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Erythrocyte Disorders in the Perinatal Period in Adverse Pregnancy Outcome and the Fetus/Neonate

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Introduction

Anemia is a common problem in the fetal and neonatal period. The differential diagnosis of fetal and neonatal anemia is broad, and includes immune-mediated hemolysis, fetal hemorrhage, intrinsic erythrocyte disorders, and erythrocyte underproduction. This review will focus on genetic conditions leading to intrinsic disorders of the erythrocyte and erythrocyte underproduction. Intrinsic disorders of the erythrocyte, such as the hemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency, are important causes of perinatal anemia and represent some of the most common inherited genetic diseases. Inherited abnormalities that lead to decreased erythrocyte production are much less common, but are important to recognize as they can cause severe anemia that is often associated with other congenital anomalies. Presentation of these disorders in the fetus and newborn is varied. Anemia may range from asymptomatic to life threatening and can be associated with other complications such as nonimmune hydrops fetalis (Table 1) and severe hyperbilirubinemia.

The Neonatal Erythrocyte

Understanding the differences between neonatal and adult erythrocytes is critical in the evaluation of perinatal erythrocyte disorders. These differences include variation in erythrocyte size, shape, globin composition, and cellular metabolism.

Size and Shape

Fetal erythrocytes are larger and have more variation in shape than those of adults. They are largest early in gestation, with an MCV of approximately 150-180 fL.[1] They slowly decrease in size reaching approximately 114 fL at term,[2] and are similar to adult cells by 1 year of age. Peripheral blood smears of neonates show greater numbers of acanthocytes, target cells, stomatocytes, and immature erythrocytes than peripheral blood smears of adults.[3] Neonatal erythrocytes also show decreased deformability and increased osmotic resistance during the first 4 to 6 weeks of life.[4] When viewed under interference microscopy, one-half of erythrocytes in preterm infants and one-quarter of erythrocytes in term infants will have surface pits, compared to only 2.6% of erythrocytes in adults. These surface pits are thought to be a consequence of the poor splenic function of the neonate.[5]

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Fetal hemoglobin

Fetal hemoglobin (Hb F) comprises 70-90% of the hemoglobin in the neonatal erythrocyte. In contrast to adult hemoglobin, fetal hemoglobin does not interact with 2,3-diphosphoglycerate (DPG), causing a shift of the hemoglobin dissociation curve to the left. This is crucial for the fetus, as the higher oxygen affinity of Hb F allows fetal erythrocytes to extract oxygen from maternal RBC. **Error! Bookmark not defined.**

In contrast to the adult RBC lifespan of 120 days, the lifespan of the neonatal erythrocyte is only 60 to 90 days with preterm infants having an even shorter life span of 35 to 50 days.[6] Hb F has a tendency to denature and damage the membrane from within, contributing to the shortened the red cell lifespan. Fetal hemoglobin is also more soluble in strong phosphate buffers and is more resistant to acid denaturation than adult hemoglobin. Its resistance to acid denaturation is the basis of the kliehauerbetke test.[7]

Metabolism of the Neonatal Erythrocyte

Neonatal erythrocytes contain higher levels of ATP and consume more glucose than adult cells. They also have lower levels of glutathione peroxidase, methemoglobin reductase, and carbonic anydrase.[3] The lower enzyme levels make neonatal erythrocytes more susceptible to oxidative damage, leading to the formation of methemoglobin and Heinz bodies,[3,8] and likely contributing to the decreased life span of the neonatal erythorcyte.[3]

Hemoglobin Disorders

Developmental differences in globin chain synthesis are responsible for the different clinical manifestations of α-chain and β-chain defects in the perinatal period. The first α-like chain, ζ, is produced in significant amounts only in the first few months of gestation. By 9 weeks, α-globin is the major α-like globin in the human fetus, and embryonic hemoglobin, hemoglobin Portland (ζ2γ2) is found only in small amounts. In contrast, β-globin production begins late in gestation, and the switch from fetal hemoglobin (α2γ2) to adult hemoglobin (α2β2) is not complete until the end of the first year of life (Figure 1). Defects in α-globin synthesis therefore manifest *in utero*, while defects in β-chain synthesis may not become apparent until late infancy.[1]

α-Thalassemia Syndromes

The α-thalassemias predominately affect patients of southeast asian, middle eastern, and mediterranean descent and carrier states are thought to provide protection from malaria.[9] α-Thalassemia results from the deletion of one or more of the four α-globin genes which reside on the short arm of chromosome 16, and the clinical manifestations of α-thalassemia are directly related to the number of functional α-globin genes present.[10] A single α-globin gene deletion results in an asymptomatic carrier state. Deletion of two genes results in α-thalassemia trait, which is characterized by microcytosis and mild anemia.

Hemoglobin H disease results from deletion of 3 α-globin genes. This leads to a significant imbalance in α– and β– like chain production and the formation of hemoglobin H (β4) and hemoglobin Barts (γ4). Patients with hemoglobin H disease are often born with a hypochromic, hemolytic anemia, and are at risk for significant neonatal hyperbilirubinemia. Hemoglobin H has been associated with hydrops fetalis.[11-13] Children and adults with hemoglobin H disease may be transfusion dependent.[14]

Patients with deletion of all 4 α-globin genes, homozygous α-thalassemia, are severely affected *in utero*. The major hemoglobin is hemoglobin Barts (γ4), and survival of the fetus depends on the presence of embryonic hemoglobin Portland (ζ2γ2). Affected infants suffer from severe

hemolytic anemia, high output cardiac failure, and hydrops fetalis.[15] Maternal complications, such as preeclampia, preterm labor, and retained placenta are common in pregnancies affected by homozygous α-thalassemia.[16-18] Without intervention, the majority of infants will die *in utero* or shortly after birth. The advent of intrauterine transfusions, long term transfusion and chelation therapy, and bone marrow transplantation has made it possible for some of these infants to survive into childhood.[19]

β-globin defects

Most β-globin chain defects, including sickle cell disease and β-thalassemia, do not present with anemia or hemolysis in the neonatal period, due to the presence of large amounts of fetal hemoglobin (α2γ2). One β-globin defect that does manifest in the perinatal period is γδβ-thalassemia. γδβ-Thalassemia is caused either by large deletions in the coding region of the β-globin cluster[20-22] or in the β-globin promoter.[23-24] Neonates with this disorder typically present with hypochromic, hemolytic anemia, and a prominent normoblastosis.[25] Older children and adults with γδβ thalassemia have variable clinical manifestations similar to patients with β-thalassemia minor.[26]

Unstable Hemoglobins

Unstable hemoglobins typically exhibit lower solubility than normal hemoglobin leading to chronic nonspherocytic hemolytic anemia. They can affect both α- and β- chains and can cause severe hemolysis and hyperbilirubinemia in the newborn. The unstable hemoglobinopathies are sometimes called congenital Heinz bony anemias, because precipitation of the insoluble globin leads to the formation of Heinz bodies, which can be viewed on peripheral smear. The absence of Heinz bodies, particularly in the perinatal period, does not rule out the presence of unstable hemoglobinopathies, as they can be difficult to detect in young patients and in patients who have a functioning spleen. The best method for diagnosing unstable hemoglobin variants is demonstration of decreased solubility using the heat stability test and/or the isopropanol precipitation test. Diagnosis of unstable hemoglobinopathy can be made with hemoglobin electrophoresis, but roughly 30% of unstable hemoglobins are not detectable using this method.[27]

Two unstable hemoglobin variants are particularly interesting in the neonatal period. In hemoglobin F Poole, a glycine is substituted for a tryptophan at position 130 of the γ-globin chain, leading to fetal hemoglobin instability and hemolysis.[27-28] Hb Hasharon results from a substitution of hisitidine for aspartate at position 47 of the α-globin gene.[29] When paired with γ-globin, it has decreased stability, leading to neonatal hemolysis. When paired with the β-globin gene, Hb Hasharon is stable and the hemolysis subsides. In both Hb F Poole and Hb Hasharon, hemolysis subsides after the first few months of life as fetal hemoglobin (α2γ2) is replaced by adult hemoglobin (α2β2).[27]

Disorders of Erythrocyte Metabolism

Several enzymatic pathways are vital for the erythrocyte to function properly. The Embden-Meyerhof pathway is the major source of ATP in the erythrocyte and metabolizes approximately 90% of ATP in the erythrocyte. The energy generated by this process is stored as glutathione, pyridine nucleotides (NADH and NADPH), and ATP. This pathway is the major source of NADH in the erythrocyte, an essential co-factor for the enzyme NADH methemoglobin reducatase, which maintains hemoglobin in the reduced state, preventing methemoglobinemia. The hexose monophosphate shunt, also called the pentose phosphate pathway, generates the majority of NADPH in the erythrocyte through the metabolism of glucose-6-phosphate. NADPH is necessary to maintain adequate amounts of reduced glutathione, critical for protecting the erythrocyte from oxidative damage.

Pyruvate Kinase Deficiency

Pyruvate kinase (PK) deficiency is the most common inherited deficiency in the Embden-Meyerhof pathway. It is inherited in an autosomal recessive manner and is seen most often in patients of Northern European descent. Heterozygotes are generally normal or only mildly affected. Homozygotes or compound heterozygotes are affected with variable degrees of clinical severity.[30] PK deficiency often presents in the neonatal period with anemia and jaundice, and patients can become transfusion dependent. More severe cases have been reported, including severe *in utero* anemia, hyperbilirubinemia requiring exchange transfusion, and hydrops.[31-36]

Diagnosis of PK deficiency may made by enzyme assay with erythrocytes exhibiting PK activity that is 5-40% of normal. Genetic studies can also reveal the diagnosis, and over 158 mutations have been described in the coding region of the PK gene.[37] The majority of these mutations are private (i.e. not shared between families or ethnic groups). Missense mutations are most common, but point mutations, deletions, or insertions leading to alterations of the spicing site, frameshift, and early termination mutations have been reported.[37-39] In addition to coding sequence mutations, disruption of the PK promoter can also lead to severe clinical manifestations.[40] Treatment for PK deficiency is generally supportive, although some transfusion dependent patients have benefited from splenectomy and successful bone marrow transplantation has been reported.[41] In the neonatal period, therapy is focused on the treatment of hyperbilirubinemia and anemia.

Glucose-6-Phosphate Dehydrogenase Deficiency

Glucose-6-phosphatase dehydrogenase (G6PD) deficiency affects nearly 400 million people.[42] It is one of the most common genetic disorders, likely due to protection of carriers from *Plasmodium falciparum* infection.[9,43] G6PD deficiency is the most common disorder of the hexose monophosphate shunt, the pathway responsible for generating NADPH, necessary for maintaining glutathione in its reduced state. Normal erythrocytes are able to significantly increase their production of NADPH in response to oxidative stress.[44-45] Although generally asymptomatic in the absence of oxidative stress, affected patients are unable to increase production of NADPH when necessary,[46] making their red cells vulnerable to oxidative damage and hemolysis. Chronic non-spherocytic hemolytic anemia occurs in a small subset of patients with such severe G6PD deficiency that they experience severe hemolysis even in the absence of oxidative stress.[42]

In the neonatal period, G6PD deficiency most commonly presents as jaundice, which can be severe enough to necessitate exchange transfusion. Rarely, severe intrauterine hemolysis and hydrops fetalis have been reported following maternal ingestion of oxidatitive agents[47-49] and severe neonatal hemolysis has been reported in breast feeding infants following maternal ingestion of fava beans.[50] There are several screening tests available for G6PD deficiency, including the dye discoloration test, the methemoglobin reduction test, and the fluorescence spot test.[51] The reticulocytosis seen during hemolysis can make these screening tests inaccurate because reticulocytes have higher G-6-PD levels than mature cells, and can falsely elevate the amount of G6PD in a sample. Screening tests are also generally unable to detect female carriers.

More definitive diagnosis can be made through direct assay of enzyme activity in erythrocytes. Molecular diagnosis is also possible and over 100 mutations have been associated with G6PD deficiency. The majority of these mutations are missense mutations in the coding region of the gene. There have been no reports of large frameshift mutations or deletions, suggesting that total deficiency of G6PD is incompatible with life.[42] Treatment for G6PD deficiency includes avoidance of oxiadative agents and supportive care during episodes of hemolysis.[4] In the

neonatal period, appropriate treatment of hyperbilirubinemia is important for the prevention of kernicterus and neonates with active hemolysis and hyperbilirubinemia have lower threshold levels for phototherapy and exchange transfusion than infants with non-hemolytic jaundice.[52]

Disorders of the Erythrocyte Membrane

The erythrocyte membrane is composed of lipids and proteins that interact to give the erythrocyte the deformability and flexibility required to endure circulatory stress. Quantitative or qualitative defects in membrane proteins lead to decreased membrane deformability, membrane instability, and subsequent hemolysis.

Hereditary spherocytosis

Hereditary spherocytosis (HS) is characterized by the presence of spherocytes on peripheral blood smear (Figure 2B). HS is the most common inherited anemia in people of northern European descent, occurring in 1/2500-5000 individuals.[53-54] Deficiencies of the membrane proteins ankyrin, band 3, α-spectrin, β-spectrin and protein 4.2 have been described. The most common cause of typical dominant HS is ankyrin deficiency,[55] followed by β-spectrin or band 3 deficiency, with nondominant cases caused by defects in protein 4.2 or α-spectrin.[56-57] The membrane instability caused by these protein defects leads to a loss of membrane surface area, decreased deformability of cells, splenic entrapment, and hemolysis. Anemia is the most common manifestation of HS in neonates. Hyperbilirubinemia is also common and nearly half of neonates with HS become jaundiced. Cases of hydrops fetalis linked to membrane defects have been described.[58]

The presence of spherocytes on peripheral smear suggests the diagnosis of hereditary spherocytosis and diagnosis can be made by osmotic fragility testing. Standard osmotic fragility testing is normal in one-fourth of patients, making incubated osmotic fragility the test of choice. Osmotic fragility testing can also be used in neonates, but it is important to use neonatal osmotic fragility curves, as neonatal RBC have greater osmotic resistance than adult cells.[4] Treatment of HS depends on severity, and splenectomy can reduce or eliminate hemolysis in severely affected patients.[27]

Hereditary Elliptocytosis

Hereditary Elliptocytosis is characterized by the presence of elliptical or oval cigar-shaped erythrocytes on peripheral blood smears of affected individuals (figure 2C). The erythrocyte life span is normal in the majority of patients with HE. Only 12% of patients with HE become symptomatic, and patients who have a shortened erythrocyte life span are the patients who tend to develop symptomatic anemia. HE is generally not symptomatic until 4-6 months of life, but neonatal jaundice, hemolysis, anemia, and hydrops fetalis have been reported.[1, 59-60] Even in symptomatic infants, hemolysis usually abates by 1-2 years of age and the infant goes on to have mild typical HE.[4]

Hereditary Pyropoikilocytosis

Hereditary Pyropoikilocytosis (HPP) (Figure 2D) is characterized by the presence of pyknocytes on peripheral blood smear and increased erythrocyte thermal sensitivity.[61] Erythrocyte morphology resembles that seen in patients with severe burns. It is most common in patients of African descent, and causes severe anemia and hemolysis in the neonatal period.[1] The hemolysis tends to gradually improve through infancy and many older patients have clinical symptoms similar to those seen in HE.[62] There is a strong relationship between HE and HPP, as many patients with HPP have a family history of typical HE.[4]

Diagnosis of HPP is generally made by reviewing the peripheral blood smear and family history. Increased incubated osmotic fragility and decreased MCV support the diagnosis. Mutations in α- and β- spectrin underlie HPP[63] and more specialized tests, such as ektacytometry, spectrin dimmer self-association studies, and genomic DNA analysis are available, but generally not necessary for diagnosis. Treatment of HPP is generally supportive, but splenectomy has been used in severe cases. In the neonatal period, phototherapy or exchange transfusion may be needed to treat hyperbilirubinemia, and transfusion may be required for anemia.

Hereditary Stomatocytosis Syndromes

The hereditary stomatocytosis syndromes are disorders of erythrocyte hydration that are generally inherited in an autosomal dominant manner.[64] Erythrocyte hydration is determined primarily by the intracellular concentration of sodium and potassium. Increased levels of intracellular sodium and potassium cause water to enter the cell, forming stomatocytes and resulting in overhydrated hereditary stomatocytosis (OHST). Decreased intracellular concentration of sodium and potassium results in cellular dehydration and the formation of xerocytes, leading to dehydrated hereditary stomatocytosis (DHST). Intermediate syndromes also exist.

OHST is characterized by stomatoctes on peripheral blood smear. The clinical presentation is variable, with some patients having pseudohyperkalemia, severe hemolysis, hyperbilirubinemia, and anemia, and other patients being asymptomatic. Hydrops fetalis has been reported.[65] Diagnosis is based on peripheral blood smear, increased MCV, increased osmotic fragility, and increased concentrations of intracellular sodium.

DHST, also known as xerocytosis, is characterized by increased MCHC and decreased osmotic fragility. The MCV is spuriously elevated when measured by flow cytometry. Erythrocyte morphology is generally normal, although stomatocytes and target cells can be seen. Diagnosis is based on decreased intracellular concentration of sodium and a characteristic osmotic gradient ektacytometric curve. The neonatal manifestations of DHST are variable, and include hemolysis and pseudohyperkalemia. Prenatally, hydrops fetalis[66]and transient perinatal ascites have been reported.[67-69] DHST has been mapped to 16q23-q24, but the precise molecular basis is not known.[69] Treatment is supportive, although unlike the other disorders of the erythrocyte membrane, splenectomy is contraindicated, as splenectomized patients have a high risk of thrombosis.[70]

Decreased Erythrocyte Production

Decreased erythrocyte production is an uncommon, but important cause of neonatal anemia, and is often associated with other congenital anomalies. The differential diagnosis for decreased erythrocyte production includes genetic causes, infectious suppression, and bone marrow replacement syndromes. This review will focus genetic causes of erythrocyte underproduction.

Diamond-Blackfan Anemia

Diamond-Blackfan anemia (DBA) is a congenital pure red cell aplasia causing a moderate to severe anemia which often presents early in infancy.[71] Hydrops fetalis is rare, but has been reported.[72-73] The most common associated anomalies are thumb and radial abnormalities with others including microcephaly, high arched palate, hypertelorism, retrognathia, webbed neck, and low set ears. Diagnosis of DBA is made on the basis of bone marrow examination, which demonstrates an absence of erythrocyte precursors in an otherwise normocellular

marrow. Elevated erythropoietin levels support the diagnosis of DBA.[1] Treatment includes chronic transfusion therapy, steroids, and bone marrow transplantation.[74]

Schwachman-Diamond Syndrome

Schwachman-Diamond Syndrome (SDS) is a rare autosomal recessive disorder characterized by bone marrow failure, exocrine pancreatic failure, and skeletal abnormalities.[75-77] Neonates with SDS can present with severe anemia, as well as overwhelming infection due to impaired immune function. Myelodysplastic syndrome develops in 10-44% of patients, and 5-24% progress to leukemia.[77] Diagnosis of SDS is generally made clinically. SDS has been localized to the Schwachman-Bodian-Diamond Syndrome Gene on chromosome 7 and confirmatory genetic testing is now available.[77] Therapy may include pancreatic enzyme replacement, G-CSF for febrile neutropenia,and monitoring for development of myelodysplastic syndrome.

Congenital Dyserythropoietic Anemia

Congenital Dyserythropoietic Anemia (CDA) is a rare group of disorders characterized by ineffective erythropoeisis, megaloblastic anemia, and secondary hemosiderosis. Three types of CDA have been described. Type I is characterized by autosomal recessive inheritance, megaloblastiod erythroid hyperplasia, and distinct nuclear chromatin bridges between cells. Type II, the most common variant, is characterized by multinuclear erythrocytes and a positive acidified serum test. Type III is characterized by multinuclear erythroblasts and macrocytosis. CDA frequently presents in the neonatal period and severe anemia,[78] hyperbilirubinemia, and pulmonary hypertension have been reported.[79] CDA can also present prenatally as hydrops fetalis.[80] Therapy for CDA has included intrauterine transfusions and transplantation.

Pearson Syndrome

Pearson Syndrome is a rare, often fatal, mitochondrial disorder. It presents early in infancy and is characterized by refractory anemia, exocrine pancreatic insufficiency, and metabolic acidosis. Hepatic and renal manifestations are also common. One-fourth of patients present with anemia in the neonatal period, and hydrops fetails has been reported.[81] Diagnosis is made based on clinical manifestations and bone marrow aspirate, which shows vaculated myeloid and erythroid precursors, decreased numbers of erythroblasts, and many sideroblasts. Pearson syndrome is caused by deletions in mitochondrial DNA and genetic diagnosis is possible.[82-83]

Aase Syndrome

Aase syndrome is a rare congenital, hypoplastic anemia associated with triphalangial thumbs.[84] Aase syndrome can also be associated with boney abnormalities, growth failure, and unilateral cleft palate. The anemia in Aase syndrome is steroid responsive and generally improves with age.[4]

Fanconi's Anemia

Fanconi's anemia (FA) is a rare autosomal recessive disorder leading to bone marrow failure and increased susceptibility to leukemia and other cancers. Fanconi's anemia is often associated with other congenital abnormalities, such as café-au-lait spots, radial ray abnormalities, thumb abnormalities, short stature, and characteristic facies (broad nose, epicanthal folds, micrognathia.). Renal and genital anomalies are also common. Fanconi's may present in the neonatal period with cytopenias, congenital malformations, or both. Diagnosis is made on the basis of increased chromosomal breakage on exposure to alkalating agents,[85] that becomes more pronounced with exposure to diepoxybutane[86] or mitomycin C.[87] Fanconi's anemia has been linked to mutations in FANCA, FANCC, FANCD1, FANCD2, FANCE, and FANCF, which are thought to form a complex important for DNA repair.[88] Therapy for Fanconi's

anemia includes androgens such as oral oxymethalone, which increase bone marrow cellularity. G-CSF and GM-CSF have also been used. Bone marrow transplantation[89] or stem cell transplant with cord blood are current therapies.[90]

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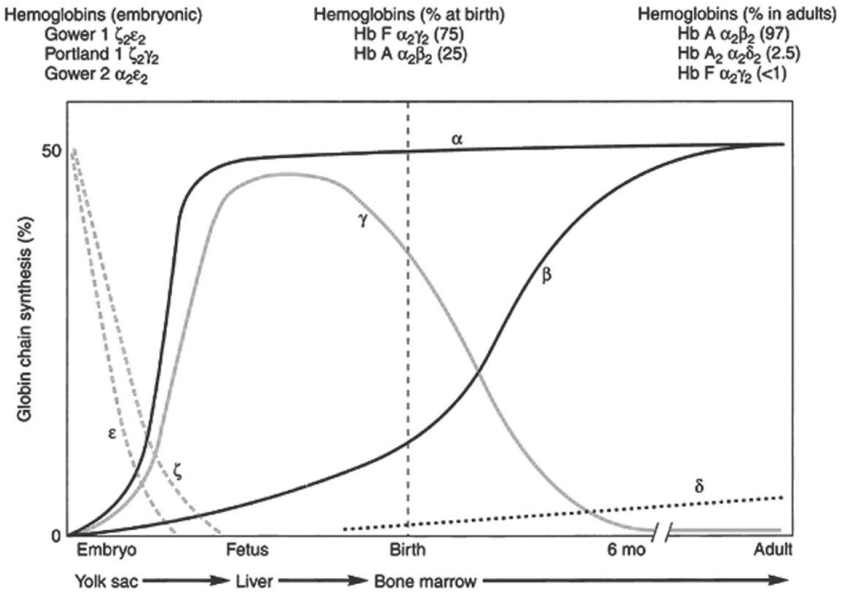

Figure 1. Hemoglobin switching during embryonic, fetal, and adult development

From: Steinberg MH, Benz Jr. EJ, Adewoye HA, and Ebert B. Pathobiology of the human erythrocyte and its hemoglobins. In Hoffman R, Benz EJ, Shattil, SJ: Hematology: Basic Principles and Practice, 4th ed., 2005. Churchill Livingston, Philadelphia. Page 442-454, with permission.

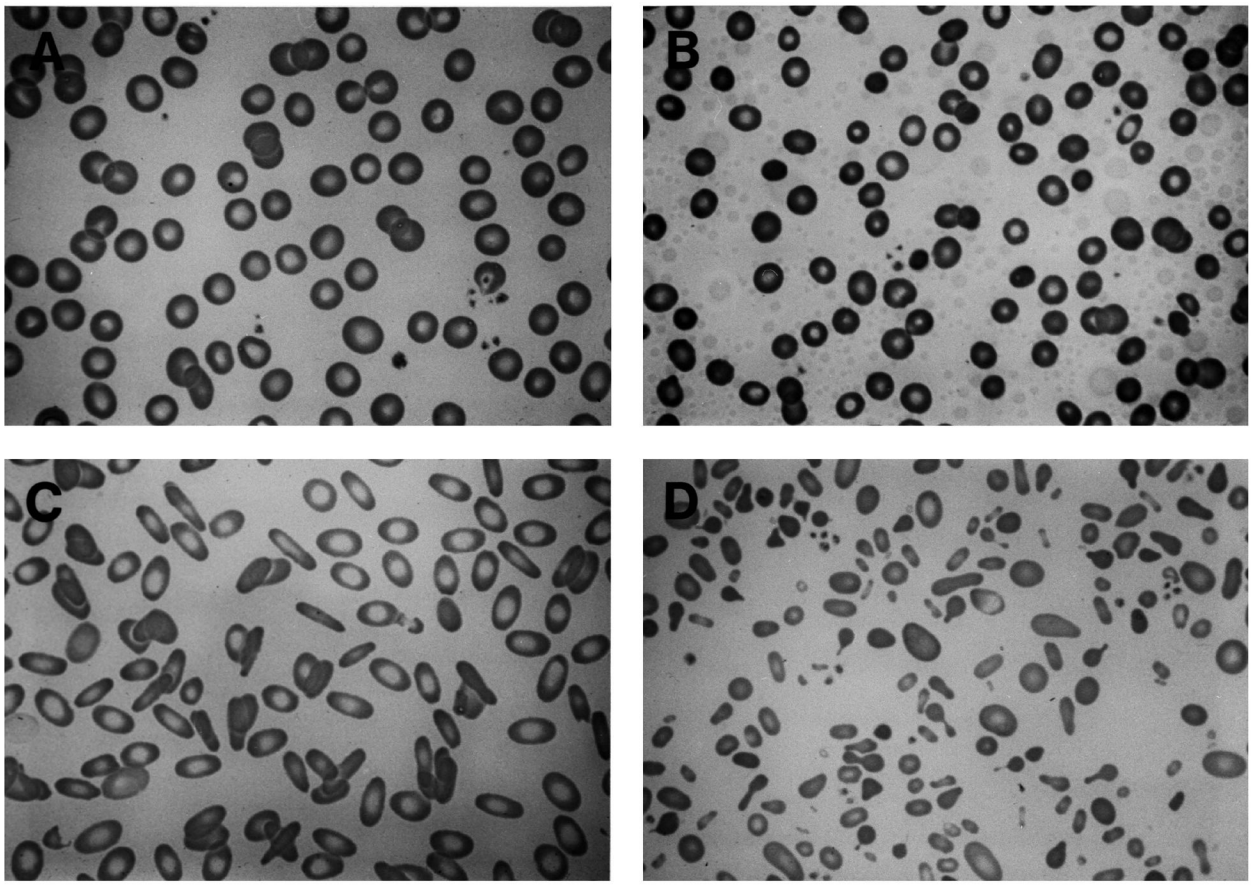

Figure 2. Wright stained peripheral blood smears

(A) Normal. (B) Hereditary Spherocytosis. (C) Hereditary Elliptocytosis. (D) Hereditary Pyropoikilocytosis. Clinical and Molecular Aspects of Disorders of the Erythrocyte Membrane Skeleton, Gallagher PG, Tse WT, Forget BG in Seminars in Perinatology, 14:351-67, 1990, with permission

Table 1

Intrinsic Disorders of the Erythrocyte Leading to Nonimmune Hydrops Fetalis

	Reference
Hemoglobin Disorders	
α-Thalassemia	
Hemoglobin H Disease	11-13
Homozygous α-Thalassemia	15
Enzyme Deficiencies	
G6PD Deficiency	47-49
Pyruvate Kinase	31-36
Membrane Defects	
Hereditary Spherocytosis	58
Hereditary Elliptocytosis	59-60
Hereditary Stomatocytosis Syndromes	65,66
Decreased Erythrocyte Production	
Congenital Dyserythopeietic Anemias	80
Pearson Syndrome	81
Diamond-Blackfan Anemia	72-73