An unusual case of an acute hemolytic transfusion reaction caused by an autoanti-I


In general, naturally occurring cold autoagglutinins react optimally at low temperatures. We describe a young child who experienced an acute hemolytic transfusion reaction by an unusual autoanti-I. The IgM autoanti-I was detected at 4°C (titer 256) and also reacted at 30°C. This case highlights the potential hazard of transfusing units of blood immediately upon removal from the blood refrigerator, especially into neonates and children of small stature.

**Key Words:** autoanti-I, acute hemolytic transfusion reaction, cold autoagglutinins

In general, naturally occurring cold autoagglutinins react optimally at low temperatures (at or below 4°C) with a titer below 64, and with low thermal amplitude (maximal thermal range at 20°C or less). Most cold autoagglutinins are benign, usually IgM antibodies, and can be detected by direct saline agglutination tests. Clinically significant cold autoagglutinins usually react at a thermal amplitude of 30°C or above and may activate complement.

Autoanti-I is commonly found to be the specificity implicated in cold hemagglutinin disease (CHD), and also after mycoplasma infection. In CHD, the cold agglutinin titer is usually higher than 1000 when tested at 4°C.

We report an acute hemolytic transfusion reaction (AHTR) in a child of small stature caused by the transfusion of two units of blood immediately upon removal from a temperature-controlled blood refrigerator, with no warming of the units to ambient room temperature. Extensive laboratory investigations revealed that the antibody implicated was an unusual autoanti-I reacting at 30°C.

This case highlights the potential hazard of transfusing cold units of blood taken from a temperature-controlled refrigerator to neonates and children of small stature.

**Case Report**

A 9-year-old boy, with acute lymphoblastic leukemia (ALL), was admitted to a district hospital with febrile neutropenia. He received intravenous (IV) antibiotics and his fever improved, but no organism was identified. His hemoglobin (Hb) fell to 7.3 g/dL (normal range, 12.2–14.2 g/dL), with a hematocrit of 19.5%, and transfusion was requested. The patient’s blood group was group A, D+, with no atypical alloantibodies detected in the plasma. The direct antiglobulin test (DAT) was positive (2+ with anti-C3d only). Two units of crossmatched compatible (by indirect antiglobulin technique [IAT] at 37°C) group A, D+, K– red blood cells (RBCs) were issued.

The first unit was taken from the temperature-controlled blood refrigerator and was immediately transfused without any adverse reactions. The second unit was also transfused without waiting for the unit to reach ambient room temperature. While receiving the second unit, the patient had classic signs of an AHTR: high fever of 40°C, rigors, loin pain, sweating, and tachycardia. The transfusion was stopped immediately (about 100 mL had been infused), and IV normal saline, hydrocortisone, and chlorpheniramine were given with oral acetaminophen. The patient also passed a quantity of dark urine soon after cessation of the transfusion, and the left kidney was tender on palpation. There was a rise in bilirubin level from 25 μmol/L to 93 μmol/L (normal, 2–17 μmol/L). The blood film showed some RBC agglutination and some atypical lymphocytes.

In view of the severe nature of the reaction and hemoglobinuria, the patient was urgently transferred to the teaching hospital the same day. The patient remained on IV fluid and antibiotics. The blood samples (pretransfusion and posttransfusion samples taken at room temperature) were referred to the reference laboratory for investigation. The Hb level the next day was 9.6 g/dL, the hemoglobinuria subsided quickly, and the bilirubin dropped to 44 μmol/L. There were no long-term clinically adverse sequelae, and the child was discharged from the hospital the following day with a Hb level of 9.2 g/dL.

A culture from the blood units showed no growth. The patient’s mycoplasma screening and Paul-Bunnell test for glandular fever were negative.

**Materials and Methods**

IATs were carried out using gel column agglutination (DiaMed Micro-ID System, BioRad Laboratories, Switzerland)
at the reference and hospital laboratories. Direct saline agglutination test and titration studies were carried out by standard tube methods, with the reactants warmed to the desired incubation temperature before mixing.

In view of the DAT findings (see Results), the hospital was asked to provide more warm-separated serum from whole blood, kept strictly at 37°C, to investigate for indirect Donath-Landsteiner test, using the method previously described.³

**Results**

Both pretransfusion and posttransfusion samples were examined macroscopically after centrifugation. The color of the supernatant plasma from the pretransfusion sample was normal (straw colored), whereas that from the posttransfusion sample was dirty brown.

Initial serologic results at the reference laboratory for both pretransfusion and posttransfusion samples showed the patient to be group AB, Rh phenotype DCcEe, and K+, with a positive DAT with anti-IgG (1+), anti-IgA (1+), anti-IgM (3+), anti-C3c (1+), and anti-C3d (3+). The reverse ABO grouping results of the plasma was consistent with group O. None of these results were reliable, however, as positive (1+) reactions were seen in all the columns containing an inert control. This suggested the presence of a cold-reactive antibody.

These results were discrepant with those found at the hospital. Although tests there were also carried out by column agglutination technology, the samples tested at the reference laboratory had been stored at 4°C, whereas those at the hospital had been tested without cooling beyond laboratory temperature. This allowed for more of the autoantibody to be adsorbed onto the RBC surface, thus giving false-positive results.

After washing of the RBCs with phosphate-buffered saline (PBS), warmed to 37°C, the tests were repeated, and the results showed the samples to be group A, probable Rh genotype RₐR₉, K-. The reverse grouping, also incubated at 37°C, showed the expected negative reaction with A, RBCs and the expected positive reaction with group B RBCs. The DAT was positive (3+) with anti-C3d only.

No RBC alloantibodies were detected by the IAT at 37°C both in the pretransfusion and posttransfusion samples. Repeat crossmatches by the IAT using pretransfusion plasma samples and RBCs from the two transfused units were compatible.

As the initial tests suggested the presence of a cold-reactive antibody, titration studies at different temperatures were undertaken by saline direct agglutination. Testing was performed using the plasma from the pretransfusion samples, after warming of the patient’s RBCs to ensure that optimal amounts of the autoantibody were eluted back into the plasma, as shown in Table 1.

The results indicate the presence of a low-titer, high thermal amplitude (reacting at 30°C) cold-reactive antibody with anti-I specificity. See Table 1. Reactions with papain-treated RBCs (not shown) were positive, thus ruling out a specificity of anti-Pr. The indirect Donath-Landsteiner test was negative, which ruled out anti-P associated with paroxysmal cold hemoglobinuria.

**Discussion**

In general, naturally occurring cold-reactive autoantibodies have a low titer (<64) at 4°C, and have a thermal amplitude of less than 20°C.¹ The most common cold autoagglutinins are directed against the I blood group system.⁴

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Cells</th>
<th>Plasma dilutions</th>
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<tbody>
<tr>
<td></td>
<td>Neat</td>
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<tr>
<td>4°C</td>
<td>Group O adult cells (H+I+)</td>
<td>5+</td>
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<tr>
<td></td>
<td>Group A1 adult cells (H–I+)</td>
<td>5+</td>
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<td>Group O ii adult cells (H+I–)</td>
<td>5+</td>
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<td>23°C</td>
<td>Group O adult cells (H+I+)</td>
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<td></td>
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¹Using different red cell phenotypes, plasma dilutions were tested by direct saline agglutination method to identify the specificity and thermal amplitude of the cold antibody.
We describe a young child who experienced an AHTR caused by an unusual cold-reactive autoantibody. In our case, the anti-I IgM cold antibody was detected at 4°C (titer 256), and the antibody reacted at 30°C. In general, anti-I is commonly found in patients with CHD, and the autoantibody titer is typically much higher (>1000) at 4°C. This was not a case of CHD, as there was no laboratory evidence of autohemolysis before the transfusion. Also the reaction was transient in nature.

Although both units were transfused immediately upon removal from the refrigerator, it was during transfusion of the second unit that the AHTR occurred. It is conjectured that the first unit lowered the peripheral body temperature, and the second unit further reduced it to a level at which the autoanti-I caused the AHTR.

Cases of autoanti-IH causing AHTR have been well described, but to our knowledge, there was only one reported case of autoanti-I with a wide thermal range causing intravascular hemolysis. In our case, autoanti-I reacted at 30°C, and this lends weight to the importance of knowing the thermal range of the antibody, rather than the specificity or titer.

British Committee for Standards in Haematology (BCSH) guidelines for the administration of blood and blood components stated that “the transfusion of blood and blood components should begin as soon as possible after delivery to the ward or operating theatre.” There is no clear guidance or clarification for the administration of RBCs in neonates and children. Fatal hypothermic reactions have been reported after receiving blood transfused immediately upon removal from a blood refrigerator.

We report a rare, unusual case of AHTR caused by autoanti-I; this case highlights the potential hazard of transfusing units of blood immediately upon removal from the blood refrigerator, especially into neonates and children of small stature.

References


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