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Detection of donor-specific-antibodies by solid phase assay and its relevance to complement-dependent-lymphocytotoxicity cross-matching in kidney transplantation

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ABSTRACT

Presensitization against a broad array of HLA is associated with prolonged waiting times and inferior kidney allogaft survival. Although the use of solid phase assay (SPA) for the detection and characterization of anti-HLA antibodies provides greater sensitivity than complement-dependent lymphocytotoxicity (CDC) assay, it often detects donor specific antibodies (DSA) which turn out to be clinically irrelevant. Our data reinforce the concept that these two types of assays should be used in parallel for pre-and post-transplantation monitoring of anti-HLA antibodies in recipients of solid organ allografts.

1. Introduction

Establishment of reliable parameters for predicting the risk for antibody-mediated rejection (AMR) is essential for selecting appropriate strategies for successful transplantation of pre-sensitized patients.

The major task of HLA laboratories in support of solid organ transplantation is to select for each transplant candidate a donor carrying HLA antigens against which the patient does not display preformed "donor-specific antibodies" (DSA).

The identification of anti-HLA antibody specificity is currently performed by measuring in solid phase assay (SPA), i.e. Luminex, the binding of antibodies contained by the recipient's serum to single antigen coated beads. The intensity of the reaction is reflected in the Mean Fluorescence Intensity (MFI). However, not all the antibodies detected by SPA may be clinically relevant as emphasized by us and other authors [1–13]. It is becoming increasingly clear that the highest level of sensitivity does not translate into the highest clinical relevance. Virtual cross-matches based the level of antibodies that would be considered unacceptable for transplantation may have a negative impact on the patient's ability to obtain a graft [3,5]. On the other hand, the virtual cross-match provides the opportunity of expanding the geographical area from which organs can be recovered within the limits of an acceptable cold ischemia time, reducing waiting time and death on the waiting list [3,5,9].

The allocation and sharing of donor organs among transplant centers remains a highly controversial issue. Some authors unconditionally support the superiority of SPA and believe Complement Dependent Cytotoxicity (CDC) testing obsolete and replaceable by flow cytometry (FC). Other authors contend that CDC has the highest clinical relevance and that SPA should be used as a complimentary technique for characterization of antibody specificity [10,12–20].

In our institution, patients' sera are screened for both cytotoxic and potentially non-cytotoxic antibodies and cross-matching of recipient sera with donor lymphocytes is routinely performed by CDC and flow cytometry.

The aims of this study were: (1) to establish whether the presence of DSA detected by SPA (i.e. the virtual cross-match) correlates with the results of the CDC and flow cross-match and (2) determine whether patients with DSA and negative CDC can be safely transplanted.

Since the MFI of DSA as a cutoff for positive crossmatch is currently selected on an empirically determined basis by each laboratory, we performed a careful analysis of its relevance to

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the outcome of the CDC cross-match and ultimately of the transplant.

2. Subject and methods

2.1. HLA typing

Recipients and donors were typed by PCR-SSP for HLA-A, -B, -C, -DR, and -DQ using reagents from One Lambda (Canoga Park, CA).

2.2. Screening of anti-HLA antibodies

Sera were obtained from each transplant candidate at monthly intervals and screened for lymphocytotoxic antibodies against HLA-A, -B, -C, -DR, and -DQ antigens on a reference panel of magnetically sorted T and B lymphocytes from 70 unrelated donors. The frequency of PRA and their HLA class I and/or class II specificity was determined by tail analysis [2,3,5–8].

To discriminate between anti-HLA and non-HLA immunoglobulin (Ig)-G, quarterly collected sera were tested by LABScreen mixed microbeads coated with purified HLA-A, -B, -C, -DR, and -DQ antigens. All sera with anti-HLA antibodies were further tested on beads coated with single HLA class I (LABScreen single antigen class I LS1 A04) and class II (LS2 A01) antigens (One Lambda), which identify SPA-S I and II, respectively. SPA were performed using a Luminex 100 LS fluoroanalyzer (Luminex, Inc., Austin, TX). Based on validation results obtained for each lot of reagents by testing sera from 20 healthy males, the MFI cutoff between negative and positive was set at 1000.

2.3. Donor cross-matching techniques

Two samples of sera with high (>10%) PRA, which had been obtained within 6 months prior to transplantation and one "current serum" collected at the time of transplantation, were cross-matched in serial doubling dilutions with magnetically sorted T and B lymphocytes from the donor. The sera were also tested for reactivity with donor and autologous T and B lymphocytes by FCXM, as described by Karpinski et al. [4]. The samples were tested within 48 h by SPA-MIX and SPA-SAB I and SPA-SAB II and the presence of DSA was considered indicative of a positive "virtual cross-match". All procedures and the interpretation of results were conducted as described before [3,5].

2.4. Diagnosis of AMR

Renal allograft biopsies were performed and processed according to established protocols. According to the Banff classification, a diagnosis of AMR requires three features: (1) morphologic evidence of acute tissue injury (e.g., acute tubular injury, neutrophils and/or mononuclear infiltrates in peritubular capillaries and/or glomeruli, and/or capillary thrombosis; intimal arteritis/fibrinoid necrosis/intramural or transmural inflammation in arteries); (2) immunopathologic staining of C4d in peritubular capillaries; and (3) documentation of DSA. Because the aim of this study was to determine the diagnostic specificity of different DSA techniques, DSA was not an a priori criterion for the diagnosis of AMR. However, all cases had morphologic tissue injury and C4d staining in peritubular capillaries (i.e., were "suspicious for AMR" by Banff criteria) [3,5].

2.5. Immunosuppressive therapy

Patients received induction therapy with 6 mg/kg antithymocyte globulin (Thymoglobulin, Genzyme Transplant, Cambridge, MA), tacrolimus (0.1 mg/kg/day), mycophenolate mofetil (2 g/day), and corticosteroids (methylprednisolone) at a dose of 500 mg intraoperatively tapered to steroid withdrawal on postoperative day 5. For treatment of AMR, plasmapheresis (1 volume exchange reconstituted with albumin) was performed on an every-other-day basis either until CDC-detectable DSA were eliminated or until clinical improvement was obtained, as determined by return to baseline serum creatinine level or urine output increase. Standard intravenous immunoglobulin or Cytogam (100 mg/kg) (CMV hyperimmune-IVIg; MedImmune, Inc., Gaithersburg, MD) was administered after each plasmapheresis treatment. Additionally, methylprednisolone boli (500 mg qd × 3) and subsequent taper were also administered upon the diagnosis of AMR. Finally, all patients received tacrolimus and either mycophenolate mofetil or mycophenolic acid immunosuppression. Tacrolimus levels were maintained at a target range of 15–20 ng/ml during the treatment of AMR. Transplants were rebiopsied after treatment as necessary.

2.6. Statistical analysis

Actuarial survival was estimated using the Kaplan–Meier method, with p values calculated by log-rank statistics [21]. Categorical variables were compared with Fisher's exact test or the χ^2 test [3] and [22]. All statistical analyses were performed with SPSS software, version 14.0 (SPSS, Inc., Chicago, IL).

3. Results

3.1. Relationship between DSA and CDC cross-match results

Within the period spanning 01/01/2012–12/31/12 lymphocytes from a total of 398 deceased donors were cross-matched by our laboratory. For each donor sera from an average of 6.24 patients were selected for direct cross-matching by CDC amounting to a total of 2485 cross-matched patients. According to the virtual cross-match results 870 out of 2485 patients had DSA and as such should have been excluded as potential candidates. However, 342/870 (39.3%) of these DSA positive population showed no cytotoxic sera to the putative donor reflecting the non-complement fixing nature of the antibodies detected by SPA and/or their low affinity for their target antigen. These 342 patients would have been denied the

Table 1
Relationship between donor-specific antibodies (DSA) and complement-dependent cytotoxicity crossmatch results.

	Complement-dependent cytotoxicity crossmatch	
	Positive (n = 528)	Negative (n = 1957)
Patients with DSA	528	342
Patients without DSA	0	1615

p-Value < 0.0001.

Table 2
Mean Fluorescence Intensity (MFI) of SPA-detected DSA and complement-dependent cytotoxicity crossmatch results.

Mean Fluorescence Intensity (MFI)	Complement-dependent cytotoxicity crossmatch	
	Positive (n = 528)	Negative (n = 1957)
10,000 < MFI	429	99
6000 ≤ MFI ≤ 10,000	55	99
1000 ≤ MFI < 6000	44	144
No DSA	0	1615

p-Value < 0.0001.