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Comparative analysis of two methods to detect donor-specific anti-HLA antibodies after kidney transplant

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ABSTRACT

Preformed anti-human leukocyte antigen (HLA) antibodies may be present in the blood of kidney transplant candidates. The production of these antibodies may occur in the post-transplant period, with the possible development of donor-specific antibodies (DSA). Luminex-based tests, such as the single antigen (SA) assay and the Luminex crossmatch (Xm-DSA) assay are the most commonly used tools to detect anti-HLA antibodies, due to their high sensitivity and specificity. This cross-sectional study aimed to compare the findings of two methods for the detection of DSAs after kidney transplant: SA and Xm-DSA. A total of 122 patients who underwent deceased donor kidney transplant at Hospital de Clínicas de Porto Alegre were included. The SA assay detected anti-class I HLA DSAs in 17 patients (13.9%) and anti-class II HLA DSAs in 22 patients (19.6%), whereas the Xm-DSA detected DSAs in 18 patients (14.8%) both against class I and class II antigens. There was agreement between the two methods for class I (kappa = 0.66, $p = 0.001$) and class II (kappa = 0.54, $p = 0.025$) antigens. The incidence of DSAs as obtained by the SA assay was 15.57%, and the most prevalent DSAs were those against HLA-DR antigens. Patient survival at 3 years was 92%. The two techniques assessed in this study provide important information on the presence of DSAs and may help in the post-transplant patient monitoring and in immunosuppressive strategy.

1. Introduction

Sensitization to human leukocyte antigen (HLA) may occur from previous blood transfusions, pregnancies, or transplantations. For this reason, the immunological status of kidney transplant candidates must be periodically assessed in order to monitor the production and titration of anti-HLA antibodies. The production of these antibodies may occur prior to or after kidney transplant, often promoting the development of donor-specific antibodies (DSA) [1,2].

Solid-phase assays have been introduced to detect anti-HLA antibodies, such as Luminex-based assays, which use synthetic microsphere beads coated with HLA antigens or with anti-HLA antibodies [3–7] and have high sensitivity and specificity [1,3,6,8–10]. Anti-HLA antibodies are detected by the LABScreen single antigen (SA) assay, in which each bead is coated with a single HLA specificity, allowing for the precise detection of anti-HLA antibodies and of their levels in recipient serum and for a virtual crossmatch to predict reactions against donor HLA antigens [8,10]. Conversely, Luminex crossmatch (Xm-DSA) allows for a real crossmatch through the preparation of a lysate containing donor HLA molecules and beads coated with anti-HLA class I and II antibodies and that will bound to antibodies from recipient serum [10].

These techniques to detect DSAs provide important immunological information, but further studies are needed to establish the best conduct and to perform the clinical evaluation of the results of these techniques [1,3,4,9,11].

Thus, this study aimed to compare the findings of these two

Abbreviations: HLA, human leukocyte antigen; DSA, donor specific antibodies; SA, single antigen; Xm-DSA, luminex crossmatch; CDC, complement dependent cytotoxicity; MFI, mean fluorescence intensity; PCR-SSO, polymerase chain reaction -sequence specific oligonucleotide; PCR-SSP, polymerase chain reaction - sequence specific primer; PRA, panel reactive antibody; FCXM, flow cytometric crossmatch

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Table 1
Pre-transplantation characteristics of recipients.

	(*n* = 122)
Age*	48.39 ± 11.44
Gender**	
Male	67 (54.9)
Female	55 (45.1)
Ethnicity**	
Caucasian	103 (84.4)
Other	19 (15.5)
Blood group (ABO)**	
A	61 (50.0)
B	12 (9.8)
AB	2 (1.6)
O	47 (38.5)
Kidney disease**	
Indeterminate cause	26 (21.3)
Systemic arterial hypertension	25 (20.5)
Polycystic kidneys	21 (17.2)
Diabetes mellitus	19 (15.6)
Glomerulopathies	5 (4.1)
Different nephropathies	4 (3.3)
Other[a]	22 (18.0)
Number of transfusions*	2.09 ± 3.25
Number of pregnancies*	1.22 ± 1.83
Prior transplants*	0.14 ± 0.37
Number of incompatibilities*	
A	1.28 ± 0.67
B	1.19 ± 0.67
DR	0.51 ± 0.58
DQ	0.70 ± 0.70
ABDR	2.97 ± 1.14
ABDRDQ	3.11 ± 1.11
Time on the waiting list (in months)***	18 (6–37)
Pre-transplant PRA**	
PRA = 0	42 (34.4)
PRA ≥ 1	80 (65.6)

* Mean ± standard deviation.
** n(%).
*** Median (25th–75th percentile).
[a] 1 nephrotic syndrome, 5 not reported, 16 other causes.

methods for the detection of DSAs after kidney transplant and evaluated the results in patient survival.

2. Material and methods

2.1. Patients

This is a cross-sectional study including 122 patients who underwent a deceased donor kidney transplant from March 2011 to February 2013 at Hospital de Clínicas de Porto Alegre, Porto Alegre, Southern Brazil. Serum samples were collected in the sixth month after transplantation and tested for the presence of DSA using SA and Xm-DSA assays. Pre-transplantation characteristics of recipients are shown in Table 1.

At the time of transplant, all patients had crossmatch by Complement-dependent cytotoxicity (CDC) T-cell negative and four patients had a positive B-cell.

All patients were regularly followed after transplantation for 3 years.

2.2. Single antigen assay

All serum samples were tested by the SA assay for the detection of anti-class I (LSA1 – HLA-A, B and C) and class II (LSA2 – HLA-DR, DQ and DP) HLA IgG antibodies, according to the manufacturer's instructions (LABScreen®, One Lambda Inc., Canoga Park, CA). In this assay, each bead was coated with a single purified HLA molecule and initially incubated with recipient serum. Subsequently, the second antibody (goat anti-human IgG conjugated to phycoerythrin [PE]) was added. The Labscan 100 flow analyzer (Luminex) was used for data acquisition and the HLA Fusion software 2.0 (One Lambda) for analysis. Result was positive for the presence of antibodies when mean fluorescence intensity (MFI) was > 500.

2.3. XM-DSA assay

The Xm-DSA assay was performed according to manufacturer's protocol (Tepnel Lifecodes Corporation, Stamford, CT).

Firstly, donor cells were isolated from peripheral blood, spleen, lymph nodes, or other tissues and dissolved with a non-ionic detergent (lymphocyte lysis buffer) that lyses the cells and solubilizes HLA molecules (lysate). The assay was performed on a filter plate (Millipore, Bedford, MA). The lysate was incubated with a mixture of beads conjugated with monoclonal antibodies specific for a constant region of class I or class II HLA molecules that capture the solubilized HLA. After the second antibody (goat anti-human IgG conjugated to PE) was added. Subsequently, data were obtained by a Labscan 100 flow analyzer. Data analysis was performed on the Quicktype for LifeMatch 2.5 software (Tepnel Lifecodes Corporation) to obtain MFI values. To determine positivity, the mean of the three negative controls was subtracted from MFI obtained from test serum. A MFI value higher than 1000 was considered positive for anti-class I and II HLA antibodies, according to manufacturer's protocol.

2.4. HLA Typing

Recipients were previously typed for HLA by polymerase chain reaction sequence-specific oligonucleotide (PCR-SSO) using the Labtype kit (One Lambda Inc., Canoga Park, CA).

Deceased donors were previously typed for HLA-A, B, DRβ1 and DQβ1 loci by single specific primer-polymerase chain reaction (PCR-SSP) using an in-house kit developed in our laboratory [12].

2.5. DATA ANALYSIS

Statistical analyses were conducted using the chi-square, Mann-Whitney, Fisher's exact tests. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 21.0. The significance level was set at $p < 0.05$. The prevalence-adjusted bias-adjusted kappa (Pabak) statistic was used for analysis of agreement between SA and Xm-DSA assays using the WinPepi software [13]. Patients survival curves were visualized using Kaplan-Meier method and log-rank test to comparison between DSA presence/absence detected by SA and Xm-DSA.

2.6. ETHICAL ASPECTS

This study was approved by the research ethics committee of Hospital de Clínicas de Porto Alegre (project no. 110026), and all patients signed an informed consent form to participate in the study.

3. Results

3.1. PATIENT DATA

A total of 122 patients were analyzed in the sixth month after deceased donor kidney transplant, most of which were male (54.9%) and Caucasian (84.4%), with a mean age of 48.39 years. The most prevalent blood group was A (50.0%). Mean time on the waiting list was 18 months. Most patients (65.6%) was sensitized to HLA (positive panel reactive antibody (PRA) by SA) before transplantation, though only 26 (21.3%) patients had DSAs detected, 11 (9.0%) had anti- class I HLA DSAs, 13 (10.6%) had anti-class II HLA DSAs and 2 (1.6%) had anti-class I and II HLA DSAs. Some patients had previous sensitization