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A paired kidney analysis on the impact of pretransplant anti-HLA antibodies on graft survival

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Abstract

Background

Pretransplant donor-specific anti-HLA antibodies (DSA) are associated with impaired kidney graft survival, while the clinical relevance of non-donor specific anti-HLA antibodies (nDSA) is more controversial. The aim of the present paired kidney graft study was to compare the clinical relevance of DSA and nDSA.

Methods

To eliminate donor and era-dependent factors, a *post-hoc* paired kidney graft analysis was performed as part of a Dutch multicentre study evaluating all transplantations between 1995-2005 with available pretransplant serum samples. Anti-HLA antibodies were detected with a luminex single antigen bead assay.

Results

Among 3237 deceased donor transplantations, we identified 115 recipient pairs receiving a kidney from the same donor with one recipient being DSA positive and the other without anti-HLA antibodies. Patients with pretransplant DSA had a significantly lower 10-year death censored graft survival (55% vs. 82%, $p=0.0001$). Among 192 pairs with one recipient nDSA positive against either class I or II and the other without anti-HLA antibodies, graft survival did not significantly differ (74% vs. 77%, $p=0.79$). Only in patients with both nDSA class I and II there was a trend towards a lower graft survival (58%, $p=0.06$). Lastly, in a small group of 42 recipient pairs 10-year graft survival in recipients with DSA was 49% compared to 68% in recipients with nDSA ($p=0.11$).

Conclusion

This paired kidney analysis confirms that the presence of pretransplant DSA in deceased donor transplantations is a risk marker for graft loss, whereas nDSA in general are not associated with a lower graft survival. Subgroup analysis indicated that only in broadly sensitized patients with nDSA against class I and II, nDSA may be a risk marker for graft loss in the long term.

Introduction

Pretransplant donor-specific anti-HLA antibodies (DSA) are a well-recognized and important risk factor for antibody mediated rejection and graft loss following kidney transplantation (1-4). DSA binding to the donor endothelium may activate the complement cascade resulting in leukocyte recruitment and endothelial cell activation inducing a proinflammatory and prothrombotic phenotype or endothelial cell lysis in the case of lytic membrane attack complex levels (5,6). Another potential mechanism by which DSA may cause graft injury is antibody-dependent cellular cytotoxicity (5,6). For non-donor specific anti-HLA antibodies (nDSA) more controversy on their clinical relevance exists. Some have suggested that nDSA resemble an intermediate risk phenotype for graft loss and antibody-mediated rejection and require an intensified immunosuppressive strategy (7,8). The magnitude of the observed impact on 5-year graft survival for patients with nDSA compared to no anti-HLA antibodies in these studies ranged from an absolute difference of 9% to 23%. In contrast, several other studies did not find an inverse association between pretransplant nDSA and graft survival (9-12). Disparities in donor characteristics and transplant era dependent factors may interfere with these analyses. Therefore, the aim of this study was to study the impact of DSA and nDSA on kidney transplant outcomes in a paired kidney graft analysis.

Methods

We have performed a post-hoc analysis on the PROCARE cohort, which includes all transplantations in the Netherlands between 1995 and 2005 with available pretransplant sera and clinical follow-up (13). All transplantations performed during that time required a negative T-cell complement-dependent cytotoxicity crossmatch with both peak and current sera. Solid phase assays for the detection of pretransplant antibodies were not used during that era. Detailed methods on the cohort have been described previously (14). In brief, pretransplant sera were tested with a Luminex screening assay (Lifecodes LifeScreen, Immucor, Norcross, GA). Positive sera were subsequently tested with a single antigen assay (Lifecodes LSA class I or class II kit, Immucor). We used the cut-off as defined by the manufacturer requiring a certain signal to noise ratio to determine bead positivity, which results in virtually identical results as when taking an absolute median fluorescence intensity (MFI) cut-off of 750. DSA were assigned for HLA-A/B/DR/DQ by comparing bead specificities on serological level with the HLA type of the donor on split level. Because HLA-C and HLA-DP typing was not routinely performed, all anti-HLA-C and -DP antibodies were assigned as nDSA. Clinical data were obtained from the Dutch Organ Transplant Registry. We identified all recipient pairs who received a kidney from the same donor within the PROCARE cohort. Subsequently, we constructed three groups based on a difference in pretransplant anti-HLA antibody status: group 1 (no HLA antibodies vs. DSA), group 2 (no HLA antibodies vs. nDSA) and group 3 (nDSA vs. DSA). Recipient pairs with a similar pretransplant antibody status were excluded from analysis. Primary outcome was 10-year death-censored graft survival; secondary outcomes were 10-year graft survival uncensored for death and 1-year rejection free survival. All patients provided written informed consent for use of their clinical data and leftover sera. The study protocol was approved by the Biobank Research Ethics Committee of the UMC Utrecht (TC Bio 13-633) and performed in accordance with the Declaration of Helsinki.

Statistical analyses were done with SAS 9.4 (SAS Institute Inc., Caru, NC) and R 3.2.2. Categorical data were analysed with the chi-square test and continuous data with the t-test or Mann-Whitney U test as appropriate. Survival analyses were performed by constructing Kaplan-Meier curves and tested for significance with the log-rank test. With Cox multiple regression we have adjusted for potential confounders including recipient age, cold ischemia time, induction therapy and calcineurin inhibitor use. To assess the robustness of the results, we additionally performed a cumulative incidence analysis and Fine and Gray competing risk regression where death with a functioning graft was considered as a competing event (15,16). The same covariates as in the Cox multiple regression analyses were entered in the Fine and Gray competing risk regression model.

Results

Between 1995 and 2005, 3237 deceased donor transplantations with available pretransplant sera were performed in the Netherlands. Within this group we identified 778 recipient pairs who received a kidney from the same donor. In the other cases one of the kidneys went to a recipient in another country, one of the kidneys was not suitable for transplantation or pretransplant DSA status was unknown for one of the recipients. In 349 recipient pairs pretransplant anti-HLA antibody status differed (Figure 1). Group 1 consisted of 115 recipient pairs with one recipient being DSA positive and the other recipient without anti-HLA antibodies; group 2: 192 recipient pairs with one recipient with nDSA and the other recipient without anti-HLA antibodies; and group 3: 42 recipient pairs with one recipient with DSA and the other recipient with nDSA, transplanted with a kidney from the same donor. Recipient, donor and transplant characteristics for the groups are summarized in Table 1. Compared to their paired recipients, patients with DSA and nDSA in groups 1 and 2 were more often female, retransplants and had a higher median peak panel reactive antibody (PRA). A trend towards a higher usage of induction therapy in patients with DSA in group 1 was observed. Lastly, patients with nDSA in group 2 were treated more frequently with tacrolimus instead of cyclosporine compared to patients without anti-HLA antibodies. Despite these small differences, overall immunosuppressive treatment among the different groups was largely comparable. Peak PRA was higher in the nDSA patients in group 3 compared to the nDSA patients in group 2.

Ten-year death-censored graft survival was significantly lower in patients with DSA compared to the paired recipients without anti-HLA antibodies (55% vs. 82%, figure 2A). Patients with DSA against both class I and II had the lowest graft survival ($n=22$, 43%), while the survival rate was 57% and 59% for patients with either class I or class II DSA ($n=58$ and $n=35$, overall $p=0.0006$; supplementary figure 1). When combining graft failure and death with a functioning transplant, graft survival remained lower for patients with DSA (44%) compared to the paired patients without anti-HLA antibodies (57%, $p=0.046$). After adjustment for recipient age, cold ischemia time and induction therapy, DSA was still associated with a lower death-censored and uncensored graft survival (table 2).

Overall 10-year death-censored graft survival did not differ significantly between recipient pairs with nDSA and without anti-HLA antibodies ($p=0.27$). However, as shown in figure 2B the curves are almost identical during the first five years, whereupon they seem to diverge slightly in disadvantage of the patients with nDSA. We next stratified patients with nDSA based on HLA classes. Patients with nDSA class I and II showed a markedly higher peak PRA (median 36, IQR 11-53) and more often had a retransplant (61%) as compared to patients with nDSA against either class I or II (supplementary table 1). Death-censored graft survival was similar for patients with nDSA class I (73%) or class II (76%) as compared to patients without anti-HLA antibodies throughout the entire follow-up period, whereas this was not the case for patients with nDSA class I and II (58%, overall $p=0.11$; figure 3). Compared to their paired recipients without anti-HLA antibodies, patients with nDSA class I and II tended to have a lower 10-year death-censored graft survival ($p=0.06$). Grouping patients with nDSA in tertiles based on their maximum or cumulative MFI of the positive beads did not

identify any significant correlation with death-censored graft survival ($p=0.21$ and $p=0.66$). Death with a functioning graft occurred slightly more often in the nDSA group, resulting in a lower overall graft survival of 50% compared to 63% in the group without anti-HLA antibodies ($p=0.02$). After adjustment for potential confounders including recipient age, which was on average slightly higher in the nDSA group, this difference was not significant anymore (HR 1.28, 95% CI 0.93-1.76; table 2).

Finally, we compared death-censored graft survival among 42 recipient pairs with one being DSA positive and the other nDSA positive (figure 1C). Within this small group, we observed a trend towards a lower death-censored graft survival in patients with DSA (49%) as to patients with nDSA (68%, $p=0.11$) and a similar trend for overall graft survival ($p=0.05$).

1-year rejection-free survival censored for death and failure unrelated to rejection did only differ between recipients with DSA (61%) compared to patients without anti-HLA antibodies (78%, adjusted HR 2.15, 95%CI 1.29-3.56), but not between the other groups (table 2).

To test the robustness of our findings we also performed a competing risk analysis to rule out any major hinder of death as a competing event on the association between pretransplant antibody status and graft survival. Cumulative incidence curves were in line with the complement of the Kaplan Meier curves (supplementary figure 2). The sub-distribution hazard ratios were also within the range of the hazard ratios that were obtained by Cox regression (supplementary table 2).

Discussion

The results of this paired kidney graft analysis confirm the association between pretransplant DSA and incidence of rejection and graft loss. For nDSA however, we did not observe such an adverse association with transplant outcomes. Subgroup analysis revealed that only the combination of nDSA against class I and II were associated with a lower death-censored graft survival in the long term, although this did not reach statistical significance.

A major strength of this study is the paired kidney design. Multivariable analyses have a limited accuracy to adjust for differences in donor organ quality and other era-dependent changes, partly due to the presence of unmeasured confounders (17). By performing a paired kidney analysis we have eliminated these factors. Within the remainder of the PROCARE cohort graft failure, including death with a functioning graft, did not differ between patients with nDSA and without anti-HLA antibodies ($p=0.33$, unpublished data). In our paired analysis, the incidence of death with a functioning graft was slightly higher in patients with nDSA. This could be the result of a higher recipient age in the nDSA group. Indeed, we did not observe a significant difference anymore after adjusting among others for recipient age.

Most studies evaluating the effect of pretransplant DSA on transplant outcomes compare DSA positive and DSA negative patients without making a distinction between patients without anti-HLA antibodies and patients with nDSA (3,18,19). The few studies that did make this distinction, had relatively small sample sized and reported conflicting outcomes on the clinical relevance of luminex defined nDSA. Several studies did not find a difference in graft survival (9-12). In contrast, Richter et al. showed in a population of 197 transplant patients that the presence of nDSA ($n=39$) was associated with a markedly lower 5-year death censored graft survival (62.2% vs. 90.8%, HR 4.91, 95%CI 1.43-16.91) (7). Malheiro et al. reported in a cohort consisting of 756 transplantations a smaller, but significant difference in 5-year graft survival between patients with nDSA and patients without anti-HLA antibodies (88% vs. 94%, HR 2.24, 95%CI 1.19-4.37) (8). Importantly, in both studies the average number of HLA-A, -B and -DR mismatches was higher than in our population (7,8). Richter et al. showed in immunized patients (nDSA and/or DSA) that graft survival was primarily affected in patients with HLA-DR mismatches (7). Because of our relatively well-matched population, we cannot exclude an effect of nDSA on graft survival in the presence of multiple HLA-DR and linked -DQ mismatches (20).

One of the hypothesized mechanisms by which the presence of nDSA is associated with impaired graft survival is that they reflect the potential to form HLA antibodies upon allorecognition (7,8). This could indicate that these patients may be more prone to develop *de novo* DSA (20). However, there is limited evidence actually supporting a correlation between pretransplant nDSA and *de novo* DSA (21). These patients could also have previously formed donor-specific antibodies that are not detectable at time of transplantation but that can become rapidly detectable posttransplantation because of resident

HLA-specific memory B-cells (20). HLA-specific B-cell ELISPOT assays could provide additional insights on this (22,23). Another suggested mechanism is that nDSA can contribute to graft injury by epitope sharing between the nDSA specificities and donor-specific antigens (24,25). The suggested reduced graft survival in patients with nDSA against class I and II, might rather be the result of the high number of retransplants and subsequent broad allosensitization as reflected by a median peak PRA of 36 (IQR 11-53). Peak PRA levels have been associated with graft survival in several studies (26-28). The chance of epitope sharing between nDSA specificities and donor-specific antigens might also be greater in these more broadly sensitized patients.

This study also has some limitations. We do not have information on the development of *de novo* DSA following transplantation, a well known risk indicator for rejection and poorer graft survival (29). Furthermore, we assigned all anti-HLA-C and -DP antibodies as nDSA since HLA-C and -DP typing was not routinely performed. Potentially, some of these antibodies were donor-specific. However, if any effect we would expect this to influence the graft survival for the nDSA group in a negative way, which was not the case. Moreover, exclusion of patients with nDSA against HLA-C or -DP from group 2 did not alter the conclusions (supplementary figure 3).

In conclusion, this paired kidney analysis shows that pretransplant nDSA per se are not an additional risk marker for graft failure in deceased donor transplantations. Only in broadly sensitized patients with nDSA against class I and II, nDSA may be a marker for graft loss on the long term. For pretransplant DSA we confirm the adverse association with graft survival, suggesting that pretransplant DSA status should be considered in deceased donor transplantations in terms of donor acceptance and immunosuppressive treatment.

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Conflict of Interest statement

The authors of this manuscript have conflicts of interests to disclose. LM is supported by an unrestricted research grant from Astellas pharma. ES is listed as inventor of a patent unrelated to this manuscript. AZ received personal fees from Astellas pharma, Novartis and Chiesi outside the manuscript. None of the other authors have any conflict of interest to disclose.

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Figures

Figure 1: Flowchart for the in- and exclusion of patients.

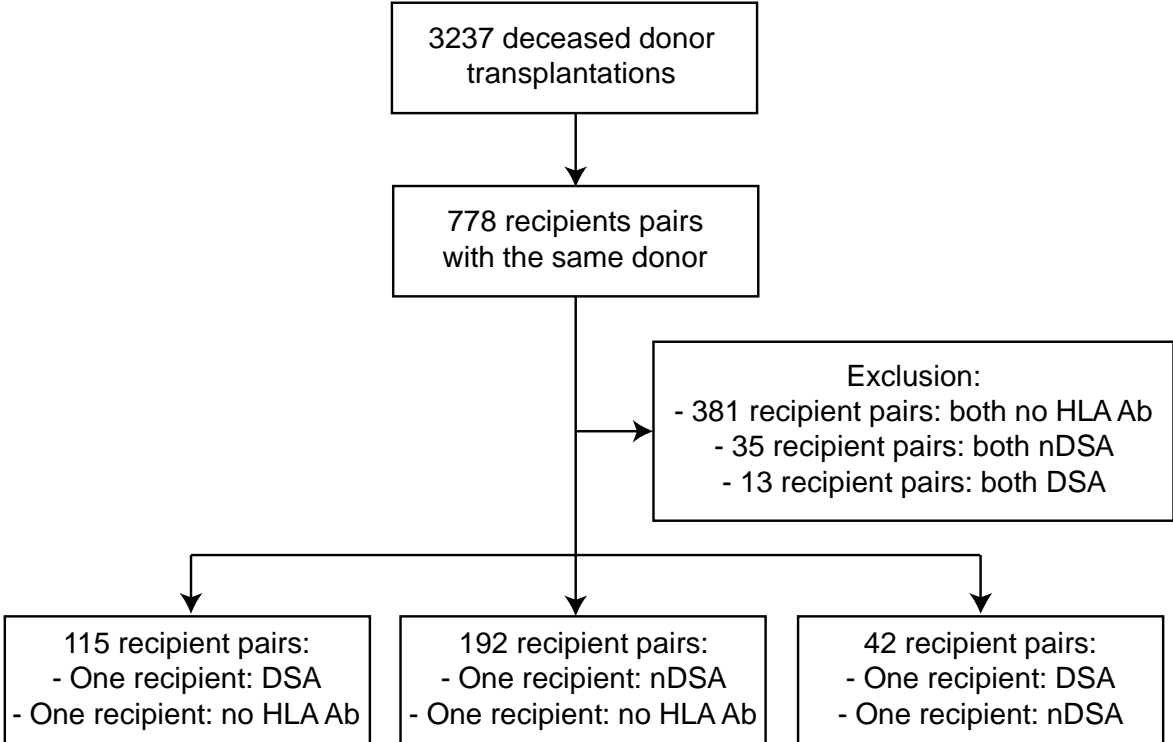
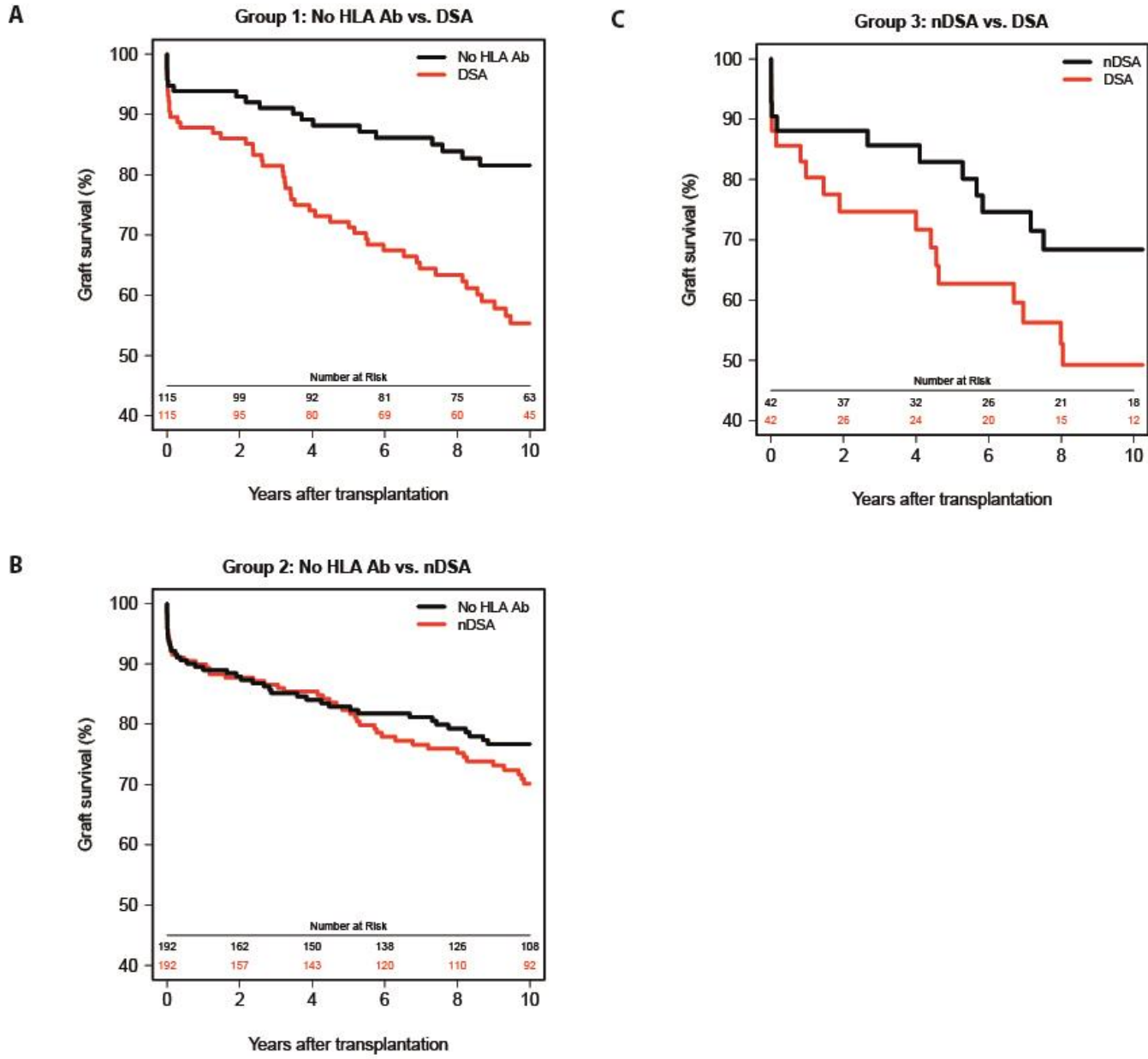
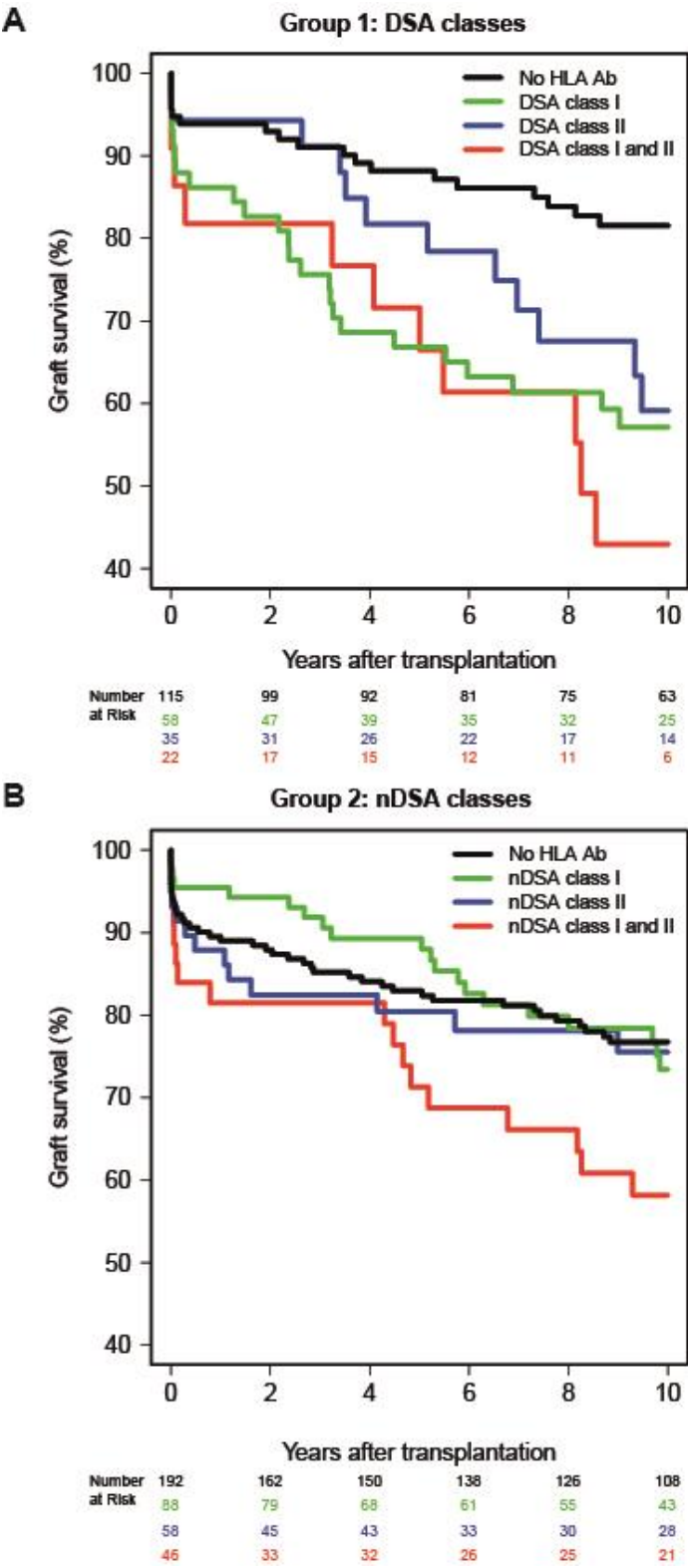


Figure 2: Death-censored 10-year graft survival stratified according to pretransplant anti-HLA antibody status.



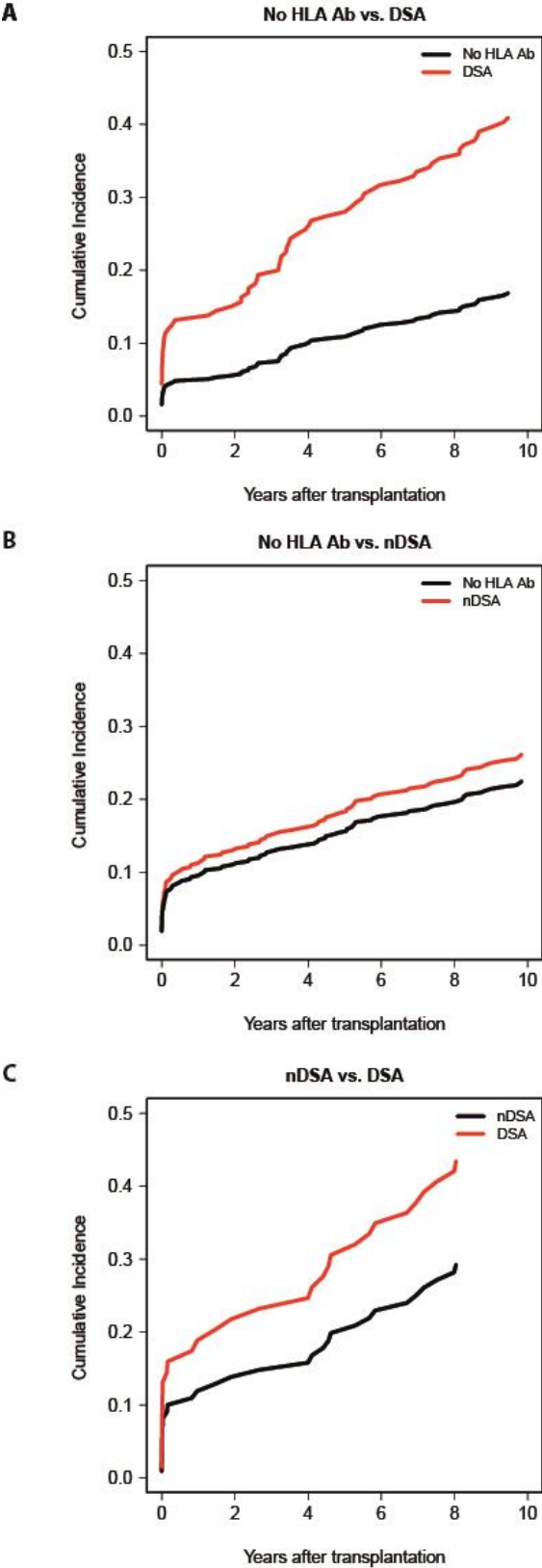
(A) DSA compared to no anti-HLA Ab (55% vs. 82%, $p=0.0001$). (B) nDSA compared to no anti-HLA Ab (70% vs. 77%, $p=0.27$). (C) DSA compared to nDSA (49% vs. 68%, $p=0.11$).

Figure 3: Death-censored 10-year graft survival stratified according to DSA and nDSA classes.



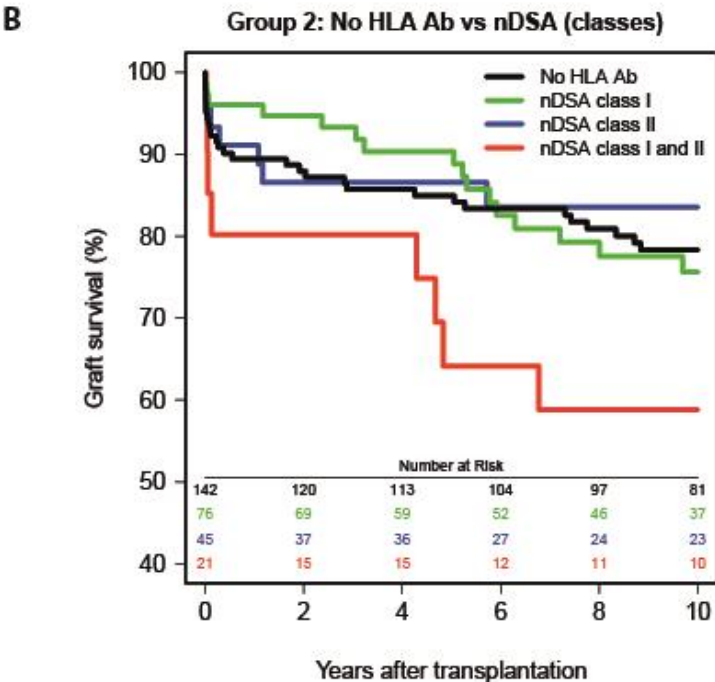
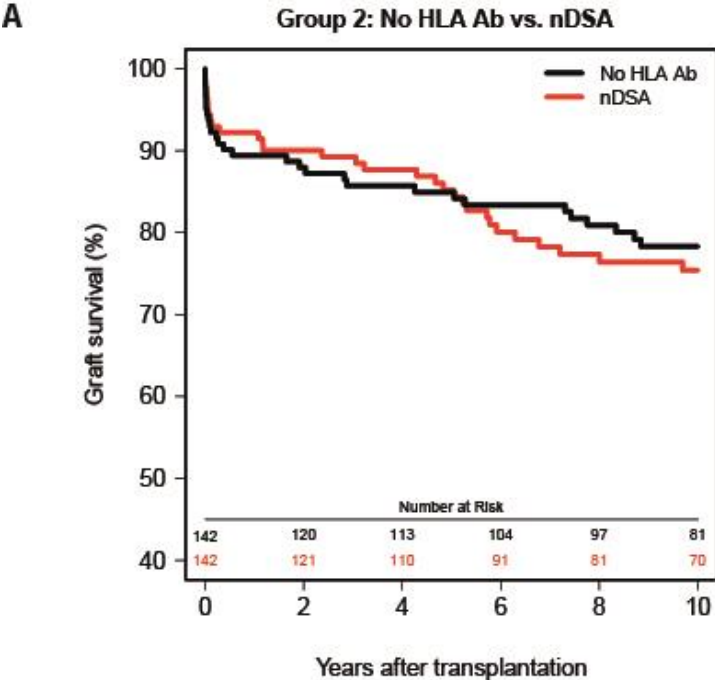
(A) Graft survival was lower in patients with DSA class I and II (43%) compared to patients with either class I or II DSA (57%, 59%) or no HLA antibodies (82%, overall $p=0.0006$). (B) Graft survival was lower in patients with nDSA class I and II (58%) compared to patients with either class I or II nDSA or no HLA antibodies (overall $p=0.11$).

Supplementary figure 1: Cumulative incidence of graft failure stratified according to pretransplant anti-HLA antibody status. Death with a functioning transplant was considered as a competing event.



(A) DSA compared to no anti-HLA Ab (0.17 vs. 0.42, $p=0.0001$). (B) nDSA compared to no anti-HLA Ab (0.26 vs. 0.22, $p=0.40$). (C) DSA compared to nDSA (0.44 vs. 0.29, $p=0.18$).

Supplementary figure 2: Death-censored 10-year graft survival for patients with nDSA compared to their paired recipients with no anti-HLA Ab, excluding patients with anti-HLA antibodies against HLA-C and/or -DP.



(A) nDSA compared to no anti-HLA Ab (75% vs. 78%, $p=0.67$). (B) Graft survival in patients with nDSA class I and II (59%) was lower compared to patients with either class I or II nDSA or no HLA antibodies (overall $p=0.18$).

Tables

Table 1: Baseline characteristics

	Group 1			Group 2			Group 3		
	No HLA Ab (n=115)	DSA (n=115)	<i>p</i> -value	No HLA Ab (n=192)	nDSA (n=192)	<i>p</i> -value	nDSA (n=42)	DSA (n=42)	<i>p</i> -value
Donor:									
Donor Age	43.7 ± 17.8	43.7 ± 17.8		41.6 ± 16.5	41.6 ± 16.5		45.7 ± 14.9	45.7 ± 14.9	
Donor Sex, male	67 (58%)	67 (58%)		108 (56%)	108 (56%)		24 (57%)	24 (57%)	
Donortype									
• DBD	76 (66%)	76 (66%)		119 (62%)	119 (62%)		29 (69%)	29 (69%)	
• DCD	39 (34%)	39 (34%)		73 (38%)	73 (38%)		13 (31%)	13 (31%)	
Recipient:									
Recipient Age	48.3 ± 13.0	46.6 ± 13.5	0.34	47.6 ± 13.0	50.4 ± 13.0	0.05	48.2 ± 13.0	46.4 ± 12.5	0.48
Recipient Sex, male	84 (73%)	50 (44%)	<0.0001	142 (74%)	89 (46%)	<0.0001	16 (38%)	18 (43%)	0.65
Retransplantation	1 (1%)	53 (46%)	<0.0001	7 (4%)	64 (33%)	<0.0001	15 (36%)	23 (55%)	0.08
Peak PRA*	0 (0-5)	46 (9-72)	<0.0001	0 (0-4)	8 (0-44)	<0.0001	18 (3-55)	66 (37-85)	0.0009
Transplantation:									
HLA-A, -B, DR mm	2.6 ± 1.2	2.5 ± 1.0	0.99	2.5 ± 1.2	2.4 ± 1.1	0.52	2.1 ± 1.3	2.1 ± 1.1	0.93
CIT (hours)	20.7 ± 7.8	21.7 ± 6.3	0.12	21.2 ± 7.3	21.9 ± 6.7	0.37	22.9 ± 7.5	24.5 ± 7.0	0.41
Induction			0.07			0.31			0.45
• II2rMoAb	24 (21%)	27 (24%)		49 (26%)	41 (22%)		8 (19%)	6 (14%)	
• T-cell depleting	2 (2%)	9 (8%)		3 (2%)	7 (4%)		2 (5%)	5 (12%)	
CNI			0.11			0.005			0.45
• Cyclosporine	61 (53%)	60 (61%)		113 (59%)	90 (47%)		27 (64%)	24 (57%)	
• Tacrolimus	44 (38%)	42 (37%)		65 (34%)	95 (49%)		12 (29%)	17 (41%)	
• None	10 (9%)	3 (3%)		14 (7%)	7 (4%)		3 (7%)	1 (2%)	
Antimetabolite			0.72			0.88			0.19
• MMF/MPA	86 (75%)	91 (79%)		139 (72%)	137 (71%)		29 (69%)	33 (79%)	
• Azathioprine	2 (2%)	2 (2%)		7 (4%)	9 (5%)		3 (7%)	0	
• None	27 (23%)	22 (19%)		46 (24%)	46 (24%)		10 (24%)	9 (21%)	

Steroids		115 (100%)	113 (98%)	0.16		188 (98%)	188 (98%)	1		42 (100%)	41 (98%)	0.31
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Data are depicted as number (percentage) or mean \pm standard deviation unless otherwise stated.

* Median (interquartile range)

Abbreviations: Ab, antibodies; CIT, cold ischemic time; CNI, calcineurin inhibitor; DBD, donation after brain death; DCD, donation after circulatory death; DSA, donor-specific anti-HLA antibodies; I12rMoAb, Interleukin 2 receptor monoclonal antibody; mm, mismatch; MMF, mycophenolic mofetil; MPA, mycophenolic acid; nDSA, non-donor specific anti-HLA antibodies; PRA, panel reactive antigen.

Table 2: Transplant outcomes compared among groups of recipients with different anti-HLA antibody status

	DSA vs. no HLA Ab	nDSA vs. no HLA Ab	DSA vs nDSA*
	HR (95% CI)	HR (95% CI)	HR (95% CI)
10-year death-censored graft failure	2.75 (1.61-4.68) ¹	1.26 (0.84-1.90) ¹	1.81 (0.87-3.76) ¹
	2.94 (1.69-5.10) ²	1.10 (0.73-1.68) ²	-
10-year graft failure including death with a functioning graft	1.46 (1.01-2.12) ¹	1.46 (1.07-1.99) ¹	1.74 (0.99-3.06) ¹
	1.56 (1.06-2.28) ²	1.28 (0.93-1.76) ²	-
Rejection within year 1	2.12 (1.29-3.50) ¹	0.79 (0.53-1.17) ¹	1.14 (0.54-2.42) ¹
	2.15 (1.29-3.56) ²	0.82 (0.55-1.23) ²	-

1 Crude analysis

2 Adjusted analysis: recipient age, cold ischemic time, calcineurin inhibitor and induction therapy

* No adjusted analyses were performed for this group because of the limited number of patients

Abbreviations: Ab, antibodies; CI, confidence interval; DSA, donor specific anti-HLA antibodies; HR, hazard ratio, nDSA, non-donor specific anti-HLA antibodies.

Supplementary Table 1: Baseline characteristics stratified according to nDSA classes for group 2

	No HLA-antibodies	nDSA class I	nDSA class II	nDSA class I and II	P-value
Number	190	88	58	46	
Donor:					
Donor Age	41.6 ± 16.5	42.4 ± 16.9	39.2 ± 17.6	42.9 ± 14.2	0.47
Donor Sex, male	108 (56%)	45 (51%)	36 (62%)	27 (59%)	0.40
Donortype					
• DBD	119 (62%)	56 (64%)	36 (62%)	27 (59%)	0.86
• DCD	73 (38%)	32 (36%)	22 (38%)	19 (41%)	
Recipient:					
Recipient Age	47.6 ± 13.0	52.2 ± 12.4	49.5 ± 13.6	48.2 ± 13.2	0.20
Recipient Sex, male	142 (74%)	36 (41%)	32 (55%)	21 (46%)	0.24
Retransplantation	7 (4%)	19 (22%)	17 (29%)	28 (61%)	<0.0001
Peak PRA*	0 (0-4)	14 (0-51)	0 (0-8)	36 (11-53)	<0.0001
Transplantation:					
HLA-A, -B, DR mm	2.5 ± 1.2	2.4 ± 1.2	2.5 ± 1.1	2.2 ± 1.1	0.58
CIT (hours)	21.2 ± 7.3	21.0 ± 6.6	22.9 ± 7.4	22.4 ± 5.8	0.14
Induction					0.12
• II2rMoAb	49 (26%)	24 (27%)	7 (12%)	10 (22%)	
• T-cell depleting	3 (2%)	5 (6%)	1 (2%)	1 (2%)	

Data are depicted as number (percentage) or mean ± standard deviation unless otherwise stated. *P*-values are obtained from an overall comparison amongst the different nDSA groups.

* Median (interquartile range)

Abbreviations: CIT, cold ischemic time; DBD, donation after brain death; DCD, donation after circulatory death; DSA, donor-specific anti-HLA antibodies; II2rMoAb, Interleukin 2 receptor monoclonal antibody; mm, mismatch; nDSA, non-donor specific anti-HLA antibodies; PRA, panel reactive antigen.

Supplementary table 2: Sub-distribution hazard ratios (Fine & Gray regression) for graft failure

	DSA vs. no HLA Ab	nDSA vs. no HLA Ab	DSA vs nDSA*
	sHR (95% CI)	sHR (95% CI)	sHR (95% CI)
10-year graft failure	2.85 (1.67-4.87) ¹	1.19 (0.79-1.79) ¹	1.65 (0.80-3.39) ¹
	3.05 (1.70-5.48) ²	1.05 (0.68-1.63) ²	-

1 Crude analysis

2 Adjusted analysis: recipient age, cold ischemic time, calcineurin inhibitor and induction therapy

* No adjusted analysis was performed for this group because of the limited number of patients

Abbreviations: Ab, antibodies; CI, confidence interval; DSA, donor specific anti-HLA antibodies; nDSA, non-donor specific anti-HLA antibodies; sHR, subdistribution hazard ratio.