



ORIGINAL CLINICAL SCIENCE

Donor-specific anti-HLA antibodies with antibody-mediated rejection and long-term outcomes following heart transplantation

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KEYWORDS:

antibody mediated rejection;
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cardiac allograft vasculopathy;
mortality

BACKGROUND: Donor-specific anti-HLA antibodies (DSA) are common after heart transplantation and are associated with rejection, cardiac allograft vasculopathy, and mortality. A noninvasive diagnostic test for pathologic antibody-mediated rejection (pAMR) does not exist.

METHODS: From January 1, 2010, through August 31, 2013, 221 consecutive adult patients underwent heart transplantation and were followed through October 1, 2015. The primary objective was to determine whether the presence of DSA could detect AMR at the time of pathologic diagnosis. Secondary analyses included association of DSA (stratified by major histocompatibility complex class and de novo status) during AMR with new graft dysfunction, graft loss (mortality or retransplantation), and development of cardiac allograft vasculopathy.

RESULTS: During the study period, 69 patients (31.2%) had DSA (24% had de novo DSA), and there were 74 episodes of pAMR in 38 patients. Sensitivity of DSA at any mean fluorescence intensity to detect concurrent pAMR was only 54.3%. The presence of any DSA during pAMR increased the odds of graft dysfunction (odds ratio = 5.37; 95% confidence interval [CI], 1.34–21.47; $p = 0.018$), adjusting for age, sex, and timing of AMR. Circulating class II DSA after transplantation increased risk of future pAMR (hazard ratio = 2.97; 95% CI, 1.31–6.73; $p = 0.009$). Patients who developed de novo class II DSA had 151% increased risk of graft loss (contingent on 30-day survival) compared with patients who did not have DSA (95% CI, 1.11–5.69; $p = 0.027$).

CONCLUSIONS: DSA were inadequate to diagnose pAMR. Class II DSA provided prognostic information regarding future pAMR, graft dysfunction with pAMR, and graft loss.

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Donor-specific anti-HLA antibodies (DSA) develop in up to 50% of patients after solid-organ transplantation.¹ DSA

are detrimental after orthotopic heart transplantation (OHT), leading to increased cellular rejection,² cardiac allograft vasculopathy (CAV),^{3–5} antibody-mediated rejection (AMR),⁴ and mortality.^{6–8} Before 2010, International Society for Heart and Lung Transplantation (ISHLT) guidelines for the diagnosis of AMR required the presence of DSA.⁹ At a 2010 ISHLT consensus conference on AMR,

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DSA were removed as a requirement from the diagnostic criteria, although the panel strongly recommended screening for DSA at the time of AMR.¹⁰ A recent study of pediatric heart transplant recipients concluded that DSA were sensitive to detect an episode of pathologic AMR (pAMR) grade 2 or 3 (area under the curve 0.75–0.79).¹¹ This study aimed to examine the role of DSA in AMR in adult heart transplant recipients.

Methods

This prospective cohort study enrolled all 221 adult (≥ 18 years old) patients who underwent heart transplantation at Columbia University Medical Center from January 1, 2010, through August 31, 2013. Patients were followed for clinical events through October 1, 2015. The study protocol was approved by the Columbia University Medical Center Institutional Review Board.

Before transplantation, patients were screened with both complement-dependent cytotoxic enzyme-linked immunoassay analysis and a solid phase assay, Luminex LABScreen Single Antigen (One Lambda, Canoga Park, CA). Patients were considered sensitized before transplant if they had anti-HLA antibodies with a mean fluorescence intensity (MFI) $> 5,000$. Highly sensitized patients were treated before transplant with intravenous immune globulin and/or plasmapheresis followed by B-cell-depleting therapies (bortezomib, cyclophosphamide). A virtual crossmatch was performed for all patients, and a prospective crossmatch was performed when technically feasible. If a prospective crossmatch was not performed, a simultaneous crossmatch was performed (concurrent with the transplant). No patients during the study period received transplants with a positive crossmatch, although highly sensitized patients were treated with perioperative intravenous immune globulin.

After OHT, routine surveillance endomyocardial biopsy (EMB) was performed weekly in the first month after transplantation, then every 2 weeks for 1 month, monthly for 4 months, bimonthly for 6 months, every 3 months for 6 months, and then every 6 to 12 months. Thereafter, EMB was performed annually unless clinical rejection was suspected. With each EMB, 4 to 5 pieces of the right ventricular endomyocardium were obtained. Each biopsy specimen was graded for AMR according to the current ISHLT guidelines (pAMR 1h, 1i, 2, or 3).¹² C4d staining was performed by immunohistochemistry on formalin-fixed paraffin-embedded tissue. Although there is no consensus on using C3d, our institution has also been staining for C3d routinely. The Cell Marque C4d (SP91) antibody was used after heat-induced epitope retrieval at pH 6. Staining was performed with the Leica Biosystems BOND-III automated stainer with Bond Polymer Refine Detection (Leica Biosystems Inc., Buffalo Grove, IL) with 3,3'-diaminobenzidine as the chromogen. Immunohistochemical staining was performed on all biopsy specimens early after transplant, on all histologically suspicious biopsy specimens, on all biopsy specimens graded ISHLT 1R/1B or greater, and when requested on clinical grounds (including symptoms of rejection, changes in ejection fraction or cardiac index [with or without new DSA], recent cellular rejection). Blood was drawn concurrently with each EMB to screen for DSA using complement-dependent cytotoxic analysis and solid phase assay Luminex LABScreen Single Antigen. C1q testing was not regularly used. De novo DSA were defined as DSA that were never detected before OHT.

Induction therapy with basiliximab was used unless a contraindication existed (infection, bleeding, or retransplantation).

Standard immunosuppressive regimens included prednisone, a calcineurin inhibitor (tacrolimus or cyclosporine), and mycophenolate mofetil. Chronic immunosuppression was modified if patients developed renal insufficiency (creatinine > 2.5 mg/dl) or CAV through the use of sirolimus or everolimus.

Screening for CAV was initially performed at 1 year after OHT and then every other year thereafter with coronary angiography. Additional angiograms were obtained if there was unexplained graft dysfunction. Dobutamine stress echocardiography was used to screen for CAV during years angiography was not performed. If patients had a creatinine > 2.0 mg/dl, dobutamine stress echocardiography was used in place of angiography. Angiograms were graded according to the 2010 ISHLT CAV recommended nomenclature by the diagnosing cardiologist.¹³

The primary objective was to determine the ability (sensitivity, specificity, negative predictive value) of DSA to predict AMR at the time of pathologic diagnosis. Secondary analyses included the association of DSA (stratified by major histocompatibility complex [MHC] class and de novo status) during AMR with graft dysfunction, graft loss (mortality or retransplantation), and subsequent development of CAV. We also investigated the future risk of AMR among patients who develop DSA. Graft dysfunction was defined as at least 2 of the following: 33% decrease in cardiac index and cardiac index < 2.2 liter/min/m², 33% increase in pulmonary capillary wedge pressure, or 33% decrease in left ventricular ejection fraction.^{14,15}

Statistical analysis

Demographic and clinical variables were summarized with standard descriptive statistics and expressed as median (with interquartile range) for skewed continuous variables and count (with percentage) for categorical variables. Group comparisons were made with the chi-square and Kruskal-Wallis test where appropriate. Sensitivity, specificity, and predictive values were determined using 2×2 contingency tables. Receiver operator characteristic curves were constructed, and the areas under the curve were calculated for the diagnostic performance of DSA to detect pAMR for MFI ranging from 1,000 to $> 10,000$. Kaplan-Meier survival analysis, unadjusted, and multivariable Cox proportional hazards regression were performed to determine survival statistics. All statistical tests were 2-tailed with statistical significance defined to be at the 0.05 level. Analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

The cohort consisted of 221 consecutive patients who had 3,790 DSA-EMB pairs. DSA were detected in 69 patients (31.2%) after OHT, with 494 DSA of any MFI detected. Among DSA detected, 340 were de novo post-OHT DSA in 53 patients (24%). Average age was similar among patients with DSA compared with patients without DSA, as was use of induction therapy. However, a greater proportion of patients with DSA were female, and mechanical circulatory support was used more frequently in patients without DSA (Table 1). During a median of 3.5 years of follow-up, AMR occurred 74 times (56 pAMR 1i, 10 pAMR 1h, 8 pAMR 2) in 38 unique patients (prevalence of 17.2% in the cohort),

Table 1 Demographic Details Based on DSA Status

| | DSA | No DSA | <i>p</i> -value |
|-------------------------------|------------------|------------------|-----------------|
| No. | 69 | 152 | |
| Age, years ^a | 53.8 (42.5–62.8) | 59.4 (47.0–64.3) | 0.17 |
| Sex | | | 0.08 |
| Male (%) | 47 (68.1) | 120 (79.0) | |
| Female (%) | 22 (31.9) | 32 (21.0) | |
| Donor age, years ^a | 46 (33.0–60.0) | 50.5 (34.5–61.0) | 0.21 |
| MCS bridge (%) | 33 (43.8) | 95 (62.5) | 0.04 |
| Induction (%) | 51 (73.9) | 102 (67.1) | 0.31 |
| AMR (%), <i>n</i> = 210 | 27 (39.1) | 11 (7.8) | <0.0001 |
| ACR (%), <i>n</i> = 210 | 41 (59.4) | 35 (24.8) | <0.001 |
| Sensitized pre-transplant (%) | 11 (15.9) | 7 (4.6) | 0.004 |
| MHC class of DSA | | | |
| Class I only | 14 (20.3) | | |
| Class II only | 34 (49.3) | | |
| Class I and II | 21 (30.4) | | |

ACR, acute cellular rejection; AMR, antibody-mediated rejection; DSA, donor-specific anti-HLA antibodies; MCS, mechanical circulatory support.

^aMedian (interquartile range).

with a greater frequency among patients with DSA. Cellular rejection was more frequent in patients with DSA.

Sensitivity and specificity of DSA at the time of pathologic AMR diagnosis

DSA were an unreliable test to screen for pAMR in this cohort. Analyzing DSA of any MFI, the sensitivity to detect concurrent pAMR was 54.3%, sensitivity was 44.4% for MFI >4,000, and sensitivity was 23.5% for MFI >10,000 (Figure 1A). The sensitivity to detect pAMR remained poor for MHC class II DSA (Figure 1B). Despite limited efficacy to rule out pAMR, the specificity of DSA for pAMR was acceptable. For DSA of any MFI, the specificity was 93.4%,

increasing to 98.1% when the MFI cutoff was 4,000. Similarly, the negative predictive value was 98.9%, but this was at least in part due to the low prevalence of pAMR on biopsy specimens (2.0% of all biopsy specimens) in this cohort.

Patients who had DSA present during pAMR had an increased risk of graft dysfunction. The presence of DSA of any MFI or MHC class during an episode of pAMR led to a 5-fold increase in the odds of concomitant graft dysfunction (odds ratio [OR] = 5.37; 95% confidence interval [CI], 1.34–21.47; *p* = 0.018), adjusting for age, sex, and timing of AMR (<1 year, >1 year).¹⁴ Comparing the effect of MHC class (adjusting for the same covariates), there was a nearly equivalent increase in the odds of accompanying graft dysfunction for patients with only class I DSA

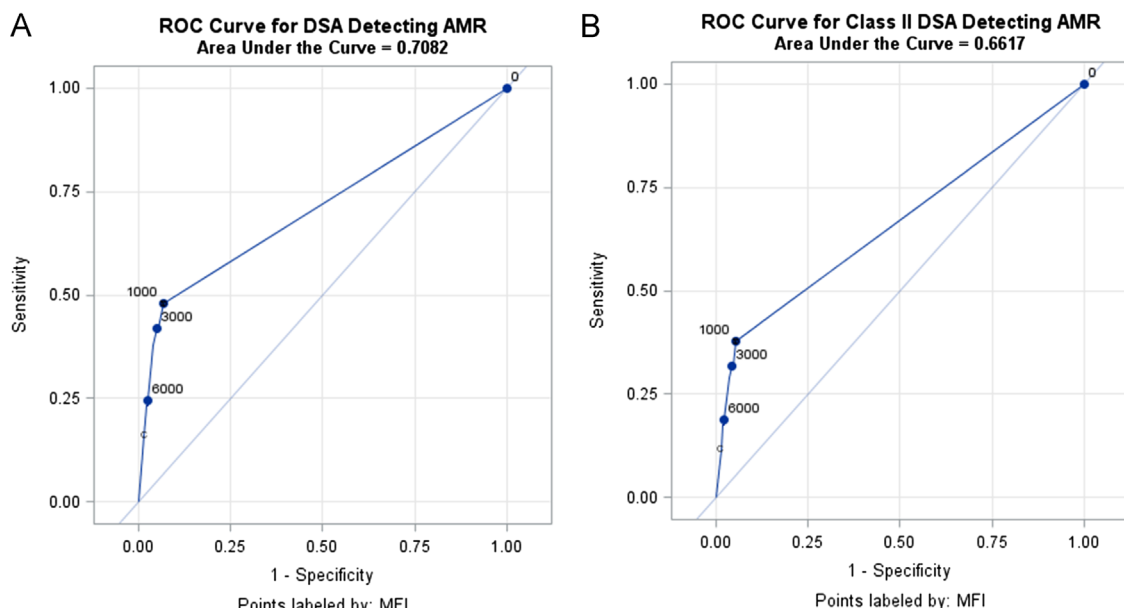


Figure 1 (A) Receiver operator characteristic curve for DSA detecting AMR. (B) Receiver operator characteristic curve for class II DSA detecting AMR.

(OR = 5.02; 95% CI, 0.63–40.3; $p = 0.13$) and class II DSA (OR = 5.44; 95% CI, 1.3–22.6; $p = 0.02$). The odds of graft dysfunction did not correlate with MFI.

DSA and risk of future AMR

Although DSA were unsuccessful in detecting concomitant pAMR in this study, they were predictive of future events. In a crude analysis, patients who made DSA at any time had >7 times the odds of developing at least 1 episode of AMR during the entire follow-up period (OR = 7.60; 95% CI, 3.47–16.62; $p < 0.0001$) compared with patients who never made DSA. A time to event analysis of patients without previous AMR and who developed circulating DSA after transplantation ($n = 54$) demonstrated that those patients had a nearly 3-fold increased risk of future AMR (hazard ratio [HR] = 2.97; 95% CI, 1.31–6.73; $p = 0.009$) (Figure 2A) compared with patients without prior AMR and who never developed DSA. Among patients with DSA, the increased risk for AMR occurred only for patients with class II DSA (Figure 2B). There was no interaction with DSA de novo status ($p = 0.23$). For those 54 patients, the risk of AMR was the greatest in the first 30 days after DSA detection (Figure 3).

DSA and Risk of Graft Loss

There was no evidence of an increased risk of graft loss between patients with AMR with DSA, AMR without DSA, DSA alone, or neither DSA nor AMR ($p = 0.24$). In every instance of graft loss associated with AMR, the DSA included class II anti-HLA antibodies. De novo DSA after transplantation have been shown to confer the greatest risk of post-transplant graft loss⁸; our findings are consistent with this observation. Patients with DSA that were present before transplantation had the lowest risk of graft loss (Figure 4A). Patients who developed de novo DSA had a 151% increase in risk of graft loss (contingent on 30-day survival) compared with patients who did not have DSA (95% CI, 1.11–5.69; $p = 0.027$). This risk remained after controlling for cellular rejection and recurrent AMR

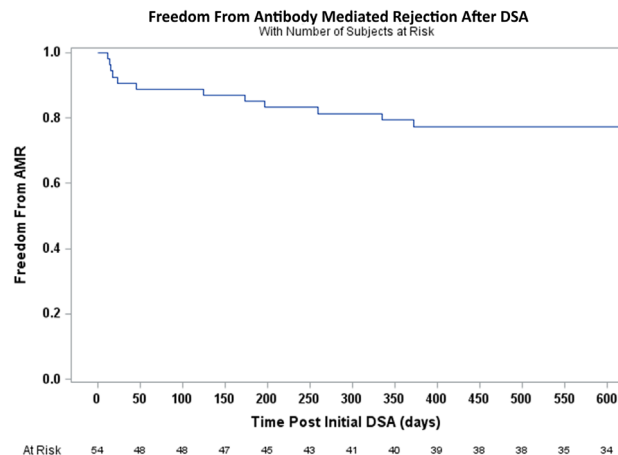


Figure 3 Freedom from initial episode of AMR after detection of DSA.

(HR = 2.79; 95% CI, 1.07–7.30; $p = 0.036$). The risk of graft loss was attributable to de novo class II DSA (Figure 4B). As for class I DSA, survival of patients with pre-transplant and de novo DSA was not diminished.

DSA and risk of CAV

Analyzing the freedom from CAV (ISHLT CAV1 or greater) after transplantation, statistically there was no significant difference, although there was a suggestion that the CAV risk may be increased for patients with AMR with DSA compared with patients without DSA or AMR (HR = 1.55; 95% CI, 0.76–3.19; $p = 0.23$) (Figure 5). This risk did not differ by class of DSA ($p = 0.37$). Freedom from CAV after the initial episode of AMR was also not different between groups ($p = 0.73$).

Discussion

The existence of AMR has been universally accepted only for the last decade, and not surprisingly diagnosis and treatment continue to evolve. This prospective cohort study of 221 consecutive adult transplant patients with >640 patient-years of follow-up sought to determine the role that

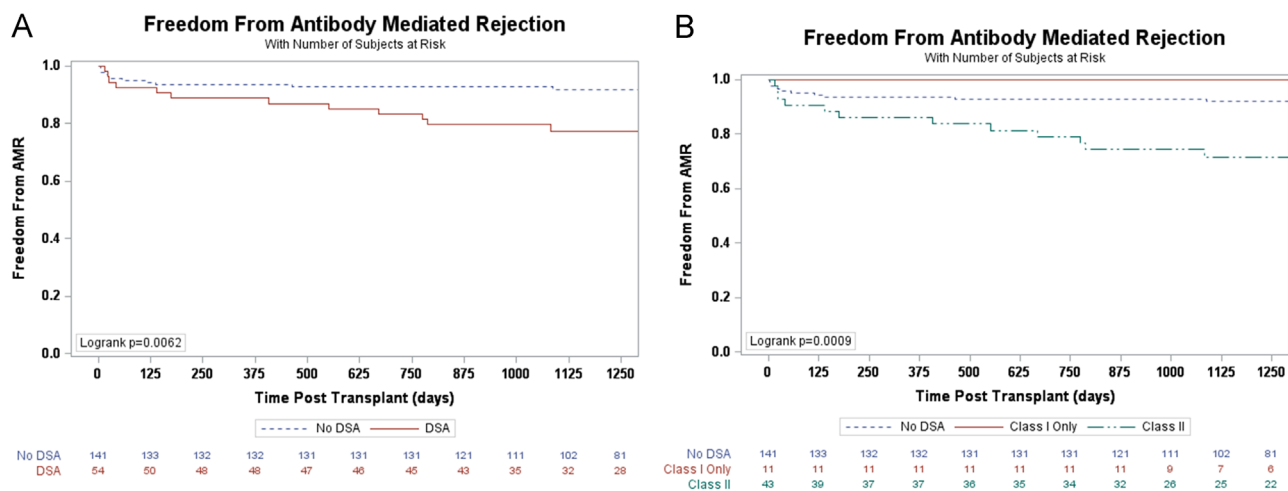


Figure 2 (A) Freedom from initial episode of AMR based on DSA status. (B) Freedom from initial episode of AMR based on DSA class.

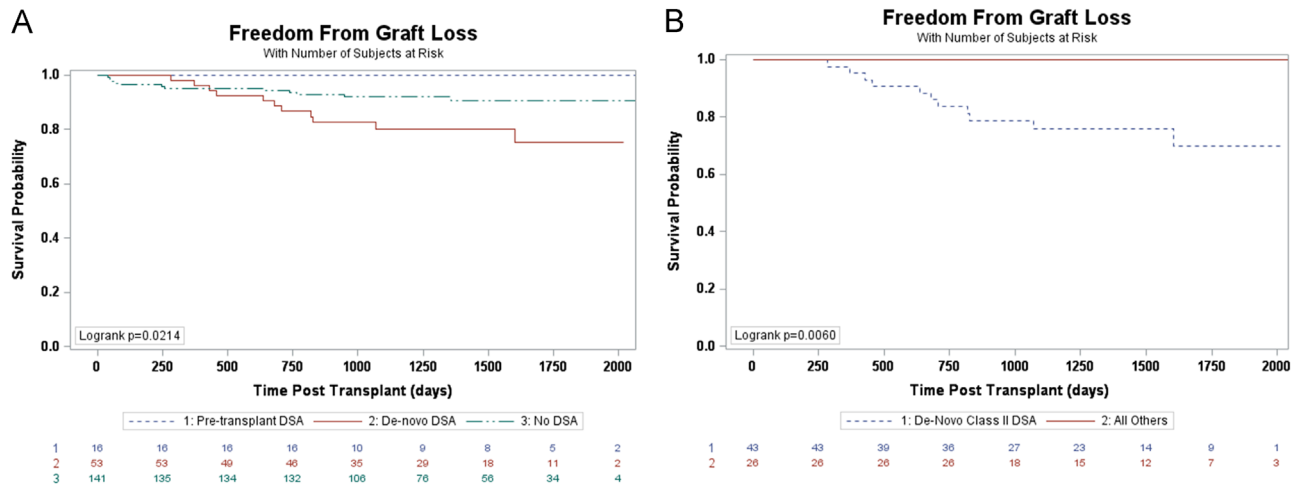


Figure 4 (A) Freedom from graft loss depending on de novo DSA status. (B) Freedom from graft loss based on de novo DSA status and MHC class.

DSA play in AMR and resulted in 4 primary findings. First, DSA were an inadequate screening test for pAMR. Second, the presence of DSA during an episode of AMR was associated with a 5-fold increase in the odds of graft dysfunction for both class I and II DSA. Third, class II DSA increased the risk of future AMR by 3 times, whereas class I DSA did not. Finally, post-transplant de novo class II DSA were associated with an increased risk of graft loss.

After heart transplantation, patients undergo frequent EMB during the first year; however, afterward, routine biopsies are performed infrequently and often are prompted by clinical symptoms. Treated AMR in the first year after transplantation was shown to have good outcomes in 1 recent study¹⁴; however, late AMR with graft dysfunction has been shown to be associated with significant 1-year mortality (50%–60%) despite treatment.^{16,17} The poor outcomes in these studies were likely due to CAV and/or preceding sub-clinical AMR. Loupy et al.¹⁸ recently described the presence of undiagnosed sub-clinical AMR in 40% of a cohort of patients that developed allograft failure. These studies demonstrate the importance of developing a reliable non-invasive screening test for AMR. The findings of

this study suggest that measurement of DSA is not sensitive enough to be that indicator, as it performed only slightly better than a coin flip in this cohort. Similarly disappointing was a recent finding that the Total AlloMap score in addition to gene subsets was unable to detect AMR.¹⁹

Complement deposition has been described as the “sine qua non” of AMR²⁰ and is needed (C4d) to diagnose immunopathologic AMR.¹² Production of C4d through the classic pathway requires antibody-antigen complex formation. However, in this study, DSA were not found to be adequate to detect concomitant pAMR, although they were found to have important prognostic significance. Patients with class II DSA had 3 times the risk of future AMR compared with patients without DSA, the odds of significant hemodynamic and echocardiographic graft dysfunction when DSA were present during an episode of AMR were 437% greater, and class II DSA were associated with an increased risk of mortality. These data signal that DSA (mostly class II) were not innocent bystanders, although they were also not sufficient to diagnose pAMR. A possible explanation may be that we are not screening for the correct antibody or enough antibodies. Antibodies to non-HLA antigens (major histocompatibility complex Class I Chain A [MICA], major histocompatibility complex Class I Chain B [MICB], endothelium, vimentin, myosin, angiotensin II type 1 receptor, and polyreactive) are capable of activating complement, although there are limited data about their role in AMR or in post-transplant care. Alternatively it may be that the episodes of anti-HLA DSA negative AMR do not require antibodies at all and are mediated by the antibody-independent lectin pathway.^{21,22}

The ideal screening test for AMR remains unknown. The current gold standard is EMB and pathologic diagnosis. This method is not without problems, as the disease may be focal and EMB itself may be inadequate to arrive at the diagnosis. This study has shown that DSA provide important information but are not independently sensitive enough to make the diagnosis. Given the prevalence of pAMR (17% in this sample, with nearly half DSA negative) and the known deleterious effects of AMR, further collaborative study is

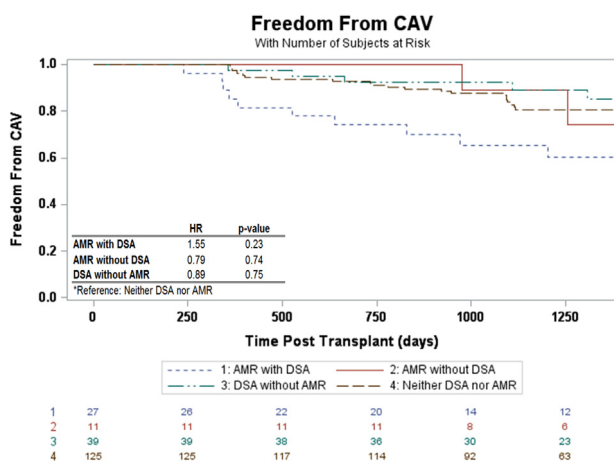


Figure 5 Freedom from CAV stratified by AMR and DSA status.

needed focusing on other non-invasive means of diagnosing AMR, such as biomarkers or non-HLA antibodies. An additional research question is whether treatment of DSA without concomitant pAMR is warranted. DSA have previously been shown to increase cellular rejection,² CAV,³⁻⁵ and mortality.⁶⁻⁸ The additional findings of this study demonstrating that class II DSA increase the risk of future AMR and increase the risk of graft dysfunction with AMR and that de novo class II DSA increase the risk of graft loss suggest there is sufficient evidence to prompt a clinical trial for the treatment of class II DSA in the absence of AMR.

This study was limited by the fact that it was conducted at only 1 large transplant center, which may potentially limit generalizability. Although the numbers of patients, biopsies, and DSA measurements in this cohort were substantial for a study of this nature, the absolute number of patients limited statistical power and subgroup analyses. Furthermore, the follow-up time was relatively short (2–5 years), which limits the number of events. Additionally, CAV was diagnosed predominantly with angiography and not intracoronary imaging, which may have limited the ability to detect more subtle disease (e.g., intimal thickening preceding coronary artery luminal narrowing). MFI measurements have been known to vary among HLA laboratories²³ and may limit the external validity of the findings related to MFI in this study, although the absence of findings for MFI > 10,000 and graft dysfunction would suggest a true lack of association. Lastly, this study was observational and not interventional; therefore, we are unable to comment on treatment strategies of asymptomatic DSA.

In conclusion, DSA were inadequate to diagnose pAMR, but class II DSA provided prognostic information regarding future AMR, graft dysfunction with AMR, and graft loss.

Disclosure statement

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