The Coombs Test

Hemolytic anemia is characterized by lysis of patients’ red blood cells (see Figure 1). Hereditary hemolytic anemias involve cell membrane defects related to abnormalities in the production of certain proteins or enzymes, such as in the case of patients with hereditary spherocytosis or lupus. Acquired hemolytic anemias are disorders that occur when antibodies either develop or are introduced by transfusion against patients’ red blood cells. These antibodies attach to the red blood cells and cause hemolysis, an abnormality that is detected with a Coombs test. The cause of the formation of these antibodies often is unidentifiable. If this is the case, the hemolytic anemia is termed idiopathic. Several other laboratory tests are included in the diagnostic workup of hemolytic anemia in addition to the Coombs test (see Table 1).

Antibodies occasionally are formed in reactions to drugs such as penicillin, methyldopa, or fludarabine (Hoffbrand, Pettit, & Moss, 2001). In these cases, the drug is interacting with red blood cell membranes either directly or with the involvement of a complement or a drug-protein-antibody complex. Withdrawal of the causative agent leads to resolution of the hemolytic anemia. In other occasions, antibodies are formed in response to an autoimmune disease, such as lupus, or in the presence of advanced cancer, such as lymphoma.

Even more rarely, primary red cell aplasia may occur with lymphoma. Although not specific to hemolysis, anemia of chronic disease is common in malignant conditions with an apparent pathogenesis related to decreased release of iron from macrophages. This results in a decrease in erythroblasts, reduced red blood cell lifespan, and inadequate responses to erythropoietin. Other contributing factors to anemia for patients with cancer are well known, including marrow infiltration by disease, cancer treatment modalities that suppress marrow function, and inadequate nutrient intake (particularly iron and vitamin C necessary for red blood cell formation). All of these may lead to the patient eventually requiring a red blood cell transfusion.

The Coombs test is helpful in detecting the antibodies that are coating the transfused red blood cells and, therefore, helpful in confirming suspected transfusion reactions. The Coombs test does not have a range; it is read as either positive if antibodies are found or negative if no antibodies are detected. If no agglutination is detected, the test is read as negative (Pagana & Pagana, 2006).

Direct Coombs Test

Two types of Coombs tests are available, direct and indirect. The direct Coombs test demonstrates whether the patient’s red blood cells have been attacked by antibodies in the blood. To perform this test, a solution called Coombs serum is mixed with the patient’s red blood cells. Coombs serum is a solution that contains antibodies to human globulin. If the red blood cells have antibodies on them, then agglutination or clumping occurs; the greater the number of antibodies, the more agglutination. Levels are read on a scale from trace (very little antibodies) to +4 (the highest level of antibodies). If agglutination does not occur, the test is read as negative.
Indirect Coombs Test

The indirect Coombs test is the screen or cross-match component. This test is performed in the laboratory to determine if the patient has minor antibodies to the blood transfusion that he or she is about to receive. The indirect Coombs test also is used to test for cold agglutinins associated with mycoplasmal infections (Huether & McCance, 2008a). Cold agglutinins are detected in autoimmune anemias where the autoantibody attaches to the red blood cells in circulating peripheral blood where the temperature is cooled (best at 4°C) and drops off in warmer parts of the body. Cold agglutinins usually are idiopathic or sequel of infectious mononucleosis or mycoplasma pneumonia (Hoffbrand et al., 2001).

The indirect Coombs test is performed on the patient’s serum instead of the red blood cells themselves. The patient’s serum is added to the blood transfusion donor’s red blood cells to test for agglutination. If agglutination occurs (i.e., a positive test result), then the patient has antibodies against the blood and he or she should not receive the transfusion. If no agglutination occurs, it is safe to proceed with the transfusion.

Antibodies that can cause hemolysis are those of the ABO blood group system. Although a number of antigens exist in the blood, the A and B antigens found on the red blood cell membrane comprising the genetic combinations of the ABO blood typing system are two of the most important. If a patient’s red blood cells have A antigens on their surface, the plasma will contain anti-B antibodies. If a patient’s red blood cells have B antigens on their surface, the plasma will have anti-A antibodies. If a patient’s red blood cells have neither A nor B antigens on the red blood cells’ surface, both anti-A and anti-B antibodies will exist in the plasma (see Table 2).

The lack of antigens on the surface of red blood cells of Type O blood is why Type O is commonly called the universal donor. Type AB patients are considered universal recipients because they lack anti-A and anti-B antibodies in their plasma.

When a patient is undergoing a workup for a hemolytic anemia that is suspicious for an underlying autoimmune etiology, the direct antiglobulin test will aid in making the determination. The test will determine if autoantibodies are present in the patient’s red blood cells. A patient may have had previous incompatible blood transfusions that have caused the circulating red blood cells to be coated with immunoglobulin G and/or complement, thus making them targets for their reticuloendothelial system (also known as the mononuclear phagocyte system). This system consists of monocyte-derived phagocytic cells that are located in special tissues (the liver, lungs, spleen, kidney, brain, and lymph nodes) that ingest and destroy circulating microorganisms, unwanted debris such as cell fragments and dead or injured cells, and foreign protein particles (Huether & McCance, 2008b). Premature destruction of red blood cells by the reticuloendothelial system is one of the potential etiologies which would fall under a diagnosis of acquired hemolytic anemia.

Case Study

Mr. P is anemic and requires two units of packed red blood cells. His red blood cells have A antigens on their surfaces and his plasma contains anti-B antibodies; therefore, his blood type is confirmed

Table 1. Laboratory Testing in the Diagnosis of Hemolytic Anemia

<table>
<thead>
<tr>
<th>FINDING</th>
<th>DESCRIPTION</th>
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</thead>
<tbody>
<tr>
<td>LDH</td>
<td>The hemolytic process releases LDH, an enzyme abundant in erythrocytes. A rising LDH may be an indicator of increasing hemolysis (Pagana &amp; Pagana, 2006).</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Released with the breakdown of hemoglobin from the erythrocytes, generating an elevated serum unconjugated bilirubin level (Hoffbrand et al., 2001)</td>
</tr>
<tr>
<td>Hemosiderin</td>
<td>Another byproduct of hemoglobin degradation and is measured in the urine (Hoffbrand et al., 2001)</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>When free hemoglobin is circulating, haptoglobin (a protein produced in the liver) binds to and inhibits its oxidative activity. The haptoglobin-hemoglobin complex is eliminated via the reticuloendothelial system (Hoffbrand et al., 2001). A haptoglobin assay will show declining levels as more haptoglobin is required to bind to free hemoglobin during hemolysis.</td>
</tr>
<tr>
<td>Shistocytes</td>
<td>The breakdown of red blood cells produces fragments of the cell membranes known as shistocytes visible on a peripheral blood smear (Lynch, 2006).</td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>Looking at hematology markers, an elevated reticulocyte count may be expected because reticulocytes or immature red blood cells are released in large numbers when the bone marrow is stimulated to respond to the anemia (Pagana &amp; Pagana, 2006).</td>
</tr>
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LDH—lactate dehydrogenase
as A. Mr. P’s blood sample is taken and spun in a centrifuge to obtain plasma for an indirect Coombs test. The plasma added to the donor’s red blood cells and is found to be a suitable match because his red blood cells have neither A nor B antigens on them. The antibodies present in Mr. P’s blood will not attack these “unmarked” cells.

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References


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