Case Report

Value of Testing at 30 ℃ for the Identification of Cold Alloantibodies in the Presence of Cold Autoantibodies

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Acute hemolytic transfusion reaction due to hemolytic anti-Lea is a rare phenomenon. We present here a case report of an acute hemolytic transfusion reaction, and demonstrated that an additional procedure of incubation at 30˚C facilitated the identification of a hemolytic anti- Lea in this diagnostic challenged case. A 46 year old man with a history of AIDS and dementia presented with symptomatic anemia. During transfusion of the 2nd unit of packed RBCs, the patient experienced high fever, back pain and dark brown urine. The blood bank and laboratory workup revealed evidence of a quickly resolved acute intravascular hemolysis. Other causes of intravascular hemolysis were ruled out with various laboratory tests. Initial blood bank antibody workup revealed a cold auto-anti-I, and a cold allo-antibody of undetermined specificity. Tests at 30˚C were described by Lawrance Petz and George Garratty for workup of cold auto-antibodies at 30˚C. We extrapolated their method to clearly identify a hemolytic anti-Lea with broad thermal amplitude. Phenotyping and crossmatching at 30˚C revealed that the 1st unit was implicated in the acute hemolytic transfusion reaction. This is the first report to successfully identify a hemolytic anti-Lea using the method at 30˚C for cold allo-antibody workup.
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Key Words: *acute hemolytic transfusion reaction, intravascular hemolysis, anti-Lea antibody, cold auto-antibody, cold allo-antibody*

INTRODUCTION

Acute hemolytic transfusion reaction (AHTR) occurs during or within 24 hours of transfusion of red blood cells. This is due to destruction of donor RBCs by preformed recipient antibodies, commonly resulting in intravascular hemolysis (IVH).[1]

AHTR symptoms include fever, chills, chest pain, back pain, dark urine. In severe cases, symptoms may also include shortness of breath, a drop in blood pressure or shock, renal function impairment, bleeding at IV site or diffuse bleeding. When suspected, transfusion should be stopped immediately, and blood testing sent to evaluate for the presence of hemolysis and implicated antibody. Treatment includes vigorous (or aggressive) intravenous fluid infusion, diuretics, and supportive care. Potential fatal complications are acute renal failure, shock and DIC. Early recognition of AHTR is important for timely treatment. Early identification of the implicated RBC antibody is important to prevent the patient from future transfusions of blood units with the implicated antigen.[1]

Laboratory findings in AHTRs include hemoglobinemia, hemoglobinuria, elevated lactate dehydrogenase (LDH), low haptoglobin, and decreased hemoglobin (Hb) / Hematocrit (Hct), with possible hyperbilirubinemia. The blood urea nitrogen and creatinine can be elevated if renal dysfunction occurs.[1]

A transfusion reaction workup in the blood bank includes: (1) clerical check, visual inspection for hemolysis (in the patient's serum or plasma), and direct antiglobulin test (DAT). The DAT detects the presence of antibody attached to red cells in the patient's circulation. If the DAT is positive, an elution is performed to identify the antibody coated on the patient's red cells. (2) testing the patient's pre-transfusion and post-transfusion blood samples for blood typing, antibody screening as well as crossmatching; and comparing those results; (3) detection of the implicated antibody in pati ent's serum or plasma.[1]

Acute hemolytic transfusion reaction (AHTR) due to anti-Le^a has been rarely reported.[2] Only a few case reports are published in the literature.[3-9] We present here a case of AHTR, which illustrated the difficulty in the detection of a clinically significant cold allo-antibody. We demonstrated that testing at 30˚C with saline facilitated the identification of a hemolytic anti-Le^a in such diagnostically challenging case.

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CASE REPORT

A 46 year old African American man with a history of AIDS and dementia presented to the emergency room (ER) with symptomatic anemia. No history of HAART administration was found. Antibody screen of the patient specimen was negative at the antihuman globulin phase (AHG) by using the anti-IgG gel card (Ortho Clinical Diagnosis, Raritan, NJ) (**Table 1**). Two units of antihuman globulin (AHG) crossmatch compatible RBCs were issued for the patient. When receiving the 2nd unit of RBCs, the patient's temperature went up to 102.7℉ from an afebrile baseline. The patient became tachycardic; other vital signs were within the normal range, but the patient reported back pain and had dark brown urine during the transfusion of the 2nd unit RBC. The transfusion was stopped. The RBC bag, which contained the remaining ¾ of the 2nd unit, was sent back to the blood bank for transfusion reaction workup. Vigorous fluid, intravenous diuretics and antibiotics were given. The patient remained clinically stable and his fever was subsided on the same day of the implicated RBC transfusion (Day 0). The patient's urine became clear yellow the following day (Day +1).

Table 1. Antibody screen for the patient's pre- transfusion specimen was tested using a anti-IgG gel card.

		Gel Card
Cells#	Rh-hr	IgG (37˚C)
1	R1R1	0
2	R2R2	0
3	rr	0

AHTR was immediately suspected. The blood bank investigation included patient examination and history inquiry, as well as blood bank and laboratory testing. Past medical history obtained from the patient did not reveal any history of hemolytic disease, transfusion or antibody detection, except that the patient reported persistent brown urine for the past 6 months. Patient also complained that he had felt very cold and had chills in ER before and during the blood transfusions. No family members of the patient could be reached for further history acquisition. No history of outside hospitals could be found or obtained from the patient.

Table 2. DAT (IgG and C3) tests performed for the patient specimens of pre- and post-transfusion (Tx).

	Pre-Tx		Post-Tx	
DAT	IgG	C3	IgG	C3
IS	3+	0	3+	0
CC		2+		2+

The blood bank workup was performed for AHTR. The clerical check revealed no discrepancies. The patient's post-transfusion (Day 0) plasma showed gross visible hemolysis with dark brown coloring; whereas the pre- transfusion (Day -1) plasma was clear yellow, suggesting hemalysis was likely due to an AHTR. The DAT results were 3+ positive for IgG and negative for C3 at immediate spin (IS) phases for both pre-transfusion and post-transfusion specimens (**Table 2**). Polyspecific DAT test was not performed in this case.

Additional laboratory tests were ordered for further workup. Urine analysis (UA) showed that the patient's post-transfusion urine on Day 0 was amber/ bloody with free hemoglobin positive, but the urine specimens of Day -1 and Day +4 were clear yellow with free hemoglobin negative. Microscopic RBC counts were 0-2/per high power field (HPF) in all the urine specimens, indicating the positive free hemoglobin in urine of Day 0 was due to hemoglobinuria, rather than due to hemoturia.

The other laboratory results were as follows:

Hb/ Hct: 6.7/22.4 (Day –1), 9.3/ 29.1 (Day 0, post-Tx), 8.8/28.1 (Day 2); LDH 1314 (day 0) 293 (day 2); Total billirubin/ Direct billirubin: 0.5/0.2 (Day –1), 1.6/0.8 (Day 0), <0.4/0.1 (Day 3); Haptoglobin: cancelled on Day 0 due to insufficient quantity of the specimen, result was normal on Day 2. BUN, creatinine, PT and PTT results were normal on Day 0 -4.

Table 3. Cold Mini-panel was run with the patient's plasma after the transfusion (Tx) using the tube method.

		Post-Tx (tube)		
Cell #	Rh-hr	IS	RT-15min	4˚C -15 min
1	R1R1	0	0	weak
2	R2R2	0	0	weak
3	rr	1+	1+	1+
4	Auto Cells	0	0	1+
5	Cord Cells	0	0	0

*RT= room temperature

Repeated blood bank antibody screen for both pre- and post – transfusion specimens were negative with Ortho anti-IgG gel cards, same as the result showed in **Table 1**. A cold mini-panel using a routine tube method rather than gel card method, revealed a cold auto-anti-I, and a cold allo-antibody (**Table 3**). Further antibody panel workup using the tube method, only showed an undetermined specificity of the cold allo-antibody at immediate spin (IS) phase, with no reactivity at 37℃ phase and AHG phase, which is also incubated at 37 ℃ (Data not shown). Cold antibodies not reacting at 37 ℃ and AHG phases with group O RBCs in antibody panels is considered not clinically significant, because they do not react with RBCs at body temperature (around 37 ℃) to cause hemolysis *in vivo.* At this point no clinically significant antibody was detected, which did not support an AHTR.

Causes of IVH unrelated to AHTR were therefore extensively investigated because an AHTR-implicated antibody was not detected, and also due to the confusing history provided by the demented patient.

The blood remaining in the returned, second unit bag and the patient's pre- and post-transfusion blood specimens were submitted for bacterial culture to rule out a septic transfusion reaction. The negative culture results for both of the patient's specimens and for the blood unit ruled out bacterial contamination of the second blood unit.

Various tests were conducted for other differential diagnoses, including autoimmune hemolytic anemia, (such as cold agglutinin syndrome (CAS), paroxysmal cold hemoglobinuria (PCH), G6PD deficiency, paroxysmal cold hemoglobinuria (PNH), microangiopathy hemolytic anemia (MAHA). The peripheral blood smear exhibited microcytic hypochromic anemia with the absence of schistocytes; a finding not supportive of MAHA. No spontaneous agglutination observed in the fresh patient's specimen at room temperature with the presence of a low titer of cold autoantibody at 4 ℃, was not consistent with cold agglutinin syndrome. A negative CD 55 and CD 59 ruled out PNH. A negative G6PD test did not support G6PD deficiency. Urine analysis for Day –1 (pre-transfusion) and Day 4 (post-transfusion) were negative for hemoglobinuria, The results were inconsistent with the patient's statement of persistent dark brown urine. Taken together, all differential diagnoses were ruled out, except AHTR. Nevertheless, it was a dilemma that no implicated antibody for AHTR was detected at this point.

Testing at an additional temperature 30˚C, rather than only at 37˚C, with saline or albumin was described by Lawrence D Petz & George Garratty to characterize cold auto-antibodies with broad thermal amplitude, which can be hemolytic *in vitro* and/ or *in vivo*.[10] A cold Auto –antibody positive at 30 ℃ is often hemolytic, as Dr. Petz and Garratty pointed out.[10]

Table 4. Antibody screen for the patient's post- transfusion specimen was tested using the tube method.

		Saline (Tube)			PEG (Tube)
Cells#	Rh-hr	IS	30˚C	AHG 30˚C	AHG 30˚C
1	R1R1	0	0	0	0
2	R2R2	0	0	0	0
3	rr	1+	2+	2+	2+

We applied their method, but with modification to use PEG instead of albumin, for our further antibody workup of clinically significant cold alloantibody, rather than cold auto-antibody. Albumin reagent was not available at our blood bank at that time. Antibody screen and identification at 30˚C, at AHG with saline and PEG, and at IS phases using the tube method clearly identified an anti-Le^a with broad thermal amplitude up to at least 30˚C in the patient's plasma (**Table 4** and **5**) and eluate (data not shown). The IgM component of the anti-Le^a was reactive at IS and 30˚C, and the IgG component at AHG-30˚C. DTT treatment was not done to further differentiate the IgM and IgG components. Although only 1 reagent cell in the panel was reactive with the patient's plasma (**Table 5**), 2 more reagent cells reactive in 2 different antibody screens (one of them was shown in **Table 4**) further supported the identification of anti-Le^a in the plasma. It was not ideal that no auto-control with the patient's own cells was tested in the antibody identification panel. However, the anti-Le^a with a high thermal amplitude was clearly identified by these tests performed at 30˚C. The same tests results were obtained with the eluate.

Table 5. Antibody Identification FOR the patient's post- transfusion specimen was tested by using the tube method.

		Rh-hr						Kell						Duffy		Kidd		Lewis		MNS				P	Lutheran			Test Results			
Cell#	Rh-hr	D	C	c	E	e	f	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b	Cell#	IS	30°C	AHG 30°C	CC
1	R1R1	+	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	0	+	+	0	+	1	0	0	0	2+
2	R1wR1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	0	+	2	0	0	0	2+
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	+	+	3	0	0	0	2+
4	Ror	+	0	+	0	+	+	0	+	0	+	0	+	0	0	+	0	0	+	+	0	+	0	+	0	+	4	0	0	0	2+
5	r'r	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	0	+	+	0	+	5	0	0	0	2+
6	rr	0	0	+	+	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	6	0	0	0	2+
7	rr	0	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	7	0	0	0	2+
8	rr	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	0	+	8	1+	2+	2+	
9	rr	0	0	+	0	+	+	0	+	+	0	0	+	+	0	+	0	0	+	+	+	+	+	0	0	+	9	0	0	0	2+
10	rr	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	0	+	0	0	+	w	0	+	10	0	0	0	2+
TC	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	0	0	+	TC				

Phenotype of the patient was Le (a-b-). Phenotyping of segments of the transfused blood units revealed the presence of Le^a antigen on the RBCs of the 1st transfused donor unit, but not the 2nd unit. Crossmatching at 30˚C showed that the 1st donor blood unit was incompatible and the 2nd was compatible. These testing at 30˚C confirmed the suspected AHTR due to an allo anti-Le^a, and revealed that the 1st transfused unit was implicated in this reaction. The fact that this antibody was not previously detected at 37˚C and AHG-37˚C phase by the routine methods indicates that the thermal amplitude of the anti-Le^a was not up to 37 ℃.

Two RBC units, which were negative for Le^a antigen and crossmatch compatible at antihuman globulin (AHG) phase at 30˚C and 37˚C, were later issued to the patient. Proper increase of Hb and Hct was observed following the transfusion and the patient's anemic symptoms improved.

DISCUSSION

AHTR of immune origin are serious, potentially life-threatening reactions, usually caused by transfusion of incompatible red cells following clerical or systems errors as well missed antiabody detection due to technical errors that result in incompatibility of the blood unit with the blood recipient. AHTR often results in the activation of complement, release of cytokines inducing a systemic inflammatory response within 24 hours of transfusion.

The typical AHTR ensues as a result of ABO errors when patient's anti-A or anti-B react with A and/ or B antigens on the transfused donor red cells triggering the binding of complement with subsequent activation of the complement cascade causing IVH. AHTR mediated by clinically significant IgG antibodies to Kell, Kidd or Duffy antigens also occur.[1]

Lewis antibodies, anti-Le^a and anti-Le^b, are naturally-occurring antibodies. They are usually adsorbed to RBCs and usually are IgM antibodies, They are generally clinically insignificant due to reactivity at cold temperatures rather than at body temperatures. However, there are a few case reports where naturally occurring, warm-reacting IgM Le^a antibodies have caused hemolytic transfusion reactions and a few reports where IgG Lewis antibodies developed following blood transfusion.[3-9]

Clinically significant anti-Le^a can be formed after blood transfusion.[9] Twenty percent of Le (a-b-) individuals develop anti-Le^a.[7] These, if active *in vitro*, rarely cause HTR *in vivo*, because they become quickly neutralized by soluble Lewis substances and dissociated Lewis antigens contained in the plasma of transfused blood units.[7] For patients with Lewis antibodies, it is usually safe to transfuse red blood cells that are crossmatch compatible at 37˚C and at the AHG phases, if the patient's anti-Le^a does not react at 37˚C, or does not cause *in vitro* hemolysis.[9]

The patient's clinical manifestation of fever, back pain, dark brown urine during RBC transfusion suggested an AHTR. The urine analyses showed hemoglobinuria in the patient's specimen of Day 0, but not in those of Day-1 and Day +4, uncovering an acute intravascular hemolysis process on Day 0.

The AHTR workup showing hemoglobinemia, hemoglobinuria and elevated LDH on Day 0 only, revealing the evidence of a quickly resolved acute intravascular hemolysis (IVH), indicating an AHTR without renal or coagulation complications.

However the culprit antibody for the AHTR was not detectable with routine antibody workup in this case.

The DAT results were 3+ positive for IgG, negative for C3 at IS phase for both pre-transfusion and post-transfusion specimens (Table 1), showing no RBC coated IgM was detected at IS phase in the patient's specimens. The DAT with no changes on pre- and post-transfusion specimens did not support an AHTR, because it was thought that the consistently 3+ positive for IgG was probably due to the patient's medical condition or medication. The fact that the implicated hemolytic anti-Le^a in this case was not detected with the DAT tests, suggesting that the detectable IgM was mostly in the plasma, but not on the RBCs in these specimens, or the IgM was easily dissociated from RBCs during DAT testing even if it did react with RBCs. Moreover, negative C3 in the post-transfusion specimen could be the consequence of hemolysis of most of the RBCs coated with anti-Le^a IgM.

Detection of anti-Le^a is usually not difficult. But in this challenging case, testing at 30˚C with saline and PEG was the turning point for the detection of the implicated anti-Le^a. Antibody screen and identification tests at 30˚C with saline and PEG clearly identified an anti-Le^a in the plasma and eluate as the culprit of his AHTR. AHTR was finally confirmed by Le^a antigen typing positive on the implicated RBC unit transfused. Anti-Le^a mediated AHTR could be then prevented by giving Le^a negative RBCs. Ideally, the patient should receive RBC of Le (a-b-), saline crossmatch compatible at both 30˚C and AHG-30˚C phases for his future blood transfusions. Individuals having potent anti-Le^a at 37˚C should be transfused with Le (a-b-), as Le (a-b+) red cells may also have some amount of Le^a.[9]

CONCLUSION

Testing at 30˚C with saline for antibody workup is an alternative way to effectively detect hemolytic cold allo-antibodies. This is the first report of successfully identifying a hemolytic anti-Le^a for AHTR using this method.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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