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Alloimmunization in patients with sickle cell disease and underrecognition of accompanying delayed hemolytic transfusion reactions

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Abstract

BACKGROUND: Patients with sickle cell disease (SCD) often require red blood cell (RBC) transfusions but alloimmunization remains a significant complication. Alloantibodies can lead to delayed hemolytic transfusion reactions (DHTRs) days to weeks after a RBC transfusion, but may be underrecognized in patients with chronic hemolysis.

STUDY DESIGN AND METHODS: This retrospective study aimed to determine the incidence and severity of DHTRs associated with new antibody detection in a cohort of 624 patients with SCD who received transfusion with C-, E-, and K-matched RBCs from primarily African American donors over a 14-year period. We identified potential DHTRs by the change in hemoglobin (Hb) and % HbS at baseline, before transfusion, and up to 30 days after the transfusion that preceded new antibody identification.

RESULTS: Laboratory evidence of a DHTR was associated with 54 of 178 evaluable antibodies at first detection (30%), among which less than half were recognized by the patient or provider at the time of the event. A DHTR was associated with 26% of Rh antibodies identified in patients receiving serologic Rh-matched RBCs, and 38% of non-Rh antibodies. Twenty-one of the 54 DHTRs (39%) were associated with a Hb decline greater than 1 g/dL lower than pretransfusion values. Among these 21 severe DHTRs, Rh specificities were identified in 10 of 12 DHTRs in chronically transfused patients, while non-Rh specificities were associated with seven of nine DHTRs in episodically transfused patients.

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AUTHOR CONTRIBUTIONS

SLC conducted research, analyzed results and edited the manuscript. CMW and DFF conducted research, analyzed results, and edited the manuscript. STC designed the study, conducted research, analyzed results and wrote the manuscript.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

CONCLUSION: High clinical suspicion and monitoring for DHTRs is warranted, as they may be more common in patients with SCD than previously appreciated.

Patients with sickle cell disease (SCD) often require red blood cell (RBC) transfusions to manage and prevent complications but alloimmunization remains a significant problem. This patient population is one of the most frequently and heavily alloimmunized, with the prevalence ranging from 7% to 59%,[1,2] compared to 2% to 3% of sporadically transfused patients from general hospital populations.[3,4] The number of transfusion exposures and RBC antigen differences between donors of primarily European decent and patients of mostly African ancestry contributes to the high rate of alloimmunization. Rh (D, C, c, E, and e) and K antibodies are the most frequent specificities encountered and, therefore, prophylactic C, E, and K (CEK) antigen-matched RBCs are recommended.[5,6] While extended matching to also include the Kidd, Duffy, and MNS systems reduces alloimmunization,[7] identifying sufficient units in the donor supply is challenging,[8,9] and extended typed units are often reserved for individuals who have formed multiple alloantibodies. Inheritance of *RH* variants that encode partial Rh antigens and result in loss of Rh protein epitopes further contributes to alloimmunization and adds additional complexity to antibody identification and donor Rh antigen matching.[9,10]

Alloantibodies can shorten the survival of transfused RBCs and lead to delayed hemolytic transfusion reactions (DHTRs) days to weeks after a transfusion. An estimated 1.6% to 11% of transfused patients with SCD develop overt DHTRs with increased fatigue, jaundice, dark urine, fever, and/or pain,[11–14] but mild DHTRs are underrecognized.[11,15] Since extravascular removal is the primary mechanism of RBC clearance, DHTRs can occur without obvious clinical symptoms. However, laboratory evaluation may demonstrate a decrease in hemoglobin (Hb) or increase in %HbS incongruent with recent transfusion, as well as hyperbilirubinemia above baseline, reticulocytosis, and/or a weakly positive direct antiglobulin test (DAT). DHTRs are also underestimated since new antibody formation is not always detectable at time of symptomatic presentation, or the anemia and hemolysis may precipitate pain and be misdiagnosed as a vaso-occlusive episode.[11,15]

The terms hyperhemolysis and bystander hemolysis are used to describe DHTRs in patients with SCD when severe hemolysis occurs and the Hb decreases to less than pretransfusion levels.[16–18] This suggests hemolysis of the patient's own RBCs in addition to transfused cells.[19] Decreased endogenous erythropoietic drive after transfusion can also exacerbate the anemia associated with a DHTR. Severe hemolysis can occur with no identifiable antibody and a negative DAT. However, recognition is critical since samples should be tested by more sensitive methods and additional transfusions be avoided if possible, as hemolysis may worsen and potentially lead to fatality.[11,20]

This study aimed to determine the incidence and severity of DHTRs associated with new antibody detection in a cohort of 624 patients with SCD after transfusion with CEK-matched RBCs from primarily African American donors. We demonstrate that 30% of new antibodies were associated with a DHTR, more than half were unrecognized at the time of the event, and the clinical significance varied with antibody specificity and transfusion setting (acute versus chronic).

MATERIALS AND METHODS

Study design

This was a retrospective analysis to determine which alloantibody specificities were associated with DHTRs. Under an institutional review board approved protocol, we reviewed the clinical records of 624 patients with SCD who received at least one RBC transfusion at The Children's Hospital of Philadelphia between January 1, 2003, and December 31, 2016. We identified all new antibody specificities and recorded the dates detected, number of units received at the time of first detection for each antibody, and complete blood counts and Hb quantifications. We excluded antibodies detected before the first transfusion at our facility and those detected more than 30 days posttransfusion.

Transfusion protocol

Patients were prospectively antigen matched for ABO, D, and CEK; patients who lack the antigen were transfused with antigen-negative units. Units were from the "Blue Tag" program consisting of donors who self-identify as African American, with some exceptions related to need for antigen-negative units. Patients received HbS-negative units less than 21 days old per protocol for patients with hemoglobinopathies. All units were leukoreduced and irradiated per institutional policy.

Laboratory testing

A three-cell antibody screen, complete blood count, and Hb quantification was performed before each transfusion or when a clinician suspected new antibody formation or a DHTR. Antibody identification was performed with a gel-based method (Ortho Diagnostics). A warm autoantibody was defined as a positive DAT with a panagglutinin in the plasma or eluate with similar strength of reactivity to all cells tested and no apparent specificity. A cold autoantibody was defined as reactivity with a panagglutinin pattern or no specificity that could be removed by prewarm testing at 37°C. A DAT was performed on samples from patients suspected of having a DHTR and a subsequent eluate with antibody evaluation if the DAT was positive. Antibody identification with a low-frequency antigen RBC panel was performed if a DHTR was suspected but no specificity identified, and 1 or more units demonstrated crossmatch incompatibility with the posttransfusion specimen. *RH* genotyping was performed with RHD and RHCE BeadChip arrays (Bioarray) and polymerase chain reaction—based assays as described previously for all patients to determine presence of partial Rh antigens.[9,10,21]

Determination of DHTR by laboratory variables

For each newly identified antibody, we compared the patient's total Hb or HbA and HbS percentages at time of antibody detection with pretransfusion values. Antibodies were excluded from DHTR analysis if there was insufficient laboratory data; if antibody detection was greater than 31 days posttransfusion; or if the antibody was detected concomitant with acute chest syndrome, splenic sequestration, or sepsis that may have exacerbated the anemia. For antibodies that occurred in chronically transfused individuals (patients transfused at regular intervals, typically every 21–28 days), we calculated the mean pretransfusion Hb and

HbA and HbS levels in the 6 to 12 months preceding antibody detection. A DHTR was defined by a Hb decrease and/or HbS% increase greater than 2 standard deviations (SDs) from their individual mean at the time of new antibody detection. For antibodies that occurred in patients who had received an episodic transfusion, the baseline Hb was calculated from outpatient visits in the preceding 12 to 24 months, and the pre- and posttransfusion Hb level and %HbS were recorded. Newly detected antibodies were considered to be associated with a DHTR that was defined by a Hb decrease below the pretransfusion or baseline Hb, and the %HbS values were used as supportive data.

Statistical analysis

Statistical analysis was performed using the t test to compare parametric data between groups, and the Mann-Whitney or Kruskal-Wallis tests for nonparametric data. Fisher's exact test was used for categorical data. A two-tailed p value of less than 0.05 was considered significant.

RESULTS

Subjects

Among 624 patients with SCD who received at least one RBC transfusion, we identified 124 individuals with one or more newly identified alloantibodies (19.9%). SCD genotypes included 111 SCD-SS (89.5%), four SCD–Sβ^0 thalassemia (3.2%), four SCD-Sβ^+ thalassemia (3.2%), four SCD-SC (3.2%), and one SCD-SO Arab (0.8%). Ninety-six patients had been on chronic transfusion therapy and 28 patients had only received episodic transfusions. Collectively, 47,601 RBC units were transfused to these 124 patients. For those receiving chronic transfusions, the mean number of exposures per patient was 493 units; median, 417; and range, 8 to 1808. For individuals who received episodic transfusions for acute complications or preoperatively, the lifetime mean was 9 units; median, 8; and range, 1 to 27.

Antibodies identified in patients with SCD transfused with Rh- and K-matched RBCs

Among 124 patients, 239 specific antibodies were identified (Fig. 1A). A total of 174 antibodies occurred during chronic transfusion therapy (72.8%) and 65 antibodies formed after an episodic transfusion (27.2%). Rh specificities were the most frequent (n = 146, 61%). Despite transfusion with D, CEK-matched RBCs, antibodies against common Rh specificities were the most frequent (n = 125): 37 anti-D, 40 anti-C, 20 anti-E, and 28 anti-e (Figs. 1A and 1B). The RBCs of individuals with *RH* variant alleles may express partial Rh antigens and lack common Rh epitopes, putting them at risk of immunization when exposed to conventional Rh proteins, and conversely, exposure to variant Rh antigens or epitopes on donor RBCs can stimulate Rh antibodies in the patient.[9,10,22] Anti-D was detected in one patient who was D− and in 36 patients with D+ RBCs. Among the D+ individuals with anti-D, five had partial D antigen and no conventional D, three had altered *RHD*DAU0*, and 28 had one or more conventional *RHD* (Fig. 1B and Table S2). Anti-C occurred in four C+ individuals with the hybrid *RHD*DIIIa-CE(4–7)-D* gene, which encodes partial C antigen, but anti-C specificity was also identified in the plasma of five C+ individuals with conventional *RHCE*Ce*. Anti-C reactivity was also detected in 31 C− patients who had

received only C− units. Anti-E was identified in the plasma of two E+ patients with conventional *RHCE*cE* and 13 patients who were E− and transfused with only E− RBCs. Five anti-E occurred in E− patients: four who had made anti-e and were switched to E+e− units for further transfusion (three chronic, one episodic) and one individual who was transfused non-CEK-matched RBCs when referred to an outside institution for a surgical procedure and presented to our institution with a DHTR. Of 28 e+ patients who formed anti-e, 11 had partial e antigen and no conventional e, eight had altered *RHCE*ce (48C)*, and nine had one or more conventional *RHCE*ce*.

In addition to antibodies directed against the common Rh specificities, three anti-hr^B and one anti-Hr^B were stimulated in individuals who lack these high-prevalence Rh antigens. Conversely, antibodies to Rh antigens that are low prevalence in most populations but frequent in African Americans included six anti-V/VS, six anti-Go^a, and two anti-RH32. Anti-C^W was also identified in two individuals; C^W is a variant Rh antigen that is present in 2% of Caucasians.[23]

Outside the Rh system, antibodies included six anti-Jk^b, 10 anti-Fy^a, three anti-Fy^b, 12 anti-M, two anti-N, 11 anti-S, one anti-s, eight anti-Lu^a, and one anti-U, which is a high-prevalence antigen absent in 2% of African Americans (Fig. 1A). No anti-K has ever been identified in our prophylactic CEK matching program. Antibodies to low-prevalence antigens included nine anti-Js^a, seven anti-Kp^a, two anti-Vw, three anti-He, and five anti-Wr^a. Js^a and He antigens are low prevalence in most populations (<1%) but have frequencies of 20 and 3%, respectively, on RBCs of African Americans. There were 25 additional specificities among this cohort that were detected prior to the study date and excluded (Table S1). Eighty-four patients (68%) demonstrated a warm autoantibody and eight a cold autoantibody on at least one occasion. Eight individuals had a DHTR reported with no specific antibody identified.

Alloimmunization and number of RBC exposures

Antibodies occurred after cumulative donor exposures ranging from 1 to 1496 units, with a mean of 173 units and median of 82 units. Antibodies against common Rh antigens, despite CEK-matched transfusions, were detected after a median of 109 units for anti-D (n = 37), 82 for anti-C (n = 40), 94 for anti-E (n = 15), and 78 for anti-e (n = 28) (Fig. 2). Among the five patients who were E− and were exposed to E+ units, the median unit exposure to anti-E formation was 5 units (noted as E* in Fig. 2; range, 2–30 units). The median unit exposure for non-Rh antibodies was lower compared to Rh: 35 for anti-Jk^b (n = 6), 35 for anti-Fy^a (n = 10), 20 for anti-S (n = 11), and 26 for anti-Le^a (n = 5). As expected based on prevalence, the median unit exposure at time of detection for antibodies against low-prevalence antigens was higher: 197 for anti-V/VS (n = 6), 169 for anti-Go^a (n = 6), 171 for anti-Kp^a (n = 7), 122 for anti-Lu^a (n = 8), and 164 for anti-Wr^a (n = 5). The median unit exposure was significantly different among Rh, non-Rh, and low-prevalence specificities ($p < 0.0001$, Kruskal-Wallis test).

Laboratory evidence of DHTRs

Patients with a DHTR may present with clinical symptoms of anemia, hemolysis, and sometimes pain. DHTRs in this patient population are also typically accompanied by an increased %HbS level and/or a decrease in Hb and may identify DHTRs without overt clinical symptoms. We evaluated whether patients experienced a DHTR at the time of antibody first detection by comparing the Hb and the %HbS with the patient's baseline and pretransfusion values (Fig. 3A). Among the 239 newly identified antibodies, we excluded 61 antibody events from DHTR analysis for either concomitant acute chest syndrome, splenic sequestration, or sepsis (n = 9); insufficient laboratory data to calculate baseline values (n = 24); or the antibody was detected over 30 days from transfusion (n = 28). There were 178 newly identified antibodies in 89 patients that were evaluable for laboratory evidence of a DHTR.

Of the 178 evaluable antibodies, 54 were associated with laboratory evidence of a DHTR at time of first detection (30.3%, Fig. 3B). Seven patients had DHTRs associated with two newly identified antibodies simultaneously, of which one or both may have contributed to transfused RBC clearance (Table 1). Among Rh antibodies identified despite receiving serologic Rh-matched RBCs, 27 of 111 (24.3%) were associated with a DHTR, compared to 24 of 63 non-Rh antibodies (38.1%; p = 0.0593, Fisher's exact test). Seven of 30 anti-D (23.3%), seven of 32 anti-C (21.9%), five of 11 anti-E (45.5%), and four of 21 anti-e (19.0%) were associated with DHTRs. Among four E− individuals exposed to E+ RBCs, three experienced a DHTR. Only one anti-D, one anti-C, and one anti-e associated with a DHTR was correlated with inheritance of partial antigen only. Among patients with a corresponding conventional allele, five of 24 anti-D (21%), two of six anti-C (33%), one of two anti-E (50%), and one of six anti-e (17%) were associated with a DHTR. However, 47% of Rh antibodies associated with a DHTR occurred in individuals with at least one allele encoding any partial Rh antigen (n = 14 of 30). Two of 6 anti-Go^a and one of two anti-C^w demonstrated a DHTR, while zero of six anti-V/VS were associated with a DHTR.

Outside the Rh system, three of four anti-Jk^b, three of seven anti-S, two of three anti-He, and three of four anti-Wr^a were associated with a DHTR (Fig. 3B). None of the five anti-Lu^a and only one of eight anti-Js^a were associated with signs of RBC clearance. Although three anti-M were associated with a DHTR, two were detected simultaneously with another newly identified specificity, Jk^b or Wr^a (Table 1). The third patient with anti-M was receiving monthly erythrocytapheresis and at time of detection the pretransfusion %HbS was 54.9%, compared to the mean pretransfusion level of 44.0% for the prior 6 months (SD, 3.3%). Overall, among the 54 cases with evidence for DHTRs, only 21 (39%) were reported to the blood bank as a suspected transfusion reaction. A DAT was obtained for 33 of the 54 DHTRs, of which 19 were positive (58%) with varying strength (5 = microscopic +, 4 = 1+, 9 = 2+, 1 = 3+).

DHTRs with no antibody specificity identified

Eight individuals had a suspected DHTR reported to the blood bank but no antibody specificity was identified (Table 2). Five cases occurred in patients receiving chronic transfusions and three followed an episodic transfusion. Five patients reported fatigue, dark

urine, increased scleral icterus, and/or jaundice and had a decreased Hb or elevated %HbS. Three patients presented for their next scheduled erythrocytapheresis visit without symptoms (unique patient identification numbers [UPIDs], 99, 93, and 119) and a DHTR was suspected due to a decreased Hb and increased %HbS (Table 2). For the chronically transfused patients, the Hb level was 1.3 to 1.6 g/dL lower and HbS was 18% to 35.4% greater than their baseline pretransfusion values. Among the episodic transfused, UPIDs 197 and 292 had 0 and 4.3% HbA remaining at 13 and 11 days after a transfusion, respectively. UPID 447 had a Hb level of 10.2 g/dL with 64.3% HbA after transfusion, which declined to 7.3 g/dL with 31.1% HbA measured 12 days later. Although the gel antibody screen was positive in four of the eight patients, no new specificity was identified. Previously identified anti-E in UPID 447 and anti-S in UPID 197 remained detectable, and antigen-negative units had been transfused to both patients. A warm and a cold autoantibody was detected in UPID 126, who was the only individual to have a positive DAT.

DHTRs resulting in Hb below pretransfusion values

Hyperhemolysis is a concern in patients with SCD experiencing a DHTR and is characterized by a Hb decrease to less than pretransfusion levels. Among the 54 antibodies associated with a DHTR, we identified 21 episodes in which the patient's Hb decreased more than 1 g/dL less than the pretransfusion Hb value after an episodic transfusion (Table 3) or 1 g/dL less than the mean pretransfusion Hb level if chronically transfused (Table 4). After episodic transfusion, nine of these events occurred in seven patients all of whom presented 6 to 30 days later with symptoms including pain, headache, dark urine, fatigue, and/or fever (Table 3). Transfusion indications included preoperative preparation for four patients, chronic pain for two, chronic hypoxia for one, anemia secondary to a prior DHTR for one, and acute chest syndrome for another. Two patients (UPIDs 109 and 292) experienced two sequential DHTRs on initiation of a period of chronic transfusions, halting transfusion therapy. Among 12 antibodies in these seven patients, three had Rh specificity and nine were non-Rh. In three cases, two specificities were detected simultaneously (Table 3). The nadir Hb ranged from 3.6 to 6.8 g/dL and was more than 2 g/dL lower than the pretransfusion level in eight of nine events (median decline, 2.7 g/dL; range, 1.1 to 4.1 g/dL). DHTR management included subsequent transfusion in four events, along with steroids and intravenous immunoglobulin (IVIG) in two cases. Two individuals were managed with steroids and IVIG alone, and the remainder received supportive care only.

In patients receiving chronic transfusion therapy, we identified 12 DHTRs in 10 patients were associated with a Hb level of more than 1 g/dL lower than their mean pretransfusion Hb (Table 4). In eight of 12 events, the Hb decline was greater than 2 g/dL. Only four individuals presented with symptoms including pain, headache, and/or dark urine. Seven patients presented to their next scheduled transfusion visit and were found to have a lower Hb and/or higher %HbS than expected on their chronic transfusion regimen. Compared to baseline pretransfusion values, the Hb decrease ranged from 1.1 to 4.6 g/dL at the time of presentation (median, 2.3 g/dL decrease) and the increase in %HbS ranged from 3.3 to 41.3% at the time of presentation (median, 21.9% increase). Among 14 new antibodies detected in 12 events, 11 had an Rh specificity.

Antibody persistence

We examined whether antibody persistence correlated with clinical significance in patients requiring chronic transfusion (n = 162 antibodies). Antibodies associated with a DHTR were detected for a median of 5.5 months from first detection, compared to 5 months for those without evidence of a DHTR (p = 0.7637, Mann-Whitney test, Fig. Fig. S1A). Antibody detection duration ranged from 0 to 119 and 0 to 179 months for those associated and not associated with a DHTR, respectively. The median duration of antibody detection for Rh antibodies associated with DHTR and those not associated with DHTR was not significant: 4 and 2 months for anti-D (p = 0.5886, Mann-Whitney test), 27 and 1 months for anti-C (p = 0.0646), 1 and 0 months for anti-E (p = 0.5130, excluding E− patients exposed to E+ RBCs), and 8 and 4 months for anti-e (p = 0.234; Fig. Fig. S1B).

DISCUSSION

We report a 14-year experience assessing the clinical significance of alloantibodies formed among patients with SCD transfused with D, CEK-matched RBCs from primarily African American donors. Laboratory evidence of a DHTR at the time of antibody first detection was found in 30% of cases (54 of 178 evaluable antibodies). Among these 54, only 21 (39%) were reported to the blood bank as a suspected DHTR. DHTRs associated with a Hb decrease of more than 1 g/dL below the patient's pretransfusion value were better recognized, as the majority (76%, n = 16 of 21) of those cases were reported to the transfusion service. These findings suggest that DHTRs are underappreciated and can be missed if the patient lacks overt symptoms. A previous study reported 11 of 23 (48%) DHTR events were misdiagnosed as a painful vaso-occlusive episode at the time of presentation.[11] Clinical vigilance for DHTRs in patients with SCD is warranted, as we also report eight additional cases without an antibody identified, but symptoms and laboratory studies were consistent with a DHTR. Specifically, a higher %HbS than expected after transfusion should also raise suspicion for a DHTR, even if the patient appears asymptomatic.

Antibodies directed against the Rh system are the most common specificities among patients with SCD despite providing D, CEK antigen-matched RBCs from primarily African American donors.[10] Among alloimmunized patients, more than half of the antibodies had common Rh specificities (52.3%), of which 26.5% were associated with a DHTR. A higher proportion of anti-E were associated with DHTRs compared to the other Rh specificities: five of 11 (46%) anti-E in E− patients transfused with E− units, compared to four of 23 (17%) anti-C that occurred in C− patients transfused with C− units and three of nine (33%) anti-C in C+ individuals, seven of 29 (24%) anti-D in D+ individuals, and four of 21 (19%) anti-e in e+ patients. Among other Rh specificities, zero of six anti-V/VS or two anti-Rh32 were associated with a DHTR, while two of six anti-Go^a and the one evaluable anti-hr^B demonstrated compromised survival of transfused RBCs. Inheritance of variant *RH* alleles in patients with SCD explain approximately one-third of cases, while the remainder are likely stimulated by variant Rh on African American donor RBCs.[9,10] The clinical significance of these anti-Rh despite serologic Rh matching appears to vary, suggesting that the specific *RH* variants that an individual inherits or is exposed to from African American donor RBCs may

have varying clinical effect. Notably, the median unit exposure at time of detection was significantly different among antibodies directed against Rh (95 units), non-Rh (19 units), and low-prevalence (154 units) antigens, likely related both to immunogenicity and to the likelihood of antigen exposure.

Delayed hemolytic transfusion reactions occurred more often with non-Rh antibodies (38.1%) compared to Rh antibodies (26.1%) that were encountered despite serologic Rh-matched RBCs. Although the number of evaluable occurrences was limited, anti-Jk^b (three of four), anti-S (three of seven), and anti-Wr^a (three of four) were associated with a higher incidence of a DHTR. Interestingly, among nine cases of severe DHTR after episodic transfusion that resulted in a lower Hb than before transfusion, non-Rh specificities were identified in seven and Rh antibodies in two (Table 3). Conversely, severe DHTRs in chronically transfused patients were associated with a Rh specificity in 10 of 12 events associated with a lower Hb level than before transfusion (Table 4). Risk of alloimmunization in patients with SCD is increased with transfusion in the acute setting and an inflammatory state.[24] However, the risk of alloimmunization against particular blood group antigens in a heightened inflammatory state has not been described, and further analysis in larger studies may be warranted.

Among the 178 evaluable antibodies, 14 of 16 antibodies (87.5%) in patients requiring transfusion for an acute complication or preoperatively were associated with a DHTR, compared to 40 of 162 antibodies (24.7%) detected in chronically transfused patients. This appears consistent with a single-institution study of 2158 transfusion episodes, in which 20 of 23 identified DHTR episodes occurred after transfusion in the acute setting.[11] However, since we excluded antibodies from the DHTR analysis if detected greater than 1 month after transfusion (n = 26), our results are biased toward antibodies found in episodically transfused patients who returned with symptoms of hemolysis or exacerbated anemia.

Although we found a 30% incidence of DHTRs with new antibody detection, our study may still underestimate both the incidence and the severity of DHTRs. First, we excluded antibodies that were detected at the time of acute chest syndrome, splenic sequestration, or sepsis since the Hb decline may be due to a DHTR or the medical complication. Second, patients who receive episodic transfusions may not return for laboratory evaluation within 1 month of transfusion if the patient does not present with overt complaints. Given that only 40% of DHTRs identified here were reported to the blood bank, mild DHTRs are likely underappreciated. Third, our patient cohort includes many who receive chronic transfusion by erythrocytapheresis, which requires multiple units of RBCs (median, 5 units). Hemolysis after new antibody formation may not be identified if only a fraction of the units transfused were antigen positive, given the relatively strict definition of DHTR requiring a change in Hb or HbS% that was greater than 2 SDs from their baseline pretransfusion values. Regarding severity, most antibodies evaluated in our study occurred in patients on chronic transfusion and were likely primary immune responses since these patients require antibody screens on a regular basis. Thus, these DHTRs may not be as severe as those from a secondary immune response, which are more likely to occur in patients who are episodically transfused given that most antibodies evanesce and, therefore, may go undetected.

Among patients who were chronically transfused for whom antibody screens were obtained regularly, antibody persistence did not correlate with the occurrence of a DHTR. Almost all antibodies became undetectable over time, with a median of 5 months for both antibodies associated with or without a DHTR. This is consistent with a prior study among non-SCD patients in which 49% of antibodies evanesced over time, with half disappearing within 6 months and all by 10 years.[25] Among 162 antibodies in the chronically transfused cohort, 40% were nondetectable within 2 months, 56% within 6 months, and 64% within a year. We observed eight antibodies that were detectable or redetected over a 10-year period, including one anti-D in a D− individual requiring chronic transfusion that has been detectable for 14 years, consistent with the persistence of the polyclonal anti-D response seen by others.[25] The commonly observed antibody evanescence underscores the importance of communication between transfusion providers to prevent DHTRs when patients receive transfusions at more than one institution.

A specific antibody is not always identified with a DHTR.[11,15] A suspected transfusion reaction was reported for eight individuals in whom no antibody specificity was identified. Five patients were symptomatic, but the remaining three presented for their next scheduled transfusion and a DHTR was suspected based on pretransfusion Hb and %HbS measurements. Half had a positive antibody screen, and the DAT was positive in only one case, similar to prior reports of DHTRs with no identifiable antibody in patients with SCD.[11,15,18] Remaining vigilant for symptoms or hematologic laboratory evidence of DHTRs despite a negative antibody evaluation is requisite. When no specificity is found during the event, sequential immunohematologic testing over the following weeks may identify the antibody to guide future donor selection.

Delayed hemolytic transfusion reactions are potentially life-threatening complications in patients with SCD.[20] Our study demonstrates a high incidence of DHTRs with antibodies at first detection (30%), among which less than half were recognized by the patient or the clinical team at the time of the event. Nearly 40% of DHTRs were associated with a Hb level of more than 1 g/dL lower than pretransfusion values. High clinical suspicion for DHTRs is warranted, as they may be more common in patients with SCD than previously appreciated, and supportive care and medical management can prevent associated morbidity and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

DHTR(s)	delayed hemolytic transfusion reaction(s)
SCD	sickle cell disease
UPID(s)	unique patient identification number(s)

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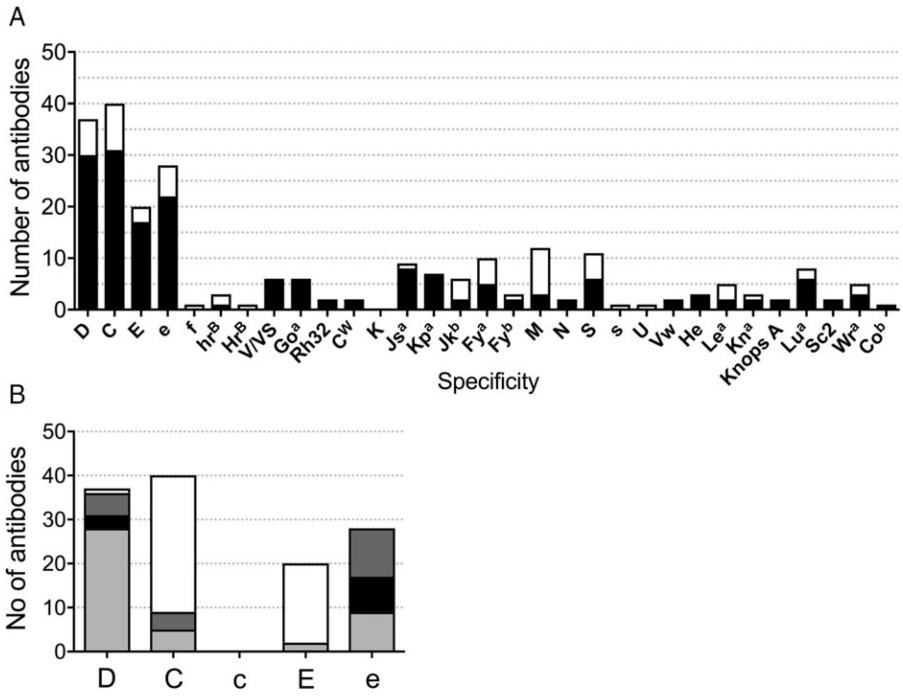

Fig. 1.

Antibody specificities identified in patients with SCD transfused with prophylactic ABO, D, C, E, and K serologic matched RBCs. (A) A total of 175 antibodies occurred during chronic transfusion therapy (■) and 64 antibodies formed after an episodic transfusion (□). (B) Antibodies detected to common Rh specificities in patients whose *RH* genotype predicts RBC expression of partial (▩), altered (■), or conventional (▨) antigen or absence of the antigen (□). *RHD*DAU0* and *RHCE*ce48C*, which encode proteins that have not been shown to lack epitopes are described as altered but not partial antigens.

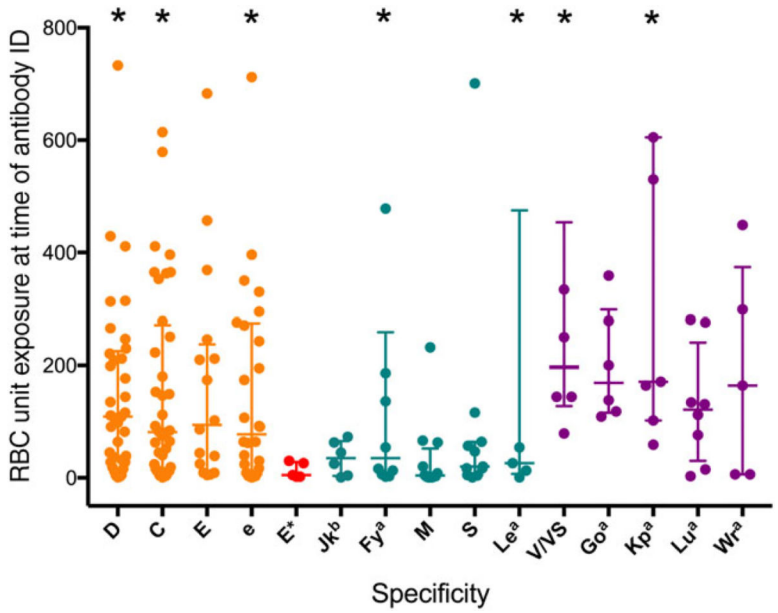

Fig. 2.

RBC unit exposure at time of first detection of new antibody. The RBC unit exposure for each antibody at first detection is shown as a circle, along with the median and interquartile range. *Seven specificities were identified with more than 800 unit exposures (882 units for anti-D, 1359 for anti-C, 864 for anti-e, 1496 for anti-Fy^a, 896 for anti-Le^a, 813 for anti-V/VS, 1492 for anti-Kp^a).

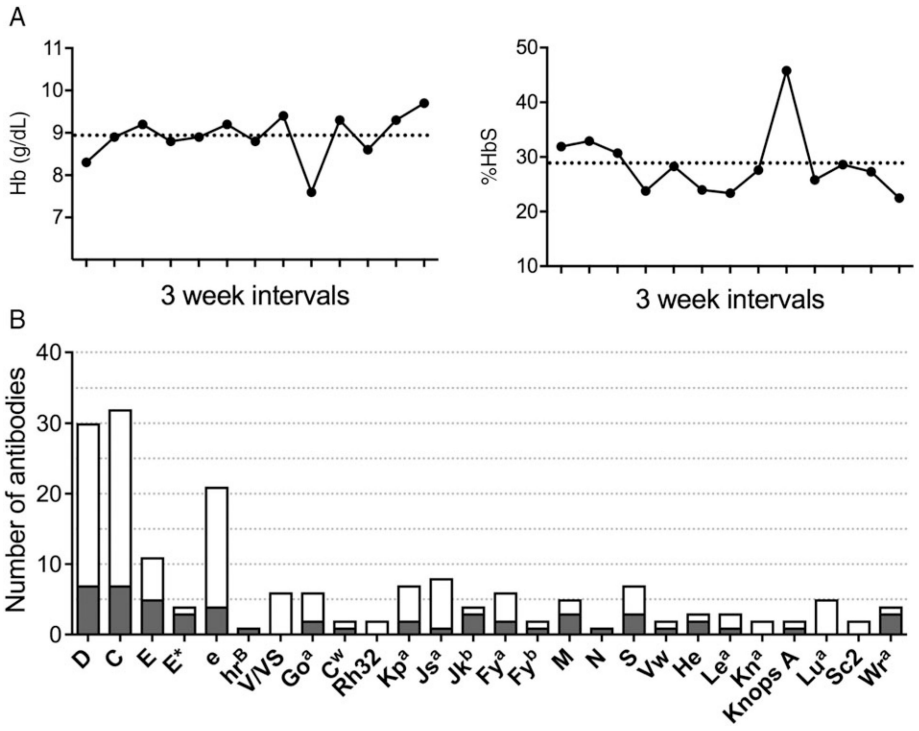

Fig. 3.

Laboratory evidence of DHTRs. (A) Laboratory evidence of a DHTR in a patient chronically receiving RBCs. Arrow denotes time at which a DHTR was suspected. Dotted line indicates the patient's mean pretransfusion Hb level and %HbS for the 12 months preceding the DHTR. (B) Number of antibodies at first detection that were (■) or were not (□) associated with a DHTR according to specificity.

TABLE 1.

Individuals with multiple antibodies identified simultaneously with evidence of a DHTR

UPID	Antibodies identified
77	Anti-C, anti-D, WAU *
109	Anti-C, anti-hr^B, WAU *
138	Anti-C^w, anti-D, WAU *
139	Anti-Jk^b, anti-M
180	Anti-Js^a, anti-Le^a, WAU *
326	Anti-Go^a, anti-Kp^a
746	Anti-Wr^a, anti-M

* Redemonstrating.

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TABLE 2.

DHTRs with no identifiable specificity*

UPID	Transfusion type	Symptoms	Hb (g/dL)		Antibody screen	Antibody identification	DAT
			% Hb at presentation	% Hb baseline mean (chronic) or post-txn (episodic)			
99	Chronic	None	7.7 65.0% S	9.3 29.6% S	Negative	Negative	Negative
93	Chronic	None	8.0 48.1% S	9.3 29.0% S	Positive	Negative	Negative
119	Chronic	None	6.8 69% S	8.3 37.5% S	Negative	Negative	Negative
326	Chronic	Dark urine, scleral icterus reported at next transfusion	7.6 45.8% S	8.9 27.8% S	Negative	Negative	Negative
126	Chronic	Jaundice, scleral icterus, dark urine	6.8 69% S	8.3 37.5% S	Positive	WAU Cold antibody	IgG 2+ C3 micro+
447	Episodic†	Jaundice, scleral icterus	*Day 12*: 7.3 63.1% S, 31.1% A	*Day 0*: 10.2 32.7% S, 64.3% A	Positive	Anti-E, no new specificity	Negative
197	Episodic	Jaundice, dark urine	Day *13*: 7.1 47.6% S, 44.2% C 0% A	*Day 0*: 8.3 8.7% S, 8.4% C 79.5% A	Positive	Anti-S, no new specificity	Negative
292	Episodic	Fatigue, dark urine, jaundice	*Day 11*: 3.9 89.2% S, 4.3% A	*Day 0*: 8.9 62.0% S, 31.4% A	Negative	Negative	Negative

*Laboratory evidence by Hb and Hb quantification in eight patients for whom a DHTR was suspected by the clinical care team but no new specificity was identified. For chronically transfused, the individuals' mean pretransfusion values were calculated from the six to 12 prior scheduled transfusion visits.

†Transfused in acute setting, but patient on chronic transfusion therapy.

micro = microscopic; txn = transfusion; WAU = warm autoantibody.

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TABLE 3.

Severe DHTRs in patients receiving episodic RBC transfusions

				Hb (g/dL)				
					Post-txn			
UPID	**Clinical presentation**	**RBC indication**	**New antibody specificity**	**Pre-txn % Hb**	**% Hb (days post-RBCs)**	**Presentation (days post-RBCs)**	**Nadir Hb quant (%) (days post-RBCs)**	**DHTR management**
109	HA, dizziness, lower back pain, jaundice	Pain (beginning trial of chronic RBCX)	Jk^b	9.4 7 U (RBCX)	10.1 (0 days) 74% A (0 days)	7.8 (+10 days)	6.1 (+12 days) 62% A (+11 days)	RBCs
109	Pain	Anemia due to DHTR (anti-Jk^b)	C, hr^B	6.1 62% A	8.5 (0 days)	6.9 (+30 days)	5.0 (+35 days) 6.5% A (+35 days)	No records
292	Fatigue and dark urine	Chronic hypoxia, awaiting T+A	D	6.5	ND	5.6 (+10 days)	4.2 (+10 days)	IVIG, steroids
292	Dark urine	Preoperative for T+A	Jk^b	7.0	ND	6.7 (+6 days)	4.5 (+6 days)	RBCs IVIG, steroids
139	BUE and BLE pain	Pain (beginning trial of chronic RBC exchange)	S	10.9 6 U (RBCX)	10.1 (0 days) 72.5% A (0 days)	10.9 (+9 days)	6.8 (+13 days) 20.6% A (+13 days)	RBCs
339	Chest, BUE, BLE pain	Preoperative for hip surgery	S	8.6	11.3 (+4 days)	6.3 (+15 days)	5.9 (+16 days)	Supportive
244	Chest pain and fever	Preoperative for cholecystectomy	Fy^a	8.3	10.1 (0 days)	6.5 (+23 days)	4.5 (+25 days)	IVIG, steroids
180	BLE pain and fever	Acute chest syndrome	Js^a, Le^a	7.4	8.0 (0 days)	7.2 (+11 days)	4.8 (+15 days)	Supportive
746	Back, BUE, BLE pain	Preoperative for dental extraction	Wr^a, M	7.7	10.8 (+1 day)	5.7 (+20 days)	3.6 (+22 days)	RBCs IVIG, steroids

BLE = bilateral lower extremities; BUE = bilateral upper extremities; HA = headache; quant = quantification; RBCX = RBC exchange; T+A = tonsillectomy and adenoidectomy; txn = transfusion; U = units.

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TABLE 4.

Severe hemolytic transfusion reactions in patients receiving chronic RBC therapy

UPID	Symptoms on presentation	Transfusion regimen in units, interval	New antibody specificity	Days after transfusion	Hb (g/dL)		HbS (%)	
					Pre-txn Hb (g/dL)*	Presentation (difference from mean pre-txn)	Pre-txn HbS (%)*	HbS (%) (difference from mean pre-txn)
50	Pain	2 units 21 days	D	11	8.5 (±0.737)	6.4 (2.1)†	44.3 (±9.2)	58.8 (14.5)
77	None	7 unit RBCX 21–28 days	E	29	9.7 (±0.41)	5.1 (4.6)†	26.9 (±3.4)	52 (25.1)†
78	None	8-unit RBCX 25–28 days	Wr^a	28	10.6 (±0.31)	9.1 (1.5)†	33.2 (±2.3)	46.9 (13.7)†
95	HA	3-unit RBCX 21 days	C	18	9.3 (±0.65)	5.0 (4.3)†	27.0 (±3.6)	31.2 (4.2)
95	None	4-unit RBCX 21 days	E	22	9.1 (±0.495)	7.5 (2.6)†	27.1 (±3.9)	68.0 (40.9)†
103	None	6-unit RBCX 21 days	E	20	10.0 (±0.36)	7.6 (2.4)†	24.3 (±4.7)	66.1 (41.3)†
108	None	2 units 28 days	e	25	7.6 (±0.32)	6.3 (1.3)†	23. (±6.3)	50.7 (27.6)†
138	None	4-unit RBCX 28 days	C^W, D†	29	10.1 (±0.52)	8.2 (1.9)†	28.2 (±4.0)	61.6 (33.4)†
326	Back pain, HA, and dark urine	2 units 28 days	Go(a), Kp^a	17	8.3 (±0.60)	6.0 (2.3)†	39.1 (±6.3)	
326	Back pain	3-unit RBCX 28 days	He	20	8.6 (±0.36)	7.5 (1.1)	30.3 (±3.9)	48.9 (18.6)†
340	None	2 units 21–28 days	E‡	22	9.0 (±0.59)	6.7 (2.3)†	63.8 (±8.0)	67.1 (3.3)
348	No history available	1 unit 35–42 days	C	31	9.2 (±0.45)	4.9 (4.3)†	42.6 (±7.0)	

*Data are reported as mean (±SD).

†Difference >2 SDs from the individual's mean Hb or HbS.

‡Anti-Rh specificities identified despite individual carrying at least one conventional corresponding *RH* gene.

HA = headache; NA = not available; RBCX = red blood cell exchange; txn = transfusion.