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Blood Transfusion and Adverse Graft-related Events in Kidney Transplant Patients

David Massicotte-Azarniouch[1], Manish M. Sood[2,3], Dean A. Fergusson[1,3], Michaël Chassé[4], Alan Tinmouth[3,5] and Greg A. Knoll[2,3]

[1]Department of Medicine, University of Ottawa, Ontario, Canada; [2]Division of Nephrology, Kidney Research Center, Department of Medicine, University of Ottawa, Ontario, Canada; [3]Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada; [4]Department of Medicine, University of Montreal, Montreal, Quebec, Canada; and [5]Department of Medicine, Division of Hematology, University of Ottawa, Ottawa, Ontario, Canada

Background: The impact of posttransplant red blood cell transfusion (RBCT) and their potential immunomodulatory effects on kidney transplant recipients are unclear. We examined the risks for adverse graft outcomes associated with post-kidney transplant RBCT.

Methods: We conducted a retrospective cohort study of all adult kidney transplant recipients at The Ottawa Hospital from 2002 to 2018. The exposure of interest was receipt of an RBCT after transplant categorized as 1, 2, 3 to 5, and >5 RBC. Outcomes of interest were rejection and death-censored graft loss (DCGL). Cox proportional hazards models were used to calculate hazard ratios (HR) with RBCT as a time-varying, cumulative exposure.

Results: Among 1258 kidney transplant recipients, 468 (37.2%) received 2373 total RBCTs, 197 (15.7%) had rejection and 114 (9.1%) DCGL. For the receipt of 1, 2, 3 to 5, and >5 RBCT, compared with individuals never transfused, the adjusted HRs (95% confidence interval [CI]) for rejection were 2.47 (1.62–3.77), 1.27 (0.77–2.11), 1.74 (1.00–3.05), and 2.23 (1.13–4.40), respectively; DCGL 2.32 (1.02–5.27), 3.03 (1.62–5.64), 7.50 (4.19–13.43), and 14.63 (8.32–25.72), respectively. Considering a time-lag for an RBCT to be considered an exposure before an outcome to limit reverse causation, RBCT was not associated with rejection; the HRs for DCGL attenuated but remained similar. RBCT was also associated with a negative control outcome, demonstrating possible unmeasured confounding.

Conclusion: RBCT after kidney transplant is not associated with rejection, but may carry an increased risk for DCGL.

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See Commentary on Page 875

Kidney transplant recipients commonly develop anemia in their posttransplant course.[1–3] Anemia is often multifactorial, with important contributors being blood loss after surgery, delayed graft function, and side effects from immunosuppressive medications.[1–5] RBCTs are often required to treat anemia, with roughly 50% of kidney transplant recipients receiving RBCTs after transplant.[6,7]

Blood transfusions may carry risks that are relevant to a kidney transplant patient. Through the exposure to non-self human leukocyte antigens (HLA), the blood recipient is at risk of sensitization to HLA through the development of anti-HLA antibodies.[8] In kidney transplant patients, such exposure to non-self HLA antigens from a RBCT may lead to the development of donor-specific antibodies (DSAs) against the kidney allograft donor.[6,9] However, this finding has not been consistently described and it is unclear whether or not an RBCT could lead to adverse graft outcomes.[6,7,9–11] Currently, there is clinical uncertainty as to whether or not an RBCT could negatively impact a kidney allograft. The most recent Kidney Disease: Improving Global Outcomes transplant guidelines make no specific recommendations with regard to RBCT in kidney transplant recipients.[12,13] Because kidney transplant patients often get exposed to RBCTs and immunological damage is one of the leading causes of graft loss,[14] a better understanding of the potential adverse graft outcomes associated with RBCT is warranted.

Correspondence: Greg Knoll, Division of Nephrology, The Ottawa Hospital, Riverside Campus, 1967 Riverside Drive, Ottawa, ON, Canada K1H 7W9. E-mail: gknoll@toh.ca

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The objective of this study was to examine the risks for adverse graft outcomes associated with the receipt of RBCT in kidney transplant patients, specifically the risk of rejection and graft loss.

METHODS

Design, Setting, and Participants

We conducted a retrospective cohort study of all adult kidney transplant recipients at The Ottawa Hospital (TOH) from January 1, 2002, until December 31, 2018. TOH is a 1200-bed, 3-campus academic teaching hospital, and is the largest tertiary care referral center for adults in a region of more than 1.2 million residents. There are no simultaneous kidney-other organ transplants that occur at TOH, so the study participants were recipients of kidney-only transplants; however, they could have a history of a previous organ transplant. There were no exclusions. The start date of January 2002 was chosen because that is when transfusion data began to be captured in our institutional data repository. Institutional research and ethics board approval was received before data gathering and study analysis. Due to the retrospective nature of our study and use of de-identified data, informed consent was waived. The study design, exposure, outcomes, and analysis were all determined before data retrieval. The reporting of this article follows the RECORD (extension of STROBE) guidelines for observational studies (Supplementary Table S1).[15]

Data Sources

The TOH Renal Transplant database, a prospectively collected database of all kidney transplants performed at TOH, updated on a monthly basis by a trained transplant clerk, was used to identify the study population and certain outcomes. The Ottawa Hospital Data Warehouse, a data repository of routinely collected health administrative data on all patients treated at all campuses of TOH, was used to identify the main exposure and baseline characteristics. Chart review was used to ascertain certain outcomes and other baseline characteristics. Data linkage between the databases was done using de-identified medical record numbers. Because all our study participants had their transplant surgeries done at TOH, there were no missing exposure or outcome data.

Exposure

The exposure of interest was the receipt of a RBCT after the date of kidney transplant (posttransplant day 1 and onward). This was chosen to avoid potential misclassification because we did not know the exact time of day of the transplant surgery and could not determine if an RBCT given on the day of surgery was given before or after transplantation. The time for the start of exposure was defined as the date and time of issuing of a unit of RBC from the blood bank, which is captured in TOH Data Warehouse. Only RBCTs that are ordered by a physician are issued from the blood bank. At our institution, all RBCTs were leukodepleted during the study period, and RBCTs are ordered at the discretion of the treating physician; therefore, these would have been done considering transfusion guidelines in effect at the time of transfusion as well as the patient's clinical condition. Kidney transplant recipients are always under the care of Nephrology during their transplant admission and for any kidney transplant patient admitted to an off-service later on in their transplant course, nonurgent RBCTs are typically always verified with the Nephrology service.

Outcomes

The outcomes were graft loss, DCGL, death with graft function (DWGF), rejection, T-cell mediated rejection (TcMR), and antibody-mediated rejection (AbMR). Graft loss was defined as the earliest occurrence of return to permanent dialysis (initiation of regular outpatient dialysis; captured in the TOH Renal Transplant database), re-transplantation, or death. DWGF is captured in the TOH Renal Transplant database. Rejection was defined as a biopsy-confirmed episode of either TcMR (acute TcMR, or vascular rejection if insufficient criteria for the diagnosis of AbMR, or borderline rejection) or AbMR (acute or chronic AbMR). We also examined the outcomes of TcMR and acute AbMR separately. At TOH, all rejection episodes are diagnosed based on histopathological criteria using the Banff criteria, which are updated every 2 years.[16] The criteria in use at the time of the rejection would have been used by the pathologist to make the diagnosis of rejection. All rejection episodes were ascertained through chart review of all pathology reports for each study participant.

Baseline Demographics and Covariates

Baseline demographics and characteristics of the study population at the time of transplantation were determined. The variables were chosen based on availability in databases and clinical relevance for the project. Supplementary Table S2 lists the baseline characteristics and data sources used to retrieve them.

Statistical Analysis

Descriptive statistics were presented for each covariate based on receipt or not of RBCT posttransplant. Means with SDs, medians with interquartile ranges (IQRs) and counts with percentages were presented, along with appropriate test statistics. Exploratory data analysis was performed to look for multicollinearity among the variables.

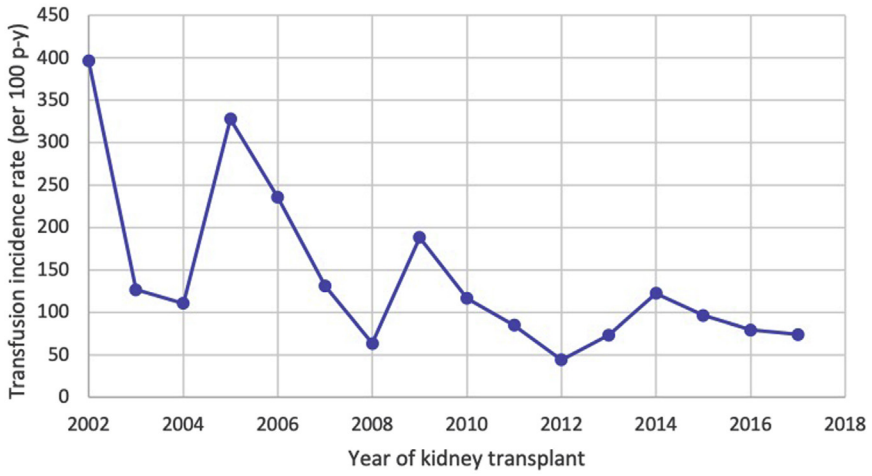


Figure 1. One-year incidence rates (per 100 person-years) of RBCT by year of transplantation. For example, individuals receiving their kidney transplant in 2012 had a 1-year incidence of blood transfusion of approximately 50 transfusions per 100 person-years of follow-up. p-y, person-year.

To analyze the association of RBCT with the outcomes of interest, a time-to-event analysis was used. Crude cumulative incidence estimates were calculated for all outcomes. Cox proportional hazards models were used to calculate crude and adjusted HRs for the outcomes of interest. The variables included in the adjusted models were those thought to be of most clinical relevance while trying to maintain an event-per-degree of freedom ratio of 5 to 10[17]; diabetes and delayed graft function (DGF) were not included because they were co-dependent with the variables "Cause of ESKD" (which included diabetes) and "*t*-cell depleting induction" (DGF is considered high-immunological risk at our institution and such patients receive thymoglobulin), respectively. The index date (time 0) for the start of follow-up was the date of kidney transplantation. Because the main exposure, RBCT, could occur at varying time points after transplantation and an individual could receive more than one RBCT posttransplant, RBCT was analyzed as a time-dependent variable,[18,19] with the Cox model constructed for repeat, time-dependent exposures.[20,21] Therefore, the cumulative exposure to RBCT was considered as the main predictor for the statistical analysis. The functional form of cumulative RBCT was nonlinear and as such, RBCT was categorized into the groups "None," "1 RBC," "2 RBC," "3–5 RBC," and ">5 RBC." This categorization provided the most equal distribution of groups among individuals who were transfused.

For each participant, the end of follow-up was the first occurrence of either the outcome of interest, a censoring event (death or graft loss), loss to follow-up (transfer to another program, captured in the Renal Transplant database) or end of study (December 31, 2018). Graft loss was considered a censoring event because once this occurs, immunosuppression is typically stopped and the patient is no longer followed by the TOH Renal Transplant clinic, so outcomes such as death would not be captured. All statistical analyses were done using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Additional Analyses

We conducted the following additional analyses: (1) To reduce the possibility of reverse causation (the development of an outcome leads to the exposure of interest), the analysis was repeated by accounting for various lag-times for a RBCT to be considered a true exposure before the occurrence of an outcome. Based on the concept that it typically takes a few days to weeks for an antigen to provoke an immune response, time frames of 3, 7, 10, and 14 days were chosen. (2) To examine the possibility for unmeasured confounding explaining a positive association between RBCT and the outcomes of interest, we examined the association of RBCT with a negative control outcome (i.e., one that should not be directly associated with an exposure other than via a confounding variable).[22] The negative control outcome was a diagnosis of osteoporotic fracture or osteoarthritis (identified by International Classification of Diseases, 10th Revision discharge codes [Supplementary Table S3]), both of which should not be caused by RBCT but would be expected to be markers of a frailer, more comorbid patient population. (3) To account for potential misclassification due to RBCT on the day of transplant not being counted, we reconducted our analyses where 50% of those transfused on the day of transplant were assigned as receiving a RBCT immediately posttransplant. (4) The occurrence of DGF may be associated with RBCT and adverse graft outcomes making it a seemingly important potential confounder, although, as explained before, we did not include it in the adjusted model due

Table 1. Baseline characteristics of the study population

Characteristic	Total cohort	Never transfused	Transfused during study period	*P* value
No. of transplant recipients (%)	1258 (100)	790 (62.8)	468 (37.2)	
Age; mean (SD)	52 (14)	50.6 (13.9)	54.0 (14.4)	<0.0001
Female; *n* (%)	454 (36.1)	235 (29.8)	219 (46.8)	<0.0001
Living donor transplant; *n* (%)	571 (45.4)	417 (52.8)	154 (32.9)	<0.0001
Race; *n* (%)				0.37
Caucasian	967 (76.8)	614 (77.7)	353 (75.4)	
Black	109 (8.7)	67 (8.5)	42 (9.0)	
Asian	68 (5.4)	35 (4.4)	33 (7.1)	
Middle-Eastern	57 (4.5)	37 (4.7)	20 (4.3)	
Other	57 (4.5)	37 (4.7)	20 (4.3)	
Cause of ESKD; *n* (%)				0.0026
GN	413 (32.8)	283 (35.8)	130 (27.8)	
Diabetes	315 (25.0)	182 (23.0)	133 (28.4)	
PCKD	180 (14.3)	122 (15.4)	58 (12.4)	
CAKUT	101 (8.0)	64 (8.1)	37 (7.9)	
Other	249 (19.8)	139 (17.6)	110 (23.5)	
Comorbidity; *n* (%)				
Diabetes	389 (30.9)	221 (28.0)	168 (35.9)	0.0033
CVD	251 (20.0)	134 (17.0)	117 (25.0)	0.0006
Kidney transplant number; *n* (%)				0.52
1	1161 (92.3)	733 (92.8)	428 (91.5)	
2	87 (6.9)	52 (6.6)	35 (7.5)	
3	8 (0.6)	4 (0.5)	4 (0.9)	
4	1 (0.1)	1 (0.1)	0 (0)	
5	0 (0)	0 (0)	0 (0)	
6	1 (0.1)	0 (0)	1 (0.2)	
Previous non-kidney transplant; *n* (%)	14 (1.1)	10 (1.3)	4 (0.9)	0.50
Recipients transplanted more than once during the study period; *n* (%)	34 (2.7)	19 (2.4)	15 (3.2)	0.40
PRA; *n* (%)				0.63
0%	590 (47)	381 (48.2)	209 (44.7)	
1%–19%	440 (35)	268 (33.9)	172 (36.8)	
20%–49%	78 (6)	51 (6.5)	26 (5.6)	
50%–79%	77 (6)	44 (5.6)	29 (6.2)	
≥ 80%	73 (6)	46 (5.8)	32 (6.8)	
Delayed graft function	309 (24.6)	116 (14.7)	193 (41.2)	<0.0001
T-cell depleting induction	542 (43.1)	265 (33.5)	277 (59.2)	<0.0001
Tacrolimus maintenance	1043 (82.9)	665 (84.2)	378 (80.8)	0.12

CAKUT, congenital anomalies of the kidney and urinary tract; CVD, cardiovascular disease (coronary artery disease, ischemic stroke, congestive heart failure, or atrial fibrillation); ESKD, end-stage kidney disease; GN, glomerulonephritis; PCKD, polycystic kidney disease; PRA, panel reactive antibodies.

to co-dependency with T-cell depleting induction. However, because it is a conceptually important potential confounder, we reconducted our analyses stratified by DGF status. (5) To account for changes in transfusion and transplantation practices that may occur over time, we performed a sensitivity analysis adjusting for the year of occurrence of kidney transplantation.

RESULTS

Patient and Transfusion Characteristics

The study cohort comprised 1258 kidney transplant recipients with a median follow-up time of 1405 days (3.8 years). In all, 2373 RBCTs were given to 468 (37.2%) study participants throughout the study period (incidence rate 33 transfusions per 100 person-years). The 1-year incidence rate of transfusion per year of transplant surgery showed a gradual decrease over time (Figure 1). Transfused participants were older, more often women, more often received a deceased donor transplant, and had more comorbidities, more DGF, and more often received T-cell depleting induction therapy (Table 1). The median (IQR) number of RBCTs among transfused participants was 3 (2 to 6) and the median (IQR) time to first RBCT was 5.7 days (2.1 to 72.2). Most study participants received <3 RBCT (Table 2). The mean hemoglobin value preceding RBCT was 7 to 8 g/dl throughout the study period, and showed a slight decrease over time (Supplementary Figure S1).

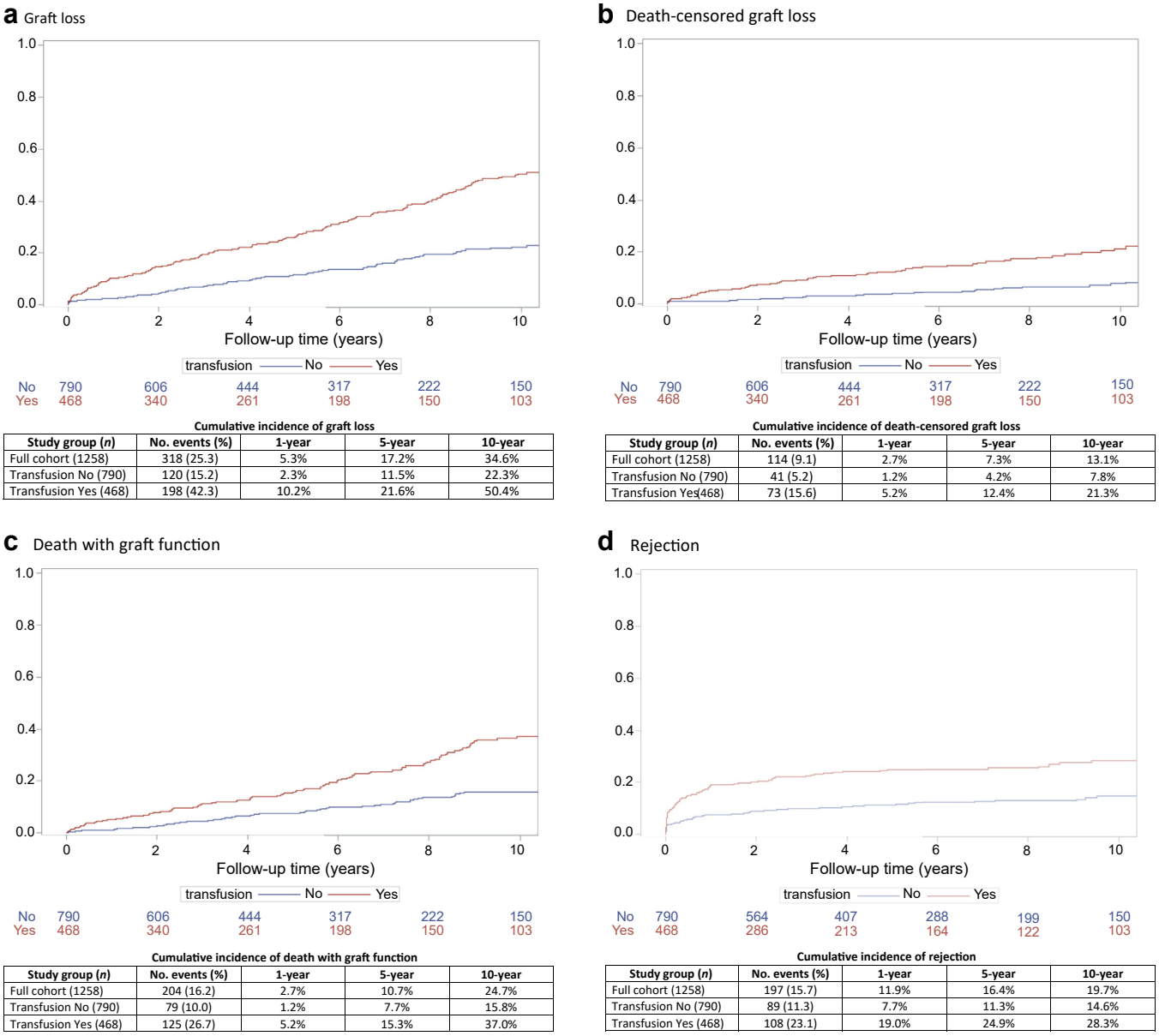
Graft and Patient Outcomes

Graft loss, DCGL, DWGF, and rejection occurred in 318 (25.3%), 114 (9.1%), 204 (16.2%), and 197 (15.7%) participants, respectively, and the 1-year cumulative incidence of rejection was 11.9%. There was a progressive increase in the cumulative incidence of graft loss, DCGL and DWGF throughout the study period (Figure 2). The HRs for graft loss, DCGL, and DWGF increased as the number of RBCTs increased. For 1, 2, 3 to 5, and >5 RBCTs, the adjusted HRs for graft loss were 1.54 (0.93–2.54), 1.99 (1.34–2.96), 3.76 (2.67–5.28), and 9.15 (6.67–12.55), respectively; for DCGL they were 2.32 (1.02–5.27), 3.03 (1.62–5.64), 7.50 (4.19–13.43), and 14.63 (8.32–25.72), respectively; for DWGF they were 1.24 (0.66–2.35), 1.44 (0.85–2.44), 2.53 (1.66–3.85), and 6.76 (4.60–9.93), respectively (Table 3). For the outcomes of rejection, TcMR and AbMR, the adjusted HRs did not increase as RBCT increased. For 1, 2, 3 to 5, and >5 RBCT, the adjusted HRs for rejection were 2.47 (1.62–3.77), 1.27 (0.77–2.11), 1.74 (1.00–3.05), and 2.23 (1.13–4.40), respectively; for TcMR they were 2.38 (1.47–3.86), 1.38 (0.79–2.40), 1.95 (1.06–3.58), and

Table 2. Transfusion characteristics

	Total cohort (*n* = 1258)	Participants transfused (*n* = 468)
No. RBCT received; median (IQR)	0 (0–2)	3 (2–6)
Total amount of RBCT received; *n* (%)		
• None	790 (62.8)	—
• 1	97 (7.7)	97 (20.7)
• 2	118 (9.4)	118 (25.2)
• 3–5	126 (10.0)	126 (26.9)
• >5	127 (10.1)	127 (27.1)
Time from transplant to 1st RBCT (days); median (IQR)	0 (0–2.7)	5.7 (2.1–72.2)

IQR, interquartile range; RBCT, red blood cell transfusion.



Cumulative incidence of graft loss

Study group (*n*)	No. events (%)	1-year	5-year	10-year
Full cohort (1258)	318 (25.3)	5.3%	17.2%	34.6%
Transfusion No (790)	120 (15.2)	2.3%	11.5%	22.3%
Transfusion Yes (468)	198 (42.3)	10.2%	21.6%	50.4%

Cumulative incidence of death-censored graft loss

Study group (*n*)	No. events (%)	1-year	5-year	10-year
Full cohort (1258)	114 (9.1)	2.7%	7.3%	13.1%
Transfusion No (790)	41 (5.2)	1.2%	4.2%	7.8%
Transfusion Yes(468)	73 (15.6)	5.2%	12.4%	21.3%

Cumulative incidence of death with graft function

Study group (*n*)	No. events (%)	1-year	5-year	10-year
Full cohort (1258)	204 (16.2)	2.7%	10.7%	24.7%
Transfusion No (790)	79 (10.0)	1.2%	7.7%	15.8%
Transfusion Yes (468)	125 (26.7)	5.2%	15.3%	37.0%

Cumulative incidence of rejection

Study group (*n*)	No. events (%)	1-year	5-year	10-year
Full cohort (1258)	197 (15.7)	11.9%	16.4%	19.7%
Transfusion No (790)	89 (11.3)	7.7%	11.3%	14.6%
Transfusion Yes (468)	108 (23.1)	19.0%	24.9%	28.3%

Figure 2. Kaplan-Meier cumulative incidence curves with 1-, 5-, and 10-year cumulative incidences for outcomes by transfusion status.

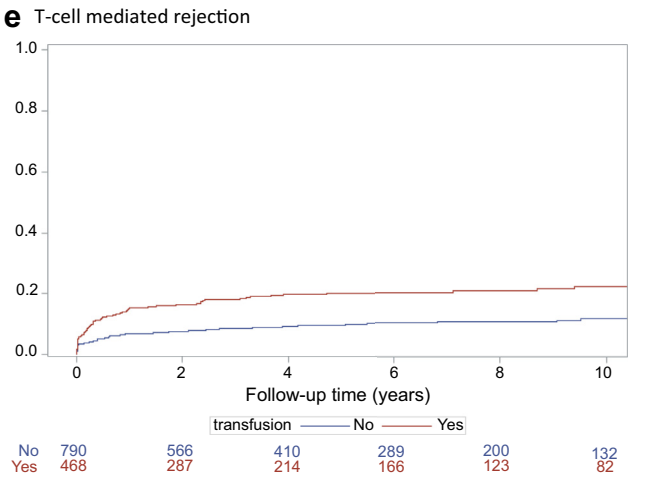
2.88 (1.39–5.94), respectively; for AbMR they were 3.01 (1.09–8.29), 2.33 (0.77–7.03), 1.85 (0.42–8.20), and 1.86 (0.24–14.46), respectively (Table 3).

Additional Analyses

When we accounted for a time-lag of 7 days for an RBCT to be considered an actual exposure before the occurrence of an outcome, RBCT was no longer associated with rejection, TcMR, and AbMR for any of the categories of RBCT (Table 4). For the outcomes of graft loss, DCGL, and DWGF, the HRs attenuated as the time-lag increased but remained significant for the higher levels of RBCT even with a 14-day time-lag (Supplementary Table S4). We found an association between RBCT and the negative control outcome osteoporotic fracture or osteoarthritis, with the HR for >5 RBCTs being 2.28 (1.08–4.83) (Supplementary Table S5). After randomly assigning 50% of study participants transfused on the day of surgery as having received their first RBCT immediately after transplant, re-analysis showed similar findings for all outcomes (Supplementary Table S6). Stratifying by DGF status and controlling for the year of transplantation also did not lead to major changes in our study conclusions (Supplementary Tables S7 and S8, respectively).

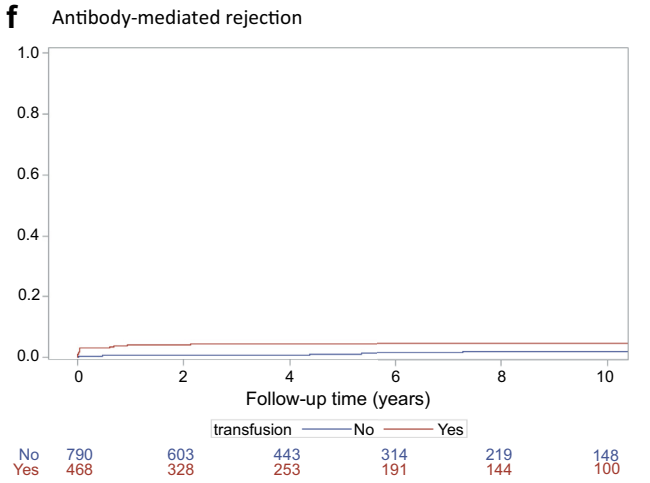
DISCUSSION

In this single-center retrospective cohort study, we found that RBCT after kidney transplant was weakly associated with rejection and that this association was lost when accounting for a biological time-lag between an RBCT and the occurrence of rejection. However,



Cumulative incidence of T-cell mediated rejection

Study group (*n*)	No. events (%)	1-year	5-year	10-year
Full cohort (1258)	158 (12.6)	10.0%	13.5%	15.6%
Transfusion No (790)	74 (9.4)	6.9%	9.6%	11.7%
Transfusion Yes (468)	84 (17.9)	15.4%	20.2%	22.3%



Cumulative incidence of Antibody-mediated rejection

Study group (*n*)	No. events (%)	1-year	5-year	10-year
Full cohort (1258)	30 (2.4)	2.0%	2.3%	2.8%
Transfusion No (790)	10 (1.3)	0.7%	1.1%	1.7%
Transfusion Yes (468)	20 (4.3)	4.2%	4.6%	4.6%

Figure 2. (Continued)

there was an association between RBCT and graft loss, DCGL and DWGF with HRs up to 9.15, 14.63, and 6.76, respectively, for the highest level of RBCT. Clinicians should be mindful of this before ordering blood transfusions, although unmeasured confounding and reverse causation may account for much of these risks.

With 37% of our study population receiving an RBCT after transplantation, fairly similar to what was found in other studies (45%–65%), we confirm that kidney transplant patients continue to be frequently exposed to RBCT.[6,7,9] We did, however, see a slight decrease over time in the yearly incidence rate of RBCT and the pre-RBCT hemoglobin level in our patients, perhaps explained by the adoption of more restrictive transfusion strategies. Our study spans a time frame during which recommendations in kidney disease regarding hemoglobin targets, use of erythropoiesis-stimulating agents, iron as well as transfusions were evolving. The circumstances leading to a RBCT in 2002 are likely to be different than in 2018. Excessive erythropoiesis-stimulating agents use may lead to adverse patient outcomes, on the other hand it may decrease transfusion requirements.[23] The changes in standard of care for anemia management throughout our study period raise the possibility of an era effect. Although we did not have information on erythropoiesis-stimulating agents or iron use pre-transplant or at time of RBCT, we were able to control for year of transplantation and found no significant changes to our findings.

The mechanism through which an RBCT could lead to adverse graft outcomes in kidney transplant patients is through immune stimulation from exposure to non-self HLA found within the RBCT. It is recognized that an RBCT is a major cause of anti-HLA antibody formation in the nontransplant population.[8] Within the transplant population, Hassan *et al.*[6] demonstrated that RBCT could lead to the formation of anti-HLA antibodies, including kidney allograft DSAs. This immunomodulatory effect could theoretically lead to immune-mediated allograft dysfunction, rejection, and eventual graft loss. In our initial analysis we found RBCT was weakly associated with rejection; however, reverse causation is an important factor to consider before concluding to a positive association. At our institution, we do not perform routine allograft biopsies after kidney transplantation; when rejection is diagnosed, significant graft dysfunction prompted the biopsy. Because anemia may be a consequence of kidney dysfunction, an RBCT may actually be the consequence of a rejection already in progress, particularly when one considers that kidney damage has been ongoing for quite some time before it becomes clinically evident.[24,25] Therefore, it is more appropriate to analyze an association between RBCT and outcomes by considering a time-lag for a RBCT to count as a true exposure. After considering a time-lag of only 3 days, the signal for rejection was nearly lost. With a time-lag of 7 days, it was completely lost for all levels of RBCT and types of rejections. This suggests that RBCT is unlikely to cause rejection because it would not have had enough time to exert its proposed immunomodulatory effect before the outcome.

We found markedly elevated risks for graft loss, DCGL, and DWGF. If an RBCT were to predispose to these outcomes through an immunologically mediated process, one would except an even greater risk for acute rejection, which we did not find. That being said, an RBCT may activate the recipient's B-lymphocytes to chronically target the allograft leading to subclinical

Table 3. Cox model HRs for outcomes based on cumulative exposure to RBCT

Outcome	No. RBC units	No. events (%)	Crude HR (95% CI)	Adjusted HR (95% CI)[a]
Graft loss	None	120 (15.2)	Reference	Reference
	1	18 (18.6)	1.44 (0.88–2.37)	1.54 (0.93–2.54)
	2	33 (28.0)	1.96 (1.33–2.89)	1.99 (1.34–2.96)
	3–5	58 (46.0)	4.45 (3.23–6.11)	3.76 (2.67–5.28)
	>5	89 (70.1)	10.07 (7.58–13.36)	9.15 (6.67–12.55)
DCGL	None	41 (5.2)	Reference	Reference
	1	7 (7.2)	1.82 (0.81–4.08)	2.32 (1.02–5.27)
	2	15 (12.7)	3.03 (1.65–5.55)	3.03 (1.62–5.64)
	3–5	21 (16.7)	5.54 (3.21–9.55)	7.50 (4.19–13.43)
	>5	30 (23.6)	12.13 (7.36–19.99)	14.63 (8.32–25.72)
DWGF	None	79 (10.0)	Reference	Reference
	1	11 (11.34)	1.27 (0.68–2.40)	1.24 (0.66–2.35)
	2	18 (15.25)	1.51 (0.90–2.52)	1.44 (0.85–2.44)
	3–5	37 (29.37)	3.97 (2.68–5.89)	2.53 (1.66–3.85)
	>5	59 (46.5)	9.18 (6.51–12.94)	6.76 (4.60–9.93)
Rejection	None	89 (11.3)	Reference	Reference
	1	18 (18.6)	2.34 (1.55–3.52)	2.47 (1.62–3.77)
	2	22 (18.6)	1.34 (0.82–2.20)	1.27 (0.77–2.11)
	3–5	28 (22.2)	1.69 (0.98–2.89)	1.74 (1.00–3.05)
	>5	40 (31.5)	2.41 (1.26–4.63)	2.23 (1.13–4.40)
TcMR	None	74 (9.4)	Reference	Reference
	1	14 (14.4)	2.16 (1.35–3.47)	2.38 (1.47–3.86)
	2	17 (14.4)	1.40 (0.81–2.42)	1.38 (0.79–2.40)
	3–5	23 (18.3)	1.83 (1.02–3.28)	1.95 (1.06–3.58)
	>5	30 (23.6)	2.82 (1.41–5.62)	2.88 (1.39–5.94)
AbMR	None	10 (1.3)	Reference	Reference
	1	3 (3.1)	3.19 (1.17–8.73)	3.01 (1.09–8.29)
	2	4 (3.4)	2.26 (0.75–6.82)	2.33 (0.77–7.03)
	3–5	3 (2.4)	1.75 (0.40–7.72)	1.85 (0.42–8.20)
	>5	10 (7.9)	1.88 (0.24–14.53)	1.86 (0.24–14.46)

AbMR, antibody-mediated rejection; CI, confidence interval; DCGL, death-censored graft loss; DWGF, death with graft function; HR, hazard ratio; RBC, red blood cell; RBCT, red blood cell transfusion; TcMR, T-cell mediated rejection;
[a]Adjusted for age, sex, transplant type, cause of end-stage kidney disease (glomerulonephritis, diabetes, polycystic kidney disease, congenital anomalies of the kidney and urinary tract, other), PRA (as a continuous variable), presence of cardiovascular disease, receipt of T-cell depleting induction and type of maintenance therapy. For outcome of AbMR, the adjusted model was only adjusted for age, panel reactive antibodies (as a continuous variable) due to the low number of events (30).

Table 4. Association of RBCT with rejection for time-lags of 3 and 7 days between exposure and occurrence of outcome (adjusted HR [95% CI])

	Original analysis	3-day lag	7-day lag
Rejection			
RBC category			
None	Reference	Reference	Reference
1	2.47 (1.62–3.77)	1.73 (1.09–2.74)	1.30 (0.79–2.15)
2	1.27 (0.77–2.11)	0.87 (0.50–1.53)	0.69 (0.38–1.25)
3–5	1.74 (1.00–3.05)	1.33 (0.73–2.40)	1.02 (0.54–1.93)
>5	2.23 (1.13–4.40)	1.95 (0.99–3.83)	1.58 (0.78–3.20)
TcMR			
RBC category			
None	Reference	Reference	Reference
1	2.38 (1.47–3.86)	1.85 (1.11–3.08)	1.42 (0.82–2.46)
2	1.38 (0.79–2.40)	0.91 (0.48–1.72)	0.68 (0.34–1.36)
3–5	1.95 (1.06–3.58)	1.62 (0.87–3.02)	1.35 (0.71–2.58)
>5	2.88 (1.39–5.94)	2.56 (1.24–5.27)	2.05 (0.96–4.36)
AbMR			
RBC category			
None	Reference	Reference	Reference
1	3.01 (1.09–8.29)	0.95 (0.22–4.09)	0.43 (0.06–3.18)
2	2.33 (0.77–7.03)	1.84 (0.62–5.43)	1.66 (0.57–4.84)
3–5	1.85 (0.42–8.20)	0.72 (0.10–5.40)	N/A
>5	1.86 (0.24–14.46)	1.43 (0.19–11.02)	1.27 (0.17–9.75)

AbMR, antibody-mediated rejection; CI, confidence interval; HR, hazard ratio; N/A, not available; RBCT, red blood cell transfusion; RBC, red blood cell; TcMR, T-cell mediated rejection.

AbMR without necessarily causing acute rejection. The aforementioned study by Hassan et al, like ours, found an increased risk for graft loss with no association with rejection.[6] Although our study did not find an association between RBCT and rejection or AbMR, there were only 11 diagnosed chronic AbMR (included in the definition of rejection). This is because at our institution we do not perform protocol surveillance biopsies, which can detect subclinical or chronic AbMR in approximately 15% of transplant recipients,[26,27] and we do not routinely investigate chronic graft dysfunction with a biopsy or DSA. Most cases of *de novo* DSA and chronic AbMR were probably never diagnosed. This means we had too few chronic immunological events to meaningfully examine the association between RBCT and this outcome, which would have been helpful to interpret the high HRs we found for graft loss and DCGL. Future studies examining the immunological effects and graft-associated risks of RBCT should incorporate more frequent biopsies and DSA testing.

There are other potential explanations for the markedly increased risks for graft loss, DCGL, and DWGF, which caution against causal inference. First, as kidney function worsens, anemia ensues and RBCT is more common, another example of possible reverse causation. Even when accounting for a time-lag of 14 days between RBCT and outcomes, the risks for graft loss, DCGL, and DWGF remained considerably elevated. Because graft loss occurs over months to years with progressive deterioration in kidney function, the time-lags used in our study would not be expected to completely account for reverse causation. We suspect many of the RBCTs given to study participants who had graft loss were given at a time when graft function was already deteriorating. Second, receipt of RBCT is probably a marker for a more comorbid patient at inherently greater risk for adverse outcomes. The strong association with DWGF suggests non-immunological processes may be implicated in the high risks for graft loss. In our negative control outcome analysis, we found a positive association between RBCT and receiving a diagnosis of osteoporotic fracture or osteoarthritis, both of which should not be directly associated with RBCT other than through confounding. Nevertheless, the magnitude of risks we found for graft

loss, DCGL, and DWGF prevent us from ignoring the possibility of RBCT truly predisposing to adverse graft outcomes, possibly through smouldering immune activation against the allograft.

Few studies have examined the association between RBCT in kidney transplant recipients and adverse graft outcomes, and results have been inconsistent. Some studies have shown no increased risk for rejections[7,28,29] or graft loss,[7] whereas others showed an increased risk for AbMR[6,9] and graft loss.[6,29] Variations in statistical methods and improper ascertainment of whether exposure occurred before outcomes could account for such inconsistencies. For an exposure such as RBCT, which may occur at any time after the start of the observation period (baseline immeasurable), considering it as a time-dependent variable is important to limit survival or time-dependent bias.[19,30] By applying a time-dependent analysis using the exact date and time of RBCT, we ensured a temporal relation between exposure and any outcome, and controlled for time-dependent bias. By considering various time-lags before an outcome for an RBCT to count as an exposure, we limited the risk for reverse causation; none of the previous studies accounted for this. We are also the first to analyze the effect of cumulative exposure to RBCT, which is important when examining the relation between an exposure and outcome.[31] Overall, the size, inclusiveness, statistical methods, and relative granularity of our data in terms of capturing the timing of exposure and ascertaining outcomes are strengths of the current study.

This study has limitations that merit discussion. First, misclassification and incomplete capture of exposure or outcomes are possible. Because this is a single-center study, any RBCT or rejection occurring outside our institution would not be captured. However, we expect this to be minimal because our transplant population is told to present to one of the TOH hospitals when they require urgent medical evaluation, and all transplant biopsies are done at our center so rejection data would be complete as long as a transplant patient is followed at our center. Also, RBCT exposure only started on day 1 posttransplant, potentially misclassifying those who received RBCT in the immediate postoperative period. However, a sensitivity analysis taking this into account revealed no difference in the study findings. Second, we lacked information on serum creatinine at time of RBCT, which could have been helpful to detect reverse causation. Third, we did not have information on DSA formation, which would have been informative when considering the potential long-term immunological effects of RBCT. Fourth, we lacked donor characteristics, such as age and type of deceased donor (Kidney Donor Profile Index/extended vs. standard criteria, cardiac vs. brain death), which could have been important confounders to consider. That being said, we suspect such variables would have been highly correlated with DGF. Finally, the observational nature and potential for unmeasured confounding prevent causal inference between RBCT and adverse graft outcomes.

CONCLUSION

In a large, single-center study, RBCT after transplantation in adult kidney transplant recipients was not associated with the development of acute rejection, often a major concern. It may be associated with a risk for graft loss and death, although reverse causation and unmeasured confounding may be contributing. Therefore, although clinicians caring for transplant recipients should be mindful of these potential adverse consequences and use transfusions judiciously, over avoidance for allograft-specific concerns when they are otherwise medically indicated is probably unwarranted. Future guidelines on the management of anemia in kidney transplant patients should take into consideration these factors in their recommendations.

DISCLOSURE

All the authors declared no competing interests.

AUTHOR CONTRIBUTIONS

DM-A, MMS, DAF, AT, and GAK came up with the research idea and conceived the study design. MC helped conceive the statistical analysis plan. DM-A carried out the data collection, statistical analysis, and interpretation. DM-A drafted the manuscript. MMS and GK provided mentorship and supervision. Each author contributed important intellectual content during manuscript drafting and revision, and approved the final manuscript version. All authors have participated in the work and have reviewed and agree with the content of the article.

DATA SHARING

De-identified raw data and programming code are available from the corresponding author on reasonable request.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. RECORD guidelines checklist.

Table S2. Data sources used to retrieve baseline characteristics.

Table S3. ICD-10 codes used to identify the negative control outcome.

Table S4. Association of RBCT with outcomes for different time-lags between exposure and occurrence of outcome (HR [95% CI]).

Table S5. Association of blood transfusions with negative control outcome osteoporotic fracture or osteoarthritis.
Table S6. Association of RBCT with outcomes accounting for RBCT occurring on day of transplant surgery (HR [95% CI]).
Table S7. Association of RBCT with outcomes, stratified by DGF status (HR [95% CI]).
Table S8. Association of RBCT with outcomes, controlling for year of transplant (HR [95% CI]).
Figure S1. Mean hemoglobin level pretransfusion by year of transfusion.

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