Austin Journal of Nephrology and Hypertension

Open Access

Austin Publishing Group

Research Article

A Case Study on Evaluation of the Correlation between Crossmatch Methods and Single Antigen Bead Test

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Received: August 24, 2015; **Accepted:** October 15, 2015; **Published:** October 20, 2015

Abstract

Introduction: The chance for transplantation from a crossmatch (XM) negative donor is low in hypersensitized patients. Single Antigen Bead (SAB) assay is one of the current tests that determine HLA antigens which may be accepted by the patients. In this case report, donor specific antibodies were investigated by flow cytometric SAB in the sera of two patients. Besides, the correlation between CDCXM (Complement-dependent cytotoxic crossmatch), FCXM (Flow cytometric crossmatch) and Luminex XM was also compared.

Methods and Materials: Flow cytometric SAB was performed by using sera from two hypersensitized male patients. Furthermore, the two sera were also tested by CDCXM, FCXM and Luminex XM tests using the cells from a healthy volunteer.

Results: CDCXM was found negative while FCXM and Luminex XM tests were positive for the first patient. All of the XM tests were found positive for the second patient. Besides, CDCXM and FCXM results were highly positive for the second patient with high MFI values in Luminex XM. As a result of flow cytometric SAB test, it was observed that acceptable HLA antigen number was lower in the patient with high MFI value.

Conclusion: SAB Assay determines the acceptable antigens that cannot be identified by the other XM techniques used for the determination of DSAs in immunology laboratories. This provides the patients advantages for graft survival and organ sharing. In addition, a second crossmatch test beside CDCXM test helps the clinical more for the identification of antibodies in low density.

Keywords: Flow cytometry-Single Antigen Bead (FC-SAB); Virtual crossmatch

Introduction

The presence of donor specific antibody (DSA) against human leukocyte antigens (HLAs) is the most significant reason of early kidney allograft rejection and graft failure. There are various techniques that can be used in determination of DSAs in graft waiting patient sera. Flow cytometric crossmatch (FCXM), can detect all of IgG types (IgG1, IgG2, IgG3, IgG4) besides the antibodies in low concentration that cannot be detected by CDC crossmatch (CDCXM). This allows FCXM be 10-100 times more sensitive than CDCXM test [1-3]. However, the specify of this test is lower, although it is more sensitive than CDCXM test. Luminex XM technology solid phase assay, can detect properly class I and II anti-HLA antibodies below the level that cannot be identified by CDCXM and FCXM. In this system, micro-beads covered by HLA antigens are used for the identification of anti-HLA antibodies in flow analyzer. Unlike FCXM, this system detects only anti-HLA antibodies (not non-HLA antibodies). Luminex technique has more sensitivity and specify than other cellular methods [4-6]. Acceptable and unacceptable HLA antigens can be detected by using the beads covered by only one recombinant HLA molecules (Single Antigen Bead: SAB). Thus, SAB method is also termed as virtual XM.

In this study, anti-HLA antibodies of two patients with >80% PRA result were detected by FC-SAB and the correlation of these results with three different crossmatch methods was investigated.

Materials and Methods

Two patients were accepted to our laboratory for their panel reactive antibody screening. The patients were 41 and 39 years-old, both of them were male and transplanted from a deceased donor in 2008 and 1999, respectively. However, the transplanted kidneys were explanted approximately 3 years after the transplantation. Besides transplantation, they had 2 and 5 unit blood transfusion after transplantation, respectively. The HLA types of deceased donors were not available from hospital registries. The tissue typings of the patients were performed by low-resolution PCR-SSO (Polymerase Chain Reaction-Sequence Specific Oligonucleotide) method (Lifecodes HLA Typing Kit Immucor Gamma, USA) and found as A*02 A*68 B*53 B*27 DRB1*11 DRB1*11, A*01 A*03 B*07 B*60 DRB1*10 DRB1*12, respectively.

The IgG antibodies produced against class I and II molecules were found 100% positive by Luminex PRA screening (LMX-Life Codes Life Screen Deluxe Kit, Immucor Gamma, USA) and identification (LM1, LM2Q- IDv2 Kit, Immucor Gamma, USA) tests during their last application to our laboratory.

Citation: Gulec D, Kılıcaslan Ayna T, Soyoz M, Kılıçoglu M and Pirim I. A Case Study on Evaluation of the Correlation between Crossmatch Methods and Single Antigen Bead Test. Austin J Nephrol Hypertens. 2015; 2(4): 1045.

Table 1: Acceptable HLA antigens for our patients according to SAB results.

Donor HLA type		A*02, A*23, B*14, B*51, DRB1*10,DRB1*11
First Patient HLA type		A*02 A*68 B*53 B*27 DRB1*11 DRB1*11
First Patient PRA Specific	**Class I / Class II**	**100% / 100% positive**
Acceptable Antigens of First Patients	**Class I**	A*02†, A*68, A*24, B*51†, B*13, B*44, B*38, B*57, B*53
	Class II	DRB1*11†, DRB1*08, DRB1*13:01, DRB1*13:03, DRB1*15:01, DRB1*15:02, DRB1*16, DRB1*03:01, DRB1*03:02, DRB1*01:02, DRB1*01, DRB3*02, DRB5*01, DQB1*04, DQB1*03:01, DQB1*03:02, DQB1*03:03
Second Patient HLA type		A*01 A*03 B* 07 B*60 DRB1*10 DRB1*12
Second Patient PRA Specific	**Class I / Class II**	**100% / 100% positive**
Acceptable Antigens of Second Patients	**Class I**	A*01, A*03, B*07, B*60
	Class II	DRB1*01:01, DRB1*01:03, DRB1*07, DRB1*10#, DRB1*12:01, DRB1*12:02, DRB1*16, DRB1*09, DRB1*15:02, DRB3*02, DRB5*01, DQB1*04, DQB1*06

†: Acceptable antigens of the first patient in terms of donor HLA type,
#: Acceptable antigens of the second patient in terms of donor HLA type

Table 2: CDC, Flow and Luminex DSA cross match test results of the patients.

	CDC XM Score		Flow XM Linear Channel Value Ratio		Luminex XM MFI	
	PBL Cell	**B Cell**	***T cell***	**B cell**	**Class I**	**Class II**
Patient 1	1 (0%)	1 (0%)	4.25	8.13	1029	1188
Patient 2	8 (100%)	8 (100%)	32.78	27.51	17741	2430

The patients' sera were tested by flow cytometry technique (FL1HD and FL2HD; One Lambda, Germany) in which beads that are covered by a single antigen are used in order to detect the most common HLA antigens and which allows virtual crossmatch. The results were evaluated by flow cytometry instrument (BD Facscalibur, USA). The acceptable HLA antigens were shown in Table 1.

The two patients with 100% PRA were crossmatched with a volunteer donor, who was 22 years-old, female and has A*02 A*23 B*14 B*51 DRB1*10DRB1*11 HLA type, by three different methods. It was determined that both of the patients had only 1DR compatibility with the donor (DRB1*10 and DRB1*11, respectively).

First crossmatch technique was Terasaki microlymphocytotoxicity technique (CDCXM) [7]. The first patient was PBL and B cell negative, while the second patient had high positive reaction (Table 2).

Second crossmatch test method was flow cytometric crossmatch. The difference between fluorescence values were evaluated by dividing patient median fluorescence value by Negative control median fluorescence value. The ratios that are 1.46 or more were accepted as positive in our laboratory. Both of the patients were T and B cell positive but second patient's results were higher than the first patient's results (Table 2).

The presence of DSA was investigated by Luminex crossmatch test (Lifecodes, Immucor Gamma, USA). Class I and II crossmatch MFI (Mean Fluorescence Intensity) values of the second patient was also higher than the first patient (Table 2) as in the FCXM.

Discussion

The chance of hypersensitized patients for finding a crossmatch negative donor is very low. Crossmatching of these patients with various donors will lead to pecuniary loss and intangible damages due to increased cost, labor and time consume in the laboratory. In various studies, it was aimed to determine acceptable donors previously to prevent this situation and SAB method was developed. There are some studies on correlation of SAB results and allograft survival and its ability to use in kidney share [8]. In various prospective and retrospective studies, it was revealed that the risk of early rejection was low and long-term allograft survival was good with SAB results [9].

First patient was found positive by FCXM, while he was found negative by CDCXM. More than 1000 MFI values in Luminex crossmatch test (class I: 1029, class II: 1188) were accepted as positive. However, the second patient was found positive by three methods (8 score by CDCXM, linear channel values more than 1.46 by FCXM and than 2000 [class I: 17741, class II: 2430] MFI values by Luminex methods). As a result, the patient with higher MFI value was found positive by CDCXM and FCXM tests, while the patient with less MFI value was found negative by CDCXM.

When the SAB results and MFI values were compared, it was observed that the patient with high MFI value had less acceptable HLA antigens (Table 2).

As it was shown in our patients, the low level of class I and II anti-HLA antibodies cannot be detected by CDCXM. Only IgG1 and IgG3 type antibodies that are complement-dependent can be detected by this method. Besides, the target lymphocytes in CDCXM test express the other molecules on their surfaces and these molecules can also lead to antibody reactivity. Non-HLA and auto antibodies, immune complexes and immunoglobulin allo-types can interfere the test [10]. It can detect all of the cellular targeted antibodies that are complement-dependent or not and in low density. However, Fc receptors and various adhesion molecules that are expressed on B lymphocytes surfaces can increase non-specific bindings [11,12]. As a result, false positive B cell crossmatch result can be observed. Solid phase tests like Luminex technology have significantly higher sensitivity and specify according to cellular methods [13-15]. The antibodies at low levels can be detected high accurately by this method.

In recent years, the studies performed with SAB techniques has come into prominence for the immunological assessment of kidney transplantations. It can support crossmatch tests by development of these techniques and increase of usage in clinic and DSAs can also be determined more precisely with SAB studies.

However, the studies should be performed punctiliously during antigen denaturation and integration on the beads since HLA antigens on cell surface bind to polypropylene beads.

Conclusion

Antibodies specify that cannot be detected by crossmatch and anti-HLA antibodies that cannot be determined by PRA tests can be determined by SAB method. We consider that SAB studies, which support crossmatch tests of hypersensitive patients and enlighten clinic will be able to be commonly used in transplantation immunology laboratories in the future.

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Austin J Nephrol Hypertens - Volume 2 Issue 4 - 2015
ISSN : 2381-8964 | www.austinpublishinggroup.com

Citation: Gulec D, Kılıcaslan Ayna T, Soyoz M, Kılıçoglu M and Pirim I. A Case Study on Evaluation of the Correlation between Crossmatch Methods and Single Antigen Bead Test. Austin J Nephrol Hypertens. 2015; 2(4): 1045.