Published in final edited form as:

Transplantation. 2013 August 15; 96(3): 324–330. doi:10.1097/TP.0b013e3182985371.

LVAD Implant as a Bridge to Heart Transplantation is Associated with Allosensitization as Measured by Single Antigen Bead Assay

Nisha Shankar¹, Richard Daly², Jennifer Geske³, Sudhir K Kushwaha⁴, Michael Timmons⁵, Lyle Joyce⁶, John Stulak⁷, Manish Gandhi⁸, Walter Kremers⁹, Soon Park¹⁰, and Naveen L Pereira¹¹

¹Participated in research design, writing the paper, performance of the research, and data analysis. University of Connecticut Health Center; Farmington, CT

²Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

³Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

⁴Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

⁵Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

⁶Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

⁷Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

⁸Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

⁹Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

¹⁰Participated in writing of the paper and performance of the research. Mayo Clinic; Rochester, MN.

¹¹Participated in research design, writing the paper, performance of the research, and data analysis. Mayo Clinic; Rochester, MN.

Abstract

Background—Left ventricular assist devices (LVAD) as a bridge (BTT) to heart transplantation (HTX) may be limited by the formation of anti-HLA antibodies. Whether sensitization occurs with

Corresponding author: Naveen L. Pereira, M.D. Mayo Clinic 200 First Street SW Rochester, MN 55905 Phone: 507-538-8151/Fax: 507-266-0103.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

continuous axial flow LVAD implant as assessed by Single Antigen Bead (SAB) assay is unknown.

Methods—Cytotoxic panel reactive antibody (PRA) and SAB assays were analyzed in HTX recipients undergoing LVAD implant as a BTT. Sensitization was defined as peak anti-HLA antibody values of >2000 mean fluorescent intensity as these values have been found to correlate with flow cytometric crossmatch results.

Results—LVADs were implanted as BTT in 30 patients. There were 7% (2/30) of patients prior to and no patients after LVAD implant with PRA >10%. However, 20% (6/30) of patients prior to and 53% (16/30) after LVAD were sensitized as measured by SAB ($p=0.024$). At HTX, 47% (14/30) of patients remained sensitized. A positive virtual crossmatch was observed in 28% (4/14) of the sensitized patients at HTX. There was no difference between the sensitized and non-sensitized groups ($p>0.4$ for all) in usage of blood products (64 11 vs. 63 39 units), time to HTX (286 63 vs. 257 48 days) and 1 year after HTX, there were no differences in rejection (total rejection score 0.30 vs. 0.37) and survival (93% vs. 88%).

Conclusion—Allosensitization after LVAD is common despite cytotoxic PRA being negative. One year after HTX, this sensitization does not translate into increased acute cellular or antibody mediated rejection or reduced survival.

Keywords

Heart transplant; HLA; single bead antigen assay; left ventricular assist device

Introduction

Left ventricular assist devices (LVAD) are increasingly being used as a bridge to heart transplantation (BTT). In 2009, for the first time, over 30% of heart transplant recipients were bridged with mechanical circulatory support¹. However, one of the proposed limitations of LVAD therapy is the higher degree of sensitization prevalent in these patients². Patients who are sensitized to foreign human leucocyte antigens (HLA) and await heart transplantation (HTX) have a longer waiting time on the HTX list than non-sensitized patients³. Despite various immunosuppression strategies targeting sensitized patients, the efficacy of these approaches appear to be limited, rendering desensitization as a procedure of limited opportunity for these unfortunate patients⁴. Furthermore after HTX, the sensitized recipient is at an increased risk for rejection and has inferior survival,⁵.

Historically, LVAD associated sensitization has been characterized by performance and measurement of panel reactive antibodies (PRA) based on a complement dependent cytotoxicity (CDC) assay, a technique that is neither specific nor sensitive for anti-HLA antibodies. Therefore, many transplant centers are increasingly using more sensitive techniques like single antigen bead (SAB) assays to assess degree of sensitization in potential HTX recipients⁴. It is now common practice to obtain anti-HLA antibody (Abs) information by using SAB in potential HTX recipients for the purposes of determining transplant eligibility, listing unacceptable antigens and determining suitability of donors. LVAD implant is also being recommended to bridge sensitized patients to transplant. However, to date there has been no data published on whether sensitization as measured by this newer technology occurs with continuous axial flow LVAD implantation in the adult population. The purpose of this study was to assess the impact of LVAD implant on sensitization as measured by SAB assays and to correlate sensitization, if it occurs, with clinical outcomes in BTT LVAD recipients.

Methods

The study was performed at Mayo Clinic, Rochester and was approved by the institutional review board.

Patient population

A total of 30 consecutive HTX recipients who underwent continuous axial flow LVAD implants as a BTT were included in this study. All clinical and demographic data at baseline, before and after LVAD implant and after HTX was retrieved from the electronic medical record. Primary immunosuppressive agents (calcineurin inhibitors or sirolimus), and secondary immunosuppressive agents mycophenolate mofetil (MMF) or azathioprine, and dose of prednisone was not modified based on the presence or absence of donor specific antibodies (DSA). All HTX recipients received induction therapy with monoclonal antibody against CD3 (OKT3) or antithymocyte globulin (ATG), as part of a standard induction protocol. Patients with a positive flow crossmatch assay underwent plasmapheresis immediately after HTX for 5 days. Total rejection score was calculated for each patient as described before⁶. Antibody mediated rejection was defined as per standard ISHLT criteria and reported as AMR 1 or 0.

Anti-HLA antibody characterization

Anti-HLA antibody levels were quantified using a combination of cell-based and solid-phase assays. HLA-Abs were measured prior to and after LVAD implantation and at the time of HTX. DSA were defined as HLA-Abs to the HLA antigens shared by the donor.

Complement Dependent Cytotoxicity (CDC) PRA was determined by a CDC-AHG assay using 56 well commercial T-lymphocyte frozen cell tray (Gentrac Inc, Liberty NC). Positive reaction was >50% cytotoxicity. All patients were tested using a T-cell AHG-CDC crossmatch assay and T-cell and B-cell flow crossmatch assay. A positive flow crossmatch result is defined as a channel shift greater than 52 or 106 for T or B lymphocytes, respectively as described previously⁷. Flow crossmatch was performed retrospectively at our institution within 24 hours of transplant.

Pretransplant sera were screened for anti-HLA antibodies using purified HLA antigen coated microspheres (LABScreen™ Single Antigen Beads, One Lambda) as described⁸. An accompanying analysis program (HLA Fusion version 1.2.1b, One Lambda) assisted in detecting the strength of HLA Class I and Class II antibodies. The analysis program determined a mean fluorescence intensity (MFI) value for each HLA specificity that corresponds to the strength of the antibody.

Sensitization and Positive SAB MFI level

In our laboratory we find a good correlation between the DSA levels and flow crossmatch, namely flow crossmatch is negative when DSA MFI is <300 and is positive when DSA MFI is >2000⁸. As the DSA level increases, there is a greater likelihood of a positive crossmatch. For example, correlating DSA MFI > 2000 as cutoff with B-flow crossmatch, the sensitivity is 84% and specificity is 100%. Thus for this study sensitization was defined when anti-HLA Abs MFI >2000.

Virtual Crossmatch

Pretransplant SAB data were compared with the potential donors antigen typing. Presence of DSA in serum sample was considered as positive virtual crossmatch where DSA MFI was >2000.

HLA antigen typing

Low resolution Class I HLA-A, B, Cw and Class II HLA-DRB1, DQB1 was performed using the LABType® SSO (One Lambda) which uses sequence-specific oligonucleotide probes bound to fluorescently coded microspheres to identify alleles encoded by the sample DNA.

Statistical Analysis

Associations of categorical variables with sensitization at time at transplant were examined using Fisher's Exact test. McNemar's test was used to test for a change in the proportion of sensitized patients at each pair of time points. Tests of change in continuous measures used paired Wilcoxon rank sum tests (nonparametric) and paired t-tests (parametric). Kaplan Meier plots are presented for time until acute rejection post-transplant. The log-rank test was used to compare time until rejection post-transplant by sensitization status at time of transplant.

Results

Patient characteristics

There were 30 patients who underwent LVAD as BTT; average age of the patient was 49 ± 2 years. There were 83% males and 17% females. There were 9 patients with ischemic and 21 patients with non-ischemic cardiomyopathy. The type of devices that were implanted included HeartMate II (67%), VentrAssist (20%), and Jarvik 2000 (13%). The mean duration of support was 264 ± 36 days.

Impact of LVAD implant on allosensitization

There were 7% of patients who had a CDC PRA $\geq 10\%$ pre-LVAD implant. After LVAD implant, none of the patients had a CDC PRA $\geq 10\%$. In contrast as shown in Figure 1, prior to LVAD implantation, anti-HLA antibodies measured by SAB assay identified 20% (6/30) of the population as being sensitized. After LVAD implant, 57% (17/30) of patients were sensitized ($p=0.024$). Hence, there were a significantly increased number of patients, 46% (11/30), who became sensitized de-novo after LVAD implantation. The number of patients who were sensitized decreased to 47% (14/30) at the time of HTX. Of these, 5 patients were sensitized pre-LVAD, the remainder 9 patients had developed de-novo sensitization after LVAD implant.

Impact of LVAD implant on anti-HLA antibody formation

Mean peak and cumulative Class I and II anti-HLA antibody MFI values as related to LVAD implant and transplant are summarized in Figures 2A and 2B respectively.

Peak anti-HLA antibody MFI values

After LVAD implantation, mean peak class I anti-HLA antibody MFI value rose significantly to 3317 ± 4041 MFI ($p=0.048$) but at the time of HTX decreased to 2624 ± 3745 MFI. The mean peak class II anti-HLA antibody MFI values after implant rose significantly to 1832 ± 3119 ($p=0.013$) and decreased to 1645 ± 3006 MFI at HTX.

Cumulative anti-HLA antibody MFI values

There was no significant change in cumulative class I anti-HLA antibody MFI values pre to post LVAD. The mean cumulative class II anti-HLA antibody MFI values rose significantly to 8099 ± 13230 MFI post-LVAD ($p=0.008$) and decreasing to 6978 ± 14286 MFI at the time of HTX.

Number of anti-HLA antibodies

To further quantify the impact of LVAD implant on anti-HLA antibody formation, the number of the different types of anti-HLA antibodies with MFI values > 2000 MFI (MFI value used to define sensitization) was measured per patient pre and post LVAD implant (as depicted in Figure 3). There were a mean of 2.6 ± 7.5 Class I anti-HLA antibodies pre LVAD that marginally increased to 4.1 ± 8.2 post LVAD ($p=0.05$), however there was a significant decrease to 2.7 ± 5.8 ($p=0.02$) at the time of HTX. There was no significant change in the number of Class II anti-HLA antibodies with LVAD implant (mean values 0.6 ± 2.3 pre LVAD, 0.9 ± 2.1 post LVAD, 0.6 ± 1.7 at HTX).

Impact of LVAD on anti-HLA antibody formation in recipients sensitized prior to LVAD implant

To assess the impact of LVAD implant on patients who are sensitized prior to LVAD implant, the change in antibody MFI values as related to LVAD implant is summarized in Table 1 for pre-LVAD sensitized and non-sensitized patients. In the patients sensitized prior to LVAD implant, a decrease in peak Class I anti-HLA antibody MFI values ($p=0.002$) but no significant change in Class II MFI values after LVAD implantation was observed as compared to the change in MFI values to the non-sensitized patients. There is a significant decrease after LVAD implant in the number of Class I anti-HLA antibodies but not Class II in the pre-VAD sensitized patients as compared to the non-sensitized patients after LVAD implant (class I -6.7 ± 10 versus 1.8 ± 4.5 p -value= 0.001, class II -0.7 ± 2.2 versus 0.2 ± 0.6 p -value= 0.523) (Figure 3).

Post LVAD sensitized versus non-sensitized recipients

The group of patients was then divided into those who were sensitized ($n=14$) and non-sensitized ($n=16$) at the time of HTX. The clinical characteristics of these patients are listed in Table 2. The mean age of the sensitized patients was 50 ± 2 years and non-sensitized patients was 47 ± 4 years. There was a trend towards more females being present in the sensitized group as compared to the non-sensitized group. There was no difference in the total usage of blood products within the two groups. The time to HTX was not significantly different indicating that sensitization by anti-HLA antibody MFI values did not affect waiting time.

Impact of LVAD implant on Virtual Crossmatch

Using anti-HLA antibody MFI values >2000 MFI, a cut off established in our laboratory based on high sensitivity for a positive flow crossmatch, a positive virtual crossmatch was seen in 28% (4/14) of the post LVAD sensitized patients and none in the post LVAD non-sensitized group. Amongst the 4 patients with a positive virtual crossmatch, 2 patients were sensitized pre-LVAD. A positive T-flow crossmatch was seen in 2 of the sensitized patients and none in the non-sensitized patients. There were no patients in either group who had a positive B-flow crossmatch.

Impact of LVAD implant associated allosensitization on HTX outcomes

The post HTX outcomes of patients based on their sensitization status after LVAD implant and at the time of transplant are outlined in Table 3. There were no differences in total rejection scores and in antibody-mediated rejection in both the sensitized and non-sensitized groups 1 year after HTX. There were no significant differences in the number of sensitized patients (7%) and non-sensitized patients (25%) who experienced severe rejection episodes that were treated ($p=0.336$). The median time to rejection in sensitized (1.74 months) and non-sensitized (1.76 months) patients was not significantly different as shown in Figure 4. Ejection fraction was preserved in both groups at 1-year post HTX and the infection risk was

low and equivalent in both groups. One patient from the sensitized group developed cytomegaloviremia and 2 patients in the non-sensitized group developed bacterial infections (*Haemophilus pneumoniae* pneumonia, Staphylococcal sternal wound infection). There was no significant difference seen in survival at 1 year after HTX between the two groups. Amongst the 4 patients with a positive virtual crossmatch antibody mediated rejection occurred in 1 patient and the total rejection score at 1 year was 0, 0.18, 0.5 and 0.62 in the 4 patients respectively. Therefore, 2 of these patients did have a higher rejection score than the average rejection score of 0.37 seen in the non-sensitized patients. All patients with a positive virtual crossmatch had a follow up coronary angiogram performed approximately 1 year after HTX that did not demonstrate significant coronary artery disease. There were no deaths in this group during the follow up period. Current immunosuppression was augmented in just one of these patients with four-drug therapy (cyclosporine, low dose sirolimus, MMF and prednisone), however the other 3 patients were either on a single agent (tacrolimus), dual agents (sirolimus, MMF) or triple therapy (prednisone, MMF, sirolimus). There were no significant differences in immunosuppressive therapy compared to non-sensitized patients.

Discussion

This is the first study to characterize the presence of anti-HLA antibodies prior to and after continuous axial flow LVAD implant as measured by SAB assays. This is also the first study to show the correlation of sensitization post LVAD measured by SAB assays with post heart transplant outcomes. SAB assays are routinely being used to assess a potential HTX recipient's candidacy, the results are being used to list unacceptable antigens limiting donor availability for the HTX candidate, and virtual crossmatch based on SAB assays is commonly being used in decision making regarding whether to proceed with HTX⁴. The older technique, CDC PRA, of screening patients prior to HTX has been routinely used to describe sensitization in LVAD recipients. However, this assay is not sensitive in detecting DSA, does not detect Class II anti-HLA antibodies and does not accurately predict a positive flow crossmatch⁹.

Allosensitization post LVAD: Prevalence versus Incidence

The prevalence of sensitization prior to LVAD implant in our study was 20% as measured by SAB assays and by CDC PRA was 7% thus demonstrating increased sensitivity of SAB assays for anti-HLA antibody detection. The prevalence of sensitization defined as an elevated CDC PRA in LVAD recipients has ranged from 0% to 66% and has been dependent on the type of device used, being more common in pulsatile devices like the HeartMate I and less prevalent in continuous axial flow devices 10-12. Many of these studies have been limited by the failure to measure baseline sensitization prior to LVAD implant and hence the true number of patients who develop de novo sensitization after LVAD implant was unclear. Most studies have also compared sensitization measured by CDC PRA in LVAD recipients to those on the HTX list without LVAD; few have characterized the temporal change in CDC PRA before and after LVAD implantation and none have described sensitization in a temporal trend based on the timing of LVAD implantation using solid phase arrays. The studies that adopted this approach of evaluating de novo development of CDC PRA after LVAD implant included patients with different types of devices (pulsatile and non-pulsatile)¹³⁻¹⁶. These and other studies suggest that patients with pulsatile LVADs are more likely to develop sensitization as compared to patients who have continuous axial flow pumps and may be related to the biomaterial used in the pulsatile LVADs¹⁷. In fact, at the time of HTX there were no patients in our study who had an elevated CDC PRA confirming the low likelihood of developing an elevated CDC PRA after implantation of continuous axial flow pumps. However, our findings do

indicate that 46% (11/24) of patients who were not sensitized pre LVAD develop de novo sensitization after LVAD implant as measured by SAB assays. Mean peak and cumulative anti-HLA antibody MFI values also increase after LVAD implant.

Allosensitization post LVAD: Risk Factors

The cause of this type of sensitization and development of anti-HLA antibodies is unclear. Prior studies have implicated female gender, younger age, high baseline CDC PRA and blood products in the development of sensitization as measured by CDC PRA^{2, 15}. In the present study, there were no significant differences in age and female gender between the sensitized and non-sensitized patients. Our study does confirm observations made by other groups that blood products are unlikely to be a cause of HLA sensitization in LVAD recipients¹⁵.

Allosensitization post LVAD: Effect of pre LVAD sensitization status

LVAD therapy can also be helpful in bridging the highly sensitized patient until an appropriately “HLA-matched” heart becomes available since traditionally these patients have a longer waiting time³. An earlier study wherein sensitization was measured by CDC PRA suggested that a greater degree of sensitization occurs after LVAD implant in the pre LVAD sensitized patient¹⁵. In our study, patients who were sensitized prior to LVAD therapy did not have a significant increase in the number of anti-HLA antibodies or a significant increase in mean peak or cumulative MFI values of Class I or Class II anti-HLA antibodies as compared to after LVAD implant. The clinical implication of this finding albeit limited by a small number of patients suggests that continuous axial flow LVADs could be used safely as a BTT in the highly sensitized patient until a suitable donor heart becomes available.

Allosensitization post LVAD: Effect on crossmatch and post HTX outcomes

Despite sensitization being present in 47% of patients at the time of transplant there was no significant effect on outcomes after HTX. This outcome is not surprising since many of these patients (n=10) did not have DSA. However, there were 4 patients who were transplanted across a positive virtual crossmatch. Three of these patients were transplanted due to DSA being 4000 MFI or less, since our TX practice at that time had adopted this value as a cut-off to transplant patients. The other patient was profoundly ill due to device malfunction and a decision was made to transplant the patient despite a positive virtual crossmatch.. A positive T cell flow crossmatch was observed only in 2 patients, a finding that may have accounted for better outcomes post HTX. There have been prior studies confirming our finding of similar rejection and survival rates between sensitized and non-sensitized patients who undergo HTX with LVAD as BTT¹¹ and is likely due to appropriate selection of HLA compatible donors as demonstrated in our study since most patients had a negative virtual crossmatch. Although sensitization as measured by CDC PRA does translate into increased mortality after HTX especially when CDC PRA values exceed 25-50%, this increase in mortality is irrespective of LVAD use^{5, 11}. None of our patients at the time of HTX had a CDC PRA > 10%.

An important limitation of this study was that the follow up period was confined to just one year after HTX and the long-term sequelae of HLA sensitization especially on transplant coronary artery disease and survival is unknown. Although HLA sensitization occurred in 14 LVAD recipients, just 4 of these patients had a positive virtual crossmatch hence the conclusion of the association of HLA sensitization with post HTX outcomes cannot be extrapolated to patients with a positive virtual crossmatch after LVAD implant due to the small number of such patients represented in this study.

In summary, sensitization as measured by CDC PRA after continuous axial flow LVAD implantation is uncommon. However, despite CDC PRA being negative, a significant subset of patients develops de novo sensitization after continuous axial flow LVAD implantation contrary to earlier reports that used CDC PRA. The degree of sensitization increases significantly after LVAD implant as measured by peak anti-HLA Class I and II antibody MFI values and number of Class I anti-HLA antibodies. However, this increase in allosensitization does not appear to be clinically significant due to HLA compatibility at the time of transplant in the majority of the patients studied, hence not translating into a positive flow crossmatch, also in part due to the temporal nature of antibody formation and a decrease in sensitization observed at the time of HTX. Sensitization post LVAD as measured by SAB assay does not appear to increase waiting time for heart transplantation. Blood products do not appear to be associated with sensitization after continuous axial flow LVAD implantation. The patients who are sensitized as measured by SAB assay do not appear to have increased rejection rates, and there is no difference in survival at 1 year between patients who are sensitized and not sensitized at the time of HTX. These results should not be extrapolated to patients with a positive virtual crossmatch at the time of HTX and furthermore the long-term consequences of such HLA sensitization are unknown at this time.

Acknowledgments

We would like to thank Kasey Muetzel for her assistance with the preparation of this manuscript.

Source of funding: Supported in part by HL 84904 (Heart Failure Clinical Research Network) (NLP), KL2RR024151 (NLP), UL1TR000135 (NLP).

Abbreviations

BTT	Bridge to transplant
CDC	Complement dependent cytotoxicity
DSA	Donor specific antibody
FXM	Flow crossmatch
HLA	Human leucocyte antigen
HTX	Heart transplantation
LVAD	Left ventricular assist device
MFI	Mean fluorescence intensity
MMF	Mycophenolate Mofetil
PRA	Panel reactive antibody
SAB	Single antigen bead

References

1. Stehlik J, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dobbels F, Kirk R, Rahmel AO, Hertz MI. The registry of the International Society for Heart and Lung Transplantation: Twenty-eight Adult Heart Transplant Report- 2011. J Heart Lung Transplant. 2011; 30(10):1078–94. [PubMed: 21962016]
2. Pak SW, Uriel N, Burke E, Cappelman SA, Sutton EM, Takayama H, Latif F, Colombo P, Naka Y, Mancini D, Jorde UP. High Incidence of Elevated Panel Reactive Antibodies in Patients Supported with HeartMate II Left Ventricular Assist Devices Sang. Circulation. 2010; 122:A20395.

3. Ross H, Tinckam K, Rao V, West LJ. In praise of ventricular assist devices-mechanical bridge to virtual crossmatch for the sensitized patient. *J Heart Lung Transplant*. 2010; 29:728–730. [PubMed: 20363155]
4. Kobashigawa J, Mehra M, West L, Kerman R, George J, Rose M, Zeevi A, Reinsmoen N, Patel J, Reed EF. Report from a consensus conference on the sensitized patient awaiting heart transplantation. *J Heart Lung Transplant*. 2009; 28:213–225. [PubMed: 19285611]
5. Nwakanma LU, Williams JA, Weiss ES, Russell SD, Baumgartner WA, Conte JV. Influence of pretransplant panel-reactive antibody on outcomes in 8,160 heart transplant recipients in recent era. *Ann Thorac Surg*. 2007; 84:1556–1562. discussion 1562-1553. [PubMed: 17954062]
6. Raichlin E, Al-Omari MA, Hayes CL, Edwards BS, Frantz RP, Boilson BA, Clavell AL, Rodeheffer RJ, Schirger JA, Kushwaha SS, Allison TG, Pereira NL. Cardiac allograft hypertrophy is associated with impaired exercise tolerance after heart transplantation. *J Heart Lung Transplant*. Oct; 30(10): 1153–60. [PubMed: 21621424]
7. Gloor JM, DeGoey S, Ploeger N, Gebel H, Bray R, Moore SB, Dean PG, Stegall MD. Persistence of low levels of alloantibody after desensitization in crossmatch-positive living-donor kidney transplantation. *Transplantation*. 2004; 78:221–227. [PubMed: 15280682]
8. Gandhi MJ, DeGoey SR, Bundy K, Kremers WK, Knauer R, Pereira N, Edwards B, Kushwaha S, Daly RC. Effect of pretransplant human leukocyte antigen antibodies detected by solid-phase assay on heart transplant outcomes. *Transplant Proc*. Dec; 2011 43(10):3840–6. [PubMed: 22172857]
9. Bishay ES, Cook DJ, Starling RC, Ratliff NB Jr, White J, Blackstone EH, Smedira NG, McCarthy PM. The clinical significance of flow cytometry crossmatching in heart transplantation. *Eur J Cardiothorac Surg*. 2000; 17:362–369. [PubMed: 10773556]
10. Joyce DL, Southard RE, Torre-Amione G, Noon GP, Land GA, Loebe M. Impact of left ventricular assist device (lvad)-mediated humoral sensitization on post-transplant outcomes. *J Heart Lung Transplant*. 2005; 24:2054–2059. [PubMed: 16364849]
11. Massad MG, McCarthy PM, Smedira NG, Cook DJ, Ratliff NB, Goormastic M, Vargo RL, Navia J, Young JB, Stewart RW. Does successful bridging with the implantable left ventricular assist device affect cardiac transplantation outcome? *J Thorac Cardiovasc Surg*. 1996; 112:1275–1281. discussion 1282-1273. [PubMed: 8911324]
12. Newell H, Smith JD, Rogers P, Birks E, Danskin AJ, Fawson RE, Rose ML. Sensitization following lvad implantation using leucodepleted blood is not due to hla antibodies. *Am J Transplant*. 2006; 6:1712–1717. [PubMed: 16827875]
13. Coppage M, Baker M, Fialkow L, Meehan D, Gettings K, Chen L, Massey HT, Blumberg N. Lack of significant de novo hla allosensitization in ventricular assist device recipients transfused with leukoreduced, abo identical blood products. *Hum Immunol*. 2009; 70:413–416. [PubMed: 19275923]
14. Drakos SG, Kfoury AG, Kotter JR, Reid BB, Clayson SE, Selzman CH, Stehlik J, Fisher PW, Merida M 3rd, Eckels DD, Brunisholz K, Horne BD, Stoker S, Li DY, Renlund DG. Prior human leukocyte antigen-allosensitization and left ventricular assist device type affect degree of post-implantation human leukocyte antigen-allosensitization. *J Heart Lung Transplant*. 2009; 28:838–842. [PubMed: 19632582]
15. George I, Colley P, Russo MJ, Martens TP, Burke E, Oz MC, Deng MC, Mancini DM, Naka Y. Association of device surface and biomaterials with immunologic sensitization after mechanical support. *J Thorac Cardiovasc Surg*. 2008; 135:1372–1379. [PubMed: 18544389]
16. Malickaite R, Rucinskas K, Staneviciene A, Miniauskas S, Maneikiene V, Zuoziene G, Sirvydis V. Sensitisation and post-transplant course after the implantation of ventricular assist device. *Interact Cardiovasc Thorac Surg*. 2009; 8:339–342. discussion 342-333. [PubMed: 19098066]
17. Schuster M, Kocher A, John R, Hoffman M, Ankersmit J, Lietz K, Edwards N, Oz M, Itescu S. B-cell activation and allosensitization after left ventricular assist device implantation is due to t-cell activation and cd40 ligand expression. *Hum Immunol*. 2002; 63:211–220. [PubMed: 11872239]

Figure 1.

Figure 2.

The graph displays the number of anti-HLA antibodies >2000 for two groups: Sensitized Pre VAD (dashed lines) and Unsensitized Pre VAD (solid lines). The Y-axis ranges from 0 to 30. The X-axis shows three time points: Pre VAD, Post VAD, and Transplant.

Legend:

- Sensitized Pre VAD
- Unsensitized Pre VAD

Approximate Data Points:

Group	Pre VAD	Post VAD	Transplant
Sensitized Pre VAD (High)	30	31	21
Sensitized Pre VAD (Medium)	15	19	14
Sensitized Pre VAD (Low)	3	8	2
Unsensitized Pre VAD (High)	1	12	5
Unsensitized Pre VAD (Medium)	1	19	19
Unsensitized Pre VAD (Low)	1	2	2
Unsensitized Pre VAD (Very Low)	1	0	0

Figure 3.

Figure 4.

Table 1

Comparison of change in peak and cumulative anti-HLA antibody MFI values stratified by pre-LVAD sensitization status. P-values represent a comparison of the changes in MFI values by sensitization status.

Type	Timing	All N=30	Sensitized N=6	Non sensitized N=24	p-value
Peak 1	Pre to Post	1384 ± 4346	-2482 ± 3844	2351 ± 3966	0.006
	Post to Tx	-694 ± 3234	-1204 ± 1079	-566 ± 3585	0.213
	Pre to Tx	690 ± 4291	-3686 ± 3744	1784 ± 3732	0.002
Peak 2	Pre to Post	509 ± 1015	585 ± 1501	490 ± 898	0.736
	Post to Tx	-186 ± 967	-635 ± 622	-73 ± 1014	0.041
	Pre to Tx	323 ± 975	-51 ± 1177	417 ± 922	0.392
Cumul. 1	Pre to Post	4167 ± 56616	-42023 ± 78760	15714 ± 44692	0.113
	Post to Tx	-5935 ± 19239	-10938 ± 12608	-4684 ± 20452	0.052
	Pre to Tx	-1768 ± 53912	-52961 ± 87971	11030 ± 33583	0.036
Cumul. 2	Pre to Post	1799 ± 11195	-7371 ± 18012	4091 ± 7750	0.287
	Post to Tx	-1122 ± 7008	-1854 ± 6860	1807 ± 7450	0.697
	Pre to Tx	677 ± 9706	-5565 ± 20408	2237 ± 3942	0.775

Table 2

Clinical characteristics of patients with LVAD implants as a bridge to transplant based on the status of sensitization at the time of heart transplantation (% , or mean $\pm$ sd).

Variable	Sensitized LVAD recipients (N=14)	Non-sensitized LVAD recipients (N=16)	p-value
Recipient age, years	50.6 $\pm$ 7.4	49.4 $\pm$ 13.9	0.950
Female gender, %	28.6	18.8	0.675
Ischemic cardiomyopathy, %	42.9	18.8	0.236
Total blood products, units	70.0 $\pm$ 43.2	57.4 $\pm$ 34.8	0.575
Total PRBCs, units	39.1 $\pm$ 26.2	28.6 $\pm$ 19.3	0.271
Total platelets, units	6.9 $\pm$ 4.2	5.9 $\pm$ 5.4	0.297
Duration of support, days	286.3 $\pm$ 237.3	244.5 $\pm$ 166.1	0.852
Duration of wait time, days	112.2 $\pm$ 94.2	158.8 $\pm$ 173.7	0.589
Donor age	24.1 $\pm$ 8.8	31.4 $\pm$ 10.3	0.053
Ischemic time, minutes	177.0 $\pm$ 45.0	212.8 $\pm$ 50.8	0.038
Positive VXM (DSA>2000 MFI)	28	0	0.466
CNI/SRL, %	92.8	100.0	0.467
MMF, %	92.8	100.0	0.467
Prednisone, %	78.6	100.0	0.090

Abbreviations: VXM – virtual crossmatch, DSA – donor specific antibody, CNI – calcineurin inhibitor, SRL – sirolimus, MMF – mycophenolate mofetil

Table 3

Clinical outcomes of patients with LVAD implants as a bridge to transplant based on the status of sensitization at the time of heart transplantation (% , or mean $\pm$ sd).

Variable	Sensitized LVAD recipients (N=14)	Non-sensitized LVAD recipients (N=16)	p-value
Ejection Fraction	62.3 $\pm$ 7.6	61.7 $\pm$ 7.1	0.837
Total rejection score	0.3 $\pm$ 0.3	0.4 $\pm$ 0.2	0.427
AMR episodes, % patients	28.6	25.0	1.000
Treated rejections, % patients	7.1	25.0	0.336
Infections *, % patients	7.1	12.5	1.000
Deaths post –transplant, %	14.3	12.5	1.000

Abbreviations: AMR – antibody mediated rejection.

* Infections included pneumonia, cytomegalovirus and sternal wound infection.