LETTER TO THE EDITORS

Anti-HLA donor-specific antibodies are not created equally. Don't forget the flow...

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Dear Editors,

The development of single antigen bead (SAB) assays has greatly improved our ability to detect anti-HLA donor-specific antibodies (DSA). However, with the recognition that not all DSA detected by the SAB assay are detrimental, a major challenge in kidney transplantation is to reliably identify those that are associated with poor graft outcome. Currently, standard guidelines for the interpretation of the SAB results are lacking [1], and some transplant physicians may feel uncertain in decision-making when adopting a "Luminex-only"-based approach to assess risk of preformed DSA [2]. In this letter, we discuss the clinical benefit of additionally performing a prospective flow cytometry crossmatch (FCXM) in conjunction with the SAB assays for improved pretransplant risk stratification.

We analyzed 175 HLA-sensitized kidney transplant recipients transplanted with a negative T-cell complement-dependent cytotoxicity crossmatch. Among them, 93 were transplanted with preformed DSA. SAB identification at baseline was performed routinely (after 2009) or retrospectively (before 2009). The positivity threshold for the beads' mean fluorescence intensity (MFI) was set at 500 [3]. FCXM was performed on donor T and B lymphocytes, looking for IgG and IgM antibodies. An autologous crossmatch was systematically performed with the recipient's peripheral blood mononuclear cells to rule out autoreactive nonspecific antibodies. The threshold for positivity was set at a mean channel shift (MCS) deviation of 50 for T-cells and 100 for B-cells, above that for the negative control serum. Graft biopsies were performed "for cause" and graded according to the current Banff classification [4].

Based on previous studies [5] we retained three profiles of patients: 41 were DSA+FCXM+, 52 were DSA+FCXM−, and 82 were DSA− (Table 1). DSA-MFI-sum was higher for DSA+FCXM+ (5544 ± 541) than for DSA+FCXM− (2581 ± 491) (P=0.02). Kaplan–Meier estimates over 24 months indicated a higher incidence of acute rejection-AR (cellular and humoral) and antibody-mediated rejection-AMR in the DSA+FCXM+ group than in the DSA+FCXM− and DSA− groups (respectively 40%, 16%, and 7.5% for AR; and 27.5%, 8%, and 2.5% for AMR, $P < 0.0001$). Two-year graft survival was lower in the DSA+FCXM+ group [DSA+FCXM+: 85%, DSA+FCXM−: 94%, DSA−: 96%, $P = 0.01$]. In the multivariate analysis, DSA+FCXM+ was an independent factor for AR (OR = 3.2 [1.2–9.3], $P = 0.02$) and AMR (OR = 6.4 [1.94–22.6], $P = 0.002$), after adjustment for MFI-DSA, previous transplant, and induction treatment. The outcome of DSA− patients with a positive or a negative FCXM was not different (data not shown). The MFI-DSA was thus insufficient for risk stratification alone. Two main limitations may participate to these results: Firstly, the anti-denatured HLA antibodies were found to be unable to recognize native HLA on the cell surface [6]. Secondly, the activation of complement on bead surface by high titer antibodies can lead to an underestimation of the DSA. Consequently, we proposed an algorithm where solid-based tests identify DSA and cell-based FCXM test discriminates between more and less pathogenic SAB-DSA, even better than the DSA-MFI. Simultaneously, promising tools for evaluating DSA pathogenicity are under intense scrutiny (C1q- [7], C3d- [8], or C4d-binding [9] SAB assays). Hopefully, they will help to gain a deeper understanding in the future.

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Table 1. Baseline characteristics of the kidney transplant recipients.

	DSA+FCXM+ $N = 42$	DSA+FCXM− $N = 51$	DSA− $N = 82$	P
Sex (men/women, *n*)	20/22	25/26	47/35	0.5
Recipient age (mean ± SD, years)	48.8 ± 10.8	49.3 ± 12.3	50.9 ± 11.1	0.6
Donor age (mean ± SD, years)	45.5 ± 14.8	47.8 ± 14.7	47.8 ± 13.8	0.7
Previous transplant (mean ± SD)	2.0 ± 0.8	1.6 ± 0.6	1.6 ± 0.7	0.002
Delayed graft function* (*n*, %)	12 (29)	18 (35)	29 (35)	0.7
Immunological data				
HLA mismatch A-B-DR-DQ (mean ± SD)	4.6 ± 1.8	4.4 ± 1.8	4.2 ± 1.7	0.6
HLA specificities of immunodominant DSA (*n*, %)				0.04
HLA-A	4 (9.5)	15 (29)	0	
HLA-B	3 (7)	5 (10)	0	
HLA-C	11 (26)	12 (24)	0	
HLA-DR	4 (9.5)	4 (8)	0	
HLA-DQ	7 (17)	1 (2)	0	
HLA-DP	13 (31)	14 (27)	0	
MFI-DSA sum	3184 [540–27490]	1300 [518–17392]	0	<0.0001
FCXM-BL pos *n* (%)	28 (67)	0	13 (16)	
FCXM-TL pos *n* (%)	25 (60)	0	3 (4)	
FCXM-BL or TL pos *n* (%)	42 (100)	0	13 (16)	
Induction treatment				
ATG/Anti-IL2R	31/11	39/12	58/24	0.8
IVIg/No IVIg	35/7	33/18	14/68	<0.0001
RTX/No RTX	10/32	19/32	1/81	<0.0001
Immunosuppressive regimen				0.6
AZA+FK+CS	1	0	1	
MMF+CsA+CS	4	2	4	
MMF+FK+CS	37	49	76	
PSI+MMF+CS	0	0	1	

Anti-IL2R, anti-interleukin 2 receptor monoclonal antibody; r-ATG, rabbit anti-thymocyte globulin; AZA, azathioprine; CS, corticosteroids; CsA, cyclosporine; DSA, donor-specific antibodies; FCXM-TL pos, flow cytometry crossmatch for T lymphocytes positive if MCS > 50; FCXM-BL pos, flow cytometry crossmatch for B lymphocytes positive if MCS > 100 unless B cell autocrossmatch was positive; FK, tacrolimus; IVIg, intravenous immunoglobulins; MFI, mean fluorescence intensity; MMF, mycophenolate mofetil; PSI, proliferator-specific inhibitors; RTX, rituximab.

*As defined by the need of hemodialysis during the first week.

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