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## RESEARCH CORRESPONDENCE

### Pathology of aortic valve remodeling after continuous-flow left ventricular assist device support

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During the normal cardiac cycle, the aortic valve (AV) leaflets are exposed to a range of shear and hemodynamic forces leading to homeostatic bending, tension, and compression of the valve tissue.[1] After continuous-flow left ventricular assist device (LVAD) implantation, blood flow through the AV is severely altered, leading to altered stresses and decreased valve opening during systole. We studied the effects of LVAD implantation on AV histology, with an emphasis on changes in extracellular matrix, valve interstitial cells (VICs), and macrophages.

We retrospectively studied AVs of 21 patients on long-term continuous-flow LVAD support (HeartMate II [Thoratec Corporation; $n = 19$] or HeartWare [HeartWare; n = 2]) after heart transplantation ($n = 15$) or at autopsy ($n = 6$). Average age of patients with LVADs was 52 years $\pm$ 12, and patients were predominantly men (76%) with an average duration of LVAD support of 731 days $\pm$ 498 (range, 7–1,922 days) (Table S1 [available at www.jhltonline.org]). Echocardiographic data during LVAD support showed valve opening in 3 patients. Mild, sub-clinical, de novo aortic insufficiency was observed in 79% of patients, and 71% showed significant ($\geq$3 mm) commissural fusion of leaflets after LVAD support. The total length and number of fused commissures displayed a positive, non-significant association with duration of LVAD support (Figure S1 [available at www.jhltonline.org]). Total leaflet thickness after LVAD support was similar to the control group. However, in valves from patients with LVADs, the ventricularis layer in the belly of the leaflet was significantly thickened (Figure S2 [available at www.jhltonline.org]). No difference in the severity of sclerotic lesions was observed between patients with LVADs and the control group.

Overall cell density was higher in patients with LVADs compared with the control group, mainly caused by an increase in cell density in the ventricularis layer (Figure 1A). Cell density was not related to the duration of support (Figure 1B) but was associated negatively with age (Figure 1C). Cell proliferation, as indicated by Ki-67 labeling, was increased in the spongiosa and ventricularis layers after LVAD support (Figure 1D and E).

We observed a large increase in myofibroblast markers alpha smooth muscle actin (αSMA) and calponin in the ventricularis layer after LVAD support (Figure 1F, G, and J), indicating an increase in VIC activation. Localization of αSMA-positive cells in the ventricularis layer was mostly diffuse in both the hinge region and the belly of the leaflet. In the fibrosa layer, a thin sub-endothelial layer of αSMA expression was occasionally observed. The presence of αSMA in the leaflets was positively associated with the duration of LVAD support and negatively associated with age (Figure 1H and I).

CD68-positive macrophages were detected in all valves and found to be significantly increased after LVAD support. All individual leaflet layers displayed increased CD68 expression (Figure 2A and C). The M2-macrophage marker CD163 expression pattern was similar to that of CD68 and showed a significant increase in the ventricularis layer of the LVAD group (Figure 2B and C). Inducible nitric oxide synthase staining showed no difference between groups (Figure S3A and D [available at www.jhltonline.org]). Inducible nitric oxide synthase–positive cells were localized predominantly in regions with adipocytes and sclerotic lesions. The broader CD45 leukocyte common antigen was significantly increased in the spongiosa and ventricularis layers after LVAD (Figure S3B [available at www.jhltonline.org]). Small numbers of single CD3-positive T lymphocytes were diffusely observed in all valves, but no difference in expression was observed (Figure S3C [available in the online version of this article at www.jhltonline.org]). No myeloperoxidase-positive neutrophils were detected in either group.

Our study demonstrates that although the thickness of the valve remained unaltered, the relative contribution of the ventricularis layer to total valve thickness was increased after LVAD implantation, accompanied by an increased cell density in this layer, and that part of this increased

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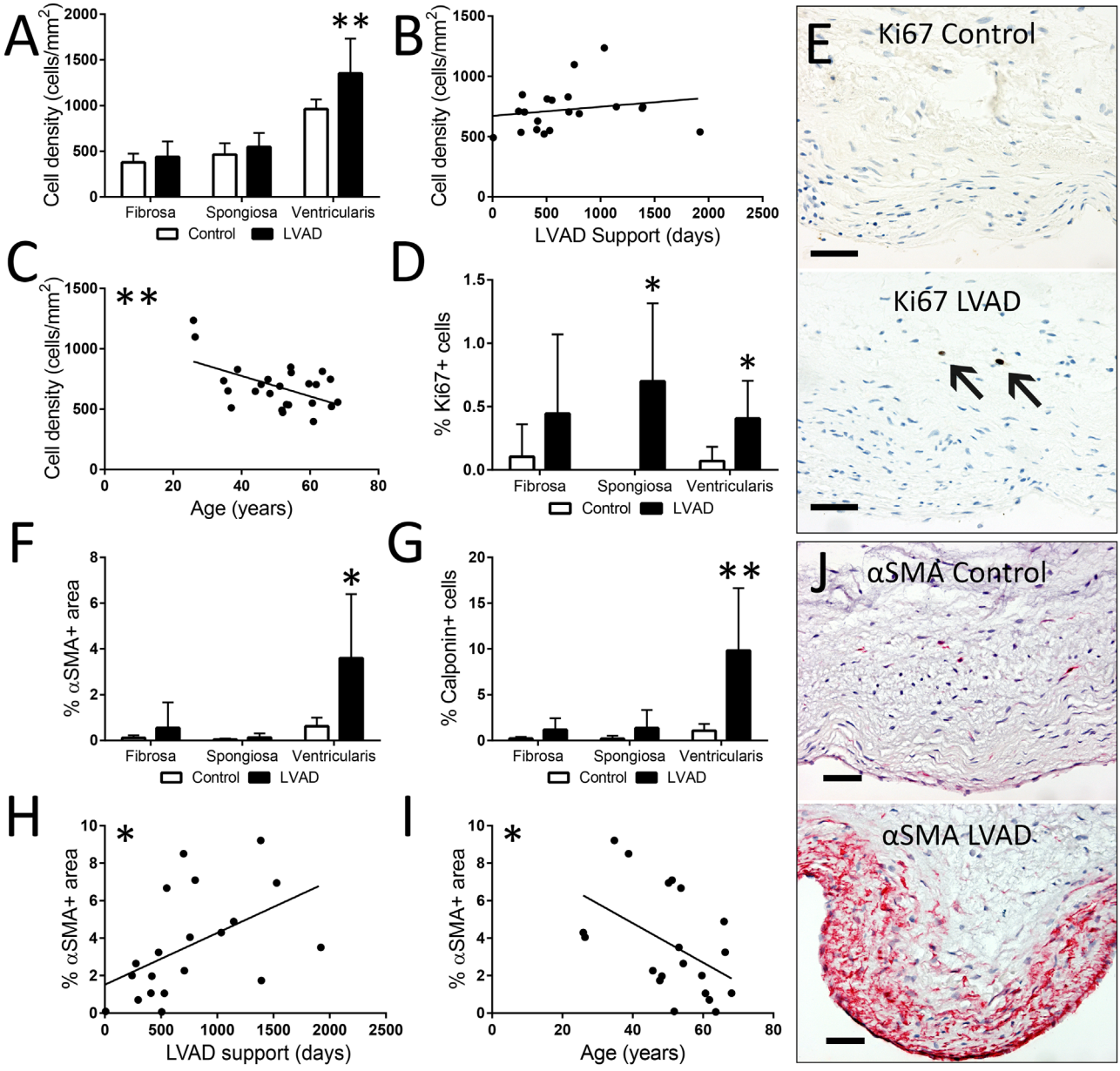


**Figure 1** Aortic valve cellularity after LVAD support. (A) Changes in cell density per layer after LVAD implantation. Relationship between leaflet cell density and (B) duration of support ($r^2 = 0.03$; $p = 0.43$) and (C) age ($r^2 = 0.27$; $p = 0.0058$). (D) Changes in proliferation as assessed by Ki-67 staining. (E) Representative photomicrographs of Ki-67 immunostain. (F) Changes in αSMA expression. Results are plotted as percentage of positive area compared with control. (G) Changes in calponin expression. Relationship between αSMA-positive (αSMA+) area in the ventricularis and (H) duration of support ($r^2 = 0.24$; $p = 0.0269$) and (I) age ($r^2 = 0.22$; $p = 0.0387$). (J) Representative photomicrographs of immunohistochemical staining of αSMA in control and LVAD valves. *$p < 0.05$, **$p < 0.01$. Scale bar = 50 μm.

cellularity could be explained by an increased amount of "activated VICs." In normal adult AVs, only 2% to 5% of the VICs have this activated αSMA-positive phenotype; however, in response to injury, the percentage may increase to >33% of the total VIC population.[2,3] This study shows that altered flow dynamics without major injury to the valve is sufficient to induce VIC activation, confirming and extending earlier in vitro and ex vivo experiments.[1]

Furthermore, we show that LVAD implantation leads to an increased M2 macrophage response in the ventricularis layer. M2 macrophages are anti-inflammatory and promote repair of damaged tissue.[4] Their scattered presence in the AV indicates an environment favoring remodeling, especially as the M1:M2 ratios were low. M2 macrophages have been shown to express transforming growth factor-β, a strong activator of VICs, and flow-induced M2 macrophage expression of transforming growth factor-β might be an important driver of VIC activation and differentiation into myofibroblast-like cells.[5] Therefore, these diffuse M2 macrophages might play an important role in VIC activation after flow alteration.

The 3 patients with LVADs with opening valves showed a trend toward less commissural fusion compared with continuously closed valves, confirming our previous results.[6]