

# Delayed hemolytic transfusion reaction and paroxysmal cold hemoglobinuria: an unusual association

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An 80-year-old female presented with melena and anemia due to bleeding from a benign gastric ulcer. Her blood group was O, D+. The serum contained anti-B and a weak anti-A (titer 2 at 18°C). She was inadvertently transfused with approximately 3.5 units of group A red blood cells with no initial ill effects. One week later, the anti-A titer increased to 8 and the direct antiglobulin test (DAT) was weakly positive (IgG and C3d). The next day, intravascular hemolysis became evident. The DAT was still weakly positive and the serum contained a weak cold autoagglutinin, which did not correlate with the severity of the hemolysis. A Donath-Landsteiner test was performed and found to be strongly positive. The antibody showed P specificity, confirming a diagnosis of paroxysmal cold hemoglobinuria (PCH). Exchange transfusion was followed by rapid recovery even though the Donath-Landsteiner test remained positive for at least a month. The patient was well when last seen 11 months after presentation. It was thought that the original low titer of anti-A reflected compromised immune homeostasis in an elderly patient and that stimulation by incompatible blood in those circumstances resulted in a delayed hemolytic transfusion reaction that triggered, exacerbated, or was accompanied by an autoimmune response manifesting as PCH. *Immunohematology* 1997;13:54-57.

Blood transfusion has been implicated in the development of red blood cell (RBC) autoantibodies and autoimmune hemolytic anemia (AIHA). Although this is not a frequently recognized phenomenon, there are a number of reports in the literature,<sup>1,2</sup> with autoantibody development being associated in particular with the production of alloantibodies and transfusion reactions.<sup>1,3-7</sup> In this report, we discuss a patient with Donath-Landsteiner antibody that developed in association with a delayed hemolytic transfusion reaction (DHTR). To our knowledge, this is the first example of paroxysmal cold hemoglobinuria (PCH) presenting in this manner.

## Case Report

An 80-year-old female patient was admitted to the hospital as an emergency with melena and severe anemia. The hemoglobin level was 69 g/L, representing a

decrease of 55 g/L during 12 days. She was group O, D+, and her serum contained anti-B but a weak anti-A (saline titer of only 2 against A<sub>1</sub> RBCs at 18°C). She was inadvertently transfused with 3.5 units of group A, D+ blood due to clerical error. There were no immediate adverse effects. The hemoglobin increased to 127 g/L 2 days later. Endoscopy showed a large gastric ulcer that appeared to be malignant. Biopsies were taken. However, histological examination showed no evidence of neoplasia, and she was treated with oral cimetidine. The transfusion error was identified within 24 hours, at which time it was noted that the anti-A titer had dropped to < 1 and her direct antiglobulin test (DAT) was weakly positive with anti-IgG and -C3d. There were insufficient RBCs for an eluate. The patient was kept under close medical observation with daily coagulation, urea, and electrolyte assessments.

One week posttransfusion, the DAT was still weakly positive with anti-IgG and -C3d. The following day, the patient collapsed; her hemoglobin level had decreased to 81 g/L and intravascular hemolysis was evident with jaundice, hemoglobinemia, hemoglobinuria, and a serum haptoglobin concentration of < 0.1 g/L; a peripheral blood film showed marked spherocytosis. The anti-A saline titer at 18°C increased to 8 and was now > 128 by the anti-IgG indirect antiglobulin test (IAT). Urea and creatinine levels rose from 6.6 to 16.1 mmol/L (normal levels are 2.5 to 5.5) and from 58 to 105 μmol/L (normally 60 to 120), respectively. The DAT was now weakly positive (C3d, only) and the serum contained a weak cold agglutinin at 18°C. These findings suggested that a DHTR alone would not account for the severity of the hemolysis. Donath-Landsteiner and Ham's tests were performed. The Ham's test was negative. The Donath-Landsteiner test

was positive with the antibody showing P specificity. This result confirmed a diagnosis of PCH. Unfortunately, the Donath-Landsteiner test could not be performed retrospectively because a pretransfusion serum sample was no longer available. There was no clinical evidence for a diagnosis of PCH prior to transfusion. The serological test for syphilis was negative. By the 9th day posttransfusion, the anti-A titer at 18°C had increased to 64, and the IgG IAT titer was > 128. Exchange transfusion with group O, D+ blood was undertaken (4 units of RBCs given, 4 removed) with good effect, followed by transfusion with 2 more units of group O, D+ RBCs.

Two days later, the patient was remarkably well; her hemoglobin was 124 g/L, urea 7.7 mmol/L, creatinine 85 µmol/L, bilirubin 10 µmol/L, and a coagulation screen was normal. She was discharged shortly afterward with maintenance doses of cimetidine. The Donath-Landsteiner test remained positive and her DAT remained weakly positive for more than 1 month, but there was no evidence of continuing hemolysis and the haptoglobin concentration remained normal. The patient was in good general health 11 months after presentation; however, the Donath-Landsteiner test was not performed at that time.

## Materials and Methods

The immunohematologic investigations conducted at this center have been described previously.<sup>8-11</sup> The blood samples examined were taken from the patient just before the initial transfusion (day 0) and on days 1, 2, 5, 6, 7, 8, 9, and 41.

Serum was tested in doubling dilutions against A<sub>1</sub> RBCs in saline at 18°C and by a 37°C IAT with anti-IgG. The reactions were examined microscopically and the

results expressed as the reciprocal of the greatest dilution at which agglutination was seen.

Indirect Donath-Landsteiner tests were carried out as described previously.<sup>11</sup> Briefly, two sets of the patient's serum (serially diluted to 16) were mixed in the presence of complement with pooled group O RBCs from normal subjects. One set of dilutions was placed in melting ice (0°C) for 1 hour before being incubated at 37°C for 1 hour; the second set was maintained at 37°C for 2 hours. After mixing and centrifugation, the supernatants were examined. Lysis, present only in samples that had been cooled and rewarmed, would denote a positive result, in which case the test would be repeated using pp (P-) RBCs.

A Ham's (acidified serum lysis) test for paroxysmal nocturnal hemoglobinuria (PNH) was performed in the standard way using acidified, complement-rich sera and patient's RBCs.

## Results

The initial tests carried out at this center (day 1) showed that the patient was group O with virtually no anti-A in the serum (saline titer 2 at 18°C). The corresponding anti-B titer was 16. Details of the hematologic and serologic findings from day 0 to day 41, after the transfusion of approximately 3.5 units of group A RBCs, are summarized in Table 1. Intravascular hemolysis became evident on day 8 posttransfusion. The Donath-Landsteiner test was strongly positive and the Ham's test for PNH was negative.

## Discussion

This case illustrates some of the fascinating and complex clinical and scientific problems that can occur in routine transfusion practice and the importance of con-

**Table 1.** Summary of hematologic and serologic findings

Days post-transfusion	Hemoglobin (g/L)	Anti-A titers			Direct antiglobulin test	Donath-Landsteiner test
		Saline 18°C	Indirect antiglobulin test (37°C)			
0	69	2				
1	117	<1		Weakly positive (IgG+C3d)		
2	127					
5	118	1	8			
6	110	2	16			
7	101			Weakly positive (IgG+C3d)		
8*	81	8	>128	Weakly positive (C3d)	Positive 8	
9	141	64	>128			
41	122			Weakly positive (IgG+C3d)	Positive 4	

\*Exchange transfusion plus transfusion of 2 units of red blood cells

stant vigilance to prevent incompatible blood from being given inadvertently. It also shows the frustrations that can be encountered, since the true sequence of events cannot be unraveled because pretransfusion samples were unavailable for Donath-Landsteiner testing.

AIHA developing in association with blood transfusions or transfusion reactions is under-recognized in humans.<sup>12</sup> The present case is unique in that the development of PCH has not been reported previously in such circumstances. The phenomenon, which illustrates a poorly understood relationship between alloantigen exposure and autoantibody development, is better known in animal work from cross-species transfusions. In fact, the production of murine AIHA by repeated injections of rat blood cells<sup>12-14</sup> is a model used for studying autoimmune hemolysis.

Previous reports have shown that the rate of RBC destruction can vary considerably. Mild autoimmune hemolysis, associated with the development of alloanti-D, -C, and -E, occurred several weeks after a compatible transfusion of group O, D+C+E+ RBCs was given to an O, D-C-E- recipient.<sup>1</sup> Severe AIHA was described in two patients with sickle cell disease and multiple alloantibodies following transfusion of serologically compatible RBCs.<sup>5</sup> Similarly, severe hemolysis was experienced by two group O, D-C-E-c+e+ patients who received group and Rh type-specific blood;<sup>6,7</sup> both autoantibodies showed Rh specificity, although in one case it mimicked an alloantibody.<sup>6</sup> The author commented that such mimicking autoantibodies were possibly more common than generally thought and could account for the serologic findings in some cases of delayed transfusion reactions.<sup>6</sup> Finally, a severe autoimmune component was demonstrated with a fatal hemolytic transfusion reaction due to anti-Jk<sup>a</sup>.<sup>3</sup>

Reduced anti-A in this elderly group O patient was not unusual and probably represents compromised immune homeostasis association with aging. In patients with hypogammaglobulinemia and low serum titers of anti-A, destruction of incompatible group A RBCs may be extravascular and relatively slow.<sup>15</sup> This may have occurred in our patient during the first week when the hemoglobin decreased from 127 g/L on day 2 post-transfusion to 101 g/L on day 7 (or it could have reflected continued bleeding from the gastric ulcer). An initial decrease in antibody titer following ABO incompatible blood transfusion has been noted previously.<sup>16</sup> The sudden onset of intravascular hemolysis on day 8 was unexpected. Although the hemolysis subsequently might have been explained as only a severe DHTR related to

the rapid rise in anti-A titers (Table 1), at the time it was felt that the hemolysis was too severe and investigations into other causes of intravascular RBC destruction were undertaken, which included Donath-Landsteiner and Ham's tests.

There was initial surprise at discovering that the Donath-Landsteiner test was strongly positive and that the antibody showed classic P specificity, findings that are pathognomonic of PCH. However, there was no other apparent cause for the positive result; serological tests for syphilis were negative, and had the symptoms occurred in the absence of the incompatible transfusion, a diagnosis of PCH would have been made without hesitation. The Donath-Landsteiner antibody was thought to be of the chronic type as it was still easily demonstrated more than 1 month posttransfusion. In our experience, the acute childhood forms of PCH are usually transient and closely associated with the period of hemolysis. Although the relative contribution to the intravascular hemolysis of the rising anti-A and the Donath-Landsteiner antibodies cannot be ascertained, we feel the latter made a significant contribution. Therefore, we conclude that stimulation of the patient's immune system by incompatible RBCs resulted in a DHTR, which either triggered, exacerbated, or was accompanied by an autoimmune response manifesting as PCH.

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