Identification of Novel Hemostatic Biomarkers of Adverse Clinical Events in Patients Implanted With a Continuous-Flow Left Ventricular Assist Device

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Abstract

Heart failure affects over 5 million people in the United States. Its rising prevalence and the limited supply of donor hearts is increasing the use of mechanical cardiac support with the implantation of continuous-flow ventricular assist devices (CF-VAD). Patients with CF-VAD implants are at risk of complications, specifically adverse hemostatic events such as nonsurgical bleeding and thrombosis. Development of a pump thrombus requires clinical intervention and/or surgical replacement significantly increasing the risk of patient morbidity and mortality. Identification of biomarkers for these events could improve current risk assessment models, subsequent treatment, and quality of life prognoses for VAD-implanted patients. The standard means for identifying thrombus in VAD patients is currently limited to monitoring levels of lactate dehydrogenase (>2× upper limit of normal), which is incapable of predicting a future event, but describes the risk of a present thrombus. Surface-enhanced laser desorption ionization time-of-flight mass spectrometry is a technique used to identify biomarkers. In this study, 3 groups of unique peaks were identified in plasma from patients with left ventricular assist devices: 8.1-kDa, 11.7-kDa, and a 15.2-/16.1-kDa pair. Unique correlations were found for each peak, respectively, with microparticles (MPs) and MP procoagulant activity, C-reactive protein, and MP-tissue factor. Furthermore, the use of 8.1-kDa peaks may be able to differentiate thrombotic events from other hemostatic events.

Keywords

ventricular assist device, LVAD, biomarkers, SELDI, mass spectrometry

Introduction

Heart failure affects over 5 million people in the United States. Its rising prevalence coupled with a limited supply of donor hearts has led to an increase in the use of continuous-flow left ventricular assist devices (CF-VAD). The CF-VAD patients are at an increased risk of nonsurgical bleeding and thrombotic events. Adherence to PREVENtion treatment guidelines still results in confirmed pump thrombus formation in 2.9% and 4.8% of Heartmate II implanted patients at 3 and 6 months postimplantation, respectively.[1] INTERMACS reports an incidence rate of 0.123 strokes per patient-year for VAD patients, of which roughly half are thrombotic and half are hemorrhagic in nature.[2] Despite technological improvements in recent generations of left ventricular assist devices (LVADs), there are still reports of hemostatic complications.[3] Currently, pump thrombus formation is indicated by elevation of blood lactate dehydrogenase (LDH) levels to 2× upper limit of normal (ULN).

The LVAD patients are at an increased risk of thrombotic and nonsurgical bleeding due to multiple factors. The artificial nature of an LVAD creates an ideal surface for activation of the coagulation cascade and platelet aggregation. To balance the risk of bleeding and thrombosis, anticoagulation with warfarin titrated to an INR of 2.5 to 3.5 is used.[4] Aspirin at 81 mg daily is used for its antiplatelet effect. Additionally, it is believed the shear stress created by the LVAD pumps create an acquired

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von Willebrand disease like that seen in patients with aortic stenosis and contributes to the unusually high rate of acquired bleeds.[5]

There exists a need to identify a biomarker capable of risk stratifying patients or indicating upcoming events in patients with VAD implants. This has led to the exploration of stress-induced products such as microparticles (MPs)[6] and integrin expression.[7] This article will present additional biomarkers and a surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF-MS) approach to identify novel hemostatic biomarkers in patients with LVAD.

Hemostasis and thrombosis are highly complex and regulated intra- and extravascular processes that utilize plasmatic coagulation enzymes, circulating blood cells, vascular compartmentalization, and inhibitors to maintain the balance between hemorrhage and thrombosis. Activation of either the intrinsic pathway via factors XII, XI, VIII, IX, or the extrinsic pathway via tissue factor (TF) and factor VII, leads to activation of the common pathway and ultimately to thrombin generation, fibrin crosslinking, and platelet plug stabilization. In addition to this central cascade, many other proteins and enzymes can be used to monitor coagulation including thrombin–antithrombin complexes, prothrombin fragment F1+2, and fibrin degradation products, such as D-dimer. Many of these biomarkers have been observed to be elevated in heart failure and LVAD patients.[8-12]

Submicron vesicles or MPs are generated in times of stress from activated white blood cells (WBCs), red blood cells (RBCs), endothelium and platelets, cancer cells and apoptotic cells and are released into the intravascular space. Depending on the activation stimulus, MPs can carry extravascular components of the cascade such as TF and contribute to a prothrombotic state.[13] Over the last few years, several studies have been published regarding the potential use of MP levels to identify adverse events in the cardiac assist device patient population. In 2010, a study comparing 3 different cardiac assist devices was one of the first to describe elevated levels of MPs in this population.[14] A few years later, conflicting data found MP levels to not be significantly elevated.[15] Then in 2015, a study assessed MP levels 3 months after implant and found elevated platelet MPs CD41/CD31$^+$, endothelium MPs CD62E$^+$, CD144$^+$ or CD31$^+$/CD41$^-$, erythrocyte MPs CD235$^+$, and leukocyte MPs CD45$^+$ in LVAD patients compared to healthy controls while platelet, white cell and red cell MPs were elevated compared to patients with coronary artery disease.[16] These processes are still in whole not well understood and with careful monitoring may eventually provide warning signs of impending hemostatic complication.

The SELDI-TOF-MS combines the work of 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE), extraction protocols, and mass spectrometry analysis into 1 system (Figure 1). Proteins are solidified into an ionization enhancing matrix on a chromatographic surface and then ionized and read by the TOF-MS. This system has been used previously to identify biomarkers for numerous types of cancers and other diseased states.[18-20] Although SELDI is capable of analyzing proteins >100 kDa, it works best for proteins of <20 kDa

molecular weight and requires smaller samples compared to 2D-PAGE.[17,21]

Materials/Methods

Sample Collection/Preparation

Sixteen Loyola University Medical Center patients who underwent the implantation of a Thoratec HeartMate II CF-VAD (Pleasanton, California) donated 71 blood samples collected perioperatively and postoperatively during regular outpatient visits. The blood samples were collected by venipuncture into 0.109 M sodium citrate tubes by surgical or outpatient staff. Samples were centrifuged at $2000 \times g$ for 15 minutes in 25°C, and the supernatant platelet poor plasma was removed via pipette, aliquoted, and stored at −80°C until assayed.

Controls

Normal human pooled plasma (NHPP) was purchased from George King Biomedical (Overland Park, Kansas). Pathological pooled human plasma was prepared in-house from plasma samples of 25 to 30 individuals exhibiting prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) in the absence of anticoagulation or increased levels of liver enzymes.

Enzyme-Linked Immunosorbant Assays

Microparticle-Tissue Factor was quantified using the ZYMU-PHEN MP-TF kit (HYPHEN-BIOMED, Neuville-sur-Oise, France). This assay uses a monoclonal antibody specific for an extracellular domain of TF that does not disrupt TF activity and measures the quantity of TF by rate of substrate conversion. Microparticle procoagulant activity was quantified using ZYMUPHEN MP-Activity kit (HYPHEN-BIOMED). The kit uses biotinylated annexin-V to sequester MPs. C-reactive protein was quantified using the ZYMUTEST CRP kit (HYPHEN-BIOMED).

All assays were performed according to the manufacturer's instructions. Automated washing was performed using a BioTek ELx405 (Winooski, Vermont). Optical densities were measured with a SpectraMax Plus spectrophotometer using SpectraMax Pro 7 software (Molecular Devices, Sunnyvale, California).

Flow Cytometric Measurement of MPs

Plasma samples were thawed and subsequently centrifuged $20\,000 \times g$ for 90 minutes at 4°C. The supernatant was removed and the pellet was resuspended in 4 μM PKH-67 (Sigma-Aldrich, St. Louis, Missouri). Samples were incubated for 5 minutes at room temperature in the dark and then 1% bovine serum albumin was added to each tube. After 1 minute incubation in the dark, samples were centrifuged at $20\,000 \times g$ for 90 minutes. The supernatant was removed and the pellet was resuspended in calcium-free Tyrode's buffer. Aliquots of resuspended pellet were incubated with saline (control) or with
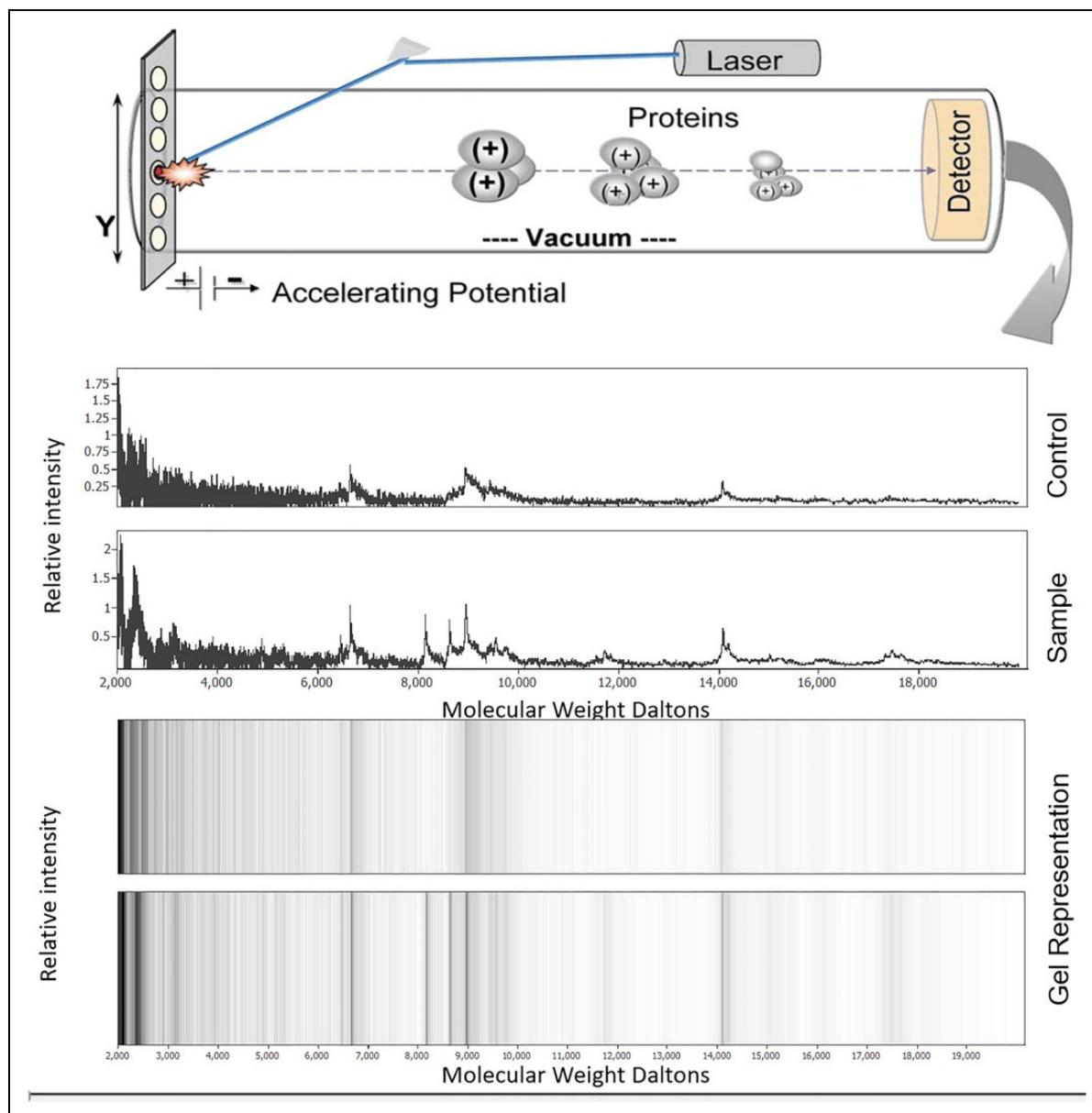
Figure 1. Top: During SELDI-TOF-MS analysis, patient serum protein samples are plated, ionized, accelerated through a vacuum, and analyzed by a detector (image taken from Issaq et al).[17] Mid: A protein peak profile or spectrum is created for visual representation of proteomic profiles in Dalton's relative intensity. Control and sample spectra are shown for peak comparisons. Bottom: Gel representation of protein peak profiles for 2D-PAGE representation. SELDI-TOF-MS indicates surface-enhanced laser desorption ionization time-of-flight mass spectrometry; 2D-PAGE, 2-dimensional polyacrylamide gel electrophoresis.

PE-labeled, anti-CD41 (eBioscience, San Diego, California). Following a 30-minute incubation at room temperature in the dark, samples were diluted in calcium-free Tyrode's buffer, and each sample was analyzed using a Beckman Coulter (Brea, California) Epics XL flow cytometer.

ProteinChip SELDI System

The SELDI-TOF-MS was acquired from Bio-Rad (Hercules, California). Plasma samples were fully thawed at 25°C. ProteinChip arrays were removed from storage in 50% methanol and serially washed in 15-mL conical tubes on a shaker plate for 6 minutes in acetone, 6 minutes in 0.01 N HCl, 6 minutes in HCl/methanol mix, and 6 minutes in methanol before being set to dry on Kimwipes for 10 minutes. Plasmas were diluted 1:10 in 0.1 M Tris-HCl buffer (pH 7.5). Matrix solutions were prepared by mixing sinapic acid powder, enough to saturate the solution, with 200 μL trifluoroacetic acid and 200 μL of acetonitrile. This solution was wrapped in foil, vortexed briefly, centrifuged for 30 seconds at 10 000 RPM, stored, and protected from light until use. Sample solutions consisting of 10 μL of matrix solution and 5 μL of diluted sample were mixed

slowly by repeatedly pipetting until the solution turned white. The sample solutions were then pipetted onto ProteinChip arrays and set to dry for SELDI-TOF-MS analysis.

Each ProteinChip array included an NHPP and PHPP sample for control and comparison, respectively. The TOF-MS analysis was conducted for the range of 0 to 20 kDa. Proteomic peak profiles were analyzed relative to NHPP normalized baseline relative frequencies to identify protein peaks in patient samples elevated above the ULN. A significant protein peak for this study was defined by NHPP relative intensity (RI) > 0.20. This cutoff was determined by compiling all NHPP peak profiles and scanning baseline areas (areas between peaks) from 5 to 20 kDa for the maximum peak intensities. Any signal that occurred only once in the set of samples and never reoccurred was not considered.

Adverse Clinical Event Identification

Historical review of patient electronic health records was conducted using the electronic health record and an internal Red-Cap database to identify adverse clinical events. Patients were identified using medical record numbers confirmed by date of birth. The CF-VAD thrombosis was characterized as cerebrovascular accident or transient ischemic attack diagnosed by a neurologist, rise in LDH or plasma-free hemoglobin, hemolysis, evidence of pump dysfunction consistent with thrombus identified by changes in pump parameters, echocardiographic or computed tomographic evidence of clot, or surgical pump exchange for thrombus. The CF-VAD bleeding events were characterized as bleeding determined by a cardiologist.

Results

Baseline characteristics for LVAD patients are displayed in Table 1. Twelve of 16 patients were male, mean age 58 years, ranging from 30 to 78. Fourteen patients experienced 18 adverse clinical events; 2 patients did not experience an adverse clinical event. There were 11 thrombotic events: 7 accounts of LDH >2× ULN (LDH$_{ave}$ = 606 IU/L), 2 confirmed thrombi, 1 event of hemolysis, and 1 transient ischemic attack (TIA). There were 4 gastrointestinal bleeds. Three septic events also occurred in 3 different patients.

Unique Peak Identification

Seven prominent peaks were observed between 6.4 and 14.1 kDa in NHPP control samples (Table 2). The intensities of these peaks ranged from 0.21 to 0.6.

Twelve PHPP controls were analyzed, and the 5 normal peaks in control samples in the 0 to 20 kDa range were identified plus a unique doublet peak at 15.2-/16.1-kDa doublet (RI$_{ave}$ = 0.438/0.423).

Inspection of the mass spectra of 71 patient samples led to identification of 3 peaks in LVAD patient samples collected prior to the date of an event that were not observed in NHPP: an 8.1-kDa singlet (8.1), 11.7-kDa singlet (11.7), and

Table 1. Baseline Characteristics of LVAD Patients During Sample Collection Periods.

Parameter	LVAD (n = 16)
Male/female	12/4
Age, years (mean: range)	58: 30-78
BMI, kg/m^2 (mean: range)	33: 23-64
Weight, kg (mean: range)	97: 62-160
INR[a] (mean: range)	2.4: 1.9-3.6
Adverse events	Thrombotic (n = 11)
	Hemorrhagic (n = 4)
	Septic (n = 3)

Abbreviations: BMI, body mass index; INR, international normalized ratio; LVAD, left ventricular assist device.
[a]INR average across adverse event period.

Table 2. Identified Control and Unique Peaks.[a]

Peak	NHPP (kDa)	RI$_{ave}$	PHPP (kDa)	RI$_{ave}$	LVAD	RI$_{ave}$
1	**6.4**	**0.21**	x	x	6.4	0.24
2	**6.6**	**0.32**	6.6	0.23	6.6	0.30
3	x	x	x	x	**8.1**	**0.73**
4	**8.71**	**0.24**	x	x	8.71	0.35
5	**8.78**	**0.64**	8.78	0.22	8.78	0.60
6	**9.4**	**0.3**	9.4	0.52	9.4	0.32
7	x	x	x	x	**11.7**	**0.26**
8	14	0.58	14	0.32	14	0.54
9	14.1	0.35	14.1	0.20	14.1	0.39
10	x	x	**15.2/16.1**	**0.43/0.42**	15.2/16.1	0.73

Abbreviations: LVAD, left ventricular assist devices; NHPP, normal human pooled plasma; PHPP, pathological human pooled plasma; RI$_{ave}$, relative intensity average.
[a]List of identified control NHPP, PHPP, and Unique LVAD peaks in kDa with RI$_{ave}$ > 0.20. Bolded numbers highlight new significant peaks from previous columns.

15.2-/16.1-kDa doublet (15/16) (Figure 2). The 8.1-kDa peak was observed 19 times, 11.7-kDa peak 11 times, and 15.2-/16.1-kDa peak was observed 15 times (8.1 kDa, RI$_{ave}$ = 0.70; 11.7 kDa, RI$_{ave}$ = 0.45; 15.2/16.1 kDa, RI$_{ave}$ = 0.26/0.37).

Although plasma samples from both patients who did not experience an adverse event were negative for the 8.1-kDa peak, samples from 8 of the 11 patients with a thrombotic event were associated with 8.1-kDa peaks while samples from 3 patients with elevated LDH (LDH$_{ave}$ = 585 IU/L, not significantly different from 8.1-kDa positive samples) levels were not. Two gastrointestinal (GI) bleeds were associated with 8.1-kDa peaks while 2 were not. No 8.1-kDa peaks occurred prior to septic events (see Table 3).

One of the 2 patients without an adverse event had a plasma sample that was positive for the 11.7-kDa peak. Two of the 11 thrombotic events were associated with 11.7-kDa peaks, whereas 1 thrombus and 6 LDH (LDH$_{ave}$ = 561 IU/L), a TIA, and hemolysis event were not. Samples from patients experiencing a hemorrhagic or septic event did not exhibit the 11.7-kDa peak.

One of the 2 patients without an adverse event was positive for the 15/16 peaks. Samples from 7 of the 11 patients with a
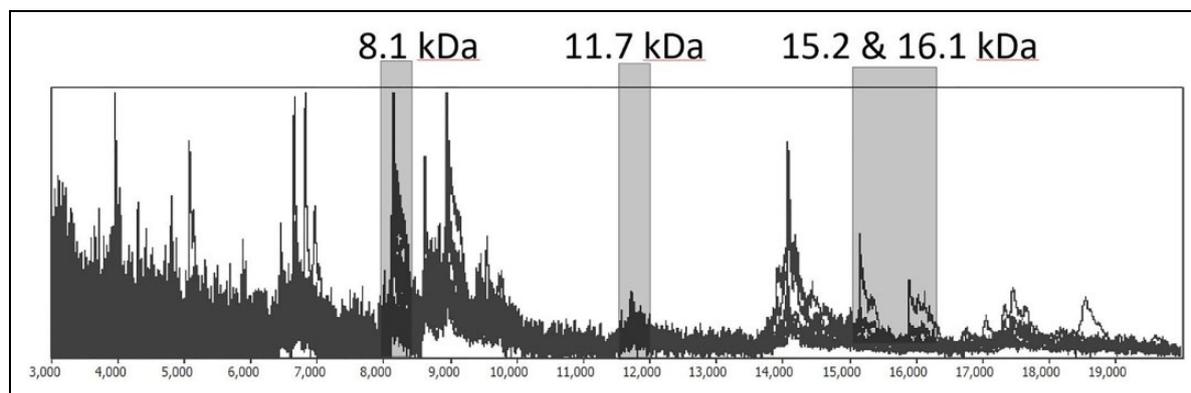
Figure 2. Overlaid spectra illustrating the 3 unique peaks from a series of samples collected from LVAD patients. These peaks had molecular weights 8.1, 11.7, and 15.2/16.1 kDa. LVAD indicates left ventricular assist devices.

Table 3. Peak Presence Prior to Event.[a]

Adverse Event	8.1 kDa (%)	11.7 kDa (%)	15.2/16.1 kDa (%)
Thrombotic, n = 11	72	18	63
Hemorrhagic, n = 4	50	0	50
Sepsis, n = 3	0	0	33
No events	0	50	50

Abbreviation: SELDI-TOF-MS, surface-enhanced laser desorption ionization time-of-flight mass spectrometry.
[a]Eighteen events from 14 patients (2 patients did not experience a hemostatic complication) were separated into categories: thrombotic, hemorrhagic, and sepsis and the percentage of events that could be defined by the presence of an 8.1-kDa peak by SELDI-TOF-MS is shown above.

thrombotic event exhibited the 15-/16-kDa peak, whereas 3 samples with elevated LDH levels (LDH$_{ave}$ = 550 IU/L) and a TIA did not. Samples from 2 patients with a GI bleed had the 15-/16-kDa peaks, whereas samples from 2 patients with GI bleeds did not. Samples from 1 of 3 patients with a septic event had the 15-/16-kDa peaks.

Flow Cytometric Analysis of Platelet MPs

Flow cytometric analysis showed elevation of CD41^{+} MPs in LVAD patients compared to healthy controls (1399 ± 1361 MP/mL, n = 74 vs 452.8 ± 405 MP/mL, n = 17, P = .0014; Figure 3, Table 4). Analysis of samples collected 30 and 45 days prior to adverse thrombotic and hemorrhagic events did not show a significant increase from controls, P = .0987 and P = .1259, respectively.

Enzyme-Linked Immunosorbent Assays

The MP-TF levels were not found to be elevated in preimplant samples from LVAD patients compared to controls. In samples collected within 30 days of identified adverse clinical events, that is, thrombotic and GI bleeds, LVAD patients were found to have elevated levels of MP-TF (n = 13) 3.73 ± 3.46 pg/mL compared to NHPP controls 0.46 ± 0.05 pg/mL, P = .019. When separated based on the type of adverse event

(thrombotic, n = 10 or hemorrhagic, n = 5), only the thrombotic had elevated MP-TF levels (4.047 ± 3.891 vs 0.6783 ± 0.4313, P = .0303). The MP-TF levels were not significantly elevated in samples collected 45 days prior hemostatic adverse events (Table 4, Figure 3).

Microparticle procoagulant activity was not found to be significantly elevated in LVAD patient samples (n = 77), in general, compared to NHPP controls (40.18 ± 21.41 ng/mL versus 24.42 ± 0.67 ng/mL), but was found to be elevated in specimens 30 days prior to hemorrhagic events (53.13 ± 9.01 ng/mL) when compared to NHPP controls, P = .022.

C-reactive protein was found to be elevated in LVAD patients (n = 77; 16.56 ± 16.72 µg/mL) compared to NHPP controls (2.24 ± 0.62 µg/mL), P = .0364.

Discussion

Biochip array profiling using SELDI-TOF-MS analysis has been used to identify unique biomarkers in various diseases.[18-20] This technique identifies unique peaks which may be generated upon the activation of proteases and subsequent generation of protein fragments specific to a certain pathophysiologic state. In the case of LVAD patients, the underlying end-stage heart failure may contribute to the generation of certain biomarkers. Furthermore, the shear forces and the contact of blood with a foreign surface may trigger activation of proteases such as factor XII and may contribute to the formation of unique peaks which may be characteristic of the endogenous pathophysiologic states in these patients. The studies reported in this communication address this and demonstrate the presence of unique peaks in SELDI mass spectra of plasma samples from LVAD patients over extended period of times.

In the cohort of LVAD patient specimens analyzed, adverse events revealed more thrombotic events than hemorrhagic, 11 versus 4. The 8.1-kDa peak was observed most often with thrombotic events compared to the 11.7- and 15-/16-kDa pair (Table 3). Besides identifying 72% of thrombotic events and 50% hemorrhagic, this is the only unique peak not associated with septic events or patients without events, a significant
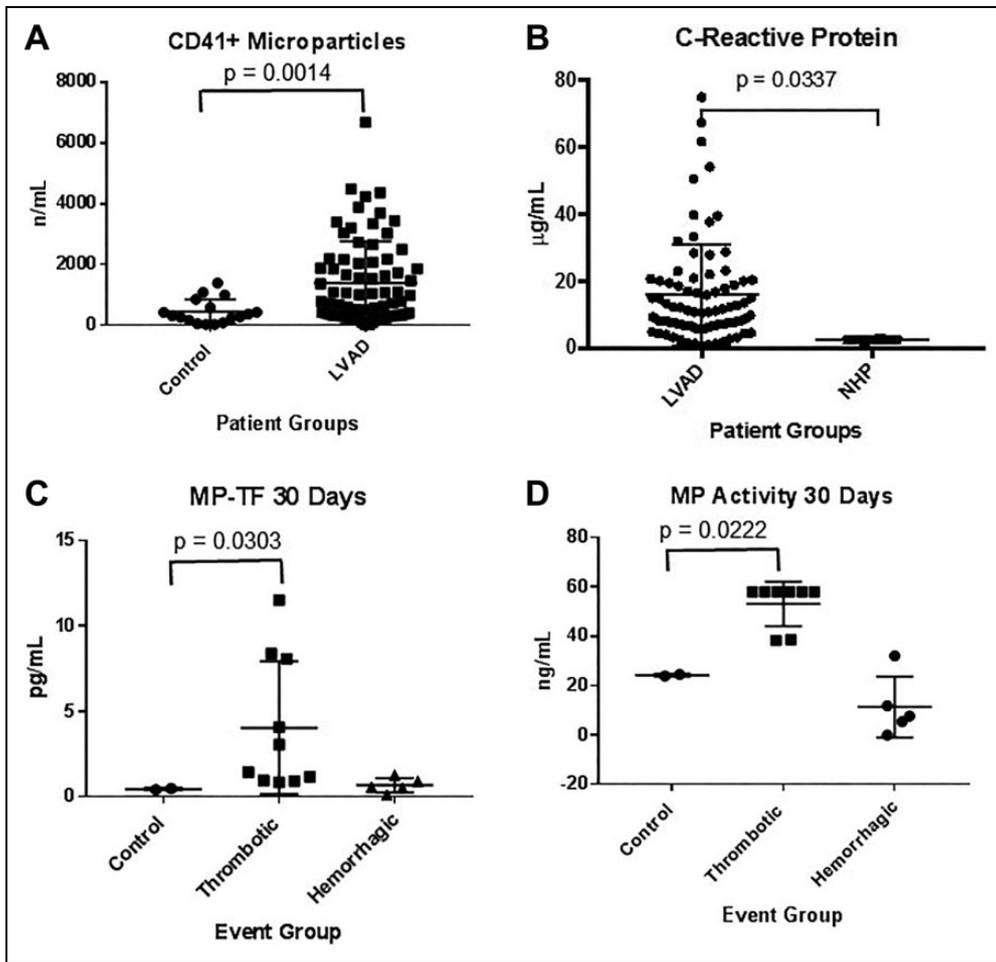
Figure 3. Scatter plot representation of (A) CD41$^+$ microparticles and (B) C-reactive protein from all samples compared to normal human pooled plasma controls. C, Microparticle-tissue factor and (D) Microparticle procoagulant activity 30 days prior to thrombotic and hemorrhagic events. Samples for hemorrhagic events were not significantly different from controls.

Table 4. Significant Elevated Hemostatic Parameters Days Prior to Events.[a]

| | | *P* Values | | | |
| | | Thrombotic | | Bleeding | |
Parameter	All Samples	30 Days	45 Days	30 Days	45 Days
CD41+ MP	.0014	NS	NS	NS	NS
MP-TF	NS	.0303	NS	NS	NS
MP-Activity	NS	.0222	NS	NS	NS
CRP	.0337	x	x	x	x

Abbreviations: CD41+ MP, platelet surface marker; CRP, C-reactive protein; MP-TF, microparticle-tissue factor; NS, not significant; x, not evaluated.
[a]Significant *P* values compared to normal human pooled plasma controls for all samples CD41$^+$ (platelet) microparticles (n = 71), microparticle-tissue factor (n = 71), microparticle activity (n = 71), and C-reactive protein (n = 71) and for samples within 30 and 45 days prior to thrombotic (n = 10, 15) and bleeding events (n = 5, 6) 30 and 45 days prior to events.

factor if this biomarker is to be used in clinical application for hemostasis and thrombosis risk stratification while avoiding medically unnecessary treatments.

The 15-/16-kDa peaks identified 63% of thrombotic events. However, their presence in sepsis and nonevent patients shows a lower specificity for the patient and event population being analyzed. Additionally, unpublished results from our laboratories have also shown the presence of the 15-/16-kDa peaks in patients with sepsis and end-stage renal disease establishing it as a nonspecific clinical biomarker. This is supported by its presence in control samples of PHPP. The 11.7-kDa peak was not as prevalent as the 8.1-kDa peak, suggesting that the 8.1-kDa marker may be specific to LVAD patients. Previously, proteomic profiling of heart failure and LVAD patient cardiac tissue has been explored extensively in humans with 2D-PAGE and isoelectric focusing,[22-24] but no one has looked at patient plasma proteomic profiles in the heart failure population.

In the past, MPs have been shown to be elevated in LVAD patients in some studies, while not elevated in others.[12-14] This may relate to particular assay conditions or the timing of sample collection. Our results support the elevation of CD41$^+$ MPs in LVAD patients. Although MP levels can be increased due to shear stress or apoptosis, increased levels of

CRP observed in these LVAD patients suggest inflammation could be playing a role.

The advantage our study has over those past is the collection of multiple samples over the course of about a year for each patient, whereas most studies collected 1 sample preoperative and 3 months postoperative. Although we observed elevated $CD41^+$ MPs ($P = .0014$), not all samples were elevated nor were they significantly elevated 30 or 45 days prior to thrombotic or bleeding events. Still MP-TF and MP procoagulant activity were elevated during this 30-day period compared to controls. This supports the idea MP-TF may be contributing to hemostatic activation in these patients, but platelets may not be their source of origin. Despite its elevation 30 days prior to an event, MP-TF was observed elevated during nonevent periods and is not supported as a good biomarker by this study. Although this provides some suggestions into the role of MPs in hemostatic activation, the question remaining is how much do MPs truly contribute to thrombus formation despite dual therapy with aspirin and warfarin. Thrombin generation studies could aid in understanding this process in the LVAD population.

Quite clearly sample size and number of comparable adverse clinical events are the limiting factors for this study. Biomarkers have been the topic of clinical discussion for decades, and this article exhibits many of the same criticisms in the past. A movement toward larger trials and interinstitutional collaboration could prove of great benefit. Although we have identified unique peaks, further studies are needed to characterize their structural and functional properties. Relating a change in biomarker expression to a clinical event can be challenging, as patients may experience multiple hemorrhagic and/or thrombotic events during the course of LVAD therapy.

References

1. Maltais S, Kilic A, Nathan S, et al; PREVENT Study Investigators. PREVENtion of HeartMate II pump thrombosis through clinical management: the PREVENT multi-center study. *J Heart Lung Transplant*. 2017;36(1):1-12.

2. Acharya D, Loyaga-Rendon R, Morgan CJ, et al. INTERMACS analysis of stroke during support with continuous-flow left ventricular assist devices: risk factors and outcomes. *JACC Heart Fail*. 2017;5(10):703-711.

3. Krabatsch T, Netuka I, Schmitto JD, et al. Heartmate 3 fully magnetically levitated left ventricular assist device for the treatment of advanced heart failure -1 year results from the Ce mark trial. *J Cardiothorac Surg*. 2017;12(1):23.

4. Nassif ME, LaRue SJ, Raymer DS, et al. Relationship between anticoagulation intensity and thrombotic or bleeding outcomes among outpatients with continuous-flow left ventricular assist devices. *Circ Heart Fail*. 2016;9(5):e002680.

5. Vincentelli A, Susen S, Le Tourneau T, et al. Acquired von Willebrand syndrome in aortic stenosis. *N Engl J Med*. 2003;349(4): 343-349.

6. Jeske WP, Walenga JM, Menapace B, Schwartz J, Bakhos M. Blood cell microparticles as biomarkers of hemostatic abnormalities in patients with implanted cardiac assist devices. *Biomark Med*. 2016;10(10):1095-1104.

7. Mondal NK, Chen Z, Trivedi JR, et al. Oxidative stress induced modulation of platelet integrin α2bβ3 expression and shedding may predict the risk of major bleeding in heart failure patients supported by continuous flow left ventricular assist devices. *Thromb Res*. 2017;158:140-148.

8. Lancé MD. A general review of major global coagulation assays: thrombelastography, thrombin generation test and clot waveform analysis. *Thromb J*. 2015;13:1

9. Jafri SM, Ozawa T, Mammen E, Levine TB, Johnson C, Goldstein S. Platelet function, thrombin and fibrinolytic activity in patients with heart failure. *Eur Heart J*. 1993;14(2):205-212.

10. Yamamoto K, Ikeda U, Furuhashi K, Irokawa M, Nakayama T, Shimada K. The coagulation system is activated in idiopathic cardiomyopathy. *J Am Coll Cardiol*. 1995;25(7):1634-1640.

11. Jafri SM, Mammen EF, Masura J, Goldstein S. Effects of warfarin on markers of hypercoagulability in patients with heart failure. *Am Heart J*. 1997;134(1):27-36.

12. John R, Panch S, Hrabe J, et al. Activation of endothelial and coagulation systems in left ventricular assist device recipients. *Ann Thorac Surg*. 2009;88(4):1171-1179.

13. Berckmans RJ, Nieuwland R, Böing AN, Romijn FP, Hack CE, Sturk A. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thromb Haemost*. 2001;85(4):639-646.

14. Diehl P, Aleker M, Helbing T, et al. Enhanced microparticles in ventricular assist device patients predict platelet, leukocyte and endothelial cell activation. *Interact Cardiovasc Thorac Surg*. 2010;11(2):133-137.

15. Birschmann I, Dittrich M, Eller T, et al. Ambient hemolysis and activation of coagulation is different between HeartMate II and HeartWare left ventricular assist devices. *J Heart Lung Transplant*. 2014;33(1):80-87.

16. Sansone R, Stanske B, Keymel S, et al. Macrovascular and microvascular function after implantation of left ventricular assist devices in end-stage heart failure: role of microparticles. *J Heart Lung Transplant*. 2015;34(7):921-932.

17. Issaq HJ, Veenstra TD, Conrads TP, Felschow D. The SELDI-TOF MS approach to proteomics: protein profiling and biomarker identification. *Biochem Biophys Res Commun*. 2002;292(3):587-592.

18. Paweletz CP, Liotta LA, Petricoin EF. New technologies for biomarker analysis of prostate cancer progression: laser capture microdissection and tissue proteomics. *Urology*. 2001;57(4 suppl 1):160-163.

19. Merchant M, Weinberger SR. Recent advancements in surface enhanced laser desorption/ionization time of flight mass spectrometry. *Electrophoresis*. 2000;21(6):1164-1177.

20. Wulfkuhle JD, McLean KC, Pawaletz CP, et al. New approaches to proteomic analysis of breast cancer. *Proteomics*. 2001;1(10):1205-1215.

21. Paweletz CP, Gillispie JW, Ornstein DK, et al. Rapid protein display profiling of cancer progression directly from human tissue using a protein biochip. *Drug Dev Res*. 2004;49:34-42.

22. Liu S, Xia Y, Liu X, et al. In-depth proteomic profiling of left ventricular tissues in human end-stage dilated cardiomyopathy. *Oncotarget*. 2017;8(29):48321-48332.

23. Su YR, Chiusa M, Brittain E, et al. Right ventricular protein expression profile in end-stage heart failure. *Pulm Circ*. 2015; 5(3):481-497.

24. de Weger RA, Schipper MEI, Siera-de Koning E, et al. Proteomic profiling of the human failing heart after left ventricular assist device support. *J Heart Lung Transplant*. 2011; 30(5):497-506.