Report

Low risk of hemolysis after transfusion of uncrossmatched red blood cells

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Transfusing uncrossmatched red blood cells (RBCs) can be a life-saving bridge until crossmatched RBCs are available. The risk of using uncrossmatched RBCs is that of hemolysis from unexpected clinically significant antibodies. This study sought to quantify the risk of hemolysis after the transfusion of uncrossmatched RBCs. The records of recipients of uncrossmatched RBCs over approximately 9 months were retrieved from the regional transfusion service. Basic immunohematologic data were recorded on all recipients including the number of uncrossmatched RBCs transfused. For recipients who had either previously identified clinically significant antibodies or those identified on the day of transfusion, clinical and biochemical data were evaluated to determine whether hemolysis had occurred after uncrossmatched RBC transfusion. There were 218 recipients of 1065 units of uncrossmatched RBCs. Most of the RBCs were administered in the emergency room (48%) followed by the operating room (24%) and intensive care unit (23%). Seven (3.2%) recipients had clinically significant antibodies that were active on the day of the transfusion, whereas in four patients a clinically significant antibody had been previously identified but was not active on the day of the transfusion. One patient with active antibodies who received three units of uncrossmatched RBCs for a gastrointestinal bleed demonstrated a reactive eluate several days later as well as positive biochemical hemolysis markers. Thus the overall rate of detectable hemolysis after uncrossmatched RBC transfusion was 1 of 218 (0.5%). The use of uncrossmatched RBCs is a relatively safe intervention, although close monitoring of recipients with clinically significant antibodies for evidence of hemolysis is recommended. ***Immunohematology* 2012;28:39–44.**

Key Words: RBC, hemolysis, uncrossmatched, antibody, transfusion

Under uncomplicated circumstances, crossmatched red blood cells (RBCs) can usually be available within approximately 60 minutes of the patient's blood sample arriving at the blood bank. However, there are circumstances under which this short delay in providing RBCs can be life threatening to the recipient. In these cases, uncrossmatched RBCs, which are RBC units whose compatibility with the recipient's plasma has not been serologically or electronically verified, can be provided almost immediately for use during an acute resuscitation while the blood bank performs a forward and reverse type and an antibody screen. As uncrossmatched RBCs are always group O, immediate hemolysis caused by naturally occurring anti-A and anti-B is avoided. The main risk of using uncrossmatched RBCs is the potential for hemolysis caused by unexpected non-ABO antibodies; thus the risk of hemolysis after receiving uncrossmatched RBCs is directly related to whether the recipient has either received a previous transfusion or been pregnant. In a situation in which uncrossmatched RBCs might be used, such as in trauma resuscitation in the emergency department, the patient's pregnancy and transfusion history are often unknown; however, the risk of immediate hemolysis caused by unexpected clinically significant antibodies should be low because the prevalence of these antibodies in the general population is quite low. In a study of almost 16,000 patients (corresponding to nearly 28,000 antibody screens) at a tertiary care hospital in Australia, only 1.9 percent of the recipients had a positive screen caused by a clinically significant antibody, of which the majority were directed toward antigens in the Rh or Kell systems.[1] When stratified by age, women generally had a higher incidence of alloimmunization than men, and patients with hematologic or oncologic diseases had higher rates of alloimmunization compared with patients in the emergency room or trauma patients. Similarly, Heddle and colleagues demonstrated that 96.5 percent of previously transfused patients at their hospital had a negative antibody screen,[2] whereas Stack et al. demonstrated a 2.4 percent alloimmunization rate among 18,750 transfused veterans.[3]

Several previous studies have evaluated patient outcomes after receipt of uncrossmatched RBCs.[4–11] In general, the risk of hemolysis was either low or absent, but it was not always clear whether the recipients in these studies had active antibodies on the day that they received their uncrossmatched RBC transfusion, thereby putting them at risk of hemolysis. A detailed study of 265 uncrossmatched RBC transfusion episodes by Goodell and colleagues found that 6.4 percent of these incidents were complicated by the presence of a clinically significant antibody.[4] However, only one of seven of the recipients with a clinically significant antibody who received at least one incompatible uncrossmatched RBC unit actually had a hemolytic reaction. Thus, we sought to determine the incidence of hemolysis after the administration of uncrossmatched RBCs throughout our hospital system.

Materials and Methods

The records of patients who were at least 16 years of age and received at least one uncrossmatched RBC unit over an approximately 9-month period were retrieved from the electronic records of a regional transfusion service. This transfusion service covers 16 hospitals in southwestern Pennsylvania including several level 1 trauma centers, active solid organ and stem cell transplantation services, and a variety of intensive care units. From the transfusion service's electronic files, basic serologic information on each recipient was recorded; this included the previous detection of alloantibodies, whether antibodies were detected on the day of receipt of the uncrossmatched RBCs, the detection of new antibodies after receipt of uncrossmatched RBCs, and the number of uncrossmatched RBC units transfused. Reports of transfusion reactions temporally associated with the uncrossmatched RBC transfusions were also recorded. Clinically significant antibodies were defined as those capable of causing hemolysis or shortening the lifespan of the transfused RBCs, and for which antigen-negative, crossmatch-compatible RBC units should be transfused.[12] Basic demographic data from the clinical records of the patients who received uncrossmatched RBCs were noted. For patients who had either known historical or active clinically significant antibodies on the day that they received uncrossmatched RBCs, biochemical variables including bilirubin, haptoglobin, lactate dehydrogenase, reticulocyte counts, and hemoglobin levels as well as the results of direct antiglobulin tests (DATs) and eluates were also analyzed, if available, to determine whether immune-mediated hemolysis had occurred at any point after the uncrossmatched RBC transfusion. The patient's clinical chart was also reviewed to determine whether there was a clinical suspicion of hemolysis. These laboratory and clinical variables were also analyzed in those recipients who subsequently produced antibodies after receipt of uncrossmatched RBCs. The physician and nursing notes from around the time of the uncrossmatched transfusions were also examined to determine whether there was a clinical suspicion of hemolysis.

Antibody detection was performed using a manual saline tube technique (Immucor, Norcross, GA) or automated techniques including column agglutination (Ortho ProVue; Ortho Clinical Diagnostics, Rochester, NY) and solid-phase (Galileo; Immucor) methodologies according to the manufacturer's specifications. The polyspecific and monospecific DATs (Ortho, Raritan, NJ) and eluates (Gamma Elu-kit II; Immucor) were performed using commercially available reagents and kits. As per our reference laboratory protocols, an eluate would have been performed on a specimen that demonstrated a newly positive DAT or one that had increased in strength from a previous test in a patient with a recent transfusion history, or if specifically ordered by a physician. Most of the uncrossmatched RBCs would not have been leukoreduced and would be stored in AS-3 or AS-5 solutions. The decision to use uncrossmatched RBCs was made by the patient's clinical team, and uncrossmatched RBCs were available from the blood bank and from remote monitored refrigerators in the emergency room and on selected wards. The prescribing physician was required to have signed and returned to the blood bank an authorization form for the use of uncrossmatched RBCs.

Descriptive statistics were used for continuous variables using the software package in Microsoft Excel 2010. Results are presented as mean ± standard deviation. This protocol was approved by the University of Pittsburgh's Total Quality Council.

Results

During the approximately 9-month period, there were 218 recipients of at least one unit of uncrossmatched RBCs. The mean age of these recipients was 54 ± 21 years, and 65 percent were male. Overall, 1065 uncrossmatched RBC units were transfused to these 218 recipients, which represents an average of 4.9 ± 4.9 uncrossmatched RBC units per recipient. Most of the uncrossmatched RBCs were administered in the emergency room (48%), followed by the operating room (24%), and the intensive care unit (23%). The remaining units were administered on medical floors, in labor and delivery suites, in the interventional radiology department, and to one patient who was receiving extracorporeal membrane oxygenation. Transfusion reactions were reported in two recipients of uncrossmatched RBCs who did not have known historical or active antibodies, and as expected, neither reaction was hemolytic in nature; one patient had hypotension in the operating room, the etiology of which was believed to be related to the patient's underlying hypovolemia from dialysis and ongoing bleeding. The signs and symptoms of the other reaction were not specific in nature, and it was reported in a patient who received the uncrossmatched RBCs during her unsuccessful resuscitation for hemorrhage.

Figure 1 presents the immunohematologic outcomes after receipt of the uncrossmatched RBCs. Of the 218 recipients of uncrossmatched RBCs, seven had active antibodies on the day of transfusion, and four others had historical

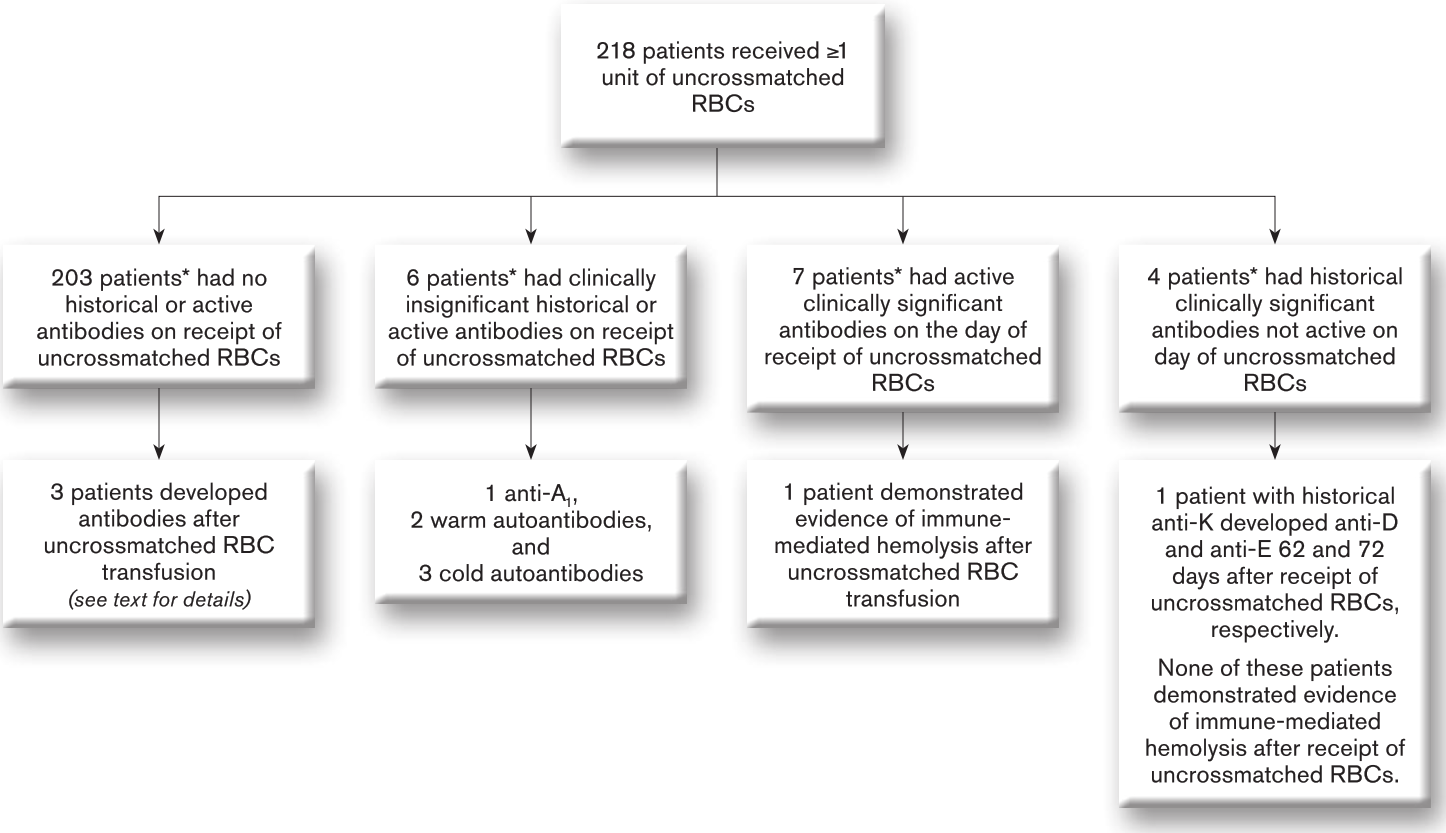

Fig. 1 Flow diagram demonstrating the immunohematologic consequences of receiving uncrossmatched RBCs. *The numbers do not add to 218 because 2 patients are counted in multiple categories: both patients had active clinically significant antibodies along with an insignificant antibody on the day of receipt of the uncrossmatched RBCs.

clinically significant antibodies that were not active on the day of the transfusion. Table 1 presents the demographic and immunohematologic details of the 11 patients who had historical or active clinically significant antibodies when they received their uncrossmatched RBC units. In one of four recipients with historical clinically significant antibodies no further antibody screens were performed after receipt of the uncrossmatched RBCs; in another recipient only K– selected antibody screening cells were used for antibody detection, so it was not possible to determine whether the historical anti-K had reappeared. In the remaining two patients, their historical anti-Jk^a and -K had not reappeared in antibody screens performed 14 and 100 days, respectively, after receipt of uncrossmatched RBCs.

Overall, only one recipient demonstrated biochemical or clinical evidence of immune-mediated hemolysis after receipt of uncrossmatched RBCs. The patient (Patient 4 in Table 1) was a 72-year-old man who received three units of uncrossmatched RBCs as a result of gastrointestinal bleeding. The antibody screen performed on a pretransfusion sample revealed anti-K, -Fy^a, -E, and -C^w, which had been detected previously. On this sample the anti-IgG DAT was 2+ with a nonreactive eluate. Because of the patient's condition, uncrossmatched RBCs had to be issued before the pretransfusion evaluation could be completed. A sample drawn 3 days later revealed a 2+ anti-IgG DAT with a weak positive anti-C3d, and on this sample the eluate revealed both anti-E and -Fy^a (the eluate was performed because of the clinical suspicion of hemolysis). The patient's hemoglobin before the uncrossmatched RBC transfusion was 7.2 g/dL (normal, 12.3–15.5 g/dL) and increased to 10.8 g/dL on the following day. His hemoglobin gradually decreased over the next 2 days to a nadir of 8.2 g/dL without further evidence of bleeding. The patient's creatinine was 1.9 mg/dL (normal, 0.5–1.17 mg/dL) before the uncrossmatched RBC transfusion and then increased to 2.4 mg/dL the day after receipt of uncrossmatched RBCs, at which time hemodialysis was instituted. The total bilirubin was only measured 6 days after the transfusion and was 16.1 mg/dL (normal, 0.1–1.2 mg/dL). The other biochemical markers of hemolysis such as reticulocyte count, lactate dehydrogenase, haptoglobin, or urine

Table 1. Demographic and immunohematologic characteristics of the patients with clinically significant historical or active antibodies at the time of the uncrossmatched RBC transfusions in this study

Patient	Age	Sex	Location of uncrossmatched RBC transfusion	Number of uncrossmatched RBCs	Transfusion reaction reported to blood bank	Previously identified clinically significant antibodies	New antibodies identified on the day of receipt of uncrossmatched RBCs	Previous RBC transfusions*	Evidence of immune-mediated hemolysis after uncrossmatched RBC transfusion
1	74	F	ICU	6	N	**D, K**		Y	N
2	67	F	ICU	2	N	S, Kp^a		Y	N
3	53	M	OR	6	N	Jk^a		Y	N
4	72	M	ICU	3	N	**K, Fy^a, E, C^w**		Y	Y
5	28	F	OR	8	N	**K, Fy^a**		Y	N
6	59	F	ED	2	N	K		Y	N
7	81	M	ED	2	N	**E**		Y	N
8	29	F	OR	5	N		**D**	N	N
9	78	F	ICU	2	N	**S**		Y	N
10	89	F	ED	2	N		**c**	Y	N
11	62	M	ICU	2	N	K		N	N

ED = emergency department; ICU = intensive care unit; OR = operating room.

*As documented in the electronic records of this regional transfusion service.

Antibodies in bold reflect those that were active on the day of the uncrossmatched RBC transfusion.

hemoglobin were not ordered on this patient. The patient died within 1 week of the uncrossmatched RBC transfusion. The reactive eluate in the setting of a decline in hemoglobin after uncrossmatched RBC transfusion, the increased bilirubin, and the worsening renal function are highly suggestive of immune-mediated hemolysis caused by the transfusion of incompatible uncrossmatched RBC units.

Several patients had clinically insignificant antibodies. There were two patients who had cold autoantibodies detected on the day of the uncrossmatched RBC transfusion, whereas a patient with four active clinically significant antibodies also had a history of a cold autoantibody that was not reacting on the day of the RBC transfusion (Patient 4 in Table 1). Two other patients had histories of anti-A_1 and warm autoantibodies, respectively, and another patient had a warm autoantibody along with anti-E that were both active on the day of the transfusion (Patient 7 in Table 1).

In total, four patients developed antibodies after receipt of uncrossmatched RBCs. Two patients developed antibodies within a short time after the uncrossmatched RBC transfusion; a 27-year-old D– male patient received two units of uncrossmatched D+ RBCs, and anti-D was detectable on an antibody screen 10 days later. This patient had a negative antibody screen 5 days after the RBC transfusion, and did not receive additional D+ RBC units between the uncrossmatched units and the detection of anti-D. A 30-year-old man developed anti-E 16 days after receipt of 18 uncrossmatched RBC units. His last negative antibody screen was 13 days after the uncrossmatched RBC transfusions, and the only addtitional RBCs he received were one crossmatched unit 5 days before the anti-E was detected, suggesting that the stimulus for the production of the antibody was the uncrossmatched RBCs themselves. Neither of these patients had a history of RBC transfusion or historical antibodies in the electronic records of the regional transfusion service; furthermore, neither patient demonstrated biochemical or clinical evidence of hemolysis after detection of the antibody. In the remaining two patients, the antibodies were detected on screens that were more remote from the time of the uncrossmatched RBC transfusion compared with the two patients described above; a 19-year-old male patient with no previous transfusion or antibody history on file at the transfusion service developed anti-Jk^a 30 days after the uncrossmatched RBCs. He had a negative antibody screen 4 days after the uncrossmatched RBC transfusion, and the anti-Jk^a was subsequently detected on his next screen. He received multiple crossmatched RBCs in the interval between the uncrossmatched RBCs and the detection of the anti-Jk^a. Similarly, a 59-year-old woman with a history of anti-K developed anti-D and anti-E 62 and 72 days, respectively, after the uncrossmatched RBC transfusion (Patient 6 in Table 1). This patient had five negative antibody screens before the anti-D and -E were detected, and the last negative screen was 47 days after the uncrossmatched RBC transfusion. She continued to receive multiple D+ RBCs in between the uncrossmatched RBCs and the detection of anti-D. As with the two patients described earlier, neither of these patients

had biochemical or clinical evidence of hemolysis after the uncrossmatched RBC transfusion.

Discussion

In our cohort, only 11 of 218 (5%) of the patients who received uncrossmatched RBCs had either a history of a clinically significant antibody or one that was active on the day of the transfusion. It is important to consider not only the patients with clinically significant antibodies that were active on the day of the RBC transfusion but also the patients with a history of a clinically significant antibody because evanescence is complicated and it is difficult to predict when or whether it will occur for a given antibody. Furthermore, recent data suggest that previously acquired antibodies seem to evanesce at a lower rate than newly formed antibodies.[13] Thus, antibodies that had been detected previously might still be present at a titer on the day of the uncrossmatched RBC transfusion sufficient to cause the immune-mediated hemolysis of incompatible units. Using the data from the current study, the risk of hemolysis after uncrossmatched RBC transfusion can be viewed in several ways. If only the patients with clinically significant antibodies that were active on the day of the uncrossmatched RBC transfusion are considered, then the risk is 1 of 7 (14%). Considering patients with either historical or active clinically significant antibodies, then the risk is 1 of 11 (9%). However, as frequently no immunohematologic information on a bleeding patient is available at the time the decision is made to use uncrossmatched RBCs, perhaps the most clinically relevant rate of hemolysis is 1 of 218 (0.5%), with the denominator including all of the patients in this study. These figures are similar to those from the study by Goodell et al. in which of the 265 uncrossmatched RBC transfusion episodes, 17 of the recipients had clinically significant antibodies and 1 patient had at least an exacerbation of an underlying immune-mediated hemolytic reaction.[4] Thus the evidence from the current study and that from the previous studies suggests that the use of uncrossmatched RBCs in the setting of a life-threatening hemorrhage is a relatively safe bridge until crossmatched RBCs become available.

As crossmatched RBCs are typically only matched for ABO and D, the use of uncrossmatched RBCs should not result in a higher incidence of anamnestic antibody responses or delayed hemolytic reactions compared with use of crossmatched RBCs. This is particularly true when the recipient's transfusion history is unknown or if there is no documentation of a historical antibody. That "new" antibodies in this study were detected relatively quickly after the uncrossmatched transfusions in two recipients without a history of antibodies suggests that these antibodies were possibly the product of a secondary immune response to D and E, respectively. Although the appearance of the anti-D could have been avoided had D– RBCs been transfused to the 27-year-old male recipient, O– RBC inventory pressures on the day of the uncrossmatched RBC transfusion required the use of D+ RBCs. Given that he did not receive other D+ blood products before the anti-D was detected, there is little doubt that the uncrossmatched RBCs were the stimuli for the reappearance of the anti-D. In the absence of any immunohematologic history, the sensitization to E in the other male recipient could not have been avoided by routine clinical practice. It is unclear whether the antibodies in the two other patients were stimulated by the uncrossmatched RBCs or by the additional RBCs that these patients received before their respective antibodies were detected.

The main limitation of this study is that we were not able to determine whether the patients with clinically significant antibodies on the day of the uncrossmatched RBC transfusion actually received RBCs that were incompatible with their antibody. Thus the observed rates of hemolysis in this study might be artificially low if the uncrossmatched RBCs were lacking the cognate antigen(s). In fact only D– uncrossmatched RBCs were transfused to two of the patients with active anti-D, although one of those recipients also had an active anti-K on the day she received uncrossmatched RBCs. Likewise, it is also possible that the recipient with the historical anti-Jk^a who had an antibody screen performed 14 days after receipt of the uncrossmatched RBCs was not reexposed to the cognate antigen; hence, the antibody did not reappear. Another limitation of the study is that because of the retrospective nature, the recipients were not specifically monitored for hemolysis after the transfusion of uncrossmatched RBCs; had each recipient of uncrossmatched RBCs been carefully followed after his or her transfusion with serial measurements of the biochemical markers of hemolysis and antibody screens, then perhaps evidence of hemolysis would have been detected in more patients. Furthermore, one patient who received uncrossmatched RBCs during resuscitation for septic shock died within a few hours of the transfusion; although no mention of an acute hemolytic event was made in the clinical notes of the resuscitation, a more thorough assessment was not possible; hence, the current rate of hemolysis might be slightly higher than reported. With the exception of the four recipients who produced an antibody after receipt of uncrossmatched RBCs, the number and results of subsequent antibody screens among the patients who did not have a known active or historical antibody on the day that they received their uncrossmatched

RBC transfusions were not recorded; thus we cannot derive the rate at which delayed serologic or hemolytic reactions occurred after the transfusion of uncrossmatched RBCs. This rate is not expected to be higher among recipients of uncrossmatched RBCs compared with recipients of crossmatched RBCs in our system because we do not routinely provide extended antigen-matched RBCs to most recipients.

Although hemolysis after the transfusion of uncrossmatched RBCs is an uncommon event that occurred in less than 1 percent of all recipients of uncrossmatched RBCs, close clinical and laboratory monitoring of recipients for evidence of hemolysis is warranted if a history of or active clinically significant antibodies are discovered on subsequent serologic investigation.

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