c DNA.

Ektacytometry

The deformability of erythrocytes (RBC) is measured in an ektacytometer,29 a laser-diffractive viscometer, as a continuous function of suspending medium osmolality. The ability of the RBC (diameter 6-8 µm) to pass through the microvasculature (open-
ings of 2-3 μm) and splenic sinuses (openings of 0.5-
1.0 μm) is referred to as deformability.

The resultant osmotic deformability profiles pro-
vide information on the viability of RBC, cellular
water content, surface area, deformability of the cell,
heterogeneity within these cellular properties, and sev-
eral pathological conditions, which are characterized
by typical shifts in this curve.

**Treatment**

**Splenectomy**

Splenectomy30-32 is very effective in reducing he-
mosis, leading to a significant prolongation of the red
cell life span. The clinical manifestations and compli-
cations (anemia and gallstones) are much reduced in se-
vere HS and abolished in milder cases but at the price
of an increased risk of life-threatening sepsis from en-
capsulated organisms, particularly *Streptococcus pneu-
monia*. Recent evidence demonstrates that splenectomy
for hereditary spherocytosis in children is very safe in
the short term with no deaths and infrequent complica-
tions (less than 1% in 1657 splenectomies).

**Folic acid supplementation**

Folic acid33 1 mg daily and consideration for blood
transfusions are recommended during periods of se-
vere anemia.

**Hereditary elliptocytosis**

Hereditary elliptocytosis (HE)34 was first reported
in 1904 by Dresbach, a physiologist at Ohio State Uni-
versity in Columbus who discovered the condition in
one of his students during examining his own blood.
Unfortunately the student died soon, and a number of
famous pathologist's supported the view that elliptocy-
tosis was a primary disorder and not a single morpho-
ic finding differentiating from sickle cell anemia.

Hereditary elliptocytosis is an autosomal dominant
disorder, characterized by the presence of elliptically
shaped red cells on peripheral blood smear, more com-
mon in malaria endemic regions in West Africa (prevalence 2%).35,36

Similar to HS, the study of erythrocyte membrane
proteins in these disorders has identified qualitative
and/or quantitative abnormalities of various erythro-
cyte membrane proteins. These include36,37 α and β
spectrin, protein 4.1 and glycophorin C. The majority
of defects occur in spectrin, the principal structural
protein of the erythrocyte membrane skeleton. Spec-
trin is composed of heterodimers of the related but non
identical proteins α and β spectrin that self-associate
into tetramers and higher-order oligomers. Spectrin in-
tegrity is critical for erythrocyte membrane stability
and erythrocyte shape and function.

Structural38 and functional defects of protein 4.1
appear to disrupt spectrin-actin contacts in the mem-
brane skeleton. Glycophorin variants are also deficient
in protein 4.1. The pathogenesis of the formation of
elliptocytes in these syndromes is unknown.

**Clinical classification and manifestations**

Most of the reported cases of HE39,40 are classified
into one of three categories: common, spherocytic el-
liptocytosis and Southeast Asian ovalocytosis (SAO).

The clinical features may appear with very severe
transfusion-dependent anemia (Hb: 2-6 g/dL), and se-
vere hemolysis accompanied by evidence of mem-
brane instability on the peripheral blood smear: budding red cells, fragments, and poikilocytes. Some-
times they develop uncompensated hemolysis in re-
ponse to stimuli that cause hyperplasia of the reticuloendothelial system especially if the spleen is
involved. In a small number of cases with typical par-
tial dysplastic and ineffective HE erythropoiesis has
been developed. The bone marrow is hyperplastic,
with decreased late erythroblasts and dysplastic fea-
tures such as asynchronous nuclear cytoplasmic mat-
uration, binuclearity, and internuclear bridges. Unfortunatley dysplasia persists after splenectomy.

Spherocytic elliptocytosis41 has been reported only
in White European families. Spherocytic elliptocytes
are osmotically fragile after incubation. Gallbladder
disease is common. Splenic sequestration is evident
and hemolysis abates after splenectomy.

Southern Asian ovalocytosis42 or Melanesian ellip-
tocytosis43 is inherited in an autosomal dominant pat-
tern. This results from the nine-amino-acid deletion
of residues 400-408 due to 27 bp deletion of the band 3
(*AE1* or *SLC4A1*, anion-exchanger 1 gene).

The abnormality is very common in Melanesia
where malaria is endemic. In vivo there is evidence
that SAO provides some protection against heavy in-
fecions of cerebral malaria. In vitro SAO red cells are
resistant to malarial parasites because the membrane
is 10 to 20 times more rigid than normal.

**Hereditary pyropoikilocytosis**

Hereditary pyropoikilocytosis (HPP)44 is a rare
case of severe hemolytic anemia characterized by
erythrocyte morphology reminiscent of that seen in
patients after a thermal burn. It appears in early child-
hood as a severe hemolytic anemia characterized by
extreme poikilocytosis. Patients typically present
splenomegaly, hyperbilirubinemia, growth retardation.

α-spectrin mutations associated with HE and HPP
are structural variants located in the heterodimer self-association region, the \( \alpha \)-domain. In some cases, HPP is associated with homozygosity or compound heterozygosity for structural defects of the \( \alpha \)-domain of spectrin. In other cases, there is compound heterozygosity for an \( \alpha \)-domain structural variant and a production-defective \( \alpha \)-spectrin allele in trans associated with markedly decreased or absent normal \( \alpha \) spectrin on the membrane. The parent who transmits the production-defective spectrin allele is clinically normal with unremarkable erythrocyte morphology because \( \alpha \) spectrin is normally synthesized in a 2- to 3-fold excess and output from a single normal \( \alpha \)-spectrin allele is sufficient to maintain membrane integrity.

Hereditary xerocytosis (high phosphatidylcholine hemolytic anemia)

Hereditary xerocytosis (HX) or high phosphatidylcholine hemolytic anemia\(^{45,46} \) is a rare disease among the types of hemolytic anemia. A dominantly transmitted chronic anemia characterized by an increase in erythrocyte membrane phosphatidylcholine and cholesterol with decrease in phosphatidylethanolamine. Stomatocytes and target cells are evident on peripheral blood smears.

Xerocytosis is characterized by a reduction in the cell potassium content, the main intracellular cation. This may be associated with a slight increase in cell sodium content, but the total cation content of the red cell is significantly lower than in normal controls.

The dehydrated cells are more resistant to lysis when incubated in hypotonic solutions. This decrease in osmotic fragility is due to the small baseline cell size. On the other hand, xerocytes exhibit a characteristic increase in cell rigidity and reduction in cell deformability. These changes are thought to play an important role in the hemolysis resulting from premature destruction of xerocytes. A genome-wide search for patients with hereditary xerocytosis has mapped this disorder to a locus on chromosome 16q24.2-16qter.\(^{47} \) Familial pseudohyperkalemia, a disorder in which red cells show a temperature-dependent leakage of potassium in collected blood, maps to the same locus. It has been suggested that about one-third of hereditary xerocytosis kindreds demonstrate pseudohyperkalemia.\(^{48} \)

Hereditary xerocytes appear to be macrocytic despite their dehydration. Splenectomy is probably not beneficial in HX.

Mediterranean stomatocytosis

Mediterranean stomatocytosis\(^{49,50} \) or Mediterranean macrothrombocytopenia is a poorly understood hematological condition that combines stomatocytic hemolysis with the presence of very large platelets. Gas chromatography mass spectrometry showed that all patients with this highly specific hematology had grossly elevated levels of phytosterols in the blood, diagnostic of phytosteroolemia. This syndrome association between stomatocytic hemolysis and macrothrombocytopenia and slight splenomegaly was first identified among Australian residents of Mediterranean extraction.

Acanthocytosis with neurologic disease (amyotrophic chorea-acanthocytosis)

Amyotrophic choreo-acanthocytosis\(^{51,52} \) is a rare disease of adult onset characterized by dyskinesias, neurogenic muscular atrophy, erythrocytary acanthocytosis with normal serum lipoproteins and elevated levels of serum creatine phosphokinase. This disease has been described among Japanese, English, Finnish and Puerto Rican people. No formal diagnostic criteria for chorea-acanthocytosis exist. The diagnosis is based on recognizing the characteristic clinical features with peripheral acanthocytosis and normal lipid studies. Particularly in male patients, testing for the McLeod blood phenotype should be considered because McLeod syndrome may present indistinguishably from chorea-acanthocytosis.

Because of the gene expression in RBCs, the western blot of a patient’s erythrocyte membranes with antichorea antisera may become a useful initial diagnostic test.\(^{53} \)

Follow-up mutational analysis of the \( VPS13A \) gene\(^{54} \) can be performed, however, this is challenging due to the large size of the gene and the heterogeneity of mutation sites. Molecular genetic testing for \( VPS13A \) mutations is not widely available.

Clinically, this disorder is characterized by the onset in young adulthood of chorea and/or Parkinsonism, oro-lingual-facial dystonias, tics, social disinhibition, seizures, areflexia, and distal muscle wasting. The mean age of onset is 35 years with a broad range from the first to the seventh decade. Initial clinical symptoms can vary. No curative or disease modifying treatment for chorea-acanthocytosis currently exists. Treatment is aimed at symptom management. A multidisciplinary approach to disease management is recommended.

References


