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Impact of persistent and cleared preformed HLA DSA on kidney transplant outcomes

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Abstract

Preformed HLA donor-specific antibodies (DSA) only detected with Luminex have been associated with increased risk of antibody-mediated rejection (ABMR) and graft failure after kidney transplantation (KT). Their evolution after KT may modify this risk. We analyzed postransplant evolution of preformed DSA identified retrospectively and their impact on outcomes of 370 KT performed 2006-2014. Antibodies were monitored prospectively at 1-3-5 years after KT and if any dysfunction. Early acute ABMR was more frequent among patients with preformed DSA class-I or I+II than isolated class-II (29.4% *vs* 4.5%,p=0.02). One year post-KT, 20 of 34 patients with functioning KT had persistent DSA. Preformed DSA class-II persisted more frequently than class-I/I+II (66.7% *vs.* 33.3%; p=0.031). The only risk factor independently associated with persistence was pretransplant MFI. Patients with *de novo* DSA had the highest risk of ABMR (HR 22.2 [CI 6.1-81.2]). Although recipients with persisting preformed DSA had significantly increased ABMR risk (HR 14.7 [CI 6.5-33.0], those with cleared preformed DSA also had a higher risk than those without DSA (HR 7.01 [CI 2.2-21.8]).

Preformed DSA are a very important risk factor for ABMR and graft loss. Patients who clear preformed DSA still show an increased risk of ABMR and graft loss after KT.

Keywords

donor-specific antibodies, preformed, kidney transplantation, antibody-mediated rejection

Abbreviations

ABMR - antibody-mediated rejection

DSA - donor-specific antibodies

dnDSA – de novo donor-specific antibodies

iDSA - immunodominant DSA

KT – kidney transplant

MFI – Mean Fluorescence Intensity

SAB – Single Antigen Beads

1. Introduction

The presence of HLA donor-specific antibodies (DSA) increases the risk of antibody-mediated rejection (ABMR) and graft failure in kidney transplant (KT) recipients [1,2]. This negative impact on graft-survival has been described associated with preformed pretransplant DSA [3,4] and *de novo* postransplant DSA [5-7]. However, the true clinical relevance of DSA remains elusive as not all patients with antibodies show the same prognosis [3-7]. The newer monitoring techniques based on single antigen beads (SAB) are revealing HLA antibodies previously undetected in KT recipients, and there is a need to identify if there are safer pretransplant DSA to open up opportunities for transplantation [8-12]. It has been suggested that some characteristics of these antibodies may differentiate those DSA which portend worse prognosis, such as the HLA class I vs. II specificity [8,12], the strength or mean fluorescence intensity (MFI) [9], the IgG subclass [10] or the complement binding ability of the antibodies [11,12].

There is no consensus on the best way to monitor HLA antibodies after kidney transplantation in patients with or without preformed DSA [2,13]. Furthermore, little is known on the capability of these antibodies to predict ABMR in the absence of clinical and analytical dysfunction. In particular, the evolution of preformed DSA after KT has been scarcely studied, and their relevance as promoters of late ABMR and graft loss is poorly defined.

Current clinical guidelines recommend avoiding transplantation across DSA unless desensitization is implemented, although often this approach only tempers the intensity of antibodies. In order to dissect the true impact of

pretransplant DSA, we selected an era in which some KT across DSA happened with unknown preformed DSA. We analyzed the evolution of these retrospectively known preformed DSA after KT and their impact on graft outcomes in this cohort of patients monitored periodically after KT. We confirmed that preformed DSA predicted worse graft-survival, those that persisted and those that cleared after KT associated significantly with ABMR diagnosis at follow-up.

2. Material and Methods

2.1. Population and design

A prospective post-transplant HLA antibody monitoring study was conducted in 370 consecutive KT recipients at our institution between August 2006 and March 2014. All were transplanted across a negative donor-specific CDC crossmatch with mixed lymphocytes (1:1 and diluted 1:4 at 4º and 22º, and DTT treated) and followed until graft loss (dialysis or death) or September 2016. A database included demographics, donor type, previous transplantations, immunosuppression, delayed or immediate graft function and acute rejection episodes.

The Hospital Research Ethical Committee approved the study and patients signed informed consents.

2.2. Immunosuppressive regimen

All patients received immunosuppression with basiliximab, tacrolimus, mycophenolate acid derivatives and prednisone (250 mg./day 0, 125 mg./day 1, 20 mg/day 2-13, 15 mg/day 14-27, 10 mg./day 28-41 and 5 mg thereafter), . Thymoglobulin was used instead of basiliximab in patients with PRA-CDC over 50% or previous renal allograft loss due to acute rejection. Acute rejection episodes were initially treated with steroids, but in case of resistance, thymoglobulin was added. In case of early acute ABMR with clinical dysfunction, plasma-exchange and low-dose polyclonal immunoglobulin or high polyclonal immunoglobulin were employed, if no improvement in renal function (decrease >25% of creatinine) or histology (decrease of microinflammation

and/or C4d deposition) was obtained after 10 days, Rituximab® was administered and plasma-exchange further extended. Late ABMR (> 1 year after transplantation) was not specifically treated.

2.3. HLA antibody detection

Luminex antibody testing was performed retrospectively on pretransplant serum pretreated with EDTA obtained immediately before KT for crossmatch and prospectively on postransplant samples obtained at dysfunction or biopsy, 1, 3 and 5 years after transplantation as previously described [12,14]. Screening for anti-HLA antibodies was performed using the Luminex Lifecodes LifeScreen Deluxe assay (Immucor Lifecodes Transplant Diagnostics, Nijlen, Belgium). Anti-HLA alloantibody IgG identification was performed using the Lifecodes LSA Class I and/or Class II assays, according to the manufacturer instructions [6]. With MatchIT software, the bead was considered positive when at least two of the results were above the specified values: Background Corrected MFI calculated for each bead or BCM> 1000, Background Corrected Ratio or BCR >5 and Antigen Density Background Corrected Ratio or AD-BCR >5 for pre and postransplant samples. For analysis purposes raw MFI were considered. Donor HLA antibody specificity was ascribed considering donor HLA A, B, DRB1 and some C and DQB typing. When typing was unknown, antibodies against C or most DQ antigens were assigned considering linkage disequilibrium for Caucasian donors.

For postransplant assessment in patients with preformed DSA, we carefully reviewed that epitope reactivity. Positivity for persistence or clearing of DSA was based on the same criteria.

Serum samples with preformed DSA were analyzed for the presence of C3d-binding anti-HLA antibodies according to the manufacturer's protocol (Immucor Lifecodes Transplant Diagnostics, Nijlen, Belgium) [6].

2.4. Renal pathology

One-hundred and sixty-five patients underwent biopsies during the study for clinical dysfunction or DSA detection. They were evaluated as previously described [15]. Conventional hematoxylin-eosin staining, PAS, silver-methenamine, and Masson´s trichrome staining were done, as well as fluorescence microscopy examination and C4d immunohistochemical staining. From each biopsy, two 1x1 mm fragments were analyzed using a transmission electron microscope. All biopsies were evaluated according to Banff 2015 classification [13]. We labeled as ABMR all cases which met criteria for any type of category 2 diagnosis of antibody-mediated changes, either suspicious for ABMR or full ABMR. Patients with cleared preformed HLA DSA at the time of biopsy could only be diagnosed as suspicious for ABMR.

2.5. Statistical analysis

We used Chi^2 or Fisher's exact tests to analyze categorical data and Student's t-test or non-parametrical Mann–Whitney U test for continuous variables. Survival was assessed using the Kaplan–Meier method and log-rank tests. Data are expressed as means±standard deviation or medians with interquartilic range (IQR) as appropriate. Logistic regression was performed to evaluate risk of factors of persistence of preformed DSA. Receiver operating characteristic (ROC) curve and area under the curve (AUC) were calculated for DSA MFI

values. The Cox proportional hazards model was used to evaluate the effect of preformed DSA (cleared and persistent) and dnDSA on graft survival and ABMR, adjusted for confounders.

Statistical analyses were performed using SPSS v.20.

3. Results

3.1. Preformed DSA: characteristics and outcomes

Among 370 patients included, 39 (10.5%) had preformed DSA at transplantation. They were more frequently female, retransplants, had higher peak and pretransplant CDC-PRA and received Thymoglobuilin® induction (35.9% vs 10.6%, p=0.001) than those without preformed antibodies (Table 1).

Patients with preformed DSA showed a significantly higher risk of ABMR (HR 10.6 [95% CI 5.5-20.4]; p<0.001) and lower ABMR-free graft-survival (41% vs 92%, p<0.001) than patients without DSA at a median follow-up of 4.6 years (IQR 3.1-6.5). In addition, they had a significantly lower death-censored graft survival (64% vs 81%, p=0.002) and higher graft loss risk (HR 2.5 [1.3-4.7]; p=0.003) than patients without antibodies.

3.2. Preformed DSA according to class

Among the 39 patients with preformed DSA, nine (23.1%) had only HLA class I antibodies, eight (20.5%) had class I and II and twenty-two (56.4%) only class II antibodies. There were no differences in baseline characteristics between patients with DSA I or mixed I-II and those with only DSA II. The strength of DSA was higher in patients with only class II DSA, considering the DSA with the

highest MFI, immunodominant DSA (iDSA), or the sum of MFI of all DSA per patient. No differences were found in the number of DSA specificities or in the capacity for C3d-binding (Table 2). All C3d binding DSA were HLA class II but one.

The group of patients with isolated class I or mixed class I-II antibodies showed more frequently early (within three months of transplantation) acute ABMR than those with preformed class II alone (29.4% vs 4.5%, p=0.02). Four patients with DSA class I and one patient with mixed class I and II -undetected by the prospective CDC XM- and acute ABMR lost their grafts, while none with DSA class II alone did (Table 2). ABMR-free survival was lower in patients with class I or mixed than in those with class II alone (65 vs 91%, p=0.043), with median follow-up of 4.6 years (IQR 3.1-6.5) (Figure 1).

3.3. Postransplant evolution of preformed DSA

Within one year after transplantation, 29.4% patients with preformed DSA class I or I-II had lost their grafts, 47.1% had cleared DSA and 23.1 % maintained DSA (mean MFI= 8581 (SD 6822)) . Of them, persistency affected almost exclusively DSA II; only one patient in this group had persistent DSA class I (without ABMR in the biopsy). In the group with isolated preformed DSA II, persistence happened in 72.7% compared with 23% in the group with preformed DSA class I or I-II (p= 0.026) (Figure 2).

When we evaluated only the 34 patients with a functioning graft at one year, preformed DSA were still detected in 20 of them. Patients with persistent DSA were younger and more frequently retransplants than those who cleared. We found no differences in persistent *vs* cleared preformed DSA regarding donor

characteristics, HLA mismatches, PRA-CDC, immunosuppression, cold ischemia time or delayed graft function. Persistent preformed DSA displayed a higher MFI of the iDSA, a higher MFI sum of DSA and more frequent C3d-binding capability than cleared preformed DSA at the time of transplant (Table 3). ROC curve analysis showed an MFI cut-off point of 5100 as predictive of DSA persistence (AUC=0.803, p=0.002) (Figure 3).

A multivariate analysis evaluated factors associated with persistence of preformed DSA one year postransplantation and identified only DSA MFI as predictor, either as a continuous variable (OR 1.01 [CI 1.001-1.010], p=0.023) or as a categorical variable setting the threshold in 5,100 (OR 6.2 [CI 1.05-36.2], p=0.004).

3.4. De novo DSA

All patients were monitored for DSA after transplantation regularly and at dysfunction or biopsy. Among 331 patients without DSA at the time of transplantation, 6 (1.9%) had developed de novo DSA (dnDSA) one year, 13 (4.5%) 3 years and 8 (8.8%) at 5 years after transplantation. No patient with preformed DSA developed de novo DSA.

3.5. Antibody-mediated rejection

One-hundred and sixty-five patients (44.6%) underwent kidney graft biopsies. Antibody-mediated changes (Category-2 Banff'2015) were diagnosed in 15 patients without DSA (11.2%). Patients with persistent preformed DSA, cleared preformed DSA and dnDSA showed similar ABMR rates in the biopsy (Table 4).

Multivariate analysis in search of risk factors for ABMR adjusted for recipient gender, retransplantation, HLA mismatch class II and pretransplant PRA CDC showed that dnDSA was the most significant risk factor (HR 7.7 [IC 2.2-27.3, p=0.008]), followed by preformed DSA, either persistent (HR 5.3 [IC 2.5-11.0], p=0.001) or cleared (HR 2.8 [IC 1.6-13.0], p=0.004) (Figure 4).

3.6. Graft loss

During follow-up, 41 patients (11.6%) lost their grafts. Graft-loss and death-censored graft-loss were higher in patients with preformed DSA and those with dnDSA than in patients with no DSA (Table 4). Cox regression analysis showed higher risk of graft loss in patients with dnDSA (HR 5.9 [3.9-25.0]; p=0.016), persistent preformed (HR 4.8 [2.3-10.4]; p=0.016) or cleared preformed DSA during follow-up (HR 3.7 (1.3-10.6); p=0.015). But when the analyses was adjusted for donor and recipient age, recipient gender and induction treatment, dnDSA [HR 4.3 (IC 0.95-17.9); p=0.05], preformed persistent [HR 2.9 (IC 0.99-8.6); p=0.058] and preformed cleared DSA [HR 2.5 (IC 0.8-7.4); p=0.105] did not associate with graft-loss.

4. Discussion

Corresponding to an era in which SAB monitoring for HLA antibodies was not systematically performed in the waiting list, we retrospectively identified that 10.5% of our patients underwent KT with unknown preformed DSA without desensitization. This prevalence is similar to 8.5-11.5% described in other series [16-18]. These patients with preformed DSA showed 10-fold risk of ABMR than those without DSA, not different to previously described [1,19,20]. In addition, they showed worse death-censored graft survival than those without DSA at transplantation [3,4,21,22]. Interestingly and opposite to other studies [23-25], we found that preformed DSA correlated with postransplant ABMR even if cleared after transplantation, although this latter group exhibited and intermediate risk.

In our cohort, antibodies involved in early graft loss were HLA class-I DSA either isolated or combined with class-II. These antibodies were detected in 43.6% of our patients with preformed DSA. Almost one third developed early acute ABMR with 100% graft loss, as no desensitization protocol was implemented because they were retrospectively identified. Contrarily, only one patient with isolated HLA class-II preformed DSA developed early ABMR without graft loss.

Some groups have published their experience in assessing differences in graft survival according to the preformed DSA HLA class. In a previous study, we noticed that preformed DSA class-I was associated with 40% early graft-loss compared with 0% in cases with DSA class-II [12]. Willicombe et al. described worse rejection-free survival in patients with preformed DSA class-I or mixed

class I-II than those with class-II alone or those without DSA [16]. Otten et al described worse graft survival only in patients with mixed class I-II DSA [8]. Similar to our experience, Caro-Oleas et al. reported that patients with either class-I or mixed classes I-II had worse graft survival when compared to those with preformed class-II alone or no DSA [17]. Contrarily, Lefaucheur et al showed similar graft survival with preformed class I-II [3]. In a different setting, Dar et al. found that among six patients who received a combined liver-kidney transplant with DSA, three with DSA I-II and one with only DSA-II suffered acute ABMR. After ABMR of the kidney, DSA-I were cleared but DSA-II were not [28]. In sensitized patients with preformed DSA treated with intravenous immunoglobulin or plasma exchange with rituximab peritransplantation, subclinical ABMR at one year was more frequent with DSA-II than with I [27]. Consequently, our experience and that of others suggest that preformed class-I DSA should discourage KT unless desensitization or combined transplantation is planned before KT. On the other hand, preformed class-II DSA confers risk for the development of late ABMR with or without peritransplant treatment.

Through monitoring with SAB one year after KT, we detected that 41.2% of patients with preformed DSA and functioning grafts cleared their antibodies without any specific treatment. Two previous very small studies described postransplant persistence of preformed DSA in some patients, but lacked systematic surveillance of all patients with preformed DSA [29,30]. No correlation with ABMR or graft survival was attempted in these studies. To our knowledge, only four recent studies have systematically assessed persistence of preformed HLA DSA employing SAB after transplantation [23-26]. They showed 62-65% clearance at follow-up. Marfo et al. and Adebiyi et al. did not

find any influence of preformed DSA on graft survival [24, 26].The other two studies reported an increase rate of graft loss at follow-up in patients transplanted with DSA only when they persisted after KT [23,25]. We did not detect impact on graft-survival in an adjusted analysis of persistent preformed or persistent cleared, but we observed a higher risk of ABMR both in patients with persistent and cleared preformed DSA. When adjusted their analysis, Caillard et al. found no differences in graft function and survival among patients with cleared preformed DSA, with persistent preformed DSA, with dnDSA and those without DSA [25].

In our cohort, patients with preformed cleared DSA showed intermediate ABMR rates between persistent DSA and no DSA. A likely explanation may be that persistent DSA are produced in higher amounts and yield graft damage, while cleared DSA might be partially controlled by immunosuppression so injury evolves slowly. Marfo et al. found that patients with preformed DSA had more acute and chronic rejection with persistent vs cleared DSA (30 vs 7%, p=0.006) without details about the type of rejection [24]. Kimball et al. found 43% ABMR in patients with persistent DSA, 3% in those who cleared DSA and 1% in those without DSA [23]. Interestingly, in patients transplanted with a negative flow crossmatch, Adebiyi and col. found that only DSA with MFI over 3000 associated with an increased risk of biopsy-proven acute rejection with indication biopsies. They saw that patients with persistent or recurrent DSA had a non-significant decrease in 5-year death-censored graft survival but provided no information on the incidence of ABMR [26]. The difference of the impact of preformed DSA on ABMR diagnosis in our study compared with those may be due to different definition of DSA and the fact that biopsies were not classified

following Banff 2015 criteria. Evolving Banff classification allows a more precise diagnosis of ABMR [15].

As persistence or clearance of DSA impacts ABMR free graft-survival, attempts to distinguish how preformed antibodies will behave after transplantation is clinically relevant. In our cohort, preformed DSA class II, with high MFI and complement binding capacity persisted more frequently, but the only factor independently associated with persistence was the strength of DSA pretransplantation with a cutoff MFI of 5000. Among the three studies mentioned before, only Caillard et al reported similar results. They found that the iDSA MFI was the most significant factor related to persistence after KT [25]. The other studies did not find any characteristic of DSA associated with clearance or persistence of DSA after KT [23,24].

Through postransplant monitoring one year after kidney transplantation, we also detected dnDSA at a low incidence of 1.9% in a low-risk population under conventional immunosuppression. It is well-known that dnDSA are associated with an increased incidence of ABMR [5-7]. Our experience confirms that in a multivariate adjusted analysis. Furthermore, this risk is also largely increased in patients with preformed DSA, either detected postrasplant or not. The incidence of ABMR in the three main types of patients (preformed DSA, de novo DSA and no DSA) is similar in our experience to that described by Caro-Oleas et al [4], with cleared preformed DSA retaining their impact.

The main limitation of our study is that our patient cohort is immunologically low-risk, as illustrated by the low incidence of de novo DSA, and full high resolution HLA typing was not available for all donors, so donor-specificity had to rely on linkage disequilibrium and interpretation consensus for some Caucasian

donors. In addition, surveillance biopsies were not available, so subclinical ABMR infraestimation is likely [31]. However, ours is a large well characterized cohort, with tight DSA monitoring during a long period after KT and evidences that not all preformed DSA that disappear after KT are innocuous. One may argue that some of these DSA may not have been completely cleared but persist very weakly with MFIs below 500, so it may be a matter of definition.

In summary, patients with preformed DSA carry a higher risk of graft loss, being early graft loss high if DSA class I alone or with class II are present and no pretransplant treatment is implemented. Preformed DSA class II are frequently associated with detection of late ABMR in the graft biopsy, regardless potential DSA clearing or minimization to very low MFI after transplantation. These findings are clinically relevant for desensitization programs. Larger studies with systematic postransplant follow-up biopsies in patients with preformed DSA are needed to characterize better patient phenotypes and design personalized management of this greater immunological risk to increase transplant opportunities.

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Conflict of interests:

The authors of this manuscript have no conflicts of interest to disclose as described by Human Immunology Journal. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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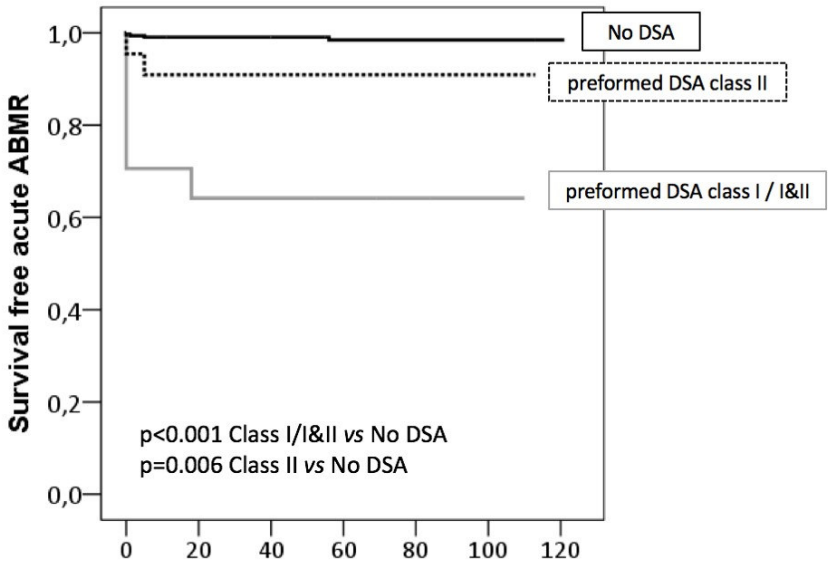
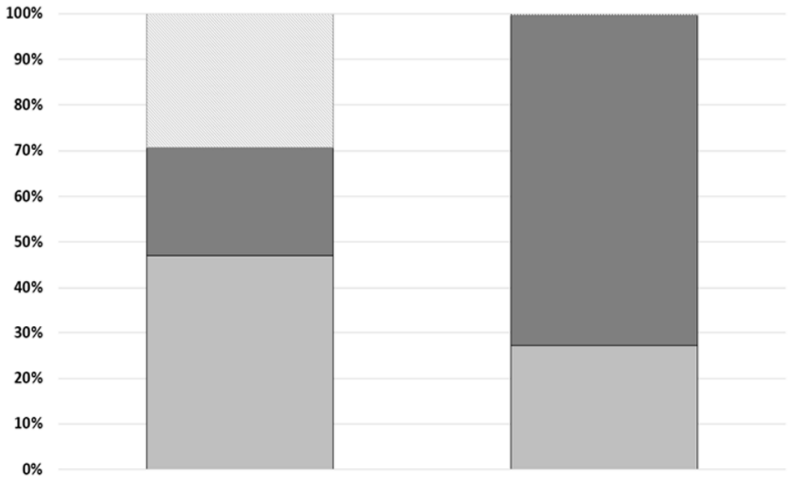


Nº at risk

No DSA	331	311	291	271	251	231	211
Preformed DSA class II	17	10	7	5	3	3	1
Preformed DSA class I/I&II	22	17	14	10	7	5	2



	DSA class I / I&II	DSA class II
Cleared DSA-1y	8 (47.1%)	6 (27.3%)
Persistent DSA-1y	4 (23.5%)	16 (72.7%)
Early acute ABMR and graft loss	5 (29.4%)	0

Figure 2. Outcome at one year after transplantation: graft-loss and persistence of preformed DSA according to HLA class.

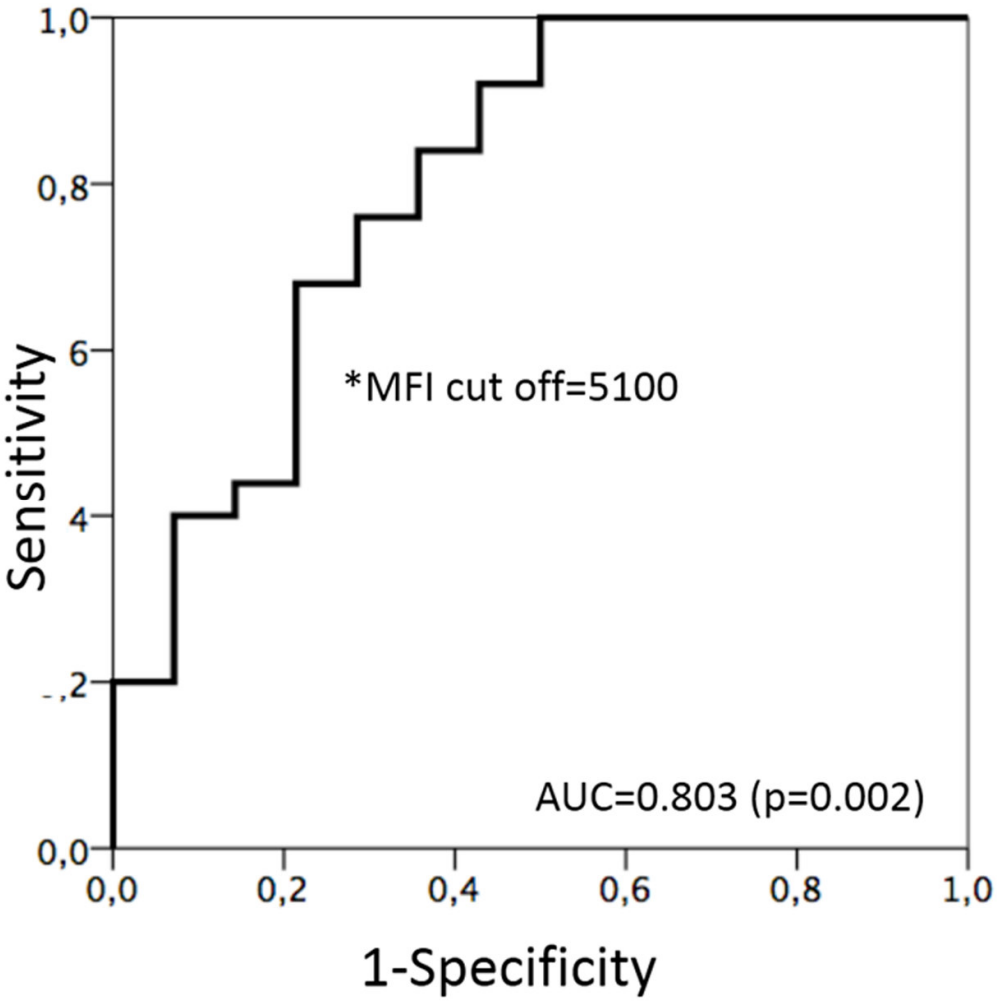
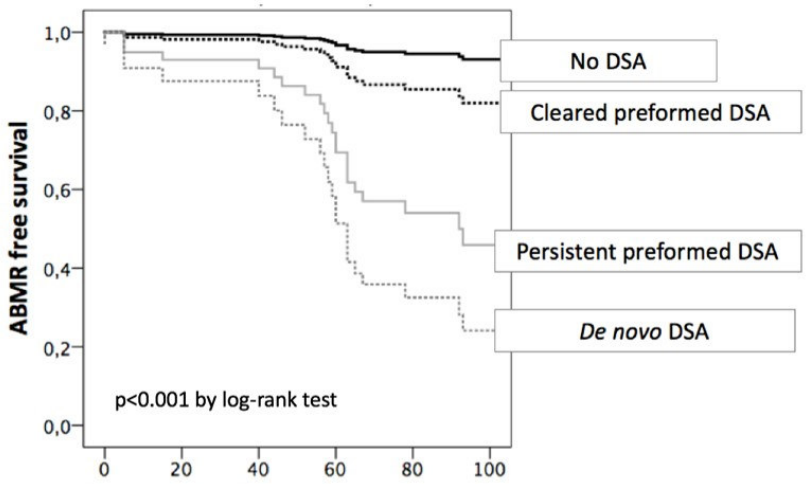


Figure 3. ROC curve: MFI cutoff for persistence of preformed immunodominant donor-specific antibodies



Nº at risk						
No Abs	306	290	227	140	78	65
Cleared preformed DSA	14	11	9	6	3	2
Persistent preformed DSA	25	18	15	8	5	3
De novo DSA	6	3	3	2	1	1

			Unadjusted analysis			Adjusted analysis*		
	N patients	N events	HR	IC (95%)	p	HR	IC (95%)	p
No DSA [no-DSA pre / no-DSA post]	306	15	ref	ref	ref	ref	ref	ref
Cleared preformed DSA [DSA pre / no DSA post]	14	4	5.309	1.59-17.65	0.006	2.768	1.58-13.01	0.004
Persistent preformed DSA [DSA pre / DSA post]	25	15	7.916	2.91-24.164	<0.001	5.307	2.55-11.035	0.001
***De novo* DSA** [no DSA pre / DSA post]	6	3	9.116	2.38-34.89	0.001	7.776	2.21-27.32	0.008

Table 1. Characteristics of patients with and without preformed HLA DSA.

	All (N=370)	**No DSA (N=331)**	**Preformed DSA (N=39)**	**p**
Recipient age (years) [mean (SD)]	53.7 (13.5)	53.8 (13.6)	53.3 (11.9)	0.71
Female sex (n, %)	146 (39.4)	116 (35.1)	30 (76.6)	**0.001**
Retransplantation (n, %)	55 (14.8)	29 (8.8)	26 (66.6)	**0.001**
Deceased donor (n, %)	325 (87.3)	289 (87.3)	36 (92.3)	0.36
Donor age (years) [mean (SD)]	52.6 (14.2)	52.6 (14.4)	52.7 (12.5)	0.97
Previous pregnancies (n, %)	112 (76.7)	88 (75.9)	24 (80)	0.99
HLA mismatch [mean (SD)]				
Class I (A/B)	2.9 (0.9)	2.8 (0.9)	2.9 (0.9)	0.69
Class II (DR)	1.2 (0.6)	1.2 (0.6)	1.4 (0.5)	0.13
preKT PRA CDC>5% (n, %)	24 (6.5)	11 (3.3)	13 (33.3)	**0.001**
Peak PRA CDC>5% (n, %)	71 (19.2)	50 (15.1)	21 (53.8)	**0.001**
Antilymphocyte induction (n, %)	49 (13.2)	35 (10.6)	14 (35.9)	**0.001**
Initial immunosuppression: Tacrolimus + Mycophenolate (n,%)	370 (100)	331 (100)	39 (100)	1
Delayed graft function (n, %)	120 (32.4)	104 (31.4)	16 (41.0)	0.27
Cold ischemia time (h) [mean (SD)]	13.9 (6.1)	14.1 (6.2)	12.8 (5.0)	0.13

DSA: donor-specific antibodies. SD: standard deviation. KT: Kidney transplantation. PRA: panel reactive antibodies

Table 2. Characteristics of patients with preformed DSA according to HLA class.

	Preformed DSA class I and class I&II (N=17)	**Preformed DSA class II (N=22)**	**p**
Recipient age (years) [mean (SD)]	50.24 (12.7)	55.64 (11.04)	0.35
Female sex (n, %)	13 (76.5)	17 (77.2)	0.62
Retransplantation (n, %)	9 (53)	17 (77.2)	0.11
Deceased donor (n, %)	16 (94.1)	20 (90.1)	0.59
Donor age (years) [mean (SD)]	50.53 (10.1)	54.3 (14.1)	0.16
Cold ischemia time (h) [mean (SD)]	12 (3.8)	13 (5.8)	0.63
Previous pregnancies (n, %)	10 (76.9)	14 (82.3)	0.50
HLA mismatch [mean (SD)]			
Class I (A/B)	3 (0.7)	3 (1.1)	0.89
Class II (DR)	1.38 (1.1)	1.44 (0.5)	0.75
Antilymphocyte induction (n, %)	8 (47)	6 (27.3)	0.33
Pretransplant PRA CDC>5% (n, %)	6 (35.3)	11 (50)	0.54
Peak PRA CDC>5% (n, %)	12 (70.6)	9 (40.1)	0.06
Initial immunosuppression: Tacrolimus + Mycophenolate (n, %)	17 (100)	22 (100)	1
Delayed graft function (n, %)	7 (41.2)	9 (40.1)	0.70
Pretransplant DSA characteristics			
MFI iDSA [mean (SD)]	7125 (6669)	11909 (13782)	**0.02**
Sum MFI all DSA specificities [mean (SD)]	9525 (8191)	15232 (11460)	**0.04**
Number of DSA specificities [mean (SD)]	1.6 (0.8)	1.5 (0.6)	0.84
DSA specificities >1 (n, %)	7 (41.2)	10 (45.4)	0.78
C3d positive DSA (n, %)	7 of 14 (50)	14 of 20 (70)	0.23
Follow up			
Early* acute ABMR (n, %)	5 (29.4)	1 (4.5)	**0.02**
Early* graft loss (n, %)	5 (29.4)	0	**<0.001**
Graft loss due acute ABMR (n, %)	5 (29.4)	0	**<0.001**

DSA: donor-specific antibodies. SD: standard deviation. KT: Kidney transplantation. PRA: panel reactive antibodies. MFI: Mean fluorescence intensity. ABMR: Antibody-mediated rejection.

iDSA: immunodominant DSA *Early was considered during the first 3 months after transplantation.

Table 3. Characteristics of patients with functioning grafts at one year after KT

	OR	95%IC	p
Recipient age (years) (mean, SD)	1.068	1.000-1.140	0.050
Female sex (n, %)	1.200	0.257-5.593	0.816
Retransplantation (n, %)	7.556	1.494-38.208	**0.014**
Deceased donor (n, %)	0.692	0.057-8.470	0.773
Donor age (years) (mean, SD)	1.043	0.985-1.104	0.148
Cold ischemia time (h) (mean, SD)	1.106	0.947-1.293	0.204
HLA mismatch (mean, SD)			
Class I (A/B)	1.273	0.552-2.932	0.571
Class II (DR)	0.857	0.172-4.267	0.851
Pretransplant PRA CDC >5% (n, %)	1.429	0.013-1.474	0.102
Peak PRA CDC >5% (n, %)	1.875	0.150-23.396	0.625
Antilymphocyte induction (n,%)	2.444	0.514-11.619	0.261
Delayed graft function (n,%)	1.469	0.350-6.168	0.599
Pretransplant DSA characteristics			
Class I or I&II	1		
Class II	5.333	1.162-24.469	**0.031**
MFI iDSA (mean, SD)	1.000	1.010-1.100	**0.007**
Sum MFI all specificities (mean, SD)	1.001	1.000-1.010	**0.016**
C3d+ (n, %)	10.010	1.781-56.150	**0.009**

according to the evolution of preformed DSA (Univariate analysis)

	No DSA (n=306)	Persistent Preformed DSA (n=25)	Cleared Preformed DSA (n=14)	De novo DSA within 1 year (n=6)
Early acute ABMR	0	6 (24%)	0	0
g+ptc≥2		5 (83.3%)		
C4d=3		6 (100%)		
IF/TA=1		1 (16.6%)		
v>1		1(16.6%)		
cg>0		0%		
Histological findings				
Late biopsy (n, %)	133 (36.9%)	19 (76%)	7 (50%)	6 (100%)
Banff´15 category				
Category 1	17 (12.8%)	1 (5.2%)	0	0
Category 2	15 (11.2%)*	10 (52.6%)	4 (57.1%)	3 (50%)
g+ptc≥2	12 (80%)	8 (80%)	3 (75%)	3 (100%)
C4d>0	6 (40%)*	8 (80%)	3 (75%)	3 (100%)
*IF/TA***	5 (33.3%)	4 (40%)	1 (25%)	2 (33.3%)
cg>0	3 (20%)	5 (50%)	2 (50%)	1 (33.3%)
PTCBM	2 (13.3%)	4 (40%)	1 (25%)	0%
Category 3	9 (6.8%)	0	0	0
Category 4	16 (12%)	0	0	0
Category 5	34 (25.6%)	6 (31.7%)	1 (14.3%)	1 (16.6%)
Category 6	42 (31.6%)	2 (10.5%)	2 (28.5%)	2 (33.4%)
Time to biopsy (months) [median (IQR)]	12 (2-20)	16 (11-32.5)	13 (8.5-33.5)	10.5 (3-14)
Follow up				
Graft loss (n,%)	50 (16.3%)*	10 (40%)	8 (57.4%)	4(66.6%)

Graft loss (death-censored) (n,%)	26 (8.5%)*	9 (36%)	4 (28.6%)	2 (33.3%)
Death with functioning graft (n,%)	24 (7.8%)*	1 (4%)[&]	4 (28.6%)	2 (33.3%)
Time of follow up (median, IQR)	58 (40-82)	59.5 (41.5-82.5)	55.5 (30-72)	24.5 (15-43)

Table 4. Biopsy data according to the presence of DSA.

DSA: Donor-specific antibodies. ABMR: Antibody-mediated rejection. g: glomerulitis. ptc: peritubular capilaritis. cg: transplant glomerulopathy. PTCBM: peritubular capillary basement membrane multilayering. IF/TA: interstitial fibrosis/tubular atrophy ** moderate and severe IF/TA

*p<0.05 No antibodies vs persistent, cleared and dnDSA. [&] p<0.05 persistent DSA vs cleared and dnDSA