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Renal transplantation in sensitized recipients with positive luminex and negative CDC (complement-dependent cytotoxicity) crossmatches

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Conflicts of Interest
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Summary

Recently, Luminex-crossmatch (LumXm) was introduced. The aim of this study was to evaluate clinical outcomes in sensitized recipients with a positive Luminex-crossmatch (LumXm (+)) and a negative complement-dependent cytotoxicity crossmatch (CDCXm (−)) after renal transplantation. Fifty-five renal transplant recipients with a CDCXm (−) and PRA class I or II ≥20% were enrolled in this study between February 2008 and December 2010 at Severance Hospital. Eighteen patients displayed LumXm (+) defined as LumXm positive class I or II and 37 patients displayed LumXm (−). Mean duration of follow-up was 18.9 ± 8.3 months. During this period, no patient death or graft loss occurred. The incidence of biopsy-proven or clinically presumed rejection was higher in the LumXm (+) group (n = 12, 66.7%) than in the LumXm (−) group (n = 6, 18.2%) (P = 0.001). All biopsy-proven acute rejections (n = 12) were diagnosed as acute cellular rejection. No significant difference in mean serum creatinine level or eGFR was observed between the groups at 18 months post-transplantation. The short-term outcome of renal transplantation in sensitized patients with a LumXm (+) and a CDCXm (−) may be considered to be acceptable. However, patients with a LumXm (+) have a substantially higher immunological risk for the development of acute cellular rejection.

Introduction

The presence of preformed antibodies against HLA (human leukocyte antigen) is considered a contraindication to renal transplantation. Preformed antibodies can be produced by sensitization via transfusion, pregnancy, or previous transplantation. Donor-specific antibody (DSA) can cause antibody-mediated graft injury and lead to graft loss [1,2]. A CDC (complement-dependent cytotoxicity) crossmatch has been used to identify donor-specific HLA antibodies for more than 40 years [3]. For recipients with a positive crossmatch to their donors, desensitization protocols have been introduced, and good short-term graft outcomes after desensitization

have been reported [4–10]. However, in a recent study, the long-term graft outcomes of positive crossmatch living donor kidney transplantation (LDKT) after desensitization was found to be lower than negative crossmatch LDKT and comparable to deceased donor kidney transplantation from a non-extended criteria donor [11]. Nevertheless, LDKT after desensitization provides a significant survival benefit for patients with DSA as compared with patients waiting for a compatible kidney [12].

Pre-transplant detection of DSA and the interpretation of clinical significance is a major issue in renal transplantation [13–18]. Recently, the Luminex-crossmatch method (DSA; GEN-PROBE, Stamford, CT, USA) was introduced. This technique has the advantage of greater sensitivity than CDC crossmatch (CDCXm). However, the clinical significance of DSA detected by LumXm is uncertain. Accordingly, the aim of this study was to evaluate clinical outcomes in renal transplantation patients with a positive Luminex-crossmatch (LumXm (+)) and a negative CDC crossmatch (CDCXm (−)).

Materials and methods

Patients

Fifty-five renal transplant recipients with a CDCXm (−) and PRA class I or II ≥20% were enrolled in this study between February 2008 and December 2010 at Severance Hospital, Yonsei University Health System. LumXm testing was performed using pretransplant recipient serum and donor lysates. The 55 patients were divided into two groups, namely, a LumXm (+) group ($n = 18$) and a LumXm (−) group ($n = 37$). The clinical outcomes of these two groups were retrospectively analyzed. Institutional Review Board approval was obtained (4-2011-0805).

Immunosuppression

Maintenance of immunosuppression consisted of a calcineurin inhibitor (tacrolimus or cyclosporine)/ mycophenolate mofetil (MMF)/steroid, or tacrolimus/sirolimus/ steroid. Target trough levels of tacrolimus were 5–12 ng/ml for the first month, and 3–7 ng/ml thereafter, whereas target trough levels of cyclosporine were 100–200 ng/ml for the first month , and 80–150 ng/ml thereafter. Basiliximab (20 mg i.v.) was administered to all patients on the day of surgery and on postoperative day 4. Anti-thymocyte globulin (ATG) was not routinely used for induction. The immunosuppressive strategy was the same in the two groups. Prior to transplantation, a single dose of rituximab (375 mg/m^2 i.v.) was administered to all patients with PRA class I or II ≥50%.

Clinical data

Acute rejection was diagnosed by graft biopsy or by clinical deterioration of graft function as determined by doppler ultrasound. If possible, a graft biopsy was performed in patients with a deteriorating graft function. No routine protocol biopsy was performed. A histologic diagnosis of acute rejection was made according to Banff 07 criteria [19]. C4d staining was performed in all biopsy samples, and interpreted as diffuse when >50% of cortical peritubular capillaries (PTCs) were linearly stained and focal when staining was <50%. Positive staining of a few capillaries was interpreted as negative. Antibody-mediated rejection (AMR) was diagnosed by the presence of circulating DSA and histologic findings including C4d deposition in PTCs. Methylprednisolone pulse therapy (500 mg/day, 3–4 times) was initiated to treat acute rejection. If there was no response to this treatment, ATG was used. Delayed graft function (DGF) was defined as the need for dialysis during the first week after transplantation. Graft function was assessed by estimated glomerular filtration rate (eGFR), which was calculated using the modification of diet renal disease (MDRD) formula. Graft loss was defined as death or conversion to maintenance dialysis.

CDC crossmatch (CDCXm)

NIH (National Institutes of Health) and AHG (anti-human globulin)-enhanced CDCXms were performed to detect antibodies against donor T-cells. The CDCXm test was employed to detect warm antibodies against B-cells. Dithiothreitol (DTT) was used to differentiate IgM from IgG antibodies.

HLA antibody screening by Luminex

All serum samples were tested using the PRA-Identification (PRA-ID) assay (LIFECODES Class I/II ID; GEN-PROBE), a multiple-antigen bead assay. Multiple class I or II antigen-coated Luminex-beads were incubated with recipient serum samples. The sensitized beads were then washed to remove unbound antibody. An anti-human phycoerythrin-conjugated IgG was then added to wells. After incubation in the dark on a rotating platform, test samples were analyzed on the Luminex instrument. The signal intensity from each bead was compared with that of beads treated with negative control sera, and positivity was determined according to the manufacturer's instructions.

Single-antigen bead assay (SABA)

To determinate the specificities of HLA antibodies, SABA (LIFECODES LSA^{TM} Class I and/or Class II; GEN-

PROBE) was performed on the sera of those positive for anti-HLA class I and/or class II antibodies by screening. SABA was performed in the same manner as PRA-ID except that single class I or II antigen was coated on the Luminex-beads. SABA (+) results were defined as the presence of donor-specific anti-HLA antibodies detected by SABA.

Luminex-crossmatch (LumXm)

Luminex-crossmatch (LIFECODES DSA; GEN-PROBE) test was used to qualitatively detect IgG antibodies to donor-specific class I and II HLA. Donor lymphocytes were isolated from peripheral blood, solubilized with a non-ionic detergent, and centrifuged to remove cell debris and fragments. Luminex capture beads consist of a single blend of beads conjugated with monoclonal antibodies specific for class I or II HLA. Donor lysates were incubated for 30 min with 5 μl of capture beads, which bound the solubilized HLA glycoprotein. The assay included control beads to monitor the amount of background in the assay and to ensure that the appropriate conjugate had been used. After capturing donor HLA, beads were transferred to a filter plate, and washed. Diluted recipient serum was then added to the beads and incubated for 30 min. Following another wash, diluted anti-human phycoerythrin-conjugated IgG was added and incubated for 30 min. Wash buffer was then added, and a LABScan 100 flow cytometer (Luminex, Austin, TX, USA) was used to detect anti-HLA IgG antibodies to the donor and to measure median fluorescent intensity (MFI). LumXm (+) was determined according to the manufacturer's instructions. The MFI values of capture beads were compared with three cutoff values (background adjustment factors; BAFs). These cutoff values were calculated from the measured backgrounds of three negative control beads in each test well. Each control bead has a separate and lot-specific equation for calculating the BAF values. The BAF values for control bead were subtracted from the MFI values of capture beads (adjusted MFI value). This process was repeated for each of the remaining two control beads to obtain three adjusted MFI values. A sample was considered positive for donor-specific antibodies if two or more adjusted MFI values were positive [20].

Statistics

Statistical analysis was performed using SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA). The chi-squared and Student's *t*-tests were used to compare categorical and continuous variables, respectively. All *P*-values were two-tailed. Statistical significance was accepted for *P*-values <0.05.

Results

Patient characteristics

Of the CDCXm (−) 55 recipients with a PRA ≥20%, 18 recipients were LumXm (+). Six were positive for class I, five were positive for class II, and seven were positive for both class I and II. Thirty-seven recipients were LumXm (−). Table 1 provides a summary of recipient and donor characteristics in the LumXm (+) and (−) groups. A single dose of rituximab was administered to 9 (50.0%) of 18 patients in the LumXm (+) group, and 8 (21.6%) of 37 patients in the LumXm (−) group (Table 1).

Table 1. Patient characteristics.

	LumXm (+) (*n* = 18)	LumXm (−) (*n* = 37)	*P*-value
Recipient age at Tx (years)	43.4 ± 9.6	42.3 ± 11.5	0.738
Donor age at Tx (years)	43.2 ± 13.2	41.7 ± 9.0	0.631
Recipient gender, female (%)	17 (94.4)	21 (77.8)	0.245
Donor gender, female (%)	4 (22.2)	25 (67.6)	0.002
Living donor (%)	15 (83.3)	37 (100)	0.031
HLA mismatch (A/B/DR)	3.2 ± 1.3	2.4 ± 1.6	0.074
Retransplantation (%)	3 (16.7)	3 (8.1)	0.381
PRA-ID class I (%)	61.0 ± 38.3	36.4 ± 32.5	0.016
PRA-ID class II (%)	48.3 ± 33.7	21.2 ± 24.0	0.005
Main immunosuppression Tac:CsA:SRL with Tac:other (%)	10:2:6:0 (55.6:11.1:33.3:0)	29:3:4:1 (78.4:8.1:10.8:2.7)	0.181
Pretransplant rituximab (%)	9 (50)	8 (21.6)	0.033
Mean follow-up time (months)	20.9 ± 8.5	18.2 ± 8.2	0.235

All values are means ± SD.

Tx, transplantation; HLA, human leukocyte antigen; PRA-ID, panel reactive antibody-identification; Tac, tacrolimus; CsA, cyclosporine; SRL, sirolimus.

Comparison of LumXm and PRA-ID/SABA results

Class I LumXm was detected in 10 of 16 (62.5%) class I SABA (+) patients and class II LumXm was detected in nine of nine class II SABA (+) patients. The sensitivity of class I LumXm was 62.5% (10/16) and class II 100% (9/9). The specificity of class I LumXm was 92.3% (36/39) and class II 93.5% (43/46). The positive predictive values of class I LumXm were 76.9% (10/13) and class II 75.0% (9/12). The negative predictive values of class I LumXm were 85.7% (36/42) and class II 100% (43/43) (Table 2).

Post-transplant outcomes

No graft loss or patient death occurred during the follow-up period in either group. DGF developed in two patients (11.1%) in the LumXm (+) group and one patient (2.7%) in the LumXm (−) group ($P = 0.247$).

Episodes of rejection were clinically suspected in 12 LumXm (+) patients and in 8 LumXm (−) patients, respectively. Graft kidney biopsies were performed in 10 of the 12 LumXm (+) patients, and in 6 of the 8 LumXm (−) patients. Biopsy-proven acute rejection occurred in nine patients (50.0%) in the LumXm (+) group and in three patients (8.1%) in the LumXm (−) group ($P < 0.001$). All biopsy-proven acute rejections were acute T-cell-mediated rejections. Biopsy-proven or clinically presumed acute rejection occurred in 12 patients (66.7%) in the LumXm (+) group and 6 patients (16.2%) in the LumXm (−) group ($P < 0.001$) (Table 3). No biopsy-proven acute AMR occurred in either group. Fifteen of the 16 patients that underwent a graft kidney biopsy were negative for C4d staining. In one LumXm (−) patient, C4d staining was focally positive in PTCs, and the histologic finding in this patient was acute cellular rejection (Type IIA). Banff scores for the two groups are provided in Table 4. The relative risk of acute rejection in the LumXm (+) group was 4.1 (1.8–9.2, 95% CI) (Table 5).

In the LumXm (+) group, episodes of acute rejection occurred in seven (77.8%) of nine patients with a single dose of rituximab administration, and in five (55.6%) of nine patients without rituximab ($P = 0.630$). In the LumXm (−) group, episodes of acute rejection occurred in two (25.0%) of eight patients with a single dose of rituximab administration, and in four (13.8%) of 29 patients without rituximab ($P = 0.591$).

Table 2. Comparison of LumXm and PRA-ID/SABA results.

	Positive	Negative
Class I LumXm		
Class I PRA-ID/SABA		
Positive	10	6
Negative	3	36
Class II LumXm		
Class II PRA-ID/SABA		
Positive	9	0
Negative	3	43

Table 3. Incidences of acute rejection.

	LumXm (+) ($n = 18$)	LumXm (−) ($n = 37$)	*P*-value
AR	12 (9;BPAR)	6 (3;BPAR)	<0.001
No AR	6	31	

LumXm, Luminex-crossmatch; AR, acute rejection; BPAR, biopsy-proven acute rejection.

Table 4. Banff types of acute T cell-mediated rejections.

Banff type	LumXm (+) ($n = 9$)	LumXm (−) ($n = 3$)
Borderline/suspicious	1	1
IA	0	0
IB	0	0
IIA	8	2
IIB	0	0
III	0	0

Table 5. Relative risks (RR) of acute rejection in the LumXm (+) group.

LumXm	RR (95% CI)	*P*-value
Negative	1	<0.001
Positive	4.1 (1.8–9.2)	

Table 6. Comparison of post-transplant renal functions.

	LumXm (+) ($n = 18$)	LumXm (−) ($n = 37$)	*P*-value
eGFR by MDRD (ml/min/1.73 m^2)			
1 month after KT	51.6 ± 20.3	62.8 ± 13.9	0.021
3 months after KT	52.2 ± 13.0	59.6 ± 10.5	0.031
6 months after KT	51.5 ± 12.8	60.3 ± 13.9	0.036
12 months after KT	51.8 ± 13.0	60.1 ± 18.1	0.017
18 months after KT	57.3 ± 10.9	64.8 ± 13.6	0.167
Serum creatinine (mg/dl)			
1 months after KT	1.44 ± 0.75	1.15 ± 0.32	0.044
3 months after KT	1.29 ± 0.30	1.18 ± 0.27	0.175
6 months after KT	1.31 ± 0.36	1.24 ± 0.68	0.694
12 months after KT	1.26 ± 0.25	1.26 ± 0.54	0.966
18 months after KT	1.18 ± 0.27	1.15 ± 0.28	0.769

Graft function

Estimated GFR values in the LumXm (+) group were significantly lower than in the LumXm (−) group at 1, 3, 6, and 12 month post-transplantation. No significant difference was observed between the groups at 18 month post-transplantation. The level of serum creatinine in the LumXm (+) group was significantly higher than that in the LumXm (−) group at 1 month post-transplantation ($P = 0.044$). No significant intergroup difference in serum creatinine was observed from 3 month post-transplantation (Table 6).

Discussion

The main objective of this study was to identify the clinical impact of LumXm (+) renal transplantation. Our results indicate that LumXm positivity in CDCXm (−) patients may not be considered a contraindication to renal transplantation. Although episodes of acute rejection developed more often in the LumXm (+) group, early post-transplant outcomes were similar to those in the LumXm (−) group. Our results are in accord with previous reports, in which DSA was detected by solid-phase assay [15,16,21]. Interestingly, in this study, all biopsy-proven cases of acute rejection were diagnosed as acute cellular rejection, and no biopsy-proven acute AMR developed. LumXm (+) with CDCXm (−) probably indicates a low-level of DSA that is insufficient to invoke acute AMR. In contrast to our results, other reports have concluded DSA detected by solid-phase assay is a risk factor of acute AMR [17,22,23], and that it is detrimental to long-term graft outcome [14,15,17,22,24]. Niederhaus *et al.* used a Luminex-based desensitization protocol in patients with DSA detected by SABA [25].

The desensitization protocol consisting of plasmapheresis and intravenous immunoglobulin (IVIG) was not used for LumXm (+) kidney transplantation at our institution. Prior to transplantation, a single dose of rituximab (375 mg/m^2) was administered to recipients with PRA >50%. The cost of a single dose of rituximab is covered by national medical insurance for patients with a PRA >50% in Korea. The incidence of rituximab use in the LumXm (+) group was higher than in the LumXm group because the proportion of recipients with a PRA >50% in the LumXm (+) group was higher. Absence of biopsy-proven acute AMR was partly attributable to rituximab. However, the risk of acute cellular rejection development was found to be significantly higher in the LumXm (+) group (relative risk 4.1). The mechanism responsible for the higher incidence of acute cellular rejection in the LumXm (+) group remains unknown. As described in the results, the majority of biopsy-proven acute cellular rejections were of Banff type IIB in both groups (88.9% in LumXM (+) vs. 66.7% in LumXm (−)). Most rejection episodes developed during the early post-transplant period. No differences in immunosuppressive strategies including, target trough level of CNI, dose of steroid, and induction therapy, were evident between the two groups. Basiliximab was used in all patients for induction therapy. We did not use ATG for induction in the LumXm (+) group because we did not have sufficient data to assess immunological risk for LumXm (+) renal transplantation during the study period. According to a recent report, ATG and IVIG induction significantly reduced clinical T-cell mediated rejection and the severity of AMR in patients with a low-level of DSA (detectable by SABA) but negative for CDCXm [26]. Our study also suggests that ATG induction may reduce the incidence of acute cellular rejection among LumXm (+) patients.

Post-transplant eGFR in the LumXm (+) group was inferior to that in the LumXm (−) group until 12 months post-transplantation, but subsequently, post-transplant renal functions were similar. Furthermore, post-transplant serum creatinine levels were non-significantly different in the two groups from 3 months post-transplantation. These findings show that renal function was similar in the two groups.

LumXm has several clinical advantages as compared with CDCXm. LumXm can detect donor-specific HLA antibodies with greater sensitivity than CDCXm. Donor lysates can be stored for a longer time and then used for DSA monitoring. LumXm detects only IgG DSA, which is primarily related to post-transplant outcome. Furthermore, LumXm can detect DSA to rare HLA not included in SABA [27]. However, LumXm has some limitations. LumXm proved to be valid for class I DSA detection, but its value for class II DSA detection was uncertain. In addition, LumXm has low sensitivity for the detection of DSA to DQ and DR [24]. On the other hand, the sensitivity of class I LumXm was low (62.5%) and sensitivity of class II LumXm was high (100%) in this study. Discrepancies between LumXm and SABA could be due to the different bead preparation protocols. For LumXm, donor HLA lysates are coated onto capture beads, whereas purified HLA are coated to beads in SABA. Therefore, the density of antigens expressed on the respective beads may differ. In bead-based immunoassays, binding of HLA molecules to bead may modify the conformation of HLA molecule, especially in SABA. This modification of structure may impair anti-HLA antibody binding, resulting in false-positive or negative reactions [24,27,28]. LumXm also detects non-complement-fixing IgG2 and IgG4 (the clinical significances of which have not been established) and complement-fixing IgG1 and IgG3 [23,29].

This study has some limitations. First, the sample size was small and the mean follow-up time was short. Second, some acute rejection episodes were not confirmed by biopsy. Third, the two groups differed with respect to donor gender and proportion of living donor, which could have confounded post-transplant outcomes. A further large-scale study with longer-term follow-up is required to confirm the clinical significance of LumXm (+).

In conclusion, the short-term outcome of renal transplantation in sensitized patient with a LumXm (+) and a CDCXm (−) may be considered to be acceptable and not to require a desensitization protocol, including plasmapheresis and IVIG. However, LumXm (+) patients were found to be at high immunological risk of acute cellular rejection. Accordingly, ATG induction may be advisable in LumXm (+) patients.

Authorship

KHH: participated in the study design, analysis of data, and writing of the manuscript. MSK: participated in the study design. HJK, DJJ, BSK, MKJ and SIK: contributed to data collection. YSK (corresponding author): designed the study, provided important intellectual contribution, and revised the manuscript.

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