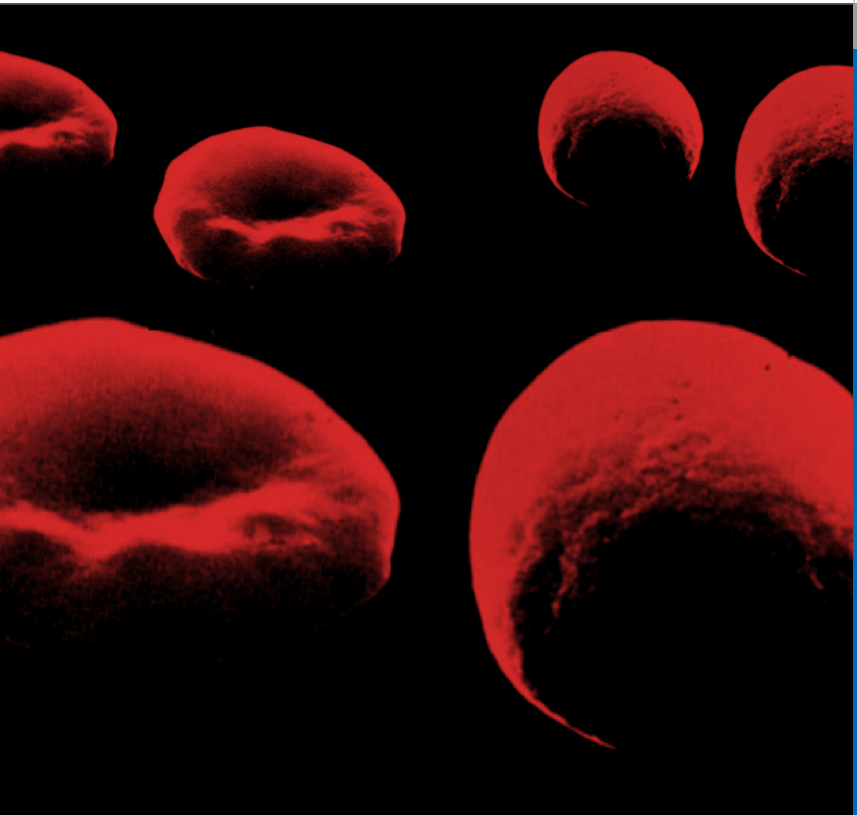
SWISS SOCIETY OF NEONATOLOGY

Icterus praecox due to hereditary spherocytosis



March 2016

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Title figure:
adapted from www.vetbook.org

Three forms of pathological hyperbilirubinemia can be distinguished in the neonatal period: 1) icterus praecox (any clinically visible jaundice within the first 24 hours of life), 2) icterus gravis (severe jaundice, cut-off values depend on gestational age and additional risk factors), and 3) icterus prolongatus (jaundice persisting after 10 – 14 days of life). Each of these manifestations of neonatal jaundice must be investigated and treated promptly to prevent potentially serious complications. The first two manifestations of problematic neonatal jaundice are caused by increased hemolysis and result in indirect hyperbilirubinemia; hemolysis is most commonly immune-mediated but can also result from erythrocyte enzyme deficiencies or membrane defects. Icterus prolongatus can be associated with indirect (e.g., in breast milk jaundice) or direct hyperbilirubinemia (e.g., in biliary atresia or neonatal hepatitis).

This male infant was born to a 26-year-old G2/P2 at 41 1/7 weeks of gestation after an uncomplicated pregnancy. The mother was GBS positive and received a total of four doses of amoxicillin prior to delivery. Delivery was by secondary Caesarean section because of failure to progress due to fetal macrosomia. Arterial and venous umbilical cord pH values were 7.30 and 7.42, respectively. The infant adapted well with Apgar scores of 8, 9 and 9 at 1, 5 and 10 minutes. The birth weight was 5140 g (> P97), birth length was 54 cm (P50-75) and head circumference was 39 cm (> P97). Routine blood glucose monitoring was within normal range.

At 12 hours of age, the infant was noted to be tachypneic without other signs of respiratory distress. Except for mild jaundice, the physical examination was also normal. Laboratory investigations were remarkable for an elevated leukocyte count (46.9 G/l) with a significant left shift, a C-reactive protein concentration of 18 mg/l and a total bilirubin concentration of 269 µmol/l. The hemoglobin concentration was 137 g/l and the platelet count was 262 G/l. The infant's blood type was B negative and the direct Coombs' test was negative. Antibiotics were administered for a total five days and then discontinued; the blood cultures remained negative.

Because of icterus praecox the infant was treated with phototherapy (maximum bilirubin concentration 354 µmol/l). There was no evidence of immune hemolysis, however, a more thorough family history revealed the presence of hereditary spherocytosis (HS) in several paternal family members (father, grandfather, aunt); therefore, HS was felt to be the likely cause of the patient's hyperbilirubinemia. Findings on peripheral blood smears were consistent with HS (Fig. 1, 2). In addition, red blood cell indices were also highly suggestive of the disorder (MCHC/MCV ratio of 0.43). The diagnosis was later confirmed when an (incubated) osmotic fragility test at the age of ten months was abnormal. In the neonatal period, the infant received two transfusions with packed red cells when hemoglobin concentrations fell to 90 g/l and 83 g/l on the 7th and 12th day of life, respectively.

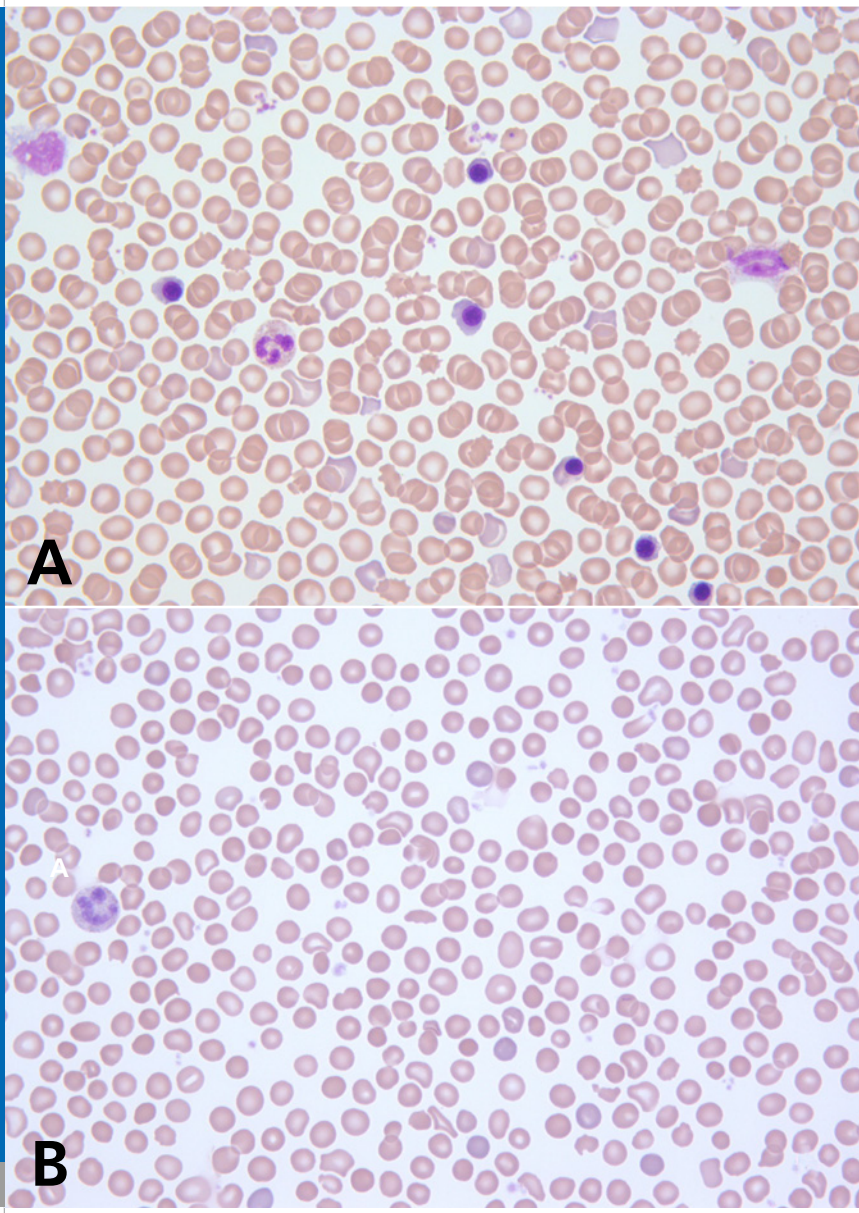

Fig. 1

Blood smears (HE stain), overview: A) control, B) index patient: note the characteristic erythrocytes lacking central pallor.

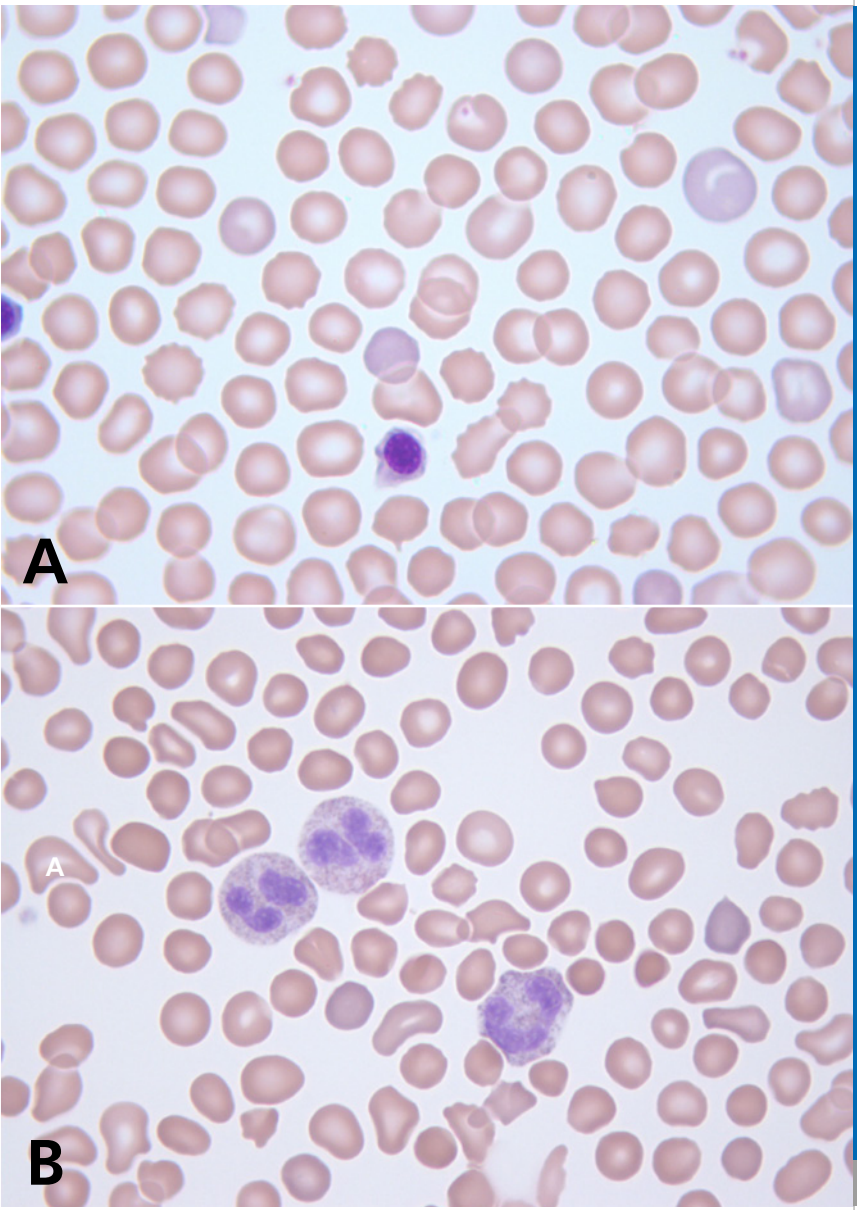

Fig. 2

Blood smears (HE stain), high power view: A) control, B) index patient: note the characteristic erythrocytes lacking central pallor.

DISCUSSION

Hereditary spherocytosis (HS) is a heterogeneous disorder with abnormalities of a variety of red blood cell structural proteins (ankyrin-1, band 3, β-spectrin, α-spectrin, and protein 4.2) (Fig. 3 and Table 1). These defects lead to a loss of erythrocyte membrane surface area and result in spherical-shaped, hyperdense, poorly deformable red blood cells with a shortened life span. Destruction in the spleen is the primary cause of hemolysis in patients with HS (1, 2). HS is most commonly inherited in an autosomal dominant pattern, but may also be inherited in an autosomal recessive pattern or occur as *de novo* mutations (Table 1). The disorder occurs worldwide and affects individuals from all ethnic and racial groups. Among white neonates from northern European ancestry, it can be as frequent as 1 in 1000 to 2000 births (2).

HS typically presents in childhood. Anemia is the most frequent finding at presentation (50%), followed by splenomegaly, jaundice, or a positive family history (3). The majority of HS patients have incompletely compensated hemolysis with mild to moderate anemia that is asymptomatic except for fatigue and pallor. Jaundice is detectable at some time in over half of HS patients, usually associated with viral infection or other stress. The most severely affected patients are transfusion-dependent and almost always have recessive HS (2).

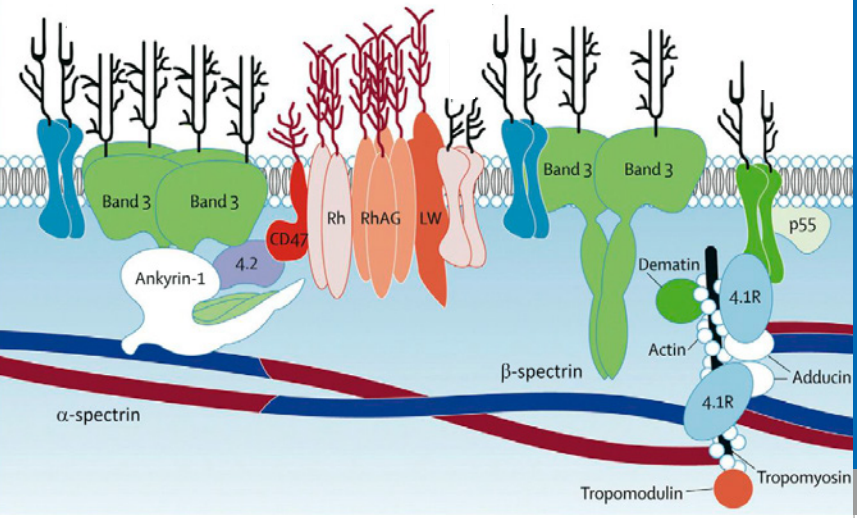

Fig. 3

Erythrocyte structural proteins; in HS, defects in ankyrin-1, band 3, β-spectrin, α-spectrin, and protein 4.2 have been described (from Ref. 1).

Table 1

Protein affected	Gene affected	Frequency of mutation among HS patients	Typical disease severity	Incidence
Ankyrin-1	ANK1	40-50	mild to moderate	autosomal dominant
Band 3	SLC4A1	20-35	mild to moderate	autosomal dominant
β-spectrin	SPTB	15-30	mild to moderate	autosomal dominant
α-spectrin	SPTA1	< 5	severe	autosomal recessive
Protein 4.2	EPB42	< 5	mild to moderate	autosomal recessive

HS is a heterogeneous disorder due to defects in various erythrocyte membrane proteins which are associated with differences in disease severity (from Ref. 1).

During the perinatal period, the clinical spectrum of HS ranges from severe fetal anemia with hydrops to the asymptomatic neonate (1). In part, this wide range of clinical severity is thought to be related to the specific mutations involved and/or co-inherited conditions (mutations or polymorphisms of genes involved in hepatic bilirubin uptake or intrahepatic bilirubin conjugation) (4 – 6). Some patients present with significant neonatal jaundice requiring phototherapy or even exchange transfusion. Of interest, among neonates listed in the USA Kernicterus Registry, HS was the third most common underlying hemolytic condition after

glucose-6-phosphate dehydrogenase deficiency and AB0 hemolytic disease (2).

The triad of anemia, splenomegaly, and jaundice, which is found in older children and adults with HS, is rare in neonates; in addition, spherocytes are less often observed on the blood smears of affected neonates. Therefore, the family history can reveal important information in neonates with non-physiological direct Coombs' test negative hyperbilirubinemia, since 65% of neonates with HS have a parent with HS (7). One should specifically ask if there are any family members with anemia, jaundice, splenectomy, early gallstones or cholecystectomy.

Spherocytes may or may not be recognizable on blood smears of neonates with HS. Typically, neonates with HS will have an elevated mean corpuscular hemoglobin concentration (MCHC) of more than 36.5-37.0 g/dl. In addition, in most neonates with HS, the mean corpuscular volume (MCV) is low. Yaish and colleagues (8) have described that if the MCHC/MCV ratio (also termed HS ratio) is > 0.36, the presence of HS is highly likely (0.43 in our patient). Based on this observation, Christensen et al. (1) have developed two algorithms that can be used in neonates with Coombs' test negative pathological hyperbilirubinemia with or without positive family history for HS (Fig. 4, 5).

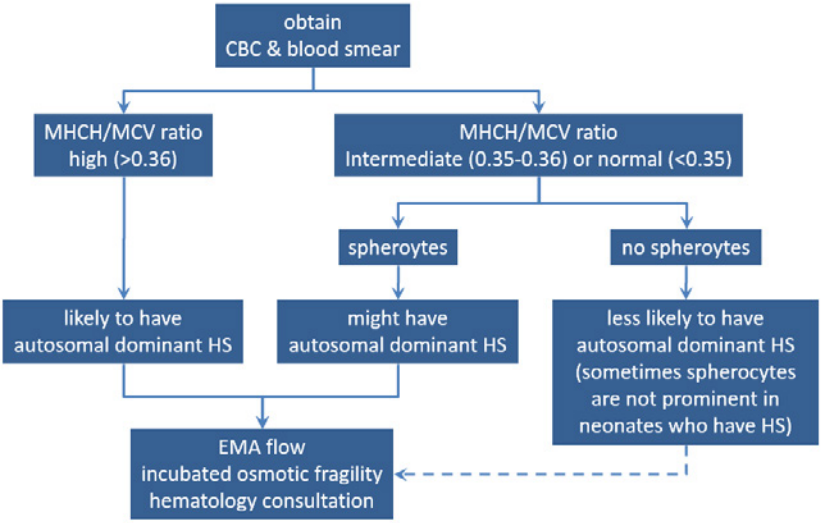

Fig. 4

Evaluation of a neonate with direct Coombs' test negative pathologic hyperbilirubinemia and a positive family history of HS (redrawn from Ref. 1).

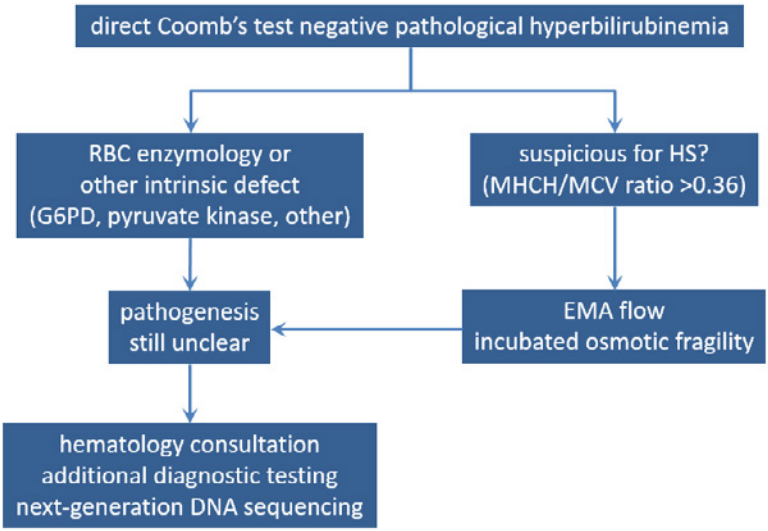

Fig. 5

Evaluation of a neonate with direct Coombs' test negative pathologic hyperbilirubinemia and a negative family history of HS (redrawn from Ref. 1).

Eosin-5-maleimide (EMA) binding or (incubated) osmotic fragility testing can be helpful in such situations (1, 2). EMA binding is a flow cytometry-based test that measures the relative amount of fluorescently labeled EMA dye bound to band 3 and Rh-related proteins in the erythrocyte membrane. In HS, reduction in band 3 and other proteins leads to decreased fluorescence intensity. Even though defects in band 3 protein are only found in about 25% of typical HS patients, decreased fluorescence is also observed in HS erythrocytes with defects in ankyrin and spectrin; apparently, such defects indirectly influence EMA binding to band 3.

Due to the loss of membrane surface area relative to intracellular volume, HS erythrocytes are unable to withstand the introduction of small amounts of free water that occurs when the are placed in increasingly hypotonic solutions. As a consequence, HS erythrocytes hemolyze more readily than normal erythrocytes at any saline concentration. Despite the fact that neonatal erythrocytes exhibit an altered response to osmotic stress compared to adult erythrocytes, osmotic fragility after incubation (rendering membranes more unstable) has been successfully used to diagnose HS in neonates.

In the neonatal period, treatment of HS should focus on phototherapy and/or exchange transfusion to avoid CNS complications and on correction of symptomatic anemia. Later in life, treatment of HS encompasses

supportive care and, when appropriate, splenectomy (9). Even though splenectomy cures anemia in most HS patients and decreases the incidence of cholelithiasis, the risk of overwhelming postsplenectomy infection with encapsulated organisms and other long-term complications (e.g., thrombosis, pulmonary hypertension) have led to re-evaluation of the role of splenectomy in the treatment of HS. It is still considered reasonable to splenectomize all patients with severe HS and all patients with severe anemia suffering from growth failure, skeletal changes, extramedullary hematopoietic tumors, and leg ulcers (2).

CONCLUSIONS

In a newborn infant with significant direct Coombs' test negative hyperbilirubinemia, careful review of the family history, evaluation of red blood cell indices (MCHC/MCV ratio) and interpretation of the peripheral blood smear may allow to make the diagnosis of HS. EMA binding and (incubated) osmotic fragility may be helpful. In problematic cases, DNA sequencing can provide the diagnosis. HS can lead to severe neonatal hyperbilirubinemia and, if not treated vigorously, CNS complications including kernicterus.

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