A Comparative Study: Flow Cytometry, Complement-dependent Cytotoxicity, and Luminex Methods

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ABSTRACT

Aim. The cell-based flow cytometric and bead-based Luminex crossmatch methods have been used alongside the standard complement-dependent cytotoxic crossmatch (CDCXM) test to detect donor specific anti-HLA antibodies. In this study, it was aimed to compare flow cytometric crossmatch (FCXM), CDCXM, and Luminex donor-specific crossmatch (LM-XM) tests for pre-transplant assessment of patients who applied to Tepecik Education and Research Hospital for kidney transplantation from related or deceased donors.

Method. HLA tissue typing of 1120 patients were tested using the sequence specific oligonucleotide probe method with low resolution. FCXM and LM-XM were performed according to the manufacturer's instructions. The CDCXM test was performed according to the standard procedure. The results were analyzed using SPSS version 21.0 software (IBM, Armonk, NY, United States). $P < .05$ was accepted as statistically significant.

Results. FCXM, CDCXM, and LM-XM tests were performed on 58.2% (n = 652), 91% (n = 1019), and 55.4% (n = 620) of 1120 patients. There were statistically significant differences between FCXM/CDCXM, LM-XM/CDCXM, and FCXM/LM-XM ($P < .0001$), although there was also a moderate correlation between them (for class I, r = .599, r = .693, and r = .507; for class II, r = .546, r = .471, and r = .495, respectively). The results obtained according to donor type were compatible with the total study group.

Conclusion. The utilization of FCXM and/or LM-XM tests together with the CDCXM method before kidney transplantations from related and/or deceased donor may facilitate the determination of target cells of donor-specific antibodies or their antibody class, which may increase the success of transplantation.

ALLOANTIBODIES against HLAs are the most important antibodies associated with allograft rejection and graft function loss [1], and it is very difficult to estimate the antibodies in recipients before transplantation. There are both cell-based and solid-phase-based assays, such as complement-dependent cytotoxic crossmatch (CDCXM), flow cytometric crossmatch (FCXM), and Luminex donor-specific crossmatch (LM-XM) tests, respectively [2]. Pre-transplant donor-specific antibodies (DSAs) may lead to hyperacute rejection and graft failure. Thus, the detection of DSAs before kidney transplantation is very important. The CDCXM technique can detect only complement-fixing DSAs, whereas the other tests can determine all of the DSAs [3]. In this study, it was aimed to compare the crossmatch techniques before kidney transplantation.

MATERIAL AND METHODS

HLA tissue typing of 1120 patient-donor couples before kidney transplantation were determined using a low-resolution, sequence-specific oligonucleotide probe test according to the manufacturer's

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Table 1. The Association Between the Tests

Tests	Number of Patients (%)	*P* (CI and CII)	r (CI)	r (CII)
FCXM/CDCXM	616 (55%)	<.0001	.599	.546
FCXM/LM-XM	167 (14.9%)	<.0001	.507	.495
CDCXM/LM-XM	551 (49.2%)	<.0001	.693	.471

Abbreviations: CDCXM, complement-dependent cytotoxic crossmatch; CI, class I; CII, class II; FCXM, flow cytometric crossmatch; LM-XM, Luminex donor-specific crossmatch.

instructions (Lifecodes HLA Typing Kit, Immucor, Norcross, Ga, United States). CDCXM and FCXM techniques were performed according to the standard procedure. LM-XM method was performed according to the manufacturer's instructions (Lifecodes Donor-Specific Antibody, Immucor). This study was conducted according to the principles expressed by the Declaration of Helsinki and approved by the Ethics Committee of İzmir Katip Celebi University.

The results were statistically analyzed using SPSS version 21.0 software (IBM, Armonk, NY, United States). The results were compared using the χ^2. $P < .05$ was accepted as statistically significant. The correlations were evaluated according to Spearman correlation.

RESULTS

In this study, a total of 1120 patients (643 were male [57.4%] and 477 were female [42.6%]) who applied to our laboratory before kidney transplantation were included. The mean ages of the patients and donors were 43.81 ± 13.634 and 44.63 ± 15.308, respectively. Of the patients, 72.9% (n = 816) and 27.1% (n = 304) were planned to be transplanted from deceased and related donors, respectively.

FCXM, CDCXM, and LM-XM tests were performed on 58.2% (n = 652), 91% (n = 1019), and 55.4% (n = 620) of 1120 patients, respectively. The coupled tests were performed on the same patients in order to analyze the associations between them (Table 1).

DISCUSSION

The determination of anti-HLA antibodies allows the most accurate selection of the desensitization protocol prior to kidney transplantation, and it is known that positive crossmatch before transplant is strongly associated with allograft rejection. CDCXM and FCXM are based on donor lymphocytes, whereas LM-XM utilizes bead technology. CDCXM can detect only complement-fixing donor specific antibodies, which may lead to false negatives. FCXM cannot distinguish anti-HLA antibodies from non-HLA antibodies, although it is more sensitive and specific than the CDCXM method. In addition, the prozone effect is the most common limitation of the LM-XM method, which is accepted as the most sensitive and specific of these 3 techniques [4].

In 2013, Ayna et al compared CDCXM and FCXM in 47 kidney patients with deceased donors and found a significant difference between the results [5]. In 2012, Huh et al investigated LM-XM (+) and CDCXM (-) patients after kidney transplantations [6] and concluded that LM-XM (+) patients might have a higher risk of acute cellular rejection. In our study, we observed that there were statistically significant differences between CDCXM/FCXM, CDCXM/LM-XM, and FCXM/LM-XM tests, similar to previous studies ($P < .0001$). However, the results were moderately correlated (for class I, r = .599, r = .693, and r = .507; for class II, r = .546, r = .471, and r = .495, respectively).

CONCLUSION

The crossmatch test protocol for pre-transplantation assessment may vary between HLA laboratories. However, the existing crossmatch techniques have certain limitations as well as advantages. At least 2 techniques can be used to overcome the limitations and to increase the rate of successful transplants.

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REFERENCES

[1] Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969;280:735–9.

[2] Graff RJ, Duffy B, Xiao H, Radell J, Lentine KL. The role of the crossmatch in kidney transplantation: past, present and future. J Nephrol Ther 2012;4:2.

[3] Konvalinka A, Tinckam K. Utility of HLA antibody testing in kidney transplantation. J Am Soc Nephrol 2015;26:1489–502.

[4] Gunawansa N, Rathore R, Sharma A, Halawa A. Crossmatch strategies in renal transplantation: a practical guide for the practicing clinician. J Transplant Surg 2017;1:8–15.

[5] Ayna TK, Soyöz M, Kurtulmuş Y, Doğan SM, Özyılmaz B, Tuğmen C, et al. Comparison of complement-dependent cytotoxic and flow-cytometry crossmatch results before cadaveric kidney transplantation. Transplant Proc 2013;45:878–80.

[6] Huh KH, Kim MS, Kim HJ, Joo DJ, Kim BS, Ju MK. Renal transplantation in sensitized recipients with positive Luminex and negative CDC (complement-dependent cytotoxicity) crossmatches. Transpl Int 2012;25:1131–7.