Brief Report

# Increases in Serum Autoantibodies After Left Ventricular Assist Device Implantation

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**ABSTRACT**

**Background**: Left ventricular assist devices (LVADs) can serve as a bridge to transplant or destination therapy for patients with advanced heart failure. Implantation of LVADs is known to be associated with increases in anti-HLA antibodies, but less is known about how autoantibody levels change with the use of these devices.
**Methods and Results**: Autoantibody levels were quantified with the use of customized antigen microarrays in 22 patients both before and after LVAD. We observed an increase (1.5- to 2-fold) in 14 IgG autoantibodies in the serum of patients after LVAD, including autoantibodies against cardiac proteins (myosin, troponin I, tropomyosin), DNA, and structural proteins (collagen, laminin). There was also a small but significant rise in total serum IgG after LVAD. Increases in autoantibodies after LVAD were positively associated with increases in calculated panel-reactive antibody class II ($P = .05$) and negatively correlated with age ($r = -0.45$; $P < .05$). Cytokines were evaluated to gain insights into the mechanism of antibody generation, and we observed a positive correlation between total IgG levels after LVAD and the level of monocyte chemoattractant protein 1 ($r = 0.60$; $P < .05$).
**Conclusions**: LVAD implantation is associated with increases in IgG autoantibodies, anti-HLA antibodies, and total IgG. Increases in IgG after LVAD implantation may relate to an inflammatory response triggered by these devices. (*J Cardiac Fail 2019;25:301−306*)
**Key Words:** Left ventricular assist device, autoantibody, sensitization, cytokines.

Left ventricular assist devices (LVADs) are mechanical circulatory devices that are used to treat refractory heart failure.[1] These devices unload the left ventricle and can promote reverse remodeling of the heart. Despite the beneficial effects of LVADs in improving heart function, there is evidence that these devices can increase systemic levels of inflammatory markers.[2,3] There is also evidence that LVADs can promote anti-HLA antibody production in a process known as sensitization.[4] LVAD recipients may become sensitized because of exposure to blood products at the time of implantation of the device. Sensitization has also been attributed to the host-device biomaterial interaction, which induces aberrant T-cell activation and B-cell hyperreactivity.[5]

Less is known about the ability of LVADs to promote autoimmune mechanisms. One prior study demonstrated that LVADs stimulate the production of autoantibodies against the angiotensin II type 1 receptor,[6] but responses against other autoantigens were not investigated. To further understand autoantibody production after LVAD implantation we used antigen microarray technology, a proteomic technology that allows for the multiplex profiling of autoantibodies.[7] We hypothesized that LVAD implantation would promote autoantibody production.

## Methods

### Patients and Sample Collection

Ethics approval (08-0732-T) for this project was obtained from the Research Ethics Board at the University

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Health Network (UHN) in Toronto. Serum samples from patients who underwent LVAD implantation from February 2009 to August 2011 were obtained from the UHN histocompatibility laboratory. Patients were excluded if they had renal failure, liver failure, or right ventricular failure. calculated panel-reactive antibody (cPRA) was determined with the use of the Canadian Blood Services web-based calculator (https://ctr2.transplantregistry.ca/otd-cpra-client/ctr2.jsp) and data from single-antigen beads (One Lambda, Canoga Park, California). A post−heart transplantation biopsy score was calculated as described previously.[8]

### Antigen Microarrays

Autoantibody profiling in plasma with the use of antigen microarrays was performed as described previously.[7] The antigen library used for the arrays is presented in Supplemental Table 1. Briefly, antigens were spotted in triplicate onto FAST nitrocellulose slides (Maine Manufacturing, Sanford, Maine). The slides were probed with patient sera diluted 1:100 and then with a pair of secondary antibodies (Cy3-labeled goat anti−human IgG and Cy5-labeled goat anti−human IgM) (Jackson Immunoresearch, West Grove, Pennsylvania). Slides were scanned with the use of an Axon 4200A scanner (Molecular Devices, Sunnyvale, California), and median fluorescent intensity (MFI) was calculated for each feature. Significance analysis of microarrays was used to determine significant changes with a false discovery rate of 5% (q value $< 0.05$).

### Serum IgG levels

Serum IgG was measured with the use of a commercially available enzyme-linked immunosorbent assay kit (eBioscience, San Diego, California).

### Biochemical Assays

Concentrations of 42 inflammatory markers were measured in plasma with the use of a multiplex kit (Linco Research, St Charles, Missouri) according to the manufacturer's directions.

### Statistical Analysis

All statistical analyses were performed with the use of the statistical package SPSS v14.0 (IBM, Armonk, New York). IgG levels were compared between groups by means of a Wilcoxon signed ranks test. A comparison of cPRA and MFI between groups was done with the use of a 2-tailed Mann-Whitney test. To examine the relationship between the levels of antibodies and inflammatory markers, the Spearman rank correlation coefficient or the Pearson product-moment correlation was performed as appropriate. To examine the relationship between levels of antibodies and clinical variables, chi-square test, Mann-Whitney test, or Pearson correlation was performed as appropriate. Data are presented as either mean ± SD or median and interquartile range (IQR). A $P$ value of $\leq 0.05$ was considered to be statistically significant.

## Results

### Measurement of Autoantibodies With Antigen Microarrays

In this retrospective study, we profiled autoantibody levels, with the use of custom antigen microarrays, in serum samples collected both before and after LVAD implantation. Characteristics of the LVAD recipients are presented in Table 1. The pre-LVAD sample was obtained in the month before LVAD implantation. The post-LVAD sample was obtained on average 101.8 ± 61.9 days after implantation. At the time of collection of the post-LVAD sample, all patients had recovered from the implantation surgery and were being followed in an outpatient clinic. A heat map showing the results of antigen microarray analysis is shown in Figure 1A. We found that 14 autoantibody reactivities were increased in the post-LVAD sample relative to the pre-LVAD sample, including reactivity to cardiac antigens (troponin I, myosin, tropomyosin), collagens, laminin, oxidized human low-density lipoprotein (LDL), and DNA. These reactivities were 1.5- to 2-fold higher in post-LVAD sample compared with the pre-LVAD sample (Supplemental Table 2). Interestingly, only increases in IgG and not IgM reactivity were observed after LVAD implantation, suggesting that there was antibody class switching with activation of the adaptive immune system. Given the increase in IgG autoantibody reactivity, we next measured total serum IgG in the patient samples. We found that serum

**Table 1.** Characteristics of Left Ventricular Assist Device (LVAD) Recipients (n = 22)

| | |
|---|---|
| Male (%) | 59% (13/22) |
| Age at LVAD implantation (y) | 50.3 ± 10.9 |
| History of pregnancy (% of women) | 78% (7/9) |
| Ischemic cardiomyopathy (%) | 14% (3/22) |
| Indication for LVAD | |
| Bridge to transplant | 91% (20/22) |
| Bridge to decision | 9% (2/22) |
| LVAD type (%) | |
| Heartmate II | 68% (15/22) |
| Heartware | 23% (5/22) |
| Duraheart | 9% (2/22) |
| Perioperative blood products (units)* | |
| Cryoprecipitate | 1.8 ± 1.7 |
| Fresh frozen plasma | 8.4 ± 6.8 |
| Platelets | 2.2 ± 1.5 |
| Packed red blood cells | 10 ± 7.8 |
| Infections (%) | 14% (3/22)† |
| Gastrointestinal bleeding (%) | 5% (1/22)‡ |
| Duration of LVAD support (d) | 101.8 ± 61.9 |
| Underwent heart transplantation (%) | 77% (17/22) |

*Total blood products received during implantation of LVAD and postoperative care.

†Three patients developed driveline infections and were on oral antibiotics when the post-LVAD blood sample was collected.

‡One patient had bleeding from a rectal polyp after LVAD implantation and did not require transfusion.

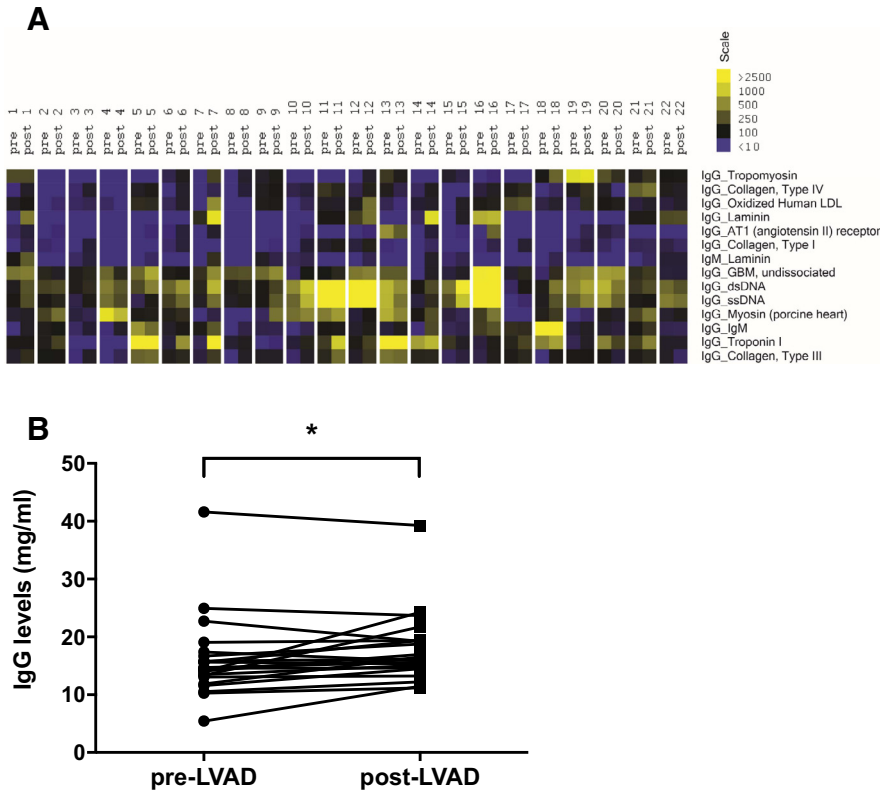


**Fig. 1.** Serum autoantibodies and total IgG are increased after LVAD implantation. (A) Heat map showing the 14 autoantibodies that are significantly increased after LVAD implantation as determined by significance analysis of microarrays. For each patient, pre- and post-LVAD antigen reactivities are shown. Yellow indicates high reactivity and blue indicates low reactivity as indicated in the scale. (B) Graph showing paired pre- and post-LVAD serum IgG levels. *$P = .0142$). dsDNA, double-stranded DNA; GBM, glomerular basement membrane; LVAD, left ventricular assist device; ssDNA, single-stranded DNA.

levels of IgG increased from 15.9 ± 7.0 mg/mL before to 17.8 ± 6.0 mg/mL after LVAD implantation ($P = .0142$; Fig. 1B).

### Correlation of Autoantibody Reactivity With Anti-HLA Antibodies

We investigated if there was a correlation between the levels of anti-HLA antibodies and autoantibodies in the LVAD recipients. Supplemental Table 3 presents changes in autoantibody levels (as measured by MFI) and anti-HLA antibodies (as measured by cPRA) for each LVAD recipient. Twelve of the 22 patients in the study had an increase in their cPRA after LVAD implantation (Supplemental Table 3). Patients who had increases in class II cPRA after LVAD implantation had larger increases in autoantibodies (as measured by increases in autoantibody MFI) than patients who did not have increases in class II cPRA ($P = .05$; Supplemental Table 4). These data suggest that the increased generation of anti-HLA and autoantibodies after LVAD implantation shared a common underlying cause. We next investigated if there were clinical variables associated with increases in antibody levels after LVAD implantation. There was a trend for the levels of anti-HLA antibodies after LVAD implantation to be positively correlated with the number of units of packed red blood cells ($P = .069$) given during device implantation. There was also a trend for a negative correlation of anti-HLA antibody levels with age ($P = .10$) (Supplemental Table 5). Increases in autoantibodies after LVAD implantation were negatively correlated with age ($r = -0.457$; $P = .033$) but were not correlated with transfusion of blood products (Supplemental Table 6). Duration of LVAD support was not a factor in increases of either anti-HLA antibodies or autoantibodies.

### Post-transplantation Outcomes

Seventeen of the 22 patients underwent heart transplantation. In the immediate post-transplantation period, 3 patients were diagnosed with primary graft dysfunction (PGD). Interestingly, one of those patients had the highest change in autoantibody MFI (Supplemental Table 3, patient 7) after LVAD implantation. The other 2 patients with PGD (9 and 18) also exhibited increases in autoantibody levels after LVAD implantation but not to the same extent. We also examined if autoantibody MFI (total or change in MFI) was associated with rejection after transplantation, but we did not find a positive association of autoantibody MFI with

biopsy score at 3, 6, or 12 months after transplantation (Supplemental Table 7).

### Correlation of IgG and Autoantibodies With Cytokines and Chemokines

Because we previously showed that LVAD is associated with higher cytokine levels, we next investigated whether antibody production was correlated with changes in cytokine or chemokine levels in our LVAD recipients. There was a positive correlation between IgG levels and monocyte chemoattractant protein 1 (MCP-1) after LVAD implantation ($r = 0.60$; $P < .05$; Fig. 2A). We also investigated if levels of individual autoantibodies correlated with cytokine or chemokine levels. After LVAD implantation, the levels of IgG-oxidized human LDL correlated with the levels of platelet-derived growth factor (PDGF) AB/BB ($r = 0.55$; $P = .05$) and PDGF AA ($r = 0.73$; $P < .01$) (Fig. 2B and C). The levels of IgG collagen (type III) after LVAD implantation were positively correlated with the levels of RANTES ($r = 0.80$; $P < .01$; Fig. 2D).

## Discussion

We investigated whether LVAD implantation was associated with autoantibody production in a cohort of heart failure patients. We found that 14 autoantibodies increased after LVAD implantation, including antibodies against cardiac proteins, DNA, and structural proteins. In addition, there was a small but significant increase in total IgG after LVAD. There was a positive correlation between autoantibody levels and increases in cPRA class II and a negative correlation between autoantibody levels and patient age. Finally, we observed that the chemokine MCP-1 was

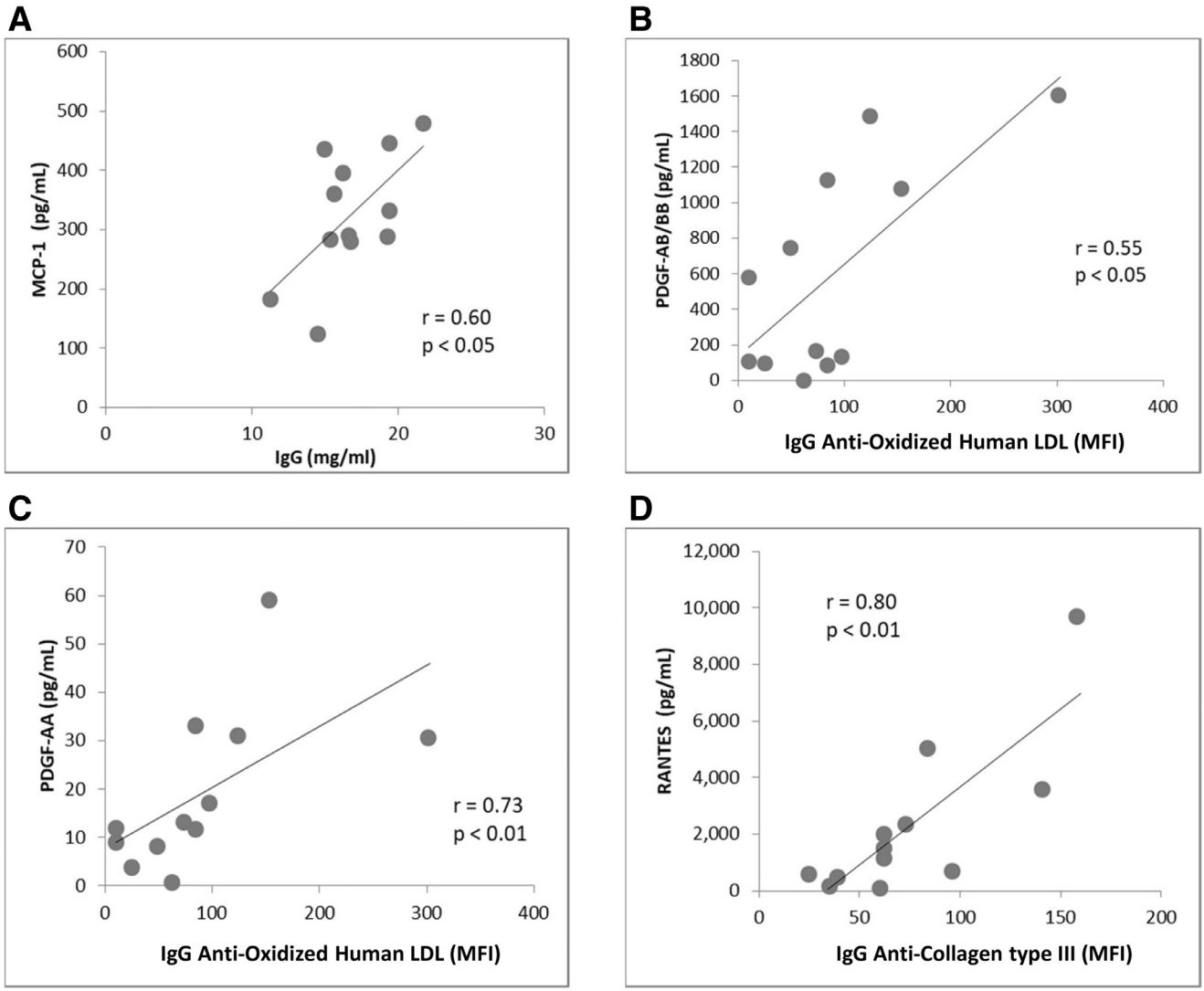


**Fig. 2.** Correlation of inflammatory cytokines with total IgG levels and individual autoantibody reactivities. (A) MCP-1 and total serum IgG. (B) PDGF-AB/BB and IgG anti−oxidized human LDL. (C) PDGF-AA and IgG anti−oxidized human LDL. (D) RANTES and IgG anti−collagen type III. Each graph includes 12 patients where there were simultaneous data on cytokine and autoantibody levels. MCP-1, monocyte chemoattractant protein 1; MFI, median fluorescent intensity; PDGF, platelet-derived growth factor.

correlated with total IgG levels after LVAD implantation. Together, these results suggest that implantation of LVADs has stimulatory effects on innate and adaptive immune mechanisms in patients.

Although LVADs are lifesaving for patients with end-stage heart failure, a number of studies have indicated that implantation of LVADs may promote inflammation. We previously showed that multiple inflammatory markers, including MCP-1 and C-reactive protein, were increased after LVAD implantation.[2,3] Here, the finding that total IgG and autoantibody levels increased after LVAD implantation provides evidence that these inflammatory changes induced by LVADs include activation of the adaptive immune system. One mechanism for this may be the contact of patient's immune cells with the foreign surfaces of the LVAD. A previous publication observed that $CD68^+$ macrophages/monocytes along with $CD34^+$ cells adhere to surfaces of the LVAD.[9] We found that MCP-1, a chemoattractant for monocytes/macrophages, correlated strongly with total IgG levels after LVAD implantation, which is consistent with monocyte recruitment being connected to antibody production. We also observed that PDGF and RANTES levels positively correlated with IgG anti−oxidized human LDL autoantibodies and IgG anti-collagen (type III) autoantibodies, respectively. Like MCP-1, RANTES and PDGF are also described as chemoattractants for monocytes/macrophages.[10,11]

We also observed that there was a correlation between autoantibody production and increases in anti-HLA antibodies (sensitization). Multiple studies have documented increases in sensitization after LVAD implantation.[4] Although sensitization may be partially related to administration of blood products during LVAD implantation, as we observed, it may also be due to a generalized antibody stimulation after LVAD implantation. Age may also be an important factor because autoantibody generation and increases in sensitization were both negatively correlated with age. This is consistent with studies demonstrating more robust immune responses in younger compared with older individuals.[12]

The majority of autoantibodies that we found to increase after LVAD implantation were specific for cardiac proteins (eg, cardiac myosin, troponin I, and tropomyosin) and extracellular proteins (eg, collagens and laminin). We also found increases in autoantibodies to the angiotensin II receptor after LVAD implantation as described previously.[6] Production of these specific autoantibodies points to local immune activation in the heart, perhaps related to the inflammation and scarring around the inflow cannula that have been reported with LVAD implantation.[13] Inflammation would lead to the recruitment of monocytes which would differentiate into macrophages at the site. These macrophages could lead to autoantibody production through activation of T cells and B cells corecruited to the site of inflammation.[14]

Autoantibodies stimulated by LVADs may not only be markers of immune activation but also promote cardiac dysfunction. In animal studies, autoantibodies against both cardiac myosin and troponin I have been shown to be directly pathogenic in heart failure.[15,16] Increases in these anti-cardiac autoantibodies after LVAD implantation may thus contribute to cardiac dysfunction, limiting the potential for myocardial recovery after mechanical unloading. This may be one reason why myocardial recovery after LVAD implantation is infrequently observed, with only 1.3% of LVAD recipients undergoing device explantation.[17] Of note, we had only 1 patient in this cohort who had significant functional left ventricular recovery during the study. Interestingly, that patient exhibited only a slight increase in autoantibody levels after LVAD implantation (MFI change 297; Supplemental Table 3).

In the 17 patients who underwent heart transplantation in this cohort, autoantibody levels did not correlate with post-transplantation rejection as measured by a biopsy score. This finding is similar to that found in a previous study that showed that anti−angiotensin II receptor autoantibodies stimulated by LVAD were not found to correlate with rejection.[6] These LVAD-stimulated autoantibodies may not promote rejection because the stimulus of the autoantibodies (ie, the LVAD) was removed at the time of transplantation. We did, however, observe that 3 patients had PGD after transplantation, including the patient who had the highest increase in autoantibodies after LVAD implantation. In lung transplantation, a study showed that autoantibodies increased the risk of PGD.[18] Larger studies will be needed to determine if autoantibodies as measured by antigen microarrays are truly associated with PGD in heart transplantation.

Limitations of this study include small sample size and recruitment of patients from a single medical center. We also did not profile autoantibodies at various time points after LVAD implantation. In a future study we will address this question by profiling autoantibodies through the LVAD implantation and post-transplantation periods. We will also address whether newer generations of LVAD devices have less immunostimulatory effects.

## Conclusion

LVAD implantation is associated with increases in IgG autoantibodies as well as increases in anti-HLA antibodies and total IgG. Increases in IgG after LVAD implantation may relate to an inflammatory response triggered by these devices.

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## Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cardfail.2019.01.002.

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