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FEATURED PAPERS

Optimizing transplantation of sensitized heart candidates using 4 antibody detection assays to prioritize the assignment of unacceptable antigens

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BACKGROUND: The virtual crossmatch relies on the assignment of unacceptable antigens (UAs) to identify compatible donors. The purpose of our study was to identify an algorithm for assignment of UAs such that a negative complement-dependent cytotoxicity (CDC) crossmatch and concomitant negative or weakly positive flow cytometric crossmatch (FXM) are obtained.

METHODS: We used 4 antibody methods: (1) Luminex single antigen (LSA), (2) LSA with a 1:8 serum dilution, (3) C1q LSA, and (4) CDC panel. The UAs were prioritized in the following order: (1) all C1q+/CDC+, (2) LSA 1:8 >7,500 median fluorescence intensity, and (3) LSA >10,000 median fluorescence intensity.

RESULTS: Of 295 heart transplants that were performed at our center, 69 (23%) recipients had detectable human leukocyte antigen specific antibody at the time of transplant. All donor specific antibodies (DSAs) were avoided for 44 of 69 (64%) (DSA−). There were 25 recipients who had DSA at the time of transplant: 12 (48%) had negative FXM (DSA+/FXM−), and 13 (52%) had positive T-cell and/or B-cell FXM (DSA+/FXM+). Lower freedom from antibody-mediated rejection was observed for the DSA+/FXM+ group compared with the DSA− group ($p < 0.0001$). DSA remained detectable after transplant in the sera of 14 recipients, and de novo DSA was detected in 32 recipients. Freedom from antibody-mediated rejection was comparable for both groups ($p = 0.53$) but was lower than the DSA− group ($p < 0.0001$). Survival was comparable for all groups at 1,200 days post-transplant.

CONCLUSIONS: Strategic prioritization of UA assignment has allowed transplantation of highly sensitized patients across the DSA barrier with survival rates comparable to DSA− heart transplant recipients.

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The assignment of unacceptable antigens (UAs) has been applied for decades, initially based on using lymphocytes from panels of donors to identify antigens that would yield a positive complement-dependent cytotoxicity (CDC) crossmatch.[1,2] Advancements in human leukocyte antigen (HLA) typing by DNA-based methods and antibody analysis by

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solid-phase multiplex technologies have allowed for a more precise definition of HLA typing of the donor antigens and of the breadth and strength of the HLA specific antibodies in the patient's serum.[3,4] By correlating results obtained from donor specific antibody (DSA) analysis and cell-based crossmatches[5–7] with graft outcome, clinically relevant antibodies can be defined for each transplant program.[8–11] Using this information, UAs can be defined with greater precision to prevent organ offers from donors with antigens that would be expected to result in an unacceptable strongly positive flow cytometric crossmatch (FXM) or CDC crossmatch.[12] When this approach is used in lieu of an actual cell-based crossmatch, it is called a virtual crossmatch.[5,8,13–16]

The Organ Procurement and Transplantation Network administered by the United Network for Organ Sharing implemented a calculated panel-reactive antibody (CPRA) based on UA assignment and their frequency in the donor population to provide a more accountable measure of sensitization and to assign priority points for kidney organ allocation. Although not used for allocation of thoracic organs, the CPRA is used to assess the probability of an incompatible donor in the donor pool for thoracic organ candidates. Transplantation of broadly sensitized heart candidates is difficult because of the need for short ischemia time, not allowing for routine prospective crossmatches. The utility of the virtual crossmatch approach allows for the identification of compatible donors for sensitized thoracic organ candidates within an expanded donor pool area,[6] decreasing time and death on the waitlist. Critical to the interpretation of the virtual crossmatch is the ability of the program to correlate the results obtained by the solid-phase assays with the corresponding cell-based crossmatch results and assign a level of immunologic risk.[5,10,11,16]

We report our approach to assign UAs based on prioritization to avoid the strongest binding antibodies likely to result in a positive CDC crossmatch. Our aims were to optimize the transplantation of sensitized heart candidates by the assignment of UAs to allow for a negative CDC crossmatch and concomitant negative or weakly positive FXM to be achieved. Our program's established clinical experience and immunosuppression protocols enable transplantation across a weakly positive donor HLA specific antibody.[17–19] The outcome results we present demonstrate the utility and feasibility of our approach.

Methods

Study population

Between May 1, 2010, and September 3, 2013, 295 heart transplants were performed at our center. The demographic characteristics of the transplanted recipients for HLA antibody-positive (HLA-Ab+) and HLA antibody-negative (HLA-Ab−) recipient groups are listed in Table 1.

Solid-phase antibody analysis

The binding level of HLA specific antibodies was determined by class I or II single antigen bead assay performed on the Luminex platform according to the manufacturer's instructions (One Lambda, Inc., Canoga Park, CA). Final HLA specific antibody assignments and binding levels were determined through HLA Fusion 3.2 software programs (One Lambda, Inc.) using a median fluorescence intensity (MFI) of 2,500 as the cutoff. The patient's serum was tested without dilution and with a 1:8 dilution. C1q testing was performed with the single antigen beads and the C1qScreen kits (One Lambda, Inc.) using purified human C1q and

Table 1 Demographic Characteristics of Transplant Recipients for HLA-Ab+ and HLA-Ab− Groups

Variable	Overall ($N = 295$)	HLA-Ab+ ($n = 69$)	HLA-Ab− ($n = 226$)	p-value
Age, years	55.5 ± 13.0	51.9 ± 12.9	56.6 ± 12.8	0.009
Donor age, years	34.7 ± 13.1	34.5 ± 12.6	34.7 ± 13.3	0.88
Female, *n* (%)	98 (33.2)	42 (60.8)	56 (24.8)	<0.0001
BMI	25.12 ± 4.50	25.24 ± 4.82	25.09 ± 4.41	0.81
CMV mismatch, *n* (%)	50 (18.8)	9/62 (14.5)	41/204 (20.1)	0.36
Ischemic time, minutes	168.4 ± 63.4	174.1 ± 68.0	166.7 ± 62.0	0.40
MCS, *n* (%)	75 (25.4)	18 (26.1)	57 (25.2)	0.88
Type of MCS, *n* (%)				0.95
LVAD	45 (15.3)	11 (15.9)	34 (15.0)	
BiVAD	27 (9.2)	7 (10.1)	20 (8.8)	
TAH	3 (1.0)	0 (0.0)	3 (1.3)	
None	220 (74.6)	51 (73.9)	169 (74.8)	
Peak PRA Pre-Tx	19.1 ± 31.3	61.1 ± 30.9	6.3 ± 16.9	<0.0001
HTN, *n* (%)	128 (48.5)	32/61 (52.5)	96/203 (47.3)	0.56
DM, *n* (%)	79 (26.8)	22 (31.9)	57 (25.2)	0.28
Serum creatinine	1.31 ± 0.77	1.33 ± 0.76	1.31 ± 0.78	0.83
Induction, *n* (%)	109 (37.2)	50/69 (72.5)	59/224 (26.3)	<0.0001
Plasmapheresis, *n* (%)	15 (5.1)	12 (17.4)	3 (1.3)	<0.0001

BiVAD, biventricular assist device; BMI, body mass index; CMV, cytomegalovirus; DM, diabetes mellitus; HLA-Ab, human leukocyte antigen antibody; HTN, hypertension; LVAD, left ventricular assist device; MCS, mechanical circulatory support; PRA, panel-reactive antibody; Pre-Tx, pre-transplant; TAH, total artificial heart.

Numerical variables (age, donor age, BMI, ischemic time, peak PRA, and creatinine) are reported as mean ± SD.