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Red Blood Cell Transfusions Are Associated with HLA Class I but not H-Y Alloantibodies in Children with Sickle Cell Disease

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Abstract

Blood transfusions can induce alloantibodies to antigens on red blood cells (RBCs), white blood cells and platelets, with these alloantibodies affecting transfusion and transplantation. While transfusion-related alloimmunization against RBC antigens and human leucocyte antigens (HLA) have been studied, transfusion-related alloimmunization to minor histocompatibility antigens (mHA), such as H-Y antigens, has not been clinically characterized. We conducted a cross-sectional study of 114 children with sickle cell disease (SCD) and tested for antibodies to 5 H-Y antigens and to HLA class I and class II. Few patients had H-Y antibodies, with no significant differences in the prevalence of any H-Y antibody observed among transfused females (7%), transfused males (6%) and never transfused females (4%). In contrast, HLA class I, but not HLA class II, antibodies were more prevalent among transfused than never transfused patients (class I: 33% vs. 13%, $p=0.046$; class II: 7% vs. 8%, $p=0.67$). Among transfused patients, RBC alloantibody history but not amount of transfusion exposure was associated with a high (>25%) HLA class I panel reactive antibody (Odds ratio 6.8, 95% confidence interval 2.1–22.3). These results are consistent with immunological responder and non-responder phenotypes, wherein a subset of patients with SCD may be at higher risk for transfusion-related alloimmunization.

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R.S.N. planned and designed the study, consented participants, analysed data and wrote the manuscript. J.T.H. and J.E.H. planned and designed the study, analysed data and were involved in the final writing of the manuscript. M.M.Y. analysed data and was involved in the final writing of the manuscript. L.S.K. directed the immunophenotype testing, analysed data and was involved in the final writing of the manuscript. R.A.B. and H.M.G. directed the HLA antibody testing, analysed data and were involved in the final writing of the manuscript. D.B.M. directed the H-Y antibody testing, analysed data and was involved in the final writing of the manuscript.

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Keywords

sickle cell disease; H-Y; HLA antibody; alloimmunization; transfusion

Introduction

Blood component transfusions are critical to the care of patients with sickle cell disease (SCD), severe aplastic anaemia, thalassaemia and other haematological diseases. Alloimmunization to red blood cell (RBC) antigens places these patients at risk for serious haemolytic transfusion reactions and can dangerously restrict their access to life-saving blood. In addition, if these patients undergo haematopoietic stem cell transplantation (HSCT), alloimmunization to histocompatibility antigens from transfusions may complicate transplantation. The capacity of RBC transfusions to cause human leucocyte antigen (HLA) alloimmunization (Balasubramaniam, *et al* 2012, Karpinski 2004, van de Watering, *et al* 2003) and of this alloimmunization to contribute to HSCT rejection has been firmly established (Brand, *et al* 2013, Ciurea, *et al* 2009, Ciurea, *et al* 2011, Cutler, *et al* 2011, Ruggeri, *et al* 2013, Spellman, *et al* 2010, Takanashi, *et al* 2010, Yoshihara, *et al* 2012). However, less is known about alloimmunization against minor histocompatibility antigens (mHA), self-peptides presented by HLA and targets of the host-versus-graft response in HLA-matched transplants. Canine and murine models have provided evidence of the capacity of transfusion-related mHA alloimmunization to induce HSCT rejection (Desmarets, *et al* 2009, Patel, *et al* 2009, Patel and Zimring 2013, Storb, *et al* 1970, Storb, *et al* 1971, Storb, *et al* 1979). While some studies suggest pre-transplant transfusions may increase the risk of HLA-identical HSCT rejection in patients with aplastic anaemia due to mHA alloimmunization (Champlin, *et al* 1989, Deeg, *et al* 1986, Sanders, *et al* 1994, Storb, *et al* 1983), it remains unclear whether, in current clinical practice, transfusions actually cause significant alloimmune responses to mHA.

Clinical investigation into this matter has been hampered by the lack of an assay suitable for assessing mHA alloimmunization. With the recent development of a multiplex antibody assay, it has become feasible to clinically study immunity to one group of mHA: H-Y antigens (Wadia, *et al* 2011). H-Y antigens are encoded by the Y chromosome and thus expressed uniquely in males. They can elicit both cellular and humoral immune responses (Porcheray, *et al* 2011, Zorn, *et al* 2004) and have been implicated in the increased HLA-identical HSCT rejection rates observed in female patients who received male grafts (Goulmy, *et al* 1982, Spierings, *et al* 2003, Stern, *et al* 2006). Previously, we have demonstrated the association of H-Y antibodies with rejection of renal allografts from male donors by female patients (Tan, *et al* 2008), secondary recurrent miscarriage (Nielsen, *et al* 2010) and chronic graft-versus-host disease in male recipients of HSC grafts from female donors (Miklos, *et al* 2005). In this study, we sought to determine if H-Y alloimmunization occurs after RBC transfusions. Though RBCs do not express H-Y antigens (Bradley, *et al* 1986, Crichton 1980, Muller, *et al* 1980) (or HLA), RBC units contain other potentially immunogenic substances in addition to RBCs, most notably residual leucocytes that remain despite leucoreduction. These leucocytes would be expected to express both HLA and mHA, including H-Y antigens if from a male blood donor.

We conducted this study of H-Y alloimmunization in children with SCD given the potential clinical importance of such immunization in a patient population who not only has a frequent need for transfusion therapy, but who also may ultimately undergo HSCT. HSCT is the only cure for this serious disease and there has been steady growth in the use of HSCT for SCD, including the extension of HSCT to adults with the use of non-myeloablative conditioning (Hsieh, *et al* 2014). A secondary objective of our study was to more fully assess transfusion-related HLA alloimmunization in SCD patients, a matter of great relevance to current efforts to extend haploidentical HSCT to these patients (Bolanos-Meade, *et al* 2012). While it has been demonstrated that HLA alloimmunization is prevalent among multiply transfused patients with SCD (Ben Salah, *et al* 2014, Friedman, *et al* 1996, McPherson, *et al* 2010), the contribution of transfusion therapy itself has not been defined in this population in the era of RBC product leucocyte reduction. This study was thus designed to provide a rigorous assessment of HLA alloimmunization secondary to leucoreduced RBC transfusions, as well as provide the first clinical assessment of transfusion-mediated mHA alloimmunization.

Methods

Study Design, Participants and Sample Acquisition

We conducted a cross-sectional study of patients age ≥ 2 years with SCD SS or S β^0 at Children's Healthcare of Atlanta (CHOA) between October 2012 and May 2013. Approval was obtained by the by the Emory University and CHOA Institutional Review Boards. Three groups of patients were recruited: 1. Female patients on chronic RBC transfusion therapy (study group; hypothesized to be at risk for alloimmunization to H-Y antigens from RBC transfusion) 2. Male patients on chronic RBC transfusion therapy (control group; hypothesized to be tolerant to H-Y antigens) 3. Female patients who had never received a transfusion (control group; thought to be not at-risk for alloimmunization to H-Y antigens). Pregnant patients or patients with a history of any pregnancy were excluded. To further avoid potential confounding sensitizing events, patients could not have received a platelet transfusion or transplant. Patients were also excluded if they had a recent acute illness (defined as fever, emergency department visit or hospitalization in the previous two weeks) or were taking an immunosuppressant medication other than hydroxycarbamide. Chronic transfusion patients had to have received greater than three consecutive RBC transfusions and had samples collected just prior to their scheduled transfusion. Patients in the never transfused group were roughly age-matched with chronic transfusion patients and specifically recruited at a routine clinic visit after a thorough record review showed that they had never received a transfusion.

Blood Bank Data

Blood bank records were reviewed to obtain the total number of RBC units received and history of RBC auto- or allo-antibodies. All patients transfused during this time at our institution received exclusively packed RBC units that were pre-storage filter leucocyte-reduced by the donor centre. These units were non-irradiated and phenotype antigen-matched for at least D, C/c, E/e, and K (patients whose RBCs lacked the antigen were transfused with antigen-negative donor units). For patients who reported a history of

transfusion at another institution at time of study consent or were identified to have received outside transfusions from review of the medical record, the patients' transfusion history from these centres was obtained, directly from the outside institution blood bank in almost all cases.

H-Y Antibody Testing

Serum was tested for immunoglobulin G (IgG) antibodies against 5 H-Y antigens (DDX3Y [DBY], EIF1AY, RPS4Y1, UTY, ZFY) using a previously validated H-Y protein microarray (Wadia, *et al* 2011). Slides were incubated with patient serum, washed and then treated with a fluorescent secondary antibody. Each H-Y-seropositive threshold was determined as a microarray mean fluorescence intensity (MFI) > the median MFI + 2.5 quartiles measured in 60 adult healthy males.

HLA Antibody Testing

Serum was evaluated for anti-HLA IgG antibodies using the FlowPRA® screening test (One Lambda, Inc., Canoga Park, CA) as described previously (McPherson, *et al* 2010). The panel reactive antibody (PRA) result from this test provides an estimate of the proportion of the general population to which the patient is alloimmunized to class I or class II HLA. As the assay contains 30 class I and class II beads, a positive result is when 1/30 or >3% of the beads show channel shift emission. To ensure accurate results, patients found to have a low positive PRA in which the fluorescent channel shift pattern was not distinct had the FlowPRA® screen repeated. These patients were also tested with LabScreen® Single Antigen Antibody Detection (One Lambda, Inc., Canoga Park, CA) for further result verification and identification of specific HLA antibodies. All HLA antibody testing was performed in the Emory University certified clinical HLA laboratory.

Immunophenotype Analysis

The quantification of 18 different immune cell counts via flow cytometry was performed on patients in this study, as previously described (Nickel, *et al* 2015).

Sample Size

This study was designed to detect a significant difference in the prevalence of transfused females with any H-Y antibodies compared to the prevalence in the two control groups (transfused males and never transfused females). Based on our previous finding that 34% of transfused paediatric SCD patients had HLA antibodies (McPherson, *et al* 2010), we hypothesized a similar prevalence of H-Y alloimmunization in transfused females and a low prevalence (5%) in each of the two control groups. To demonstrate this proposed 29% absolute prevalence difference with 80% power and 95% confidence, a sample size of 50 transfused females and 25 patients in each of the control groups was required using Fisher's exact method.

Statistical Analysis

Fisher's exact test or chi-square test was used when appropriate to compare the prevalence of H-Y antibodies in the three patient groups and to compare the prevalence of HLA class I

or HLA class II antibodies in transfused versus non-transfused patients. Comparison of patients who had HLA class I or HLA class II antibodies with those patients who did not have antibodies was also performed for patient-, disease- and treatment-related variables using a Fisher's exact test or chi-square test for categorical variables and two-sample *t*-test or Wilcoxon rank-sum test when appropriate for continuous variables. Multivariate analysis was performed via logistic regression. Statistical calculations were performed with SAS 9.3 (SAS Institute Inc., Cary, NC.) and graphics created using GraphPad Prism version 6.02 (GraphPad Software, La Jolla California USA, www.graphpad.com).

Results

Patient characteristics

A total of 117 patients with SCD were enrolled. Three patients were excluded because they did not meet eligibility criteria upon further review of the medical record, yielding 58 female patients on chronic transfusion, 32 male patients on chronic transfusion, and 24 never transfused female patients. Patients' age ranged from 2.1 to 22.1 years and was similar across groups (mean age for transfused female 12.0 years, transfused male 12.0 years, never transfused female 10.5 years, $p=0.37$). More patients in the chronic transfusion groups had a history of splenectomy (27% vs. 0%, $p=0.004$), while more patients in the never transfused group were taking hydroxycarbamide (46% vs. 8%, $p<0.0001$).

Among the chronic transfusion patients, the number of RBC units transfused varied considerably (range 6–543 units, median 93 units). The main indications for chronic transfusion therapy were primary (59%) and secondary stroke prevention (32%). Regarding RBC antibody history, 29/90 (32%) had a history of a RBC alloantibody, 25/90 (28%) had a history of a RBC autoantibody and 19/90 (21%) had a history of both. The majority of patients, 56/90 (62%), were exclusively transfused at our institution. Of the patients who had received a transfusion outside of our institution, only two patients had received a non-leucoreduced RBC transfusion (neither of these patients had H-Y or HLA antibodies).

H-Y antibodies were not associated with RBC transfusion

The prevalence of patients with any H-Y antibodies was low in each of the three study groups: 4/58 (7%) of transfused female patients, 2/32 (6%) of transfused male patients and 1/24 (4%) of never transfused female patients ($p=1.0$, Supplemental Table 1). Mean MFI was also not significantly different between the three study groups for any of the H-Y antigens (Supplemental Figure 1). The few patients classified as antibody positive had MFI values just above the positive threshold and much lower than the MFI values from positive control reference samples obtained from male HSCT patients who had received a female graft (F → M) who were known to have H-Y alloantibodies (Figure 1).

HLA class I antibodies were associated with RBC transfusion

Figure 2 shows the distribution of HLA class I and II PRA values for the transfused and never transfused patients. 30/90 (33%) of transfused and 3/24 (13%) of never transfused patients had HLA class I antibodies ($p=0.046$). 16/90 (18%) of transfused and none of the never transfused patients had a HLA class I PRA value $>25\%$ ($p=0.022$). HLA class II

antibodies were less common and not different between the two groups: 6/90 (7%) of transfused and 2/24 (8%) of never transfused patients had HLA class II antibodies ($p=0.67$). Only three patients (all transfused patients) had both HLA class I and class II antibodies.

Table I shows the analysis of HLA class I alloimmunization among all patients. Both of the transfused study groups had a higher odds ratio (OR) of having HLA class I antibodies compared to the reference female never transfused patient group, although this difference was only significant for the male chronic transfusion study group. When the two transfused groups were combined in that only the dichotomous transfusion variable (history of any RBC transfusion, yes/no) was evaluated, transfusion was significantly associated with HLA class I alloimmunization after adjustment for age, splenectomy and hydroxycarbamide (Table I). In contrast, no variable was significantly associated with HLA class II alloimmunization (Supplemental Table 2).

HLA antibodies in never transfused patients

To validate the finding that 5/24 (21%) of never transfused, nulliparous patients had a positive HLA antibody screen (class I or class II), these positive results were confirmed by two methods. First, re-testing banked serum from the original collection with a repeat antibody screen yielded identical positive PRA results. Second, a different HLA antibody assay involving single antigen bead testing was also performed on these samples and identified antibodies to unique HLA antigens for each patient (Supplemental Table 3). In addition, a second serum sample was collected from these patients 6.0–18.1 months after the first sample for another HLA antibody screen. On this repeat testing, 3 patients had similar positive results and 2 patients had a distinct change in the flow emission pattern and a decreased PRA consistent with diminishing antibody (Supplemental Figure 2).

In chronic transfusion patients, history of a RBC alloantibody but not transfusion burden was associated with HLA class I alloimmunization

Among the chronic transfusion patients, alloimmunization to HLA was not associated with the number of lifetime transfusion exposures. While patients who had received the most number of transfusions had a higher prevalence of RBC alloimmunization, a similar trend did not exist for HLA class I alloimmunization (Supplemental Figure 3). Of note, two patients who had received less than 20 RBC units had among the highest HLA class I PRA values (91% and 97%), while 12/16 (75%) of the patients who had received the most transfusions (>200 RBC units) did not have any HLA antibodies.

To further investigate why some transfused patients became alloimmunized to class I HLA, a HLA class I PRA >25% was used to define a group of patients who were strongly HLA alloimmunized. Chronic transfusion patients with a HLA class I PRA >25% ($n=16$) were thus compared with chronic transfusion patients who had a HLA class I PRA ≤25% ($n=74$). In both the univariate and multivariate analyses, the only variable significantly associated with HLA class I alloimmunization was a history of a RBC alloantibody (Table II).

Immunophenotype analysis of chronic transfusion patients

We have previously reported on the immune phenotype of SCD patients that included the patients we describe in this study (Nickel, *et al* 2015). In the current analysis, we compared the 18 studied immune cell counts between chronic transfusion patients with a HLA class I PRA >25% (n=13) vs. chronic transfusion patients with HLA class I PRA ≤25% (n=59) who had samples collected for this testing and were not on concurrent hydroxycarbamide therapy (Supplemental Table 4). The total number of white blood cells, neutrophils, monocytes, lymphocytes, natural killer, B, naïve B, T, CD4+, CD8+, CD4+ T-naïve, CD4+ T-memory, CD4+ T-regulatory, CD8+ T-naïve, CD8+ T-memory, CD4+ Ki67 and CD8+ Ki67 cells were not significantly different between the two groups. Only the number of memory B cells was significantly different between the groups, with the highly sensitized patients having more memory B cells (0.044 vs. 0.029×10^9 cells/l, $p=0.019$).

Longitudinal analysis of HLA Antibodies in chronic transfusion patients

16 chronic transfusion patients in this study had data on HLA alloimmunization available from our previous study (McPherson, *et al* 2010). At the time of this previous study, 14 patients were negative for HLA antibodies and 2 patients were positive for HLA antibodies. Despite a median of 146 additional RBC units transfused over a median of 5.5 years, 11/14 (79%) of patients with initially negative HLA antibody screens continued to have no HLA antibodies detected. After a median of 78 additional RBC units transfused over a median of 5.1 years, 3/14 (21%) of patients developed new HLA antibodies. The 3 patients who developed interval positive HLA antibody screens had the following positive PRA results: Patient 1, 8% class I; Patient 2 17% class I; Patient 3 class II 41%. Both of the 2 patients who initially had positive HLA antibody screens (both with class I PRA >25%) continued to have a class I PRA value >25% on repeat testing 5.6 and 7.0 years later.

Discussion

Our results suggest that leucocyte-reduced RBC transfusions promote HLA antibody formation, but they do not cause detectable H-Y alloantibodies. Thus, while leucoreduced RBC transfusions can lead to HLA alloimmunization, they may be an inadequate stimulus for H-Y alloimmunization. Supporting this idea, Desmerets et al (2009) found in a murine model that leucocyte reduction of pre-transplant RBC transfusions nearly obviated subsequent H-Y directed HSCT rejection. An important caveat to this interpretation is that in the current study only humoral immunity was assessed. Thus, it is possible that leucoreduced RBC transfusions could induce an H-Y response involving only T cells, as appears to be characteristic of immunity to other mHAs (Patel, *et al* 2009, Patel, *et al* 2012). Moreover, it should not be inferred from the lack of an association between RBC transfusions and H-Y antibody formation in our study that leuco-reduced RBC transfusions are weakly or non-immunogenic for all mHAs. Previous murine work suggests that pre-transplant leucocyte-reduced RBC transfusions mismatched for multiple autosomal mHAs can strongly induce HSCT rejection (Desmerets, *et al* 2009), and immunity to mHAs other than H-Y antigens was not evaluated in the current study.

This study builds on our previous description of HLA alloimmunization in SCD (McPherson, *et al* 2010) by more definitively linking RBC transfusion to the production of HLA class I antibodies. While pregnancy can definitively cause HLA alloimmunization, no patient in this study had a known pregnancy or spontaneous abortion. It is very unlikely that undocumented pregnancy-induced alloimmunization affected our results as most females studied were premenarchal, older patient age was not associated with alloimmunization and males actually had a higher prevalence of HLA antibodies. Our findings add to the growing body of evidence that HLA alloimmunization after RBC transfusion remains a problem despite leucocyte reduction (Balasubramaniam, *et al* 2012, Yabu, *et al* 2013). The source of the HLA class I antigens in RBC units causing sensitization is currently unknown. However, even with modern leucocyte reduction, as many as 5×10^6 leucocytes may remain in a RBC unit, and these residual leucocytes may lead to sensitization. Residual platelets (which express class I HLA) and free-floating HLA molecules also contaminate all RBC units. Furthermore, RBCs themselves may also contain adsorbed HLA antigens on their surface (Everett, *et al* 1987, Rivera and Scornik 1986). Future work should seek to characterize the HLA-sensitizing source in RBC units, with a goal of decreasing this cell subset from processed leuco-reduced blood products.

Our finding that some non-transfused SCD patients had HLA antibodies is consistent with others' reports of "natural" or "spontaneous" HLA alloimmunization in "unsensitized" individuals (Aston, *et al* 2014, Morales-Buenrostro, *et al* 2008). The aetiology of these antibodies is unknown; their production may be triggered by a non-allogeneic stimulus, such as immunization (Alberu, *et al* 2007, Katerinis, *et al* 2011, Roddy, *et al* 2005) or cross-reactivity to a foreign antigen, such as a microorganism (Hirata and Terasaki 1970, Ogasawara, *et al* 1986, Raybourne, *et al* 1988). While also found in healthy individuals (Morales-Buenrostro, *et al* 2008), it is unclear if these antibodies are more common in patients with SCD. Our limited data suggests that these apparently unprovoked antibodies may be more transient than those elicited by RBC transfusion, as the reactivity of two of these five patients waned over months, compared to the stability of HLA antibodies observed in two transfused patients for more than five years.

In this study, as in our previous work (McPherson, *et al* 2010), the lone factor associated with HLA alloimmunization among multiply transfused patients was a history of a RBC alloantibody. Our results add further to the evidence linking HLA and RBC alloimmunization (Brantley and Ramsey 1988, van de Watering, *et al* 2003). Recent studies suggest that genetic and immunological differences distinguish these immunological "responders" (patients who make alloantibodies) from "non-responders" in SCD (Bao, *et al* 2013, Tatari-Calderone, *et al* 2013, Zhong, *et al* 2014). Our observation that strongly HLA alloimmunized patients had higher memory B cell counts suggests that an underlying difference in the B cell biology of these patients may explain their tendency to form or not form alloantibodies. However, it is also possible that this association was found by chance or that the higher memory B cell counts are a result rather than a cause of the HLA immunity. In addition, while SCD transplant studies to date have not found a clear association between the number of transfusions received and graft rejection (Panepinto, *et al*

2007), it is possible that an individual's unique response to transfusion (more so than the total number of transfused units) may impact graft rejection.

One consideration in interpreting our data is that in a *post hoc* fashion we used an HLA class I PRA >25% to define an allo-antibody "responder." We chose this threshold after observing that none of the never transfused patients had a HLA class I PRA value above this level. A high PRA value has clinical significance in that it suggests that a patient has HLA antibodies that react with a large percentage of the general population. However, a high PRA does not necessarily mean that a patient has many unique HLA antibodies or has antibodies at a high titre.

Given the relatively high prevalence of patients with HLA class I or class II antibodies in our transfused group (37%) and the known association of donor-specific HLA antibodies with graft rejection (Brand, *et al* 2013, Ciurea, *et al* 2009, Ciurea, *et al* 2011, Cutler, *et al* 2011, Ruggeri, *et al* 2013, Spellman, *et al* 2010, Takanashi, *et al* 2010, Yoshihara, *et al* 2012), our results provide strong rationale for performing HLA antibody testing in patients with SCD being evaluated for HSCT using HLA-mismatched related or unrelated donors. With the promising early results (Bolanos-Meade, *et al* 2012, Talano and Cairo 2014) of haploidentical transplant for SCD and potential of this approach to cure many patients with SCD, the role of HLA alloimmunization could become increasingly important in SCD as HLA antibody testing should be used to help select donors (so as to avoid donors that have HLA mismatches that would react against a patient's specific HLA antibodies). Strategies also need to be developed to counter alloimmunization for those patients whose only option is a donor they are sensitized against. One area that should be explored further is desensitization treatment, as various protocols (including plasmapheresis and intravenous immune immunoglobulin) (Gladstone, *et al* 2013) have been implemented in which successful HSCT was achieved despite donor-specific antibodies (Zachary and Leffell 2014). Furthermore, all SCD patients undergoing HSCT may benefit from HLA antibody testing as HLA alloantibodies predispose transplant patients to platelet refractoriness (Balduini, *et al* 2001, Fasano, *et al* 2014, Klumpp, *et al* 1996, Markt, *et al* 2010).

In conclusion, this study demonstrated that, in paediatric SCD, leucocyte-reduced RBC transfusions are associated with HLA class I alloimmunization but not H-Y antibody development. Importantly, some individuals ("responders") had a high risk of becoming immunized to both RBC and HLA antigens, while others ("non-responders") never become immunized despite large transfusion burdens. Future studies investigating responder versus non-responder patients with SCD in the context of transfusion and HSCT outcomes are needed. These studies will require close collaboration between haematology, transplant and transfusion services, with a common goal of improving outcomes for patients with SCD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. H-Y Antibody MFI for three study groups compared to positive controls
 Banked plasma from reference male haematopoietic stem cell transplantation (HSCT) patients who received a female graft (Female→Male) who were known to have H-Y antibodies was used as a positive control. Results are only shown for DBY, ZFY and UTY2 because these HSCT patients did not make antibody against EIFIAY, RPS4Y, UTY1 or UTY3. The solid lines for each group depict the group mean mean fluorescence intensity (MFI) value. The dashed line represents the positive MFI threshold that defined

seropositivity, calculated from median MFI + 2.5 quartiles measured in 60 adult healthy males.

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Figure 2. HLA PRA Values in Transfused vs. Never Transfused Patients

Distribution of panel reactive antibody (PRA) values in the 90 transfused (black) and 24 never transfused (gray) patients. Bar heights depict the percentage of patients in that group (transfused or never transfused) having the specific PRA range. Numbers above the bar denote the absolute number of patients within that PRA range.

HLA, human leucocyte antigen.

Table 1

Analysis of HLA class I alloimmunization among all study patients

	HLA class I positive (n=33)	HLA class I negative (n=81)	P-value*	OR (95% CI)	Adjusted OR [†] (95% CI)
Study group:					
Female chronic transfusion	16 (27.6%)	42 (72.4%)	0.037	2.7 (0.70–10.2)	3.3 (0.77–14.3)
Male chronic transfusion	14 (43.8%)	18 (56.3%)		5.4 (1.3–22.0)	9.0 (1.8–44.6)
Female never transfused	3 (12.5%)	21 (87.5%)		1.0 (reference)	1.0 (reference)
RBC transfusion, n (%) (reference: no transfusion)	30 (90.9%)	60 (74.1%)	0.046	3.5 (0.97–12.7)	4.3 (1.1–18.0)
Age (years), mean (range) (reference: per year of age)	12.1 (2.1, 20.7)	11.5 (2.1, 22.1)	0.49	1.03 (0.94–1.1)	1.03 (0.93–1.1)
Splenectomy (reference: no splenectomy)	5 (15.2%)	19 (23.5%)	0.32	0.58 (0.20–1.7)	0.42 (0.14–1.3)
Hydroxycarbamide (reference: no hydroxycarbamide)	4 (12.1%)	14 (17.3%)	0.49	0.66 (0.2–2.2)	1.1 (0.29–4.4)

HLA, human leucocyte antigen; OR, odds ratio; 95% CI, 95% confidence interval; RBC red blood cell.

* P-value calculated from univariate analysis Fisher's exact test or chi-square test for categorical variables and two-sample *t*-test for continuous variable.[†] With the exception of the adjusted OR for the study groups, adjusted OR were calculated from logistic regression model with the variables RBC transfusion, age, splenectomy and hydroxycarbamide. The adjusted OR values for the study groups were calculated from logistic regression model with the variables study group, age, splenectomy and hydroxycarbamide.

Table II

Analysis of HLA class I PRA high positive among chronic transfusion patients

	HLA class I PRA high positive (n= 16)	HLA class I PRA low positive or negative (n= 74)	P-value*	OR (95% CI)	Adjusted OR [†] (95% CI)
Female (reference: male)	9 (56.3%)	49 (66.2%)	0.45	0.66 (0.22–2.0)	0.79 (0.22–2.8)
Age (years), mean (range) (reference: per year of age)	12.6 (4.6, 20.7)	11.8 (2.1, 22.1)	0.49	1.0 (0.92–1.2)	1.1 (0.71–1.8)
RBC units transfused (n), mean (range) (reference: per unit transfused)	130 (9, 324)	119 (6, 543)	0.55	1.0 (0.996–1.006)	1.0 (0.98–1.01)
Age began chronic transfusion (years), mean (range)	6.5 (1.1, 10.8)	6.2 (0.5, 17.5)	0.38	1.0 (0.89–1.2)	0.96 (0.67–1.4)
Clinical stroke (reference: no stroke)	8 (50.0%)	21 (28.4%)	0.093	2.5 (0.84–7.6)	1.9 (0.48–7.3)
Splenectomy (reference: no splenectomy)	3 (18.8%)	21 (28.4%)	0.54	0.58 (0.15–2.3)	0.42 (0.068–2.6)
RBC alloantibody (reference: no RBC alloantibody)	11 (68.8%)	18 (24.3%)	0.0006	6.8 (2.1–22.3)	6.3 (1.7–22.6)
Outside institution transfusion (reference: no outside transfusion)	6 (37.5%)	28 (37.8%)	0.98	0.99 (0.32–3.0)	0.45 (0.096–2.1)
Transfusion during ACS (reference: no transfusion during ACS)	4 (25.0%)	29 (39.2%)	0.29	0.52 (0.15–1.8)	0.47 (0.12–1.9)

HLA, human leucocyte antigen; PRA, panel reactive antibody; OR, odds ratio; 95% CI, 95% confidence interval; RBC red blood cell; ACS, acute chest syndrome.
PRA high positive defined as >25%.

* P-value calculated from univariate analysis Fisher's exact test or chi-square test for categorical variables and two-sample *t*-test or Wilcoxon rank-sum test for continuous variables.

[†] Adjusted OR was calculated from logistic regression model with all variables shown.