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Association between cell-derived microparticles and adverse events in patients with nonpulsatile left ventricular assist devices

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Abstract

BACKGROUND—Continuous-flow left ventricular assist devices (LVADs) expose blood cells to high shear stress, potentially resulting in the production of microparticles that express phosphatidylserine (PS+) and promote coagulation and inflammation. In this prospective study, we attempted to determine whether PS+ microparticle levels correlate with clinical outcomes in LVAD-supported patients.

METHODS—We enrolled 20 patients undergoing implantation of the HeartMate II LVAD and 10 healthy controls who provided reference values for the microparticle assays. Plasma was

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collected before LVAD implantation, at discharge, at 3-month follow-up, and when an adverse clinical event occurred. We quantified PS+ microparticles in the plasma using flow cytometry.

RESULTS—During the study period, 8 patients developed adverse clinical events: ventricular tachycardia storm (n=1), non-ST-elevation myocardial infarction (n=2), arterial thrombosis (n=2), gastrointestinal bleeding (n=2), and stroke (n=3). Levels of PS+ microparticles were higher in patients at baseline than in healthy controls ($2.11\% \pm 1.26$ vs 0.69 ± 0.46 , $P=0.007$). After LVAD implantation, patient PS+ microparticle levels increased to $2.39\% \pm 1.22$ at discharge and then leveled to $1.97\% \pm 1.25$ at 3-month follow-up. Importantly, patients who developed an adverse event had significantly higher levels of PS+ microparticles than did patients with no events ($3.82\% \pm 1.17$ vs $1.57\% \pm 0.59$, $P<0.001$), even though the 2 patient groups did not markedly differ in other clinical and hematologic parameters.

CONCLUSIONS—Our results suggest that an elevation of PS+ microparticle levels may be associated with adverse clinical events. Thus, measuring PS+ microparticle levels in LVAD-supported patients may help identify patients at increased risk for adverse events.

Keywords

ventricular assist device; cell-derived microparticles; phosphatidylserine

Left ventricular assist devices (LVADs) have become a mainstream therapy for patients with end-stage heart failure who are considered ineligible for cardiac transplantation. Left ventricular assist devices are increasingly used as a bridge to transplant or destination therapy because these devices are readily available and can be used in patients with a wide spectrum of heart failure symptoms. Compared to first-generation pulsatile LVADs, the new continuous-flow LVADs have a lower rate of pump failure and have improved the overall patient survival rate.¹ However, device-related complications, especially those involving the extremes of the hemostasis spectrum (ie, hemorrhage and thrombosis), may pose challenges to patient recovery after implantation and are a cause of morbidity in long-term support therapy.

The study of LVAD-induced hemostatic defects has predominantly focused on the loss of high-molecular-weight von Willebrand factor (VWF) multimers.² This loss of VWF multimers is believed to be caused by persistently high shear stress generated by the LVAD pump.³ In addition, de novo arteriovenous malformations that are prone to bleeding can occur after LVAD implantation and spontaneously resolve after LVAD explantation.^{4, 5} These arteriovenous malformations occur primarily in the gastrointestinal tract,⁶ but the underlying pathogenesis is poorly understood.⁷ Patients also have an increased incidence of ischemic and hemorrhagic stroke.^{8–10}

The clinical and pathologic effects of high shear stress generated by the LVAD pump on red blood cells, platelets, leukocytes, and endothelial cells are poorly understood. Blood cells and endothelial cells that have been activated or have undergone apoptosis produce membrane fragments known as microparticles, which are less than 1 μm in diameter.^{11–15} Microparticles from apoptotic and mechanically disrupted cells express anionic phospholipids, especially phosphatidylserine (PS). Phosphatidylserine is essential for

initiating and propagating the coagulation cascade at sites of vascular injury,^{16, 17} but PS can also induce endothelial damage in inflammatory conditions^{11, 16, 18, 19} and stimulate neo-angiogenesis.²⁰ A persistent and systemic generation of microparticles can, therefore, lead to a sustained proinflammatory and procoagulant state and can cause activation of the endothelium, which responds to microparticle-bound tissue factor.^{21–23} The levels of microparticles from platelets, leukocytes, and endothelial cells have been reported to be significantly higher in patients with LVADs than in healthy controls.²⁴ However, it is unclear whether the elevated level of circulating microparticles found in these patients contributes to the development of adverse events such as thrombosis and bleeding diathesis.

In this pilot, prospective study, we assessed the levels of PS+ microparticles in patients before and after LVAD implantation to determine whether longitudinal changes in circulating PS+ microparticles are associated with the development of adverse events in LVAD-supported patients. Our findings suggest that PS+ microparticle levels may have prognostic value in predicting future clinical events.

Methods

Patients and study design

Approval for this study was obtained from our center's institutional review board, and informed consent was obtained from participants before enrollment. Twenty patients who were eligible for LVAD implantation at the Texas Heart Institute were enrolled in this prospective, observational study. In addition, 10 healthy volunteers were enrolled to provide normal reference values for microparticle assays. All patients received a HeartMate II LVAD (Pleasanton, CA), which was implanted according to standard surgical procedure, as destination therapy or as a bridge-to-heart transplant to treat the patients' New York Heart Association (NYHA) class IV symptoms. Patients on LVAD support received standard antithrombotic therapy (ie, aspirin, 81 mg/day; warfarin with a targeted international normalized ratio [INR] of 2 to 3; and dipyridamole, 75 mg 3 times per day).

Blood samples (30 to 40 mL) were collected via peripheral venipuncture at the time of admission (baseline), at discharge after LVAD implantation (on average 28 days after implantation), and at 3 months after implantation. At each time point, we analyzed basic hematology and biochemistry variables (ie, blood cell count, platelet count, renal function, and liver function), platelet consumption, coagulation profile, and hemolysis markers (ie, lactic dehydrogenase [LDH], hemoglobin, hematocrit, bilirubinemia, and free plasma hemoglobin). Left ventricular assist device function (ie, power output, power index, and impeller speed) and mean arterial pressure (MAP) on support were assessed during each follow-up visit. If a patient was readmitted for an adverse clinical event (as defined below), we repeated all tests. Clinical events were recorded at each outpatient visit and in the event of readmission.

Inclusion criteria—All patients who were eligible for LVAD implantation at the end of an institutional evaluation at the Texas Heart Institute, according to current consensus and guidelines,²⁵ were considered for inclusion in this study.

Exclusion criteria—Patients with known malignancy, autoimmune disease, or hypercoagulable conditions were excluded, regardless of whether they were eligible for LVAD implantation. These exclusion criteria were instated to minimize confounding factors known to be associated with thrombotic risk independent of LVAD implantation.

Clinical adverse events—Adverse events were defined according to the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) adverse event definitions. Major bleeding was defined as an episode of suspected internal or external bleeding that resulted in 1 or more of the following: death, reoperation, hospitalization, or transfusion of red blood cells. Arrhythmias were defined as any documented arrhythmia that resulted in clinical compromise (eg, diminished VAD flow, oliguria, presyncope, or syncope) and required hospitalization or occurred during a hospital stay. Cardiac arrhythmias were noted if they presented as sustained ventricular arrhythmia requiring defibrillation or cardioversion or sustained supraventricular arrhythmia requiring drug treatment or cardioversion. Pump thrombosis was defined as the presence of thrombus within the device or its conduits that resulted in or could have potentially induced circulatory failure. Hemolysis was defined by a plasma-free hemoglobin value greater than 40 mg/dl with clinical signs associated with hemolysis (eg, anemia, low hematocrit, hyperbilirubinemia) that occurred after the first 72 hours post implant. Documented nondevice-related causes (eg, transfusion or drug) were excluded from this definition. Nonperioperative myocardial infarction was defined by the presence of 2 of the 3 following criteria more than 7 days post implant: chest pain characteristic of myocardial ischemia, ECG with a pattern or changes consistent with a myocardial infarction, and troponin or creatine kinase levels (measured by using standard clinical pathology/laboratory medicine methods) greater than the normal range for the local hospital with a positive MB fraction ($\geq 3\%$ total creatine kinase). In addition, a new regional left ventricular or right ventricular wall motion abnormality should have been detected on a myocardial imaging study. Stroke was defined as any ischemic or hemorrhagic cardiovascular accident/cerebrovascular accident persisting beyond 24 hours that is associated with infarction on an imaging study performed to investigate a new, clinically diagnosed neurologic deficit. Arterial thrombosis was defined as an acute systemic arterial perfusion deficit in any noncerebrovascular organ system due to thromboembolism that was confirmed by standard clinical and laboratory testing operative findings.

Platelet activation and function

Extensive platelet function analysis was performed for the first 15 patients prospectively enrolled in this pilot study. Briefly, whole blood samples were collected in 3.2% sodium citrate vacutainers (0.32% final concentration). Platelet activation state and turnover were determined by using an EPICS XL-MCL flow cytometer with Expo32 software (Beckman Coulter, Miami, FL). Briefly, citrated whole blood samples were stimulated with 0.5 μ M adenosine diphosphate (ADP, Helena Laboratories, Beaumont, TX) for 5 minutes in the presence of Tyrode's buffer containing 1% bovine serum albumin (BSA, EMD Biosciences, Inc., La Jolla, CA). Control samples were not stimulated with ADP. The samples were then incubated for 20 minutes at room temperature with fluorescently labeled antibodies to P-selectin (CD62P-PE, BD Biosciences, San Jose, CA) or PAC-1 (PAC-1-FITC, BD

Biosciences), which binds activated integrin $\alpha\text{IIb}\beta 3$. The samples were then fixed with 1% paraformaldehyde in phosphate-buffered saline. To measure baseline levels of platelet-leukocyte aggregates and platelets bound with von Willebrand factor, we performed flow cytometry analysis of samples incubated with fluorescence-conjugated antibodies specific for platelets (CD41-FITC, Beckman Coulter, Miami, FL), leukocytes (CD11b-PE, Dako, Carpinteria, CA), and von Willebrand factor (von Willebrand-PE, Dako, Carpinteria, CA) in Tyrode's/1% BSA buffer for 20 minutes. Platelet-leukocyte aggregates were identified by detecting CD41 staining in cells gated for CD11b positivity, and platelets bound with von Willebrand factor were identified by detecting CD41 staining in cells gated for von Willebrand positivity, as previously described.²⁶

To globally evaluate hemostatic activity, we measured the ability of blood to form a hemostatic plug on a collagen-adenosine diphosphate (CADP) cartridge in a platelet function analyzer-100 (PFA-100, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY), as previously described.²⁶ Platelet turnover was measured by quantifying the number of reticulated platelets, as previously reported.²⁷ Briefly, 5 μl of citrated whole blood was added to Isoflow solution (Beckman Coulter, Miami, FL), either alone (control tube) or with 100 μl of Retic-COUNT reagent (BD Retic-COUNT Kit, BD Biosciences, San Jose, CA). After a 30-minute incubation, samples were centrifuged at $3,500 \times g$ for 2.5 minutes. We discarded the supernatant and gently rinsed the pellets with 1 ml Isoflow solution. Reticulated platelet counts were measured by flow cytometry within 1 hour of sample preparation.

Microparticle analysis

For all 20 patients enrolled in this study, we determined the quantity of PS+ microparticles by double-labeling microparticles from cell-free plasma with allophycocyanin (APC)-conjugated Annexin V, which specifically binds PS. The presence of microparticles was detected by using flow cytometry, as previously described.²⁸ Briefly, 50 μl of cell-free plasma was mixed with 5 μl of APC-conjugated Annexin V and 5 μl of antibody specific for 1 of the parental cell types for 30 minutes at room temperature and then analyzed by flow cytometry. Microparticles were first gated according to particle size (forward scatter); 0.5-, 1.0-, and 1.5- μm polystyrene standard beads were used as references to exclude intact cells. We used phorbol myristate acetate (PMA)-stimulated monocytes or THP-1 cells, which bind strongly to Annexin V, as a positive control. For negative controls, we used blood samples without staining and samples with Annexin V binding in the presence of calcium-chelating EDTA.

Statistical analysis

All statistical analyses were performed by using SAS statistical software (Version 9.1, SAS Institute, Cary, NC). Categorical (frequency) variables were expressed as percentages, and continuous variables were expressed as the mean $\pm$ standard deviation (SD). The Kolmogorov-Smirnov test was used to test normality of the data. A paired *t* test was used to compare values from healthy subjects and patients at various times of sample collection, and one-way repeated measures analysis of variance was used to compare data from patients before LVAD implantation (baseline), at discharge after implantation, and at 3-month

follow-up. We compared data from patients at baseline, patients who had an adverse event, and patients who had no adverse event by using a two-tailed *t* test. A *P* value of <0.05 was considered to be statistically significant.

Results

Clinical parameters and outcomes

Of the 20 patients in this pilot study, 3 were excluded after baseline enrollment because of death or improper sample collection. Among the remaining 17 patients, we observed 10 clinical adverse events in 8 patients: ventricular tachycardia storm (n=1), non-ST-segment elevation myocardial infarction (n=2), arterial thrombosis (n=2), gastrointestinal bleeding (n=2), and stroke (n=3). No significant hemolytic episodes were observed during the follow-up period. At the time of LVAD implantation, all patients were NYHA class IV, which was reduced to NYHA class I or II after hospital discharge. The clinical parameters of patients at baseline before LVAD implantation, at discharge after implantation (ie, approximately 1 month after LVAD implantation), and at 3-month follow-up were not significantly different, with the exception of INR, which increased after implantation as the result of introducing oral anticoagulation (Table 1). When clinical data were compared between patients at baseline and patients with or without events, LDH levels were significantly higher in patients after implantation than at baseline (Table 2). More importantly, LDH levels were significantly higher in patients who developed events than in those who did not (Table 2). No significant differences were observed in LVAD parameters (ie, impeller speed, power index, and power outputs) between patients who developed events and those who did not (Table 2).

Platelet studies

The reference values for mean PFA-100 closure time have previously been defined as 71–118 seconds in healthy subjects and 57–120 seconds in patients who have previously undergone an aortocoronary bypass and are receiving long-term oral aspirin therapy (81–325 mg/day).²⁹ Mean PFA-100 closure time in our cohort of patients was 99.5 ± 25.9 seconds at baseline (Table 1), which is consistent with previous reports.²⁹ However, the PFA-100 closure time was significantly prolonged after LVAD implantation, both at discharge and 3-month follow-up ($P < 0.001$; Table 1), most likely due to anticoagulation and dual antiplatelet therapy. Nevertheless, we did not detect a significant difference in PFA-100 closure time between patients who had adverse events and those who did not (Table 2). We also measured markers of platelet activation: P-selectin (CD62P) expression, PAC-1 binding, the formation of platelet-leukocyte aggregates, and the presence of von Willebrand factor bound to platelets; we found no difference in the levels of these markers in patients at baseline, discharge, or 3-month follow-up (Table 1). We also did not detect a significant difference in the levels of these markers between patients who had adverse events and those who did not (Table 2).

Although we found no statistically significant difference in patient platelet counts at baseline and after LVAD implantation, we observed a general reduction in levels of reticulated platelets (ie, newly produced platelets) in patients after LVAD implantation when compared

with baseline levels (Table 1). Furthermore, the reduction in the percentage of reticulated platelets from baseline levels reached statistical significance in patients who had no adverse event after LVAD implantation (Table 2).

Cell-derived microparticles

We compared the levels of PS+ microparticles between healthy controls and patients at baseline, discharge, and 3-month follow-up. We also compared the levels of PS+ microparticles in patients at baseline, patients who had an adverse event, and patients who had no adverse event. Overall, PS+ microparticle levels were higher in patients at baseline than in healthy controls (2.11 ± 1.26 vs 0.69 ± 0.46 , $P=0.007$; Figure 1, Table 3), which is consistent with the notion that end-stage heart failure constitutes a prothrombotic state. After LVAD implantation, patient PS+ microparticle levels increased to $2.39\% \pm 1.22$ at discharge and then leveled to $1.97\% \pm 1.25$ at 3-month follow-up; however, PS+ microparticle levels were not significantly different in patients at baseline, at discharge after implantation, and at 3-month follow-up (Table 3). In contrast, PS+ microparticle levels after implantation were significantly higher in patients who had an adverse event than in patients who had no adverse event (3.82 ± 1.17 vs 1.57 ± 0.59 , $P<0.001$, Figure 2 and Table 4). These results suggest an association between the level of PS+ microparticles and the development of major adverse events in patients with LVAD implants.

Discussion

Although platelet dysfunction and the production of cellular microparticles have been implicated in LVAD-associated pathophysiology, no prospective studies have correlated these parameters with clinical outcomes in LVAD-supported patients. To our knowledge, this longitudinal pilot study is the first to link levels of microparticles to adverse clinical outcomes in LVAD-supported patients and indicates that microparticle levels after LVAD implantation may potentially be used to predict adverse events in these patients. It is not yet clear what specific values of microparticles can be used to predict the occurrence and severity of adverse clinical events in LVAD-supported patients, but the results from our pilot study show that all post-implant events occurred in patients with a microparticle titer $>3\%$.

In our study, we found that the established markers of platelet activation varied considerably among a small cohort of LVAD-supported patients. Because of this large variation, the markers did not distinguish between LVAD patients who had adverse events and those who did not. A larger cohort is most likely necessary to identify potential correlations between the established markers of platelet activation and clinical outcomes in patients on LVAD support. In contrast, LDH levels were significantly higher in patients who developed clinical events after LVAD implantation than in patients at baseline, suggesting that ongoing sub-clinical hemolysis occurred in the absence of a rise in levels of well-established markers of red blood cell destruction. Thus, LDH levels may provide a sensitive, though non-specific, signal of major adverse events.

Two parameters were used to indicate the state of coagulation: INR and plasma levels of PS + microparticles. INR values were higher in patients after LVAD implantation than at

baseline, which indicated an increase in bleeding tendency potentially caused by anti-platelet medications and anticoagulants. However, PS+ microparticles, which are considered to be procoagulant, were at the highest levels for patients who developed events after LVAD implantation—levels higher than those observed in patients at baseline or in patients who did not develop an adverse event after implantation.

Whether PS+ microparticles are released before or after an event has occurred remains unknown, but the presence of PS+ microparticles at high levels suggests that blood cells either are activated (and vesiculated) or undergo apoptosis. Continuous-flow pumps induce high fluid shear stress that may lead to the production of microparticles from shear-sensitive cells such as platelets, endothelial cells, and erythrocytes. Furthermore, because PS+ microparticles have a negatively charged surface, they are considered to be procoagulant. Therefore, it is conceivable that PS+ microparticles contributed to the development of thrombotic and consumptive bleeding events observed in our patients after LVAD implantation. In addition, because PS+ microparticles express distinct biochemical markers of their cell of origin, including adhesive receptors,¹¹ they may also deliver inflammatory mediators, transcriptional factors, and mRNA to target cells through membrane fusion or endocytosis. In this regard, microparticles from different parental cells may play distinct roles in endothelial activation and dysfunction and neo-angiogenesis/angiodysplasia associated with LVAD implants.

Limitations

The connection observed between PS+ microparticle levels and the occurrence of clinical events in LVAD-supported patients is intriguing, but the interpretation of our data is limited by the small number of patients in this study and by the duration of follow-up. To validate the use of microparticle assessment as a predictor of adverse events, a prospective study involving a larger cohort of LVAD-supported patients and a longer follow-up period is currently underway.

Conclusions

The use of LVADs as either destination therapy or bridge to transplantation has revolutionized the treatment of end-stage heart failure. As these devices become smaller, shear stress could become a limiting factor to their further development. Identifying potential biochemical markers of shear stress, such as PS+ microparticles, that could be used to determine which patients are at high risk of developing adverse clinical events after LVAD implantation could improve patient care.

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Figure 1.

Box plot showing the percentage of phosphatidylserine-positive (PS+) microparticles in healthy controls and in patients at baseline, discharge (approximately 1 month after LVAD implantation), and 3-month follow-up. A paired *t* test was used to compare values between patients and healthy subjects. Vertical axis measurements represent the mean percentage of PS+ microparticles in cell-free plasma.

Figure 2.

Box plot showing the percentage of phosphatidylserine-positive (PS+) microparticles in healthy controls (Healthy), in patients before LVAD implantation (Baseline), in patients after LVAD implantation when an adverse event occurred [Events (+)], and in patients who had no adverse events after LVAD implantation (No events). Vertical axis measurements are given as the mean percentage of PS+ microparticles in cell-free plasma. A two-tailed *t* test was used to compare values between groups.

Table 1

Comparison of Clinical Data, LVAD Parameters, and Platelet Function in All Patients at Baseline, Discharge, and 3-Month Follow-up^a

	Baseline	Discharge	3 Months	P value
Clinical data				
Hemoglobin, g/dl	11.95 ± 1.82	11.39 ± 1.32	12.03 ± 1.71	0.83
Platelets/mm ³	245 ± 124	250 ± 80	205 ± 55	0.08
International normalized ratio	1.28 ± 0.42	2.27 ± 0.92	2.14 ± 0.99	0.014
Serum lactic dehydrogenase	248 ± 95	300 ± 58	266 ± 87	0.15
Mean arterial pressure, mmHg	n/a	81 ± 13	85 ± 13	n/a
LVAD parameters				
Impeller speed, rpm	n/a	7964 ± 2675	8792 ± 575	n/a
Power index	n/a	5.50 ± 0.96	5.46 ± 1.10	n/a
Power output, watts	n/a	6.02 ± 1.34	5.58 ± 1.12	n/a
Platelet function^b				
PFA-100 closure time, s	99.5 ± 25.9	265.9 ± 42.8	290.3 ± 19.77	<0.001
Reticulated platelets, % of total platelets	1.49 ± 0.72	1.33 ± 0.81	0.51 ± 0.63	0.025
Reticulated platelets/mm ³	310 ± 169	292 ± 143	113 ± 146	0.005
PAC-1, %	2.38 ± 1.06	2.33 ± 1.27	2.31 ± 0.98	0.81
Anti-VWF, %	2.36 ± 4.05	2.17 ± 2.43	4.11 ± 3.56	0.15
P-SELC, %	8.52 ± 2.55	7.97 ± 2.86	7.15 ± 2.20	0.11
PLT-Leuko, %	39.23 ± 16.08	39.30 ± 15.50	31.02 ± 16.21	0.31
Therapy at time point				
LVAD implanted	No	Yes	Yes	
Anticoagulation	No	Yes	Yes	

^aData are presented as the mean ± SD (n=20 unless otherwise indicated).

^bPlatelet function analyses were performed only for the first 15 patients enrolled in this study. Baseline, before LVAD implantation; discharge, approximately 1 month after LVAD implantation; and 3 months, 3-month follow-up after LVAD implantation. Anti-VWF, percentage of platelets with von Willebrand factor bound; n/a, data not available (ie, no LVAD implanted); PAC-1, percentage of platelets positive for PAC-1, which was detected by using a specific monoclonal antibody that binds activated integrin αIIbβ3; PFA-100, platelet function analyzer-100; PLT-Leuko, percentage of leukocytes with platelets attached; P-SELC, percentage of platelets positive for P-selectin. One-way repeated measures analysis of variance was used to compare data from patients at baseline before implantation, at discharge after implantation, and at 3-month follow-up.

Table 2

Comparison of Clinical Data, LVAD Parameters, and Platelet Function in Patients Who Had Adverse Events Versus Patients Who Had No Adverse Events After LVAD Implantation

	Baseline	Events (+)	No events	Baseline vs Events (+) <i>P</i> value	Baseline vs No Events <i>P</i> value	Event (+) vs No Events <i>P</i> value
Clinical data						
Hemoglobin, g/dl	11.95 ± 1.83	11.14 ± 1.54	11.87 ± 1.49	0.25	0.88	0.20
Platelets/mm ³	245 ± 124	185 ± 56	229 ± 72	0.19	0.66	0.08
International normalized ratio	1.28 ± 0.42	2.01 ± 1.16	2.68 ± 0.96	0.025	<0.001	0.37
Total bilirubin, mg/dl	0.95 ± 0.59	0.67 ± 0.63	0.51 ± 0.15	0.27	0.008	0.22
Serum lactic dehydrogenase	248 ± 95	511 ± 298	288 ± 51	<0.001	0.011	0.001
Mean arterial pressure, mmHg	n/a	93 ± 17	82 ± 11	n/a	n/a	0.10
LVAD parameters						
RPM of impeller	n/a	8756 ± 523	8359 ± 1934	n/a	n/a	0.85
Power index	n/a	4.90 ± 1.70	5.54 ± 0.80	n/a	n/a	0.69
Power output, watts	n/a	5.52 ± 1.14	5.71 ± 1.22	n/a	n/a	0.38
Platelet function						
PFA-100 closure time, sec	99.5 ± 25.9	259 ± 68	278 ± 35	<0.0001	<0.0001	0.36
Reticulated platelets, %	1.49 ± 0.72	1.24 ± 0.80	0.92 ± 0.82	0.49	0.05	0.41
Reticulated platelets/mm ³	310 ± 169	232 ± 164	202 ± 168	0.36	0.08	0.70
PAC-1, %	2.38 ± 1.06	1.73 ± 0.86	2.32 ± 1.10	0.21	0.88	0.24
Anti-VWF, %	2.36 ± 4.05	-0.42 ± 4.79	3.14 ± 3.12	0.35	0.64	0.06
P-SELC, %	8.52 ± 2.55	8.13 ± 3.94	7.56 ± 2.52	0.80	0.29	0.67
PLT-Leuko, %	39.23 ± 16.08	38.6 ± 12.6	35.2 ± 16.0	0.93	0.48	0.63
Therapy at time point						
LVAD implanted	No	Yes	Yes			
Anticoagulation	No	Yes	Yes			

^aData are presented as the mean ± SD (n=20 unless otherwise indicated).

^bPlatelet function analyses were performed only for the first 15 patients enrolled in this study. Baseline, before LVAD implantation; events (+), when an adverse event occurred; no events, any time-point after implantation in patients with no events. Anti-VWF, percentage of platelets with bound von Willebrand factor; PAC-1, percentage of platelets with bound PAC-1, which was detected by using a specific monoclonal antibody that binds activated integrin αIIbβ3; PFA-100, platelet function analyzer-100; PLT-Leuko, percentage of leukocytes with platelets attached; P-SELC, percentage of platelets that expressed P-selectin (another marker for platelet activation); n/a, data not available (ie, no LVAD implanted). A two-tailed *t* test was used for comparisons between groups.

Table 3

Phosphatidylserine-positive Microparticles in Cell-free Plasma from Healthy Controls and from Patients at Baseline, Discharge, and 3-Month Follow-up*

	Healthy	Baseline	Discharge	3 Months	Healthy vs Baseline <i>P</i> value	Healthy vs Discharge <i>P</i> value	Healthy vs 3 Months <i>P</i> value	Baseline vs Discharge <i>P</i> value	Baseline vs 3 Months <i>P</i> value	Discharge vs 3 Months <i>P</i> value
MPs (%)	0.69 ± 0.46	2.11 ± 1.26	2.39 ± 1.22	1.97 ± 1.25	0.007	0.009	0.073	0.65	0.65	0.69
LVAD implanted	No	No	Yes	Yes						
Anticoagulation therapy	No	No	Yes	Yes						

* Data are presented as the mean ± SD. Baseline, before LVAD implantation; Discharge, approximately 1 month after LVAD implantation; 3 Months, 3-month follow-up after LVAD implantation; MPs, cell-derived phosphatidylserine-positive microparticles. One-way repeated measures analysis of variance was used to compare data from patients at baseline before implantation, at discharge after implantation, and at 3-month follow-up, and a paired *t* test was used for comparing data between healthy subjects and patients.

Table 4

Phosphatidylserine-positive Microparticles in Cell-free Plasma from Healthy Controls, Patients at Baseline, and Patients With or Without Adverse Events^a

	Healthy	Baseline	Events (+)	No Events	Healthy vs Baseline <i>P</i> value	Healthy vs Events (+) <i>P</i> value	Healthy vs No Events <i>P</i> value	Baseline vs No Events <i>P</i> value	Events (+)vs No Events <i>P</i> value
MPs (%)	0.69 ± 0.46	2.11 ± 1.26	3.82 ± 1.17	1.57 ± 0.59	0.007	<0.001	0.02	0.23	<0.001
LVAD implanted	No	No	Yes	Yes					
Anticoagulant	No	No	Yes	Yes					

^a Data are presented as the mean ± SD. Baseline, before LVAD implantation; events (+), when an adverse event occurred; no events, any time-point after implantation in patients with no events; MPs, cell-derived phosphatidylserine-positive microparticles. A two-tailed *t* test was used to compare values between groups.