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Research Paper

The impact of pre-existing HLA and red blood cell antibodies on transfusion support and engraftment in sickle cell disease after nonmyeloablative hematopoietic stem cell transplantation from HLA-matched sibling donors: A prospective, single-center, observational study

Robert Sheppard Nickel[a,b,*], Willy A. Flegel[c], Sharon D. Adams[c], Jeanne E. Hendrickson[d], Hua Liang[e], John F. Tisdale[f], Matthew M. Hsieh[f]

[a] Children's National Hospital, Division of Hematology, 111 Michigan Ave NW, Washington, DC 20010, United States
[b] The George Washington University School of Medicine and Health Sciences, Washington, DC, United States
[c] Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, MD, United States
[d] Departments of Laboratory Medicine and Pediatrics, Yale School of Medicine, New Haven, CT, United States
[e] The George Washington University, Department of Statistics, Washington, DC, United States
[f] Cellular and Molecular Therapeutics Branch, National Institutes of Health, Bethesda, MD, United States

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ABSTRACT

Background: Hematopoietic stem cell transplantation (HSCT) is curative for patients with sickle cell disease (SCD). Prior to HSCT, patients with SCD commonly receive RBC transfusions with some becoming RBC or HLA alloimmunized. This alloimmunization may impact post-HSCT transfusion requirements and donor engraftment.

Methods: The study population included patients with SCD transplanted on a single-center nonmyeloablative, HLA-matched sibling HSCT trial at the National Heart, Lung, and Blood Institute (NHLBI) who had a pre-HSCT sample available for HLA class I antibody testing. We evaluated transfusion requirements and engraftment outcomes comparing patients with and without pre-existing HLA and RBC antibodies.

Findings: Of 36 patients studied, 10 (28%) had HLA class I antibodies and 11 (31%) had a history of RBC alloantibodies. Up to day +45 post-HSCT, patients with HLA antibodies received more platelet transfusions (median 2.5 vs 1, $p = 0.042$) and those with RBC alloantibodies received more RBC units (median 7 vs 4, $p = 0.0059$) compared to respective non-alloimmunized patients. HLA alloimmunization was not associated with neutrophil engraftment, donor chimerism, or graft rejection. However, RBC alloimmunization correlated with a decreased donor T cell chimerism at 1 year (median 24% vs 55%, $p = 0.035$).

Interpretation: Pre-existing HLA and RBC alloantibodies are clinically significant for patients undergoing HLA-matched nonmyeloablative HSCT. Testing for both HLA and RBC antibodies is important to help estimate transfusion needs peri-HSCT. The association of lower donor T cell chimerism and pre-existing RBC alloantibodies needs further investigation.

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1. Introduction

Hematopoietic stem cell transplantation (HSCT) is now an established curative treatment option for patients with sickle cell disease (SCD) [1]. Traditionally HSCT for SCD involved myeloablative conditioning, but myeloablation is not necessary given that stable donor chimerism of only 25% achieves a hematologic "cure" [2,3]. To minimize HSCT-related toxicity, the National Heart, Lung, and Blood

Abbreviations: GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; NIH, National Institutes of Health; NHLBI, National Heart, Lung, and Blood Institute; RBC, red blood cell; SCD, sickle cell disease

Statement of disclaimer: The views expressed do not necessarily represent the view of the National Institutes of Health, the US Food and Drug Administration, the Department of Health and Human Services, or the U.S. Federal Government.

* Corresponding author at: Children's National Hospital, Division of Hematology, 111 Michigan Ave NW, Washington, DC 20010, United States.
E-mail address: rnickel@childrensnational.org (R.S. Nickel).

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Research in Context

Evidence before this study

HLA class I antibodies are known to cause platelet transfusion refractoriness, and donor-specific HLA antibodies are known to increase the risk of HLA-disparate hematopoietic stem cell transplantation (HSCT) graft rejection. The clinical significance of HLA antibodies in the context of HLA-matched HSCT is not well described, particularly for patients with sickle cell disease (SCD) undergoing nonmyelablative HSCT. We searched PubMed for studies published up to April 20, 2020 using the search terms "sickle cell," "transplant," and "antibodies." One study was identified that evaluated pre-existing HLA antibodies in HLA-matched HSCT for SCD. This study involved children who received myeloablative conditioning and found that patients with HLA class I antibodies received more platelet transfusions and had a higher incidence of acute graft-versus-host disease. No studies have investigated this issue in the context of non-myelablative HSCT.

Added value of this study

We studied the impact of pre-exisiting HLA class I antibodies on transfusion support and engraftment outcomes in patients with SCD enrolled on the NIH HLA-matched sibling nonmyelablative HSCT clinical trial. Patients with HLA class I antibodies received significantly more platelet transfusions in the first 45 days post-HSCT compared to patients without antibodies.

Implications of all the available evidence

Pre-exisiting HLA class I antibodies are clinically significant for patients with SCD undergoing HLA-matched HSCT. Testing patients for HLA class I antibodies before HSCT may help predict platelet transfusion need and assist with the management of platelet transfusion support post-HSCT.

Institute (NHLBI) has studied a chemotherapy-free, nonmyeloablative HLA-matched sibling HSCT regimen for patients with SCD [4,5]. The outcomes of this approach, which have been replicated by other institutions, are excellent: no graft-versus-host disease (GVHD) or transplant-related mortality with a disease-free survival reaching 90% [6–8]. Nonetheless, some patients transplanted with this approach have low donor chimerism that may lead to graft rejection or require prolonged immunosuppression.

Prior to undergoing HSCT, most patients with SCD have received multiple red blood cell (RBC) transfusions that can cause alloimmunization to blood group antigens. Transfused patients with SCD have an increased risk for becoming RBC alloimmunized compared to other transfused patient groups [9]. In some cohorts of adults with SCD, more than 1 in 4 patients had RBC alloantibodies [10,11]. Additionally, even leukocyte-reduced RBC units can induce HLA alloimmunization in patients with SCD [12]. Further, females may become RBC or HLA alloimmunized from pregnancy [13,14].

In the context of the NIH nonmyeloablative HLA-matched sibling HSCT regimen for SCD [4,5], we have previously described immunohematologic complications resulting from RBC antibodies [15]. Patients with pre-existing RBC alloantibodies against donor RBC antigens can develop prolonged reticulocytopenia post-HSCT, requiring protracted RBC transfusion support [15]. Patients can also develop new RBC antibodies in the peri-transplant period which may cause acute, life-threatening hemolysis [15]. The significance of pre-existing HLA alloimmunization in this unique HSCT setting, however, is unknown. In myeloablative HSCT, pre-existing HLA class I antibodies appear to increase the platelet transfusion need [16–18]. And, in HLA-disparate HSCT, HLA alloimmunization increases the risk of graft rejection [19–23].

We designed this study to primarily evaluate the impact of pre-existing HLA class I alloimmunization on transfusion support and donor engraftment after nonmyeloablative HLA-matched sibling HSCT in patients with SCD. As a secondary analysis, we also evaluated the significance of pre-existing RBC alloimmunization on these outcomes.

2. Methods

2.1. Patients

Inclusion criteria for this study were enrollment in the NIH trial NCT00061568 [4,5], and availability of a stored pre-HSCT plasma sample. NCT00061568 was a single-center, single-arm phase 1 and 2 study of nonmyeloablative HSCT with HLA-matched related donors for patients with hemoglobinopathies. For this study, 1 patient with a diagnosis other than SCD (β thalassemia) was excluded. All donor-recipient pairs were identical or minor incompatible for ABO antigens. Patients initially were not excluded because of RBC alloimmunization; but the protocol was later amended to exclude patients with any RBC antibodies against their donor. This exclusion was added because two patients with anti-donor RBC alloantibodies developed RBC aplasia post-HSCT [15]. Written informed consent was obtained from all participants.

2.2. Transplantation regimen

The transplantation regimen has been described previously [4,5]. Briefly, patients received alemtuzumab on days −7 to −3, 300 cGy total body irradiation on day −2, and sirolimus for at least 1 year. The graft source was granulocyte colony-stimulated factor mobilized peripheral blood stem cells [24].

2.3. Transfusion support

Referring hospitals were contacted to obtain RBC antibody histories of all patients, and a RBC antigen matching plan was established [25]. Prior to the start of conditioning, patients underwent a RBC exchange transfusion to achieve a hemoglobin S fraction of 30% or less. In the peri-transplant period, patients were transfused to maintain hemoglobin between 9 and 10 g/dL and platelet counts greater than 50×10^9/L whenever possible. Patients received leukocyte-reduced and irradiated RBC and apheresis platelet products. RBC transfusions were ABO matched to donors and recipients, with antigen-negative units provided when one or both individuals were phenotypically negative. At minimum, RBC transfusions were matched for D, C, E, c, e, and K; patients with a history of a RBC alloantibody received units also matched for the corresponding antigen and for Jk^a, Jk^b, Fy^a, Fy^b, S, and s. Platelet transfusions were not initially HLA-matched, but HLA compatible platelets may have been ordered later in the setting of platelet transfusion refractoriness as part of standard of care. The details of the immunohematology testing that was done as part of the transfusion support of these patients has been previously described [15]. The primary outcomes were the number of platelet transfusions and the number of RBC units transfused from the start of the conditioning regimen to day 45 post-HSCT. RBC units transfused as part of the pre-HSCT RBC exchange were not included.

2.4. HLA and red cell antibody testing

Stored patient plasma samples collected before transplantation were evaluated for HLA class I IgG antibodies (LABScreen PRA Class I; One Lambda, Canoga Park, CA, USA) [26]. Testing was limited to HLA class I, because platelets do not express HLA class II antigens. As the

HLA antibody testing was performed specifically for this study on prospectively collected and banked samples, the antibody results were not available clinically and could not have influenced patient management. Red cell antibody screening, antibody identification, and direct antiglobulin tests were tested by standard hemagglutination in gel matrix method (ID-Micro Typing System; Ortho Clinical Diagnostics, Raritan, NJ, USA) [15].

2.5. Chimerism analysis

Peripheral blood cells CD3 and CD14 or CD15 were positively selected using immunomagnetic beads and short tandem repeats by DNA PCR to determine T cell and myeloid chimerism, respectively. Patients with donor T cell 0% and myeloid 0% were recorded as having 0% donor chimerism at future time points if no chimerism was obtained at the specified time.

2.6. Statistical analysis

As this study was a post hoc analysis of data available from NCT00061568, no a priori sample size calculation was performed. Categorical data was analyzed with the chi-square or Fisher exact test. Continuous data was analyzed using the Wilcoxon rank-sum test. To compare the T cell chimerism among groups of patients over time, a linear mixed-effect model was applied. Statistical calculations were performed with SAS University Edition (SAS Institute Inc., Cary, NC) and R (R Core Team, Vienna, Austria); graphics created using GraphPad Prism version 8.1 (GraphPad Software, La Jolla, CA).

2.7. Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report.

3. Results

3.1. Characteristics of cohort

Fifty-one pre-HSCT stored plasma samples were available for HLA class I antibody testing from 41 eligible patients (10 patients had two pre-HSCT samples tested). Of these 51 specimens, 11 initially had invalid HLA antibody testing results and repeat testing was performed. With repeat testing that included adsorption treatment, 2 of these 11 samples had valid results. Thus a total of 42 specimens had valid HLA antibody test results. Among the 10 patients with two pre-HSCT samples tested, 6 had two valid test results that were concordant, 2 only had invalid test results, and 2 patients and one valid and one invalid test result for which the valid test result was used. Excluding the 5 patients with only invalid HLA antibody testing, the final study cohort involved a total of 36 patients (Supplemental Figure 1). The 5 excluded patients did not differ significantly from the 36 studied patients (Supplemental Table 1).

The 36 studied patients included 11 females and 25 males transplanted between March 2008 and August 2018. The median age was 28 years (range 10 to 65), with only 3 patients less than the age of 18 years (age 10, 16, 17 years). Before HSCT, 11 patients (31%) had a history of RBC alloimmunization (range 1 to 8 RBC alloantibodies) with the most common alloantibody being anti-E. Seven patients (64% of the RBC alloimmunized patients) had a positive RBC antibody screen at the time of HSCT. Three patients had a history of an RBC alloantibody against a donor RBC antigen (these patients were transplanted before the protocol amendment that excluded these patients) [15]. All patients had follow-up past 1 year.

3.2. HLA class I antibody testing

The pre-HSCT sample for HLA antibody testing was obtained a median of 16 days before HSCT. Ten patients (28%) had a positive HLA class I antibody screen. When comparing patients with HLA class I antibodies to patients without these antibodies, sex, age, and RBC alloimmunization were not significantly different (Supplemental Table 2).

3.3. Increased transfusion requirements for patients with pre-existing HLA and RBC alloantibodies

Patients with HLA class I antibodies received significantly more platelet transfusions (median 2.5 vs 1, $p = 0.042$), but not RBC units (median 5 vs 5, $p = 0.49$) (Fig. 1A) in the first 45 days after HSCT. The proportion of patients who did not require any platelet transfusions was not significantly different between patients with and without HLA class I antibodies: 2/10 (20%) vs 12/26 (46%), $p = 0.25$. The graft CD34+ count did not correlate with the number of platelet transfusions (r^2=0.0002, $p = 0.93$). Patients with a history of RBC alloantibodies received significantly more RBC units (7 vs 4, $p = 0.0059$), but not platelet transfusions (1 vs 1, $p = 0.94$) (Fig. 1B) in the first 45 days post-HSCT. The three patients with a history of an RBC alloantibody against a donor RBC antigen received 4, 7, and 10 RBC units during this early post-HSCT time-period. Even after excluding these three patients, patients with a history of an RBC alloantibody received significantly more RBC units than non-alloimmunized patients (6.5 vs 4, $p = 0.0096$). There was a strong correlation between the number of RBC units and platelet transfusions received among patients without HLA antibodies (coefficient of determination r^2=0.68); such a correlation did not occur (r^2=0.04) among patients with HLA antibodies (Fig. 2).

3.4. Decreased donor T cell chimerism for RBC alloimmunized patients

All 36 patients initially had donor engraftment. There was no association of neutrophil engraftment and donor myeloid chimerism with HLA or RBC alloimmunization (Table 1). There was a significant association between RBC alloimmunization with decreased donor T cell chimerism levels at day 365 (Table 1). T cell chimerism over time was significantly different for RBC alloimmunized versus non-alloimmunized patients, $p = 0.048$ (Fig. 3). Specifically regarding donor T cell chimerism at day 365, in univariate analysis the only significant variable was RBC alloimmunization (Supplemental Table 3). Day 365 median donor T cell chimerism was lowest (17.5%) for patients with both RBC and HLA antibodies (Fig. 4). Day 365 donor T cell chimerism for the three patients with a history of an RBC alloantibody against a donor RBC antigen was 8%, 11%, and 29% with a corresponding donor myeloid chimerism of 93%, 97%, and 89%.

Three patients had graft rejection at day +49, 119, and 154. None of these 3 patients had a history of RBC alloantibodies and only one was positive for pre-existing HLA class I antibodies. None of the 36 patients in this study experienced any acute or chronic GVHD.

4. Discussion

We analyzed the RBC and platelet transfusions of patients with SCD treated with nonmyeloablative HSCT from a HLA-matched sibling. Pre-existing alloimmunization correlated with an increased peri-transplant transfusion need. Patients with HLA class I antibodies required significantly more platelet transfusions, and patients with a history of RBC alloantibodies significantly more RBC unit transfusions. Further, RBC alloimmunized patients appeared to have lower donor T cell chimerism at the first year after HSCT.

The association between HLA class I alloimmunization and increased platelet transfusion support has been reported in the myeloablative HSCT setting where children with SCD commonly receive greater than 10 platelet transfusions [17]. We now confirmed this

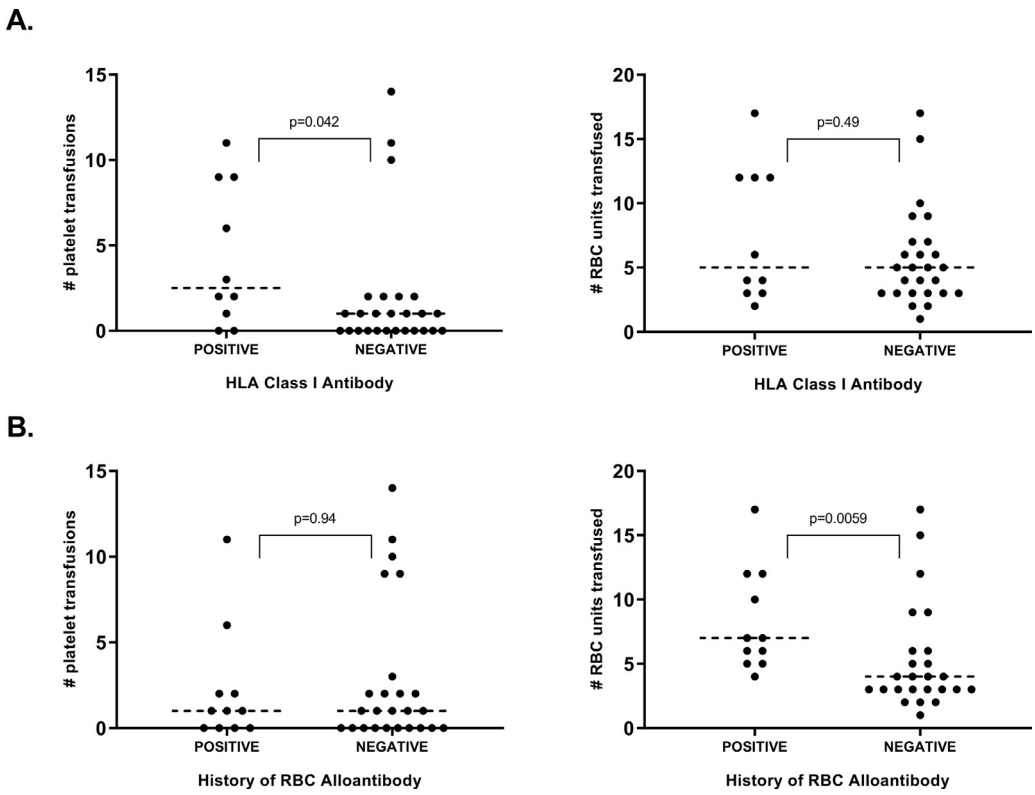

Fig. 1. Transfusion need in patients with and without antibodies to HLA or RBC antigens. RBC, red blood cell. Dashed lines represent the group median. A. HLA Alloimmunization. From the start of conditioning to day +45, patients with pre-existing HLA class I antibodies received significantly more platelet transfusions (2.5 vs 1). B. RBC Alloimmunization. From the start of conditioning to day +45, patients with a history of RBC alloantibodies received significantly more RBC units (7 vs 4).

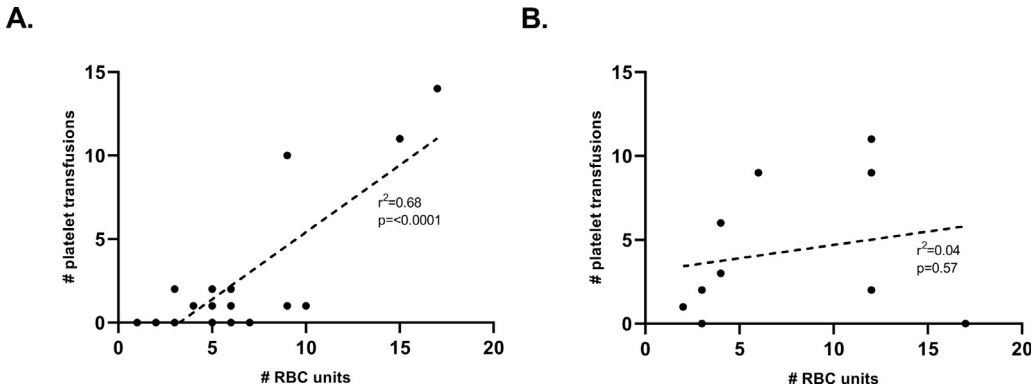

Fig. 2. Relationship between number of RBC units and platelet transfusions during first 45 days post-transplant. RBC, red blood cell. Dashed lines represent the line of best fit. *P*-values represent whether the slope of the line of best fit is non-zero from linear regression. A. Patients without HLA alloimmunization. Among patients with no pre-existing HLA class I antibodies, there was a positive linear association between the number of RBC units and platelet transfusions received. B. Patients with HLA alloimmunization. Among patients with pre-existing HLA class I antibodies, there was no significant association between the number of RBC units and platelet transfusions received.

same association in the nonmyeloablative setting in adults who, however, received a median of only 1 platelet transfusion. The duration of and depth of thrombocytopenia from myeloablation likely explain this difference in platelet transfusion requirements. In both of these HSCT settings, it is unclear if HLA alloimmunized patients require increased platelet transfusion support because of antibody-mediated destruction of transfused platelets or if other immunologic mechanisms are causing increased consumption of transfused platelets. The clinical implication of our results is that it may be useful to obtain HLA antibody testing as part of the pre-HSCT work-up to identify patients at risk for increased platelet transfusion requirements. Identifying HLA antibodies pre-HSCT could also help the transfusion service to more readily obtain HLA-compatible platelets in the setting of future platelet transfusion refractoriness.

Furthermore, HLA alloimmunization was not associated with any adverse engraftment outcome or GVHD in this study. These results are in contrast with our previous results in pediatric myeloablative HSCT for SCD where HLA alloimmunized patients had a higher incidence of acute GVHD [17]. This apparent inconsistency is reflective of the very different conditioning as no GVHD was observed with this nonmyeloablative regimen. Other studies have reported that HLA alloimmunization was associated with graft rejection in the context of HLA-disparate HSCT, but this association was generally restricted to donor-specific HLA alloimmunization [19–23]. Since our study

Table 1
Association of HLA and RBC alloimmunization with engraftment outcomes.

	HLA Alloimmunization			RBC Alloimmunization		
	Yes	No	p-value	Yes	No	p-value
Day of neutrophil engraftment	21.5 (13, 37)	22 (15, 32)	0.33	22 (14, 25)	22 (13, 37)	0.62
Day 100 donor myeloid chimerism	99% (81, 100)	100% (0, 100)	0.47	100% (89, 100)	100% (0, 100)	0.86
Day 100 donor T cell chimerism	3.5% (0, 30)	7.5% (0, 100)	0.38	5% (0, 30)	7% (0, 100)	0.24
Day 365 donor myeloid chimerism	97% (0, 100)	99% (0, 100)	0.50	97% (79, 100)	98% (0, 100)	0.75
Day 365 donor T cell chimerism	28.5% (0, 79)	50% (0, 90)	0.37	24% (8, 62)	55% (0, 90)	**0.035**

RBC, red blood cell.
Values shown are the medians and ranges (minimum, maxium).

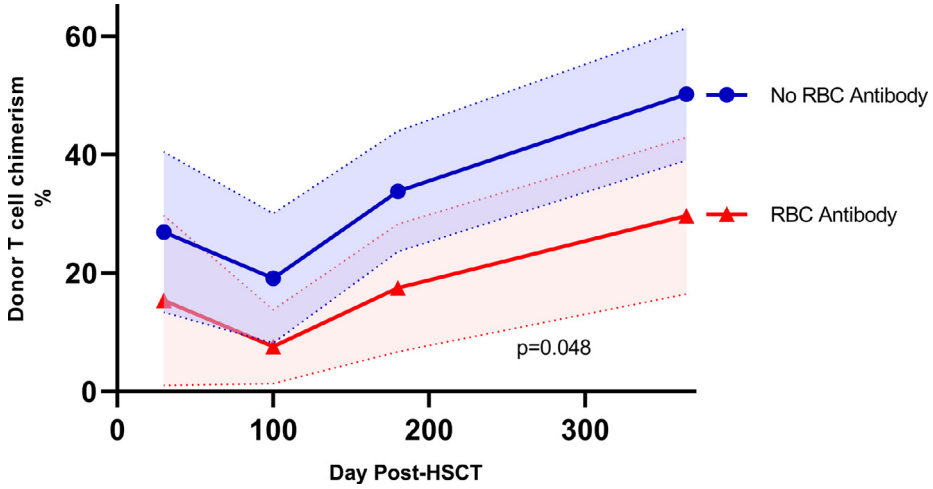

Fig. 3. Comparison of T cell chimerism between patients with and without RBC alloantibodies. HSCT, hematopoietic stem cell transplant. RBC, red blood cell. Points represent the mean donor T cell chimerism for patients with and without a history of pre-existing RBC alloantibodies at day 30, 100, 180, and 365 post-HSCT. Dotted lines and shaded regions represent the corresponding 95% confidence intervals. P-value represents the comparison of T cell chimerism between the two groups over time with a linear mixed-effect model.

involved HLA-matched donors, no patient had donor-specific HLA antibodies. Our results thus confirm the concept that patients with HLA antibodies that are not against donor antigens do not have worse engraftment outcomes.

Patients with delayed engraftment or clinical complications after HSCT generally require more platelet *and* RBC transfusions [27]. We found this expected association between number of platelet and RBC transfusions (i.e. patients who received an increased number of platelet transfusions also received an increased number of RBC units, Fig. 2) among patients without HLA class I antibodies. Interestingly, among HLA alloimmunized patients, this association was not present, possibly because it was confounded by their alloimmunization status.

The finding of an association between HLA class I alloimmunization and increased platelet transfusion support is expected given that platelet products were not empirically HLA matched. On the other hand, our finding of an association between RBC alloimmunization and an increased RBC transfusion burden is more intriguing as all transfused RBC units were negative for antigens for which patients had RBC alloantibodies. The mechanism for this increased transfusion requirement is unclear, as it cannot be caused by known donor specific antibodies. In this patient group we have previously reported that patients with RBC antibodies incompatible with donor antigens, including pre-existing RBC antibodies, were dependent on RBC transfusions for a significantly longer time period than other patients [15]. Currently, however, we demonstrate that RBC alloimmunization that does not involve any known donor specific antibodies was associated with an increased RBC transfusion burden in the first 45 days post-HSCT. Of note, a prior study of patients with SCD undergoing *myeloablative* transplant found that pre-existing RBC antibodies were not associated with increased RBC transfusions post-HSCT [27]. It is possible that the more intensive myeloablative conditioning may negate recipient immunologic characteristics among RBC alloimmunized patients that contribute to increased transfusion requirements among this group post-nonmyeloablative HSCT. Our current findings are consistent with a study that reported patients with SCD and RBC alloantibodies on chronic transfusion therapy had a shorter circulatory half-life of transfused RBCs that were negative for the cognate antigens [28]. Taken together, these findings suggest that the recipient's immune system may impact transfusion responses to matched donor RBCs by yet to be determined characteristics.

Our finding of decreased donor T cell chimerism among RBC alloimmunized patients was novel and requires further study. RBC alloimmunized patients can be considered immunologic "responders," as many transfused patients exposed to most foreign minor RBC antigens do not form alloantibodies. RBC alloimmunized "responder" patients are immunologically distinct from "non-responders" with differences in B and T cells as well as genes involved with immune regulation [29–36]. It is not surprising that these underlying immunological differences persist after nonmyeloablative HSCT and could impact T cell chimerism. In our study, patients who had both RBC and HLA alloantibodies had the lowest T cell chimerism levels (Fig. 4). This interesting finding needs to be substantiated by additional work involving a larger number of patients. The clinical implication of this result is that these patients theoretically could be at higher risk for graft rejection. In reduced intensity

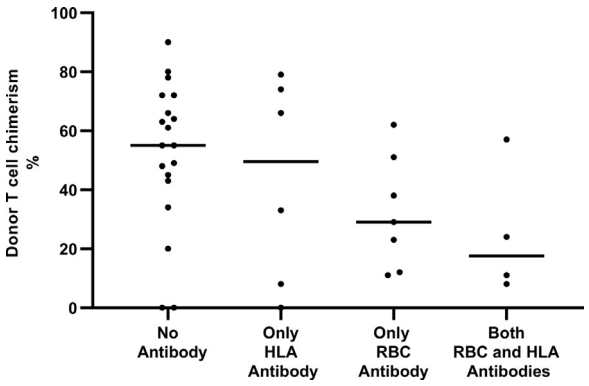

Fig. 4. Comparison of T cell chimerism by alloimmunization group at 1 year post-transplant. RBC, red blood cell. Solid lines represent the group median. Day 365 median donor T cell chimerism for patients with neither pre-existing RBC nor pre-existing HLA antibodies (n = 19) was 55%, for patients with only HLA antibodies (n = 6) was 49.5%, for patients with only RBC antibodies (n = 7) was 29%, and for patients with both RBC and HLA antibodies (n = 4) was 17.5%.

conditioning HSCT for patients with hematologic malignancies, low donor chimerism early post-HSCT is an independent risk factor for relapse and impaired long-term survival [37]. In the nonmalignant HSCT setting where a graft-versus-tumor effect is not needed, the relevance of low donor chimerism is less clear. Patients transplanted for SCD who have mixed chimerism above a certain donor chimerism threshold have normal hematologic parameters [2–7,38]. The long-term stability of grafts with low donor chimerism levels, however, remains unknown.

In this study we did not observe an association of alloimmunization with graft rejection or with decreased donor myeloid chimerism. As such, it is likely that other patient or donor characteristics that have not yet been identified are likely more important in driving graft rejection. Better understanding graft rejection as well as any possible consequences of decreased donor T cell chimerism are important, as more intensive conditioning regimens could be planned for patients deemed to be at higher risk for graft rejection.

This study has several limitations. Five patients were excluded because of invalid HLA antibody testing results, however, this is within the range of expected high background for stored samples and exclusion of these patients should not bias our results. Our HLA antibody testing did not include antibody titer and we did not analyze mean fluorescence intensity or antibody specificity, which may all have clinical implications. Furthermore, we did not measure corrected count increments with all platelet transfusions to evaluate for platelet transfusion refractoriness. In addition, we did not control for multiple comparisons in our statistical analysis of engraftment outcomes as this was an exploratory analysis. Our finding of a significant association of RBC alloimmunization with decreased day 365 donor T cell chimerism thus needs to be validated with future research designed to investigate this issue. Our analysis of chimerism was also restricted to the first year post-HSCT because the management of patients after 1 year changed during the course of the clinical trial. Patients initially transplanted on this trial were all maintained on sirolimus after 1 year; however the protocol was later amended to allow tapering of sirolimus at 1 year if donor T cell chimerism was greater than 50%.

RBC and HLA alloimmunization can prevent some patients from pursuing HSCT because of no compatible stem cell donor secondary to donor-specific antibodies, difficulties finding compatible RBCs for transfusion, or complications encountered during RBC exchange transfusion before HSCT [39,40]. Our study demonstrates that this alloimmunization continues to have implications for patients able to undergo HSCT. The data from our patients with SCD undergoing nonmyeloablative HSCT provide evidence to further support the notion that alloimmunized patients have unique immunologic characteristics. As nonmyeloablative transplant approaches become more widely adopted, the impact of pre-existing alloimmunization on various outcomes, as well as the underlying immunologic mechanisms behind these outcomes, warrant further study.

Declaration of Competing Interest

The authors have nothing to disclose.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2020.100432.

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