Acute Hemolytic Transfusion Reaction Caused by a Red Cell Antibody That Was Missed by Pretransfusion Testing Using Tube Method

*Aaron Shmookler, MD, Diane Hamad, MLS(ASCP), Scott Scrape, MD, Jian Chen, MD, PhD**

Laboratory Medicine 48:3:258–261

DOI: 10.1093/labmed/lmx037

ABSTRACT

Pretransfusion testing is very important to prevent transfusion of incompatible red cells, which might result in a hemolytic transfusion reaction. This includes the detection of antibodies in recipients' serum and compatibility testing between donor cells and recipient serum. The most commonly used methods include gel and tube techniques. We present a case in which an anti-E alloantibody was detected by gel method but not by tube testing. As a result, red cells that were retrospectively phenotyped as positive for E antigen were inadvertently selected and transfused after crossmatch using the same tube method. After transfusion, the patient developed signs of hemolytic transfusion reaction. This case highlights the potential risk of transfusion of incompatible red cells when alloantibody detection is solely relied on tube testing.

Keywords: blood banking, transfusion medicine, pretransfusion testing, hemolytic transfusion reaction, gel testing, tube testing

Clinical History

A 48-year-old female with IgG lambda multiple myeloma that was diagnosed 1 month earlier presented to an outside hospital with acute pulmonary embolism, for which she was started on a therapeutic heparin drip, and anemia of unclear etiology with hemoglobin (Hb) 4.7 g/dL. Initial pretransfusion testing was performed at the outside hospital using tube method with low-ionic strength-saline (LISS) enhancement, which showed nonspecific reactivity with all clinically significant alloantibodies ruled out. She received 2 units of red cells (RBCs) and was transferred to our institution. Upon arrival her Hb was 4.9 g/dL (12.0–16.0 g/dL).

A blood sample was sent for a type and cross, which was initially performed with automated gel column-agglutination technology (ORTHO VISION Analyzer) and revealed a positive antibody screen (Table 1). Antibody detection was performed manually with gel technique, which suggested an anti-E antibody (Table 2). However, the blood bank technologist still felt uncertain about the antibody detection results partially because of reactions with E-negative cells (Table 1) and went on with tube testing. Conventional tube testing using LISS was negative at immediate spin, incubation at 37°C for 15 minutes after dispersing rouleaux with the serum saline replacement (SSR) technique, and at anti-human globulin (AHG) phase (Table 3). Direct antiglobulin testing (DAT) performed using polyspecific AHG in tube was negative. Based on these results, the antibody workup was interpreted as nonspecific with all clinically significant alloantibodies being ruled out.

Three units of RBCs were selected to be transfused to the patient. They were found to be compatible after testing with the same tube method using LISS. On the day of admission (hospital day 1), the first 2 units were administered consecutively from 9:00 PM to 1:00 AM. On hospital day 2, her Hb was 7.4 g/dL in the morning but dropped to 6.7 g/dL at noon, prompting the administration of the third unit of RBCs that afternoon. Prior to administering the third unit, a new blood sample was sent for a type and cross to perform additional crossmatches. An anti-E alloantibody was identified in the plasma using gel. The DAT became weakly

Abbreviations

Hb, hemoglobin; LISS, low-ionic strength-saline; RBCs, red cells; SSR, serum saline replacement; AHG, anti-human globulin; DAT, Direct antiglobulin testing; IS, immediate spin; TPE, therapeutic plasma exchange

Department of Pathology, The Ohio State University Wexner Medical Center, Columbus, Ohio.

*To whom correspondence should be addressed.
jian.chen2@osumc.edu

Table 1. Automated Gel Antibody Screen

Cell	D	C	E	c	e	f	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub	Result
1	+	+	0	0	+	0	0	0	+	+	0	+	/	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	1+
2	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	2+
3	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	+	0	+	0	+	0	0	0	+	0

In cell 1 the E antigen is absent, but there is a positive reaction, suggesting either different antibody specificity or a nonspecific reaction.

Table 2. Antibody Identification (Manually Performed Using Gel)

Cell	D	C	c	E	e	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	Result
1	+	+	0	+	+	0	0	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+	1+
2	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	2+
3	+	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	0
4	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	+	0	+	0	2+

Table 3. Antibody Screen Using Tube Method With Low-Ionic-Strength Saline

Cell	D	C	E	c	e	f	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub	IS*	37°C/ SSR	AHG
1	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	0	w+/0	0
2	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	0	+	+	+	+	0	0	+	0	+	+	0	+	0	1+/0	0
3	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	0	+	0	+	0	w+/0	0

In cells 1 and 3 the E antigen is absent, but there is weak reactivity at 37°C that is not seen after SSR treatment.
Reagent cells were incubated with patient's plasma at 37°C for 15 minutes before the reactions were read.
IS, immediate spin; SSR, serum saline replacement; AHG, antihuman globulin.

positive (barely visible and with a turbid background)[1] with anti-IgG and negative with anti-C3bd. Allo anti-E was recovered in the eluate using polyethylene glycol.

This patient also required urgent therapeutic plasma exchange (TPE) for hyperviscosity syndrome with a serum monoclonal protein level of 6175.5 mg/dL. TPE was performed with 5% albumin 2 hours after the last unit of the 3 initially crossmatched RBCs was administered (hospital day 2). She tolerated the treatment well. However, her plasma in the collection container was noted to be Coca-Cola in color (**Image 1**). Her urine was also found to be red in color. Given the suspicion for a hemolytic transfusion reaction additional laboratory testing was performed in the evening of hospital day 2. Her urinalysis showed free hemoglobin 11.6 mg/dL (0.0–1.0 mg/dL) and urobilinogen 2.0 EU per dL (<2.0 EU per dL). Compared to laboratory results on admission, her lactate dehydrogenase increased from 259 units/L (100–190 units/L) to 757 units/L; haptoglobin decreased from 196 mg/dL (44–215 mg/dL) to <30 mg/dL; total and indirect bilirubin levels increased from 0.1 mg/dL (<1.5 mg/dL) and 0.0 mg/dL (<0.3 mg/dL) to 0.7 mg/dL and 0.3 mg/dL, respectively.

The 3 units of RBCs that were transfused in our institution were subsequently retrospectively phenotyped on hospital day 3, and 2 of them were positive for the E antigen. Based on these findings, a diagnosis of acute hemolytic transfusion reaction was made. Fortunately, this patient was clinically stable and did not have any symptoms such as fever or pain. Her shortness of breath that was attributed to hyperviscosity syndrome remained unchanged during this period. She received another treatment of TPE on hospital day 4 in which her plasma had become clear. She stayed in our institution for about a month to receive treatment for her multiple myeloma. During this period, she received another unit of RBCs and 1 unit of platelets without complications.

Discussion

Standard pretransfusion testing methods include both gel column agglutination and tube techniques.[1] Among the

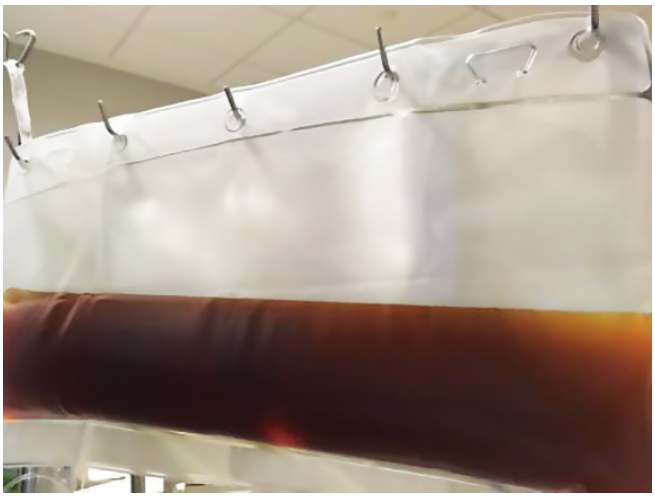

Image 1

Coca-Cola colored plasma in the collection container after therapeutic plasma exchange procedure.

advantages of gel technique over tube testing is its higher sensitivity, probably due to the lack of need for wash step.[2-5] However, it is well known that this technique is associated with nonspecific reactions. The product insert of the gel card states, "Rouleaux caused by serum or plasma with abnormally high concentrations of protein (such as in patients with multiple myeloma …) may infrequently cause difficulties in the Gel Test interpretation. False positive results or hazy reactions may occur with these samples…."[6] Therefore some transfusion services, including our facility, use it for initial antibody screening, which, if positive, is followed by antibody identification using a gel panel. Furthermore, a common approach to confirm equivocal reactions is to perform repeat tube testing with LISS enhancement. The interpretation of the results can be subjective, and we cannot say the technologist's interpretation was wrong.

However, upon review by both a senior technologist and physician in blood banking, the results were likely indicative of anti-E, and E-negative products would have been issued in the first place as a precaution. A teaching point here is that if there is any doubt regarding the result of a test, the technologist should consult a senior technologist or physician, which did not happen in this case.

As for pretransfusion crossmatch, tube testing is still the most commonly used method in many blood banks.

Currently, most RBC transfusions are only matched for ABO and D antigens, which can result in alloimmunization to other RBC antigens.[1] Recent studies showed that the alloimmunization rate in patients received multiple transfusions ranges from 1.4 to 8 per 100 patients, with anti-E being the most common alloantibody.[7-10] These alloantibodies, if not detected by pretransfusion testing, can result in the administration of mismatched RBCs and hemolysis. As shown in a recent study, the alloantibody detection rate using tube methods is estimated to be as low as less than 30% in a real-world practice setting. The authors attribute this low detection rate to the rapid evanescence of some alloantibodies and/or to the performance of antibody testing before antibody induction.[11] In the United States, non-ABO hemolytic transfusion reaction was the third leading cause of transfusion-related fatalities (14%).[12] Therefore, antibody detection techniques with high sensitivity are desirable in order to improve transfusion safety. In general, studies have shown the gel technique is more sensitive than tube techniques.[2-5] In a study comparing 5 different detection methods utilizing various enhancement media,[13] gel technique detected 70/70 (100%) samples containing anti-E. In contrast, tube method using LISS only detected 47/70 (67%) samples containing anti-E.

In our patient, the initial antibody screen using gel technique was positive. Further testing on the gel suggested an anti-E antibody, but a reagent cell negative for E was also reacting with the patient's plasma. Testing with tube technique and LISS enhancement was nonreactive, which was consistent with the results obtained a day before from the outside hospital. Based on the tube results including crossmatching,

DOI:10.1093/labmed/lmx037

E-positive RBCs were inadvertently transfused and patient had signs of acute hemolysis. In this case, if the antibody workup interpretation had been based on the results obtained using the gel technique, a possible allo anti-E antibody would have been reported and E antigen-negative RBCs selected for crossmatching and subsequent transfusions, based on our policies. As the final step of pretransfusion testing, crossmatch is designed not only to verify ABO compatibility but also to confirm that the selected antigen-negative RBCs are indeed compatible with the patient. In another words, there is no unexpected reactivity. Therefore, tube techniques which are less sensitive but relatively more specific are often employed in crossmatching. If an antibody with a very low titer slips through the more sensitive antibody detection phase, it would not demonstrate during crossmatching either. Based on the participants' survey response from the College of American Pathologists proficiency testing program, approximately 40% of the over 3000 participating immunohematology testing laboratories use tube techniques, and more than 20% of these laboratories also use LISS.[14] Therefore, there may be a potential risk to transfusion safety, considering that many laboratories, including reference laboratories, use tube techniques as the main method for antibody detection and crossmatch.

Conclusion

Both gel and tube techniques are acceptable methods for RBC antibody screening and identification. However, as shown in previous studies, tube testing techniques are less sensitive than the gel technique. When the antibody detection results are equivocal, techniques known for their higher sensitivities should be applied and transfusion decision should rely on the results obtained by more sensitive techniques. **LM**

References

1. Fung MK, Grossman BJ, Hillyer CD, et al. American Association of Blood Banks. *Technical manual*. 18th ed. Bethesda, Maryland: American Association of Blood Banks; 2014.
2. Mikesell KV, George MR, Castellani WJ, et al. Evaluation of different testing methods for identification of RhIG in red blood cell antibody detection. *Transfusion*. 2015;55(6 Pt 2):1444–1450.
3. Das SS, Chaudhary R, Khetan D. A comparison of conventional tube test and gel technique in evaluation of direct antiglobulin test. *Hematology*. 2007;12(2):175–178.
4. Thakur MK, Marwaha N, Kumar P, et al. Comparison of gel test and conventional tube test for antibody detection and titration in D-negative pregnant women: study from a tertiary-care hospital in North India. *Immunohematology*. 2010;26(4):174–177.
5. Novaretti MC, Jens E, Pagliarini T, et al. Comparison of conventional tube test technique and gel microcolumn assay for direct antiglobulin test: a large study. *J Clin Lab Anal*. 2004;18(5):255–258.
6. ID-Micro Typing System, Instruction for use. (Pub. No. J32848) Version 4.0, Ortho Clinical Diagnostics. October 2016: 6.
7. Caamaño J, Musante E, Contreras M, et al. Frequency and specificity of red blood cell alloimmunization in Chilean transfused patients. *Transfus Med Hemother*. 2015;42(1):4–7.
8. Sood R, Makroo RN, Riana V, et al. Detection of alloimmunization to ensure safer transfusion practice. *Asian J Transfus Sci*. 2013;7(2):135–139.
9. Ngoma AM, Mutombo PB, Ikeda K, et al. Red blood cell alloimmunization in transfused patients in sub-Saharan Africa: a systematic review and meta-analysis. *Transfus Apher Sci*. 2016;54(2):296–302.
10. Schonewille H, Honohan Á, van der Watering LM, et al. Incidence of alloantibody formation after ABO-D or extended matched red blood cell transfusions: a randomized trial (MATCH study). Transfusion. 2016;56(2):311–320.
11. Stack G, Tormey CA. Detection rate of blood group alloimmunization based on real-world testing practices and kinetics of antibody induction and evanescence. *Transfusion*. 2016;56(11):2662–2667.
12. US Food and Drug Administration Center for Biologics Evaluation and Research. Fatalities reported to FDA following blood collection and transfusion: annual summary for fiscal year 2015. http://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/UCM518148.pdf. Accessed February 1, 2017.
13. De Castilho LM, Pellegrino J Jr, Bechelli AP, et al. Evaluation of recent techniques for detection of red blood cell antibodies in sera of reference samples, patients, pregnant women, and blood donors. *J Clin Lab Anal*. 1996;10(5):250–256.
14. Downes KA, Shulman IA. Pretransfusion testing practices in North America, 2005–2010: an analysis of the College of American Pathologists Interlaboratory Comparison Program J-survey data, 2005–2010. Arch Pathol Lab Med. 2012;136(3):294–300.AQ2