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Clinically-relevant threshold of preformed donor-specific anti-HLA antibodies in kidney transplantation

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ABSTRACT

Background: Pretransplant anti-HLA donor-specific antibodies (DSA) are recognized as a risk factor for acute antibody-mediated rejection (AMR) in kidney transplantation. The predictive value of C4d-fixing capability by DSA or of IgG DSA subclasses for acute AMR in the pretransplant setting has been recently studied. In addition DSA strength assessed by mean fluorescence intensity (MFI) may improve risk stratification. We aimed to analyze the relevance of preformed DSA and of DSA MFI values.
Methods: 280 consecutive patients with negative complement-dependent cytotoxicity crossmatches received a kidney transplant between 01/2008 and 03/2014. Sera were screened for the presence of DSA with a solid-phase assays on a Luminex flow analyzer, and the results were correlated with biopsy-proven acute AMR in the first year and survival.
Results: Pretransplant anti-HLA antibodies were present in 72 patients (25.7%) and 24 (8.6%) had DSA. There were 46 (16.4%) acute rejection episodes, 32 (11.4%) being cellular and 14 (5.0%) AMR. The incidence of acute AMR was higher in patients with pretransplant DSA (41.7%) than in those without (1.6%) ($p < 0.001$). The median cumulative MFI (cMFI) of the group DSA+/AMR+ was 5680 vs 2208 in DSA+/AMR− ($p = 0.058$). With univariate logistic regression a threshold value of 5280 cMFI was predictive for acute AMR. DSA cMFI's ability to predict AMR was also explored by ROC analysis. AUC was 0.728 and the best threshold was a cMFI of 4340. Importantly pretransplant DSA > 5280 cMFI had a detrimental effect on 5-year graft survival.
Conclusions: Preformed DSA cMFI values were clinically-relevant for the prediction of acute AMR and graft survival in kidney transplantation. A threshold of 4300–5300 cMFI was a significant outcome predictor.

1. Introduction

The presence of pretransplant donor-specific antibodies (DSA) is recognized to be a risk factor for antibody-mediated rejection (AMR) and worse kidney allograft survival, even with a negative complement-dependent cytotoxicity (CDC) cross-match [1,2]. Since highly sensitive analytical technologies based on solid phase assays (SPA) have been routinely used in the transplant immunology laboratory, anti-HLA antibody detection has become much more sensitive than cell-based crossmatches but their precise clinical relevance (MFI, number, IgG subclass, complement fixing ability) remains to be established [2–8]. Over recent years, studies to identify which characteristics of pretransplant DSA (classes, strength, IgG-subclasses, capacity to activate complement, etc.) were predictive of adverse outcomes have been performed in order to improve our understanding of immunological risk stratification. As previously shown, not all pretransplant DSA, especially those at low levels, have a negative impact on graft function or acute

Abbreviations: AIC, Akaike's information coefficient; AMR, antibody-mediated rejection; CDC, complement dependent cytotoxicity; cMFI, cumulative mean fluorescence intensity; CsA, cyclosporine; DSA, donor-specific antibodies; FCXM, flow cytometric crossmatch; HLA, human leukocyte antigen; ivIg, intravenous immunoglobulin; MMF, mycophenolate mofetil; PRA, panel reactive antibody; SPA, solid phase assay; Tx, transplantation.

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rejection [9,10]. However, the amount of DSA, as measured by mean fluorescence intensity (MFI) with the Luminex, could play a role [4]. The analysis of IgG subclasses and the ability to activate complement may be important for identifying high-risk pretransplantation DSA in kidney and lung transplantation, although this remains controversial [10–15]. A recent study has suggested that the amount of pre-transplant DSA, as measured by MFI, may predict post-transplant AMR and kidney allograft survival [16]. Furthermore, recent data from our own program have indicated that cumulative MFI values above 5000 are highly predictive of a positive flow cytometric crossmatch (FCXM) [17]. Interestingly, a recent editorial discussed the issue and significance of clinically-relevant DSA in organ transplantation [18]. In the current study, we analyzed whether the strength of pre-transplant DSA, as measured by cumulative MFI (cMFI), may correlate with the incidence of acute AMR and allograft survival.

2. Materials and methods

2.1. Patients

All 280 consecutive adult patients undergoing kidney transplantation at our Transplantation Center at the CHUV in Lausanne, between January 2008 and March 2014, were analyzed. No patient was excluded from the analysis. Baseline patient characteristics are shown in Table 1. All patients had a negative pretransplant T- and B-cell CDC crossmatch on current and peak sera. Data regarding pretransplant FCXM were available in 59% of cases (n = 166) and pretransplant FCXM were positive in 10 patients (6.0% of patients). Patient data were obtained by analyzing the clinical records and electronic databases of the CHUV Transplantation Center. All patients were regularly followed in the outpatient clinic from the time of transplantation until death, allograft failure (defined as the need of chronic renal replacement therapy), up to April 2015. Allograft survival was analyzed after censoring for death with a functioning graft.

Table 1
Patients characteristics.

	All	DSA negative	DSA positive	p-value (DSA+ vs DSA−)
Patients, n (%)	280	256 (91.4)	24 (8.6)	
Male, %	66.8	69.1	41.7	0.011
Age, mean ± SD	54.6 ± 15.8	54.5 ± 16.0	55.4 ± 14.5	0.788
Kidney disease, %				
Diabetes	8.6	8.6	8.3	1.00
Hypertension	11.4	12.5	0.0	–
Glomerulonephritis	31.1	30.5	37.5	0.494
Genetic diseases	19.6	18.7	29.2	0.279
Other	29.3	29.7	25.0	0.815
Living donor, %	49.3	51.6	25.0	0.018
HLA mismatches, %				
0	4.6	4.7	4.2	1.00
1	2.1	2.3	0.0	–
2	9.3	9.0	12.5	0.476
3	18.2	18.0	20.8	0.782
4	27.8	26.6	29.2	0.811
5	25.0	25.0	25.0	1.00
6	13.9	14.4	8.3	0.548
HLA DQ mismatches, n				
0	11	10	1	1.00
1	22	21	1	0.705
2	14	10	4	0.023
Cytotoxic PRA I at tx, %				
0%	91.1	93.0	70.8	0.002
<10%	4.6	3.9	12.5	0.089
10–49%	3.6	2.3	16.7	0.006
50–79%	0.7	0.8	0.0	–
⩾80%	0.0	0.0	0.0	–
Ac anti-HLA, %				
No	74.3	81.2	0.0	–
Class I	6.8	5.9	16.7	0.067
Class II	6.8	6.2	12.5	0.215
Class I + II	12.1	6.6	70.8	<0.001

DSA. = donor-specific antibody; HLA. = human leucocyte antigen; PRA. = panel reactive antibody; SD. = standard deviation; tx. = transplantation.

2.2. Detection of anti-HLA antibodies and definition of DSA

All pretransplant sera were tested for the presence of anti-HLA antibodies using the multiplex technology SPA. Anti-HLA class I (*i.e.*, HLA-A/B/Cw) and anti-HLA class II (*i.e.*, HLA-DR/DP/DQ) antibodies were tested using LabScreen LS1A04 Lot 007/008 and LS2A01 Lot 008/009/010 (OneLambda, Canoga Park, CA) commercialized in Switzerland by InGen. Briefly, 20 µl of serum samples were incubated with HLA class I–coated and HLA class II–coated microspheres, respectively, for 30 min in the dark under gentle agitation. The specimens were then washed five times before being incubated with anti-human phycoerythrine-conjugated IgG in the same conditions as in the first incubation. The Labscan 100 flow analyzer (Luminex, Austin, TX) was used for beads and data acquisition. Data were then exported to HLA Visual software (One Lambda) for analysis. The cut-off level was defined as a baseline normalized 500 mean fluorescence intensity units (MFI). The presence of DSA was assigned by comparing the various HLA specificities proposed by the software analysis with the HLA typing of the donor for all transplanted patients. The analysis was carried out on the last available pre-transplant serum. DSA were considered positive if MFI was superior to 500. If several DSAs were present, we calculated the cMFI by adding together the MFI of each single antibody.

2.3. Donor HLA typing and virtual crossmatch

Between 2008 and 2011, HLA typing was done without DQ specificity. A retrospective HLA-DQ typing was realized using donor's DNA in cases of anti-HLA DQ antibody production in the recipient, to look for the presence of DSA. Since 2012, all donors were typed for class I and class II HLA molecules using PCR-SSP technology (Olerup SSP HLA-A-B-DR-DQ Combi-tray, Stockholm, Sweden) distributed in Switzerland by Milan Analytica AG or PCR-SSO technology (LabType SSO, One Lambda Canoga Park, CA, USA distributed in Switzerland by InGen). No detection of DSA against HLA-Cw and HLA-DP could be performed.

2.4. Complement-dependent cytotoxicity crossmatch

Prospective donor T- and B-cell crossmatches were performed at the time of transplantation using the classical CDC-technique. Patient sera were incubated with donor's T and B cells, respectively for 20 and 30 min. The cells were then incubated and stained with rabbit complement sera mixed with acridine orange/ethidium bromide for 35 min (T cells) and 45 min (B cells), and observed for cytotoxicity in a fluorescence microscope. The CDC crossmatch results were considered positive when cell death exceeded 20%.

2.5. Induction protocol and maintenance immunosuppression

Induction therapy was used for all patients. For low-risk patients, it consisted of an anti-IL-2 receptor monoclonal antibody (Basiliximab Novartis®, 20 mg twice, at day 0 and 4). For high-risk patients (defined by the presence of high levels of lymphocytotoxic antibodies (PRA > 50%), previous transplantations or delayed graft