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# Factors influencing transfusion-associated HLA sensitization in patients bridged to heart transplantation using ventricular assist device

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## Abstract

**Background:** Bridging heart failure patients with mechanical ventricular assist devices (VAD) enables access to transplantation. However, VAD is associated with increased risk for anti-HLA antibodies associated with rejection of subsequent allografts. Factors determining alloantibody formation in these patients remain undefined.

**Methods:** We performed a single-center retrospective cohort study of 164 patients undergoing heart transplantation from 2014 to 2017. Medical records including use of VAD, transfused blood products, anti-HLA antibody testing, crossmatch, and time to transplant were evaluated.

**Results:** Patients received an average of 13.8 red blood cell and 1.9 single-donor platelet units associated with VAD. There was a 28.7% increase in the incidence of anti-HLA antibodies after VAD. Development of anti-HLA antibodies did not correlate with volume or type of blood products, but with pre-VAD HLA sensitization status; relative risk of new alloantibodies in patients with pre-VAD antibodies was 3.5-fold higher than those without prior antibodies ($P$ = .008). Development of new anti-HLA antibodies was associated with an increased time to transplant (169 vs 330 days, $P$ = .013).

**Conclusions:** Our findings indicate that the presence of anti-HLA antibodies pre-VAD was the most significant risk factor for developing additional antibodies post-VAD, suggesting that a subset of patients may be predisposed to alloantibody formation.

**KEYWORDS**

antibodies, heart transplant, HLA, sensitization, transfusion, ventricular assist device

## 1 | INTRODUCTION

The limited availability of suitable donor organs impedes transplantation as a curative therapy for heart failure.[1] The ventricular assist device (VAD) is increasingly used as a bridge to transplantation to increase survival while waiting for a compatible organ. VAD is effective in prolonging survival of patients with heart failure, enabling 70% of patients to bridge to subsequent heart transplant.[1] The effectiveness of VAD has led to increased use, with 19% of heart transplants in the United States between 2004 and 2014 bridged to

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transplant using VAD. Though VADs are an effective bridge therapy, they are associated with distinct complications that affect subsequent transplantation. In addition to complications associated with a major surgery such as bleeding and infection, there is a debated immunologic risk. VAD support has been associated with development of antibodies against HLA alloantigens.[2-8]

The presence of anti-HLA antibodies has serious adverse consequences for patients awaiting heart transplantation. Antibodies against donor HLA class I and class II antigens are definitively associated with antibody-mediated rejection and allograft failure and associated with increased mortality.[9-13] Reported rates of alloimmunization following VAD placement are variable, ranging from 28% to 66% of patients.[2,4,14,15] Transfusion of cellular blood products is known risk factors for HLA-allosensitization, with reported sensitization rates of 17%-15% for packed red blood cell (PRBC) units and 28%-55% for single-donor platelet (SDP) units.[16] Patients undergoing VAD surgery have a nearly universal exposure to blood products, receiving a median of 8 PRBCs and 2 SDPs.[6,17-19] This provides ample exposure to alloantigens, though direct associations between blood product exposure and risk for development of alloantibodies in the setting of VAD remain elusive.[14,20-22] However, reported rates of HLA alloimmunization associated with VAD are higher than for general transfusion, with reports of sensitization rates up to 66%.[2,4,14,15] Similar effects have not been observed for extracorporeal membrane oxygenation, suggesting a unique effect of VAD to promote alloantibody formation. Given the undefined effects of VAD on alloimmunization, we investigated transfusion associated with VAD implantation with the goal of identifying risk factors for anti-HLA antibody formation.

## 2 | METHODS

### 2.1 | Study population

Records for 164 adult patients undergoing heart transplantation (n = 107 patients undergoing VAD implantation as a bridge to transplantation, n = 57 non-VAD patients) at the University of California San Diego between January 2014 and September 2017 were retrospectively reviewed (Table 1). Transfusion data were available for all 164 heart transplant patients. Of the 107 VAD patients, both pre-VAD and subsequent post-VAD antibody data were available in 59 patients (n = 84 pre-VAD antibody data, n = 61 post-VAD antibody data). Electronic medical records and laboratory records were reviewed for perioperative (day 0-3 following VAD insertion) blood product utilization, and anti-HLA antibody testing (most recent pre-VAD placement and 2-12 weeks after VAD placement) and cellular crossmatch testing results. Clinical records were reviewed for patient demographics and clinical events related to VAD implantation and heart transplantation. All study procedures were conducted under the approval and supervision of institutional IRB.

**TABLE 1** Patient demographics

| | VAD patients | Heart transplant patients not receiving VAD |
|---|---|---|
| N | 107 | 57 |
| Age (mean, range) | 58.7 (19-80) | 52.0 (19-73) |
| Gender (% female) | 15.0 | 14.0 |
| Ethnicity (%) | | |
| Asian | 6.5 | 10.5 |
| African American | 12.1 | 8.8 |
| Caucasian | 42.0 | 33.3 |
| Hispanic | 31.8 | 43.9 |
| Native American | 2.8 | 3.5 |
| Other/Unknown | 4.7 | 0 |
| Diagnosis (%) | | |
| Ischemic cardiomyopathy | 51.4 | 28.1 |
| Non-ischemic cardiomyopathy | 45.8 | 43.9 |
| Congenital | 0 | 14.0 |
| Other | 2.8 | 14.0 |

### 2.2 | Anti-HLA antibody testing

All patient serum samples were collected and tested as part of standard of care treatment according to the existing protocols. Patients were screened for anti-HLA antibodies using FlowPRA Class I and Class II assays (One Lambda) using FACSCanto or FACSCalibur instruments (BD Biosciences). Anti-HLA antibodies detected by FlowPRA were identified using LABScreen Single Antigen HLA Class I and Class II bead assays using a Labscan 200 (Luminex). Data were analyzed using HLA Fusion software (One Lambda). Antibodies with normalized mean fluorescence intensity (MFI) ≥3000 were identified as positive, based upon likelihood of causing a positive flow cytometric crossmatch.[23]

### 2.3 | Cellular crossmatch testing

Donor lymphocytes isolated from peripheral blood, spleen, or lymph node samples by density gradient separation using Rosette-Sep Lymphocyte Enrichment kit (StemCell Technologies) were treated with 2 mg/mL pronase (Sigma) for 20 minutes at 37°C. Donor cells were incubated in duplicate with current (typically <30 days old) and historical peak (maximum cPRA within the last 12 months) recipient serum for 20 minutes at room temperature. Cells were labeled with anti-CD3 PerCP (SK7; BD Biosciences) anti-CD19 PE (SJ25-C1; BD Biosciences), and goat F(ab')2 anti-human IgG FITC (Jackson) for 20 minutes at 4°C, washed, and analyzed using a FACSCalibur or FACSCanto. Alloantibody binding was determined by calculating mean channel shift (MCS) of cells incubated with patient serum as compared to cells incubated with

control normal human serum; MCS ≥16 was considered positive for T-cell FCXM and MCS ≥32 was considered positive for B cell FCXM.

## 2.4 | Statistical analysis

Calculated PRA (cPRA) percentages were calculated entering all unacceptable antigens for HLA-A, -B, -C, -DR, and -DQ in the UNet computer system at the US Department of Health and Human Services Organ Procurement and Transplantation Network website (http://optn.transplant.hrsa.gov). Categorical data were analyzed using Fisher's exact test and chi-square test for multifactor analysis, numerical data were analyzed using the Mann-Whitney test and the Wilcoxon signed-rank test for paired samples, and survival analysis was performed by the Gehan-Breslow-Wilcoxon test using Prism 7 (Graph Pad).

## 3 | RESULTS

### 3.1 | VAD placement results in increased anti-HLA antibodies

To examine the effect of VAD on sensitization against allogeneic HLA, we compared the incidence of anti-HLA antibodies in patients before (n = 84) and after VAD implantation (n = 61) (Figure 1A). The proportion of patients testing positive for any anti-HLA antibodies predicted to cause a positive cellular crossmatch (MFI ≥3000) increased from 34.5% in the pre-VAD population to 63.2% in the post-VAD population (P = .001). Direct comparison of pre-VAD and post-VAD anti-HLA antibodies by individual patients (n = 59) demonstrated that VAD placement was associated with the development of new antibodies against both HLA class I and class II antigens (Figure 1B). This resulted in a significant increase in risk for immunologic incompatibility with potential donors as measured by increased cPRA (Figure 1C).

### 3.2 | Development of anti-HLA antibodies after VAD significantly delays subsequent transplantation

Development of anti-HLA antibodies decreases the pool of immunologically compatible donors, potentially hindering subsequent transplant. To evaluate the potential effect of new anti-HLA antibodies formed after VAD placement on subsequent transplantation, we compared the time from VAD placement to transplant between patients who developed new anti-HLA antibodies (n = 19) and those who did not (n = 40) (Figure 2). Patients developing new alloantibodies had a significant delay in subsequent transplantation. Patients who developed new anti-HLA antibodies post-VAD implantation waited an average of 330 days for a heart transplantation vs the average wait time of 169 days for patients who did not develop new

anti-HLA antibodies (P = .013). Review of crossmatch testing demonstrated that patients having developed anti-HLA antibodies after VAD were twice as likely to have a positive crossmatch against a potential donor as compared to patients who did not develop antibodies after VAD (36.4% vs 15.6%, P = .031). This resulted in having to evaluate more potential donors in order to find an immunologically compatible organ (2.6 ± 1.6 donors tested/transplanted organ) in patients with new anti-HLA antibodies as compared with those who did not develop additional antibodies after VAD implantation (1.1 ± 1.1). This increase does not take into account organ offers that may have been deemed unlikely to be immunologically compatible based on UNET avoid antigen listing or virtual crossmatch analysis. VAD patients had a higher rate of false-positive reactivity that was eliminated with DTT serum treatment compared with heart transplant patients that did not receive VAD therapy (30.4% in VAD patients vs 5.9% in patients without VAD, P = .107). However, the increased frequency of positive crossmatches for patients developing anti-HLA antibodies after VAD was not attributable to non-specific antibody reactivity, as both groups had similar rates of conversion of positive cellular crossmatch to negative with serum DTT treatment (31.3% in patients with new antibodies vs 28.6% in patients without new antibodies, P = 1.000).

### 3.3 | Transfusion associated with VAD

Formation of antibodies against HLA alloantigens results from specific sensitizing events such as transplantation, transfusion, or pregnancy. Given the high degree of exposure to blood products associated with thoracic surgery for VAD placement, we sought to evaluate whether there is a product-specific or dose-dependent relationship between peri-VAD surgery transfusion and anti-HLA antibody formation. Review of transfusion records for all VAD patients demonstrated that 100/107 (93.5%) of patients received at least one PRBC or SDP unit temporally associated with VAD placement (defined as either during surgery or within 3 days after surgery). Average transfusion support included 9.6 ± 9.9 total cellular blood products including 7.8 ± 9.1 PRBC units and 1.8 ± 1.9 SDP units (Figure 3A), similar to reported rates of transfusion associated with VAD placement. These data confirm the significant exposure risk for alloimmunization by transfusion in patients receiving VAD therapy.

To evaluate the risk of anti-HLA antibody formation associated with VAD-associated transfusion, we correlated formation of new anti-HLA antibodies in the 59 patients for which pre- and post-VAD anti-HLA antibody testing was available with PRBC and SDP transfusion. New anti-HLA antibodies were detected in 25/56 (44.6%) of patients receiving PRBCs (468 total units transfused), 18/44 (40.9%) of patients receiving SDPs (117 total units transfused), and 27/59 (45.8%) of transfused patients. Patients that developed new anti-HLA antibodies associated with VAD placement did not demonstrate any differences in exposure to transfused PRBC and SDP compared to patients that did not develop antibodies (Figure 3B). The number of new anti-HLA antibodies did
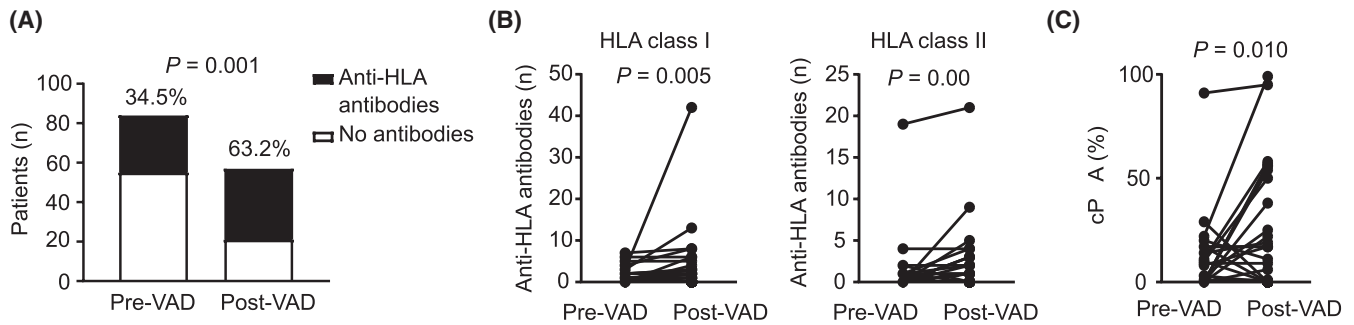
**(A)**



**(B)**

HLA class I

HLA class II



**(C)**



**FIGURE 1** Ventricular assist device (VAD) implantation is associated with increased anti-HLA antibodies. A, Comparison of anti-HLA antibodies detected by solid-phase immunoassays in patients prior to VAD implantation or in patients 1-3 mo after VAD placement. B, Comparison of antibodies against HLA class I and class II antigens detected in pre- and post-VAD samples for individual patients. Groups compared using the Wilcoxon signed-rank test. C, Change in overall allosensitization evaluated by cPRA calculated from individual alloantibodies detected in pre- and post-VAD samples. Groups compared using the Wilcoxon signed-rank test
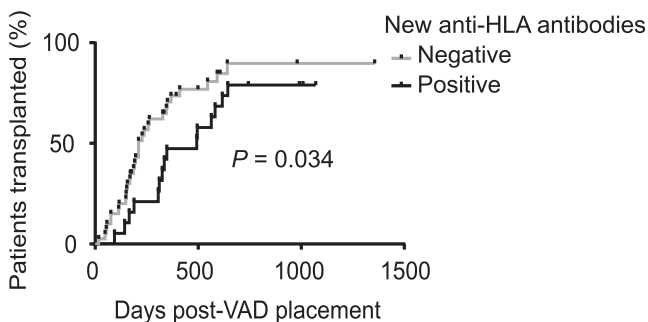


**FIGURE 2** Development of anti-HLA antibodies after VAD placement is associated with an increased time to subsequent transplant. Death-censored time to transplant for patients developing or not developing additional anti-HLA antibodies after VAD placement. Curves compared using the Gehan-Breslow-Wilcoxon test

not demonstrate significant correlation with the unit exposure to PRBCs ($r$ = .014, $P$ = .912), SDPs ($r$ = .075, $P$ = .651), or total transfusion ($r$ = .072, $P$ = .586).

### 3.4 | Factors influencing alloantibody formation

Given the absence of a dose-dependent correlation between cellular blood product transfusion and formation of anti-HLA antibodies, we next investigated patient-specific factors that could explain differential alloantibody formation (Table 2). There were no significant differences in age, gender, ethnicity, underlying diagnosis, or type of VAD used between patients that developed new anti-HLA antibodies compared to those that did not (n = 19 patients who developed new anti-HLA antibodies, n = 40 patients who did not develop new anti-HLA antibodies). However, 10/19 (52.6%) of patients developing new anti-HLA antibodies after VAD placement had other pre-existing anti-HLA antibodies compared with only 6/40 (15.0%) of patients who did not develop new alloantibodies ($P$ = .004). Given that our laboratory uses a cutoff value of 3000 MFI to identify positive anti-HLA antibodies, it is

conceivable that antibodies evident as positive after VAD placement were present at lower levels and increased in response to allogeneic stimulation by transfusion. To evaluate this, we compared the MFI for each antibody detected by solid-phase immunoassay on samples prior to and following VAD placement (Figure 4). Of the 19 patients who were new anti-HLA antibody formers post-VAD implantation, a total of 90 new alloantibodies were detected. A majority (65/90, 72.2%) of these new antibodies developed in the absence of previously detectable antibodies as indicated by MFI values <1000 on pre-VAD testing. Of the remaining 25 antibodies (27.8%), 9/90 (10.0%) had pre-VAD MFIs between 2000 and 3000 and 16/90 (17.8%) had pre-VAD MFIs between 1000 and 2000. We interpret the second group as reflecting a memory immune response against alloantigens to which the patient had been previously sensitized. Together, these data suggest that patients who have a history of forming anti-HLA antibodies after prior alloimmunization are at increased risk (OR = 6.30, 95% CI 1.24-4.34) for development of anti-HLA antibodies associated with VAD placement.

## 4 | DISCUSSION

Alloantibodies present a significant obstacle to heart transplantation. Anti-HLA antibodies are present in as much as 43% of patients awaiting heart transplantation, reducing the number of immunologically compatible organs from among the already limited pool of potential donors.[1] VAD devices are an effective and widely used method for improving cardiac function in patients with heart failure. Unfortunately, VAD use is associated with development of anti-HLA antibodies which hinder subsequent transplantation.[2-8] Experience from our center mirrors this, with significantly increased incidence of anti-HLA antibodies after VAD placement (Figure 1). Importantly, development of new anti-HLA antibodies after VAD placement presented a significant obstacle to identification of an immunologically compatible organ, delaying subsequent transplantation wait times twofold compared to those without new
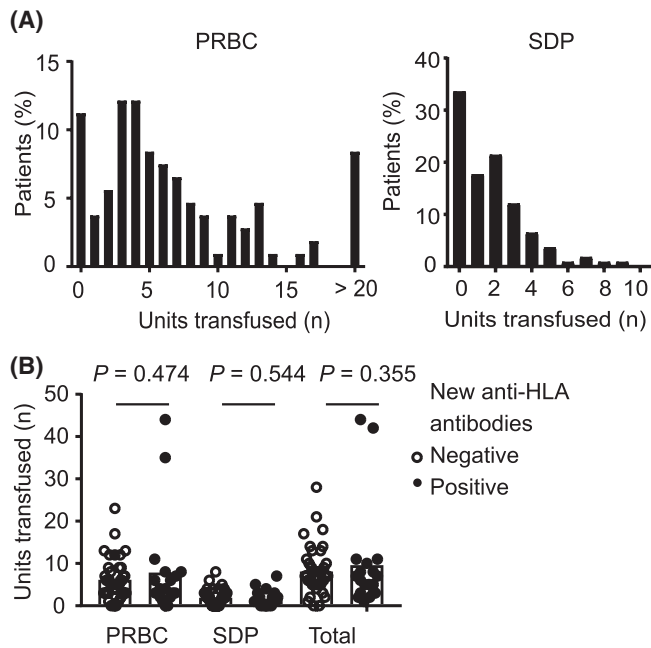
**FIGURE 3** Differential exposure to cellular blood components does not explain development of anti-HLA antibodies associated with VAD placement. A, Frequency of patients receiving indicated doses of PRBC and SDP products. B, Comparison of blood product exposure for patients developing or not developing new anti-HLA antibodies. Groups compared using the Mann-Whitney test
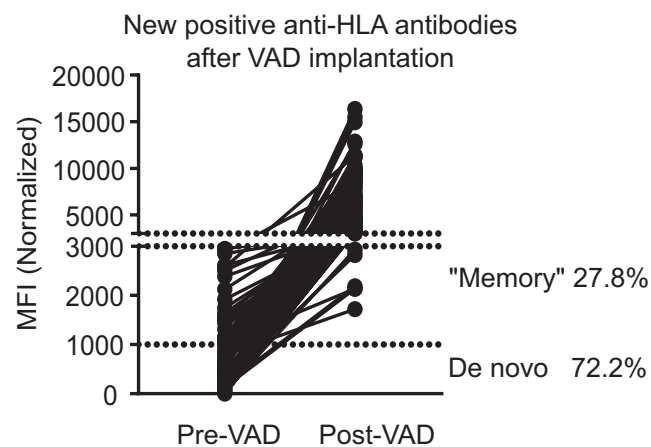


**FIGURE 4** New anti-HLA antibodies detected after VAD represent both de novo and memory immune responses. Comparison of MFI for individual HLA specificities detected by single-antigen immunoassay in pre- and post-VAD samples for patients with new antibodies detectable after VAD placement. Dotted line at 1000 MFI represents upper limit for antibodies considered not present in pre-VAD samples (de novo). Dotted line at 3000 MFI represents upper limit for antibodies considered to be present but not "positive" in pre-VAD samples (memory)

anti-HLA antibody formation (Figure 2), similar to effects in other studies.[6,21] Thus, we sought to identify underlying risk factors that might enable risk stratification or risk avoidance measures.

Ventricular assist device-associated antibodies can be split into two categories, non-specific antibodies and HLA-specific

**TABLE 2** Risk factors for alloimmunization

| | No new antibodies n = 40 | New anti-HLA antibodies n = 19 |
|---|---|---|
| Age (y) | 56.2 ± 12.3 | 55.9 ± 13.5 |
| | | P = .838 |
| Female (%) | 12.5 | 21.1 |
| | | P = .438 |
| Ethnicity (%) | | |
| Asian | 5.0 | 5.3 |
| African American | 12.5 | 5.3 |
| Caucasian | 40.0 | 47.4 |
| Hispanic | 35.0 | 26.2 |
| Native American | 5.0 | 5.3 |
| Other/Unknown | 2.5 | 10.5 |
| | | P = .757 |
| Diagnosis (%) | | |
| Ischemic cardiomyopathy | 35.0 | 52.6 |
| Non-ischemic cardiomyopathy | 55.0 | 47.4 |
| Congenital | 0 | 0 |
| Other | 10.0 | 0 |
| | | P = .220 |
| VAD type (%) | | |
| HVAD | 40.0 | 57.9 |
| HM II | 35.0 | 21.1 |
| HM III | 7.5 | 10.5 |
| BiVAD | 17.5 | 10.5 |
| | | P = .465 |
| Pre-VAD anti-HLA Antibodies (n, %) | 6 (15.0) | 10 (52.6) |
| | | P = .004 |

alloantibodies. Production of non-specific antibodies associated with VAD has been attributed to bystander activation of B cells due to a "pro-inflammatory" environment caused by turbulent blood flow and reactivity to the VAD. Non-specific antibodies can cause positive reactions in cellular crossmatch and alloantibody immunoassays, though their clinical significance appears to be limited.[4,5,22,24] Consistent with this, we observe non-specific antibodies as evidenced by high background in solid-phase immunoassays and cellular crossmatching, though the effects of these are limited by routine use of EDTA serum treatment in solid-phase immunoassay and DTT serum treatment in cellular crossmatch. Of greater concern are antibodies with definable specificities against allogeneic HLA which can mediate AMR and allograft loss.[9-13]

Development of antibodies against allogeneic HLA requires sensitization against specific alloantigen. Alloimmunization can occur through pregnancy, transfusion of cellular blood products, or prior

transplant.[16] Given the nearly ubiquitous exposure risk as well as the viability of blood product exposure as a modifiable risk factor, we investigated cellular blood product exposure at the time of VAD placement as a risk factor for development anti-HLA antibodies. Analysis of cellular blood product exposure for patients in our study did not reveal a relationship between exposure dose and risk of alloimmunization (Figure 3). Direct association between cellular blood product exposure and development of antibodies following VAD implantation has proven similarly elusive in other studies for several reasons, including the nearly universal exposure to cellular blood products and variability in the origin and composition of cellular blood products (ie, leukoreduced or non-leukoreduced PRBCs, SDPs vs multi-donor platelet units, irradiated or non-irradiated units). The most suggestive of these studies have indicated a higher risk of allommunization in VAD patients resulting from platelets[20,21] and a decreased risk associated with leukoreduced PRBCs.[14,16,20,25.] However, other studies saw no relationships between transfusion exposure and alloimmunization.[22]

Comparison of previously studied risk factors for anti-HLA antibody incidence did not demonstrate significant differences between patients developing anti-HLA antibodies and those not developing antibodies related to gender, ethnicity, or type of VAD used (Table 2). Much like other studies in thoracic organ transplantation, our study is limited by sample size, especially when comparing transplantation wait times (data available for 55% of patients, 59/107). This increases the probability of a type II error, which could lead to rejection of possibly relevant clinical factors that our study is not powered to confidently identify. However, we observed significantly increased risk for new anti-HLA antibodies in patients with prior evidence of alloimmunization. Other studies have observed similarly increased risk for anti-HLA antibody formation in patients with a history of detectable anti-HLA antibodies.[6,25] Our findings that 72.2% of new anti-HLA antibodies having pre-VAD MFIs 0-1000 (Figure 4) are suggestive that a majority of anti-HLA antibodies that arise after VAD placement and associated transfusion may do so as new immune responses to VAD-associated sensitization. Our data cannot definitively rule out the possibility that these newly detected antibodies are not a result of memory immune responses, particulary since they occur in patients with other anti-HLA antibodies (demonstrating prior sensitization). Together, these data support a conclusion that the detection of anti-HLA antibodies is predictive of an increased risk for subsequent development of additional anti-HLA antibodies. This suggests there may be factors that predispose some patients to form alloantibodies, in the context of VAD but also in a more general manner. Future studies identifying such factors would be of particular interest to facilitate focused risk reduction strategies as well as possibly identify actionable targets to inhibit alloimmunization. Given the absence of a well-defined association between cellular blood product exposure, VAD placement, and alloimmunization, it is likely prudent to continue with current best practice guidelines for general blood conservation in thoracic surgery[26] and exclusive use of leukoreduced PRBCs[27] to minimize potential risk for alloimmunization.

It is worth noting that primary outcomes in this study included anti-HLA antibody formation as well as time to transplant. We do not have complete post-transplant anti-HLA antibody and clinical outcomes data for this cohort, and were thus unable to analyze mortality and the incidence of antibody-mediated rejection. Questions regarding the impact of pre-transplant alloimmunization on post-transplant outcomes are of interest for future study. We hypothesize that patients who are sensitized to alloantigens as a result of VAD therapy could be at risk for post-transplant donor-specific alloantibody formation and resulting higher morbidity and mortality. A recent study[28] reported the outcome of patients with high immunological risk defined by pre-transplant donor-specific antibodies that were managed with a post-transplant prophylactic strategy instead of desensitization. The results of our study suggest that it may be of interest to propose applying such a strategy to patients identified as "at risk" based on immunologic response to pre-transplant alloimmunization against non–donor-specific HLA.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

## AUTHORS' CONTRIBUTIONS

Jae Elkind, Juliana Sobczyk, Oscar Ostberg-Braun, Jorge Silva Enciso, Eric Adler, and Gerald P. Morris: Designed study, analyzed data, and revised the manuscript; Jae Elkind, Juliana Sobczyk, and Oscar Ostberg-Braun: Collected data; and Jae Elkind, Juliana Sobczyk, and Gerald P. Morris: Analyzed data and drafted manuscript.

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