

# ARE ALL DONOR-SPECIFIC ANTIBODIES DETECTED BY SOLID PHASE ASSAY BEFORE TRANSPLANTATION CLINICALLY RELEVANT ?

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## BACKGROUND

Since new technologies based on solid phase assays (SPA) have been routinely used in the transplant immunology laboratory, the presence of pre-transplant donor-specific antibodies (DSA) against HLA antigens has been considered as a risk factor for acute rejection (AR). To investigate the clinical relevance of pre-transplant DSA we screened renal transplant recipients for circulating anti-HLA antibody and DSA before transplantation.

## PATIENTS

Patients (n=113) transplanted at the Centre Hospitalier Universitaire Vaudois in Lausanne between April 2003 and April 2007 were investigated. The demographic characteristics of the patient population are shown in Table 1. All transplanted patients had current and peak negative T-cell and B-cell CDC-crossmatch. No prospective flow-cytometric or Luminex crossmatches were performed. All retrospective data were obtained by carefully reviewing the patient case-history.

All sera were stocked at -80 °C and thawed just before analyses were performed.

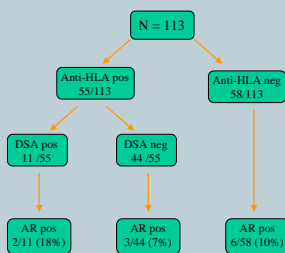


## METHOD

Sera were tested for anti-HLA antibodies using the multiplex technology SPA. Class I (i.e. HLA-A/B) and class II (i.e. HLA-DR/DP/DQ) HLA antibodies were tested using reagents from One Lambda, Inc., Canoga Park CA, (LabScreen LS1A01 Lot 007 and LS2A01 Lot 004, commercialized in Switzerland by Ingens). Briefly, 20 µl of serum samples were incubated with HLA class I and HLA class II coated microspheres respectively for 30 min at dark and under gently agitation. The specimens were then washed 5 times before being incubated with anti-human IgG-conjugated phyco-erythrin 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 the HLA Visual software (One Lambda) for analysis. The cut off level was defined as a baseline normalized > 500 MFI. Presence of DSA was assigned by comparing the several HLA-specificities proposed by the software analysis with the HLA typing of the donor for all transplanted patients. The Luminex results did not influence transplant management (retrospective study).

## RESULTS :

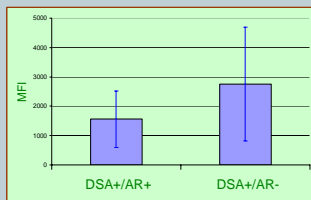
**Figure 1** Anti-HLA, DSA before transplantation and acute rejection



**Table 3** Anti-HLA antibodies and rejection

	All (n=113)	No rejection (n=102)	Acute rejection (n=11)	P-Level
anti-HLA positive	55 (48.6%)	50 (49%)	5 (45.5%)	ns
Class I anti-HLA	18 (16%)	15 (14.7%)	3 (27.3%)	ns
Class II anti-HLA	14 (12.3%)	13 (13.7%)	1 (9%)	ns
Class I/II anti-HLA	23 (20.3%)	22 (21.5%)	1 (9%)	ns
anti-HLA negative	58 (51.3%)	52 (51%)	6 (54.5%)	ns

**Figure 2** Mean DSA MFI between patients with or without AR



**Table 1** Patients characteristics

	All	DSA neg	DSA pos	P level
<b>Patients number</b>	113	102	11	
Male, n (%)	79 (70)	76 (74)	3 (27)	P < 0.005
Mean age at time of tx ± std	46.2 ± 15.1	46.6 ± 15.5	42.8 ± 10.2	ns
Age - median at tx [range]	49 [3-78]	50 [3-78]	39 [31-65]	
<b>Kidney disease n (%)</b>				
Diabetes	8 (7)	8 (8)	0	ns
Hypertension	9 (8)	9 (9)	0	ns
Glomerulonephritis	33 (29)	28 (27)	5 (45)	ns
Genetic disease	25 (22)	24 (24)	1 (10)	ns
Others	38 (34)	33 (32)	5 (45)	ns
<b>Sensitizing events n (%)</b>				
Prior tx	21 (19)	16 (16)	5 (45)	P < 0.01
Blood transfusions	63 (56)	56 (55)	7 (64)	ns
Pregnancies	20 (18)	13 (13)	7 (64)	P < 0.0001
Living donor (%)	45 (40)	41 (40)	4 (36)	ns
<b>HLA mismatches n (%)</b>				
0	9 (8)	9 (9)	0	ns
1	2 (2)	2 (2)	0	ns
2	12 (11)	11 (11)	1 (9)	ns
3	24 (21)	21 (20)	3 (27)	ns
4	35 (31)	32 (31)	3 (27)	ns
5	18 (16)	15 (15)	3 (27)	ns
6	13 (11)	12 (12)	1 (9)	ns
<b>HLA A/B mismatches n (%)</b>				
0	9 (8)	9 (9)	0	ns
1	7 (6)	7 (7)	0	ns
2	38 (34)	34 (33)	4 (36)	ns
3	34 (30)	30 (29)	4 (36)	ns
4	25 (22)	22 (21)	3 (27)	ns
<b>HLA DR mismatches n (%)</b>				
0	27 (24)	23 (22)	4 (36)	ns
1	51 (45)	49 (48)	2 (18)	P < 0.03
2	35 (31)	30 (29)	5 (45)	ns
<b>Current PRA I at tx, n (%)</b>				
0%	102 (90)	92 (90)	10 (91)	ns
< 10%	4 (3)	4 (4)	0	ns
10% - 49%	4 (3)	4 (4)	0	ns
50% - 79%	1 (1)	1 (1)	0	ns
> 80%	2 (2)	1 (1)	1 (9)	P < 0.03

**Table 2** Baseline immunosuppression, acute rejection and survival

	All	DSA neg	DSA pos	P level
<b>Patients number</b>	113	102	11	
<b>Induction</b>				
Basiliximab, n (%)	81 (72)	76 (75)	5 (45)	P < 0.03
Thymoglobulin, n (%)	25 (22)	19 (19)	6 (55)	P < 0.005
Basiliximab + Thymo n (%)	7 (6)	7 (6)	0	ns
<b>Immunosuppression</b>				
Tac, n (%)	108 (96)	97 (95)	11 (100)	ns
CsA, n (%)	5 (4)	5 (5)	0	ns
<b>Rejection</b>				
All acute rejection, n (%)	11 (9.7)	9 (8.8)	2 (18)	ns
Acute cellular rejection, n (%)	6 (5)	6 (6)	0	ns
Borderline, n (%)	2 (2)	1 (1)	1 (9)	ns
Ia, n (%)	4 (3)	5 (5)	0	ns
Acute humoral rejection, n (%)	1 (1)	0	1 (9)	P < 0.005
Clinically suspected	1 (1)	1 (1)	0	ns
Graft survival at 1 yr, n (%)	112 (99)	101 (99)	11 (100)	ns
Patient survival at 1 yr, n (%)	113 (100)	102 (100)	11 (100)	ns

- Baseline characteristics of the 113 patients (all, pre-transplant DSA negative or positive recipient) enrolled in the study are shown in Table 1.
- Pre-sensitizing events (prior transplants, pregnancies and female gender) were significantly more common in the group of patients with DSA than in the group without DSA (Table 1).
- Approximately half of our patient population (55/113, 48.7%) had pre-transplant circulating anti-HLA antibodies. (fig.1)
- 11 of 113 patients (9.7 %) had pre-transplant DSA (fig.1)
- Out of a total of 11 AR episodes post-transplant, 6 had no anti-HLA antibodies and 5 were anti-HLA positive, but only 2 had pre-transplant DSA, of whom one developed AHR (C4d positive) at day 7 post-transplantation (fig. 1)
- 9 from 11 recipients had pre-transplant DSA and did not present any post-transplant rejections episodes. (Table 2)
- There are no differences between patients with only class I or only class II pre-transplant DSA compared to patients with both or more in predicting outcome (Table 3)
- Pre-transplant mean DSA-MFI values of the two kidney transplant recipients with acute rejection were at 1557 ± 954 (patient 1: anti-DR11 (1004), and patient 2: anti-A26 (1008) and anti-A11 (2659)). On the other hand, the pre-transplant DSA-MFI values of the 9 kidney transplant recipients without any rejection episodes ranged from 694 up to 5717 (mean: 2758 ± 1938) (fig.2)

## CONCLUSIONS :

- Our data confirm the high sensitivity of solid phase assays (SPA) of the Luminex type to detect circulating DSA antibodies.
- The presence of pre-transplant anti-HLA or DSA antibody were correlated with pre-existing sensitizing events before transplantation, but not with a high risk of AR or worse clinical outcomes.
- The number, class and intensity of pre-transplant DSA as well as pre-sensitizing events could not predict post-transplant acute rejection episodes.
- No hyperacute rejection occurred and patient and graft survival were excellent in anti-HLA or DSA positive and negative groups
- One possible explanation of the low AR rates in the DSA positive group was the predominant use of a potent induction regimen with thymoglobulin, because of prior transplants or high PRA at transplantation.
- If we would have taken to consideration the presence of pre-transplant DSA as an absolute contra-indication to transplant, 10 % (11/113) of the patients would not have received a kidney transplant.
- Pre-transplant DSA (especially at low-levels) detected by SPA multiplex technology should not be used alone to discourage transplantation, as most patients with DSA had good outcomes.