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The role of positive flow cytometry crossmatch in late renal allograft loss

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Abstract

Many studies relating flow cytometery crossmatch (FCXM) results to kidney transplant outcomes have examined risk in the first 3 to 12 months. We used OPTN registry data for 66,594 kidney transplants in 1995-2007 to investigate associations of T cell positive (T+) and T-cell negative/B-cell positive (T-B+) FCXM with graft failure risk early (years 0-1) and late (years >1-5) after transplant. Compared to transplants with T-cell negative/B-cell negative (T-B-) FCXM, living-donor transplants performed after T+ FCXM had significantly higher adjusted, relative risks of both early (adjusted hazards ratio (aHR) 1.71, P<0.0001) and late (aHR 1.36, P=0.017) graft loss. T-B+ FCXM was associated with approximately 40% higher relative risk of graft loss in the late period only. Patterns were similar for deceased-donor transplants. The risks of positive FCXM persist beyond the peri-transplant period for years after transplant. Damage by memory effector cells may explain the long-term risks associated with positive FCXM.

Keywords

Crossmatch; Flow cytometry crossmatch; Graft failure; Kidney transplant; Registries

Introduction

In a classic review of 248 transplants, Patel and Terasaki reported immediate allograft loss in 24 of 30 kidneys transplanted after positive crossmatch results [1]. As an outgrowth of that study, prospective crossmatch has become a standard practice before kidney transplantation

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and a positive crossmatch, particularly when performed by complement-dependent microcytotoxicity (CDC), is generally considered a contraindication to transplantation. However, some transplants do proceed with positive flow cytometry crossmatch (FCXM) – for example, during 1995-2007, nearly 5,000 of approximately 66,000 kidney transplants performed after FCXM had positive FCXM results for at least one target cell [2].

In their 1969 review, Patel and Terasaki stated: “It appears that a preformed antibody has its effect early and does not necessarily affect the long-term outcome . . .” [1]. However, no data were offered to support this assertion. Based on that opinion and data presented in several single center studies (reviewed in [3]), a dogma has developed that patients transplanted after a positive crossmatch who avoid early graft loss face no greater long-term risks than patients transplanted after a negative crossmatch.

In a recent study, we evaluated a national sample of kidney transplants performed after FCXM, with attention to target cell utilization, FCXM results patterns, and associated graft outcomes over five years of follow-up [2]. We observed that positive FCXM results for either T-cell or B-cell targets were associated with inferior outcomes compared to when both T-cell and B-cell FCXM targets were included and yielded negative results. Further, we found no significant statistical interactions of these effects with duration of time from transplantation, an observation supporting long-term persistence of the detrimental effects of positive FCXM.

In the current study, we sought to refine description of the outcome implications of positive FCXM results beyond the peri-transplant period. Using data for a national cohort of transplant recipients recorded in the Organ Procurement and Transplant Network (OPTN) registry data, we formally compared the associations of positive FCXM results and graft loss risk within the first year after transplant to risk in the interval between the first and fifth transplant anniversaries.

Methods

Data were drawn from the OPTN Standard Transplant Analysis and Research Files describing kidney transplants performed from January 1995 to November 2007. At the time of transplant, information is transmitted from each transplant center to the OPTN on the crossmatch technique (CDC, FCXM, etc), cellular target used (T-cell, B-cell, undifferentiated lymphocytes, etc) and the type of antibody detected (IgG only, IgM only, both or undefined). The OPTN surveys post-transplant follow-up information for patient and graft vital status at six months, 1 year and then annually. We studied transplants performed after FCXM tests that were submitted from 30 days prior to transplant through the day of transplantation and identified IgG antibody using B-cell and/or T-cell targets. The FCXM technique has been previously described [4]. Briefly, fluorescent labels are used to identify T-cells and B-cells that have or have not bound antibody. Although the test is very sensitive, FCXM cannot characterize antibody specificity and can only crudely identify antibody class by the pattern of T-cell and B-cell labeling. T-cells possess HLA class I and non-HLA antigens. B-cells possess larger amounts of HLA class I antigen, class II antigen and non-HLA antigens.

In a recent study we found that categories of positive FCXM results associated with significantly higher risk of graft failure compared to a reference of T-cell negative/B-cell negative (T-B-) FCXM included: T-cell positive/B-cell positive (T+B+), T-cell positive/B-cell not performed or not evaluable, and T-cell negative/B-cell positive (T-B+) [2]. A fourth category, T-cell positive/B-cell negative (T+B-) was not significantly associated with increased graft failure risk. In the current study, we analyzed four groups. 1) We combined T+B+ and T-cell positive/B-cell not performed/not evaluable groups, both of which may possess any combination of antibodies against HLA class I, class II or non-HLA antigens and had similar

outcomes in the prior analysis, into a group called T+. 2) The T-B+ group may possess any combination of low-titer anti-HLA class I antibodies, HLA class II antibodies or non-HLA antibodies. 3) The T+B- may represent non-HLA antibodies only. 4) T-B- was considered as the reference. We restricted the analysis to groups with definitive positive or negative FCXM results. Analyses were performed separately for deceased and living donor grafts.

The primary outcome of interest was all-cause allograft loss from graft failure or patient death. Graft failure is indicated by the permanent return of the transplant patient to dialysis or re-transplantation according to OPTN reports. Death events were identified by OPTN reporting and supplemented with the Social Security Death Master File.

Statistical analyses were performed with SAS for windows software, version 9.1 (SAS Institute, Inc., Cary, N.C.). Time-to-all-cause graft loss was censored at five years after transplant or end of study (November 2007). We compared graft survival over time in patients transplanted after T+, T-B+, T+B- and T-B- FCXM by the Kaplan-Meier method and applied the Log-Rank test to assess the statistical significance of differences in absolute survival from transplant until the first and fifth anniversaries, respectively. We used multivariate Cox regression to estimate the relative risks of graft loss associated with positive FCXM early (0-1 years) and late (years >1-5) after transplant. Regression models were adjusted for the following factors as covariates: recipient age category, gender, race, Hispanic ethnicity, cause of end-stage renal disease, duration of pre-transplant dialysis, peak panel reactive antibodies, re-transplant status, and comorbidities; donor gender, race, hypertension, diabetes, and subtype for deceased donors (expanded criteria, donation after cardiac death); degree of HLA mismatch, cold ischemia time, and transplant year

Results

From January 1995 to November 2007, 66,590 kidney transplants in the OPTN registry were performed after FCXM. Of 37,524 transplants from deceased donors, 27,051 met selection criteria based on FCXM results, comprising 24,429 T-B-, 733 T+, 649 T-B+, and 249 T+B-. Of 29,066 transplants from living donors, 27,051 met selection criteria based on FCXM results, comprising 18,440 T-B-, 668 T+, 1,096 T-B+ and 237 T+B-.

Kaplan-Meier estimates of graft survival over time according to FCXM results and donor type are displayed in Figure 1. Both living and deceased donor transplants performed after T+ and T-B+ FCXM showed an early decrement in survival compared to T-B- FCXM, but survival curves continued to diverge beyond the peri-transplant period. This was not true for transplants performed after T+B- FCXM. Among recipients from living donors, graft survival with T+ FCXM was 6.2% lower in absolute terms than in recipients with T-B- FCXM at one year and 12.4% lower at 5 years ($P<0.0001$ at both times). T-B+ FCXM was associated with absolute reductions in living-donor graft survival of 1.8% and 6.5% at one-year ($P=0.005$) and five-years ($P<0.0001$), respectively. Recipients of living donor kidneys with T+B- FCXM had 2.1% and 1.9% reductions at 1-year ($P=0.13$) and 5-years ($P=0.29$), respectively.

Among recipients from deceased donors, graft survival with T+ FCXM was 5.2% lower than in recipients with T-B- FCXM at one year and 7.8% lower at five years ($P<0.0001$ at both times). Recipients of deceased-donors kidneys with T-B+ FCXM had 2.4% and 5.3% absolute reductions in at one-year ($P=0.0008$) and five-years ($P<0.0001$), respectively. Finally, recipients of deceased donor kidneys with T+B- FCXM had 4.9% and 3.5% reductions at 1-year ($p=0.008$) and 5-years ($p=0.29$), respectively.

Table 1 displays the relative risks of graft loss associated with T+, T-B+ and T+B- FCXM, each compared to T-B- FCXM, during the first year and during years 1-5 after transplant for living and deceased-donor grafts. Covariate-adjusted estimates are shown in Table 1B. Living

donor transplants performed after T+ FCXM had significantly higher adjusted, relative risks of both early and late graft loss: 0-1 year adjusted hazards ratio (aHR) 1.71 (95% CI 1.33–2.19, P<0.0001), 1-5 year aHR 1.36 (95% CI 1.06–1.75, P=0.017). Kidney transplants from living donors with T-B+ FCXM did not have increased relative risk of graft loss during the early time period, but did face increased risk during the late period: 0-1 year aHR 1.10 (95% CI 0.90–1.49, P=0.25), 1-5 year aHR 1.39 (95% CI 1.14–1.69, P=0.001). Transplants from living donors after T+B- FCXM did not experience significantly increased relative risk of graft loss during the early or late time periods: 0-1 year aHR 1.24 (95% CI 0.74-2.07, P=0.41), 1-5 year aHR 0.92 (95% CI 0.55-1.54, P=0.76).

Relative risks of graft loss associated with T+ FCXM in deceased donor transplants was of lesser magnitude than that observed for living donor transplants, and only the early risk was statistically significant: 0-1 year aHR 1.29 (95% CI 1.05–1.58, P=0.014), 1-5 year aHR 1.19 (95% CI 0.98-1.45, P=0.08). Deceased donors transplants performed after T-B+ FCXM showed no increase in the relative risk of early graft loss but significantly increased late risk: 0-1 year aHR 1.10 (95% CI 0.95–1.28, P=0.22), 1-5 year aHR 1.19 (95% CI 1.05–1.35, P=0.009). The adjusted relative risk of graft loss was not significantly elevated for deceased donor transplants performed after T+B- FCXM in either time period, although there was trend of borderline significance in the early period: : 0-1 year aHR 1.41 (95% CI 1.00-1.99, P=0.05x), 1-5 year aHR 0.97 (95% CI 0.66-1.43, P=0.89).

Discussion

Early observations of kidney transplant outcomes after positive crossmatch suggested that the associated risk of graft loss is limited to the early post-transplant period [1]. In 2003, Gebel, Bray and Nickerson reviewed 23 studies that examined associations of positive crossmatch results with early graft loss [3]. With one exception, observation time in the summarized studies was limited to a maximum of one year after transplant. In the study notable for longer follow-up, Mahoney et al reported that of 22 transplants performed after positive FCXM at one center, 12 were lost in the first two months, but the remaining 10 were still functioning at two years [5]. Based on these observations, it is commonly believed that if early graft loss in crossmatch-positive transplants can be avoided (as by use of aggressive immunosuppression), a positive crossmatch result loses detrimental effects over time.

In the current study of registry data for a recent national sample of kidney transplants performed after FCXM, we characterized the graft survival implications of positive FCXM results over five-years of follow-up. Contrary to common beliefs, our analyses indicate a continued detrimental effect of positive FCXM results beyond the first transplant anniversary. As with the relative impact of positive FCXM in general, late relative effects were more pronounced in living compared to deceased-donor transplants. T+ FCXM was associated with significantly higher relative risks of both early and late graft loss – specifically, the relative risk of graft loss compared to T-B- FCXM was approximately 70% higher in the first year after transplant and 40% higher in the next four years.

Surprisingly, the detrimental effect of T-B+ FCXM appeared greater in the late period, increasing from no significant association to approximately 40% and 20% relative risk increases in the late period for living and donor transplants, respectively. A recent prospective study of 471 transplants in 1987-2005 at one center found that T-B+ CDC crossmatch was associated with increased risk of graft loss only if donor specific antibody detected by luminex technology was present [6]. The crossmatch cannot distinguish between tests that are positive because of anti-HLA class II antibodies and those positive because of low titers of anti-HLA class I or non-HLA antibodies. A role of HLA class II antibodies in rejection has been previously described [7]. Recent reports, which have characterized the responsible antibodies

with solid state antibody screening techniques, have placed particular significance on anti-HLA class II antibodies [8].

T+B- FCXM had no late effect on graft outcomes. The equivocal early effects of T+B- FCXM, particularly in deceased donor recipients, may reflects decreased sensitivity (ie, false negative) of the B-cell crossmatch due to lower B-cell viability. Alternatively, T+B- FCXM may indicate the presence of only non-HLA antibodies, such that our data support the concept that antibodies against non-HLA antigens are not detrimental to transplants. Further clarification of the significance of T+B- FCXM awaits specific antibody detection with solid-state technology.

The rationality of long-term risk with positive crossmatch results derives from consideration of the complete immune response to the peptide antigens of a transplant. This response starts with T-cell recognition, progresses to the production of antibody, mobilization of effector mechanisms and production of memory cells, and finally activates negative feedback mechanisms to terminate the immune response. An individual who has been sensitized to the extent of producing antibodies is likely to have produced memory cells, which can emerge to trigger a new response in the future. The ability of most clinical histocompatibility laboratories to identify a state of sensitization is limited to showing the presence of serum antibody, which captures only one dimension of the immune response and notably does not characterize the cellular participants, particularly the presence or absence of memory cells.

Antibodies, when present, can function both as augmenters of the effector mechanism and also as mediators of negative feedback at a later point in time. The negative feedback capacity of antibodies has been demonstrated in experimental models using tumor allografts [9]. The brief attempt to protect kidney allografts with donor specific transfusion [10] and the more recent use of intravenous immunoglobulin [11] might represent antibody-mediated negative feedback. Our current “advanced” immunosuppressive drugs have clearly reduced the incidence of early graft loss, but it is unclear what these agents do to the “off switch” of the immune response.

Limitations of this analysis include its retrospective design. Although we analyzed crossmatches performed no later that the day of transplant, it is possible that results of some tests submitted on the day of transplant were not known until after transplant. Such cases would not impact associations between positive crossmatch results and outcome but would reduce the frequency of transplants knowingly performed in the context of a positive crossmatch. Any analysis of graft outcomes in relation to crossmatch results is limited by selection, as a positive crossmatch result may lead to the clinical decision to decline to a given transplant scenario. The OPTN analysis files do not describe the number of transplants turned down based on positive crossmatch and these counts would not define outcomes, but presumably some turndown scenarios would have resulted in early graft loss due to preformed donor-specific antibody. Thus, significant risks identified in this analysis are likely under rather than over-estimates. Although a positive FCXM constitutes a long term risk to outcome, the moderate magnitudes of risk estimates indicate that the mitigating factors that resulted in decision to transplant despite positive FCXM were well-considered.

In conclusion, this analysis of national registry data demonstrates that both T+ and T-B+ FCXM are detrimental to renal graft survival, and that risks persist beyond the peri-transplant period for years after transplant. Until we learn to more effectively exploit the negative feedback abilities of antibodies, we must assess their presence and be cognizant of the presence of both short-term and long-term associated risks. Although long-term graft outcomes are most favorable for transplants performed after T-B- FCXM, risk elevations may be acceptable in some cases given the limited organ supply. The decision to accept the risks of transplant with positive FCXM must be individualized

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Abbreviations

aHR
: Adjusted hazards ratio

CDC
: Complement dependent microcytotoxicity crossmatch

FCXM
: Flow cytometry crossmatch

HR
: Hazards ratio

OPTN
: Organ Procurement and Transplant Network

T-B-
: T-cell negative, B-cell negative

T-B+
: T-cell negative, B-cell positive

T+
: T-cell positive

T+B-
: T-cell positive, B-cell negative

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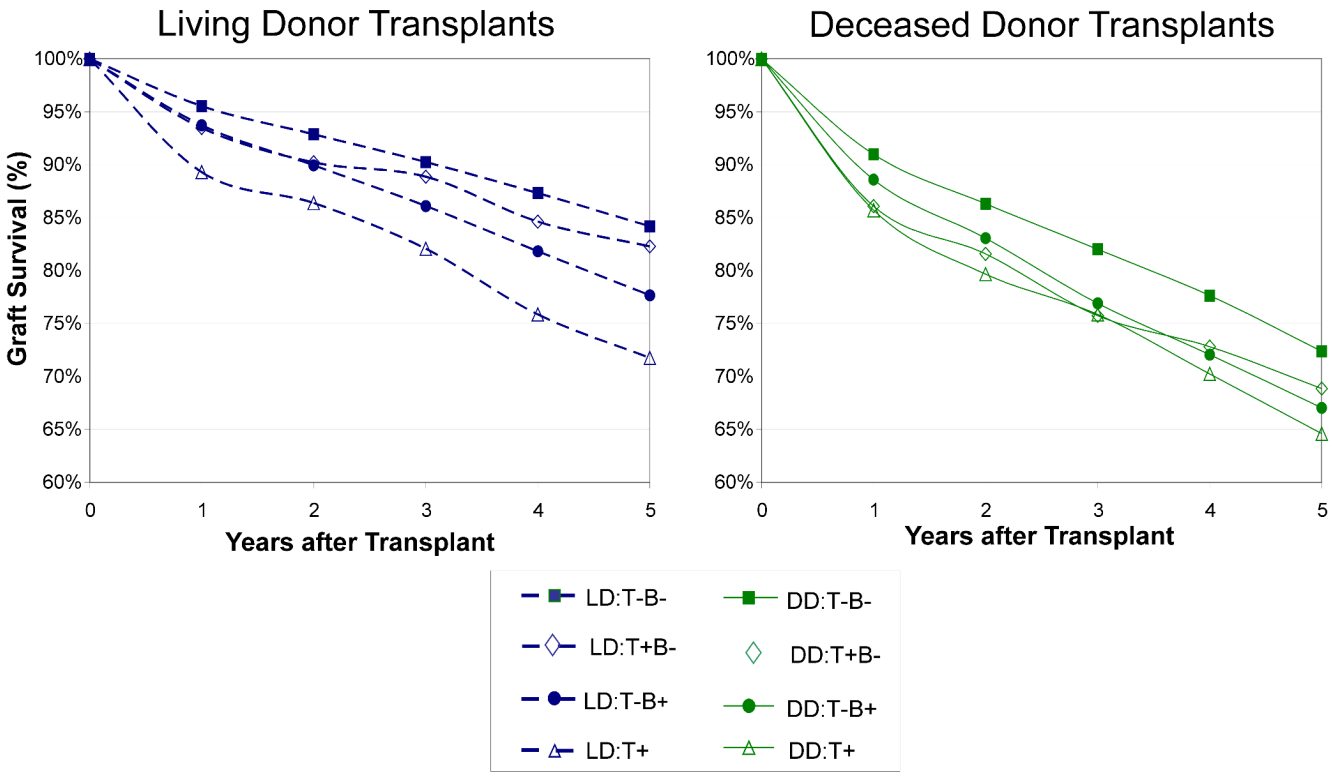

Figure.
Kaplan-Meier estimates of graft survival over five years according to FCXM results for living and deceased donor transplants.

Table 1

Table 1A Unadjusted relative risks of allograft loss associated with positive FCXM by time period after transplantation.

	Living Donor Transplants					Deceased Donor Transplants				
	Number	0-1 year		1-5 years		Number	0-1 year		1-5 years	
		HR	P-value	HR	P-value		HR	P-value	HR	P-value
T-B-	18,440	reference		reference		24429	reference			
T+	668	2.48	<.0001	1.66	<.0001	733	1.58	<.0001	1.22	0.047
		(1.94-3.17)		(1.29-2.13)			(1.29-1.93)		(1.00-1.48)	
T-B+	1,096	1.41	0.007	1.50	<.0001	1649	1.23	0.007	1.22	0.003
		(1.10-1.81)		(1.23-1.82)			(1.06-1.43)		(1.07-1.38)	
T+B-	237	1.49	0.13	1.05	0.84	249	1.57	0.01	1.00	0.99
		(0.90-2.49)		(0.63-1.76)			(1.11-2.21)		(0.68-1.48)	

Table 1B. Covariate-adjusted relative risks of allograft loss associated with positive FCXM by time period after transplantation.*

	Living Donor Transplants					Deceased Donor Transplants				
	Number	0-1 year		1-5 years		Number	0-1 year		1-5 years	
		aHR	P-value	aHR	P-value		aHR	P-value	aHR	P-value
T-B-	18,440	reference		reference		24429	reference			
T+	668	1.71	<.0001	1.36	0.02	733	1.29	0.014	1.19	0.08
		(1.33-2.19)		(1.06-1.75)			(1.05-1.58)		(0.98-1.45)	
T-B+	1,096	1.16	0.25	1.39	0.001	1649	1.10	0.22	1.19	0.009
		(0.90-1.49)		(1.14-1.69)			(0.95-1.28)		(1.05-1.35)	
T+B-	237	1.24	0.41	0.92	0.76	249	1.41	0.05	0.97	0.89
		(0.74-2.07)		(0.55-1.54)			(1.00-1.99)		(0.66-1.43)	

* Regression models were adjusted for the following factors as covariates: recipient age category (≤18, 19-30, 31-45, 46-60, or >61), gender, race (black, white, or other), Hispanic ethnicity, cause of end-stage renal disease (diabetes, hypertension, glomerulohephritis, other), duration of pre-transplant dialysis, peak panel reactive antibodies (<10%, 11%-30%, or >30%), retransplant status, and comorbidities (diabetes, hypertension, peripheral vascular disease, chronic obstructive pulmonary disease); donor category, gender, race, hypertension, diabetes, and subtype for deceased donors (expanded criteria, donation after cardiac death); degree of HLA mismatch (0 ABDR, 0 DR, or DR-mismatch), cold ischemia time. All models were stratified by transplant year.