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ORIGINAL ARTICLE

Impact of *de novo* donor-specific HLA antibodies detected by Luminex solid-phase assay after transplantation in a group of 88 consecutive living-donor renal transplantations

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Summary

De novo donor-specific HLA antibodies (DSA) after renal transplantation are known to be correlated with poor graft outcome and the development of acute and chronic rejection. Currently, data for the influence of *de novo* DSA in patient cohorts including only living-donor renal transplantations (LDRT) are limited. A consecutive cohort of 88 LDRT was tested for the occurrence of *de novo* DSA by utilizing the highly sensitive Luminex solid-phase assay for antibody detection. Data were analyzed for risk factors for *de novo* DSA development and correlated with acute rejection (AR) and graft function. Patients with *de novo* DSA [31 (35%)] showed a trend for inferior graft function [mean creatinine change (mg/dL/year) after the first year: 0.15 DSA (+) vs. 0.02 DSA (−) ($P = 0.10$)] and a higher rate of AR episodes, especially in case of *de novo* DSA of both class I and II [6 (55%), ($P = 0.05$)]. Antibody-mediated rejection (AMR) appeared in five patients and was significantly correlated with *de novo* DSA ($P = 0.05$). Monitoring for *de novo* DSA after LDRT may help to identify patients at risk of declining renal function. Especially patients with simultaneous presence of *de novo* DSA class I and class II are at a high risk to suffer AR episodes.

Introduction

Kidney transplantation is the gold standard for therapy of end-stage renal disease. Living-donor renal transplantation (LDRT) provides excellent allograft and patient survival rates. Despite excellent outcome in LDRT, chronic allograft dysfunction in LDRT has remained relatively constant over recent years and is a major reason for graft loss [1]. Chronic allograft dysfunction is due to interaction of time-dependent immunologic and nonimmunologic causes [2]. Based on the humoral theory of transplantation, HLA antibodies (Ab) play a detrimental role in immunologic processes leading to allograft rejection [3,4]. The reevaluation of the role of HLA Ab in allograft rejection was possible with the introduction of Luminex solid-phase assay as a standard method for HLA Ab detection with more specificity and higher sensitivity [5,6].

The prevalence of HLA Ab before and the new appearance of HLA Ab after kidney transplantation are well-

known risk factors for poor graft outcome in renal transplantation [7,8]. The presence of *de novo* DSA after transplantation can cause acute AMR [9,10] and is also known to be associated with the development of chronic rejection in renal allografts [11–14]. The majority of former studies analyzed the influence of *de novo* DSA for cohorts of either deceased donors alone or deceased *and* living donors. Although the role of *de novo* DSA in LDRT has been investigated [15], the data remain limited. The intention of this study was to investigate the clinical relevance of *de novo* DSA in a consecutive cohort of LDRT from a single center, utilizing the sensitive Luminex solid-phase assay for antibody detection. We analyzed risk factors for *de novo* DSA development and correlation of *de novo* DSA with acute rejection and graft function.

Patients and methods

Patients

A total of 105 consecutive LDRT were performed between January 2008 and October 2011 at the Transplant Center Cologne, Cologne, Germany. All transplantations were carried out after a negative CDC cross-match and had a minimum follow-up of one year after transplantation. Three patients were excluded from analysis because of death before a follow-up of HLA Ab detection could be performed. Five patients were previously transplanted and were also excluded from analysis. Nine of the remaining 97 patients had pretransplant DSA detected at time of final cross-match. Finally, a study group of 88 patients was retrospectively analyzed in relation to *de novo* DSA status, including eight children under the age of 18 years.

HLA typing

Patients and corresponding donors were typed for the HLA-A, -B, -C, -DR and -DQ locus. Typing was performed via molecular techniques either by low resolution PCR-SSP (HLA-Ready Gene; Inno-Train, Kronberg, Germany) and/or rSSO (One Lambda LABType SSO Bead Mix; BmT, Merbusch, Germany), according to the manufacturer's protocols.

Screening for HLA-specific antibodies by CDC and ELISA

Prior to transplantation, patient sera were screened for the presence of complement fixing HLA-specific IgG-Ab by complement dependent cytotoxicity assay (CDC) using a well-characterized selected donor cell panel covering most of the defined HLA antigens (CTS 60-well plates, Transplantation Immunology, Heidelberg, Germany). CDC screening was performed by following the standard guidelines of EFI (European Immunogenetics and Histocompatibility Conference). Assessment of positive wells was carried out using the ASHI score. A value of 2–4 was considered to be weak positive. CDC was initially performed without dithiothreitol (DTT), and in case of a positive result, the assay was repeated with DTT (likewise according to EFI standards). The evaluation of a certain specificity required at least 75% of the appropriate wells to react positive. Further, the generic ELISA assay was performed to determine the general presence of class I and/or class II HLA-specific antibodies. The assay was conducted fully automated using the QuickStep instrument with AbScreen class I and II reagents (Bio-Rad, Dreieich, Germany).

Screening for HLA-specific antibodies by Luminex solid-phase assay

For determination of HLA-specific Ab, all patients' sera underwent Luminex solid-phase assay (One Lambda LABScreen Single Antigen class I and II; BmT, Merbusch, Germany) following the manufacturer's instructions. The Luminex technology makes use of microbeads coated with HLA antigens. Every bead population carries a certain single HLA antigen and has an unique fluorescent signal to discriminate between different bead populations. After incubating the bead solution with the serum of interest, HLA antibodies bind to the appropriate beads. Bound HLA antibodies are marked with a PE-labeled secondary antibody. Beads are analyzed in a modified flow cytometer containing two different lasers. The first laser can discriminate between the differently dyed microparticles while the other one measures the presence and amount of PE and hence the presence and amount of the HLA-specific antibodies. For the evaluation of DSA, beads with normalized mean fluorescence intensity (MFI) values more than 100 were considered to be positive.

HLA Ab screening protocol

Patients were screened pretransplant for the presence of HLA-specific Ab by CDC, ELISA and Luminex solid-phase assay. Transplantation was only carried out in case of a prospective negative CDC cross-match. For determination of post-transplant HLA-specific Ab, all patients underwent Luminex solid-phase assay, at least once after transplantation. Serum sampling took place when clinically indicated or at routine visits during the first year after transplantation in case of stable renal function.

Biopsies and diagnosis of AR

Graft biopsies were performed when clinically indicated in case of renal dysfunction, but not as routine protocol biopsies. Therefore, patients with stable renal function after

transplantation did not undergo biopsies. Clinical indication for a graft biopsy was a creatinine increase of more than 25% of the minimum creatinine after transplantation. BANFF 2007 criteria were used for diagnosis and grading of AMR and T-cell-mediated rejection (TCMR). AMR was defined as focal or diffuse C4d positivity and the histologic evidence of tissue injury.

Immunosuppression and treatment of rejection

All patients underwent induction therapy by administration of interleukin (IL)-2 receptor monoclonal Ab (basiliximab) before and on the fourth day after transplantation. Furthermore, according to their risk profile prior to transplantation, the patients received an immunosuppressive regimen based on cyclosporin (CyA) or tacrolimus (TAC) in combination with mycophenolate (M) and prednisolone (P) to maintain immunosuppression. Treatment of rejection was individually adapted to the respective clinical case. In case of a CyA-based immunosuppressive regimen, most of the patients received a change to TAC-based regimen when an AR episode occurred. TCMR was basically treated with a steroid pulse (SP) over 3 days with or without administration of antithymocyte globulin (ATG) based on a daily CD3 lymphocyte count. Furthermore, the standard treatment protocol of AMR included a selection of daily plasmapheresis (PP) or immunoadsorption (IA), and the administration of rituximab (RM). Bortezomib (BZ) was reserved for failed clinical response, and its administration was discussed for the individual clinical case by the treating physicians.

Allograft function and patient outcome

Graft function was assessed by levels of creatinine (mg/dL) at discharge (baseline creatinine), 1 year after transplantation and at the end of follow-up. Based on the creatinine levels at these points, the annual rate of mean creatinine change for defined periods after transplantation was determined. The first of these periods lasted from discharge until 1 year after transplantation. The second period reached from 1 year until the end of follow-up, while the length of follow-up varied between one and up to 4 years. Graft failure was defined as a return to hemodialysis. Patients who died with or without a functioning graft were also included in the study if they had at least one Luminex solid-phase-assay-based HLA antibody detection after transplantation.

Statistical analysis

Qualitative data were summarized by count and percentage, quantitative data by mean ± standard deviation (SD). Correspondingly, patient groups (i.e., DSA- and DSA+) were compared by Fisher's exact test and Mann–Whitney *U*-test, respectively. Multivariable logistic regression analysis was performed to determine the association of risk factors for *de novo* DSA development. The following factors were considered: immunosuppression (TAC/M/P, CyA/M/P), total ABCDRDQ mismatches >5, donor–recipient relation (related/unrelated), recipient age, recipient gender. Multivariable linear regression analysis was performed to determine the association of risk factors for annualized change in creatinine for the first year and after the first year post-transplant. The following factors were considered: *de novo* DSA, baseline creatinine (for first year analysis)/mean creatinine at 1 year (for after the first year analysis), immunosuppression (TAC/M/P, CyA/M/P), total ABCDRDQ mismatches >5, donor–recipient relation (related/unrelated), donor age, recipient gender. A *P*-value lower or equal 0.05 was considered statistically significant. All calculations were performed using SPSS Statistics (IBM Corp., Armonk, NY).

Results

Eighty-eight patients were included in the final analysis in relation to *de novo* DSA, which was performed in October 2012. The mean follow-up was 659 (± 323) days for all transplants. At time of final analysis, 84 patients showed a functioning graft. One patient was back on hemodialysis because of graft failure and three patients died with a functioning graft. The baseline characteristics for the primary study group are shown in Table 1.

Incidence and characterization of *de novo* DSA development

Thirty-one (35%) patients developed *de novo* DSA after transplantation [DSA (+)]. Within the remaining 57 (65%) patients, no *de novo* DSA were detected [DSA (−)]. Ten patients of these 31 DSA (+) patients had *de novo* DSA class II. Ten showed positivity for *de novo* DSA class I, whereas 11 displayed both, *de novo* DSA class I and DSA class II. The majority of *de novo* DSA positive patients showed positivity over several measurements. Table 2 displays a list of all DSA (+) patients with specific type of *de novo* DSA against each type of class I and class II molecules with the correspondent MFI range.

According to Table 1, showing the baseline characteristics for DSA (+) and DSA (−) patients, no significant differences in age of donor and recipient, gender, and CMV status existed between patients with and without *de novo* DSA in univariable analysis. Additionally, there was no discrepancy for the number of HLA mismatches, the primary immunosuppressive regimen, which was based either on CyA or TAC, and the relationship between donor and recipient. In addition, a multivariate logistic regression analysis

Table 1. Baseline characteristics for all patients and the *de novo* DSA (+) and DSA (−) groups.

	All patients, *n* (%)	DSA (+), *n* (%)	DSA (−), *n* (%)	*P*-value
Total number of patients	88 (100)	31 (35)	57 (65)	
Donor age (years), mean ± SD	51.31 ± 10.65	51.45 ± 10.22	51.23 ± 10.96	0.87
Recipient age (years), mean ± SD	40.78 ± 18.09	40.94 ± 19.22	40.70 ± 17.62	0.92
Recipient gender				
Female	25 (28)	5 (16)	20 (35)	0.06
Male	63 (72)	26 (84)	37 (65)	
ABDR mismatches, (mean ± SD)	3.32 ± 1.63	3.71 ± 1.40	3.11 ± 1.72	0.12
ABCDRDQ mismatches, (mean ± SD)	5.10 ± 2.45	5.74 ± 2.02	4.75 ± 2.61	0.07
Total ABCDRDQ mismatches >5	36 (41)	16 (52)	20 (35)	0.17
Living related transplants	51 (58)	15 (48)	36 (63)	0.26
Specific relation donor/recipient				
Donation between parents and children	34 (39)	13 (42)	21 (37)	0.06
Father donating to child	12	4	8	
Mother donating to child	21	8	13	
Adult child donating to father	1	1	0	
Adult child donating to mother	0	0	0	
Sibling	17 (19)	2 (7)	15 (26)	
Couple	28 (32)	14 (45)	14 (25)	
Woman recipient/men donor	5	2	3	
Men recipient/woman donor	23	12	11	
Other	9 (10)	2 (7)	7 (12)	
Immunosuppression*				
TAC/M/P	36 (41)	15 (48)	21 (37)	0.29
CyA/M/P	52 (59)	16 (52)	36 (63)	
CMV donor/recipient				
Neg/Neg	30 (34)	12 (39)	18 (32)	0.92
Pos/Pos	27 (31)	9 (29)	18 (32)	
Pos/Neg	15 (17)	5 (16)	10 (17)	
Neg/Pos	16 (18)	5 (16)	11 (19)	

DSA, donor-specific antibody; CMV, cytomegalovirus; CyA, cyclosporin; M, mycophenolate; P, prednisone; TAC, tacrolimus; SD, standard deviation.
*Shows groups of initial immunosuppressive regimen.

was performed (detailed results are shown in Table 3). None of the factors recipient age, female recipient, total ABCDRDQ mismatches >5, related transplant and TAC/M/P as primary immunosuppression was statistically significant.

Correlation of *de novo* DSA with graft function

The baseline graft function defined by mean creatinine according to DSA (+) or DSA (−) status at time of discharge is shown in Table 4. At time of discharge, there was no significant difference between the two groups [mean creatinine (mg/dL): 1.47 in DSA (+) vs. 1.41 in DSA (−), ($P = 0.59$)]. In addition, Table 4 shows the development of graft function based on annualized change in mean creatinine. In the first year, a trend for a higher annual rise of mean creatinine was assessed for the *de novo* DSA (+) group, but did not reach statistical significance [mean annual creatinine change first year (mg/dL/year): 0.07 DSA (+) vs. 0.01 DSA (−), ($P = 0.82$)]. This trend was also seen in multivariate analysis (DSA (+): coefficient 0.07, 95% CI: −0.09–0.22, $P = 0.38$). This difference between the DSA (+) and DSA (−) patients can be followed in Fig. 1 (mean slope), which further allows to follow the graft function of the individual patient in case of DSA positivity. After the first year, the trend for a higher annual rise of mean creatinine persisted [mean annual creatinine change after first year (mg/dL/year): 0.15 DSA (+) vs. 0.02 DSA (−), ($P = 0.02$)], which was also analyzed by multivariate analysis: DSA (+): coefficient 0.13, 95% CI: −0.03–0.29, $P = 0.10$.

Correlation of *de novo* DSA with AR episodes

Graft dysfunction was observed in 44 cases, prompting an indicated biopsy to determine the cause of renal dysfunction (Table 5). No biopsy was taken in all other patients due to a stable renal function.

Table 5 shows that more patients in the DSA (+) (17 (55%)) group had an indication to undergo biopsy, as compared with 27 (47%) in the DSA (−) group. Pathological findings of AR were found in a total of 20 patients, with the majority of these AR episodes occurring within the first

Table 2. Characterization of *de novo* DSA (+) patients ($N = 31$): Class I and II Ab status; specific type of class I and class II Ab; range of MFI; HLA mismatch; time of first detection of DSA after transplantation.

DSA (+) patient ID	Class I and II Ab status	Time of first detection of DSA [days]	HLA MM with donor (A/B/C/DR/DQ)	DSA class I (MFI)	DSA class II (MFI)
1	+/–	1310	0/1/0/1/1	B47 (215)	–
2	–/+	1393	1/2/1/1/1	–	DQ7(3) (1825)
3	+/+	1178	1/1/1/1/1	B14 (440)	DQ6(1) (160)
4	+/–	1064	1/1/1/1/0	A1 (840)	–
5	+/–	834	2/2/0/1/1	B8 (150)	–
6	+/+	697	2/1/1/2/1	A24(9) (2640), A29(19) (240), B44(12) (1125)	DR7 (7910), DR11(5) 2130, DR53 (11750), DQ7(3) (18020)
7	+/–	643	2/2/2/0/1	Cw6 (dl)	–
8	+/–	588	2/2/2/1/0	Cw15 (dl)	–
9	–/+	540	0/0/0/1/1	–	DR4 (140)
10	+/–	503	0/0/0/1/1	Cw6 (140)	–
11	–/+	495	1/2/2/2/1	–	DR53 (925)
12	–/+	736	2/2/2/1/1	–	DR17(3) (dl)
13	+/+	412	1/1/1/1/1	A1 (520), B8 (495)	DQ2 (18870)
14	+/+	12	1/1/1/1/1	A3 (dl), B35 (dl)	DR1 (dl-380), DQ5(1) (dl-515)
15	+/–	10	2/2/1/1/1	A2 (dl)	–
16	–/+	400	1/0/0/1/1	–	DR10 (460)
17	+/+	411	0/2/1/1/1	Cw7 (285)	DQ8(3) (dl)
18	+/–	279	1/0/1/1/0	A11 (425)	–
19	+/+	212	2/2/1/1/1	B8 (dl)	DR4 (dl)
20	+/+	146	1/1/1/1/1	B57(17) (210), Cw6 (105)	DQ6(1) (560)
21	–/+	163	1/1/1/1/1	–	DR7 (390)
22	+/+	21	1/1/1/1/1	B13 (dl), Cw6 (dl-130)	DQ2 (dl-170)
23	+/+	167	1/2/0/1/1	A31(19) (670), B7 (340)	DQ5(1) (6480)
24	+/–	272	2/2/1/1/1	A1 (200–200), A3 (100–300), B57(17) (100–300), B35 (100–300)	–
25	+/+	149	2/2/0/2/2	A2 (200–500), B27 (200–600)	DQ7(3) (600–1200)
26	+/+	29	2/1/2/1/2	A1 (100–200), A23(9) (100–300), Cw6 (100–500)	DQ7(3) (1000–4000), DQ9(3) (1000–3000)
27	–/+	314	0/0/0/1/1	–	DR14(6) (100)
28	–/+	21	1/2/2/2/2	–	DQ7(3) (500–1000)
29	–/+	31	2/2/2/2/1	–	DR8 (dl-200)
30	+/–	127	1/1/1/0/0	A1 (100), B27 (100)	–
31	–/+	120	2/1/2/2/1	–	DR11(5) 100–500, DQ2 100–300

DSA, donor-specific antibody; dl, detection limit; MFI, median fluorescence intensity; DSA (+) patient ID, unique ID for individual DSA positive patient.

2 month after transplantation. With 10 AR episodes in the DSA (+) group, there was a higher rate of AR compared with patients with no DSA (32% vs. 18%, ($P = 0.18$), not statistically significant). After dividing the DSA (+) group, according to their class I and class II Ab status, 6 of these 10 patients with AR were positive for both *de novo* DSA classes. This shows a correlation with a significant higher AR rate for patients with *de novo* DSA in class I and class II [DSA (+) simultaneously: 6 (55%), ($P = 0.05$)].

Splitting the group of AR in AMR and TCMR, AMR was observed in five cases in total and showed significant positivity for the presence of *de novo* DSA (four of these patients were positive for DSA ($P = 0.05$)). Three of them displayed a diffuse C4d positivity, two a focal C4d positivity. The only patient with AR and no detected *de novo* DSA showed histologic chronic AMR and lost his graft despite rejection therapy including bortezomib (BZ). The individual treatments of the AR episodes, occurred within the study group, are listed in Table 6.

Discussion

In this study, a systematic analysis of post-transplant *de novo* DSA development was conducted in a cohort of 88 LDRT. A total of 31 (35%) patients developed *de novo* DSA after transplantation. Among former studies, which also investigated the development of alloantibodies after transplantation, there was a high variability in the frequency of alloantibodies, ranging from 1.6% to 60% of the patients [16]. This great variation is most likely due in part to

Table 3. Multivariable logistic regression analysis of risk factors for *de novo* DSA development.

Variable	Odds ratio	95% CI	*P*-value
Recipient age (per year)	0.99	0.96–1.02	0.39
Recipient female (versus male)	0.38	0.12–1.16	0.09
Immunosuppression TAC/M/P (versus CyA/M/P)	1.13	0.4–3.15	0.82
Total ABCDRDQ mismatches >5 (versus ≤5)	1.61	0.41–6.43	0.50
Related transplant (versus unrelated)	0.73	0.16–3.26	0.68

DSA, donor-specific antibody; CI, confidence interval; CyA, cyclosporin; M, mycophenolate; P, prednisone; TAC, tacrolimus.
The dependent variable in this analysis is *de novo* DSA status [DSA (+) vs. DSA (−)].

Table 4. Follow-up of renal function according to *de novo* DSA status.

	All patients	DSA (+)	DSA (−)
Baseline creatinine (mg/dL) mean ± SD (time of discharge)	1.43 ± 0.71	1.47 ± 0.71[a]	1.41 ± 0.71
Mean annual increase in creatinine first year (mg/dL/year)	+0.03	+0.07[b]*	+0.01
Mean annual increase in creatinine after first year (mg/dL/year)	+0.06	+0.15[c]†	+0.02

DSA, donor-specific antibody; SD, standard deviation; CI, confidence interval.
[a]$P = 0.59$, DSA(+) vs. DSA(−).
[b]$P = 0.82$, DSA(+) vs. DSA(−).
[c]$P = 0.02$, DSA(+) vs. DSA(−).
*DSA (+): coefficient 0.07, 95% CI: −0.09–0.22, $P = 0.38$.
†DSA (+): coefficient 0.13, 95% CI: −0.03–0.29, $P = 0.10$ for multivariable linear regression analysis including the factors: *de novo* DSA, baseline creatinine (for first year analysis)/mean creatinine at 1 year (for after the first year analysis), immunosuppression (TAC/M/P, CyA/M/P), total ABCDRDQ mismatches >5, donor–recipient relation (related/unrelated), donor age, recipient gender.

different MFI thresholds, which are used for the assessment of positive and negative reactions and which differ among different studies and centers [17]. Therefore, the rate of *de novo* DSA (35%) observed in this study group was higher than reported in many former studies. This is possibly due to a lower and more sensitive MFI threshold of ≥100 considered to be *de novo* DSA positive. Although many centers use a MFI threshold of ≥1000 or higher for *de novo* DSA positivity, a strong correlation between *de novo* DSA positivity and transplant outcome, assessed with a low MFI threshold of ≥300 for *de novo* DSA positivity, was recently reported by Wiebe *et al.*[14]. These findings underline the clinical importance of *de novo* DSA even at low levels. Nevertheless, to date, no clear consensus for MFI thresholds has been established [17], even though there is a strong need for developing standardized guidelines for testing and management of DSA in solid organ transplantation [18]. Unless this situation has markedly changed, we acknowledge DSA even at lower levels as important reports for clinical management and as relevant information to analyze in our study group.

As shown in Table 4 and Fig. 1, the presence of *de novo* DSA was not correlated with poor graft function in the early period after transplantation. Comparing the mean annual creatinine change between the DSA (+) and DSA (−) group for the first year after transplantation (Table 4 and mean slope in Fig. 1), there was a trend for inferior graft function in case of *de novo* DSA positivity, but it did not reach statistical significance. The negative effects of *de novo* DSA on graft function may first be seen in long term outcome, as *de novo* DSA are known to be involved in the processes leading to chronic allograft failure [14,19]. By looking at the individual patient (Fig. 1) and at the overall function of the DSA (+) and the DSA (−) group, a tendency of impairment of graft function after the first year can be reported for the study group [mean annual creatinine change after first year (mg/dL/year): 0.15 DSA (+) vs. 0.02 DSA (−), ($P = 0.02$); multivariate regression analysis: DSA (+): coefficient 0.13, 95% CI: −0.03–0.29, $P = 0.10$]. This result should be seen with the limitation that the length of follow-up varied between one and up to 4 years (Fig. 1), and therefore, not all of the patients were part of the analysis after the first year. However, for the future, it will be of great interest if this trend could be confirmed for the study group.

A very similar effect of *de novo* DSA was recently reported by Everly *et al.*[20]. They report on 189 patients that were transplanted between 1999 and 2006. About 20% of the patients developed *de novo* DSA. 24% of these grafts with development of *de novo* DSA failed within the following 3 years.

None of the variables that were analyzed as risk factors for appearance of the *de novo* DSA was statistically significant. In particular, a correlation between *de novo* DSA development and HLA antigen mismatches or the kind of immunosuppressive regimen, as could be seen in former studies, was not observed in this study group (Tables 1 and 3) [14,21].

De novo DSA detected at the time of AR are known to be associated with reduced allograft survival, and the strong correlation between the appearance of *de novo* DSA and AR was shown in recent studies [10,14]. As summarized in Table 5, a trend for a higher rate of AR in case of

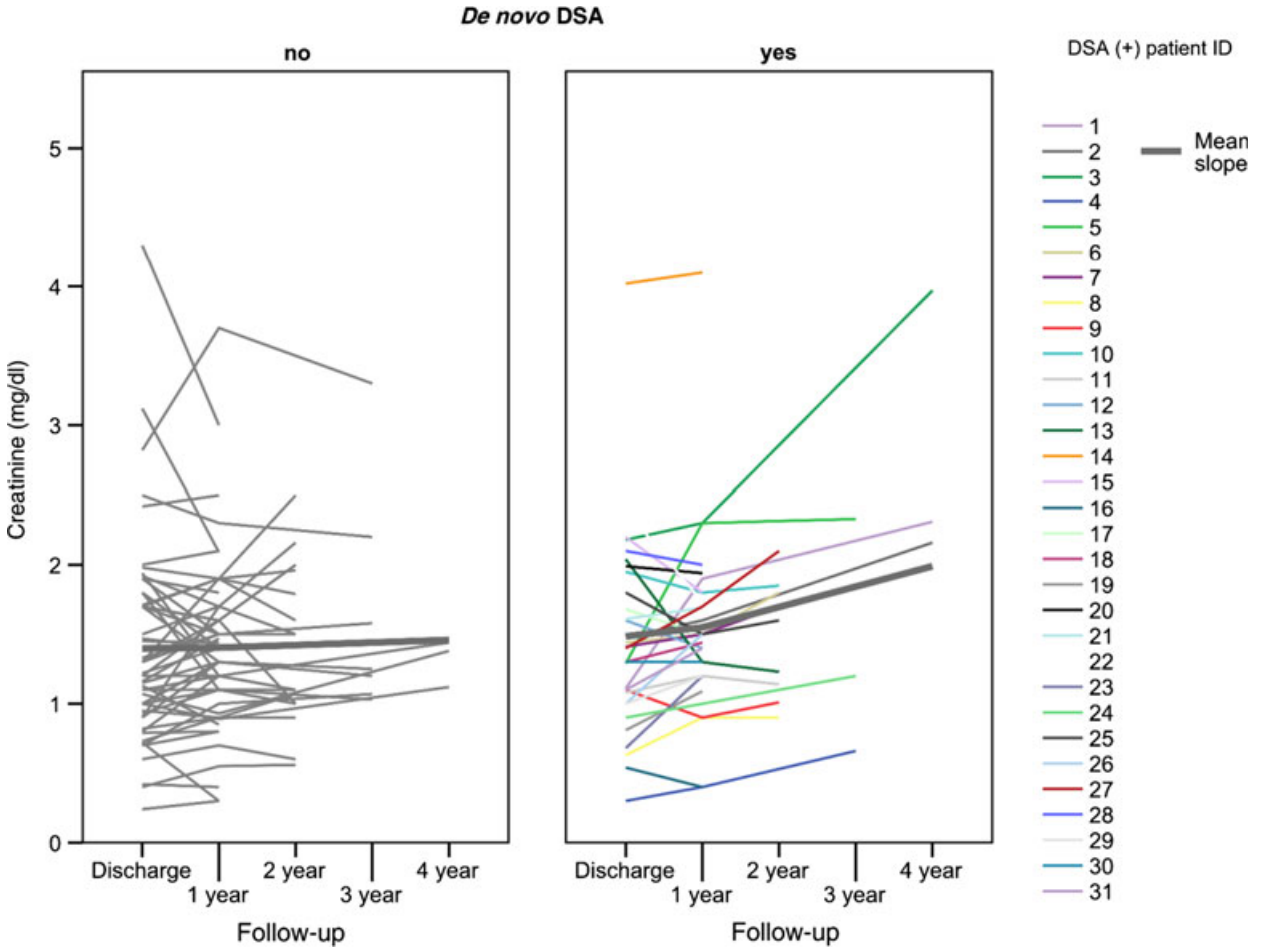

Figure 1 Renal function according to *de novo* DSA status at different points of follow-up; mean annual creatinine change for DSA (+)/ DSA (–) groups. No: patients without *de novo* DSA; yes: patients with *de novo* DSA; creatinine (mg/dL): values measured at the time of discharge (baseline creatinine), 1 year after transplantation and at the end of follow-up; mean slope = bold gray line: mean annual creatinine change first year and after first year (mg/dL/year) anchored at 1 year mean; DSA (+) patient ID: unique ID for individual DSA positive patient.

Table 5. Incidence of AR episodes according to *de novo* DSA status.

	All Patients (n = 88)	DSA(+) (n = 31)	DSA (–) n (n = 57)	DSA class I (n = 10)	DSA class II (n = 10)	DSA class I + II (n = 11)
Number of patients received biopsy (%)	44 (50)	17 (55)	27 (47)			
AR episode in total (%)	20 (23)	10 (32)	10 (18)	1 (10)	3 (30)	6 (55)[a]
AMR (%)	5 (6)	4 (13)[b]	1 (2)	0 (0)	1 (10)	3 (27)
TCMR (%)	16 (18)	7 (23)	9 (16)	1 (10)	2 (20)	4 (36)

DSA, donor-specific antibody, AR, acute rejection; AMR, antibody-mediated rejection; TCMR, T-cell-mediated rejection.
[a]P = 0.05, DSA(–)/DSA class I/DSA class II vs. DSA class I+II.
[b]P = 0.05, DSA(+) vs. DSA(–).

de novo DSA positivity was seen in the study group (32% vs. 18%, (P = 0.18)). Further, there was a significant correlation between the simultaneous presence of *de novo* DSA class I and class II and AR episodes [6 (55%), (P = 0.05)]. Therefore, among the large number of patients with *de novo* DSA, patients with coexistence of *de novo* DSA against HLA antigens class I and class II are at higher risk of developing clinical relevant rejection. Three of the six AR episodes in the group of patients with simultaneous presence of *de novo* DSA were classified as AMR (Table 5), whereas in the group of all *de novo* DSA (+) patients, without splitting in groups according to DSA

Table 6. Characterization of AR episodes: *de novo* DSA status, timing, pathological findings, therapy.

DSA status	DSA (+) patient ID	Class I and II Ab status	Interval NTX until AR	TCMR	Banff	AMR	C4d	Therapy
DSA (+)	3	pos/pos	2 weeks	pos	IA	neg		ATG, CIR, SP
DSA (+)	6	pos/pos	4 month	pos	IIA	neg		ATG, IA, PP, SP
DSA (+)	12	neg/pos	1 week	neg		pos	f-pos	IA, BZ, SP
DSA (+)	14	pos/pos	1 week	pos	IIA	pos	d-pos	ATG, BZ, IA, SP
DSA (+)	15	pos/neg	1 week	pos	BC	neg		ATG, SP
DSA (+)	21	neg/pos	4 weeks	pos	BC: IA	neg		SP
DSA (+)	22	pos/pos	3 weeks	neg		pos	d-pos	IA, SP, RM
DSA (+)	25	pos/pos	2.5 month	pos	II A	neg		ATG, CIR, SP
DSA (+)	26	pos/pos	1 month	neg		pos	f-pos	ATG, CIR, RM, PP, SP
DSA (+)	31	neg/pos	1 month	pos	BC	neg		CIR, SP
DSA (–)		neg/neg	1 week	pos	IB	neg		ATG, CIR, SP
DSA (–)		neg/neg	2 weeks	neg		pos	d-pos	BZ, PP, SP
DSA (–)		neg/neg	8 weeks	pos	IIA	neg		ATG, CIR, IA, SP
DSA (–)		neg/neg	1 week	pos	BC	neg		ATG, BZ, CIR, IA, SP
DSA (–)		neg/neg	4 weeks	pos	IA + IIA	neg		ATG, SP
DSA (–)		neg/neg	2 weeks	pos	IA	neg		SP
DSA (–)		neg/neg	2 weeks	pos	IIA	neg		SP
DSA (–)		neg/neg	2 month	pos	II B	neg		CIR, SP
DSA (–)		neg/neg	5 month	pos	I A	neg		CIR, SP
DSA (–)		neg/neg	1 month	pos	BC	neg		ATG, SP

NTX, kidney transplantation; DSA, donor-specific antibody; AR, acute rejection; TCMR: T-cell-mediated rejection; AMR, antibody-mediated rejection; BC, borderline changes; f-pos, fokal positivity: d-pos, diffuse positivity; CIR, change of immunosuppressive regimen; ATG, antithymocyte globulin; SP, steroid pulse; PP, plasmapheresis; BZ, bortezomib; IA, immunoadsorption; RM, rituximab; DSA (+) patient ID, unique ID for individual DSA positive patient.

subclasses, four of five patients developed AMR ($P = 0.05$).

The limitations of this study are the limited time of follow-up for parts of the study group, the variable period of time of HLA antibody detection after transplantation, and the lack of surveillance biopsies.

The frequency and timing of DSA monitoring is still in discussion. Concordantly with a recently published consensus recommendation [18], we recommend to perform an initial screening in low risk patient within the first 3 month after transplantation and 1 year after transplantation. Patients that developed *de novo* DSA should get a biopsy and treated if any changes are found. However, those patients that are positive for *de novo* DSA and do not show any alteration on biopsy should be offered a prospective intervention trial. In our opinion, this trial should evaluate B-cell directed therapies, which have been shown by single-center reports to reduce DSA [22].

In conclusion, our results show that monitoring *de novo* DSA after LDRT may help to identify patients at risk of declining renal function. Patients with simultaneous presence of *de novo* DSA against HLA antigens class I and class II displayed the highest risk to suffer from AR episodes. Randomized studies are needed to address the question of optimized immunosuppression in these patients with an increased risk of a decline in graft function.

Authorship

GD and VD: Contributed equally to the manuscript and are joint first authors. GD and VD: Participated in performing HLA antibody testing, collecting clinical data, analyzing data, and writing the article. AH, TK, and WA: Participated in collecting patient clinical data. MH: Participated in analyzing the data. UB: Participated in supervising the HLA antibody testing and in developing the study design. DLS: Participated in developing the study design, supervising the study, and writing the article. All authors read and approved the final manuscript.

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