

Value of Donor-specific Antibody Detection in First-Graft Renal Transplant Recipients with a Negative Complement-dependent Cytotoxic Crossmatch

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Abstract

Objectives: The clinical significance of pretransplant donor specific antihuman leukocyte antigen antibodies that occur despite negative cytotoxicity crossmatches is still unclear. In this study, we assessed the impact of those antibodies on the outcome of renal transplants.

Materials and Methods: Our study subjects consisted of 153 living-donor kidney transplant recipients whose pretransplant sera were available. All subjects had a negative complement-dependent cytotoxic crossmatch and were retrospectively evaluated for antihuman leukocyte antigen antibodies and their donor specificities by means of LABScan 100 Flow analyzer (Luminex Corporation, Texas, USA). The follow-up data of all subjects were reviewed.

Results: Antihuman leukocyte antigen antibodies were detected in 49 patients, donor nonspecific antihuman leukocyte antigen antibodies were found in 33, and donor specific antihuman leukocyte antigen antibodies were identified in 16. There was a trend toward more acute rejection in the patients with antihuman leukocyte antigen antibodies (22%) than in those without antihuman leukocyte antigen antibodies (17%), but that difference had no statistical significance ($P = .378$). Patients with donor specific antihuman leukocyte antigen antibodies had a significantly higher incidence of acute cellular rejection (19% vs. 6%, respectively) and vascular rejection (25% vs. 6%,

respectively) than did patients with donor nonspecific antihuman leukocyte antigen antibodies ($P = .04$).

Conclusions: Our results suggest that there is a higher incidence of acute rejection in patients with donor specific antihuman leukocyte antigen antibodies and a negative complement-dependent cytotoxic crossmatch; however, those factors had no statistically significant impact on patient or graft survival.

Key words: *Crossmatch, Donor-specific antibodies, Kidney transplant, Luminex, Rejection*

The value of detecting pretransplant donor specific antihuman leukocyte antigen (HLA) antibodies that occur despite negative cytotoxicity remains unclear (1). Pretransplant donor specific anti-HLA antibodies can be detected via several methods (2-4). Since Patel and Terasaki (5) first reported the relevance of the pretransplant lymphocyte crossmatch to post-transplant outcomes, the available test options for crossmatching potential transplant recipients have increased dramatically in number. The antihuman globulin complement-dependent cytotoxic crossmatch improved the sensitivity of the standard complement-dependent cytotoxic crossmatch and thus became the preferred crossmatch for use before kidney transplant (2). Similarly, the flow cytometry crossmatch improved the sensitivity of screening for antidonor antibodies and has replaced the antihuman globulin complement-dependent cytotoxic assay as the crossmatch of choice in many centers. Vigorous debate has accompanied the use of the flow cytometry crossmatch. Although studies have shown worse clinical and immunologic outcomes in patients with a positive flow cytometry crossmatch, some investigators have argued that the routine flow cytometry crossmatch is too sensitive

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for nonsensitized recipients and will exclude potentially acceptable donor-recipient pairs (6,7). While clinicians struggle with uncertainty about the appropriate use of those established technologies, new antibody-detection techniques continue to evolve. Luminex xMAP technology (Luminex Corporation, Texas, USA) is based on proven existing technologies that have been combined in a unique way. Featuring a flexible, open-architecture design, xMAP technology can be configured to perform a wide variety of bioassays quickly, cost-effectively, and accurately (8-10).

At least theoretically, recipients with repeatedly negative crossmatches should exhibit either no titer or a low titer of donor specific anti-HLA antibodies. In this retrospective study, we used a highly sensitive method (Luminex technology) to detect those donor specific anti-HLA antibodies in transplant recipients from whom sufficient pretransplant sera could be obtained, and we describe the impact of those antibodies on the clinical outcomes of the renal transplant recipients.

Materials and Methods

Study design: The study subjects consisted of first-graft recipients of living-donor kidney transplants performed in the Urology and Nephrology Center at Mansoura University in Mansoura, Egypt. The study was approved by the ethics committee of the institution before its initiation, and the protocols used conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from the participants (153 transplant recipients whose pretransplant sera were available between March 2000 and November 2005). All patients studied received triple immunosuppressive therapy (a steroid, azathioprine plus calcineurin inhibitors, or sirolimus without induction therapy), and all had a negative complement-dependent cytotoxic crossmatch. Crossmatching was performed with the standard complement-dependent cytotoxicity test to detect, in the serum of the transplant recipients, preformed HLA antibodies against the lymphocytes of the proposed donor (11). Flow cytometry crossmatching was not performed because none of the subjects was considered a high-risk recipient for a living-donor transplant. The patients were retrospectively evaluated for the presence of anti-HLA antibodies and their donor

specificities by means of Luminex technology.

Luminex method: Mixed class I and class II coated beads were incubated 30 minutes with patient serum in the dark at room temperature with gentle shaking. Negative control serum was tested for each test batch, washed twice with diluted wash buffer, and centrifuged. Phycoerythrin: fluorescent dye-conjugated antihuman immunoglobulin G was added to the beads, which were incubated 30 minutes at room temperature in the dark and were then washed twice with diluted wash buffer and centrifuged. Finally, phosphate-buffered saline was added to the beads, which were analyzed with the Luminex machine. To determine whether an individual bead was positive, the median fluorescent intensity of that bead was divided by the median fluorescent intensity of 3 negative control beads, and a background adjustment factor was subtracted to provide 3 different adjusted ratios. A mathematical negative control calculation yielded a fourth negative ratio. A specific bead was considered positive if 2 or more adjusted ratios were positive.

According to the presence or absence of anti-HLA antibodies, the patients were classified into 3 groups as follows: group 1, no detected antibodies (104 patients); group 2, detected donor nonspecific antibodies (33 patients); and group 3, detected donor specific anti-HLA antibodies (16 patients). All data and medical records of the study subjects were reviewed to determine their baseline characteristics and clinical outcomes.

Statistical analysis: The *t* test was used to compare continuous data, and the chi-square test was used to compare simple proportions. Patient and graft survival were computed with the Kaplan-Meier method. A *P* value of less than .05 was considered statistically significant.

Results

The demographic characteristics of the 3 groups are summarized in Table 1. All groups were similar with regard to demographic and tissue-matching characteristics. Of the 153 patients studied, anti-HLA antibodies were detected in 49 patients (32%), 33 patients (67%) had donor nonspecific anti-HLA antibodies, and 16 patients (33%) had donor specific anti-HLA antibodies. There was a higher incidence

Table 1. Characteristics of the patients studied.

Variables	Negative (n = 104)	Nonspecific (n = 33)	Specific (n = 16)	P Value
Recipient age (y)	28.7 ± 11.2	29.6 ± 12.2	30.3 ± 12.3	.870
Donor age (y)	35.6 ± 9.9	34.2 ± 11.1	39.8 ± 12.4	.121
Blood transfusions (no. of units)				.546
1 - 2	8	4	2	
3 - 4	0	2	0	
≥ 5	1	2	0	
HLA mismatch				.975
0	7	3	2	
1	4	1	0	
2	16	4	2	
3	57	18	9	
4	11	3	2	
5	4	3	1	
Haplotype mismatch				.754
0	8	3	2	
1	70	22	11	
2	26	8	3	
DR 1 match/2 matches	84/20	29/4	13/3	.534
Recipient sex (Male/female)	81/23	23/10	11/5	.946
Donor sex (Male/female)	51/53	14/19	8/8	.617
Relationship to patient				.613
Related	87	27	14	
Unrelated	17	6	2	
Blood group (identical/compatible)	89/20	29/4	13/3	.534
Immunosuppression				.128
Azathioprine	10	5	1	
Cyclosporine	37	13	7	
Triple**	39	10	6	
Tacrolimus	18	3	2	
Sirolimus	0	2	0	
Total steroid dose (3 mo)	3.2 ± 1.5	3.1 ± 1.4	3.8 ± 1.5	.171
Serum creatinine level	1.5 ± 0.8	1.4 ± 1	2.4 ± 2.0	.143
Calcineurin nephrotoxicity	12	5	0	.111
Chronic rejection	0	0	1	.146
Diabetes	3	2	1	.979
Pretransplant hypertension	61	20	9	.771
Blood pressure level after transplant				.471
Normotensive	61	17	5	
Sustained hypertension	32	12	7	
Newly developed hypertension	11	4	4	
Malignancy	2	0	0	.422

*All continuous variables are presented as the mean ± SD, and categorical variables are presented as the total number of patients.

**Azathioprin, Cyclosporine and steroid

of acute rejection in the subjects in whom anti-HLA antibodies were detected (22%) than in those without anti-HLA antibodies (17%), but that difference was not statistically significant ($P = .378$). Subjects with donor specific antibodies had a significantly higher incidence of acute cellular rejection episodes than did those with donor nonspecific antibodies (19% vs.6%, respectively; $P = .04$). Furthermore, a significantly higher incidence of humoral rejection was found in the group with donor specific antibodies than in the group with donor nonspecific antibodies (25% vs.6%, respectively; $P = .04$) or the group without anti-HLA antibodies (25% vs.5%, respectively; $P = .038$). However, the cumulative dose of steroids required during the first 3 months and/or the 5-year period after transplantation was not higher in the groups with anti-HLA antibodies (either donor specific anti-

HLA antibodies or donor nonspecific anti-HLA antibodies). The number of rejection episodes in the subjects with donor specific anti-HLA antibodies during the first 3 months of the study and the entire study was higher than the other 2 groups, but that difference was not statistically significant (Tables 2 and 3). However, humoral rejection was highly significant in the subjects with donor specific anti-HLA antibodies ($P = .006$) in the first 3 months of the study (Table 4).

At the conclusion of follow-up, the 3 groups exhibited almost the same incidences of chronic allograft nephropathy, posttransplant hypertension, and diabetes mellitus (Table 1). There was no significant difference among the 3 groups regarding graft function as determined by the serum creatinine level (Table 1). In patients with or without anti-HLA

Table 2. Number of rejection episodes per patient during the first 3 poststudy months.

Variables	Negative antibodies (n = 104)	Nonspecific antibodies (n = 33)	Specific antibodies (n = 16)
One episode of rejection	26	8	3
Two episodes of rejection	3	1	2
More than 2 episodes of rejection	0	0	1
Total no. of rejection episodes	32	10	10
No. of patients with no rejection	75	24	10

Table 3. Number of rejection episodes per patient during follow-up.

Variables	Negative antibodies (n = 104)	Nonspecific antibodies (n = 33)	Specific antibodies (n = 16)
One episode of rejection	27	8	5
Two episodes of rejection	9	2	2
More than 2 episodes of rejection	0	1	1
Total no. of rejection episodes	45	15	13
No. of patients with no rejection	68	22	8

Table 4. Types of rejection episodes during first 3 poststudy months .

Type of Rejection	Negative antibodies (n = 104)	Nonspecific antibodies (n = 33)	Specific antibodies (n = 16)	P Value
Humoral	5	2	5	.006
Cellular	27	8	5	
BLR	10	2	1	
Group 1	14	5	1	
Group 2	3	1	2	
Group 3	0	0	1	

BLR, Border Line Rejection

antibodies, respectively, the 5-year actuarial survival rate was 85% vs. 95% ($P = .245$) for graft survival (Figure 1) and the patient survival rate was 100% vs. 97% ($P = .485$)

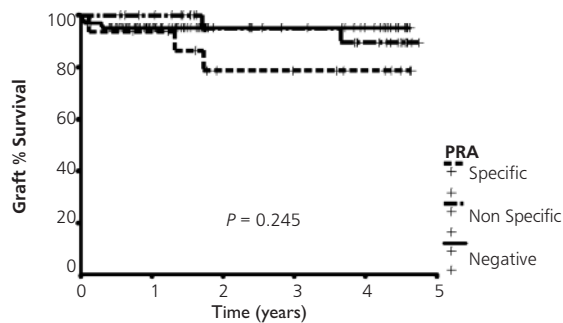


Figure 1. Actuarial graft survival in the study subjects.

Discussion

There is no doubt that donor specific antibodies in patients with a positive crossmatch adversely affect the outcome and survival of the graft (12,13). The clinical impact of donor specific anti-HLA antibodies in the presence of negative complement-dependent cytotoxicity is not certain and questionable.

Furthermore, there are few data on the significance of antibodies detected solely by solid-phase testing. Perhaps the effect of those low titers could be determined with a sensitive technique like that provided by Luminex, and the information yielded would resolve this debatable issue. In this study, we addressed that issue by using the Luminex technique to detect donor specific anti-HLA antibodies in the pretransplant sera of all available recipients with negative complement-dependent cytotoxic to determine the clinical significance of those antibodies on graft outcome. The use of that sensitive solid-phase assay enabled us to detect anti-HLA antibodies in 32% of our complement-dependent cytotoxic negative patients, which is almost the same incidence reported by Gupta and colleagues (14) and Lefaucheur and colleagues (15) (31% and 27.4%, respectively) (14,15).

It seems that the presence or absence of antibodies is not the problem in patients with complement-dependent cytotoxic negative crossmatch. We found that when we compared the incidence of acute rejection in the group with anti-HLA antibodies (22%) with that in the group without anti-HLA antibodies (17%), the results were not statistically significant. The important factor in that finding is the specificity of the antibodies. We found a higher significant incidence of both acute cellular and humoral rejection in the group with donor specific antibodies. However, that higher significant incidence did not have an impact on graft or patient survival; a finding also reported by van den Berg-Loonen and colleagues (16). This mutual finding may have occurred because the low titer of specific or nonspecific antibodies (which can be detected only by the highly sensitive Luminex technique) was insufficient to activate the complement in quantities large enough to cause tissue injury or because those antibodies were noncomplement-activating subtypes or isotypes (14). Other studies must be conducted to verify the impact of those antibodies on graft and patient survival.

In contrast to our results, those of Gupta and colleagues (14) showed no significant difference in the incidence of either cellular or humoral acute rejection in patients without anti-HLA antibodies and those with specific or nonspecific antibodies. This may be because Gupta and colleagues (14) also performed flow cytometry crossmatches for their patients, but we did not do so in our study.

Perhaps a number of subjects larger than that in our study should be evaluated and a relatively longer follow-up might be needed, but given the retrospective nature of our investigation, that was not possible.

Although our study included a relatively small number of patients, we concluded that the presence of donor specific anti-HLA antibodies (as detected with the Luminex method in patients with a negative complement-dependent cytotoxic crossmatch) carried a higher risk of acute rejection in the early posttransplant period without exerting a significant impact on 5-year patient or graft survival.

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