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Recurrent Donath-Landsteiner hemolytic anemia: a pediatric case report

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Abstract

BACKGROUND—Paroxysmal cold hemoglobinuria (PCH) is a form of autoimmune hemolytic anemia caused by the Donath-Landsteiner antibody (D-L antibody). In children, this is typically a transient immune-mediated hemolysis that follows a viral illness and does not recur. Recurrent acute or chronic PCH due to D-L antibody is very rare.

CASE REPORT—We have reported a unique case of recurrent PCH in a 5-year-old boy with two acute episodes of hemolysis separated by 21 months of hematologic remission. Each episode was severe requiring red blood cell transfusions, intravenous methylprednisolone, and intravenous immunoglobulin during the second episode. Testing identified recurrence of the D-L antibody with the classic anti-P biphasic hemolysis.

CONCLUSION—This demonstrates that PCH can be a recurrent disease in the pediatric population (in the absence of syphilis) with the classical D-L antibody.

Paroxysmal cold hemoglobinuria (PCH) is a form of autoimmune hemolytic anemia historically associated with syphilis that is caused by the Donath-Landsteiner antibody (D-L antibody). PCH can also occur after febrile illnesses in the pediatric population.¹ Some studies report that 30% to 40% of pediatric patients presenting with autoimmune hemolytic anemia demonstrate the pattern of complement binding at cold temperatures that defines the D-L antibody.²

The classic presentation of PCH is an acute episode of immune-mediated hemolysis that starts 1 to 3 weeks after a viral infection.^{1,3,4} Children present with hemoglobinuria, jaundice, pallor, and severe anemia with hemoglobin (Hb) levels reported to be less than 5 g/dL. The key laboratory findings associated with this diagnosis are 1) a positive direct antiglobulin test (DAT), 2) biphasic testing for the D-L antibody, and 3) spherocytes and other signs of immune-mediated hemolysis on the peripheral blood smear. The D-L antibody is typically a polyclonal autoantibody of the immunoglobulin (Ig)G class often with anti-P specificity and with a characteristic “biphasic hemolysin” behavior.^{1,4}

D-L antibody binds to red blood cell (RBC) surface antigens at temperatures below core body temperature and fixes early complement components. With warming to body temperature, the terminal complement sequence is activated and RBCs are lysed, causing massive intravascular hemolysis. The C3 DAT may be positive, but the complement-fixing IgG antibody dissociates from the RBC surface and is classically not detectable in the IgG DAT performed at 37°C.¹

Recurrent acute or chronic PCH due to D-L antibody is very rare. The four cases in the pediatric population in which recurrent episodes were reported did not have the classic anti-P specificity. We present a case of recurrent acute PCH in a 5-year-old boy with proven anti-P specificity and characteristic biphasic hemolysis during both episodes.

CASE REPORT

A 3-year-old previously healthy male presented to the emergency department with 2 days of fever, emesis, jaundice, and dark urine. Vital signs demonstrated a temperature of 36.6°C, heart rate of 104 beats/min (normal, 70–110 beats/min), blood pressure of 116/58 mmHg (normal, 76–109/42–65 mmHg), respiratory rate of 20 breaths/min, and pulse oximetry readings of 96% to 99% in room air. His physical examination was notable for scleral icterus, dry mucous membranes, and no hepatosplenomegaly. Urine dipstick noted large blood with microscopy showing only 0 to 2 RBCs suggesting hemoglobinuria. His serum total bilirubin was elevated to 5.1 mg/dL (normal, 0.6–1.4 mg/dL), with aspartate aminotransferase of 307 IU/L (normal, 20–60 IU/L), and lactate dehydrogenase (LDH) of 8816 IU/L (normal, 500–920 U/L). Hb was 7.4 g/dL (normal, 11.5–13.5 g/dL) on admission, but decreased to 4.1 g/dL within 30 hours. He received a transfusion of 10 mL/kg RBCs at that time. The DAT was 2+ positive for both polyspecific and C3, while the monospecific IgG DAT was negative. The serum antibody screen was negative. A sample was sent to the immunohematology reference laboratory and demonstrated presence of the D-L antibody (see Table 1). The PCH was presumed to be secondary to a viral illness that occurred several weeks before presentation. However, no respiratory or gastrointestinal viral testing was performed. He was treated with 1 mg/kg/day methylprednisolone divided every 6 hours, and provisions were made to maintain a warmed hospital room and bed. LDH steadily decreased, and the reticulocyte count increased to a maximum of 22.9% over the subsequent 7 days after initiation of steroids (Fig. 1A). His hemoglobinuria resolved before discharge. Methylprednisolone was changed to oral prednisone and was slowly tapered over a 6-week period as an outpatient. The patient returned to his normal state of health with recovery of his Hb level to 13.0 g/dL and normal reticulocyte count of 1.1%. An interval DAT was not obtained.

Twenty-one months later, the patient, now 5 years old, presented with recurrent fevers, emesis, and dark urine for 2 days. DAT was microscopically 3+ positive for C3 reagent, while negative for monospecific IgG and polyspecific reagents. He was initially restarted on a 5-day course of 1 mg/kg/day prednisone and followed as an outpatient with Hb level of 10.9 gm/dL (hematocrit [Hct] 32.2%). However, he continued to experience emesis, including after doses of oral steroids. He presented again 2 days later with a history of black urine, increased lethargy, altered mental status, and extreme pallor. Repeat laboratory

evaluation at the referring institution demonstrated a Hct level of between 6 and 7% and a Hb value that could not be quantified. He was tachycardic with heart rate of more than 150 beats/min with pulse oximetry measurements of 85% on nonrebreather mask with 100% oxygen.

At this point he was transferred to a tertiary care hospital for further management in the pediatric intensive care unit. He received an emergent 10 mL/kg uncrossmatched group O D– RBCs during transport and his Hb level increased to 3.9 g/dL. Peripheral smear suggested recurrence of immune-mediated hemolysis with findings seen in Fig. 2. His urine dipstick demonstrated large blood; however, urine microscopy again showed just 0 to 2 RBCs. Additional laboratory measures were significant for serum total bilirubin of 3.7 mg/dL and LDH of 6240 IU/L. Intravenous (IV) methylprednisolone was started at 2 mg/kg/day divided every 6 hours and intravenous immunoglobulin (IVIG) at 1 g/kg was administered. He received an additional 17.5 mL/kg RBCs over the next 24 hours with appropriate increase in Hb level to 9.8 g/dL (Fig. 1B). DAT was 2+ positive for polyspecific, 3+ positive for C3, and negative for monospecific IgG. Repeat D-L antibody testing was completed (after administration of IVIG and RBC transfusions) at the immunohematology reference laboratory (Table 1) demonstrating a recurrent anti-P specific IgG D-L antibody.

After transfusions and treatment with IVIG and methylprednisolone, the patient's Hb, LDH, and reticulocyte count improved, as shown in Fig. 1B. He was transitioned to 1 mg/kg oral prednisone BID before discharge. During this admission, he was found to be positive for the respiratory syncytial virus, a possible trigger for the recurrence of PCH. Approximately 1 week after discharge, his Hb level remained stable at 10.9 g/dL (still with an elevated reticulocyte count of 9.4%). His steroid wean was initiated at that time with a 25% decrease in his dose, which was subsequently decreased weekly over the course of the month. His Hb level increased to 13.8 g/dL (reticulocyte count, 5.2%) 3 weeks after discharge and then to 15.1 g/dL at the time his steroids were discontinued.

MATERIALS AND METHODS

The Donath-Landsteiner test was performed according to the method described by Mallory⁵ based on the original method of Dacie and Lewis.⁶ Blood samples were drawn, kept warm, and walked directly to the blood bank per hospital blood bank protocol where separation of the serum from the RBCs was performed. Separated samples were used for the Donath-Landsteiner test. Briefly, fresh serum from the patient, separated at 37°C, was tested with type O screening cells, I– and PP₁P^K– RBCs with fresh inert serum as a source of complement. Two sets of tests were prepared as demonstrated in Table 1. The first set, the patient sample, was incubated at 4°C for 60 minutes, centrifuged and read for hemolysis, incubated at 37°C for 30 minutes, centrifuged, and read for agglutination and hemolysis, and then RBCs were washed and tested with polyspecific antiglobulin sera. The second set, the controls, was warmed to 37°C, incubated at 37°C for 90 minutes, centrifuged, and read for hemolysis and agglutination, and then RBCs were washed and tested with polyspecific antiglobulin sera.

DISCUSSION

PCH is a cold autoimmune hemolytic anemia causing transient hemolysis often after an upper respiratory viral syndrome. The anemia can be mild and self-limited or severe, requiring RBC transfusions and treatment with corticosteroids or IVIG. When associated with chronic syphilis, PCH can be recurrent. However, in the pediatric population, the hemolysis mediated by D-L antibody is almost always an acute event that does not recur.^{1,2,4}

RBC destruction occurs primarily by intravascular hemolysis with activation of the full complement cascade, intravascular cell lysis, and liberation of free Hb into the serum. However, extravascular hemolysis with complement fixation and reticuloendothelial system clearance of RBCs coated with C3b and C4b is also involved. Because of both extravascular and intravascular hemolysis, peripheral blood smears can exhibit 1) RBC rosetting around neutrophils, 2) erythrophagocytosis by neutrophils, and 3) spherocytosis.²

The previous literature contains four pediatric cases of recurrent PCH. Two of these cases did not demonstrate the anti-P specificity and in the third case the antibody specificity was not reported. Our patient and the patients in the previous case reports showed a similar age of onset, each consistent with the finding that the majority of children with autoimmune hemolytic anemia initially present at age less than 4 years old.³ The first case was a report of a 3-year-old girl who had a D-L antibody of the IgG subtype, but with specificity other than anti-P. She had a recurrence 8 months later, again with an IgG antibody and specificity other than anti-P, but the D-L antibody testing was negative at the time of recurrence. The second case was a 3-year-old boy who was D-L antibody positive (without further details) who had a recurrence 22 months later that was D-L antibody negative. It is possible that testing at the time of recurrence was a false-negative result due to low antibody titers or to a testing technique without addition of normal serum as a source of complement.^{7,8}

The third report describes a 3-year-old boy who had a recurrence of PCH 7 months after initial presentation. The IgM antibody did not have anti-P or anti-I specificity at either time point despite positive D-L antibody testing. Ogoose and colleagues⁹ suggested that there was production of a new D-L antibody because of different temperature thresholds for hemolysis at initial presentation and recurrence. They postulated that D-L antibody with specificities other than anti-P are more prone to recur.

Karabin and colleagues⁴ discuss a pediatric case of chronic PCH in a 5-year-old girl with an IgM antibody and anti-I specificity. Her course began as a recurrence of hemolysis 1 year after an initial PCH episode, but became chronic, requiring steroids, rituximab, and IVIG over 39 months.

In the case we present there were two acute episodes of hemolysis separated by 21 months of hematologic remission. In the few recurrent cases discussed, the recurrences occurred 7 to 22 months after initial presentation of hemolysis. We were able to demonstrate the same D-L antibody showing the classical anti-P specificity in both the initial and the recurrent presentation unlike the previous case reports of biphasic antibodies with specificities other than anti-P.² Both episodes of hemolysis were life-threatening for our patient, with Hb values reaching nadirs of 4.1 g/dL in the first episode and approximately 2 g/dL in the

second episode, with the patient requiring RBC transfusions, high-dose steroid treatment, and IVIG. He had reticulopenia at diagnosis, which has been described in previous cases and is hypothesized to be due to attack on RBC precursors which also express the P antigen.¹⁰ As the reticulocyte count began to increase, the Hb recovered appropriately.

The treatment for Donath-Landsteiner PCH is generally supportive including environmental warming and RBC transfusions as needed. Steroids are typically most effective for warm antibody autoimmune hemolytic anemia; however, they remain the standard treatment in cold antibody autoimmune hemolytic anemia as well. Plasmapheresis can be used for both cold and warm antibody autoimmune hemolytic anemia although it is presumed to be more effective in cold agglutinin disease since IgM is primarily intravascular. Roy-Burman and Glader¹¹ report a case of Donath-Landsteiner hemolytic anemia responsive to plasmapheresis, suggesting that perhaps this may be helpful for future cases. Other immunosuppressive agents, such as rituximab and cyclophosphamide, have also been used in refractory cases.^{10,12} In addition to immune suppression, there are case reports of the use of eculizumab in adults with cold agglutinin-mediated hemolysis, but the efficacy of complement blockade in D-L hemolysis is unknown.^{13,14} To our knowledge, there are no prior case reports of acute recurrence of such severity in the pediatric population.

In conclusion, we have reported a unique case of PCH in a child with well-documented recurrence of the Donath-Landsteiner anti-P biphasic hemolysin. This demonstrates that PCH can be a recurrent disease in the pediatric population (in the absence of syphilis) with the classical D-L antibody. Outpatient follow-up after an initial episode of PCH is imperative to ensure resolution of the primary event. Counseling of the patient and family should include the rare possibility of recurrence and should emphasize the signs and symptoms for which medical evaluation should be sought. Although it might be of interest to repeat the antibody testing during remission, once the Hb has stabilized we cannot conclude that a negative D-L antibody during remission would be reassuring or conversely that persistent detection of the antibody would necessarily predict clinical recurrence.

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ABBREVIATIONS

D-L antibody	Donath-Landsteiner antibody
PCH	paroxysmal cold hemoglobinuria.

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Fig. 1.

Hb (■) and reticulocyte (▲) trends (left vertical axis) and LDH (●) trends (right vertical axis) with time from initial presentation on the horizontal axis. Treatments with IV methylprednisolone, IVIG, and transfusions of RBCs are noted with arrows at the time of administration. (B) Hb value at 2 g/dL is highlighted to indicate that it was estimated from the measured Hct of 6% to 7%. [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 2.
Two representative fields of the peripheral blood smear demonstrating prominent RBC rosetting around neutrophils, remarkable hemophagocytosis, and spherocytes. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Defining serologic characteristics of the D-L antibody, hemolysis with a biphasic temperature incubation and P antigen specificity, were demonstrated in samples from both episodes as shown*

Untreated cells	Test 4 to 37°C		Control, 37°C only
	4°C	37°C	
First episode			
Screening Cell I	No hemolysis	Complete hemolysis	No hemolysis
Screening Cell II	No hemolysis	Complete hemolysis	No hemolysis
Screening Cell III	Not tested	Not tested	Not tested
I–	No hemolysis	Complete hemolysis	No hemolysis
PP ₁ P ^K –	No hemolysis	No hemolysis	No hemolysis
Second episode			
Screening Cell I	No hemolysis	Complete hemolysis	No hemolysis
Screening Cell II	No hemolysis	Complete hemolysis	No hemolysis
Screening Cell III	No hemolysis	Complete hemolysis	No hemolysis
I–	No hemolysis	Partial hemolysis	No hemolysis
PP ₁ P ^K –	No hemolysis	No hemolysis	No hemolysis

* Hemolysis was observed when the test was moved from 4 to 37°C and no hemolysis was observed when the control was maintained at 37°C. No hemolysis was observed in biphasic test conditions when PP₁P^K– cells were tested. The possibility of anti-I specificity was excluded, since I– P+ RBCs were hemolyzed. Tubes with no visible hemolysis were tested for agglutination after addition of polyspecific anti-human globulin (not included in table). Weak reactivity was observed with Screening Cells I and II during the first episode at 37°C. Screening Cell III was not tested during the first episode (as it was not available at that time).