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De Novo Donor-Specific HLA Antibodies Decrease Patient and Graft Survival in Liver Transplant Recipients

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Abstract

The role of de novo donor-specific HLA antibodies (DSA) in liver transplantation remains unknown as most of the previous studies have only focused on preformed HLA antibodies. To understand the significance of de novo DSA, we designed a retrospective cohort study of 749 adult liver transplant recipients with pre- and posttransplant serum samples that were analyzed for DSA. We found that 8.1% of patients developed de novo DSA 1 year after transplant; almost all de novo DSAs were against HLA class II antigens, and the majority were against DQ antigens. In multivariable modeling, the use of cyclosporine (as opposed to tacrolimus) and low calcineurin inhibitor levels increased the risk of de novo DSA formation, while a calculated MELD score >15 at transplant and recipient age >60 years old reduced the risk. Multivariable analysis also demonstrated that patients with de novo DSA at 1-year had significantly lower patient and graft survival. In conclusion, we demonstrate that de novo DSA development after liver transplantation is an independent risk factor for patient death and graft loss.

Keywords

Allograft rejection; de novo donor-specific HLA antibodies; graft survival; liver transplantation; patient survival; risk factors

Introduction

The detrimental effect of alloantibodies directed against donor HLA has been widely demonstrated and accepted in all solid organ transplantation except the liver (1–4). Studies have shown that preformed donor-specific HLA antibodies (DSA) increase the risk of acute rejection and lower graft survival (5–7), leading this to be commonly considered a contraindication to transplant. In addition, many studies have shown that de novo DSA after

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transplantation is associated with higher rates of rejection and lower rates of survival (3,4,8,9).

In sharp contrast, livers are transplanted regardless of the crossmatch result. This is because posttransplant outcomes were thought to be similar regardless of the pretransplant DSA status (10–12). These perceived clinically equivalent outcomes in crossmatch positive and negative patients were supported by experiments in rats from the 1980s where DSA was thought to be an integral part of tolerance development (13,14).

Despite the early clinical evidence showing no difference in patient or graft survival, later studies demonstrated that patients transplanted with a positive crossmatch had an increased risk of early graft loss (15,16). This has been further supported by later evidence in rats that hyperacute rejection can be induced by adoptively transferred DSA (17), and the group previously claiming DSA was an integral part of tolerance (13,14) later found another protein (other than DSA) in serum to explain their tolerance induction (18). However, since consistent results are lacking, practice has not changed (19).

Although pre-transplant DSA's impact on outcome has been evaluated numerous times, the importance of DSA after liver transplantation has more limited data. Only in recent years have studies suggested that post-liver transplant DSA may play a role in acute and chronic rejection (20–25). One of the first studies to link the role of alloantibodies and chronic rejection was published by Demetris et al. (20) where they showed 7 of 22 patients with chronic rejection had high PRA (panel reactive antibodies) peri-transplant and the majority showed deposition of Ig or complement components in the rejected liver tissue. Later, Piazza et al. (21) showed that 65% of liver transplant recipients had DSA after transplant, and this was associated with rejection. Kasahara et al. (22) demonstrated that if patients had DSA within the first month after transplant, 100% experienced rejection, compared to only 17% if no DSA was found. Similarly, Kozłowski et al. (23) found that preformed DSA that persists after transplant was associated with severe early rejection. Recently, Musat et al. (24) demonstrated that DSA is present in up to 75% of patients experiencing rejection, and both DSA and C4d staining was present in 54% of patients diagnosed with rejection, showing a previously unrecognized humoral component to these rejections. Likewise, 70% of patients with ductopenia had DSA and 60% had both DSA and C4d staining in their biopsy. We similarly showed an association between DSA in serum and biopsy-proven chronic rejection (25). Despite these reports on the association between DSA in serum and rejection, to date no group has investigated the frequency of de novo DSA formation after liver transplantation in a large cohort. Therefore, we sought to define the frequency and consequences of de novo DSA formation after liver transplantation.

Methods

Patients

Patients' serum samples and data were obtained from the biorepository and liver transplant research database system (LTRDS) of the Annette C. and Harold C. Simmons Transplant Institute. The biorepository and LTRDS were initiated in 1985 and were designed to prospectively collect biological samples, clinical, laboratory, and pathology information in a

protocolized fashion from all donors and recipients at the time of transplant and 1, 2, 5, 10, 15, and 20 years after transplantation. Some patients also have event-driven samples collected at variable time points. All patients are consented to participate in this prospective collection at the time of the transplant with the understanding that the samples and data will be used for research purposes. Between January 1, 2000 and May 31, 2009, a total of 1326 adult patients received a primary liver allograft without another organ. The following criteria were used to select patients for this study: (1) available serum sample just prior to surgery, (2) available serum sample between 10 and 14 months after transplant, and (3) absence of pre-transplant DSA (defined as absence of any DSA with MFI >5000 detected by LABScreen single antigen beads). A total of 577 patients were excluded (no samples available, n = 55; lack of posttransplant sample, n = 161; posttransplant sample <10 months or >14 months, n = 204; preformed DSA with MFI >5000, n = 157) from the analysis, leaving 749 patients with available data and sera to study de novo DSA formation by 1-year posttransplant.

HLA typing

All patients and donors were typed for HLA-A, -B, -DRB1, -DRB345, and -DQ. Only patient class I typing was performed by serology using commercially available serologic typing trays (Terasaki HLA Tissue Typing Trays, One Lambda Inc., Canoga Park, CA), while patient class II and donor class I and class II HLA typing was performed by molecular methods (Micro SSP or LabTypeQR SSO, One Lambda Inc.). The typing information for HLA-C and -DP loci was not available for all patients and donors, and therefore was not used in the study.

HLA antibody determination

All sera were blinded and sent to the Terasaki Foundation Laboratory for evaluation. The detection of HLA antibodies was performed using LABScreenQR Single Antigen beads class I (Lot 7) and class II (Lot 9) (One Lambda Inc.). The assay was performed according to the manufacturer's protocol. Trimmed mean values were normalized for background and expressed as mean fluorescence intensity (MFI). Antibodies detected with a MFI >5000 were considered positive. The antibody results of patients were compared with the corresponding donor's HLA to define if they were donor specific or not. DSAs were defined based on serological equivalences. If a patient had more than one DSA, the MFI of the highest DSA was used.

Statistical analysis

Categorical variables are expressed as percentages and compared between groups using the chi-squared test. Continuous variables were expressed as medians and ranges, and compared using the Mann–Whitney test. Cumulative probability of patient and graft survival was calculated according to the Kaplan–Meier method and curves were compared using the log-rank test. Logistic regression analysis was used to investigate predictors of de novo DSA formation. A Cox proportional hazards analysis for predicting patient and graft loss was used. Variables with a p-value < 0.2 in the univariate analysis were entered into the stepwise multivariable analyses. However, two variables—de novo DSA and hepatitis C virus (HCV) viremia posttransplant—were both forced into the final multivariable model for patient and

graft survival. De novo DSA was built as a time-dependent covariate; patients were at risk for patient or graft loss from de novo DSA only after it was detected. In addition, because of the potential for immunosuppression to play a significant role in de novo DSA production, the final multivariable analysis for predictors of de novo DSA formation had the following immunosuppression variables forced in: cyclosporine versus tacrolimus treatment at 1-year, sirolimus treatment at 1-year, steroid treatment at 1-year, mycophenolate versus azathioprine/no treatment at 1-year, and “low” calcineurin inhibitor level (tacrolimus level <3 ng/mL or cyclosporine level <75 ng/mL on protocol levels stored in our data-base). p-values < 0.05 were considered significant. All statistical analysis was performed with SAS 9.1.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Baseline characteristics

All patients were adults who received a primary liver transplant without another organ, and their characteristics are outlined in Table 1. The majority were Caucasian males receiving a liver transplant for cirrhosis secondary to HCV. The median recipient and donor ages were 52 and 41 years, respectively. The median number of HLA-A, -B, -DRB1, -DRB345, -DQ mismatches was seven. Cytomegalovirus infection was observed in 19% of the cohort. Forty-one percent received induction therapy (primarily with daclizumab), 40% had at least one episode of biopsy proven acute rejection in the first-year and 4% had at least one episode of biopsy proven steroid-resistant rejection. Immunosuppression at 1-year included tacrolimus as the calcineurin inhibitor (CNI) of choice in 65% of the patients, mycophenolate in 50%, and oral steroids in 42%.

Prevalence of de novo DSA

We observed that 61 of 749 (8.1%) patients developed de novo DSA at 1 year post-liver transplantation. Three of 61 (5%) patients developed DSA against HLA class I antigens only, 58 of 61 (95%) developed DSA against HLA class II antigens only, and no patient developed DSA against both classes. Forty-seven of 61 (77%) patients developed only one DSA, 12 of 61 (20%) patients developed two DSAs, and 2 of 61 (3%) patients developed three or more DSAs. The locus-specificity of de novo DSA was as follows: 3 of 61 (5%) against B locus, 7 of 61 (12%) against DRB1 locus, 8 of 61 (13%) against DRB345 locus and 52 of 61 (85%) against DQ locus. Table 2 depicts the frequency of de novo DSA by HLA class and locus.

HLA mismatches and de novo DSA production Analysis of the relationship between the number of mismatches for each HLA locus and the development of de novo DSA against that same locus was undertaken to establish if the high prevalence of DSA against class II—especially against HLA-DQ locus—was simply due to a higher number of HLA-DQ mismatches. We observed that more patients had A, B and DRB1 mismatches than DQ mismatches, but the latter showed significantly higher frequency of de novo DSA formation than all of the other loci: 7.7% of patients with DQ mismatches, 1.8% of patients with DRB345 mismatches, 0.9% of patients with DRB1 mismatches, 0.4% of patients with B mismatches, and no patients with A mismatches (Figure 1A). No significant difference was

observed in de novo DSA development rates between patients with one and two DRB1, DRB345 and DQ mismatches (Figure 1B).

Predictors of de novo DSA production

Logistic regression analysis was undertaken to study factors predicting the production of de novo DSA. Since immunosuppression drugs and levels may have a profound impact on this, we forced these variables into the multivariable model regardless of their statistical significance in univariate analysis. HLA mismatches to each locus were analyzed in univariate analysis as continuous variables. However, only HLA-DQ mismatches was entered into stepwise multivariable modeling as 85% of the de novo DSAs were against this locus, and it was not found to be significant in the final model. Ten other variables with p-values < 0.2 in univariate analysis were entered into stepwise multivariable modeling. The results of the multivariable logistic regression model showed that patients receiving cyclosporine instead of tacrolimus at 1 year (OR = 2.50, p = 0.004), and patients with low calcineurin inhibitor levels (tacrolimus <3 ng/mL or cyclosporine <75 ng/mL) in the first-year (OR = 2.66, p = 0.015) had an increased likelihood of de novo DSA formation, while patients with a calculated MELD score >15 (OR = 0.47, p = 0.021) at the time of transplantation and recipients >60 years old (OR = 0.26, p = 0.03) had a significantly lower likelihood of de novo DSA production (Table 3).

Impact of de novo DSA on patient and allograft survival

We first investigated the effect of de novo DSA on survival and observed that patients with de novo DSA had a significantly lower patient (p = 0.002) and graft survival (p = 0.005) than patients without de novo DSA (Figure 2). The role of de novo DSA in predicting patient and graft survival was then analyzed using Cox proportional hazard analysis (Table 4). In the univariate analysis for patient survival, seven variables were found to have a p-value <0.2 and were entered into the stepwise multivariable model. The final multivariable model identified de novo DSA (HR = 1.99, p = 0.005), HCV viremia posttransplant, donor age >50 years old, recipient age >60 years old, and recipient African-American race as independent predictors of patient death.

Similarly, seven variables were found to have a p-value <0.2 in the univariate analysis for graft loss and were entered into the stepwise multivariable model. Again, the results of the multivariable model showed that de novo DSA (HR = 1.85, p = 0.01), HCV viremia posttransplant, donor age >50 years old, recipient age >60 years old, and recipient African-American race were independent predictors of graft loss.

Table 5 depicts the outcome of patients during the study period, and highlights that a higher proportion of patients without de novo DSA remain alive (81.1% vs. 67.2%) at the end of follow-up. In addition, those with de novo DSA at 1-year experienced death from or with a failing graft more commonly than those without de novo DSA (14.8% vs. 9.6%).

Discussion

With the improvement in patient and allograft survival that has occurred over the last several decades in liver transplantation, the re-evaluation of DSA's importance in liver

transplantation is essential and has demonstrated a higher risk of rejection and decreased allograft survival in most, but not all studies (12,19,26,27). However, most prior studies focused on the impact of preformed DSA, treated class I and II DSA equally, and used a low MFI cutoff adopted from renal transplantation. As a result, the impact of DSA on patient and liver allograft survival may have been under appreciated. Specifically, one area not investigated is the impact of de novo DSA formation on patient and liver allograft survival. We found that 8.1% of liver transplant recipients develop de novo DSA with a MFI >5000. These findings are similar to the only other report using modern technology to screen for DSA after liver transplantation by Fontana et al. (28) where they observed DSA in 4.2% of the 95 patients analyzed. Our findings are also similar to previous observations in kidneys, where the prevalence of de novo DSA ranges from 6% to 27% (29–32). This supports the hypothesis that the liver allograft is able to induce a humoral response in a similar fashion and frequency to other solid organ allografts.

We observed that DSA against HLA class II antigens was more common than class I and represented 95% of all de novo DSA. The higher prevalence of DSA against class II over class I observed in this study has also been shown in other solid organs but the explanation is still not clear (32–34). It is known that there is a differential expression of class I and II antigens on the surface of liver cells, including endothelial cells, hepatocytes and biliary epithelial cells; and that this expression can be altered by different events, such as rejection episodes, infections, and inflammation related to transplantation (35–37). Knowing that there is a higher constitutive expression of some HLA molecules (especially class I) in the liver, one may expect to find more alloantibodies against such molecules, as higher expression of alloantigen could translate into a stronger humoral response. Conversely, the higher expression of HLA antigens may facilitate more complete absorption thereby inhibiting one's ability to detect DSA in serum. Since DQ has the least expression in liver of all the HLA molecules, we cannot rule out the possibility that the higher prevalence of HLA-DQ antibodies is secondary to their inability to be absorbed by the liver (35,36,38). However, if this were the case one would not necessarily find them to be detrimental, and we have shown that they are in some cases.

Another finding of our study is the discovery of four factors associated with the development of de novo DSA. First, the use of cyclosporine as opposed to tacrolimus carries more than twice the risk of de novo DSA formation. Since DSA and cyclosporine use have independently been linked to higher rates of acute and chronic rejection (39), it is no surprise that cyclosporine is now associated with an increased rate of DSA formation. Second, low levels of calcineurin inhibitors increase the risk of de novo DSA formation. It is not surprising that under immunosuppression increased the likelihood of mounting an alloimmune response (40). Third, a MELD score >15 at the time of transplant reduce the risk of de novo DSA formation in half. Patients transplanted with high MELD scores are known to be sicker and at higher risk for infection pre-transplant.

However, it is intriguing that they have a lower risk of de novo DSA formation by 1-year posttransplant. Lastly, recipients over 60 years of age displayed a lower likelihood of de novo DSA formation, which likely results from a less reactive immune system found in older patients (41).

Finally, we show that de novo DSA is an independent predictor of patient death and liver allograft loss, as shown in the Cox proportional hazards multivariable analysis. Although significant, the small difference in survival rates between groups observed in the Kaplan–Meier analysis (only 7% difference at 5 years for patient survival and 6% for graft survival) shows that the impact of DSA alone is not as pronounced as in other organs during the 5 years of follow up for this study, and opens the possibility for future research to find other cofactors or diagnostic tools to better identify patients at the highest risk for losing their allografts from DSA. Complement-fixing DSA, IgG subclasses of DSA (42), IgM DSA, and antibodies against non-HLA targets are some potential markers to examine in the near future. However, recent data has shown us that longer-term studies may be needed to fully understand the implications of post-liver transplant DSA, as slowly progressive idiopathic fibrosis may be a previously unrecognized consequence (43). Therefore, while we found an association between DSA with MFI >5000 and adverse outcomes, this does not rule out the possibility that DSA at lower MFI may be deleterious.

Even though this is a large cohort study of 749 patients, there are limitations. First, in an effort to achieve a homogeneous cohort, we did exclude patients without samples available for analysis at the predetermined time points and those with preformed DSA. Second, sample acquisition did not occur frequently enough for us to determine a temporal-relationship between the formation of de novo DSA and graft loss or patient death. Third, the lack of complete donor and recipient HLA-C and HLA-DP typing information did not allow for a complete DSA determination of all potential HLA loci, since these two loci can result in graft rejection (44,45).

In conclusion, de novo DSA at 1-year post-liver transplant occurred in 8.1% of patients and was found to be an independent predictor of patient death and allograft loss. Future research will need to focus on improved mechanisms to identify pathologic DSA, outline monitoring and diagnostic algorithms, and design prospective treatment trials.

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Abbreviations

DSA	donor-specific HLA antibodies
HCV	hepatitis C virus
MELD	model for end-stage liver disease
MFI	mean fluorescence intensity

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Figure 1.

Frequency of locus-specific de novo DSA according to HLA mismatches. (A) Frequency of de novo DSA by HLA locus. The number in parenthesis indicates the number of patients with mismatches to the corresponding locus. The table shows the p-values comparing the frequency of DSA in different HLA loci. (B) Frequency of de novo DSA by the number of HLA mismatches to each HLA locus.

Figure 2.

Five-year patient and graft survival by the presence of de novo DSA. Patient (A) and graft survival (B) curves are shown from the time of transplant.

Table 1**Patients' characteristics**

Number		749
Donor age, median (range)		41 (7–82)
Donor race, n (%)	Caucasian	687 (63.3)
	Hispanic	220 (20.3)
	African-American	161 (14.8)
	Asian	11 (1)
	Other	7 (0.6)
Recipient age, median (range)		52 (19–74)
Recipient race, n (%)	Caucasian	482 (65)
	Hispanic	142 (19.2)
	African-American	108 (14.6)
	Other	9 (1.2)
Recipient gender, M/F (%)		528/221 (70.5/29.5)
Calculated MELD, median (range)		16 (6–40)
Cold ischemia time, median hours (range)		7.7 (2.1–16.7)
HLA-A, -B, -DRB1, -DRB345, -DQ mismatches, median (range)		7 (0–10)
Etiology of liver disease, n (%)	HCV	350 (46.7)
	Alcoholic cirrhosis	115 (15.4)
	Autoimmune conditions (AIH, PSC, PBC)	97 (13)
	NASH/cryptogenic cirrhosis	91 (12.1)
	Metabolic/congenital	17 (2.3)
	Other	79 (10.5)
CMV infection, n (%)		139 (18.6)
Hepatic artery thrombosis in first 3 months, n (%)		9 (1.2)
Primary cause of death, n (%)	De novo or recurrent disease	46 (30.7)
	Cancer	35 (23.3)
	Infection	13 (8.7)
	Renal failure	11 (7.3)
	Cardiovascular/pulmonary	8 (5.3)
	Surgical complications	6 (4)
	Rejection	5 (3.3)
	Other/unknown	26 (17.4)
Patients with at least one rejection episode in the first-year, n (%)		300 (40.1)
Patients with at least one steroid-resistant rejection episode in the first-year, n (%)		30 (4)
Induction therapy, n (%)		310 (41.4)
Calcineurin inhibitor at 1 year, n (%)	Cyclosporine	217 (29)
	Tacrolimus	485 (64.8)
	none	47 (6.3)
Anti-proliferative at 1 year, n (%)	Mycophenolate mofetil	377 (50.3)
	Azathioprine	9 (1.2)

	none	363 (48.5)
Sirolimus at 1 year, n (%)		119 (15.9)
Steroids at 1 year, n (%)		315 (42.1)

MELD, model for end-stage liver disease; HCV, hepatitis C virus; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; NASH, non-alcoholic steatohepatitis; CMV, cytomegalovirus.

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Table 2

Frequency of de novo DSA by HLA class and locus

All patients (n = 749)	
DSA positive, n (%)	61 (8.1%)
By HLA class	
DSA class I, n (%)	3 (5%)
DSA class II, n (%)	58 (95%)
DSA class I+II, n (%)	0
By HLA locus	
HLA-A, n (%)	0
HLA-B, n (%)	3 (5%)
HLA-DRB1, n (%)	7 (12%)
HLA-DRB345, n (%)	8 (13%)
HLA-DQ, n (%)	52 (85%)

Table 3

Logistic regression analyses of predictors of de novo DSA formation

Variables	Univariate analysis			Multivariable analysis		
	Odds ratio	95% CI	p Value	Odds ratio	95% CI	p Value
Cyclosporine (compared to tacrolimus) at 1 year ^I	2.61	1.48–4.62	<0.001	2.5	1.35–4.63	0.004
Sirolimus at 1 year ^I	1.83	0.99–3.4	0.055	0.63	0.23–1.7	0.359
Steroids at 1 year ^I	0.51	0.28–0.9	0.021	0.67	0.35–1.28	0.229
Mycophenolate vs Azathioprine/none at 1 year ^I	1.07	0.63–1.81	0.805	1	0.54–1.86	0.998
Low level of calcineurin inhibitor in the first year ^I	2.3	1.14–4.66	0.02	2.66	1.21–5.84	0.015
MELD >15	0.65	0.38–1.1	0.109	0.47	0.25–0.89	0.021
Patient's age >60 years old	0.39	0.14–1.1	0.074	0.26	0.08–0.87	0.03
Statins at 1 year	2.62	1.25–5.47	0.01	–	–	–
Induction therapy	1.63	0.96–2.75	0.069	–	–	–
African-American recipient	2.11	0.95–4.7	0.068	–	–	–
HCC	1.87	1.08–3.23	0.024	–	–	–
Non-liver autoimmune disease (RA, SLE, thyroid disease)	1.81	0.98–3.36	0.06	–	–	–
Biliary complications between 3 and 12 months	1.76	0.85–3.62	0.125	–	–	–
# HLA-DRB345 mismatches	1.42	0.95–2.12	0.086	–	–	–
# HLA-DQ mismatches	1.59	1.02–2.47	0.04	–	–	–

MELD, model for end-stage liver disease; HCC, hepatocellular carcinoma; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; low level of calcineurin inhibitor (tacrolimus <3 ng/mL or cyclosporine <75 ng/mL).

^IVariables forced in the multivariable model.

Table 4

Cox regression analysis to predict patient and graft survival

	Univariate analysis			Multivariable analysis		
	Hazard ratio	95% CI	p Value	Hazard ratio	95% CI	p Value
Patient survival						
De novo DSA ^I	2.07	1.29–3.32	0.002	1.99	1.23–3.22	0.005
HCV viremia posttransplant ^I	1.61	1.16–2.22	0.004	1.68	1.21–2.32	0.002
Donor age >50 years old	1.55	1.12–2.16	0.009	1.52	1.09–2.13	0.01
Patient's age >60 years old	1.55	1.03–2.34	0.037	1.76	1.16–2.67	0.008
African-American patient	1.86	1.15–3.01	0.011	1.77	1.08–2.88	0.02
HCC	1.27	0.88–1.83	0.196	–	–	–
ACR in the first year	1.33	0.97–1.84	0.081	–	–	–
Graft survival						
De novo DSA ^I	1.93	1.21–3.09	0.006	1.85	1.15–2.98	0.01
HCV viremia posttransplant ^I	1.52	1.11–2.08	0.009	1.58	1.15–2.17	0.005
Donor age >50 years old	1.63	1.18–2.25	0.003	1.59	1.16–2.2	0.004
Patient's age >60 years old	1.44	0.96–2.17	0.08	1.6	1.06–2.43	0.03
African-American patient	1.89	1.18–3.02	0.008	1.79	1.11–2.88	0.02
ACR in the first year	1.37	1–1.87	0.051	–	–	–
Steroids at 1 year	0.81	0.59–1.12	0.198	–	–	–

DSA, donor-specific HLA antibodies; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; ACR, acute cellular rejection.

^I Variables forced in the multivariable model.

Table 5

Causes of death in patients with and without de novo DSA (p = 0.044)

	Without de novo DSA (n = 688)	With de novo DSA (n = 61)
Alive	558 (81.1%)	41 (67.2%)
Death with a functioning graft	64(9.3%)	11 (18%)
Death with a non-functioning graft	66 (9.6%)	9 (14.8%)