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Transfusion of leukoreduced blood products and risk of antibody-mediated rejection of renal allografts

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Abstract

Background—Antibody-mediated rejection (AMR) is a major barrier to the long-term function of renal allografts. Platelets and leukocytes, which may be present in red blood cell (RBC) units, express HLA antigens that may increase the risk of AMR by inducing or increasing humoral sensitization to HLA.

Methods—A retrospective cohort study of HLA-incompatible renal transplant recipients between 2004-2015 was conducted. Data on apheresis platelet and leukoreduced RBC transfusions within four weeks of transplantation, demographic information, and biopsy-proven AMR were collected from medical records and the Scientific Registry of Transplant Recipients (SRTR). Patients were evaluated until they showed evidence of AMR or until one year post-transplant, whichever came first. Multivariable analysis with Cox modeling was performed.

Results—Of 244 individuals, 182(74.6%) received RBCs and 20(8.2%) of those also received platelets. During the first year post-transplant, 97(39.8%) had AMR. RBC alone or RBC and platelet transfusions were not associated with increased risk of AMR after adjustment for panel reactive antibody, years on dialysis, HLA antibody strength, and number of therapeutic plasma exchange treatments (adjusted hazard ratio [adjHR]=1.00,95% confidence interval [95%CI]=0.59-1.69 and adjHR=0.68,95%CI=0.28-1.68, respectively). For each one unit increase in RBC transfusions, there was no association with AMR (adjHR=0.94,0.85-1.05). Only HLA antibody strength prior to transplantation was associated with AMR (adjHR=2.23,95%CI=1.10-4.52, cytotoxic crossmatch compared to crossmatch negative but detectable donor-specific HLA antibodies).

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Disclosures

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Conclusions—Patients who receive an HLA-incompatible transplant who are transfused with leukoreduced RBCs or platelets in the peri-transplant period are at no higher risk of AMR than non-transfused patients.

Introduction

Over 100,000 patients are waiting for a kidney transplant in the United States. In 2012, only 15,967 patients received a kidney and 5,209 died waiting for one.¹ Among those waiting, approximately 14% were sensitized to HLA antigens due to exposures from prior transplants, transfusions, or pregnancies.¹

Alloimmunization to HLA is well-recognized as a potentially serious and relatively common complication of transfusions.² HLA antigens are present on both platelets and leukocytes. Red blood cells (RBCs) do not generally express HLA antigens, but Bennett–Goodspeed antigens may occasionally be present and lead to HLA alloimmunization. More importantly, RBC units contain a large number of leukocytes, which historically led to high rates of HLA alloimmunization.³ While leukoreduced RBCs are often transfused, these units still may contain up to five million leukocytes and stimulate HLA antibody formation.⁴ Platelet transfusion may lead to HLA antibodies directed against class I HLA epitopes and exposure to leukocytes from RBC transfusion may lead to HLA antibodies directed against class I and II HLA epitopes.^{5,6} HLA sensitization resulting from transfusion is less robust and generally shorter lived than that resulting from transplantation. However, IgG HLA antibodies to a transplanted organ negatively impact graft function and graft survival, regardless of the route of sensitization.

Unfortunately, many renal transplant patients require transfusion support, either during the peri-operative period due to falling hemoglobin associated with therapeutic plasma exchange (TPE) desensitization to undergo an HLA-incompatible (HLAi) renal transplant or while the patient is on hemodialysis. The role of transfusion in HLA antibody development has long been widely recognized by the transfusion, transplant and nephrology communities;^{7,8} however, data are limited on whether blood transfusions are associated with increased risk of AMR.⁹ Furthermore, transfusion has been shown to increase both the breadth and strength of HLA antibodies in sensitized patients.¹⁰ This retrospective cohort study evaluated the impact of leukoreduced RBC and platelet transfusions on AMR in sensitized patients undergoing HLAi renal transplantation.

Materials and Methods

Study Population

This retrospective cohort study evaluated 244 consecutive patients who received an HLAi kidney transplant using a protocol approved by the Johns Hopkins Medical Institutions Institutional Review Board. The study included all patients who underwent an HLAi live donor kidney transplantation between January 2004 and June 2015 at The Johns Hopkins Hospital. Since almost all transfusions occur peri-operatively, patients were evaluated for either RBC and/or platelet transfusion within four weeks of transplantation. However, patients were subsequently assessed until they showed evidence of AMR or at one year post-

transplant, whichever came first. Patients with graft failure or death were censored at that point to ensure that the transfusions occurred prior to these events. Data were extracted retrospectively from the medical records at The Johns Hopkins Hospital and augmented with data from the Scientific Registry of Transplant Recipients (SRTR).

The SRTR includes information on all donors, waitlisted transplant candidates, and transplant recipients in the United States as provided by members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services, provides oversight to the activities of the OPTN and SRTR contractors. The SRTR supplements death ascertainment through linkage to the Social Security Death Master File and death and graft loss ascertainment through linkage to data from the Centers for Medicare & Medicaid Services.

All participants in this study had HLA antibodies. HLA incompatibility was defined by three measures of antibody strength: complement-dependent cytotoxic crossmatch (CDC), flow-cytometric crossmatch (FCXM) or detectable donor-specific HLA antibodies on multiplex bead assay performed on the Luminex platform. Participants were excluded if the transplant donor was also ABO-incompatible (ABOi), or if the patient currently or previously had documented non-HLA anti-endothelial antibodies.

Immunosuppression and desensitization

Alternate day, single volume TPE and 100mg/kg intravenous immunoglobulin (IVIg) (cytogam, MedImmune, Gaithersburg, MD) were utilized to decrease HLA titers to a preoperative DSA below a cytotoxic cross-match level, as previously described.^{11,12} Mycophenolate mofetil (2g/day) and tacrolimus (to a serum level of 8-10 ng/mL) were initiated at the same time as TPE treatments.^{13,14} Pre-operative rituximab was used selectively for those who had a high-risk donor-recipient phenotype, such as those starting with a high titer cross-match, with multiple repeated mismatches, and with high levels of donor HLA-specific B cells.¹⁵ Intraoperatively, induction therapy included IL-2 receptor antibody (anti-CD25, daclizumab 2 mg/kg, or thymoglobulin, 1.5 mg/kg per day $\times$ 5 days).^{13,14} Triple drug immunosuppression was comprised of tacrolimus, mycophenolate mofetil and steroids. When the tacrolimus level reached 8-12 ng/mL, the prednisone dose was decreased to 20 mg/day and after the first month, tapered to 5 mg/day by three months post-transplant. TPE was continued postoperatively until the DSA strength was below a flow cytometric cross-match level. After TPE was discontinued, it was restarted if indicated by clinical data including biopsy results, rising DSAs, increasing serum creatinine, and/or a diagnosis of AMR.

Transfusions

Leukocyte reduced RBC transfusions were given strictly based on clinical need, following standard transfusion practices.¹⁶ However, patients were maintained with a hematocrit $>22\%$ while receiving TPE. Apheresis platelet transfusions were also given based on clinical need, following standard transfusion practices.¹⁷ Patients with chronic stable thrombocytopenia were transfused at a threshold based on their degree of bleeding, in general transfusing to keep the level greater than $10 \times 10^9/L$ in a non-bleeding patient. Before transplant, patients

were transfused to a platelet count of $50 \times 10^9/L$.^{17,18} The vast majority of study subjects who were transfused (93%, 169/182), were under immunosuppression during all times of their transfusions.

Histopathologic analysis of biopsies and diagnosis of rejection

Biopsies were obtained when clinically indicated or by protocol. Surveillance biopsies were performed at 1, 3, 6, and 12 months after transplantation. All biopsies were analyzed by routine light microscopy using hematoxylin-eosin, periodic acid-Schiff, methenamine silver and Masson's trichrome stains. For all biopsies, a small (3-4 mm) piece of cortical or medullary tissue was also taken for C4d staining, which was performed by indirect immunofluorescence or cryostat sections using a mouse monoclonal anti-human C4d antibody (Quidel, San Diego, CA) at 1:40 dilution, followed by fluorescein isothiocyanate conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA).¹⁹ Staining for C4d in peritubular capillaries (PTCs) was graded as diffuse (positive in >50% of specimen), focal (10-50%) or absent (<10%). Clinical and subclinical acute cellular rejections were graded according to the 2013 International Banff classification.²⁰ AMR (clinical or subclinical) was diagnosed as being present when the following criteria were met: 1) positive (diffuse or focal) C4d staining in PTC and 2) at least one of the following: margined leukocytes (neutrophils or mononuclear cells) in at least 10% of cortical PTC, moderate or severe glomerulitis (Banff *g* score >2), or transmural necrotizing arteritis and 3) Donor specific antibodies (DSAs) were detectable in the serum.

Antibody testing

HLA antibody levels were determined before and after the first, pre-transplant TPE. Thereafter, HLA antibody levels were determined after each TPE. HLA class I and II DSAs were identified by using a color-coded multi-analyte bead immunoassay on the Luminex platform, which uses beads coated with soluble HLA molecules as targets (LABScreen Single Antigen Class IVCombi and LABScreen Single Antigen Class II Antibody Detection TestVGroup 1; One Lambda, Thermo Fisher Scientific, Canoga Parc, CA) supplemented with phenotype panels (LIFECODES class I ID, LIFECODES class II IDv2; Immucor, Stamford, CT). The multianalyte immunoassays were performed according to the manufacturer's instructions as reported elsewhere.^{21,22}

Statistical analysis

The 244 consecutive patients who received HLAi kidney transplant were categorized into three groups based on transfusion within four weeks before or after the transplant: no transfusion (N=62), RBC-only (N=162), and RBC & platelets (N=20). The Cox model was used to assess the association between transfusion and AMR. Items considered as potential confounders included age at transplant, gender, race, primary cause of end stage renal disease (ESRD), panel reactive antibody (PRA), previous transplant, time on dialysis, HLA antibody strength, and TPE before transplant. For the adjusted model, potential confounders that showed statistical significance ($p < 0.20$) in univariate analyses were included.

The primary analysis did not include the impact of TPE events post-transplant or post-transfusion since post-transplant TPE may be initiated for possible concern for AMR prior to

a biopsy being performed and we did not want to bias the results. However, an additional multivariate analysis was performed that included post-transplant TPE.

Two sensitivity analyses were performed. A sensitivity analysis was performed to evaluate only individuals who were transfused during the day of surgery and within four weeks of transplantation. A second sensitivity analysis was performed among those who received platelets. These individuals were categorized by the types of platelets they received and compared with those who received no transfusion. HLA-selected platelets were those in which all donor antigens were avoided, HLA-unselected platelets were those for which donor antigens were not avoided, and HLA unknown platelets were those for which the platelet antigens were unknown. The Cox model was used to assess the association between level of match and AMR. The adjusted model included the following potential confounders: transfusion, PRA, time on dialysis, HLA antibody strength, and number of TPE treatments prior to transplant.

Results

Patients and Matched Control Subjects

For the 244 HLAi kidney transplant patients, the demographic and clinical data are summarized in Table 1. Of the study population, 62 (25.4%) received no transfusions, 162 (66.4%) received RBC transfusions only and 20 (8.2%) received both RBCs and platelet transfusions. Individuals who were transfused both RBCs and platelets received significantly more units of RBCs than those individuals who only received RBCs ($p<0.0001$). The individuals who received platelets received a median of 1 (IQR 1 - 2.25) apheresis unit.

Among patients who received no transfusions, only RBCs or RBC and platelet transfusions, there were no significant differences in age at transplant, male-to-female ratio, or racial ethnicity. The most common cause of ESRD was glomerular disease, with other common causes including diabetes mellitus, hypertensive nephrosclerosis, polycystic kidney disease, and vascular disease. There was no significant difference in the PRA, with a median (interquartile range, IQR) of 86% (52-96%) for those who didn't receive transfusion and 93% (70-100%) for those who received RBC only and 93% (66-97%) for those who received both RBCs and platelets. A previous kidney transplant had occurred in 48% of patients who were not transfused, in 54% of those who received RBCs, and in 65% of those who received RBC & platelets. Average time on dialysis was also similar among the groups, with most patients on dialysis between 2 and 6 years and very few not on dialysis prior to transplant. HLA antibody strength was significantly different among the three groups, with the highest level (pre-transplant CDC positive) occurring most often in those who received both RBC and platelet transfusions ($p=0.026$). The number of TPE treatments was also significantly higher among those transfused ($p<0.001$).

Antibody-mediated rejection

The number of patients with clinical or subclinical AMR was similar between the three groups, with 34% of those who did not receive transfusion developing AMR, 43% of those who received RBCs only developing AMR, and 35% of those who received RBCs and

platelets developing AMR. Patient characteristics associated with AMR are shown in Table 2.

In the unadjusted model, a PRA > 80, 6 years on dialysis, higher HLA antibody strength, and greater number of TPE treatments showed statistically significant risk associated with AMR. After adjustment, however, only HLA antibody strength remained statistically significant (adjHR=2.23, 1.10-4.52, CDC positive compared to luminex positive). Differences in age at transplant, gender, race, cause of ESRD, and history of previous transplant were not associated with a significant risk for AMR. Compared to those who received no transfusions, patients who received RBCs only were no more likely to develop AMR (adjHR=1.00, 0.59-1.69). For each one unit increase in RBC transfusions, there was no association with increased risk of AMR (adjHR=0.94, 0.85-1.05, p=0.3). Receiving both RBCs and platelets were not associated with developing AMR (adjHR=0.68, 0.28-1.68). One unit increase in platelet transfusion was not associated with any change in AMR risk (adjHR=0.97, 0.90-1.05, p=0.5).

In an additional multivariate analysis including post-transplant TPE, post-transplant TPE procedures were highly associated with AMR (3-5 procedures adjHR=1.73, 0.92-3.24, 5 procedures adjHR=5.25, 2.85-9.66, compared to 2 procedures). In this model, however, transfusions were still not associated with AMR (RBC-only transfusion adjHR=0.80, 0.47-1.37, RBC and platelet transfusion adjHR=0.70, 0.28-1.74).

In a sensitivity analysis which focused on only those under immunosuppression at the time of transfusion and excluded the 13 individuals transfused prior to the day of transplantation, transfusions were not associated with increased risk of AMR (compared to those not transfused, RBC transfusion on the date of transplant aHR=1.33, 0.84-2.12, RBC transfusion post-transplant aHR=0.85, 0.50-1.43).

A sensitivity analysis was performed for patients who received platelets, which is summarized in Table 3. There were a total of 11 patients who received platelets which avoided donor HLA antigens; 3 of these developed AMR and 8 of them did not (adjHR=0.43, 0.12-1.50, compared to no transfusion group). Three patients received platelets that were known to have donor antigens; one of these developed AMR and 2 did not (adjHR=1.38, 0.18-10.46, compared to no transfusion group). For six patients, the platelet HLA type was not known; three of these developed AMR and 3 did not (adjHR=0.95, 0.27-3.33, compared to no transfusion group).

Discussion

Patients undergoing HLAi renal transplants have a survival benefit compared to those who wait for deceased donors.²³ However, patients undergoing HLAi renal transplantation often require blood transfusions. Many surgeons hesitate to transfuse their patients in the perioperative period due to a presumed increased risk of AMR with transfusion from HLA antigen exposure from blood products. This retrospective cohort study of 244 renal transplant patients who had donor-specific anti-HLA antibodies who underwent desensitization with a standardized protocol including TPE and IVIg found no association

between transfusion and increased rate of AMR. A multivariate analysis controlling for PRA, number of years on dialysis, HLA antibody strength, and number of TPE treatments and transfusions showed that only HLA antibody strength prior to transplantation was associated with the development of AMR.

Although it is well established that prior transfusions, pregnancy, and transplants can lead to HLA sensitization,²⁴ the role of perioperative transfusion in HLA incompatible transplants and its effect on long-term outcome after transplant has been unclear. Because these transfusions are given under the cover of immunosuppression, a B-cell response may be blunted or prevented. Known factors that contribute to transplant outcomes include those based on the donor (i.e. quality of the transplanted kidney), those based on the recipient (i.e. age, ethnicity, time spent on dialysis, and cardiovascular complications at the time of transplant); and, finally those based on the compatibility of the donor and the recipient (i.e. HLA compatibility and anti-HLA immunization).²⁵ Other factors that have bearing on graft function and survival include recurrence of native disease, delayed graft function in the immediate post-transplantation period, glomerular filtration rate, proteinuria, chronic allograft dysfunction, and types of immunosuppression.²⁵

Of note, some of the patients in our study received platelets selected to avoid donor antigens with the intention of reducing the risk of inciting an immune response and increasing a patient's DSA and, therefore, the risk of AMR. The patients who received HLA selected platelets had a lower incidence of AMR than those who did not receive HLA selected platelets; however, because the numbers were small the results did not reach statistical significance. Based on these study results, apheresis platelets and leukocyte reduced RBCs administered in the peri-transplant period do not appear to be associated with AMR in HLAi renal transplant recipients; however, platelets should be given cautiously and an attempt should still be made to give HLA-selected platelet products.

This study has several limitations. This is a retrospective, single center study in a large referral center; therefore, these findings should be replicated in a prospective, multicenter study with a more heterogeneous population. We evaluated only HLAi live donor transplants, and it would be of interest to study all kidney transplant recipients, including deceased donor transplants with DSA. The majority of renal transplant AMR is caused by HLA antibodies, but other antibodies, such as anti-endothelial cell antibodies or angiotensin II receptor type 1 antibodies, may also lead to AMR. While this study focused on HLA, the findings of this study suggest that blood transfusions are not associated with an increased risk of AMR from any type of antibody. Since we did not find an increased risk of AMR associated with transfusion, we did not evaluate the possible etiology. The final multivariable model also did not incorporate post-transplant factors, such as medication non-compliance or infection. Although we have a large referral service, the final number of patients who received platelets in our study is small, and approximately half of these patients received platelets known to contain no donor HLA antigens. When these patients were considered separately, differences in AMR rates did not reach statistical significance. Patients who have higher antibody titers tended to receive more TPE treatments. Furthermore, those receiving TPE during desensitization for an HLAi kidney transplant commonly require blood products. The more TPE sessions they receive, the more likely they will need to be

transfused. In the perioperative transplant period when these transfusions occur the patients were receiving induction therapy and high doses of maintenance immunosuppression. The patients were receiving both humoral (IVIg, rituximab, mycophenolate mofetil) and cellular mediated (IL-2 receptor antibody, tacrolimus, mycophenolate mofetil) immunosuppression. Thus, the immunosuppression might offer protection from the additional HLA exposure triggering an AMR episode.

In patients who are already highly sensitized against HLA – that is, those who have already proven they can make anti-HLA antibodies – leukocyte reduced RBC and platelet transfusions in the perioperative period do not place them at any higher risk for AMR. Although we recognize that selecting platelets that avoid donor antigens may be particularly difficult, if not impossible, in these already highly sensitized patients, this study also suggests that when HLA-selection can be accomplished with the resources available, it is reasonable to attempt to provide these products for highly sensitized patients. Further prospective, controlled multi-center studies, including studies of patients who receive HLA-compatible organs, will be necessary to determine if RBC or platelet transfusions in the peritransplant period increase sensitization or negatively impact outcomes.

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References

1. OPTN/SRTR 2012 annual data report. United Network for Organ Sharing; 2012. <http://www.unos.org>. Accessed December 15, 2016
2. Blajchman MA. Platelet transfusions: a historical perspective. Hematology Am Soc Hematol Educ Program. 2008; 197:32–34.
3. Snyder EL. Prevention of HLA alloimmunization: Role of leukocyte depletion and UV-B Irradiation. Yale Journal of Biology and Medicine. 1990; 63:419–427. [PubMed: 2293501]
4. Sniecinski I, O'Donnell MR, Nowicki B, et al. Prevention of refractoriness and HLA-alloimmunization using filtered blood products. Blood. 1988 May; 71(5):1402–1407. [PubMed: 3282572]
5. International Forum. Detection of platelet-specific antibodies in patients who are refractory to platelet transfusions, and the selection of compatible donors. Vox Sang. 2003; 84:73–88. [PubMed: 12542737]
6. Yankee RA, Grumet FC, Rogentine GN. The selection of compatible platelet donors for refractory patients by lymphocyte HLA typing. N Engl J Med. 1969; 281:1208–12. [PubMed: 5347838]
7. Leffel MS, Kim D, Vega RM, et al. Red blood cell transfusions and the risk of allosensitization in patients awaiting primary kidney transplantation. Transplantation. 2014; 97(5):525–533. [PubMed: 24300013]
8. Pavenski K, Freedman J, Semple JW. HLA alloimmunization against platelet transfusions: pathophysiology, significance, prevention and management. Tissue Antigens. 2012; 79:237–245. [PubMed: 22385314]
9. Hickey MJ, Valenzuela NM, Reed EF. Alloantibody generation and effector function following sensitization to human leukocyte antigen. Front Immunol. 2016; 7:30. [PubMed: 26870045]

10. Locke JE, Zachary AA, Warren DS, et al. Proinflammatory events are associated with significant increases in breadth and strength of HLA-specific antibody. *Am J Transplant*. 2009 Sep; 9(9): 2136–9. [PubMed: 19663896]
11. Montgomery RA, Lonze BE, King KE, et al. Desensitization in HLA-Incompatible Kidney Recipients and Survival. *N Engl J Med*. 2011; 365:318–26. [PubMed: 21793744]
12. Szczepiorkowski ZM, Bandarenko N, Kim HC, et al. Guidelines on the use of therapeutic apheresis in clinical practice: Evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis. *J Clin Apheresis*. 2007; 22:106–175. [PubMed: 17394188]
13. Jackson AM, Kraus ES, Orandi BJ, et al. A closer look at rituximab induction on HLA antibody rebound following HLA incompatible kidney transplantation. *Kidney Int*. 2015 Feb; 87(2):409–416. [PubMed: 25054778]
14. Montgomery RA, Zachary A. Transplanting patients with a positive donor-specific crossmatch: a single center's perspective. *Pediatr Transplant*. 2004; 8:535–542. [PubMed: 15598320]
15. Iyer HS, Jackson AM, Zachary AA, et al. Transplanting the highly sensitized patient: trials and tribulations. *Curr Opin Nephrol Hypertens*. 2013; 22:681–688. [PubMed: 24076558]
16. Carson JL, Guyatt G, Heddle NM, et al. Clinical practice guidelines from the AABB: red blood cell transfusion thresholds and storage. *JAMA*. 2016; 316(19):2025–2035. [PubMed: 27732721]
17. Kaufman RM, Djulbegovic B, Gernsheimer T, et al. Platelet transfusion: A clinical practice guideline from the AABB. *Ann Intern Med*. 2015; 162(3):205–213. [PubMed: 25383671]
18. Guidelines for the use of platelet transfusions. *British Journal of Haematology*. 2003; 122:10–23. [PubMed: 12823341]
19. Haas M, Segev DL, Racusen LC, et al. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am J Transplant*. 2006; 6:1929–1940.
20. Haas M, Sis B, Racusen LC, et al. Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant*. 2014 Feb; 14(2): 272–83. [PubMed: 24472190]
21. Zachary AA, Vega RM, Lucas DP, et al. HLA antibody detection and characterization by solid phase immunoassays: methods and pitfalls. *Methods Mol Biol*. 2012; 882:289–308. [PubMed: 22665241]
22. Zachary AA, Sholander JT, Houpp JA, et al. Using real data for a virtual crossmatch. *Hum Immunol*. 2009 Aug; 70(8):574–9.
23. Orandi BJ, Luo X, Massie AB, et al. Survival benefit with kidney transplants from HLA-incompatible live donors. *N Engl J Med*. 2016 Mar 10; 374(10):940–50. [PubMed: 26962729]
24. Lopes D, Barra T, Malheiro J, et al. Effect of different sensitization events on HLA alloimmunization in kidney transplantation candidates. *Transplantation proceedings*. 2015 May; 47(4):894–897. [PubMed: 26036480]
25. Legendre C, Canaud G, Martinez F. Factors influencing a long-term outcome after kidney transplantation. *Transplant international*. 2014 Jan; 27(1):19–27. [PubMed: 24138291]

Table 1

Study population characteristics

	No transfusions (N=62)	RBC-only (N=162)	RBC & platelets (N=20)	P value
Age at transplant (Mean, SD)	45.6 (11.7)	45.0 (14.1)	47.8 (14.3)	0.8
Female (%)	59.7	69.1	75.0	0.3
African American (%)	14.5	14.8	20.0	0.8
Cause of ESRD (%)				0.2
Glomerular disease	40.3	42.6	25.0	
Diabetes mellitus	11.3	7.4	5.0	
Hypertensive nephrosclerosis	8.1	14.2	10.0	
Polycystic kidney disease	14.5	8.6	5.0	
Renovascular & other vascular disease	3.2	1.9	0.0	
Other or missing	22.6	25.3	55.0	
Pre-transplant PRA, median (IQR)	86.0 (52-96)	92.5 (70-100)	93.0 (66-97)	0.2
Previous transplant (%)	48.4	53.7	65.0	0.4
Zero HLA-mismatch (%)	0.0	0.0	0.0	-
Time on dialysis (%)				0.2
Preemptive	6.4	11.1	0.0	
2 years	35.5	19.8	20.0	
2-6 years	37.1	39.5	45.0	
6 years	21.0	29.6	35.0	
Pre-transplant HLA antibody strength (%) ^I				0.026
Complement-dependent cytotoxic (CDC) cross-match positive	12.9	27.2	45.0	
Flow cytometric crossmatch positive	46.8	44.4	40.0	
Luminex-positive	40.3	28.4	15.0	
Units of RBCs (Median, IQR)	0 (0-0)	3 (2-4)	8 (4-14)	<0.0001
Number of pre-transplant TPE treatments (SD)	2.3 (2)	3.7 (3.7)	4.0 (3.3)	0.0002
Number of AMR (%)	33.9	42.6	35.0	0.4

[†] RBC: Red Blood Cells; SD: Standard Deviation; AA: African American; ESRD: End Stage Renal Disease; PRA: Panel Reactive Antibody; IQR: Interquartile Range; HLA: Human Leukocyte Antigen; CDC: Complement-dependent Cytotoxic; AMR: Antibody Mediated Rejection; TPE: therapeutic plasma exchange

^I In general, positive flow cytometric cross-match (FCXM) tests correlated with the following MFI values from the solid-phase immunoassays: 5000 on phenotype panels and 10 000 to 15 000 on single-antigen panels. Positive CDC cross-match results were associated with 10 000 MFI on phenotype panels.

Table 2

Population characteristics associated with AMR

	AMR Proportion (%)	Hazard Ratio (95% CI)	
		Unadjusted	Adjusted ^I
Transfusion			
None	21/62 (33.9)	1.00 (reference)	1.00 (reference)
RBC-only	69/162 (42.6)	1.26 (0.77-2.05)	1.00 (0.59-1.69)
RBC & platelets	7/20 (35.0)	1.02 (0.43-2.40)	0.68 (0.28-1.68)
Age at transplant			
40	44/92 (47.8)	1.00 (reference)	1.00 (reference)
41-60	41/112 (36.6)	0.72 (0.47-1.10)	0.73 (0.47-1.14)
>60	12/40 (30.0)	0.63 (0.33-1.19)	0.71 (0.35-1.42)
Gender			
Male	30/80 (37.5)	1.00 (reference)	
Female	67/164 (40.8)	1.15 (0.75-1.77)	
Race			
Non-African American	87/207 (42.0)	1.00 (reference)	1.00 (reference)
African American	10/37 (27.0)	0.58 (0.30-1.11)	0.59 (0.30-1.18)
Cause of ESRD			
Glomerular disease	39/99 (39.4)	1.00 (Reference)	
Diabetes mellitus	6/20 (30.0)	0.78 (0.33-1.86)	
Hypertensive nephrosclerosis	13/30 (43.3)	1.25 (0.67-2.34)	
Polycystic kidney disease	8/24 (33.3)	0.87 (0.41-1.87)	
Renovascular & other vascular disease	2/5 (40.0)	1.01 (0.24-4.19)	
Other or missing	29/66 (43.9)	1.10 (0.68-1.79)	
PRA			
<80	23/86 (26.7)	1.00 (Reference)	1.00 (Reference)
81-100	74/158 (46.8)	2.00 (1.25-3.19)	1.27 (0.75-2.15)
Previous transplant			
No	38/114 (33.3)	1.00 (Reference)	1.00 (reference)
Yes	59/130 (45.4)	1.43 (0.95-2.15)	0.98 (0.61-1.58)
Time on dialysis (%)			
Preemptive	5/22 (22.7)	1.00 (Reference)	1.00 (Reference)
2 years	18/58 (31.0)	1.51 (0.56-4.08)	1.36 (0.49-3.77)
2-6 years	41/96 (42.7)	2.19 (0.86-5.54)	1.81 (0.67-4.92)
6 years	33/68 (48.5)	2.66 (1.04-6.82)	1.67 (0.57-4.86)
HLA antibody strength (%)			
CDC	35/61 (57.4)	3.19 (1.80-5.64)	2.23 (1.10-4.52)
Flow	44/109 (40.4)	1.79 (1.03-3.10)	1.52 (0.84-2.73)

	AMR Proportion (%)	Hazard Ratio (95% CI)	
		Unadjusted	Adjusted ^I
Luminex	18/74 (24.3)	1.00 (Reference)	1.00 (Reference)
Number of pre-transplant TPE treatments			
2	42/136 (30.9)	1.00 (Reference)	1.00 (Reference)
3-5	36/74 (48.6)	1.77 (1.13-2.76)	1.26 (0.74-2.14)
>5	19/34 (55.9)	2.26 (1.31-3.88)	1.29 (0.63-2.62)

[†] AMR: Antibody Mediated Rejection; CI: Confidence Interval; RBC: Red Blood Cell; AA: African American; ESRD: End Stage Renal Disease; PRA: Panel Reactive Antibody; HLA: Human Leukocyte Antigen; CDC: Complement-dependent Cytotoxicity; TPE: therapeutic plasma exchange

^I Adjusted model includes transfusion, PRA, time on dialysis, HLA antibody strength, and number of TPE treatments prior to transplant.

Table 3

Risk of AMR based on HLA matching of platelets

	Unadjusted HR	Adjusted HR ^I
No transfusion	Reference	Reference
HLA selected platelets with avoidance of donor antigens	0.70 (0.21-2.34)	0.43 (0.12-1.50)
HLA-unselected platelets with donor antigen mismatches	1.12 (0.15-8.34)	1.38 (0.18-10.46)
Platelets with unknown HLA type	1.80 (0.53-6.03)	0.95 (0.27-3.33)

[†]HR: Hazard Ratio; HLA: Human Leukocyte Antigen

^I Adjusted model includes transfusion, PRA, time on dialysis, HLA antibody strength, and number of therapeutic plasma exchange treatments prior to transplant.