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Significance of C1q-fixing Donor-Specific Antibodies After Kidney Transplantation

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ABSTRACT

Objectives. De novo donor-specific HLA antibodies (DSA) are associated with allograft rejection and allograft loss. However, not all DSA are equally detrimental to allograft function. The ability to activate complement may be an important factor differentiating clinically relevant DSA from nonrelevant DSA. The C1q assay detects a subset of HLA antibodies that can fix complement. This study aimed to investigate the correlation between C1q-fixing de novo DSA (dnDSA) and clinical outcomes posttransplant.

Methods. This retrospective study included 193 sera from kidney transplant recipients who underwent posttransplant DSA testing and/or kidney biopsy for clinical causes. Thirty-five of the 193 (18.1%) had immunoglobulin G DSA. Seventeen of the 35 patients were excluded owing to the presence of pretransplant HLA antibodies. We then analyzed C1q DSA at the time of biopsy in 18 recipients who developed dnDSA. The clinical outcomes of patients with C1q-positive DSA and C1q-negative DSA were compared.

Results. C1q-positive DSA were detected in 10 of 18 patients (55.6%). The incidences of transplant glomerulopathy were significantly higher among patients with C1q-positive DSA than patients with C1q-negative DSA (80% vs 0%; $P = .001$). Although patients with C1q-positive DSA experienced more chronic antibody-mediated rejection and graft loss (80% vs 37.5% [$P = .145$]; 60% vs 25% [$P = .188$]), the differences were not significant. The receiver operating characteristic curve analysis showed that the C1q assay was an excellent predictor of transplant glomerulopathy with area under the curve of 0.9 (95% CI, 0.769–1.000).

Conclusion. The presence of C1q-positive dnDSA was associated with an increased risk of transplant glomerulopathy. The C1q assay is potentially a powerful method for identifying patients at risk for transplant glomerulopathy.

ALTHOUGH SHORT-TERM ALLOGRAFT OUTCOME has significantly improved in recent years, long-term graft loss among kidney transplant recipients remains substantial. The development of de novo donor-specific HLA antibodies (dnDSA) has been shown to be associated with allograft rejection, decreased allograft function, and increased risk of graft loss [1,2]. A recent study showed that the 10-year graft survival for patients with dnDSA was lower than for patients without dnDSA [3]. However, all DSA may not be equal in terms of their deleterious effects on allograft function. A significant number of patients with dnDSA had stable graft function and no graft loss. The

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reasons behind the slow rate of progression to graft dysfunction in these patients remain unknown, but the ability to activate complement may be a critical factor differentiating clinically relevant and nonrelevant DSA.

The classical complement pathway is activated by binding of the globular domains of C1q with immunoglobulin (Ig)G or IgM bound to antigen epitopes on the graft endothelium. Once activated by C1q, the classical complement cascade leads to the generation of the key effector molecule of the complement system, the terminal membrane attack complex, which causes cell lysis. A single antigen bead assay (SAB) to detect HLA antibodies capable of binding to C1q has recently been developed [4]. It has been shown that C1q-positive DSA was highly correlated with rejection and graft loss in kidney transplant recipients [5]. The aims of this study were to determine the impact of de novo C1q-fixing DSA on the outcomes of kidney transplantation and to evaluate the ability of the C1q assay to predict those outcomes.

METHODS

Patients

We included 193 kidney transplant recipients at Ramathibodi Hospital, Bangkok, Thailand, who had posttransplant HLA antibody testing and/or kidney biopsy for clinical causes between January 2009 and February 2013. Only patients who had stored serum samples taken at the time of biopsy were included. Of the 193 patients, 158 had no detectable DSA and 35 had detectable DSA posttransplantation. Stored pretransplant serum samples of these 35 patients with posttransplant DSA were retrospectively tested for HLA antibody testing. Seventeen patients were identified to have HLA antibodies pretransplant and were then excluded. This left a study group of 18 patients who developed dnDSA for the analysis. The medical records, pathologic reports and allograft biopsies of these 18 patients with dnDSA were reviewed to obtain their baseline characteristics and clinical data. This study was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Detection of HLA Antibodies

LABScreen SAB assay (One Lambda, Canoga Park, CA) on a Luminex platform was used to identify HLA specificities as previously described [6]. In brief, multiplexed microbeads, each coated with a single antigen, were incubated with patient serum for 30 minutes and washed to remove unbound antibody. Anti-human immunoglobulin antibody conjugated to phycoerythrin was added for 30 minutes and then washed. Then the microbeads were examined for fluorescence by LABScan 100 flow analyzer (Luminex, Austin, TX) and data were analyzed using HLA Visual software (One Lambda). The cutoff for a positive reaction in IgG SAB assay was set at a normalized mean fluorescence intensity (MFI) value of 1000 or greater. Antibodies against HLA molecules of the donor were assigned as DSA considering low resolution of HLA typing data.

C1q SAB

Detection of antibodies capable of fixing complement was performed using SAB and C1qScreen (One Lambda) according to the manufacturer's instructions. The same samples used to detect posttransplant IgG SAB assay were used for the C1q assay. Briefly, 5 μL of heat-inactivated sera were spiked with human C1q and then incubated with antigen-coated beads for 20 minutes at room temperature. Then, 5 μL of phycoerythrin labeled anti-C1q antibody were added and incubated for a further 20 minutes at room temperature with gentle shaking. The beads were washed twice, then resuspended in 80 μL phosphate-buffered saline and analyzed on the Luminex. The cutoff for a positive reaction was set at the normalized MFI value of 500 or greater.

Definition of Clinical Outcomes

All 18 allograft biopsies were evaluated under light microscope and scored according to the Banff schema [7]. C4d staining was performed using the immunoperoxidase technique in all biopsies. The diagnosis of antibody-mediated rejection was based on the Banff 09 classification [8]. Graft loss was defined as return to dialysis, graft removal, retransplantation of the recipient, or patient death.

Statistical Analysis

Continuous variables were described as mean values (SD) and median values (range) for data with normal distribution and nonnormal distribution respectively. Categorical variables were described as frequency and percentage. Student t test (or Mann–Whitney U test) was used to compare the difference between groups for continuous data. A χ^2 test (or Fisher's exact test) was used to compare the difference between groups for categorical data. The area under receiver operating characteristic curve was used to assess the ability of the C1q assay in predicting the outcomes of kidney transplantation. All analyses were performed using Stata statistical software, version 12.0 (Stata Corp., Collage Station, TX). $P < .05$ was considered significant.

RESULTS

Patient Characteristics and Clinical Outcomes

Eighteen patients with dnDSA were identified and analyzed with the C1q assay. Of the 18 patients with dnDSA, 10 (55.6%) had C1q-positive DSA and 8 (44.4%) had C1q-negative DSA. Patient characteristics and clinical information for these two groups are presented in Table 1. There were no differences between the two groups with regard to age at transplantation, gender, donor type, donor age, panel-reactive antibody levels, HLA mismatch, induction therapy, or immunosuppressive regimens.

Transplant glomerulopathy occurred more frequently in patients with C1q-positive DSA than in patients with C1q-negative DSA (80% vs 0%; $P = .001$). Although patients who had C1q-positive DSA experienced more chronic antibody-mediated rejection (80% vs 37.5%; $P = .145$) and graft loss (60% vs 25%; $P = .188$) than patients with C1q-negative DSA, the differences were not significant.

Patients with C1q-positive DSA had significantly higher MFI of IgG DSA (IgG DSA MFI) than those with C1q-negative DSA (10,176 vs 2651.5; $P = .008$). Given that some patients had more than one DSA, the MFI value of DSA that gave the highest MFI in each patient was used for the comparative analysis of MFI of IgG DSA. In the univariate analysis between C1q-positive and C1q-negative groups, the results demonstrated both MFI of IgG DSA and transplant glomerulopathy as significant variables. However, after we put these variables into the multiple

Table 1. Patient Characteristics and Clinical Data

Variable	C1q Positive (n = 10)	C1q Negative (n = 8)	*P* Value
Age at transplant (y), mean (SD)	37.5 (21.3)	34 (15.7)	.704
Female recipients	5 (50%)	2 (25%)	.367
Deceased donors	6 (60%)	5 (62.5%)	.999
Donor age, mean (SD)	38.8 (11.8)	34.6 (12.2)	.474
First transplant	10 (100%)	8 (100%)	
PRA class I ≤ 10%	10 (100%)	8 (100%)	
PRA class II ≤ 10%	10 (100%)	8 (100%)	
HLA mismatch, mean (SD)	2.7 (1.06)	3.13 (0.64)	.335
Induction therapy	6 (60%)	5 (62.5%)	.999
Baseline immunosuppression			.227
CsA/MMF/Pred	5 (50%)	1 (12.5%)	
CsA/Aza/Pred	2 (20%)	1 (12.5%)	
Tac/MMF/Pred	3 (30%)	5 (62.5%)	
CsA/Pred	0 (0%)	1 (12.5%)	
IgG DSA class			
I	4 (40%)	5 (62.5%)	.637
II	9 (90%)	5 (62.5%)	.275
IgG DSA MFI, median (IQR)	10176 (2580, 16620)	2651.5 (1053, 9274)	.008
Chronic antibody-mediated rejection	8 (80%)	3 (37.5%)	.145
Transplant glomerulopathy	8 (80%)	0 (0%)	.001
Graft loss	6 (60%)	2 (25%)	.188

Aza, azathioprine; CsA, cyclosporine; DSA, donor-specific antibodies; Ig, immunoglobulin; IQR, interquartile range; MFI, mean fluorescence intensity; MMF, mycophenolate mofetil; PRA, panel-reactive antibodies; Pred, prednisolone; SD, standard deviation; Tac, tacrolimus.

logistic regression model simultaneously, the result showed that transplant glomerulopathy was the only variable which was associated with C1q positivity ($P = .001$).

Comparison of dnDSA by IgG and C1q Assays

Of the 10 patients with C1q-positive DSA, one had class I IgG DSA, six had class II IgG DSA, and three had both class I and class II IgG DSA (Table 2). Among these 10 patients, a total of 24 DSA were detected by the IgG assay, but only 14 DSA (58.3%) were detected by the C1q assay. Interestingly, of the 14 C1q-positive DSA, 12 (85.7%) were antibodies to HLA class II. Comparison of DSA-MFI between the IgG and the C1q assays revealed no correlation. IgG DSA with MFI as low as 1199 had the capability to bind C1q. On the other hand, IgG DSA with MFI as high as 12,760 did not (Table 2, patient 6). Analysis of the percentage of C1q positivity in IgG DSA, according to HLA class in the 18 studied patients, demonstrated that 16.7% of IgG DSA against HLA class I were able to bind C1q, whereas 52.2% of IgG DSA against HLA class II were able to bind C1q.

Assessment of Predictive Ability of the C1q Assay for Clinical Outcomes

To evaluate the predictive ability of the C1q assay for clinical outcomes, receiver operating characteristic curve analysis was performed. The predictive performance of the C1q assay was evaluated by using the area under the curve. The C1q assay had the highest area under the curve value of 0.9 (95% CI, 0.769–1.000) for transplant glomerulopathy (Table 3). The ability of the C1q assay to predict chronic antibody-mediated rejection and graft loss were lower with area under the curve values of 0.721 (95% CI, 0.493–0.948) and 0.675 (95% CI, 0.448–0.902), respectively.

Table 2. Comparison of MFI Values of De Novo DSA by IgG and C1q Assays in C1q-Positive Patients

Patient No.	DSA Class I	MFI IgG	MFI C1q	DSA Class II	MFI IgG	MFI C1q	TG	Graft Loss
1	Neg	–	–	DR11	12,140	22,866	Yes	Yes
				DQ7	9671	22,990		
2	Neg	–	–	DR51	8542	23,572	Yes	Yes
				DQ6	1108	0		
3	B58	3687	164	DQ6	11,810	10,163	Yes	Yes
	A33	1828	0	DR13	11,191	12,278		
				DR52	1705	0		
4	Neg	–	–	DQ7	2580	16,676	Yes	No
5	Neg	–	–	DR53	13,458	22,798	No	No
				DR4	1169	0		
6	A11	1199	967	DR51	12,760	105	Yes	Yes
	B60	1032	0	DR12	922	0		
7	A2	8207	20,355	Neg	–	–	Yes	Yes
8	Neg	–	–	DR53	6034	22,310	Yes	No
				DR9	3928	0		
				DQ9	2692	21,804		
9	Neg	–	–	DQ7	6577	11,135	No	No
				DR13	1877	0		
10	B27	2322	3	DR53	16,620	2474	Yes	Yes
				DQ9	5768	12,451		

DSA, donor-specific antibodies; Ig, immunoglobulin; MFI, mean fluorescence intensity; Neg, negative; TG, transplant glomerulopathy.

DISCUSSION

This study demonstrated the association of C1q-positive dnDSA and transplant glomerulopathy. Furthermore, our results showed that the C1q assay was an excellent predictor for transplant glomerulopathy in patients with dnDSA. The C1q assay was a better predictor for transplant glomerulopathy than it was for chronic antibody-mediated rejection or graft loss. Yabu et al [9] were the first to report the clinical significance of C1q-positive dnDSA in adult kidney transplant recipients. They showed that DSA testing with the C1q assay had higher levels of specificity for transplant glomerulopathy and graft loss than testing with the standard IgG DSA assay. All patients who developed transplant glomerulopathy, in their study, were tested positive for

Table 3. Area under ROC curve for predicting ability of C1q according to different outcomes

Outcome	AUC	95% CI
Transplant glomerulopathy	0.900	0.769–1.000
Graft loss	0.675	0.448–0.902
Chronic antibody-mediated rejection	0.721	0.493–0.948

AUC, area under the curve; ROC, receiver operating characteristic.

C1q-fixing antibodies. Sutherland et al [5] also reported the clinical relevance of C1q-positive dnDSA though in pediatric kidney transplant recipients. They focused on a cohort of 35 patients who had dnDSA, 15 with C1q-positive DSA and 20 with C1q-negative DSA. They found that patients with C1q-positive dnDSA were significantly more likely to have peritubular capillary C4d deposition on biopsy, acute rejection, and allograft loss than patients with C1q-negative dnDSA. In our study, we did not find an association between the presence of C1q-positive dnDSA and graft loss. This may be owing to the small sample size in our cohort. Nevertheless, the greater incidence of transplant glomerulopathy in the C1q-positive dnDSA group in our data was consistent with the result from Yabu et al [9]. Together these results suggested that C1q-positive dnDSA was associated with an increased risk of transplant glomerulopathy.

The correlation between transplant glomerulopathy and the presence of C1q-positive dnDSA in our data supported the notion that the complement pathway may play a critical role in endothelial injury and pathogenesis of transplant glomerulopathy. However, the effector mechanisms of tissue injury in transplant glomerulopathy remain poorly understood. Some researchers have postulated a complement-independent pathway for microcirculation inflammation [10,11]. A recent study by Hidalgo et al [12] reported that macrophages, natural killer (NK) cells, and NK-specific transcripts in peritubular capillaries were significantly increased in biopsy specimens of patients with transplant glomerulopathy and late antibody-mediated rejection versus biopsy specimens showing T-cell mediated rejection. These results suggested that NK cells and macrophages may contribute to allograft injury. It is also possible that the recruitment of leukocytes through complement receptors and/or Fc-γ receptors may be involved in inducing endothelial injury [13,14]. Whether and how the complement pathway participates in the development of transplant glomerulopathy remains an open question.

It is important to highlight the different findings of clinical significance of C1q-positive DSA between pretransplant and posttransplant settings. Although C1q-positive dnDSA detectable posttransplant was highly specific for adverse clinical sequelae, as found in our study and others [5,9], C1q-positive DSA in pretransplant sera was not correlated with impaired graft outcome. Otten et al [15] reported a significant association between pretransplant IgG DSA and graft survival, but no correlation between pretransplant C1q-positive DSA and graft survival. Another study from Spain showed that pretransplant C1q-positive DSA was not able to predict antibody-mediated rejection or graft loss [16].

On univariate analysis, we found that the MFI of IgG DSA in the C1q-positive group was higher than the MFI of IgG DSA in the C1q-negative group. However, the association was not significant on subsequent multivariate analysis. This finding was in accordance with the result from another group [4], who found no correlation between MFI of IgG DSA and the ability to bind C1q. Contrarily, several researchers showed that the MFI of immunodominant IgG DSA in C1q-positive patients were significantly higher than that in C1q-negative patients [15,16]. More studies with larger sample sizes are required to resolve these conflicting results.

In conclusion, our study showed that the presence of C1q-positive dnDSA was associated with transplant glomerulopathy. The C1q assay is potentially a useful tool to identify patients at risk for transplant glomerulopathy.

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