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SUPPLEMENT ARTICLE

TRANSFUSION

Anti-D alloimmunization in Rh(D) negative adults with severe traumatic injury

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Abstract

Introduction: Widely varying rates of alloimmunization associated with transfusing uncrossmatched RBC products to trauma patients as part of hemostatic resuscitation have been reported. We characterized the rates of RBC alloimmunization in our severely injured Rh(D) negative trauma population who received uncrossmatched Rh(D) positive RBC products.

Methods: In a 10-year retrospective analysis to assess Rh(D) alloimmunization risks, Rh(D) negative adult trauma patients initially requiring uncrossmatched group O Rh(D) positive RBC products with either RBC units or low titer group O whole blood as part of massive transfusion protocol (MTP) activation were identified. Only those Rh(D) negative patients whose initial antibody screenings were negative were included. Duration of serologic follow-up from date of MTP activation to either date of anti-D detection or most recent negative antibody screening was calculated.

Results: There were 129 eligible Rh(D) negative trauma patients identified. Median injury severity score was 25. Anti-D was detected in 10 (7.8%) patients after a median of 161.5 days; the median duration of serologic follow-up in those who did not have anti-D detected was 220 days. Patients who had anti-D detected were less severely injured and received fewer Rh(D) positive RBC products versus those who did not.

Discussion: In our severely injured adult trauma patients with MTP activation requiring uncrossmatched group O Rh(D) positive RBC products, the rate of anti-D detection was low. Additional studies are necessary to determine generalizability of these findings and fully characterize alloimmunization risks in trauma patients with varying extents of injury.

KEYWORDS

immunohematology (RBC serology, blood groups), transfusion practices (adult)

1 | INTRODUCTION

Trauma patients may require hemostatic resuscitation, and these blood products can be rapidly provided with massive transfusion protocol (MTP) activation.[1] Due to the emergent nature of treatment, a blood group

Abbreviations: HDFN, hemolytic disease of the fetus and newborn; ISS, injury severity score; LTOWB, low titer group O whole blood; MTP, massive transfusion protocol.

determination is usually unavailable at the onset of transfusion. Such patients usually receive group O RBC products, which include RBC units and whole blood units. Due to the relative scarcity of group O Rh(D) negative RBC products, many trauma centers provide group O Rh(D) positive RBC products to these injured patients. A potential long-term complication of receiving such uncrossmatched RBC or low titer group O whole blood (LTOWB) units in those who survive their traumatic injuries is RBC alloimmunization. Rh(D) negative patients are at risk of developing anti-D, and such alloimmunized females of childbearing potential who become pregnant may experience hemolytic disease of the fetus and newborn (HDFN).[2] Since the risks of RBC alloimmunization associated with receipt of uncrossmatched, Rh(D) positive RBC products in traumatic injury are debated, we sought to characterize this phenomenon in trauma patients at our medical center.

2 | METHODS

This was a 10-year retrospective, single center, IRB-approved study conducted at the only level 1 trauma center in our state. Trauma patients who presented to our level 1 trauma center from January 1, 2010, to December 31, 2019 were evaluated. Patients were included if at least 18 years of age, suffered traumatic injury, which prompted MTP activation, transfused with at least 1 unit of RBC products, and received valid type and screenings at presentation and at least 14 days after injury. Patients with an initially positive antibody screen were excluded. Detections of anti-D within the first 14 days after MTP activation were not counted because such occurrences were deemed to be anamnestic responses from prior immunizing events.

All patients had at least 1 unit of either group O Rh(D) positive RBCs or LTOWB transfused immediately prior to or during initial MTP activation. Up to 6 units of LTOWB are available in our center's trauma bay, and each MTP round issued by our institution's blood bank for adult patients consists of 6 units of group O Rh(D) positive RBCs, 6 units of group A or AB plasma, and 1 unit of any group apheresis platelets. Only uncrossmatched group O Rh(D) positive RBC products transfused prior to resulting a valid type and screen were tallied.

Patient demographics, mechanism of injury, injury severity score (ISS), Rh(D) positive blood products transfused, and immunohematologic data were all collected on eligible Rh(D) negative patients. Duration of serologic follow-up was calculated as the time interval between first transfusion of Rh(D) positive RBC products and either the first antibody screening with anti-D detected or the most recent negative antibody screening. Serologic follow-up was performed through December 31, 2020. Data are presented as median (interquartile range) or as number (percentage). Anti-D alloimmunization rates are accompanied by binomial probability of 95% confidence intervals (www.statpages.info). Continuous and categorical variables were analyzed by the two-tailed Mann–Whitney test and Fisher's exact test, respectively, with statistical significance defined as $p < .05$ (GraphPad Software, Prism version 5.0, San Diego, CA).

3 | RESULTS

A total of 1654 trauma patients had MTP activation in the study period. Ultimately, 129 Rh(D) negative patients were identified who met all eligibility criteria at day 14 after injury (see Figure 1). Demographics, injury, transfusion, hospitalization, and antibody screening data for all patients are presented in Table 1. The duration of serologic follow-up was 212 (131.5) days for all 129 Rh(D) negative patients. None of the patients received Rh(D) immune globulin.

A total of 10 (7.8%, 3.8%–13.8%) patients had anti-D detected. These 10 alloimmunized patients received a range of 1–4 units of RBCs and/or LTOWB, 1–5 units of plasma, and 0–1 units of platelets. The anti-D detection rate per unit of uncrossmatched Rh(D) positive RBC product transfused for the entire group was 1.1% (0.5%–2.0%; 10 antibodies detected after 905 units transfused). Six (60%) patients who had anti-D detected were males, and the time to detection of anti-D was 161.5 (167.8) days; the duration of serologic follow-up for those who did not have anti-D detected was 220 (127) days.

When comparing those trauma patients who did have anti-D detected versus those who did not, no significant differences in sex, age, race, ethnicity, mechanism of injury, intensive care unit days, mechanical ventilator days, hospital days, or antibody screenings were observed ($p > .05$ for all; see Table 2). However, a significant difference was identified in ISS, with those who had anti-D detected having less severe injuries compared with those who did not (16 vs. 26, respectively; $p = .0023$). These less severely injured patients who had anti-D detected also received significantly fewer Rh(D) RBC products than those who did not alloimmunize (2 vs. 5 units, respectively; $p \leq .0001$).

4 | DISCUSSION

We identified a low anti-D alloimmunization rate in this largest-to-date analysis in Rh(D) negative patients with

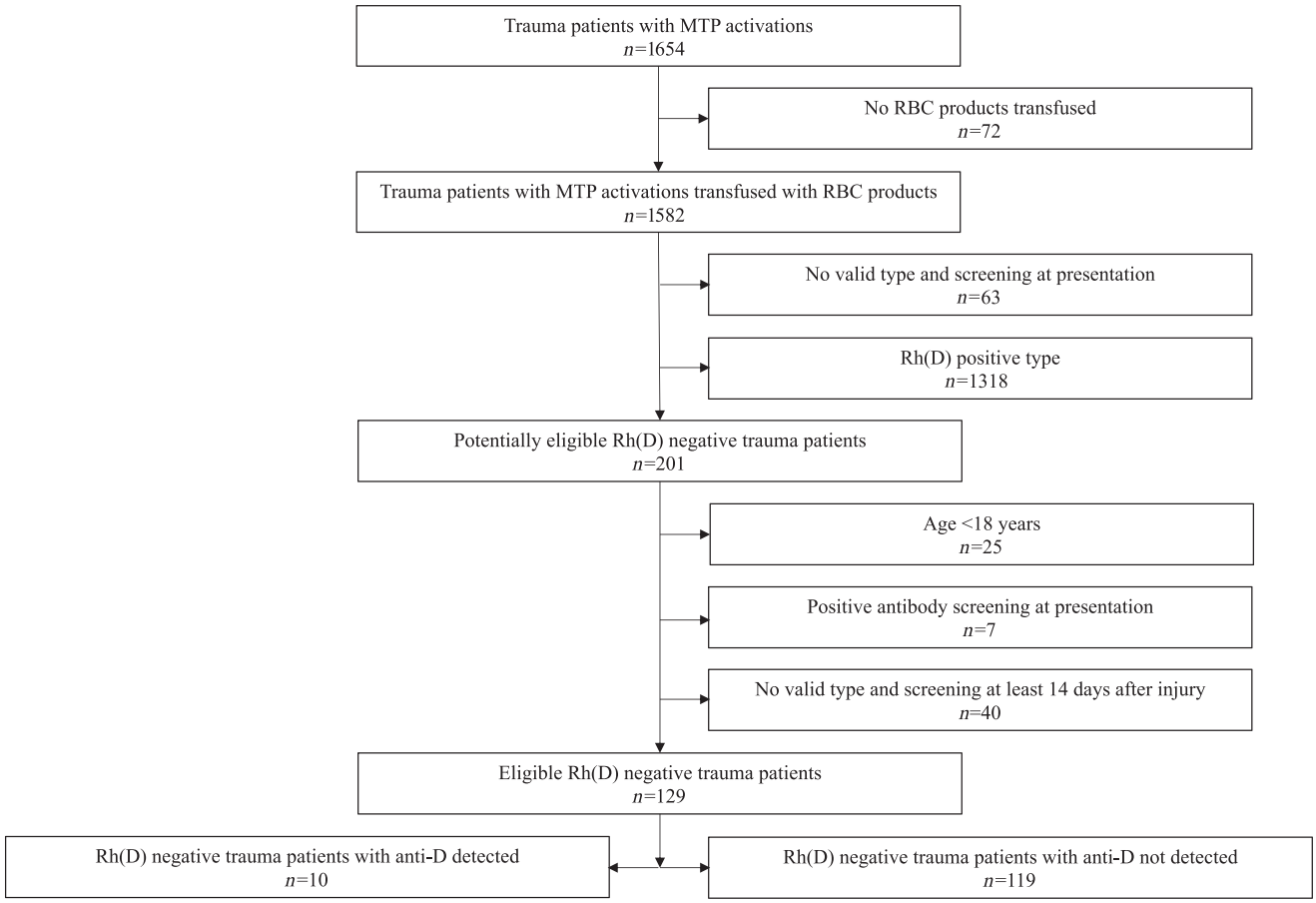

FIGURE 1 CONSORT flow diagram displaying inclusion and exclusion criteria applied to trauma patients for whom massive transfusion protocols were activated in the study period. MTP, massive transfusion protocol

severe traumatic injury receiving uncrossmatched group O Rh(D) positive RBC products. The detection of anti-D in patients longer than 14 days after injury supports the premise that these alloantibodies were due to the uncrossmatched Rh(D) positive RBC products transfused during hemostatic resuscitation for traumatic injury and not to an anamnestic response from a prior immunizing event. Our findings suggest that selective allocation of group O Rh(D) negative RBC products to patients who have suffered severe traumatic injury to reduce the risk of developing anti-D would not be a resource-conscious or effective strategy. Previous descriptions of RBC alloimmunization in Rh(D) negative trauma patients receiving Rh(D) positive RBCs have reported highly variable rates ranging from 11% to 50%;[3-6] the alloimmunization rate in the current study of 7.8% was consistent with a recent REDS-III registry analysis that demonstrated overall RBC alloimmunization rates in all patients subsequent to transfusion to be 6.67%.[7]

The patients in whom anti-D was detected were significantly less severely injured and received fewer Rh(D) positive RBC products than those who did not. Interestingly, all patients who did have anti-D detected received 4 units or fewer of RBCs or LTOWB, the equivalent of less than one round of blood products issued in our MTP. It has been previously noted that there may be an inverse correlation between number of RBC units transfused and antibody development;[8] indeed, such an observation has also been noted in the sickle cell disease population.[9-12] Mechanisms such as immune tolerance, altered inflammatory profiles of the patient, or immunomodulatory characteristics of the RBC products may explain such a relationship.[13-17]

Our study has a number of limitations. This was a retrospective analysis and thus has all inherent limitations associated with such a study design. It is also important to recognize that this was a single-center analysis at a large tertiary academic medical center, and our results may not be generalizable to other institutions with different patient populations. Notably, the median ISS of 25 for our patients in the current study just met the criteria for a severely injured trauma population; however, alloimmunization risks may not be the same for patients

TABLE 1 Demographic, injury, transfusion, hospitalization, and antibody screening data for Rh(D) negative trauma patients ($n = 129$)

Age (years)	Male	Caucasian	Hispanic	Blunt injury	Injury severity score	Rh(D) positive RBC products transfused	Intensive care unit days	Mechanical ventilator days	Hospital days	Antibody screenings performed
41 (36)	93 (72.1%)	88 (68.2%)	56 (43.4%)	103 (79.8%)	25 (17.5)	5 (2)	5 (11)	3 (9)	16 (25)	4 (3)

Note: Continuous data are presented as median (interquartile range) and categorical data are presented as number (percentage).

TABLE 2 Demographic, injury, transfusion, hospitalization, and antibody screening data for Rh(D) negative trauma patients stratified by those who had anti-D detected ($n = 10$) versus those who did not ($n = 119$)

	Age (years)	Male	Caucasian	Hispanic	Blunt injury	Injury severity score	Rh(D) positive RBC products transfused	Intensive care unit days	Mechanical ventilator days	Hospital days	Antibody screenings performed
Anti-D	37.5 (28.5)	6 (60.0%)	6 (60.0%)	5 (50.0%)	7 (70.0%)	16 (9.25)	2 (2)	4.5 (5.5)	2 (6.75)	20.5 (24.25)	5 (2)
No anti-D	41 (42)	87 (73.1%)	82 (68.9%)	51 (42.9%)	96 (80.7%)	26 (19)	5 (2)	5 (11)	3 (9)	16 (26)	4 (3)
p value	.4743	.4640	.7248	.7457	.4204	.0023	<.0001	.7301	.7665	.4672	.5647

Note: Continuous data are presented as median (interquartile range) and categorical data are presented as number (percentage).

with different extents of injury severity. There are also factors for selection bias. Since alloimmunization status could only be confirmed for those patients who had antibody screenings performed after injury, we do not know what happened to those who survived but did not have repeat antibody screenings or were lost to follow up. Furthermore, we could not account for comorbidities in individuals for whom providers may have been compelled to order more frequent serial type and screens, particularly in the outpatient setting. While none of the Rh(D) negative patients in the current study had received Rh(D) positive RBC products or platelets prior to their traumas according to our blood bank records, we could not definitively exclude a prior history of transfusion from outside our system. We also identified potential subjects for this study using the records of MTP activation to select for trauma patients injured to a greater extent that would require uncrossmatched group O Rh(D) positive RBC products. Though the decision to activate MTP for traumatic injury can be subjective, the patients in the current study for whom MTPs were ordered were severely injured. While it is possible that massive quantities of blood products for trauma patients could have been ordered outside of the MTP, this is not the typical ordering practice from our trauma service, and Rh(D) positive RBC units would not have been routinely issued from our blood bank to such patients. Furthermore, it is not our usual practice to issue Rh(D) positive RBC products to trauma patients who do not have serious injuries requiring MTP activation. Lastly, no children with severe traumatic injuries were included in the current analysis.

Only approximately 10% of RBC collections in the world are group O Rh(D) negative, and the needs for these units are increasing.[18,19] Given the relatively low rate of anti-D detection observed in the current study along with the small risk of less than 0.5% of fetal demise due to HDFN in a future pregnancy from anti-D development in Rh(D) negative female trauma patients transfused with uncrossmatched Rh(D) positive RBC products,[2] our findings support the general practice of adult patients with severe traumatic injury receiving group O Rh(D) positive RBC products during the initial stages of hemostatic resuscitation.[20] These data also justify our level 1 trauma center's current practice of initial uncrossmatched group O Rh(D) positive RBC product allocation as part of our MTP. In conclusion, the risk of anti-D detection in this select group of severely injured Rh(D) negative trauma patients is low, but those with less serious injuries may be at greater risk of alloimmunization. Multicenter studies are necessary to more completely characterize anti-D alloimmunization risks across multiple demographic groups with a broad range of injury severities.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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