Title

Understanding the Correlation Between DSA, Complement Activation, and Antibody-Mediated Rejection in Heart Transplant Recipients.

Journal

Transplantation, 102(10)

ISSN

0041-1337

Authors

Zhang, Qiuheng
Hickey, Michelle
Drogalis-Kim, Diana
et al.

Publication Date

2018-10-01

DOI

10.1097/tp.0000000000002333

Peer reviewed

HHS Public Access

Author manuscript

Transplantation. Author manuscript; available in PMC 2019 October 01.

Published in final edited form as:

Transplantation. 2018 October ; 102(10): e431–e438. doi:10.1097/TP.0000000000002333.

Understanding the correlation between DSA, complement activation and antibody mediated rejection in heart transplant recipients

Qiuheng Zhang[a], **Michelle Hickey**[a], **Diana Drogalis-Kim**[b], **Ying Zheng**[a], **David Gjertson**[a], **Martin Cadeiras**[c], **Tam Khuu**[c], **Arnold S. Baas**[c], **Eugene C. Depasquale**[c], **Nancy J. Halnon**[c], **Gregory Perens**[c], **Juan Alejos**[c], **Daniel Cruz**[c], **Ali Nsair**[c], **Richard Shemin**[c], **Murray Kwon**[d], **Michael C. Fishbein**[c], **Abbas Ardehali**[d], **Mario Deng**[c], and **Elaine F. Reed**[a]

[a]UCLA Immunogenetics Center, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles 90095

[b]Rainbow Babies and Children's Hospital in Cleveland, OH

[c]Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles 90095

[d]Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles 90095

Abstract

Background—Donor specific HLA antibodies (DSA) are associated with increased rates of rejection and of graft failure in cardiac transplantation. The goal of this study was to determine the association of preformed and posttransplant development of newly detected DSA (*nd*DSA) with antibody mediated rejection (AMR) and characterize the clinical relevance of complement activating DSA in heart allograft recipients.

Methods—The study included 128 adult and 48 pediatric heart transplant patients transplanted between 2010 to 2013. Routine posttransplant HLA antibody testing was performed by IgG-Single Antigen Bead (SAB) test. The C3d−SAB assay was used to identify complement activating antibodies. Rejection was diagnosed using ISHLT criteria.

Results—In this study, 22 patients were transplanted with preexisting DSA, and 43 patients developed *ndDSA* posttransplant. Pretransplant ($p<0.05$) and posttransplant ($p<0.001$) *nd*DSA were associated with higher incidence of AMR. Patients with C3d+DSA had significantly higher incidence of AMR compared to patients with no DSA ($p<0.001$) or patients with C3d−DSA ($p=0.02$). Nine out of 25 (36%) patients with AMR developed transplant coronary artery disease (TCAD) compared to 17/107 (15.9%) patients without AMR ($p<0.05$). Among the 47 patients who received ventricular assistant device (VAD), 7/9 VAD+ patients with preformed DSA

Address for correspondence from readers: Qiuheng Zhang, Ph.D., UCLA Immunogenetics Center, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, University of California Los Angeles, 1000 Veteran Ave, Los Angeles, CA 90095, Phone: 310-794-4943, Fax: 310-206-3216, jqzhang@mednet.ucla.edu.

Disclosure statement

The authors have no conflicts of interest to disclose.

experienced AMR compared to 7/38 VAD+ patients without preformed DSA, indicating presensitization to donor HLA significantly increased the risk of AMR ($p<0.015$).

Conclusion—Preformed and posttransplant *ndDSA* were associated with AMR. C3d+DSA correlates with complement deposition on the graft and higher risk of AMR which may permit the application of personalized immunotherapy targeting the complement pathway.

Introduction

Despite improvements in patient care and immunosuppressive regimens, allograft rejection remains a formidable risk factor for morbidity and mortality in heart transplant recipients[1–3]. It is widely accepted that donor-specific HLA antibodies (DSA) are associated with antibody-mediated rejection (AMR) and poor graft survival[4–15]. Therefore, implementing a scheme of routine monitoring for DSA following transplantation has the potential to identify patients for early intervention and improve long-term outcomes. To develop an optimal posttransplant immune assessment protocol, it is important to understand the tempo of HLA DSA development after heart transplant and whether or not it differs in patients with a prior history of allosensitization and/or placement of a mechanical circulatory device. Approximately 30% of heart transplant recipients are sensitized to HLA antigens prior to transplant[16]. Mechanisms of sensitization to HLA are pregnancy, blood transfusion, surgery to implant a homograft or prior transplant. Heart transplant patients with implanted ventricular assistant devices (VAD)-are particularly prone to developing HLA antibodies due to multiple platelet and blood transfusions[17,18].

DSA mediate graft damage by binding to target HLA antigens expressed on the endothelium of the allograft and activating complement via the classical pathway. Activation and deposition of various complement components, including C1q, C4b, C4d, C3a, C3d and C5, contribute to endothelial cell injury and microvascular inflammation during AMR [6,10,11,19]. HLA DSA may also activate and injure endothelial cells via complement independent pathways, by transducing signals that promote endothelial cell migration, proliferation, adhesion molecule expression and recruitment of leukocytes to the allograft [20–22]. C4d deposition on the graft endothelium and presence of intravascular activated monocytes are criteria for diagnosis of AMR in cardiac transplantation [1].

The cell based complement-dependent cytotoxicity test has been in use for over 50 years to detect complement fixing antibodies. However, weaker HLA antibodies can go undetected due to low sensitivity, and nonspecific binding in this assay can result in false positive results. In contrast, the solid phase IgG Single Antigen Bead (IgG-SAB) test detects HLA antibodies with increased sensitivity and specificity. The IgG-SAB test is a semiqualitative assay that provides a measurement of the strength of IgG HLA antibodies reported as median fluorescence intensity (MFI). Currently, the results of the IgG-SAB test are widely used to aid risk assessment at the time of a deceased donor offer through virtual crossmatches. The IgG-SAB has also being used for the identification of posttransplant DSA in supporting the diagnosis of AMR [23]. However, the IgG-SAB test cannot distinguish between complement fixing noncomplement fixing antibodies that may differ in pathogenicity after solid organ transplantation [24]. Two solid phase assays have been

developed that allow for the detection of complement-fixing HLA antibodies. The C1q-SAB test measures the binding of the C1q molecule to the Fc regions of HLA antibody and is a necessary step to initiate the complement cascade [25–29]. The C3d−SAB assay detects the complement split product of C3d [30,31].

DSA not present prior to transplant but develop for the first time after transplantation in response to HLA alloantigen exposure are considered de novo HLA DSA (*dn*DSA). The identification of *dn*DSA can be difficult without a complete history of pretransplant antibody monitoring or when the HLA antibody falls below the detection threshold of the IgG-SAB test[15]. Therefore, early identification of posttransplant HLA DSA, in particular between 2 weeks and 3 months, can reflect either a memory response to prior allo-antigen exposure or can be truly de novo[32]. Because we are unable to distinguish between true *dn*DSA and DSA that are developed early post transplant in an amnestic response, we adopted the use of the term newly detected DSA (*nd*DSA)[15]. Posttransplant *nd*DSA are defined as any DSA that were not identified in pretransplant tests but are identified any time after transplantation.

The goal of this study was to determine the association of preformed and *nd*DSA with AMR and patient survival. The second goal of the study was to evaluate the clinical significance of complement activating antibodies using C3d−SAB in heart transplant recipients.

Material and Methods

Study Population

The study included 128 adult and 48 pediatric heart transplant patients transplanted between 2010–2013 at UCLA. 47 patients received a VAD as bridge to transplantation (BTT). Demographic information was collected by reviewing the patient's medical record during the period of 2014–2016. 50 patients received induction therapy in this cohort. All patients received triple-drug immunosuppression (tacrolimus, mycophenolate mofetil, and corticosteroids). Research approval for this study was granted by the UCLA Institutional Review Board (IRB#01-08-015-21).

Diagnosis of rejection and TCAD

Adult cardiac transplant recipients underwent routine surveillance endomyocardial biopsies weekly in the first month, biweekly in month 2, and at months 3, 4, 5, 6, 9, 12. Additional biopsies were performed when there was clinical suspicion of rejection. Protocol biopsies of pediatric patients were obtained at weeks 1, 3, 6, months 3, 6 and annually thereafter unless clinical concerns for rejection arose. For patients under age 1 year old, the first biopsy occurred no earlier than 3 months posttransplant. A total of 1465 endomyocardial biopsies were obtained from 176 patients. Acute cellular rejection (ACR) and AMR were diagnosed according to ISHLT criteria [33]. Coronary angiography was performed annually posttransplant. A diagnosis of transplant coronary artery disease (TCAD) was made when new luminal stenosis ≥ 30%, or significant distal pruning of the coronary arteries was identified in comparison with baseline angiogram.

HLA typing and Evaluation of HLA antibodies

HLA typing of patients and donors was performed for HLA-A, -B, -C, -DRB1, and -DQB1 loci using LABType SSO (One Lambda, Canoga Park, CA). HLA typing of the -DQA1 and -DPA1, -DPB1 loci were performed if recipients displayed HLA antibodies to these antigens.

Undiluted pre and posttransplant sera samples were treated with DTT and tested for HLA antibodies using the IgG-SAB Assay from both One Lambda (Canoga Park, CA) and Immucor (Stamford, CT) as previously described [34]. The results were highly concordant between the companies. Antibodies were considered positive if the MFI >1000 for HLA-A, B, DR, DQ and >2000 was used for HLA-C and DP. In correlation with our clinical practice, a higher cutoff for HLA-C and DP is used because there is a greater amount of HLA-C and DP antigen bound to the Luminex IgG-SAB beads. Routine posttransplant HLA antibody testing was performed at weeks 1, 2, 3, 4, 6, at months 2, 3, 4, 5, 6, 8, 10, 12, and quarterly thereafter and when clinically indicated. Pretransplant antibody status was based on the presence (preformed DSA+) or absence (DSA−) of DSA in the pretransplant sample closest to day of transplantation. Posttransplant *nd*DSA were defined as posttransplant DSA identified any time after transplantation when the pretransplant tests were DSA negative.

C3d−SAB test

Undiluted sera positive for IgG HLA antibodies were tested for C3d binding antibodies by using C3d−SAB kit according to the manufacturer's protocol (Immucor, Stamford, CT). Briefly, 40 μl of LSA bead mix were incubated with 10μl of serum for 30 minutes at room temperature (RT) in the dark on a rotating platform. After incubation, 30μl of complement containing serum was added to each well and incubated for another 30 minutes at RT. After 4 washes, 50 μl of C3d conjugate was added to each well and incubated for 30 minutes at RT. After a final wash, 200 μl of wash buffer was added and data was collected using the Luminex platform. Antibodies with MFI > 1000 were considered positive. The Immunodominant DSA was defined as the DSA with the highest normalized MFI value.

Statistical analysis

Contingency tables were analyzed for differences in proportions among categorical variables between recipients with and without rejection using Fisher's exact test. Differences in mean values of continuous variables were analyzed via Wilcoxon's rank-sum test. Logistic regression models were used to evaluate odds ratio (OR). Statistical significance was defined as a p value < 0.05, allowing for multiple comparisons of main variables via Bonferroni's procedure. All p values were 2-sided, and all estimates were done via the STATA statistical software (StataCorp. 2003. Stata Statistical Software. College Station, TX: Stata Corporation). Actuarial graft survival and freedom from TCAD was estimated using Kaplan–Meier analysis and statistical differences calculated with the log-rank statistic.

Results

176 cardiac transplant recipients comprised 48 children and 128 adults were prospectively monitored for the development of HLA DSA following transplantation (Table 1). The

median follow-up time was 17.9 months for patients without AMR and 15.7 for patients with AMR ($p = 0.63$). 34/176 (19.3%) patients were diagnosed with AMR. 4/34 AMR+ patients had concomitant ACR ≥ 2R. Six patients had an isolated ACR episode ≥ 2R. Statistical analysis showed no association between diagnosis of AMR and recipient age, gender, race, or number of HLA mismatches between recipient and donor. Interestingly, patients that developed AMR were more likely to be recipients of combined heart/liver or heart/kidney transplants ($p < 0.01$). 17 patients received combined heart/kidney transplant, 7/17 had diagnoses of AMR in the transplanted hearts, 2/17 had concomitant AMR in the kidney allografts, AMR was never diagnosed in the kidney alone. 47 patients had VAD placed as BTT. AMR was diagnosed in 14/47 (29.8%) VAD+ patients compared to 20/129 (15.5%) in VAD− patients, indicating the placement of VAD is a risk factor for AMR ($p < 0.05$, Table 1).

Preformed HLA DSA and AMR

22/176 patients were transplanted with preformed DSA (Table 2a). Preformed DSA were targeted to either HLA class I (n = 10; 6 against A; 2 against B; 1 against A+C; 1 against A +B), class II (n = 9; 4 against DR; 4 against DQ; 1 against DR+DQ), or both class I/II antigens (n = 3; 1 against A+DQ; 1 against A+DR+DQ; 1 against B+DR+DQ). Diagnosis of AMR was significantly increased in patients transplanted with preformed DSA. 8/22 (36%) patients transplanted with preformed DSA experienced AMR compared to 26/154 (17%) of patients without DSA ($p < 0.05$). In the 47 patients who received VAD as BTT, 9 VAD+ patients were transplanted with preformed DSA. Among them, 7/9 experienced AMR compared to 7/38 VAD+ in VAD− patients without preformed DSA ($p < 0.01$, Table 2b). Presensitization to donor HLA was associated with AMR in VAD+ patients.

nd*DSA* and AMR

All patients waiting for heart transplant were monitored by routine pretransplant solid phase antibody testing either biannually, quarterly or monthly according to the patients' wait list status and sensitization history. Routine posttransplant immune assessment provided an opportunity to characterize the evolution of *nd*DSA in primary heart allograft recipients. In the 154 patients transplanted without preformed DSA, 43 patients developed *nd*DSA. The 3-year cumulative incidence of *nd*DSA development was 28% (Table 2a). Majority of the patients made *nd*DSA to class II (n = 24; 14 of them made *nd*DSA to DQ; 5 to DR; 4 to DR +DQ; 1 to DP), 6 to class I (2 to A; 2 to B; 2 to C) and 13 to both class I/II (10 of 13 patients had *nd*DSA to DQ in addition to DSA of other specificities). Development of *nd*DSA was significantly associated with AMR. Among the *nd*DSA producers, 15/43 (35%) patients experienced AMR compared to 11/111 (9.9%) in *nd*DSA− patients ($p < 0.001$, Table 2a). Of the 47 VAD+ patients, 9 patients had preformed DSA, 38 patients had no preformed DSA. Seven out of 9 VAD+ patients with preformed DSA had AMR compared to 7/31 VAD+ patients without preformed DSA ($p < 0.01$, Table 2b). Of the 43 patients that developed *nd*DSA, 13/43 patients had VAD before transplant, 6/13 (46%) VAD+ patients experienced AMR compared to 9/30 (30%) in VAD− patients ($p = 0.49$, Table 2b).

The cumulative incidence *nd*DSA was illustrated in Figure 1. There was no significant difference in the incidence of *nd*DSA in pediatric and adult transplant recipients (Figure 1a,

p = 0.72). The time to identification of the first *nd*DSA was evaluated in the 43 *nd*DSA+ patients and in the 13/22 patients transplanted with preformed DSA that developed *nd*DSA to additional HLA specificities. The median time of *nd*DSA development in patients transplanted with preformed DSA was 1.6 months, much earlier than sensitized patients transplanted with third party antibodies (Sensitized DSA−, 7.7 months) and nonsensitized patients (9.4 months; $p < 0.05$, Figure 1b). In patients transplanted with preformed DSA, 5/13 developed *nd*DSA within 1 month, and 11/13 developed *nd*DSA within 6 month of transplant. However, 11/23 nonsensitized patients and 8/20 sensitized DSA− patients developed *nd*DSA within 6 months respectively, indicating the importance of early posttransplant monitoring in all group of patients. The cumulative incidence of *nd*DSA stratified by patient sensitization was shown in Figure 1c. Patients with preformed DSA developed *nd*DSA to additional mismatched donor HLA antigens at a faster rate than sensitized patients without HLA DSA or nonsensitized patients ($p < 0.01$). Analysis of the cumulative incidence of *nd*DSA showed there was no significant difference in patients who received induction therapy compared to patients without induction therapy (p = 0.65, Figure 1d).

Correlation of C3d+DSA with AMR

We next determined if complement activating DSA detected by the C3d−SAB assay correlated with AMR. The C3d−SAB test was performed on the 22 patients transplanted with preformed DSA and 43 patients who developed posttransplant *nd*DSA. Univariate logistic regression analysis was used to evaluate odds of developing AMR in patients with and without complement activating DSA in comparison to DSA− patients. DSA+C3d− patients had 2.8 odds ratio for higher risk of developing AMR compared to DSA negative patients ($p < 0.05$, Table 3). The risk of AMR dramatically increased 33 times for C3d+DSA patients compared to patients without DSA ($p < 0.001$).

To determine if C3d+DSA correlated with C4d deposition in the graft, we evaluated 35 DSA + patients who had DSA 7 days before and up to 2 days after their endomyocardial biopsies. Biopsies were categorized into C4d+AMR+ (n = 10), C4d−AMR+ (n = 6) and C4d−AMR− (n = 19) groups. 13/35 (37%) patients developed C3d+DSA. Among them, 7 were diagnosed with C4d+AMR+, 4 had C4d−AMR+, and 2 had no AMR (Table 4). While in the 22 patients with C3d−DSA, only 3 had C4d+ deposition indicating the presence of C3d+ DSA was significantly associated with C4d deposition in AMR+ biopsies ($p < 0.01$).

To further correlate C3d+DSA to AMR, the immunodominant MFI of IgG+DSA was plotted against C3d DSA positivity in AMR+ and AMR− patients (Figure 2). Patients with C3d +DSA had higher IgG+DSA MFI (10,383 ± 1497) compared to patients with C3d−DSA (3,474 ± 537, $p < 0.01$). Notably, when the MFI's of the IgG+DSA were compared between the 2 groups, there was a wider range of MFI's in the C3d+DSA group compared to the C3d −DSA group. Furthermore, 5 AMR+ patients with IgG+DSA < 10,000 MFI were C3d+ and experienced AMR, suggesting that the C3d−SAB assay may be a useful tool to identify patients at risk of AMR, even in the context of weak to moderate IgG+DSA.

Correlation of DSA with TCAD

Among 132 patients who had a follow-up greater than 1 year, 9/25 (36%) patients with AMR developed TCAD compared with 17/107 (15.9%) patients without AMR ($p < 0.05$, Table 5). The TCAD incidence rate was 2.3 cases per month per 100 cases in patients with AMR compared with 1.0 cases per month per 100 cases in patients without AMR ($p < 0.05$, Table 5).

HLA DSA and graft survival

Patients with AMR experienced a trend toward more graft failure than patients without AMR. The 3-year TCAD free survival rate was 81.4% in patients without AMR and 58.9% in patients with AMR ($p = 0.09$, Figure 3a). To study the long-term effect of preformed DSA and *nd*DSA on graft survival, preformed DSA and *nd*DSA were correlated with TCAD in patients who had more than 1 year of follow-up. Patients with *nd*DSA had a 68% TCAD-free survival at 3 years posttransplant, compared with 82% for patients with preformed DSA and 84% for patients without DSA. However, there was no significant difference in TCAD free survival comparing patients with *nd*DSA, preformed DSA and no DSA (p = 0.31, Figure 3b).

Discussion

The goal of this study was to determine the evolution of posttransplant development of DSA and characterize the clinical relevance of complement activating DSA in heart allograft recipients. The main findings of this study showed both pretransplant and posttransplant DSA were associated with higher incidence of AMR in heart transplant recipients. In particular, VAD+ patients transplanted with preformed HLA DSA had a higher risk of AMR. Notably, the median time of *nd*DSA development in patients transplanted with preformed DSA was 1.6 months; significantly earlier than sensitized patients transplanted without DSA. However, regardless of pretransplant sensitization status, nearly half of the heart transplant recipients developed DSA by 6 months posttransplant. We demonstrated that patients with preformed DSA developed *nd*DSA to additional HLA specificities at a much faster rate than sensitized patients without HLA DSA or nonsensitized patients suggesting that donor specific memory B cells were likely present in these sensitized recipients at the time of transplantation. This finding was consistent with studies by Han et al that investigated the presence of memory B cells in sensitized allograft recipients. They found that HLA antibody-producing B cells detected in sensitized individuals were mainly comprised of memory B cells[35]. This supports the premise that alloreactive memory B cells exhibit recall responses with faster kinetics compared to naïve B cells [36].

Patients with C3d+DSA had a significantly higher risk of AMR compared to patients with C3d−DSA. A significantly higher number of patients with AMR also developed TCAD compared to patients without AMR. There was no significant difference found between the pediatric and adult cohorts in *nd*DSA development, the incidence of AMR and patient survival, probably due to the relatively small population of the pediatric cohort.

In our previous report, *dn*DSA was found in 20% of the patients and the development of *dn*DSA was strongly linked with AMR and TCAD in heart transplant recipients [5]. Similarly, in an adult cardiac transplant study, *dn*DSA was reported in 23.4% of patients and was significantly associated with poor patient survival [9]. A slightly higher incidence of *dn*DSA was reported in 2 pediatric cardiac transplant cohorts [13,37]. In both reports, ~40% of the patients developed *dn*DSA and the development of *dn*DSA was significantly associated with higher incidence of TCAD and poor graft survival. Patients who received VAD as BTT are considered high risk for developing HLA DSA in part due to frequent blood and platelet transfusions[17]. In our cohort, VAD+ patients, particularly those transplanted with preformed DSA, had significantly higher risk of AMR compared to VAD− patients. Ko et al recently reported among 113 VAD+ patients, 25% of them became sensitized after VAD implantation. These patients had a higher risk of ACR and AMR compared to VAD− patients [38].

The incidence of *dn*DSA in heart transplant patients is similar to what is reported for kidney transplantation [30,39–41]. In an adult kidney transplant study, Everly et al [40] reported a 5-year accumulative incidence of *dn*DSA is 20% in a cohort of 189 nonsensitized primary kidney transplant recipients. The authors reported the annual incidence of DSA is highest in the first year posttransplant (11%) accounting for more than 50% of the patients who developed *dn*DSA Similarly, Wiebe et al [41] studied 315 consecutive renal transplants without preformed HLA DSA. They showed that 47/315 (15%) patients developed *dn*DSA and demonstrated that the 10-year graft survival for patients with *dn*DSA was significantly lower than patients without DSA (59% vs. 96%). The time between the development of DSA and graft loss provides an opportunity for clinical interventions. Walsh et al showed that early therapeutic intervention and rapid reversal of the rejection is associated with improved graft survival compared with late intervention that leads to refractory rejection and irreversible kidney damage [42].

HLA DSA-induced complement activation not only damages the allograft as an effector system; it also promotes the adaptive immune responses [43–46]. We demonstrated C3d+DSA was strongly correlated with C4d deposition in endomyocardial biopsies, suggesting C3d +DSA is more pathogenic compared to C3d−DSA. We found that patients with C3d+DSA had 33 times higher risk of developing AMR compared to DSA− patients, and 2.8 times higher risk of developing AMR compared to patients with C3d−DSA. Currently there are 2 solid phase assays to detect complement-fixing HLA antibodies, the C1q−SAB [24–26,29,47] and the C3d−SAB assays [30,31]. The C1q−SAB test detects the initial binding of the C1q molecule to the Fc regions of HLA antibody. The C3d−SAB assay measures the complement activation product C3b. Recently, Comoli et al reported that C3d+DSAs were better predictors for AMR and graft loss as compared to C1q+DSA test in a pediatric kidney transplant cohort [30]. Sicard et al showed that patients with C3d+DSA had worse estimated glomerular filtration rate and increased risk of AMR and allograft loss in an adult kidney transplant [31]. These data suggests that the C3d−SAB test is useful for identifying patients at higher risk of graft rejection and may benefit from therapeutic intervention with complement inhibitors such as such as the C5 inhibitor (eg, Eculizumab) and C1 inhibitors [48]. Publications on complement fixing DSA in heart transplant patients are small and limited. A study of 18 pediatric heart transplant recipients showed that C1q+DSA had better predictive

value for AMR early posttransplant compared to IgG−SAB [26]. Similarly, Zeevi et. al reported persistent C1q+DSA was associated with early clinical AMR in 15 cardiac transplant recipients. [28].

One limitation of the solid phase C3d−SAB assays is that these tests are unable to detect the synergistic effect of multiple DSA as measured by cell-based assays. In addition, the production of HLA antibodies is dynamic; noncomplement fixing antibodies can become complement-fixing antibodies or vice versa [26]. Therefore, although these early C3d−SAB studies are promising, clinical usage of the test needs to be further evaluated.

In conclusion, patients with both preformed and posttransplant *nd*DSA had significantly higher incidence of AMR and relatively lower graft survival as compared to patients without DSA. Patients who had complement-activating DSA were likely to develop C4d+AMR and earlier graft failure compared to patients with noncomplement fixing antibodies. These results suggest routine posttransplant monitoring by IgG−SAB and C3d−SAB testing may be beneficial for identifying patients at risk of rejection and patients that may benefit from specific therapeutic intervention targeting the complement pathway.

Acknowledgments

Funding

This work was supported by a grant (R01 AI042819) and (UO1 AI124319) to E.F.R from the National Institute of Allergy and Infectious Diseases

Abbreviations

ACR	Acute cellular rejection
AMR	antibody-mediated rejection
BTT	bridge to transplantation
DSA	Donor specific HLA antibodies
***dn*DSA**	de novo *DSA*
ndDSA	newly detected DSA
IgG-SAB	Single Antigen Bead test
ISHLT	International Society for Heart and Lung Transplantation
MFI	median fluorescence intensity
OR	odds ratio
RT	room temperature
VAD	ventricular assistant device
TCAD	transplant coronary artery disease

References

1. Colvin MM, Cook JL, Chang P, et al. Antibody-mediated rejection in cardiac transplantation: emerging knowledge in diagnosis and management: a scientific statement from the American Heart Association. Circulation. 2015; 131:1608–1639. [PubMed: 25838326]
2. Michaels PJ, Espejo ML, Kobashigawa J, et al. Humoral rejection in cardiac transplantation: risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. J Heart Lung Transplant. 2003; 22:58–69. [PubMed: 12531414]
3. Uber WE, Self SE, Van Bakel AB, Pereira NL. Acute antibody-mediated rejection following heart transplantation. Am J Transplant. 2007; 7:2064–2074. [PubMed: 17614978]
4. Reed EF, Hong B, Ho E, Harris PE, Weinberger J, Suciu-Foca N. Monitoring of soluble HLA alloantigens and anti-HLA antibodies identifies heart allograft recipients at risk of transplant-associated coronary artery disease. Transplantation. 1996; 61:566–572. [PubMed: 8610382]
5. Zhang Q, Cecka JM, Gjertson DW, et al. HLA and MICA: targets of antibody-mediated rejection in heart transplantation. Transplantation. 2011; 91:1153–1158. [PubMed: 21544036]
6. Peng DM, Law YM, Kemna MS, Warner P, Nelson K, Boucek RJ. Donor-specific antibodies: can they predict C4d deposition in pediatric heart recipients? Pediatr Transplant. 2013; 17:429–435. [PubMed: 23551503]
7. Tan CD, Sokos GG, Pidwell DJ, et al. Correlation of donor-specific antibodies, complement and its regulators with graft dysfunction in cardiac antibody-mediated rejection. Am J Transplant. 2009; 9:2075–2084. [PubMed: 19624562]
8. Tambur AR, Pamboukian SV, Costanzo MR, et al. The presence of HLA-directed antibodies after heart transplantation is associated with poor allograft outcome. Transplantation. 2005; 80:1019–1025. [PubMed: 16278580]
9. Smith JD, Banner NR, Hamour IM, et al. De novo donor HLA-specific antibodies after heart transplantation are an independent predictor of poor patient survival. Am J Transplant. 2011; 11:312–319. [PubMed: 21219570]
10. Frank R, Molina MR, Goldberg LR, Wald JW, Kamoun M, Lal P. Circulating donor-specific anti-human leukocyte antigen antibodies and complement C4d deposition are associated with the development of cardiac allograft vasculopathy. Am J Clin Pathol. 2014; 142:809–815. [PubMed: 25389335]
11. Frank R, Molina MR, Wald JW, Goldberg LR, Kamoun M, Lal P. Correlation of circulating donor-specific anti-HLA antibodies and presence of C4d in endomyocardial biopsy with heart allograft outcomes: a single-center, retrospective study. J Heart Lung Transplant. 2013; 32:410–417. [PubMed: 23498162]
12. Hodges AM, Lyster H, McDermott A, et al. Late antibody-mediated rejection after heart transplantation following the development of de novo donor-specific human leukocyte antigen antibody. Transplantation. 2012; 93:650–656. [PubMed: 22245878]
13. Irving CA, Carter V, Gennery AR, et al. Effect of persistent versus transient donor-specific HLA antibodies on graft outcomes in pediatric cardiac transplantation. J Heart Lung Transplant. 2015; 34:1310–1317. [PubMed: 26123951]
14. Nath DS, Angaswamy N, Basha HI, et al. Donor-specific antibodies to human leukocyte antigens are associated with and precede antibodies to major histocompatibility complex class I-related chain A in antibody-mediated rejection and cardiac allograft vasculopathy after human cardiac transplantation. Hum Immunol. 2010; 71:1191–1196. [PubMed: 20868717]
15. Dipchand AI, Webber S, Mason K, et al. Incidence, Characterization and Impact of Newly Detected Donor Specific Anti-HLA Antibody in the First Year after Pediatric Heart Transplantation: A Report From the CTOTC-04 Study. Am J Transplant. Published online February 14, 2018.
16. Colvin M, Smith JM, Skeans MA, et al. Heart. Am J Transplant. 2016; 16(Suppl 2):115–140. [PubMed: 26755266]
17. Kwon MH, Zhang JQ, Schaenman JM, et al. Characterization of ventricular assist device-mediated sensitization in the bridge-to-heart-transplantation patient. J Thorac Cardiovasc Surg. 2015; 149:1161–1166. [PubMed: 25702320]

18. Hong BJ, Delaney M, Guynes A, et al. Human leukocyte antigen sensitization in pediatric patients exposed to mechanical circulatory support. ASAIO J. 2014; 60:317–321. [PubMed: 24469294]
19. Rodriguez ER, Skojec DV, Tan CD, et al. Antibody-mediated rejection in human cardiac allografts: evaluation of immunoglobulins and complement activation products C4d and C3d as markers. Am J Transplant. 2005; 5:2778–2785. [PubMed: 16212640]
20. Valenzuela NM, Reed EF. Antibodies to HLA Molecules Mimic Agonistic Stimulation to Trigger Vascular Cell Changes and Induce Allograft Injury. Curr Transplant Rep. 2015; 2:222–232. [PubMed: 28344919]
21. Zhang X, Valenzuela NM, Reed EF. HLA class I antibody-mediated endothelial and smooth muscle cell activation. Curr Opin Organ Transplant. 2012; 17:446–451. [PubMed: 22710387]
22. Jin YP, Valenzuela NM, Ziegler ME, Rozengurt E, Reed EF. Everolimus inhibits anti-HLA I antibody-mediated endothelial cell signaling, migration and proliferation more potently than sirolimus. Am J Transplant. 2014; 14:806–819. [PubMed: 24580843]
23. Stehlik J, Islam N, Hurst D, et al. Utility of virtual crossmatch in sensitized patients awaiting heart transplantation. J Heart Lung Transplant. 2009; 28:1129–1134. [PubMed: 19782589]
24. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med. 2013; 369:1215–1226. [PubMed: 24066742]
25. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. Hum Immunol. 2011; 72:849–858. [PubMed: 21791230]
26. Chin C, Chen G, Sequeria F, et al. Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. J Heart Lung Transplant. 2011; 30:158–163. [PubMed: 20951058]
27. Sutherland SM, Chen G, Sequeira FA, Lou CD, Alexander SR, Tyan DB. Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allograft loss. Pediatr Transplant. 2012; 16:12–17. [PubMed: 22093755]
28. Zeevi A, Lunz J, Feingold B, et al. Persistent strong anti-HLA antibody at high titer is complement binding and associated with increased risk of antibody-mediated rejection in heart transplant recipients. J Heart Lung Transplant. 2013; 32:98–105. [PubMed: 23142561]
29. Guidicelli G, Guerville F, Lepreux S, et al. Non-Complement-Binding De Novo Donor-Specific Anti-HLA Antibodies and Kidney Allograft Survival. J Am Soc Nephrol. 2016; 27:615–625. [PubMed: 26047793]
30. Comoli P, Cioni M, Tagliamacco A, et al. Acquisition of C3d-Binding Activity by De Novo Donor-Specific HLA Antibodies Correlates With Graft Loss in Nonsensitized Pediatric Kidney Recipients. Am J Transplant. 2016; 16:2106–2116. [PubMed: 26725780]
31. Sicard A, Ducreux S, Rabeyrin M, et al. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. J Am Soc Nephrol. 2015; 26:457–467. [PubMed: 25125383]
32. Tambur AR, Campbell P, Claas FH, et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. Am J Transplant. Published online March 30, 2018.
33. Berry GJ, Burke MM, Andersen C, et al. The 2013 International Society for Heart and Lung Transplantation Working Formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. J Heart Lung Transplant. 2013; 32:1147–1162. [PubMed: 24263017]
34. Blumberg JM, Gritsch HA, Reed EF, et al. Kidney paired donation in the presence of donor-specific antibodies. Kidney Int. 2013; 84:1009–1016. [PubMed: 23715120]
35. Han M, Rogers JA, Lavingia B, Stastny P. Peripheral blood B cells producing donor-specific HLA antibodies in vitro. Hum Immunol. 2009; 70:29–34. [PubMed: 19026703]
36. Chong AS, Sciammas R. Memory B cells in transplantation. Transplantation. 2015; 99:21–28. [PubMed: 25525921]
37. Tran A, Fixler D, Huang R, Meza T, Lacelle C, Das BB. Donor-specific HLA alloantibodies: Impact on cardiac allograft vasculopathy, rejection, and survival after pediatric heart transplantation. J Heart Lung Transplant. 2016; 35:87–91. [PubMed: 26422083]

38. Ko BS, Drakos S, Kfoury AG, et al. Immunologic effects of continuous-flow left ventricular assist devices before and after heart transplant. J Heart Lung Transplant. 2016; 35:1024–1030. [PubMed: 27316382]
39. Mizutani K, Terasaki P, Hamdani E, et al. The importance of anti-HLA-specific antibody strength in monitoring kidney transplant patients. Am J Transplant. 2007; 7:1027–1031. [PubMed: 17391143]
40. Everly MJ, Rebellato LM, Haisch CE, et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. Transplantation. 2013; 95:410–417. [PubMed: 23380861]
41. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant. 2012; 12:1157–1167. [PubMed: 22429309]
42. Walsh RC, Brailey P, Girnita A, et al. Early and late acute antibody-mediated rejection differ immunologically and in response to proteasome inhibition. Transplantation. 2011; 91:1218–1226. [PubMed: 21617586]
43. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science. 1996; 271:348–350. [PubMed: 8553069]
44. Jindra PT, Zhang X, Mulder A, et al. Anti-HLA antibodies can induce endothelial cell survival or proliferation depending on their concentration. Transplantation. 2006; 82(1 Suppl):S33–35. [PubMed: 16829793]
45. Li F, Zhang X, Jin YP, Mulder A, Reed EF. Antibody ligation of human leukocyte antigen class I molecules stimulates migration and proliferation of smooth muscle cells in a focal adhesion kinase-dependent manner. Hum Immunol. 2011; 72:1150–1159. [PubMed: 22001078]
46. Vieyra M, Leisman S, Raedler H, et al. Complement regulates CD4 T-cell help to CD8 T cells required for murine allograft rejection. Am J Pathol. 2011; 179:766–774. [PubMed: 21704012]
47. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. Transplantation. 2011; 91:342–347. [PubMed: 21116220]
48. Thomas KA, Valenzuela NM, Gjertson D, et al. An Anti-C1s Monoclonal, TNT003, Inhibits Complement Activation Induced by Antibodies Against HLA. Am J Transplant. 2015; 15:2037–2049. [PubMed: 25904443]

Authorship

Qiuheng Zhang:	• Participated in research design • Participated in the writing of the paper • Participated in data acquisition and analysis • Participated in the performance of the research • Final approval of the version to be published
Michelle Hickey	• Participated in the writing of the paper • Participated in data analysis • Participated in the performance of the research
Diana Drogalis-Kim	• Participated in data acquisition
Ying Zheng	• Participated in data analysis
David Gjertson	• Participated in data analysis
Martin Cadeiras	• Participated in the performance of the research
Tam Khuu	• Participated in the performance of the research

Arnold S. Baas	• Participated in the performance of the research
Eugene C. Depasquale	• Participated in the performance of the research
Nancy J. Halnon	• Participated in the performance of the research
Gregory Perens	• Participated in the performance of the research
Juan Alejos	• Participated in the performance of the research
Daniel Cruz	• Participated in the performance of the research
Nsair, Ali	• Participated in the performance of the research
Richard Shemin	• Participated in the performance of the research
Murray Kwon	• Participated in the performance of the research
Michael C. Fishbein	• Participated in the performance of the research
Abbas Ardehali	• Participated in the performance of the research
Mario Deng	• Participated in the performance of the research
Elaine F. Reed	• Participated in the writing of the paper • Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved • Final approval of the version to be published

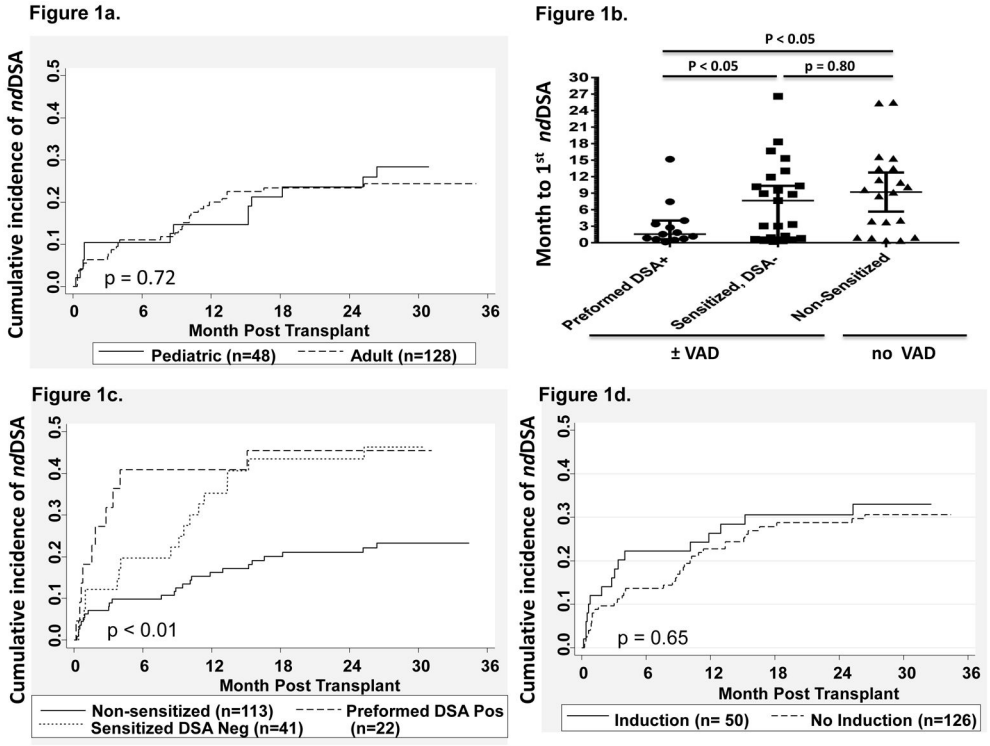

Figure 1. Incidence of *nd*DSA

1a) Cumulative incidence of *nd*DSA stratified in pediatric and adult patients. Solid line: pediatric patients; Dashed line: adult patients (log-rank: p = 0.72).

1b) Time to the first appearance of posttransplant *nd*DSA.

The time to the first appearance of *nd*DSA was plotted on 56 patients. Patients that were transplanted with or without VAD were categorized into 3 groups. 1) Patients transplanted with preformed DSA that developed additional *nd*DSA (Preformed DSA+; n=13); 2) Sensitized patients with no HLA DSA (Sensitized DSA−; n=23); 3) Nonsensitized patients (n=20). y axis: Month to the development of first *nd*DSA. Whiskers indicate 95% confidence interval. Median values indicated by horizontal lines. Significance was calculated using 1-way Anova among the 3 groups or unpaired *T* test between 2 groups.

1c) Cumulative incidence of *nd*DSA stratified according to patient's sensitization history. The cumulative incidence of *nd*DSA was plotted on 3 groups. 1) Nonsensitized patients; 2) Sensitized patients transplanted without preformed DSA; 3) Patients transplanted with preformed DSA (log-rank: p < 0.01).

1d) Cumulative incidence of *nd*DSA stratified according to induction therapy. Solid line: patient received induction therapy; Dashed line: patient without induction therapy (log-rank: p = 0.65).

VAD: ventricular assistant device; DSA: donor specific antibody; *nd*DSA: newly developed DSA

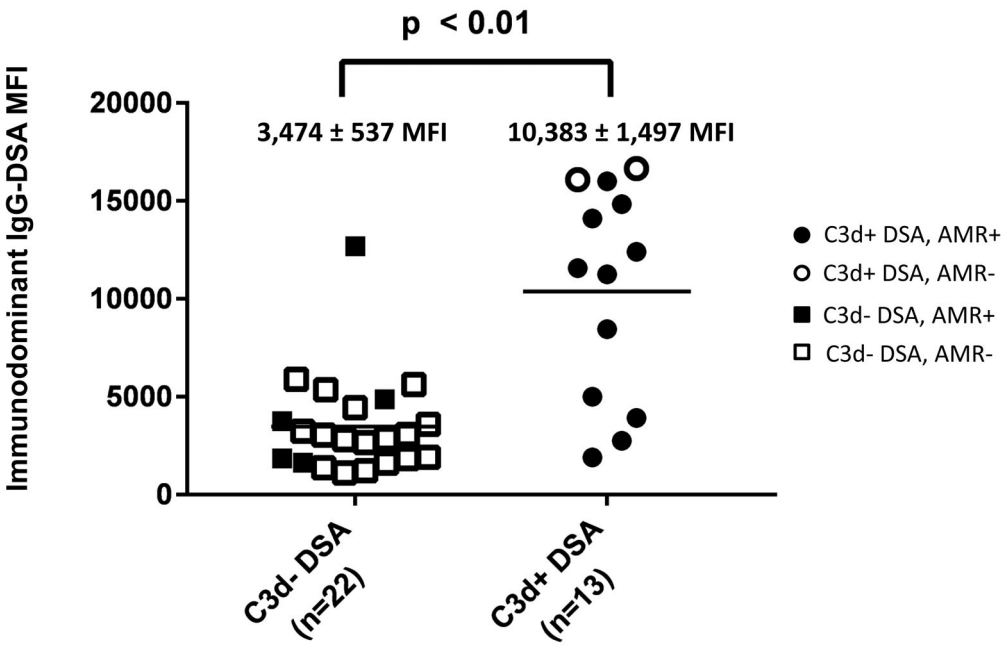

Figure 2. Correlation of Immunodominant IgG DSA with C3d DSA positivity

The MFI of the Immunodominant IgG DSA was plotted according to C3d DSA positivity of 35 patients with concurrent biopsy results. Patients with C3d−DSA had median IgG−DSA MFI of 3474 ± 537. Patients C3d+DSA had had median IgG−DSA MFI of 10383 ± 1497 ($p < 0.01$). The statistical different of the IgG−DSA MFI values between C3d+DSA and C3d −DSA was performed using unpaired *T* test. DSA: donor specific antibody; MFI: median fluorescence intensity.

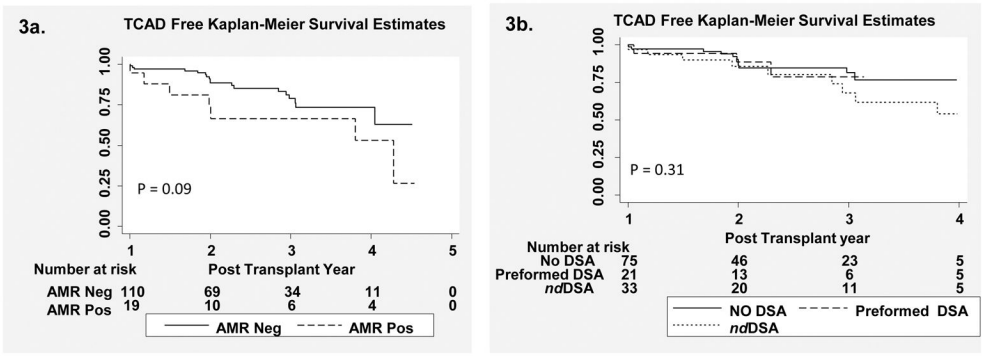

Figure 3.

Kaplan-Meier curve comparing survival between heart transplant recipients with and without AMR in all patients. 3a). Solid line: AMR negative; dotted line: AMR positive. 3b). Kaplan-Meier curve comparing survival in heart transplant recipients with preformed DSA, *nd*DSA and no DSA. Solid line: no DSA; dotted line: *nd*DSA; dashed line: preformed DSA. Kaplan-Meier survival analysis was performed using multiple-record survival data showing freedom from TCAD 1 year after transplantation. AMR: antibody mediated rejection; DSA: donor specific antibody; *nd*DSA: newly developed donor specific antibody; TCAD: transplant coronary artery disease.

Table 1

Patient demographics

	AMR Neg (n=142)	AMR Pos (n=34)	*p value*
Adult Age, years (mean ± SD) (n=128)	52.6 ± 12.3 (n = 101)	48.6 ± 13.6 (n = 27)	0.13
Pediatric Age, years (mean ± SD) (n=48)	8.3 ± 7.0 (n = 41)	12.9 ± 6.5 (n = 7)	0.11
Gender (Male/Female)	100/42	19/15	0.11
ACR ≥ 2R	6	4	
VAD+ (n=47)	33	14	**< 0.05**
VAD− (n=129)	109	20	
Race			
Asian	20	4	
African American	17	7	0.3
White	98	23	
Other	7	0	
Heart Transplant Only (n=146)	131	25	
Multiorgan transplant (n=20)	11	9	**< 0.01**
Heart/Kidney (n=17)	10	7	
Heart/Liver (n=3)	1	2	
HLA-A mismatches (mean ± SD)	1.6 ± 0.5	1.7 ± 0.5	0.68
HLA-B mismatches (mean ± SD)	1.8 ± 0.4	1.9 ± 0.5	0.16
HLA-DR mismatches (mean ± SD)	1.5 ± 0.6	1.6 ± 0.6	0.49
HLA-DQ mismatches (mean ± SD)	1.4 ± 0.6	1.4 ± 0.6	0.71
Median follow-up time (mo)	17.9	15.7	0.63

AMR: antibody mediated rejection; ACR: acute cellular rejection; VAD: ventricular assistant device

Table 2

Association of HLA DSA and AMR

2a. Association of preformed and *nd*DSA with AMR

DSA	N	AMR Neg	AMR Pos	*p Value*
Preformed DSA+	22	14 (64%)	8 (36%)	
Preformed DSA−	154	128 (83%)	26 (17%)	< 0.05
*nd*DSA+	43	28 (65%)	15 (35%)	
DSA−	111	111 (90.1%)	11 (9.9%)	< 0.001

2b. Association of HLA DSA and AMR stratified by VAD patients

DSA	N	AMR Neg	AMR Pos	*p Value*
Preformed DSA+	9 VAD+	2 (22%)	7 (78%)	
Preformed DSA−	38 VAD+	31 (81.5%)	7 (18.5%)	< 0.01
*nd*DSA+ (n=43)	13 VAD+	7	6	
	30 VAD−	21	9	0.49

AMR: antibody mediated rejection; DSA: donor specific antibody; *nd*DSA: newly detected DSA ACR: acute cellular rejection; VAD: ventricular assistant device

Table 3

Univariate logistic regression analysis for the risk of AMR

	n	Logistical Model, AMR Adjusted	95% Confidence Interval	*p Value*
DSA−	111	OR= 1		
DSA+ C3d−	51	OR = 2.8	[1.1, 6.9]	< 0.05
DSA+ C3d+	14	OR= 33	[8.1, 138.0]	< 0.001

AMR: antibody mediated rejection; DSA: donor specific antibody; OR, Odds ratio;

Table 4

Correlation of Immunodominant DSA with Endomyocardial Biopsy Results

DSA	C4d+ AMR+ (n=10)	C4d− AMR+ (n=6)	C4d− AMR− (n=19)	*p value*
DSA+ C3d+ (n=13)	7	4	2	$p < 0.05$
DSA+, C3d− (n=22)	3	2	17	

AMR: antibody mediated rejection; DSA: donor specific antibody

Table 5

Association of AMR with TCAD in 132 patients who had follow-up greater than one year

	N (n = 132)	AMR Neg (n = 107)	AMR Pos (n = 25)	*p Value*
TCAD Pos	26	17 (65.4%)	9 (34.6%)	< 0.05
TCAD Neg	106	90 (84.9%)	16 (15.1%)	
TCAD incident rate (case/mo/100 Transplant)	132	1.0	2.3	< 0.05

AMR: antibody mediated rejection; TCAD: transplant coronary artery disease