HEMOLYTIC ANEMIA

APPROACH TO DIAGNOSIS

An essential feature of hemolytic anemia is a reduction in the normal red cell survival of 120 days. Premature destruction of red cells may result from corpuscular abnormalities (within the red cell corpuscle), that is, abnormalities of membrane, enzymes, or hemoglobin; or from extracorpuscular abnormalities, that is, immune or nonimmune mechanisms. Tables 7-1 and 7-2 list the causes of hemolytic anemia due to corpuscular and extracorpuscular defects, respectively.

The approach to the diagnosis of hemolytic anemia should include:

1. Consideration of the clinical features suggesting hemolytic disease
2. Laboratory demonstration of the presence of a hemolytic process
3. Determination of the precise cause of the hemolytic anemia by special hematologic investigations.

Clinical Features

The following clinical features suggest a hemolytic process:

1. **Ethnic factors:** Incidence of sickle gene carrier in the African-American population (8%), high incidence of thalassemia trait in people of Mediterranean ancestry, and high incidence of glucose-6-phosphate dehydrogenase (G6PD) deficiency among Sephardic Jews
2. **Age factors:** Anemia and jaundice in an Rh-positive infant born to a mother who is Rh negative or a group A or group B infant born to a group O mother (setting for a hemolytic anemia)
3. History of anemia, jaundice, or gallstones in family
4. Persistent or recurrent anemia associated with reticulocytosis
5. Anemia unresponsive to hematinics
6. Intermittent bouts or persistent indirect hyperbilirubinemia
7. Splenomegaly
8. Hemoglobinuria
9. Presence of multiple gallstones
10. Chronic leg ulcers
11. Development of anemia or hemoglobinuria after exposure to certain drugs
12. Cyanosis without cardiorespiratory distress
13. Polycythemia
14. Dark urine due to dipyrroluria.
Table 7-1. Causes of Hemolytic Anemia Due to Corpuscular Defects

I. Membrane defects
   A. Primary membrane defects with specific morphologic abnormalities
      1. Hereditary spherocytosis
      2. Hereditary elliptocytosis/pyropoikilocytosis
      3. Hereditary stomatocytosis with:
         a. Increased osmotic fragility (high Na\(^+\), low K\(^+\))
         b. Decreased osmotic fragility (high Na\(^+\), low K\(^+\))
         c. Normal osmotic fragility
         d. Rh\(_{null}\)
      4. Congenital hemolytic anemia with dehydrated red cells (high Na\(^+\), low K\(^+\),
         decreased osmotic fragility)
   B. Secondary membrane defects: abetalipoproteinemia

II. Enzyme defects
   A. Energy potential defects (Embden–Meyerhof: anaerobic; ATP-producing pathway
deficiencies)
      1. Hexokinase
      2. Glucose phosphate isomerase
      3. Phosphofructokinase
      4. Triosephosphate isomerase
      5. Phosphoglycerate kinase
      6. 2,3-Diphosphoglyceromutase (polycythemia and no hemolysis)
      7. Pyruvate kinase
   B. Reduction potential defects (hexose monophosphate: aerobic; NADPH-producing
      pathway deficiencies)
      1. G6PD
      2. 6-Phosphogluconate dehydrogenase (6PGD)
      3. Glutathione reductase
      4. Glutathione synthetase
      5. 2,3-Glutamyl-cysteine synthetase
   C. Abnormalities of erythrocyte nucleotide metabolism
      1. Adenosine triphosphatase deficiency
      2. Adenylate kinase deficiency
      3. Pyrimidine 5'-nucleotidase (P5N) deficiency
      4. Adenosine deaminase excess

III. Hemoglobin defects
   A. Heme: congenital erythropoietic porphyria
   B. Globin
      1. Qualitative: hemoglobinopathies (e.g., Hb S, C, H, M)
      2. Quantitative: α- and β-thalassemias

IV. Congenital dyserythropoietic anemias
   A. Type I
   B. Type II
   C. Type III
   D. Type IV

Abbreviations: ATPase, adenosine triphosphatase; G6PD, glucose-6-phosphate dehydrogenase.

*World Health Organization (WHO) classification of G6PD variant: Class I variant: Chronic hemolysis due to
severe G6PD deficiency, e.g., G6PD deficiency Harilaou. Class II variant: Intermittent hemolysis in spite of severe
G6PD deficiency, e.g., G6PD Mediterranean. Class III variant: Intermittent hemolysis associated usually with
drugs/infections and moderate G6PD deficiency, e.g., G6PDA variant. Class IV variant: No hemolysis, no G6PD
deficiency, e.g., normal G6PD (B\(^+\) variant).
Laboratory Findings of hemolytic anemia consist of:

1. Reduced red cell survival and evidence of accelerated hemoglobin catabolism
2. Evidence of increased erythropoiesis.

Accelerated Hemoglobin Catabolism

Accelerated hemoglobin catabolism varies with the type of hemolysis as follows:

- Extravascular hemoglobin catabolism (see Figure 7-1)
- Intravascular hemoglobin catabolism (see Figure 7-2).

Table 7-2. Causes of Hemolytic Anemia Due to Extracorpuscular Defects

<table>
<thead>
<tr>
<th>I. Immune</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Isoimmune</td>
<td></td>
</tr>
<tr>
<td>1. Hemolytic disease of the newborn</td>
<td></td>
</tr>
<tr>
<td>2. Incompatible blood transfusion</td>
<td></td>
</tr>
<tr>
<td>B. Autoimmune: IgG only; complement only; mixed IgG and complement</td>
<td></td>
</tr>
<tr>
<td>1. Idiopathic</td>
<td></td>
</tr>
<tr>
<td>a. Warm antibody</td>
<td></td>
</tr>
<tr>
<td>b. Cold antibody</td>
<td></td>
</tr>
<tr>
<td>c. Cold–warm hemolysis (Donath–Landsteiner antibody)</td>
<td></td>
</tr>
<tr>
<td>2. Secondary</td>
<td></td>
</tr>
<tr>
<td>a. Infection, viral: infectious mononucleosis—Epstein–Barr virus (EBV), cytomegalovirus (CMV), hepatitis, herpes simplex, measles, varicella, influenza A, coxsackie virus B, human immunodeficiency virus (HIV); bacterial: streptococcal, typhoid fever, Escherichia coli septicemia, Mycoplasma pneumoniae (atypical pneumonia)</td>
<td></td>
</tr>
<tr>
<td>b. Drugs and chemicals: quinine, quinidine, phenacetin, p-aminosalicylic acid, sodium cephalothin (Keflin), penicillin, tetracycline, rifampin, sulfonamides, chlorpromazine, pyradone, dipyrone, insulin; lead</td>
<td></td>
</tr>
<tr>
<td>c. Hematologic disorders: leukemias, lymphomas, lymphoproliferative syndrome, associated idiopathic thrombocytopenic purpura (Evans syndrome), paroxysmal cold hemoglobinuria, paroxysmal nocturnal hemoglobinuria</td>
<td></td>
</tr>
<tr>
<td>d. Immunopathic disorders: systemic lupus erythematosus, periarthritis nodosa, scleroderma, dermatomyositis, rheumatoid arthritis, ulcerative colitis, agammaglobulinemia, Wiskott–Aldrich syndrome, dysgammaglobulinemia, IgA deficiency, thyroid disorders, giant cell hepatitis, Evans syndrome, autoimmune lymphoproliferative syndrome (ALPS), common variable immune deficiency</td>
<td></td>
</tr>
<tr>
<td>e. Tumors: ovarian teratomata, dermoids, thymoma, carcinoma, lymphomas</td>
<td></td>
</tr>
</tbody>
</table>

II. Nonimmune

| A. Idiopathic                  |                                      |
| B. Secondary                   |                                      |
| 1. Infection, viral: infectious mononucleosis, viral hepatitis; bacterial: streptococcal, E. coli septicemia, Clostridium perfringens, Bartonella bacilliformis; parasites: malaria, histoplasmosis |                                      |
| 2. Drugs and chemicals: phenylhydrazine, vitamin K, benzene, nitrobenzene, sulfones, phenacetin, acetaminamide; lead |                                      |
| 3. Hematologic disorders: leukemia, aplastic anemia, megaloblastic anemia, hypersplenism, pyknocytosis |                                      |
| 4. Microangiopathic hemolytic anemia: thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, chronic relapsing schistocytic hemolytic anemia, burns, post cardiac surgery, march hemoglobinuria |                                      |
| 5. Miscellaneous: Wilson disease, erythropoietic porphyria, osteopetrosis, hypersplenism |                                      |
Fig. 7-1. Extravascular hemoglobin catabolism following extravascular destruction of the RBC.
Intravascular disruption of red blood cell (RBC) membrane and release of hemoglobin in circulating blood

Free Hemoglobin

Hemoglobin-Haptoglobin
Haptoglobin
Hemopexin-Heme
Hemopexin
Heme
Albumin
Methemalbumin

Liver (Hepatocyte)

Oxygen (O₂)
Methemoglobin

Kidney

Hemoglobinuria
Oxyhemoglobin
Methemoglobin
Urobilinogen

Hemoglobin-haptoglobin, hemopexin-heme, and methemalbumin are cleared by hepatocytes. Heme is converted to iron and bilirubin. The common pathway for both extravascular and intravascular hemolysis is the conjugation of bilirubin (bilirubin glucuronide) by the hepatocytes, its excretion in bile, and ultimately formation of the urobilinogen by the bacteria in the gut. Part of urobilinogen enters in enterohepatic circulation and part is excreted by the kidney in urine, and the remainder of urobilinogen is excreted in stool (see Fig. 7-1).
Signs of extravascular hemolysis:

1. Increased unconjugated bilirubin
2. Increased fecal and urinary urobilinogen
3. Increased rate of carbon monoxide production.

Signs of intravascular hemolysis:

1. Raised plasma hemoglobin level (normal value <1 mg hemoglobin/dL plasma, visibly red plasma contains >50 mg hemoglobin/dL plasma)
2. Hemoglobinuria (Table 7-3 lists the causes of hemoglobinuria)
3. Hemosiderinuria (due to sloughing of iron-laden tubular cells into urine)
4. Low or absent plasma haptoglobin (normal level, 128 ± 25 mg/dL)
5. Raised plasma methemalbumin (albumin bound to heme; unlike haptoglobin, albumin does not bind intact hemoglobin)
6. Raised plasma methemoglobin (oxidized free plasma hemoglobin) and raised levels of hemopexin-heme complex in plasma.

Increased Erythropoiesis

Erythropoiesis increases in response to a reduction in hemoglobin and is manifested by:

1. Reticulocytosis: Frequently up to 10–20%; rarely, as high as 80%
2. Increased mean corpuscular volume (MCV) due to the presence of reticulocytosis and increased red cell distribution width (RDW) as the hemoglobin level falls
3. Increased normoblasts in peripheral blood
4. Specific morphologic abnormalities: Sickle cells, target cells, basophilic stippling, irregularly contracted cells (schistocytes), and spherocytes

Table 7-3. Causes of Hemoglobinuria

<table>
<thead>
<tr>
<th>I. Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Mismatched blood transfusions</td>
</tr>
<tr>
<td>B. Drugs and chemicals</td>
</tr>
<tr>
<td>1. Regularly causing hemolytic anemia</td>
</tr>
<tr>
<td>a. Drugs: phenylhydrazine, sulfones (dapsone), phenacetin, acetanilid (large doses)</td>
</tr>
<tr>
<td>b. Chemicals: nitrobenzene, lead, inadvertent infusion of water</td>
</tr>
<tr>
<td>c. Toxins: snake and spider bites</td>
</tr>
<tr>
<td>2. Occasionally causing hemolytic anemia</td>
</tr>
<tr>
<td>a. Associated with G6PD deficiency: antimalarials (primaquine, chloroquine), antipyretics (aspirin, phenacetin), sulfonamides (Gantrisin, lederkyn), nitrofurans (Furadantin, Furacin), miscellaneous (naphthalene, vitamin K, British antilewisite [BAL], favism)</td>
</tr>
<tr>
<td>b. Associated with Hb Zürich: sulfonamides</td>
</tr>
<tr>
<td>c. Hypersensitivity: quinine, quinidine, \textit{para}-aminosalicylic acid (PAS), phenacetin</td>
</tr>
<tr>
<td>C. Infections</td>
</tr>
<tr>
<td>1. Bacterial: \textit{Clostridium perfringens}, \textit{Bartonella bacilliformis} (Oroya fever)</td>
</tr>
<tr>
<td>2. Parasitic: malaria</td>
</tr>
<tr>
<td>D. Burns</td>
</tr>
<tr>
<td>E. Mechanical (e.g., prosthetic valves)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Paroxysmal cold hemoglobinuria; syphilis; idiopathic</td>
</tr>
<tr>
<td>B. Paroxysmal nocturnal hemoglobinuria</td>
</tr>
<tr>
<td>C. March hemoglobinuria</td>
</tr>
<tr>
<td>D. Cold agglutinin hemolysis</td>
</tr>
</tbody>
</table>
5. **Erythroid hyperplasia of the bone marrow:** Erythroid:myeloid ratio in the marrow increasing from 1:5 to 1:1
6. Increased red cell creatine levels
7. Erythroid hyperplasia in bone marrow
8. Expansion of marrow space in chronic hemolysis resulting in:
   a. Prominence of frontal bones
   b. Broad cheekbones
   c. Widened intratrabecular spaces, hair-on-end appearance of skull radiographs
   d. Biconcave vertebrae with fish-mouth intervertebral spaces
9. Decreased red cell survival demonstrated by $^{51}$Cr red cell labeling.

Table 7-4 lists the investigations used to demonstrate the presence of a hemolytic process.

**Determination of Cause**

Once the presence of a hemolytic process has been established, the precise cause of the hemolytic anemia must be determined. Table 7-5 lists the tests used to establish the cause of hemolytic anemia.

**CORPUSCULAR HEMOLYTIC ANEMIAS**

**Membrane Defects**

Hereditary spherocytosis, elliptocytosis, stomatocytosis, acanthocytosis, xerocytosis, and pyropoikilocytosis can be diagnosed on the basis of their characteristic morphologic abnormalities. Spectrin is responsible for maintaining red cell shape and is composed of two subunits, $\alpha$- and $\beta$-spectrin, which are structurally distinct and are encoded by separate genes. A variety of mutations in $\alpha$- and $\beta$-spectrin have been reported. Spectrin regulates the lateral mobility of integral membrane proteins and provides structural support for the lipid bilayer. Disruption of spectrin self-association leads to disorders characterized by abnormally shaped red cells. Enzyme defects and many hemoglobinopathies have nonspecific morphologic abnormalities.

<table>
<thead>
<tr>
<th>Table 7-4. Tests Used to Demonstrate a Hemolytic Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <strong>Accelerated hemoglobin catabolism</strong></td>
</tr>
<tr>
<td>1. Serum bilirubin level</td>
</tr>
<tr>
<td>2. Urinary urobilinogen excretion</td>
</tr>
<tr>
<td>3. Fecal urobilinogen excretion</td>
</tr>
<tr>
<td>4. Haptoglobin level</td>
</tr>
<tr>
<td>5. Plasma hemoglobin level</td>
</tr>
<tr>
<td>6. Methemoglobin level</td>
</tr>
<tr>
<td>7. Methemalbumin level</td>
</tr>
<tr>
<td>8. Carboxyhemoglobin</td>
</tr>
<tr>
<td>9. Urinalysis for hemoglobinuria and hemosiderinuria</td>
</tr>
<tr>
<td>10. Blood smear: red cell fragments (schistocytes), spherocytes</td>
</tr>
<tr>
<td>11. Red cell survival studies: $^{51}$Cr, difluorophosphate-32 ($^{32}$DFP)</td>
</tr>
<tr>
<td>12. Increased erythropoiesis</td>
</tr>
<tr>
<td>13. Reticulocyte count/reticulocyte index</td>
</tr>
<tr>
<td>14. Macrocytosis</td>
</tr>
<tr>
<td>15. Normoblastemia</td>
</tr>
<tr>
<td>16. Bone marrow examination for erythroid hyperplasia</td>
</tr>
<tr>
<td>17. Radiography: hair-on-end appearance</td>
</tr>
</tbody>
</table>
Hereditary Spherocytosis

Genetics

1. Autosomal dominant inheritance (75% of cases). The severity of anemia and the degree of spherocytosis may not be uniform within an affected family.
2. No family history in 25% of cases. Some show minor laboratory abnormalities, suggesting a carrier (recessive) state. Others are due to a de novo mutation.
3. Most common in people of northern European heritage, with an incidence of 1 in 5000.

Pathogenesis

In hereditary spherocytosis (HS), the primary defect is membrane instability due to dysfunction or deficiency of a red cell skeletal protein. A variety of membrane skeletal protein defects have been found in different families. These include:

1. Ankyrin mutations: Account for 50–67% of HS. In many patients, both spectrin and ankyrin proteins are deficient. Mutations of ankyrin occur in both dominant and recessive forms of HS. Clinically, the course varies from mild to severe. Red cells are typically spherocytes.

---

Table 7-5. Tests Used to Establish a Specific Cause of Hemolytic Anemia

<table>
<thead>
<tr>
<th>Corpuscular defects</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood smear: spherocytes, ovalocytes, pyknocytes, stomatocytes&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Osmotic fragility (fresh and incubated)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Autohemolysis&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cation permeability studies</td>
<td></td>
</tr>
<tr>
<td>Membrane phospholipid composition</td>
<td></td>
</tr>
<tr>
<td>Scanning electron microscopy</td>
<td></td>
</tr>
</tbody>
</table>

| Hemoglobin defects | Blood smear: sickle cells, target cells (Hb C)<sup>a</sup> |
| Sickling test<sup>a</sup> |
| Hemoglobin electrophoresis<sup>a</sup> |
| Quantitative fetal hemoglobin determination<sup>a</sup> |
| Kleihauer–Betke smear<sup>a</sup> |
| Heat stability test for unstable hemoglobin |
| Oxygen dissociation curves |
| Rates of synthesis of polypeptide chain production |
| Fingerprinting of hemoglobin |

| Enzyme defects | Heinz-body preparation<sup>a</sup> |
| Osmotic fragility<sup>a</sup> |
| Autohemolysis test<sup>a</sup> |
| Screening test for enzyme deficiencies<sup>a</sup> |
| Specific enzyme assays<sup>a</sup> |

| Extracorpuscular defects | Coombs’ test: IgG (gamma), C’3 (complement), broad-spectrum (both gamma and complement)<sup>a</sup> |
| Acidified serum lysis (Ham’s) test<sup>a</sup> |
| Donath–Landsteiner test<sup>a</sup> |
| Flow cytometric analysis of red cells with monoclonal antibodies to GP1-linked surface antigens (for PNH) |

<sup>a</sup>Tests commonly employed and most useful in establishing a diagnosis.
2. α-Spectrin mutations occur in recessive HS and account for less than 5% of HS. Clinical course is severe. Contracted cells, poikilocytes, and spherocytes are seen.

3. β-Spectrin mutations occur in dominant HS and account for 15–20% of HS. Clinical course is mild to moderate. Acanthocytes, spherocytic elliptocytes, and spherocytes are seen.

4. Protein 4.2 mutations occur in the recessive form of HS and account for less than 5% of HS. Clinical course is mild to moderate. Spherocytes, acanthocytes, and ovalocytes are seen.

5. Band 3 mutations occur in the dominant form of HS and account for 15–20% of HS. Clinical course can be mild to moderate. Spherocytes are occasionally mushroom-shaped or pincerred cells.

Deficiency of these membrane skeletal proteins in HS results in vertical defect, which causes progressive loss of membrane lipid and surface area. The loss of surface area results in characteristic microspherocytic morphology of HS red cells. The sequelae are as follows:

1. Sequestration of red cells in the spleen (due to reduced erythrocyte deformability)
2. Depletion of membrane lipid
3. Decrease in membrane surface area relative to volume, resulting in a decrease in surface area-to-volume ratio
4. Tendency to spherocytosis
5. Influx and efflux of sodium increased; cell dehydration
6. Rapid adenosine triphosphate (ATP) utilization and increased glycolysis
7. Premature red cell destruction.

Hematology

1. Anemia: Mild to moderate in compensated cases. In erythroblastopenic crisis, hemoglobin may drop to 2–3 g/dL.
2. MCV usually decreased; mean corpuscular hemoglobin concentration (MCHC) raised and RDW elevated.*
3. Reticulocytosis (3–15%).
4. Blood film: Microspherocytes† (vary in number); hyperdense cells,‡ polychromasia.
5. Coombs’ test negative.
6. Increased red cell osmotic fragility (spherocytes lyse in higher concentrations of saline than normal red cells) occasionally only demonstrated after incubation of blood sample at 37°C for 24 hours. In spite of normal osmotic fragility, increased MCHC or an increase of hyperdense red cells is highly suggestive of HS.
7. Autohemolysis at 24 and 48 hours increased, corrected by the addition of glucose.
8. Survival of ⁵¹Cr-labeled cells reduced with increased splenic sequestration.
9. Marrow: Normoblastic hyperplasia; increased iron.

*The MCHC is only raised in hereditary spherocytosis, hereditary xerocytosis, hereditary pyropoikilocytosis, and cold agglutinin disease. The presence of elevated RDW and MCHC (performed by aperture impedance instruments, e.g., Coulter) makes the likelihood of hereditary spherocytosis very high, because these two tests used together are very specific for hereditary spherocytosis.

†The percentage of microspherocytes is the best indicator of the severity of the disease but not a good discriminator of the HS genotype.

‡Hyperdense cells are seen in HbSC disease, HbCC disease, and xerocytosis. In HS, hyperdense cells are a poor indicator of disease severity but an effective discriminating feature of the HS phenotype.
Biochemistry

1. Raised bilirubin, mainly indirect reacting
2. Obstructive jaundice with increased direct-reacting bilirubin; may develop due to gallstones, a consequence of increased pigment excretion.

Clinical Features

1. Anemia and jaundice: Severity depends on rate of hemolysis, degree of compensation of anemia by reticulocytosis, and ability of liver to conjugate and excrete indirect hyperbilirubinemia.
2. Splenomegaly.
3. Presents in newborn (50% of cases) with hyperbilirubinemia, reticulocytosis, normoblastosis, spherocytosis, negative Coombs’ test, and splenomegaly.
4. Presents before puberty in most patients.
5. Diagnosis sometimes made much later in life by chance.
6. Co-inheritance of HS with hemoglobin S-C disease may increase the risk of splenic sequestration crisis.
7. Co-inheritance of $\beta$-thalassemia trait and HS may worsen, improve, or have no effect on the clinical course of HS.
8. Iron deficiency may correct the laboratory values but not the red cell life span in HS patients.
9. HS with other system involvement:
   b. HS may be associated with neurologic abnormalities such as cerebellar disturbances, muscle atrophy, and a tabes-like syndrome.

Classification

Table 7-6 lists a classification of hereditary spherocytosis in accordance with clinical severity and indications for splenectomy.

Diagnosis

1. Clinical features and family history
2. Hematologic features.

Complications

1. Hemolytic crisis: With more pronounced jaundice due to accelerated hemolysis (may be precipitated by infection)
2. Erythroblastopenic crisis: Dramatic fall in hemoglobin level (and reticulocyte count); usually due to maturation arrest and often associated with giant pronormoblasts in the recovery phase; usually associated with parvovirus B19 infection*
3. Folate deficiency: Caused by increased red cell turnover; may lead to superimposed megaloblastic anemia. Megaloblastic anemia may mask HS morphology as well as its diagnosis by osmotic fragility

*Parvovirus B19 infects developing normoblasts, causing a transient cessation of production. The virus specifically infects CFU-E and prevents their maturation. Giant pronormoblasts are seen in bone marrow. Diagnosis is made by increased IgM antibody titer against parvovirus and PCR for parvovirus on bone marrow.
<table>
<thead>
<tr>
<th>Classification</th>
<th>Trait</th>
<th>Mild spherocytosis</th>
<th>Moderate spherocytosis</th>
<th>Severe spherocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>Normal</td>
<td>11–15</td>
<td>8–12</td>
<td>6–8</td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
<td>≤3</td>
<td>3.1–6</td>
<td>≥6</td>
<td>≥10</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>≤1.0</td>
<td>1.0–2.0</td>
<td>≥2.0</td>
<td>≥3.0</td>
</tr>
<tr>
<td>Reticulocyte production index</td>
<td>&lt;1.8</td>
<td>1.8–3</td>
<td>&gt;3</td>
<td></td>
</tr>
<tr>
<td>Spectrin per erythrocyte(^b) (% of normal)</td>
<td>100</td>
<td>80–100</td>
<td>50–80</td>
<td>40–60</td>
</tr>
<tr>
<td>Osmotic fragility</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh blood</td>
<td>Normal</td>
<td>Normal to slightly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
</tr>
<tr>
<td>Incubated blood</td>
<td>Slightly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
</tr>
<tr>
<td>Autohemolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without glucose (%)</td>
<td>&gt;60</td>
<td>&gt;60</td>
<td>0–80</td>
<td>50</td>
</tr>
<tr>
<td>With glucose (%)</td>
<td>&lt;10</td>
<td>≥10</td>
<td>≥10</td>
<td>≥10</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>Not necessary</td>
<td>Usually not necessary during childhood and adolescence</td>
<td>Necessary during school age before puberty</td>
<td>Necessary, not before 3 years of age</td>
</tr>
<tr>
<td>Symptoms</td>
<td>None</td>
<td>None</td>
<td>Pallor, erythroblastopenic crises, splenomegaly, gallstones</td>
<td>Pallor, erythroblastopenic crises, splenomegaly, gallstones</td>
</tr>
</tbody>
</table>

\(^a\) Value before transfusion.

\(^b\) Normal (mean ± SD): 226 ± 54 × 10\(^3\) molecules per cell.

4. **Gallstones:** In approximately one-half of untreated patients; increased incidence with age. Occasionally, HS may be masked or improved in obstructive jaundice due to increase in surface area of red cells and formation of targets cells.

5. **Hemochromatosis:** Rarely.

**Treatment**

1. Folic acid supplement (1 mg/day)
2. Leukocyte-depleted packed red cell transfusion for severe erythroblastopenic crisis
3. Splenectomy* for moderate to severe cases. Most patients with less than 80% of normal spectrin content require splenectomy. Splenectomy should be carried out early in severe cases but not before 5 years of age, if possible. The management of the splenectomized patient is detailed in Chapter 26. Although spherocytosis persists postsplenectomy, the red cell life span becomes essentially normal and complications are prevented, especially transient erythroblastopenia and persistent hyperbilirubinemia, which leads to gallstones
4. Ultrasound should be carried out before splenectomy to exclude the presence of gallstones. If present, cholecystectomy is also indicated.

**Hereditary Elliptocytosis**

Hereditary elliptocytosis (HE) is clinically and genetically a heterogeneous disorder.

**Pathogenesis**

HE is due to various defects in the skeletal proteins, spectrin, and protein 4.1. The basic membrane defects consist of:

1. Defects of spectrin self-association involving the α-chains
2. Defects of spectrin self-association involving the β-chains
3. Deficiency of protein 4.1
4. Deficiency of glycophorin.

Deficiencies of these skeletal proteins result in decreased horizontal stability and reduced pliability of red blood cells. Thus, a red blood cell is unable to regain its biconcave shape after its distortion in the microcirculation.

The membrane defect results in decreased cellular deformability as a result of increased membrane rigidity, which is a consistent feature in all cases. In addition, cell dehydration is present. In HE, cell fragmentation is the result of the loss of mechanical integrity of the skeleton. The cell remnants contain a full complement of various membrane proteins and represent a true fragmentation process in which microcytic red blood cells with decreased hemoglobin content are generated. In HS, however, continuous loss of the lipid-rich and skeleton-free domains of the membrane during the red cell life span results in spherocytic red cells with near-normal hemoglobin content and the absence of fragmented cells with markedly decreased hemoglobin content.

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*Laparoscopic splenectomy is safe in children. Although it requires more operative time than open splenectomy, it is superior with regard to postoperative analgesia, smaller abdominal wall scars, duration of hospital stay, and more rapid return to a regular diet and daily activities. It is not known if accessory spleens are readily identified with the laparoscope although the magnification afforded by the laparoscope might be advantageous in some cases.
Genetics
HE is characterized by an autosomal dominant mode of inheritance (with variable penetrance), affecting about 1 in 25,000 of the population. Two types of inheritance occur:

1. Non-Rh-linked, associated with a high incidence of severe anemia in the homozygote
2. Rh-linked, usually associated with a milder disorder.

Clinical Features
1. Varies from patients who are symptom free to severe anemia requiring blood transfusions. The percentage of microcytes best reflects the severity of the disease.
2. About 12% have symptoms indistinguishable from hereditary spherocytosis.
3. The percentage of elliptocytes varies from 50% to 90%. No correlation has been established between the degree of elliptocytosis and the severity of the anemia.
4. HE has been classified into the following clinical subtypes:
   a. Common HE, which is divided into several groups: silent carrier state, mild HE, HE with infantile pyknocytosis
   b. Common HE with chronic hemolysis, which is divided into two groups: HE with dyserythropoiesis and homozygous common HE, which is clinically indistinguishable from hereditary pyropoikilocytosis (see later discussion)
   c. Spherocytic HE, which clinically resembles HS; however, a family member usually has evidence of HE
   d. Southeast Asian ovalocytosis, in which the majority of cells are oval; however, some red cells contain either a longitudinal or transverse ridge.

Laboratory Findings
1. Blood smear: 25–90% of cells elongated oval elliptocytes
2. Osmotic fragility normal or increased
3. Autohemolysis usually normal but may be increased and usually corrected by the addition of glucose or ATP.

Treatment
The indications for transfusion, splenectomy, and prophylactic folic acid are the same as for hereditary spherocytosis.

Hereditary Pyropoikilocytosis
Definition
Hereditary pyropoikilocytosis (HPP) is a congenital hemolytic anemia associated with in vivo red cell fragmentation and marked in vitro fragmentation of red cells at 45°C. Because of the similarities in the membrane defect in this condition and HE, it is viewed as a subtype of HE.

Genetics and Etiology
1. Homozygous or doubly heterozygous for the spectrin chains (e.g., Sp-α$^{1/74}$ and Sp-α$^{2/46}$). The spectrin chain defects found in HPP are similar to those found in HE.
2. Increased ratio of cholesterol to membrane protein.
3. Decreased cell deformability.

Clinical Features

1. Anemia characterized by extreme anisocytosis and poikilocytosis
   a. Red cell fragments, spherocytes, and budding red cells (the red cells are exquisitely sensitive to temperature and fragment after 10 minutes of incubation time at 45–46°C in vitro; heating for 6 hours at 37°C explains in vivo formation of fragmented red cells and chronic hemolysis)
   b. Hemoglobin level, 7–9 g/dL
   c. Marked reduction in MCV and elevated MCHC
2. Jaundice
3. Splenomegaly
4. Osmotic fragility and autohemolysis increased
5. Mild HE present in one of the parents or siblings.

Differential Diagnosis

Similar cells are seen in microangiopathic hemolytic anemias, after severe burns or oxidant stress, and in pyruvate kinase deficiency.

Treatment

Patients respond well to splenectomy with a rise in hemoglobin to 12 g/dL. Following splenectomy, hemolysis is decreased but not totally eliminated.

Hereditary Stomatocytosis

Definition and Genetics

The stomatocyte has a linear slit-like area of central pallor rather than a circular area. When suspended in plasma, the cells assume a bowl-shaped form. This hereditary hemolytic anemia of variable severity is characterized by an autosomal dominant mode of inheritance.

Etiology

The cells contain high Na⁺ and low K⁺ concentrations. The disorder is probably due to a membrane and protein defect. The cells are abnormally rigid and poorly deformable, contributing to their rapid rate of destruction. There are many biochemical variants.

Clinical Features

1. Very variable
2. Jaundice at birth
3. Pallor: marked variability depending on severity of anemia
4. Splenomegaly
5. Hematology
   a. Anemia
   b. Smear, 10–50% stomatocytes
   c. Reticulocytosis
   d. Increased osmotic fragility and autohemolysis.
Differential Diagnosis

Stomatocytosis may occur with thalassemia, some red cell enzyme defects (glutathione peroxidase deficiency, glucose phosphate isomerase deficiency), Rhnull red cells, viral infections, lead poisoning, some drugs (e.g., quinidine and chlorpromazine), some malignancies, liver disease, and alcoholism.

Treatment

Splenectomy may be beneficial if hemolysis is severe.

Hereditary Acanthocytosis

Definition

Acanthocytes have thorn-like projections that vary in length and width and are irregularly distributed over the surface of red cells.

Genetics

The mode of inheritance is autosomal recessive.

Clinical Features

1. **Steatorrhea**: Only fat malabsorption
2. **Neurologic symptoms**: Weakness, ataxia and nystagmus, atypical retinitis pigmentosa with macular atrophy, blindness
3. **Anemia**: Mild hemolytic anemia; 70–80% acanthocytes; slight reticulocytosis.

Diagnosis

1. Clinical syndrome
2. Absent β-lipoprotein in plasma
3. Diagnostic findings on small intestine biopsy.

Differential Diagnosis

During the neonatal period, hereditary acanthocytosis may have to be distinguished from the benign nonhereditary disorder of infantile pyknocytosis. Acquired acanthocytosis occurs under the following conditions: renal failure, cirrhosis, microangiopathic hemolytic anemia, hypothyroidism, pyruvate kinase deficiency, and in association with some neoplasms.

Hereditary Xerocytosis

Definition

Hereditary xerocytosis is a familial condition characterized by red cells that appear to be shrunken, with hemoglobin puddled at the periphery or center of the cell. The defect in these cells permits increased permeability of univalent cations Na⁺ and K⁺. The accompanying cell water loss results in dehydrated red cells. There is an increased proportion of phosphatidylcholine in the membrane.

Genetics

The mode of inheritance of this rare condition is autosomal dominant.
Clinical Features

1. Few symptoms, moderate anemia; red cell morphology, stomatocytic
2. Elevated reticulocytes
3. Splenomegaly and gallstones
4. MCHC elevated, MCV increased
5. Osmotic fragility reduced
6. Increased heat stability (46 and 49°C for 60 minutes).

Treatment

Transfusions are generally not required. The benefit of splenectomy is slight.

Enzyme Defects

There are two major biochemical pathways in the red cell: the Embden–Meyerhof anaerobic pathway (energy potential of the cell) and the hexose monophosphate shunt (reduction potential of the cell). Figure 7-3 illustrates the enzyme reactions in the red cell.

![Diagram of Enzyme Reactions](Image)

Fig. 7-3. Enzyme reactions of Embden–Meyerhof and hexose monophosphate pathways of metabolism. Documented hereditary deficiency diseases are indicated by enclosing dotted lines.)
Pyruvate Kinase Deficiency

Pyruvate kinase (PK) is an enzyme active in the penultimate conversion in the Embden–Meyerhof pathway. Although deficiency is rare, it is the most common enzyme abnormality in the Embden–Meyerhof pathway.

Genetics
1. Autosomal recessive inheritance
2. Significant hemolysis seen in homozygotes
3. Found predominantly in people of northern European origin
4. Deficiency not simply quantitative; probably often reflects the production of PK variants with abnormal characteristics.

Pathogenesis
1. Defective red cell glycolysis with reduced ATP formation
2. Red cells rigid, deformed, and metabolically and physically vulnerable (reticulocytes less vulnerable because of ability to generate ATP by oxidative phosphorylation).

Hematology
1. Features of nonspherocytic hemolytic anemia: macrocytes, oval forms, polychromatophilia, anisocytosis, occasional spherocytes, contracted red cells with multiple projecting spicules, rather like acanthocytes or pyknoocytes
2. Erythrocyte PK activity decreased to 5–20% of normal; 2,3-diphosphoglycerate (2,3-DPG) and other glycolytic intermediary metabolites increased (because of two- to threefold increase in 2,3-DPG, there is a shift to the right in \( P_{50} \))
3. Autohemolysis markedly increased, showing marked correction with ATP but not with glucose.

Clinical Features
1. Variable severity; can cause moderately severe anemia (not drug induced)
2. Usually presents with neonatal jaundice
3. Splenomegaly common but not invariable
4. Late: gallstones, hemosiderosis (from multiple transfusions), bone changes of chronic hemolytic anemia
5. Erythroblastopenic crisis due to parvovirus B19 infection.

Treatment
1. Folic acid supplementation
2. Transfusions as required
3. Splenectomy (if transfusion requirements increase); splenectomy does not arrest hemolysis, but decreases transfusion requirements.

Other Enzyme Deficiencies
1. Hexokinase deficiency, with many variants
2. Glucose phosphate isomerase deficiency

*Because of the right shift of \( P_{50} \), patients do not exhibit fatigue and exercise intolerance proportionate to the degree of anemia.
3. Phosphofructokinase deficiency, with variants
4. Aldolase
5. Triosephosphate isomerase deficiency
6. Phosphoglycerate kinase deficiency
7. 2,3-DPG deficiency due to deficiency of diphosphoglycerate mutase
8. Adenosine triphosphatase deficiency

These enzyme deficiencies have the following features:

1. **General hematologic features:**
   a. Autosomal recessive disorders except phosphoglycerate kinase deficiency, which is sex linked
   b. Chronic nonspherocytic hemolytic anemias (CNSHAs) of variable severity
   c. Osmotic fragility and autohemolysis normal or increased
   d. Improvement in anemia after splenectomy
   e. Diagnosed by specific red cell assays

2. **Specific nonhematologic features:**
   a. Phosphofructokinase deficiency associated with type VII glycogen storage disease and myopathy
   b. Triosephosphate isomerase deficiency associated with progressive debilitating neuromuscular disease with generalized spasticity and recurrent infections (some patients have died of sudden cardiac arrest)
   c. Phosphoglycerate kinase deficiency associated with mental retardation and a behavioral disorder.

Note the three exceptions to the general hematologic features listed above: (1) Adenosine deaminase excess (i.e., not an enzyme deficiency) is an autosomal dominant disorder. (2) Pyrimidine 5'-nucleotidase deficiency is characterized by marked basophilic stippling, although the other chronic nonspherocytic hemolytic anemias lack any specific morphologic abnormalities. (3) Deficiency of diphosphoglycerate mutase results in polycythemia.

**Glucose-6-Phosphate Dehydrogenase Deficiency**

Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme in the pentose phosphate pathway of glucose metabolism. Deficiency diminishes the reductive energy of the red cell and may result in hemolysis, the severity of which depends on the quantity and type of G6PD and the nature of the hemolytic agent (usually an oxidation mediator that can oxidize NADPH, generated in the pentose phosphate pathway in red cells).

**Genetics**

1. Sex-linked recessive mode of inheritance by a gene located on the X chromosome (similar to hemophilia).
2. Disease is fully expressed in hemizygous males and homozygous females.
3. Variable intermediate expression is shown by heterozygous females (due to random deletion of X chromosome, according to Lyon hypothesis).
4. As many as 3% of the world’s population is affected; most frequent among African Americans and those of Mediterranean origin.

The molecular basis of G6PD deficiency and its clinical implications follow:

1. Deletions of G6PD genes are incompatible with life because it is a housekeeping gene and complete absence of G6PD activity, called hydeletions, will result in death of the embryo.
2. Point mutations are responsible for G6PD deficiencies. They result in:
   a. *Sporadic mutations:* They are not specific to any geographic areas. The same
      mutation may be encountered in different parts of the world that have no
      causal (e.g., encountering G6PD Guadalajara in Belfast) relationship with
      malarial selection. These patients manifest with chronic nonspherocytic
      hemolytic anemia (CNSHA WHO Class I).
   b. *Polymorphic mutations:* These mutations have resulted from malaria selection;
      hence, they correlate with specific geographic areas. They are usually WHO
      Class II or III and not Class I.

The World Health Organization (WHO) classification of G6PD variants on the
basis of magnitude of the enzyme deficiency and the severity of hemolysis are shown
here:

<table>
<thead>
<tr>
<th>WHO Class</th>
<th>Variant</th>
<th>Magnitude of enzyme deficiency</th>
<th>Severity of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Harilaou, Tokyo, Guadalajara, Stonybrook, Minnesota</td>
<td>2% of normal activity</td>
<td>Chronic nonspherocytic hemolytic anemia</td>
</tr>
<tr>
<td>II</td>
<td>Mediterranean</td>
<td>3% of normal activity</td>
<td>Intermittent hemolysis</td>
</tr>
<tr>
<td>III</td>
<td>A−</td>
<td>10–60% of normal activity</td>
<td>Intermittent hemolysis usually associated with infections or drugs</td>
</tr>
<tr>
<td>IV</td>
<td>B (Normal)</td>
<td>100% of normal activity</td>
<td>No hemolysis</td>
</tr>
</tbody>
</table>

Pathogenesis

1. Red cell G6PD activity falls rapidly and prematurely as red cells age
2. Decreased glucose metabolism
3. Diminished NADPH/NADP and GSH/GSSG ratios
4. Impaired elimination of oxidants (e.g., $\text{H}_2\text{O}_2$)
5. Oxidation of hemoglobin and of sulfhydryl groups in the membrane
6. Red cell integrity impaired, especially on exposure to oxidant drugs and chemicals.

Clinical Features

Episodes of hemolysis may be produced by:

- Drugs (Table 7-7)
- Fava bean (broad bean, *Vicia fava*): ingestion or exposure to pollen from the
  bean’s flower (hence favism)
- Infection (in more susceptible subjects).

1. Drug-induced hemolysis
   a. Typically in African Americans but also in Mediterranean and Canton types
   b. List of drugs (see Table 7-7); occasionally need additional stress of infection
      or the neonatal state
   c. Acute self-limiting hemolytic anemia with hemoglobinuria
   d. Heinz bodies in circulating red cells
   e. Blister cells, fragmented cells, and spherocytes
   f. Reticulocytosis
   g. Hemoglobin normal between episodes
2. Favism
   a. Acute life-threatening hemolysis, often leading to acute renal failure caused by ingestion of fava beans
   b. Associated with Mediterranean and Canton varieties
   c. Blood transfusion required
3. Neonatal jaundice
   a. Usually associated with Mediterranean and Canton varieties
   b. Infants may present with pallor, jaundice (can be severe and produce ker-
      nicterus*), and dark urine.

   Often no exposure to drugs; occasionally exposure to naphthalene (mothballs),
   aniline dye, marking ink, or a drug. In a majority of neonates, the jaundice is not
   hemolytic but hepatic in origin.

4. Chronic nonspherocytic hemolytic anemia
   a. Occurs mainly in people of northern European origin
   b. Hematologic picture
      (1) Chronic nonspherocytic anemia
      (2) Reticulocytosis
      (3) Shortened red cell survival
      (4) Increased autohemolysis with only partial correction by glucose
      (5) Slight jaundice
      (6) Mild splenomegaly.

Treatment
1. Avoidance of agents that are deleterious in G6PD deficiency
2. Indication for transfusion of packed red blood cell in children presenting with
   acute hemolytic anemia:
   a. Hemoglobin (Hb) level below 7 g/dL
   b. Persistent hemoglobinuria and Hb below 9 g/dL
3. Chronic nonspherocytic hemolytic anemia (NSHA):
   a. In patients with severe chronic anemia: transfuse red blood cells to maintain
      Hb level 8–10 g/dL and iron chelation, when needed
   b. Indications for splenectomy
      (1) Hypersplenism
      (2) Severe chronic anemia
      (3) Splenomegaly causing physical impediment
   c. Genetic counseling and prenatal diagnosis for severe CNSHA if the mother
      is a heterozygote.

Other Defects of Glutathione Metabolism

Glutathione Reductase
In this autosomal dominant disorder, hemolytic anemia is precipitated by drugs hav-
ning an oxidant action. Thrombocytopenia has occasionally been reported. Neurologic
symptoms occur in some patients.

Glutamyl Cysteine Synthetase
In this autosomal recessive disorder, there is a well-compensated hemolytic anemia.

Glutathione Synthetase
In this autosomal recessive disorder, there is a well-compensated hemolytic anemia,
exacerbated by drugs having an oxidant action.

*The excessive jaundice is not only due to hemolysis but may be due to reduced glucuronidation of
bilirubin caused by defective G6PD activity in the hepatocytes.
Glutathione Peroxidase

In this autosomal recessive disorder, acute hemolytic episodes occur after exposure to drugs having an oxidant action.

**HEMOGLOBIN DEFECTS**

**Sickle Cell Disease**

**Incidence**

Sickle hemoglobin is the most common abnormal hemoglobin found in the United States (approximately 8% of the African-American population has sickle cell trait). The expected incidence of sickle cell disease (SCD) at birth is 1 in 625.

**Genetics**

1. Sickle cell disease is transmitted as an incomplete autosomal dominant trait.
2. Homozygotes (two abnormal genes) do not synthesize hemoglobin A (HbA); red cells contain 90–100% hemoglobin S (HbS).
3. Heterozygotes (one abnormal gene) have red cells containing 20–40% HbS.
4. HbS arises as a result of spontaneous mutation and deletion of the β-globin gene on chromosome 11, which results in selective advantage against *Plasmodium falciparum* malaria in carriers (balanced polymorphism).
5. α-Thalassemia (frequency of 1–3% in African Americans) may be co-inherited with sickle cell trait or disease. Individuals who have both α-thalassemia and sickle cell anemia are less anemic than those who have sickle cell anemia alone. However, α-thalassemia trait does not appear to prevent frequency or severity of vaso-occlusive complications.

Results of DNA polymorphism linked to the β* gene suggest that it arose from three independent mutations in tropical Africa:

1. Benin–Central West African haplotype (the most common haplotype)
2. Senegal–African West Coast haplotype
3. Bantu–Central African Republic (CAR) haplotype

The Benin type is also found in Ibadan, Algeria, Sicily, Turkey, Greece, Yemen, and southwest Saudi Arabia. In Caribbean and North American patients of African heritage with SCD, 50–70% of chromosomes are Benin, 15–30% are Bantu-CAR, and 5–15% are Senegal. The Benin and Senegalese patients have higher levels of fetal hemoglobin (Hbf) and fewer dense cells compared with Bantu-CAR patients. Patients with Senegal haplotype have the least severe disease, whereas patients with Bantu-CAR haplotype have most severe disease.

**Pathophysiology**

Figure 7-4 depicts the pathophysiology of sickle cell disease.
A single amino acid substitution (valine for glutamic acid) occurs in the β-polypeptide chain. This simple alteration has the following consequences:

1. Hemoglobin S has a higher net electrical charge than that of hemoglobin A and hence a different electrophoretic mobility.
2. Hemoglobin S in the reduced form (deoxygenated) is less soluble than hemoglobin A. The molecules form rod-like tactoids; these in turn distort the red cell, which takes on the sickle form (Figure 7-4).