Case report: serologic confirmation of paroxysmal cold hemoglobinuria

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A child with a history of recent viral infection entered the hospital with severe anemia, hemoglobinuria, and suspected autoimmune disease. Serologic findings included a positive direct antiglobulin test and incompatible crossmatches. Extensive studies, including a Donath-Landsteiner test, confirmed paroxysmal cold hemoglobinuria. The child was transfused several times with washed red blood cells compatible by prewarm technique. Although hemolysis continued after each transfusion, he stabilized and went home 5 days after his hospital admission. He reportedly made a complete recovery. Immunohematology 1992;8:19-21.

Historically, paroxysmal cold hemoglobinuria (PCH) was seen as a chronic disease in patients with syphilis. Today it is usually seen in an acute, transient form in children under 5 years of age with a recent history of viral infection. It is often considered to be a rare condition, and, because of its transient nature, may be frequently undiagnosed.1,2

The most common clinical signs of PCH, severe anemia and hemoglobinuria, are caused by an IgG autoantibody. This antibody is a biphasic hemolysin because it binds to red blood cells (RBCs) at cooler temperatures in the patient’s extremities, then causes complement-mediated lysis of the RBCs at the warmer core body temperature. Since the process may deplete the patient’s own complement, a source of fresh complement is often needed to recreate the scenario in the in vitro Donath-Landsteiner (D-L) test. This test is generally considered the confirmatory test for PCH. Historically, hemoglobinuria was seen most often after the patient was exposed to cold temperatures, but the acute type most often seen today in children is apparently not related to exposure to the cold.1

Although a positive D-L test is usually specific for PCH, especially in children under 5, it can also be positive in cold hemagglutinin disease (CHD).1 One differentiating factor is the antibody specificity in the two diseases. The PCH antibody is usually anti-P, while CHD often has anti-I, -i, or -Pr specificity.1

The mechanism for the development of the PCH antibody is unknown. It may be due to viral alteration of the RBC membrane. Another possibility is alteration of suppressor T-cell function. The excellent review of PCH by Heddle1 is highly recommended for additional information.

Case Report

A 17-month-old male was admitted to the hospital with severe anemia on the evening of July 17, 1990. He was transferred from another hospital where his last reported hematocrit (Hct) was 15 percent. The patient’s physician ordered a transfusion of 120 cc of red blood cells (RBCs). A direct antiglobulin test (DAT) was also ordered, since autoimmune hemolytic anemia was suspected. The child had a history of recent viral infection, and hemoglobinuria was present at admission.

Materials and Methods

Initial testing, including ABO and Rh type, antibody screen, crossmatches, and DAT, was performed according to the reagent manufacturer’s instructions (Gamma Biologicals, Houston, TX), using standard tube methods. LISS was used as an enhancement medium in the antibody screen and crossmatch procedures. Prewarm technique was performed according to the standard reference,3 with the exception (noted in the Results section) of the initial prewarm testing done by the hospital blood bank.

The D-L test was performed using recommendations from two references.1,3 An ABO-compatible laboratory employee donated blood, and the serum was used as a source of fresh complement and as a negative control. Reagent manufacturer’s antibody screening cells (Gamma) were used as a source of P-positive cells. The test was performed as follows: a specimen
was carefully drawn and kept at 37°C until the serum was separated. The patient's serum and P+ cells were incubated both with and without fresh complement in a bath of melting ice for 60 minutes, then incubated at 37°C for 30 minutes. The negative control serum was likewise incubated with P+ red cells. An identical set of tubes was also incubated at 37°C for 60 minutes with no exposure to cold. All tubes were centrifuged and examined for presence of hemolysis at the end of the incubation periods.

Testing with rare P- cells to confirm the presence of anti-P was done by the reference laboratory (Pacific Northwest American Red Cross Regional Blood Services, Portland, OR).

Results

Initial testing by the hospital blood bank technologist showed the patient to be B positive. The antibody screen and autocontrol were weakly positive both at 37°C after incubation with LISS and in the antihuman globulin (AHG) phase. Crossmatches with RBC samples from six different donors were incompatible. The DAT results were anti-IgG: 1+, anti-C3: 2+. The anti-IgG was not heavy chain specific. A prewarm technique was attempted on the antibody screen and crossmatches, but polyspecific AHG was used and weak positive reactions remained.

A new specimen was drawn and sent to the reference laboratory for further studies. A conversation with the reference laboratory evening shift technologist brought up the possibility of PCH. The reference laboratory staff found the DAT on their specimen to be positive only with anti-C3d. Using strict prewarm technique with anti-IgG AHG, they got negative reactions. The hospital blood bank repeated the prewarm technique using anti-IgG AHG and found all tubes except the autocontrol to be negative. It was then early on the morning of July 18, 1990, and an attempt was made to transfuse the child with 60 cc of RBCs. See Table 1 for laboratory values and transfusion history during the first 24 hours of the patient's hospital stay.

Following the first transfusion of 60 cc of RBCs, the patient's Hct was 12.3 percent. There was some suspicion that the blood had infiltrated, so it was likely that none of it actually entered the child's circulation.

During the morning of July 18, 1990, the day shift staff at the hospital blood bank and at the reference laboratory ran special tests to prove the suspected PCH. Using instructions received from the reference laboratory, the hospital ran a D-L test. The results are seen in Table 2. Only when the patient's serum was first incubated in the melting ice bath with fresh complement, then warmed to 37°C, did hemolysis occur. This confirmed the presence of a biphasic hemolysin typical in PCH. The reference laboratory was able to test the serum with rare P- cells and confirm the anti-P specificity.

With the diagnosis of PCH established by late morning of July 18, 1990, another attempt at transfusion was made. It was unknown whether the child would hemolyze transfused RBCs as quickly as or more so than his own. Since literature on this disease indicated that transfusion with rare P- blood was seldom required,1,2 the child was given washed RBCs compatible by prewarm technique. Two aliquots of 55 cc each of washed RBCs were transfused through a blood warmer over a 4-hour period. One hour after the last aliquot, the Hct was 23.0 percent and the plasma Hb was 43.2 mg/dL. Five hours later the Hct was 20.3 percent and the plasma Hb was 39.0 mg/dL (Table 1), indicating that hemolysis was still occurring.

Table 1. Laboratory test results and transfusion history during the initial 24 hours

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal values</th>
<th>7/17/90 6:00 p.m.</th>
<th>7/18/90 12:59 a.m.</th>
<th>7/18/90 8:21 a.m.</th>
<th>7/18/90 4:30 p.m.</th>
<th>7/18/90 9:30 p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10³)</td>
<td>4-10</td>
<td>25.7</td>
<td>—</td>
<td>25.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RBC (×10⁵)</td>
<td>4.4-6.4</td>
<td>1.57</td>
<td>—</td>
<td>1.46</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>14-18</td>
<td>5.1</td>
<td>—</td>
<td>4.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>42-52</td>
<td>13.8</td>
<td>—</td>
<td>12.3</td>
<td>23.0</td>
<td>20.3</td>
</tr>
<tr>
<td>Plasma Hb (mg/dL)</td>
<td>2-6</td>
<td>—</td>
<td>26.4</td>
<td>—</td>
<td>43.2</td>
<td>39.0</td>
</tr>
</tbody>
</table>

Transfusion history
7/18/90 @ 2:30 a.m., 60 cc RBCs transfused (infiltrated)
7/18/90 @ 11:00 a.m., 55 cc washed RBCs transfused
7/18/90 @ 1:20 p.m., 55 cc washed RBCs transfused
Table 2. Donath-Landsteiner test results

<table>
<thead>
<tr>
<th>Tube contents</th>
<th>60 min. in melting ice bath followed by</th>
<th>30 min. at 37°C</th>
<th>60 min. at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's serum + P+ RBCs</td>
<td>NH*</td>
<td>NH*</td>
<td></td>
</tr>
<tr>
<td>Control serum + P+ RBCs</td>
<td>NH*</td>
<td>NH*</td>
<td></td>
</tr>
<tr>
<td>Patient's serum + P+ RBCs + complement</td>
<td>H†</td>
<td>NH*</td>
<td></td>
</tr>
</tbody>
</table>

* No hemolysis  † Hemolysis

Following the transfusions on July 18, 1990, the Hct reverted to 15.0% over the course of 3 days (Table 3). Another 200 cc of washed RBCs were transfused on July 22, 1990, and the patient was discharged from the hospital. Additional laboratory results were not available. The patient went to a private physician and was reported to have made a complete recovery.

Table 3. Subsequent laboratory values and transfusion history

<table>
<thead>
<tr>
<th>Test</th>
<th>7/19/90 4:30 p.m.</th>
<th>7/19/90 6:28 p.m.</th>
<th>7/20/90 6:00 a.m.</th>
<th>7/21/90 3:00 p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct(%)</td>
<td>23.8</td>
<td>20.9</td>
<td>18.3</td>
<td>15.0</td>
</tr>
<tr>
<td>Plasma Hb(mg/dL)</td>
<td>33.4</td>
<td>8.9*</td>
<td>27.4</td>
<td>49.8</td>
</tr>
<tr>
<td>WBC (x 10^3)</td>
<td>49.8</td>
<td>43.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Transfusion history

7/19/90 @ 12:15 a.m., 100 cc washed RBCs transfused
7/22/90 @ 9:00 a.m., 100 cc washed RBCs transfused
7/22/90 @ 5:00 p.m., 100 cc washed RBCs transfused

* Specimen clotted

Discussion

This was the first diagnosed case of PCH seen at this hospital within the recollection of the technologists working here; however, several authors suggest that the disease is more common than diagnosed. It is helpful if PCH is suspected early in the disease state, as the antibody may disappear rapidly once recovery is in progress.

It was only through a series of unusual circumstances that this case was recognized by the blood bank. If the hospital technologist who performed the original prewarm technique had remembered to use anti-IgG AHG instead of the polyspecific AHG, the only remaining serologic abnormality would have been a positive DAT. If the anti-IgG had been heavy chain specific, the DAT would have been positive with anti-C3 only. This would have been reported to the physician, but no further workup would have been initiated by the hospital technologists, and the reference laboratory would not have been contacted for assistance.

After the child was discharged, it was difficult to obtain background information on the patient or to gather all laboratory testing done. It would have been desirable to explore more thoroughly some of the interesting phenomena, such as erythrophagocytosis associated with PCH disease states. Although reportedly common in PCH, erythrophagocytosis was not reported in this case.

This experience resulted in greater awareness by the blood bank staff of the possibility of PCH when confronted with unusual laboratory test results, and it also added a new procedure, the D-L test, to our manual. Future cases may be more quickly recognized and greater attention given to the patient's history as a result of this unique case.

Acknowledgments

The author acknowledges the dedicated staff at Emanuel Hospital Blood Bank, which rose to the challenge of new procedures to help confirm the diagnosis, the staff at the Pacific Northwest Regional Blood Services Red Cell Reference Laboratory, which first suggested the possibility of PCH and assisted in making the diagnosis, and N.G. Orfanakis, MD, Blood Bank Medical Director, Emanuel Hospital, for reviewing the manuscript.

References


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