



Human Leukocyte Antigen Compatibility and De Novo Donor-Specific Antibodies in Long-term Renal Transplant Patients With Stable Graft Function

E. Kasimatis^{a,*}, A. Fylaktou^b, T. Karampatakis^b, M. Schoina^a, C. Zarras^b, A. Anastasiou^b, M. Papachristou^b, A. Boukla^b, M. Daoudaki^c, I. Fouzas^c, and A. Papagianni^a

^aDepartment of Nephrology, Aristotle University Medical School, Hippokration General Hospital, Thessaloniki, Greece; ^bNational Peripheral Histocompatibility Center, Immunology Department, Hippokration General Hospital, Thessaloniki, Greece; and ^cDivision of Organ Transplantation, Department of Surgery, Aristotle University Medical School, Hippokration General Hospital, Thessaloniki, Greece

ABSTRACT

Purpose. De novo donor-specific antibodies (DSA) are associated with antibody-mediated rejection leading to late renal transplant failure. The aim of this study was to evaluate whether HLA compatibility is associated with sensitization along with other risk factors.

Methods. Eighty-nine stable renal transplant recipients (47 men) were studied. Patients were classified into 2 groups according to HLA compatibility between donor and recipient, group A (1–4/8 matches) and group B (5–8/8 matches). Cold ischemia time (CIT) and delayed graft function (DGF) were recorded along with time with a functional graft. Anti-HLA antibodies were detected using a Luminex single-antigen bead assay and were further classified into DSA and non-DSA.

Results. HLA group A consisted of 49 (56%) transplant recipients while 38 (44%) were classified to group B, with functional grafts for 10.9 ± 6.7 and 14.8 ± 8.5 years, respectively ($P = .019$). Group A patients had more anti-HLA antibodies than group B ($P = .001$) and this correlation was retained for DSA patients. De novo anti-HLA were detected in 40 patients; DSA were detected in 19 (21.8%). DSA (+) patients had recorded with functional renal grafts for 11 ± 5 years, compared to 14.4 ± 8.6 years ($P = .048$) for anti-HLA negative patients. Increased CIT and DGF were associated with anti-HLA antibodies detection but no with DSA.

Conclusion. HLA compatibility is probably correlated with DSA in a context of a more general anti-HLA sensitization, and both have a negative effect on long-term renal graft outcome.

IMPROVED immunosuppression and other advances in patient management have led to a debate whether HLA matching should keep its principal role in organ allocation policies, in favor of other priorities, such as diminishing cold ischemia time (CIT) [1]. There are studies showing decreased significance of HLA mismatch regarding graft survival [2]. However, more recent studies have re-emphasized the influence of HLA compatibility between donor and recipient in kidney allograft survival [3] and its correlation with risk of death with a functioning graft [4].

Donor-specific antibodies (DSA) are associated with antibody-mediated rejection leading to late renal transplant failure [5]. In the present study, we evaluated whether HLA

*Address correspondence to Efstratios Kasimatis, MD, Department of Nephrology, Aristotle University of Thessaloniki, Hippokration General Hospital, 49 Konstantinoupoleos Str 54642, Thessaloniki, Greece. Tel: +30 2313312530; Fax: +30 2313312382. E-mail: frasci@outlook.com.gr

Table 1. Patient Demographics and Transplantation Characteristics

	All	Anti-HLA (-)	Anti-HLA (+)	DSA	P	
					(+)/(−)	DSA/(−)
Number of patients (%)	89	49 (55)	40 (45)	19 (22)		
Mean (SD) recipient age at transplantation	34.0 (12.4)	35.1 (12.9)	32.6 (11.9)	28.7 (8.8)	.34	.021
Male, n (%)	47 (53)	28 (60)	19 (40)	9	.37	.46
Deceased donor, n (%)	32 (36)	14 (44)	18 (56)	6	.11	.98
Mean (SD) donor age at transplantation	50.3 (14.7)	51.9 (14.6)	48.3 (14.8)	50.3 (15.3)	.27	.83
Pre-emptive transplantation, n (%)	11 (12)	8 (77)	3 (23)	1	.22	.40
Retransplantation, n (%)	14 (16)	5 (36)	9 (64)	2	.11	.80
Low HLA compatibility, n (%)	49 (56)	19 (39)	30 (61)	14	.001	.014
Presensitization, n (%)	9 (10)	1 (11)	8 (89)	3	<.001	.020
CIT > 12 hours, n (%)	17 (19)	5 (29)	12 (71)	4	.022	.37
DGF, n (%)	10 (11)	2 (20)	8 (80)	3	.028	.34
ACMR, n (%)	12 (14)	6 (50)	6 (50)	3	.71	.39
Mean (SD) time with functional grafts (years)	12.9 (7.9)	14.4 (8.6)	11.4 (7.3)	11 (5)	.11	.048

Abbreviations: ACMR, acute cellular rejection; anti-HLA, antibodies against human leukocyte antigens; CIT, cold ischemia time; DGF, delayed graft function; DSA, donor-specific antibodies; SD, standard deviation.

compatibility is a risk factor for sensitization, even in the era of potent immunosuppression regimens and its importance in graft survival. Furthermore, we estimated whether presensitization and increased CIT and delayed graft function (DGF) are also risk factors for de novo sensitization.

METHODS

We studied 89 renal transplant recipients with stable renal function, up to 27 years after renal transplantation. Patients were classified into 2 groups according to HLA compatibility for HLA-A, -B, -DR, and -DQ between donor and recipient, group A (1–4 out of 8 matches) and group B (5–8 out of 8 matches). Anti-HLA antibodies were detected using Luminex single antigen bead assay (Luminex Corporation, Austin, Tex.) and were further classified into DSA and non-DSA. CIT was recorded and classified as prolonged if it lasted for more than 12 hours. DGF and biopsy proven acute cellular rejection episodes were also recorded along with time with a functional graft. Patients were defined as presensitized if their pretransplant panel-reactive antibody score was $\geq 10\%$. Categorical variables were reported as percentages and compared using χ^2 or Fisher's exact test. Quantitative data were expressed as mean \pm standard deviation or medians with quartiles 1 and 3, as appropriate. Differences between the groups were statistically compared using *t* test for normally distributed data or the Mann-Whitney *U* test for non-normally distributed data. Independent predictors of DSA were identified by binary logistic regression model. Statistical analyses of clinical data were performed using SPSS version 20 software (IBM, Armonk, NY).

RESULTS

Patient demographics and transplantation characteristics are listed in Table 1. HLA group A consisted of 49 (56%) transplant recipients while 38 (44%) were classified in group B, with functional grafts for 10.9 ± 6.7 and 14.8 ± 8.5 years, respectively ($P = .019$). Less compatible group A patients had more anti-HLA antibodies than group B ($P = .001$), and this correlation was retained, particularly for DSA patients ($P = .014$). De novo anti-HLA were detected in 40

patients, DSA in 19 (21.8%) and non-DSA in 19, as well. In 2 more patients, anti-HLA-DQ antibodies were detected, but it was not possible to further classify them due to a lack of recorded donor HLA-DQ typing that also excluded them from classification into HLA groups. DSA were detected against class I loci in 6 cases, class II loci in 11 cases, and both classes in the other 3 cases. Antibodies against HLA-DQ were the prominent type of DSA as they were detected in 12 out of 19 patients (63.2%). Mean fluorescence intensity was 3625 (range 1243–14,411) and 12,009 (range 2855–21,613) for DSA against HLA class I and II, respectively. Patients with DSA were younger at transplantation than those with no anti-HLA ($P = .021$) and also younger than non-DSA patients at that time (median 27 vs 37 years old, $P = .025$). DSA patients had functional renal grafts for 11 ± 5 years, while patients with no detectable anti-HLA had recorded functional grafts for 14.4 ± 8.6 years ($P = .048$). Presensitization, CIT, and DGF were associated with anti-HLA detection but only the former correlated with DSA ($P = .02$) (Table 1). In multivariate logistic regression analysis including HLA compatibility, patient age at transplantation, CIT and DGF as predictive factors, HLA compatibility was an independent predictor for DSA ($P = .034$, odds ratio = 6.49, confidence interval: 1.15–36.6) (Table 2).

DISCUSSION

Risk factors for sensitization would either increase graft immunogenicity, as in less HLA compatibility, or the recipient's immune system ability to respond to alloantigens, as is the case in inadequate immunosuppression [6]. Young patients with more potent immune response are most likely to develop DSA [5], and this was confirmed in our de novo sensitized patients. Most of the patients in this study were of low immunologic risk, defined as primary transplant recipients who were free of DSA, with negative crossmatch prior to transplantation. In these patients, it has been

Table 2. Predictors of DSA: Multivariate Analysis

Independent Variable	Odds Ratio	95% CI	P
Low HLA compatibility	6.49	1.15–36.6	.034
Patient age at transplantation	0.92	0.83–1.004	.06
CIT	5.73	0.38–86.2	.21
DGF	3.81	0.19–74.4	.38

Abbreviations: Anti-HLA, antibodies against human leukocyte antigens; CI, confidence interval; CIT, cold ischemia time; DGF, delayed graft function; DSA, donor-specific antibodies.

shown that incidence of de novo DSA is increased along with increased number, up to 8, of HLA mismatches [7]. In particular, HLA-DQ mismatch has been found to be predictive of DSA [5,7], and most of the DSA detected in our patients were against donor HLA-DQ. In this study, apart from HLA compatibility, other factors, like DGF and increased CIT, that could increase graft immunogenicity were indeed correlated to de novo anti-HLA. However, these correlations were weaker and not statistically significant for DSA in particular. Correlation with presensitization seems to be strong enough to be retained for DSA but the actual number of presensitized patients with detectable de novo DSA was too small to permit conclusions. Although non-DSA HLA immunization has been correlated with poor transplant function and lower graft survival [8], a causal connection to chronic rejection is supported only with DSA [9]. Nevertheless, HLA immunization, in view of recent advances in HLA Epitopes definition [10], could be considered as a broader process in which HLA mismatches play a leading role. In conclusion, HLA compatibility was probably correlated with DSA in the context of a more general anti-HLA sensitization, and

both of them have a negative effect on long-term renal graft outcome.

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