ORIGINAL ARTICLE

Pre-existing donor-specific antibodies are detrimental to kidney allograft only when persistent after transplantation

Sophie Caillard[1], Camille Becmeur[1], Gabriela Gautier-Vargas[1], Jerome Olagne[1,2], Clotilde Muller[1], Noelle Cognard[1], Peggy Perrin[1], Laura Braun[1], Francoise Heibel[1], Francois Lefebre[3], Veronique Renner[4], Christian Gachet[4], Bruno Moulin[1] & Anne Parissiadis[4]

1 Department of Nephrology, Division of Renal Transplantation, University Hospital of Strasbourg, Strasbourg, France
2 Department of Nephropathology, University Hospital of Strasbourg, Strasbourg, France
3 Public Health Department, University Hospital of Strasbourg, Strasbourg, France
4 Histocompatibility Laboratory, UMR_S949 Inserm, Strasbourg, France

Correspondence
Sophie Caillard MD, PhD, Nephrology Department, Hôpitaux Universitaires de Strasbourg, 1, place de l'Hopital, 67091 Strasbourg, France.
Tel.: 0033369551320;
fax: 0033369551723;
e-mail: Sophie.caillard@chru-strasbourg.fr

SUMMARY

Donor-specific antibodies (DSA) increase the risk of allograft rejection and graft failure. They may be present before transplant or develop *de novo* after transplantation. Here, we studied the evolution of preformed DSA and their impact on graft outcome in kidney transplant recipients. Using the Luminex Single Antigen assay, we analyzed the sera on the day of transplantation of 239 patients who received a kidney transplant. Thirty-seven patients (15.5%) had pre-existing DSA detected the day of transplantation. After 5 years, the pre-existing DSA disappeared in 22 patients whereas they persisted in 12. Variables associated with DSA persistence were age <50 years ($P = 0.009$), a history of previous transplantation ($P = 0.039$), the presence of class II DSA ($P = 0.009$), an MFI of preformed DSA >3500 ($P < 0.001$), and the presence of two or more DSA ($P < 0.001$). DSA persistence was associated with a higher risk of graft loss and antibody-mediated rejection. Previously undetected preformed DSA are deleterious to graft survival only when they persist after transplantation.

Transplant International 2017; 30: 29–40

Key words
antibody-mediated rejection, donor-specific antibody, graft survival, kidney transplantation

Received: 31 January 2016; Revision requested: 21 March 2016; Accepted: 22 September 2016; EV Pub Online 17 October 2016

Introduction

It is widely recognized that anti-HLA antibodies, particularly donor-specific antibodies (DSA), increase the risk of allograft rejection and graft failure after kidney transplantation [1–3]. Both pre-existing and *de novo* DSA may have a negative impact on graft outcome. Recently, the detection of DSA has improved through the use of new technologies such as the Luminex assay, which is much more sensitive than complement-dependent cytotoxicity cross-match (CDCXM) and ELISA tests. The Luminex technology enables the detection of antibodies and DSA that cannot be identified by other techniques. Pre-existing DSA could be retrospectively identified using the Luminex assay in patients in whom CDCXM and ELISA failed to detect DSA [4,5]. In a study by Lefaucheur *et al.* [6], pre-existing DSA were identified *a posteriori* by ELISA and Luminex assays in kidney transplant recipients with negative IgG CDCXM. Patients with pre-existing DSA had an increased risk of acute humoral rejection and graft failure, and the higher the mean fluorescence intensity (MFI) in SA, the greater the risk. Nevertheless, the evolution of pre-existing DSA after transplantation was not described in the

doi:10.1111/tri.12864

Lefaucheur's study [6], nor in most of the published literature.

Therefore, we retrospectively investigated the sera from the day of transplantation in kidney transplant recipients using the Luminex assay to describe the evolution and the impact of undetected pre-existing DSA on graft outcome in patients who did not undergo antibody reduction therapy.

Patients and methods

Patients

We retrospectively included all patients who underwent kidney transplantation at the University Hospital of Strasbourg (France) between January 2005 and December 2007. The inclusion criteria were as follows: age >18 years, kidney or simultaneous pancreas kidney transplantation, living or deceased donor, and IgG- and IgM-negative CDCXM (current and historical sera). Patients transplanted with heart, lung or liver, graft failure due to a surgical cause, death in the first month, treatment with IVIg, plasmapheresis or rituximab on the day of transplantation were excluded. The following recipient-related variables were collected: age, sex, kidney disease, dialysis type and duration, HLA type, immunizing events, EBV and CMV status. The type, age, cause of death, HLA type, EBV and CMV status were determined in all donors. The collected parameters related to transplantation included cold ischemia time, immunosuppression protocol, and post-transplant blood transfusions or pregnancies.

Immunosuppression regimen

After transplantation, the immunosuppressive treatment consisted of induction therapy with either basiliximab (Simulect®; Novartis, Basel, Switzerland) or antithymocyte globulins (ATG, Thymoglobulins®; Genzyme, Lyon, France), calcineurin inhibitors (cyclosporine or tacrolimus), mycophenolate mofetil, and steroids. Steroids were initially given at a dose of 1 mg/kg/day and then progressively tapered off during the first four post-transplant months. In immunologically high-risk patients or those with a history of acute rejection, steroids were continued at a dose of 0.1 mg/kg/day. Target trough levels of tacrolimus were 10–12 ng/ml in the first 3 months, 8–10 ng/ml from 4 to 6 months, and 6–8 ng/ml thereafter. Target trough levels of cyclosporine were 150–200 ng/ml in the first 6 months, 125–150 ng/ml from 6 to 12 months, and 75–125 ng/ml thereafter. The target for MPA $AUC_{0-12\ h}$ at M3 was 30–60 h.mg/l. In cases of acute cellular rejection, steroid pulses were given for 3 days, followed by oral steroids at a dose of 1 mg/kg/day, in addition to a switch to tacrolimus in patients treated with cyclosporine.

Treatment of antibody-mediated rejection consisted in steroids pulse during three consecutive days, IVIg 2 g/kg total dose, three cycles every 3 weeks, and rituximab 375 mg/m^2 one or two injections (Roche, Basel, Switzerland). Plasmapheresis was performed in two patients. Of them, one patient had preformed DSA and lost its graft because of acute antibody-mediated rejection in the first post-transplant month. The other patient (with no pre-existing DSA) developed *de novo* DSA 2 years after transplantation. Treatment was adapted according to general status, comorbidities, and infectious risk of the recipient.

The study was approved by the Institutional Review Board, and all participants provided their written consent in accordance with the Declaration of Helsinki.

CDCXM and HLA typing

With regard to organ allocation, we considered as forbidden antigens all of the antigens with corresponding antibodies characterized by a MFI reactivity >1000 in SAB. Complement-dependent cytolysis cross-matching was performed using current and historical serum samples. The sera were initially incubated with donor's T and B spleen lymphocytes separated by magnetic beads tests (Dynabeads; Life Technologies, Villebond sur Yvette, France) for 30 min at +25 °C. Subsequently, we performed an additional 45-min incubation with rabbit complement (Cedarlane, Burlington, NC, USA) at +25 °C. All of the tests were performed either in the presence or absence of dithiothreitol (DTT) to distinguish between IgG and IgM reactivity. After lysis, the FluoroQuench dye (One Lambda Inc., Canoga Park, CA, USA) was used to differentiate live from lysed cells. When at least 50% of target cells were lysed, the test was defined as positive. All recipients and donors were molecularly typed for HLA-A*, HLA-B*, HLA-DRB1*, and HLA-DQB1* antigens at low resolution. Donors were further typed for other loci corresponding to recipient antibodies. Molecular typing was performed by reverse sequence-specific oligonucleotides (SSO) hybridization (LABtype SSO; One Lambda Inc.) or by PCR amplification with sequence-specific primers (SSP; Olerup SSP; West Chester, PA, USA).

Detection of anti-HLA antibodies and definition of DSA

Luminex antibody testing was performed on sera obtained at J0, M12, M24, M36, M48, and M60 after

transplantation. Antibodies to HLA-A, B, C, DRB1, DRB5, DRB3, DRB4, DQA1, DQB1, and DPB1 alleles were analyzed. The sera were tested for the presence of anti-HLA antibodies using Luminex LABScreen LS1A04 and LS2A01 (One Lambda Inc.). Samples were incubated 15 min at room temperature (+20–25 °C) and then centrifuged before testing in bead assay. Twenty microliters of sera was added to 5 µl of antigen beads and was incubated for 30 min in the dark under gentle agitation at room temperature. After a washing step (five times), samples were incubated with 100 µl of PE-conjugated goat anti-human IgG under the same conditions used for the first incubation. The Labscan 100 flow analyzer (Luminex, Austin, TX, USA) was used for data acquisition and analysis. Data were also analyzed using the HLA-FUSION software (One Lambda). To account for the intralaboratory variability, a quality control sample containing a known amount of antibodies was included in each series. The mean fluorescence intensity (MFI) values for each bead were computed, and the coefficients of variation (CV) were calculated. A CV < 25% was deemed acceptable. EDTA was added to serum to avoid the prozone effect. EDTA pretreatment was performed by the addition of 10 µL disodium EDTA solution (0.1 M, pH 7.5) every 90 µl of serum [7]. MFI positivity was defined as a normalized MFI > 500, a value in line with that found to be associated with a higher risk of AMR in previous studies [6,8]. In patients with several DSAs, the DSA with the highest MFI (immunodominant DSA: iDSA) was identified. The sum of DSA MFIs (sDSA) was calculated. The evolution of MFI of the DSAs was examined during a 5-year follow-up. Disappearance of DSAs was defined by a MFI < 500 at 5 years post-transplantation (or on the date of graft failure or patient's death if they occurred within the first 5 years after transplantation).

Renal pathology and kidney function

Kidney biopsies were performed based on clinical findings (increased serum creatinine, onset of proteinuria) or upon the first detection of DSA by Luminex, the only exception being patients in whom biopsy was contraindicated. All biopsy specimens were reclassified according to the Banff 2009 classification [9]. Creatininemia, proteinuria, and glomerular filtration rate measured by iohexol clearance at 1 and 5 years post-transplantation were recorded.

Statistical analysis

Descriptive statistics are presented as means and standard deviations or medians and ranges (as appropriate). Patients with pre-existing DSA were compared with patients without using Student's *t*-test for continuous variables and chi-square test for categorical variables. The analyses of (i) patient and graft survival, (ii) the cause-specific cumulative incidence of DSA clearance (taking into account the competing risk of graft loss or death), and (iii) biopsy-proven antibody-mediated (acute and chronic) rejection were performed using the Kaplan–Meier method (log-rank test). The date of event onset was defined as the first date of DSA clearance, documented rejection, graft loss, or patient death. Multivariate Cox regression analyses were performed to adjust for clinically relevant variables that may affect DSA clearance. Cox regression models with time-dependent DSA parameters (persistence or disappearance) were constructed to evaluate the effect of DSA clearance on graft survival and the occurrence of antibody-mediated rejection (with the exclusion from the latter analysis of patients with *de novo* DSA). Receiver operating characteristic (ROC) curves were plotted for MFI of pre-existing iDSA or sDSA and DSA persistence. Statistical analyses were performed using SPSS 11.5 (SPSS, Inc., Chicago, IL, USA). The competing risk models have been run with the CMPRSK R package, with R 3.3.1 (Vienna, Austria).

Results

Patients at the time of transplantation

A total of 260 patients underwent kidney or SPK transplantation during the study period. Twenty-one patients were excluded (i.e., eight patients who received another transplant organ; four patients with vascular thrombosis; three deaths occurring during the first post-transplant year; six patients who received plasmapheresis, IVIg, or rituximab due to a positive historical cross-match or FSGS recurrence prophylaxis). Finally, 239 patients were enrolled (Fig. 1). The characteristics of the donors and recipients are summarized in Table 1. A total of 136 patients (56.9%) had sensitizing events before the graft transplant: 92 received blood transfusions, 59 women had a pregnancy (65.5% of all women; mean of 3.2 pregnancies per woman [range: 1–13]), and 55 patients (23%) had undergone previous transplantations.

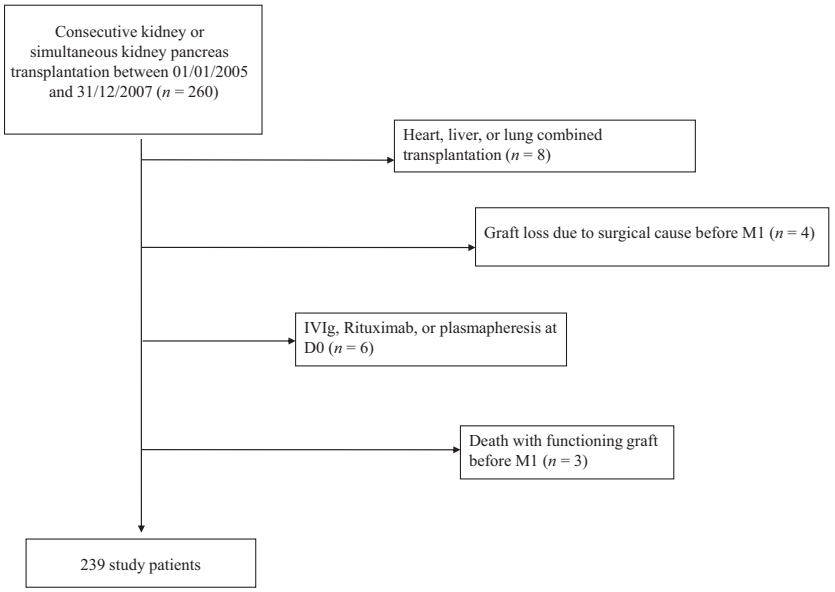

Figure 1 Patients flowchart.

Using the Luminex assay, we identified previously undetected DSA in D0 sera in 37 patients (15.5%), non-DSA anti-HLA antibodies in 46 patients (19.2%), and no HLA antibodies in 156 patients (65.3%). The two latter groups were combined to form the non-DSA group (n = 202). Patients with pre-existing DSA were slightly older than those without DSA (50 ± 9.6 vs. 47.2 ± 13.8 years, P = 0.18). Thirty-five patients had a sensitizing event before transplantation: 23 had blood transfusions, 13 women had pregnancies (4.2 per woman), and 24 had already undergone previous transplantation (11 graft removals). Patients with pre-existing DSA had more pre-transplant sensitizing events than those without (Table 1). Two male patients had no pre-transplant sensitizing events. Patients with preformed DSA received thymoglobulin and tacrolimus more frequently. Among the 37 patients with pre-existing DSA detected in D0 sera, 22 had class I DSA (A, B, C) (59.5%), eight had class II DSA (DR, DQ, DP) (21.6%), and seven had class I and class II DSA (18.9%). iDSA had class I and class II specificity in 23 and 14 patients, respectively. The median MFI of iDSA was 2205; 1378 for class I iDSA [range: 621–16 534] and 5279 for class II iDSA [range: 989–11 114]. DSA specificities and MFI for each patient are shown in Table 2.

Post-transplant events and DSA evolution

The mean post-transplantation follow-up time for the 239 study patients was 6.8 ± 2.2 years. During this period, 23 patients died and 47 patients lost their graft. The patient 5-year survival and graft survival rates were 93% and 82%, respectively. Five-year death-censored graft survival was 89%. Among the 37 patients with pre-existing DSA, we were able to follow DSA MFI after transplantation in 34 patients (two died and one returned to dialysis during the first year) (Table 2). iDSA disappeared in 22 cases (66%) and persisted in 12 cases (44%) during the 5 post-transplant years. The characteristics of patients with preformed DSA according to the evolution of DSA are described in Table 3. The majority of DSA (84%) disappeared during the first post-transplant year. ROC curve analysis showed that a MFI cutoff of 3500 was a good predictor of the post-transplant DSA evolution (Fig. 2).

Univariate analysis identified the following variables as significantly associated with DSA persistence (Fig. 3): age <50 years (P = 0.009), a history of previous transplantation (P = 0.039), the presence of class II DSA (P = 0.009), D0 iDSA and sDSA MFI >3500 (P < 0.001), and a number of DSA per patient ≥2 (P < 0.001). In multivariate analysis, a number of DSA per patient ≥2 (hazard ratio [HR] = 4.9, 95% confidence interval [CI] = 1.23–19.59, P = 0.025) and D0 iDSA MFI > 3500 (HR = 9.74, 95% CI = 1.50–63.13, P = 0.017) were independently associated with DSA persistence (Table 4). Among patients without DSA on the day of transplantation (n = 202), 32 (16%) developed *de novo* DSA in the 5 years post-transplant. In addition, five patients with pre-existing DSA developed *de novo* DSA. Six patients developed class I DSA, 25 developed class II DSA, and six developed both class I and II DSA. *De novo* DSA appeared during the first post-transplant year in 11 patients, the second year in

Table 1. Characteristics of the entire study cohort (n = 239) and comparison of patients with preformed DSA (n = 37) and patients without preformed DSA (n = 202). n (%) or mean (±SD).

	Entire cohort (n = 239)	Patients with preformed DSA (n = 37)	Patients without preformed DSA (n = 202)	P
Male sex	149 (62.3%)	22 (59.4%)	127 (62.8%)	0.69
Age at KT, years	48 (± 13)	50 (± 9.6)	47 (± 13.8)	0.18
SPK	6 (2.5%)	0 (0%)	6 (3%)	0.15
Preemptive KT	11 (4.6%)	0 (0%)	11 (5.4%)	0.05
Deceased donor	223 (93.3%)	37 (100%)	186 (92.1%)	0.02
Living donor	16 (6.7%)	0 (0%)	16 (7.9%)	
Kidney disease				
Diabetic	26 (10.9%)	1 (2.7%)	25 (12.4%)	0.19
Glomerular	87 (36.4%)	15 (40.5%)	72 (35.6%)	
Vascular	21 (8.8%)	1 (2.7%)	20 (9.9%)	
APKD	38 (15.9%)	7 (18.9%)	31 (15.3%)	
TICN	34 (14.2%)	6 (16.3%)	28 (13.9%)	
Other	33 (13.8%)	7 (18.9%)	26 (12.9%)	
Pretransplant events				
Blood transfusion	92 (38.5%)	23 (62.1%)	69 (34.1%)	0.004
Pregnancies	59 (65.5%)	13 (86.7%)	46 (61.3%)	0.04
Transplant	55 (23.0%)	24 (64.8%)	31 (15.3%)	<0.001
Graft removal	26 (47.3%)	11 (45.8%)	15 (48.4%)	0.85
HLA mismatch				
Class 1	2.6 (±1.1)	2.5 (±1.1)	2.6 (±1.1)	0.62
Class 2	1.1 (±0.8)	1.1 (±0.7)	1.1 (±0.8)	0.74
CMV status				
CMV D+/R+	102 (42.7%)	19 (51.4%)	83 (41.1%)	0.18
CMV D−/R−	42 (17.6%)	4 (10.8%)	38 (18.8%)	
CMV D−/R+	60 (25.1%)	6 (16.2%)	54 (26.7%)	
CMV D+/R−	35 (14.6%)	8 (21.6%)	27 (13.4%)	
EBV status				
EBV D+/R+	229 (95.8%)	36 (97.3%)	193 (95.5%)	0.51
EBV D−/R−	0 (0%)	0 (0%)	0 (0%)	
EBV D−/R+	6 (2.5%)	1 (2.7%)	5 (2. 5%)	
EBV D+/R−	4 (1. 7%)	0 (0%)	4 (2%)	
Induction therapy				
ATG	168 (70.3%)	34 (91.9%)	134 (66.3%)	0.001
Anti-RIL2	71 (29.7%)	3 (8.1%)	68 (33.7%)	
Maintenance therapy at discharge				
Tacrolimus	87 (36.4%)	28 (75.7%)	59 (29.2%)	<0.001
Cyclosporine	152 (63.6%)	9 (24.3%)	143 (70.8%)	
Anti-HLA Ab detected by ELISA at D0	72 (30.1%)	26 (70.3%)	46 (22.8%)	<0.001

KT, kidney transplantation; SPK, simultaneous kidney pancreas transplantation; APKD, autosomal dominant polycystic kidney disease; TICN, tubulointerstitial chronic nephropathy.

six patients, the third year in 10 patients, the fourth year in three patients, and the fifth year in seven patients. The mean delay of *de novo* DSA occurrence was 2.7 ± 1.5 years.

Clinical outcome according to DSA evolution

The 5-year patient survival rate was not different between patients with and without D0 DSA. The 5-year death-censored graft survival rate was lower in patients with preformed DSA compared with those without DSA, albeit not significantly so (P = 0.13, Fig. 4a). When we analyzed graft survival according to the evolution of preformed DSA after transplantation, the results of univariate analysis indicated that the presence of preformed DSA that subsequently persisted increased the risk of graft loss by 6.5-fold (P < 0.001) and the risk of death-censored graft loss by 5.7-fold (P < 0.001).

Table 2. Characteristics of the patients with preformed DSA (immunizing events and DSA specificities) and evolution of the DSA MFI at 5 years post-transplant.

	Sex	BT	Preg	KT	ELISA I	ELISA II	HLA I MM	HLA II MM	DSA specificity	MFI at D0	MFI at last FU
DSA that disappeared (n = 22)											
1	F	0	1	0	1	0	4	1	B8	1370	250
									A1	900	0
2	M	0		1	1	0	3	1	A11	1260	0
3	F	1	1	0	0	0	3	2	DR51	1750	500
									DR53	1110	150
4	M	1		1	1	1	2	1	B7	3330	400
									B35	1420	20
5	M	1		0	0	0	3	1	B18	800	0
6	M	1		1	0	0	1	1	DQ6	1000	0
7	M	0		1	0	0	2	2	B51	620	160
8	M	0		0	0	1	0	0	C*02	900	0
9	F	1	1	0	1	1	3	1	C*01	2390	140
10	F	1	1	0	0	0	4	2	DR15	1140	0
11	M	0		1	1	1	0	0	C*16	1000	160
12	M	0		1	1	0	4	1	DQA1*05:05	5460	0
13	M	1		0	0	0	4	1	A1	800	0
14	F	1	1	1	1	0	1	2	C*07	4700	0
15	M	1		1	1	0	3	1	B62	1140	0
16	M	1		1	1	1	3	0	B62	1510	300
17	M	0		1	0	1	2	2	DQ7	1580	60
18	F	1	1	0	1	0	4	0	B62	3470	420
19	M	1		1	1	1	2	2	B51	730	0
20*	F	1	1	0	0	0	3	2	B52	1000	0
21*	M	1		0	0	0	3	2	C*17	1250	0
22*	M	0		0	0	0	3	1	B44	1470	0
DSA that persisted (n = 12)											
23	F	1	0	1	1	0	2	1	B52	3870	770
24	F	1	1	1	1	1	3	1	DP5	5650	490
									A26	1240	1920
25	F	0	1	0	1	1	3	1	DQ8	5100	13 140
									B7	1280	260
									DQ4	1210	2480
									DR4	5100	470
									DR53	560	1410
26	M	0		1	1	1	2	0	C*03:03	1380	170
									B18	710	700
									B62	860	640
27	M	1		1	1	0	3	1	DQ5	11 410	8770
									A*02*01	1430	1130
									Cw7	8700	4260
28	M	1		1	1	1	1	0	A26	2210	660
29	F	1	1	1	1	1	1	0	C*02	16 530	990
									A31	800	0
30	M	1		1	0	1	3	2	DR53	10 500	1250
31	M	1		1	0	0	2	2	DQ6	11 110	660
									DQ8	7690	810
32	F	0	0	1	1	1	4	1	DPB1*04:01	4780	690
									B38	2510	1570
									A26	2570	500
33*	F	0	1	1	1	1	0	0	C*07	7520	7400
									C*16	5710	1720
34*	M	0		1	1	1	3	1	DRB1*14:54	6840	2380

BT, blood transfusions; preg, pregnancy; KT, previous kidney transplantation; MM, mismatch; MFI, mean fluorescence intensity; FU, follow-up.

*In patients 20, 21, 22, 33, and 34, *de novo* DSA developed after transplantation.

Transplant International 2017; 30: 29–40

Multivariate analysis revealed that having an ECD donor (HR = 1.86, 95% CI = 1.05–3.43, $P = 0.048$) and the persistence of preformed DSA (HR = 5.71, 95% CI = 3.27–9.97, $P < 0.001$) were independently associated with an increased risk of graft loss (Table 5).

During follow-up, 63 patients underwent an allograft biopsy for clinical reasons. Among them, 32 recipients developed isolated lesions of cellular rejection and 29 showed features of humoral rejection (18 with acute and 11 with chronic lesions). In the latter group, 14 patients showed mixed rejection. The presence of previously undetected preformed DSA was associated with a higher risk of antibody-mediated rejection ($P < 0.001$, Fig. 4b). Moreover, antibody-mediated rejection (both acute and chronic) occurred more frequently in patients with long-lasting DSA (HR = 3.31; 95% CI = 0.88–12.5, $P = 0.07$).

There was no statistical difference between the groups with respect to creatininemia and glomerular filtration rate at 1 and 5 years post-transplantation; however, patients with D0 DSA tended to have lower GFR and higher proteinuria at one and 5 years post-transplantation (Table 6).

Table 3. Characteristics of patients with preformed DSA that disappeared ($n = 22$) and preformed DSA that persisted ($n = 12$). Data are given as n (%) or mean ± SD.

	Preformed DSA that disappeared $n = 22$	Preformed DSA that persisted $n = 12$
Male sex	15 (68.2%)	6 (50%)
Age at transplantation, years	53 ± 8	44 ± 7
HLA mismatches		
Class 1	2.6 ± 1.2	2.2 ± 1.1
Class 2	1.2 ± 0.7	0.8 ± 0.7
Induction		
ATG	20 (91%)	11 (92%)
Anti-IL2R	2 (9%)	1 (8%)
IS therapy		
Tacrolimus	15 (68%)	10 (83%)
Cyclosporine	7 (32%)	2 (17%)
Pretransplant events		
Blood transfusion	13 (59%)	7 (58%)
Pregnancies	7 (100%)	4 (67%)
Transplantation	11 (50%)	11 (92%)
Graft removal	6 (27%)	4 (33%)
More than 1 pretransplant sensitizing event	11 (50%)	8 (67%)
D0 anti-HLA Ab detected by ELISA (non-DSA)	13 (59%)	11 (92%)
DSA class		
Class I	17 (77%)	5 (42%)
Class II	5 (23%)	3 (25%)
Class I and II	0	4 (33%)
iDSA MFI	1757 ± 1317	7241 ± 4407
sDSA MFI	1912 ± 1442	10 605 ± 6388
Nb of DSA specificities	1.1 ± 0.3	2.1 ± 1.2

iDSA, immunodominant DSA; sDSA, sum of MFI of all specificities of DSA; MFI, mean fluorescence intensity.

Discussion

Here, we have shown that certain DSA can be detected *a posteriori* in the serum on the day of transplantation using the Luminex assay in transplant patients with negative B and T IgG and IgM CDCXM. Importantly, our findings indicate that 66% of all preformed DSA disappeared after transplantation without hampering the graft. The better sensitivity and specificity of the Luminex assay compared with ELISA or lymphocytotoxicity assay have been well-documented and correlate with a better prediction of acute rejection and risk of graft loss [8]. In our study, one-third of the patients had a negative ELISA test for class I and II antibodies. Lee and coworkers [10] performed a retrospective analysis of 48 samples and detected the presence of DSA in eight patients by ELISA and 27 patients by the Luminex assay. Nevertheless, the improved sensitivity of the Luminex technique could lead to false-positive results or the detection of DSA that are not deleterious for graft survival, ultimately limiting the transplantation access. Among the 239 patients included in our study, 37 (15.5%) had DSA in the serum on the day of transplantation, but the clinicians were unaware of the information. This prevalence was close to that described in the Lefaucheur's cohort in 2010 [6], in which 83 of 402 kidney recipients (20.6%) had preformed DSA detected by the Luminex technique. This percentage was lower than that in the Otten's series, in which 35% of patients had preformed DSA [11]. These higher rates could be explained by the use of historical sera in the Lefaucheur's and Otten's series, whereas we only studied sera on the day of transplantation. In our study, most of the patients with preformed DSA were sensitized. Two patients did not have any known sensitizing events. We speculate that such patients were carrying natural antibodies as previously described in nonalloimmunized healthy males [12]. In our series, the majority of preformed DSA were class I DSA; moreover, class I DSA

	Area under the curve	*P*	Sensitivity for a MFI of 1500 (%)	Specificity for a MFI of 1500 (%)	Specificity for a MFI of 3500 (%)	Specificity for a MFI of 3500 (%)	Specificity for a MFI of 5500 (%)	Specificity for a MFI of 5500 (%)
iDSA	0.932	<0.001	92	60	83	91	58	100
sDSA	0.947	<0.001	100	68	83	86	75	100

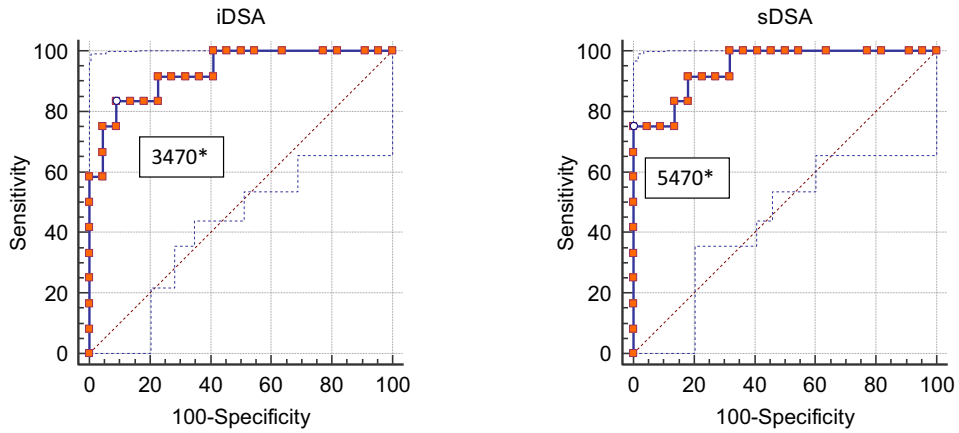

Figure 2 Receiver operating characteristic curves of the risk of persistence of the preformed DSA at 5 years according to the MFI of preformed DSA (immunodominant DSA = iDSA MFI and sum of MFI of all DSA = sDSA). *Youden point.

had a lower MFI than class II DSA. The predominance of preformed class I antibodies was previously described by Ling, in which 251 patients of 1069 kidney transplant patients had preformed DSA detected by Luminex, with the majority of them being class I [13]. In the Lefaucheur's cohort, 32% of the patients had class I preformed DSA, 13% had class II preformed DSA, and 55% had class I and II preformed DSA [14]. In contrast to our findings, the MFI of class I preformed DSA was higher than the MFI of class II DSA in these two series, whereas in the Crespo's series, the maximum MFI was higher for class II than for class I DSA [15].

In our study, we followed the evolution of preformed DSA annually until a minimum of 5 years post-transplantation. No patients received specific antibody reduction therapy at the time of transplantation, which allowed us to describe the evolution of preformed DSA under standard sequential quadruple immunosuppression. We showed that the majority of preformed DSA disappeared, most of them during the first post-transplant year (84%). In a recent study, Kimball *et al.* [16] demonstrated that 65% of patients who were DSA and FCXM positive on the day of transplantation had their DSA cleared during the first post-transplant year. However, the monitoring of preformed DSA was stopped at 1 year, whereas in our present investigation, the sera were analyzed for 5 years post-transplant. Moreover, the occurrence of *de novo* DSA was not taken into account [16]. In our study, the variables associated with DSA persistence were age <50 years, a history of previous transplantation, and DSA characteristics (e.g., class II, higher MFI, and DSA numbers). These findings indicate that the robustness of sensitization is likely to influence the persistence of preformed DSA. We were also able to estimate a high predictive threshold for MFI persistence of 3500. Specificity does not seem to have a major impact on DSA evolution even though our sample size is too small to draw firm conclusions. One hypothesis that may explain the disappearance of DSA is a process of accommodation, even though its mechanisms remain unclear [17]. Thirty-seven patients (15%) developed *de novo* DSA 5 years after transplantation in our cohort. Based on the published literature, the rates of DSA detection between 1- and 5-year post-transplant vary from 5% to 35% (depending on the center and the immunological risk) [18–25].

In this study, the presence of DSA on the day of transplantation and their detection by the Luminex assay was associated with an increased risk of graft loss at 5 years only when they persisted after transplantation. Conversely, patients with pre-existing DSA that disappeared after transplantation had nearly the same prognosis as patients without DSA. This is in accordance with the Kimball's series [16]. Otten *et al.* [11]

Transplant International 2017; 30: 29–40

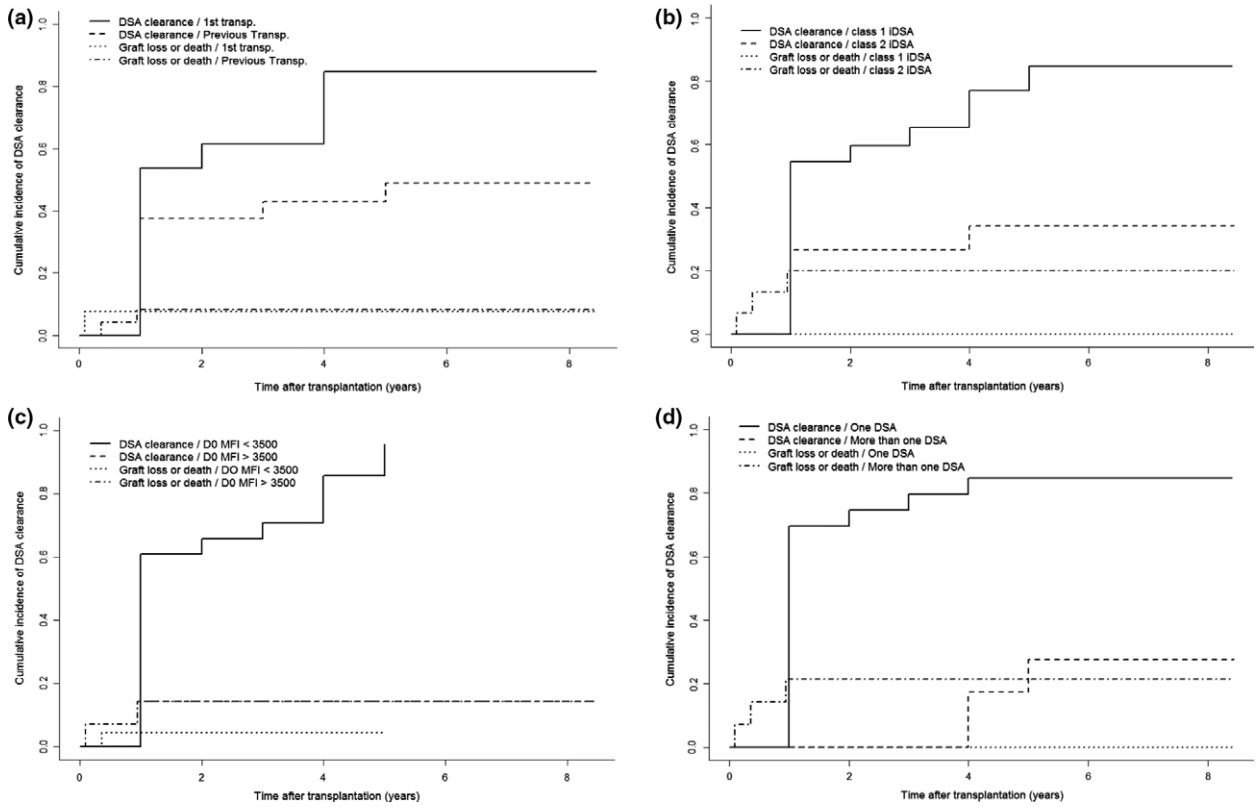

Figure 3 (a) Cause-specific cumulative incidence of DSA clearance according the history of previous transplantation. Group 1: patients without previous transplantation (n = 12); group 2: patients with previous transplantation (n = 22); P = 0.039. (b) Cause-specific cumulative incidence of DSA clearance according to the iDSA class. Group 1: patients with class I iDSA (n = 22); group 2: patients with class II iDSA (n = 12); P = 0.009. (c) Cause-specific cumulative incidence of DSA clearance according to the MFI of sDSA at D0. Group 1: patients with preformed sDSA MFI < 3500 (n = 22); group 2: patients with preformed sDSA MFI > 3500 (n = 12); P < 0.001. (d) Cause-specific cumulative incidence of DSA clearance according to the number of DSA per patient. Group 1: patients with one preformed DSA (n = 23); group 2: patients with more than one preformed DSA (n = 11); P < 0.001.

Table 4. Multivariate analysis of variables associated with post-transplant DSA persistence.

	P value	HR	95% CI
Female sex	0.911	0.945	0.347–2.5
Age at transplantation <50 years	0.645	1.286	0.44–3.75
Preformed iDSA MFI >3500	0.017	9.738	1.50–63.13
Preformed class II iDSA	0.60	0.731	0.23–2.35
Presence of a pretransplant sensitizing event	0.919	1.074	0.27–4.23
Presence of more than one preformed DSA	0.025	4.90	1.23–19.6

HR, hazard ratio; 95% CI, 95% confidence interval.

described a deleterious effect of preformed DSA only when DSA were class I and II. Patients with class I DSA showed a long-term graft survival comparable to that of patients with non-DSA HLA antibodies. Lefaucheur *et al.* [14] and Wu *et al.* [25] correlated the impact of preformed DSA with MFI level before transplantation. Lefaucheur reported that pre-existing DSA were associated with an increased risk of graft loss when MFI level was higher than 3000 [14]. Nevertheless, the authors did not analyze the evolution of the preformed DSA after transplantation. In our cohort, patients with class I DSA, one specificity, and a MFI < 3500 the day of transplantation had a good prognosis, a result which may be explained by the fact that DSA disappeared rapidly after transplantation. In such a case, HLA-incompatible kidneys could be accepted and the graft could be performed without specific reduction in antibody therapy.

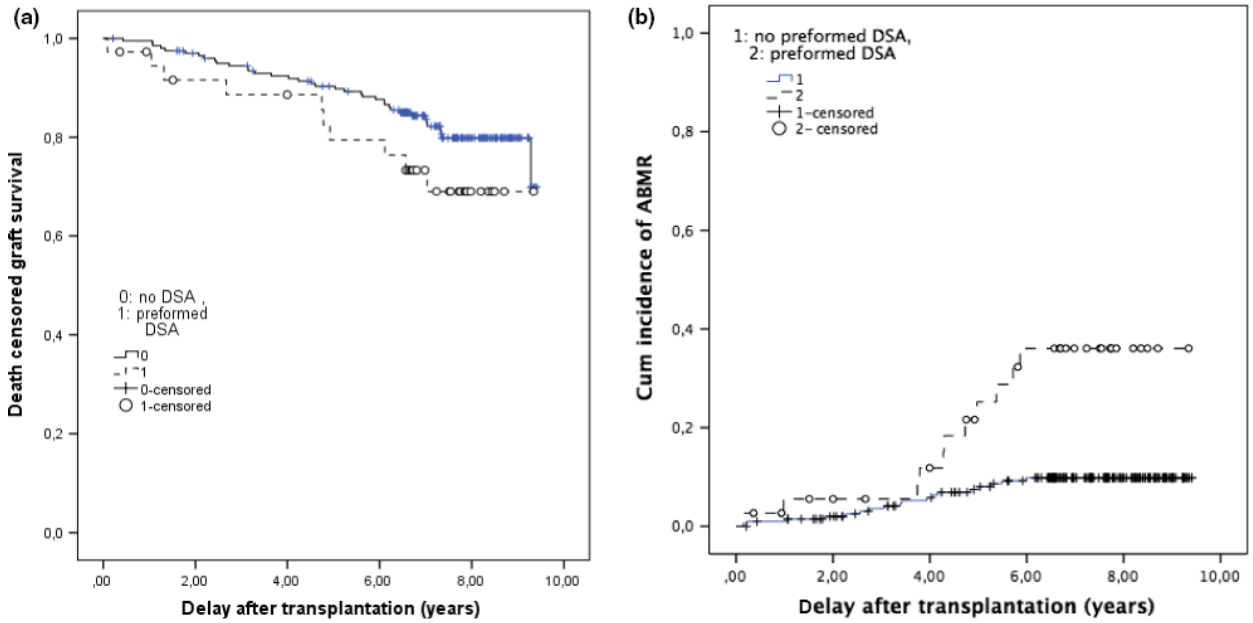

Figure 4 (a) Death-censored graft survival according to the presence of preformed DSA at D0. Group 1: patients without DSA at the time of transplantation (n = 202), group 2: patients with previously undetected preformed DSA (n = 37); P = 0.132. (b) Cumulative incidence of antibody-mediated rejection according to presence of previously undetected DSA at D0. Group 1: patients without previously undetected DSA (n = 202); group 2: patients with previously undetected DSA (n = 37), $P < 0.001$.

	P value	HR	95% CI
Female sex	0.88	0.958	0.54–1.69
Age at transplantation <50 years	0.98	0.99	0.57–1.75
ECD donor	0.048	1.86	1.05–3.43
Donor origin (living vs. deceased)	0.90	0.92	0.27–3.13
History of a pretransplant sensitizing event	0.64	0.87	0.49–1.55
DSA persistence*	<0.001	5.71	3.27–9.97

HR, adjusted hazard ratio; 95% CI, 95% confidence interval; ECD, expanded criteria donor; DSA, donor-specific antibody.

*As a time-dependent variable.

Table 5. Multivariate analysis of risk factors for graft loss according to the evolution of DSA after transplantation and the transplant characteristics.

In our series, 29 patients who underwent biopsy showed lesions suggestive of acute or chronic ABMR. Huang and colleagues studied the impact of preformed or *de novo* DSA on protocol biopsies at M3, M6, and M12 [26]. In this series, 14% of 150 patients had preformed DSA and 7.8% developed *de novo* DSA. The incidence of biopsy-proven rejection was 34% at 1 year in patients without DSA, 48% in those with preformed DSA, and 70% in patients with *de novo* DSA [26]. In a study by Loupy [8], protocol biopsies were performed at M3 in 54 patients with preformed DSA. The presence of subclinical humoral lesions increased the risk of chronic active humoral rejection and lower GFR at 1 year. Although we could have underestimated the frequency of rejection due to the absence of protocol biopsies, our long follow-up enabled the evaluation of the role of preformed DSA in the development of chronic ABMR after the first post-transplant year. Finally, no significant differences were found in graft function at 1 and 5 years post-transplant between patients with and without DSA. Nevertheless, we identified a higher rate of proteinuria at 5 years in patients with persistent DSA, indicating the existence of glomerular lesions and suggesting less favorable outcomes.

Some caveats of our study merit comment. First, it is a retrospective and single-center study. We only analyzed sera on the day of transplantation and not historical sera. Although we used a low MFI cutoff to define DSA positivity, this was in line with two published studies [6,8] and gives us the opportunity to study the impact of preformed DSA with low MFI. Moreover,

Table 6. Creatininemia, GFR (iohexol clearance), 24-h proteinuria at 1 year and 5 years after transplantation in the four groups (patients with functional graft were considered). Mean ± SD.

	Group 1 (n = 170)	Group 2 (n = 22)	Group 3 (n = 12)	Group 4 (n = 32)	Entire cohort (n = 236)	P
1-year creatininemia (µmol/l), n = 233	142.2 ± 69.4	153.8 ± 51.4	181.3 ± 76.2	150 ± 72.8	146.4 ± 68.9	0.26
1-year GFR (ml/min) N = 204	56 ± 18	50 ± 17.5	45 ± 19	56 ± 21	55.3 ± 18.2	0.15
1-year proteinuria (g/24 h), n = 220	0.43 ± 0.77	0.70 ± 0.94	0.92 ± 1.1	0.312 ± 0.34	0.47 ± 0.77	0.06
5-year creatininemia (µmol/l), n = 191	137.2 ± 55.1	156 ± 77	176 ± 84	149.4 ± 52.9	142.5 ± 58.8	0.17
5-year GFR (ml/min) N = 158	51.9 ± 16.2	45.3 ± 20	42.5 ± 20.8	51.4 ± 22.1	50.8 ± 17.6	0.36
5-year proteinuria (g/24 h), n = 173	0.49 ± 0.8	0.82 ± 1.1	1.32 ± 0.9	0.51 ± 0.51	0.56 ± 0.85	0.06

Group 1 = nonsensitized patients plus patients with non-DSA antibodies (n = 170); Group 2 = patients with preformed DSA that disappeared (n = 22); Group 3 = patients with preformed DSA that persisted (n = 12); Group 4 = patients with *de novo* DSA only (n = 32).

there is no consensus on an ideal MFI threshold. In our study, we pretreated sera with EDTA to avoid the prozone effect; unfortunately, serial dilutions—that can significantly improve accuracy of MFI titration [27]—were not performed. Conversely, the use of serial dilutions requires a greater number of Luminex tests and significantly increases the analytical costs. Finally, kidney biopsies were performed only when medically indicated; therefore, we may have misdiagnosed subclinical kidney rejections.

In conclusion, many preformed DSA detected only by the Luminex assay in the serum on the day of transplantation disappeared after kidney transplantation without any antibody reduction therapy. Under these circumstances, the prognosis of kidney recipients is close to that of nonsensitized patients (at least in the midterm).

Authorship

SC: designed the study, performed research, collected data, analyzed data, and wrote the paper. CF: collected the data, performed research, and contributed to the writing, revision, and approval of the paper. AP: designed the study, performed research, collected data and contributed to the writing, revision and approval of the paper. GV, JO, CM, and NC: collected data and contributed to the writing, revision and approval of the paper. PP, LB, FH, CG, and BM: contributed to the writing, revision and approval of the paper. VR: performed the Luminex tests and contributed to the approval of the paper. FL: contributed to the statistical analysis, revision and approval of the paper.

Funding

This study received no specific funding.

Conflicts of interest

The authors of this manuscript have no conflict of interests to disclose.

REFERENCES

1. Terasaki PI. Humoral theory of transplantation. *Am J Transplant* 2003; **3**: 665.
2. Mao Q, Terasaki PI, Cai J, *et al.* Extremely high association between appearance of HLA antibodies and failure of kidney grafts in a five-year longitudinal study. *Am J Transplant* 2007; **7**: 864.
3. Caro-Oleas JL, Gonzalez-Escribano MF, Gentil-Govantes MA, *et al.* Clinical relevance of anti-HLA donor-specific antibodies detected by Luminex assay in the development of rejection after renal transplantation. *Transplantation* 2012; **94**: 338.
4. Patel AM, Pancoska C, Mulgaonkar S, Weng FL. Renal transplantation in patients with pre-transplant donor-specific antibodies and negative flow cytometry crossmatches. *Am J Transplant* 2007; **7**: 2371.
5. Picascia A, Infante T, Napoli C. Luminex and antibody detection in kidney transplantation. *Clin Exp Nephrol* 2012; **16**: 373.
6. Lefaucheur C, Loupy A, Hill GS, *et al.* Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 1398.
7. Guidicelli G, Anies G, Bachelet T, *et al.* The complement interference phe nomenon as a cause for sharp fluctuations of serum anti-HLA antibody strength in kidney transplant patients. *Transpl Immunol* 2013; **17**: 17.
8. Loupy A, Lefaucheur C, Vernerey D, *et al.* Complement-binding anti-HLA

antibodies and kidney-allograft survival. *N Engl J Med* 2013; **369**: 1215.
9. Haas M, Sis B, Racusen LC, *et al.* Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant* 2014; **14**: 272.
10. Lee PC, Ozawa M. Reappraisal of HLA antibody analysis and crossmatching in kidney transplantation. *Clin Transpl* 2007; **21**: 219.
11. Otten HG, Verhaar MC, Borst HP, Hene RJ, van Zuilen AD. Pretransplant donor-specific HLA class-I and –II antibodies are associated with an increased risk for kidney graft failure. *Am J Transplant* 2012; **12**: 1618.
12. Morales-Buenrostro LE, Terasaki PI, Marino-Vazquez LA, *et al.* "Natural" human leukocyte antigen antibodies found in nonalloimmunized healthy males. *Transplantation* 2008; **86**: 1111.
13. Ling M, Marfo K, Masiakos P, *et al.* Pretransplant anti-HLA-Cw and anti-HLA-DP antibodies in sensitized patients. *Hum Immunol* 2012; **73**: 879.
14. Lefaucheur C, Suberbielle-Boissel C, Hill GS, *et al.* Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. *Am J Transplant* 2008; **8**: 324.
15. Crespo M, Torio A, Mas V, *et al.* Clinical relevance of pretransplant anti-HLA donor-specific antibodies: does C1q-fixation matter? *Transpl Immunol* 2013; **29**: 28.
16. Kimball PM, Baker MA, Wagner MB, King A. Surveillance of alloantibodies after transplantation identifies the risk of chronic rejection. *Kidney Int* 2011; **79**: 1131.
17. Tang AH, Platt JL. Accommodation of grafts: implications for health and disease. *Hum Immunol* 2007; **68**: 645.
18. Wiebe C, Gibson IW, Blydt-Hansen TD, *et al.* Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant* 2012; **12**: 1157.
19. Sawinski D, Forde KA, Trofe-Clark J, *et al.* Persistent BK viremia does not increase intermediate-term graft loss but is associated with de novo donor-specific antibodies. *J Am Soc Nephrol* 2015; **26**: 966.
20. Kim JJ, Balasubramanian R, Michaelides G, *et al.* The clinical spectrum of de novo donor-specific antibodies in pediatric renal transplant recipients. *Am J Transplant* 2014; **14**: 2350.
21. Willicombe M, Roufosse C, Brookes P, *et al.* Acute cellular rejection: impact of donor-specific antibodies and C4d. *Transplantation* 2014; **97**: 433.
22. Devos JM, Gaber AO, Teeter LD, *et al.* Intermediate-term graft loss after renal transplantation is associated with both donor-specific antibody and acute rejection. *Transplantation* 2014; **97**: 534.
23. Croze LE, Tetaz R, Roustit M, *et al.* Conversion to mammalian target of rapamycin inhibitors increases risk of de novo donor-specific antibodies. *Transpl Int* 2014; **27**: 775.
24. Hirai T, Furusawa M, Omoto K, *et al.* Analysis of predictive and preventive factors for de novo DSA in kidney transplant recipients. *Transplantation* 2014; **98**: 443.
25. Wu P, Jin J, Everly MJ, Lin C, Terasaki PI, Chen J. Impact of alloantibody strength in crossmatch negative DSA positive kidney transplantation. *Clin Biochem* 2013; **46**: 1389.
26. Huang Y, Ramon D, Luan FL, Sung R, Samaniego M. Incidences of preformed and de novo donor-specific HLA antibodies and their clinicohistological correlates in the early course of kidney transplantation. *Clin Transpl* 2012; **26**: 247.
27. Tambur AR, Herrera ND, Haarberg KM, *et al.* Assessing antibody strength: comparison of MFI, C1q, and titer information. *Am J Transplant* 2015; **9**: 2421.