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Acquired platelet defects are responsible for non-surgical bleeding in left ventricular assist device recipients

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Abstract

Background: Left ventricular assist devices (LVADs) have been used as a standard treatment option for patients with advanced heart failure. However, these devices are prone to adverse events. Non-surgical bleeding (NSB) is the most common complication in patients with continuous flow (CF) LVADs. The development of acquired von Willebrand syndrome (AVWS) in CF-LVAD recipients is thought to be a key factor. However, AVWS is seen across a majority of LVAD patients, not just those with NSB. The purpose of this study was to examine the link between acquired platelet defects and NSB in CF-LVAD patients.

Methods: Blood samples were collected from 62 CF-LVAD patients at pre- and post-implantation timepoints. Reduced adhesion receptor expression (GPIbα and GPVI) and activation of platelets (GPIIb/IIIa activation) were used as markers for acquired platelet defects.

Results: Twenty-three patients experienced at least one NSB episode. Significantly higher levels of platelet activation and receptor reduction were seen in the post-implantation blood samples from bleeders compared with non-bleeders. All patients experienced the loss of high molecular

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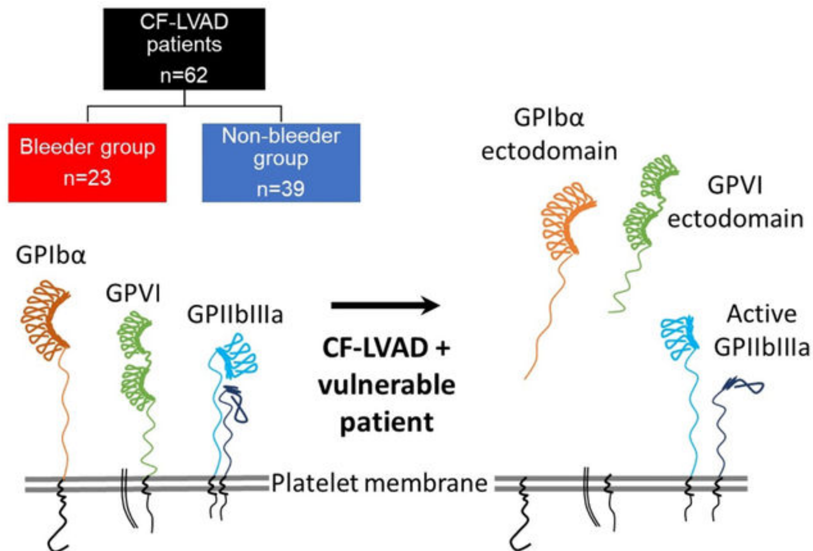
Conflict of Interest
ENS, EF, and DK were paid HeartWare/Medtronic consultants. All other authors declare that they have no conflict of interest in the subject matter or materials discussed in this study.

weight monomers (HMWM) of von Willebrand Factor (vWF), but no difference was seen between the two groups. Multivariable logistic regression showed that biomarkers for reduced platelet receptor expression (GPIbα and GPVI) and activation (GPIIb/IIIa) have more predictive power for NSB, with the area under curve (AUC) values of 0.72, 0.68, and 0.62, respectively, than the loss of HMWM of vWF (AUC: 0.57).

Conclusion: The data from this study indicated that the severity of acquired platelet defects has a direct link to NSB in CF-LVAD recipients.

Graphical Abstract



This study demonstrated that some CF-LVAD patients are more vulnerable to device-induced platelet defects, in terms of GPIbα and GPVI shedding and GPIIbIIIa activation.

Keywords

Heart failure; mechanical circulatory support; left ventricular assist devices; non-surgical bleeding; acquired platelet defects

Introduction

Heart failure (HF) is expected to affect over 8 million Americans by 2030 and cost over $69.8 Billion, due to the increased incidence of HF and the improved survival of those with HF. Currently, 21 out of 1000 people over the age of 65 are diagnosed with HF, with a lifetime risk of 20–45% over the age of 45.[1] Throughout the past two decades, LVADs have been used to provide mechanical circulatory support to patients with advanced HF. As of October 2021, there are 35,316 patients enrolled in the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) database.[2] These patients have been implanted with devices either as a bridge to native heart recovery and heart transplantation or as destination therapy. With recent advances in the continuous flow (CF) LVAD technology, one and two-year survival rates for patients who are supported

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with contemporary CF-LVADs are approaching those who receive heart transplantation.[3] However, these devices are accompanied by a myriad of complications such as bleeding, infection, thrombosis, and stroke. Postoperative NSB has become the most common complication in CF-LVAD patients, with incidences anywhere from 20–40%.[4,5] NSB is defined as any bleeding event that is not surgically related.

Clinical observations have shown that CF-LVAD patients develop acquired von Willebrand syndrome (AVWS) which is manifested as the loss of HMWM of vWF.[6] Thus, AVWS has been suggested as the underlying cause of NSB in CF-LVAD patients.[7,8] Interestingly, this loss is universally seen across CF-LVAD patients, however, only 20–40% of patients experience NSB.[4,5,9] This suggests that there are other factors at play that may contribute more crucially to NSB. Hemostasis is based on a delicate balance of pro- and anticoagulant forces in which complex interactions occur between coagulation proteins, cellular elements, and the vessel wall. It encompasses platelet aggregation and plug formation, coagulation, and fibrinolysis. Platelets play a crucial role in initiating the primary hemostasis via adhesion, activation, and aggregation at vascular injury sites.[10] Thus, it is reasonable to assume that NSB in CF-LVAD patients is inherently related to acquired platelet defects that lead to abnormalities in hemostasis.

Contemporary CF-LVADs are based on rotary pump technology. The high-speed rotation of the impeller inside a CF-LVAD inevitably introduces non-physiological shear stress into the blood circulation in HF patients. It has been shown that non-physiological shear stress causes platelet activation and reduced platelet receptor expression.[11–14] The activation of platelets causes hypercoagulability that can lead to thrombosis and platelet consumption/clearance. The reduced expression of adhesive platelet receptors may impair hemostasis which can increase the propensity for bleeding. As platelets play one of the most important roles in coagulation, we believe that the acquired platelet defects induced by CF-LVADs lead to an impairment in hemostasis. GPIbα and GPVI are two key platelet receptors that bind vWF and collagen, respectively, to initiate hemostasis at sites of vascular injury. Reduced expression levels of GPIbα and GPVI receptors have been shown to be induced by high shear stress.[13,14] Similar platelet defects have been reported in LVAD patients and more pronounced in patients who experienced NSB.[15,16] GPIIb/IIIa activation induces its ligand-binding function that mediates stable platelet adhesion, aggregation, and thrombus formation. GPIIb/IIIa activation is also seen in LVAD patients and may be indicative of potential thrombus formation.[17,18] In this study, the platelet integrity (GPIIb/IIIa activation, GPIbα, and GPVI expressions) and vWF multimeric profile in blood from sixty-two patients on CF-LVAD support were characterized and the link between device-induced platelet defects and postoperative NSB was examined.

Materials and Methods

Patient Enrollment

Sixty-two HF patients implanted with a CF-LVAD at the University of Maryland Medical Center (UMMC) (Baltimore, MD) were recruited from June 2013 to February 2020. All patients were informed of the purpose of the study and gave their informed consent. Blood samples were collected from the participants before LVAD implantation (baseline) and at

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1-, 2-, 3-, and 4-weeks post-implantation. Baseline blood was collected one day prior to LVAD implantation surgery. All blood was collected in vacuette sodium citrate (3.2%) tubes from venipuncture or intravenous lines. Demographic data, clinical complications, hemodynamics, and LVAD operating conditions were gathered as well as relevant medical information. Laboratory hematology and blood chemistry data was measured with standard clinical instruments were also collected from the patients' treatment records. Four types of LVADs were implanted, Jarvik 2000 (Jarvik Heart, New York, NY), HVAD (Medtronic, Framingham, MA), HeartMate II, and HeartMate III (Abbott, Abbott Park, Illinois). Patients were placed on an anticoagulation/antiplatelet regimen. Patients were followed up for two years or until heart transplant, explant, or death. During LVAD support, each patient was placed on an anticoagulation/antiplatelet regimen. Patients were divided into two groups: (a) Bleeder group (n=23) and (b) Non-bleeder (n=39) group. The bleeder group were patients that experienced at least one postoperative NSB event within their two-year follow-up period. The NSB events for these CF-LVAD patients were determined according to the INTERMACS MCS-ARC Bleeding adverse event definition in Appendix A.[2] This study was approved by the University of Maryland's Institutional Review Board and in compliance with the ethical standards of the Declaration of Helsinki.

Platelet Monitoring

Platelet function was measured using platelet function analyzer PFA-100 (Dade Behring, Inc., Deerfield, IL) and a TEG® Hemostasis Analyzer System (Haemonetics Corp., Braintree, MA). The PFA-100 measures the time it takes for an aperture in a membrane coated by collagen and epinephrine or adenosine diphosphate (ADP) to be occluded by a platelet plug. The TEG® system monitors the phases of hemostasis in whole blood and measures the time and viscoelasticity of a clot formation under low shear stress.

Patient Management

The anticoagulation and antiplatelet therapy guidelines for LVAD patients at our center have been previously reported.[19] Briefly, TEG and PFA-100 were used to guide antiplatelet titration with goals of a normal TEG maximum amplitude (MA) of 50–70mm and a prolonged PFA-100 (160–250s for PFA-ADP and >200s for PFA-EPI). Dipyridamole (50mg 3x daily) is started post-implantation. Aspirin, Omega-3, and P2Y12 inhibitors are added as needed. Anticoagulation titration was guided with partial thromboplastin time (PTT) and INR; TEG R-time was occasionally used for further guidance. The goals for these were PTT range of 45–55s, INR range of 2.5–3.0, and TEG R-time range of 5–10min. Heparin is started once drainage is low and continued until the INR goal is reached for two days. Tests were performed until patients were on a stable regimen. Outpatients with INR < 2.0 are given enoxaparin.

Detection of Acquired Platelet Defects with Flow Cytometric Assays

Whole blood flow cytometry was utilized to characterize the activation level of GPIIb/IIIa and the expressions of GPIbα and GPVI on the platelet surface. Platelets were identified using anti-human CD41a antibody conjugated with allophycocyanin (CD41a-APC, IgG1κ, clone HIP8) (BD Biosciences, San Jose, CA, USA). APC-labeled immunoglobulin IgG1κ antibody served as the negative control for CD41a (IgG1κ-APC clone MOPC-21) (BD

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Biosciences). Activated GPIIb/IIIa was detected by measuring PAC-1 levels using anti-human PAC-1 antibody conjugated with fluorescein isothiocyanate (FITC) (PAC-1 FITC, IgMκ, clone PAC-1) (BD Biosciences). FITCl-abeled immunoglobulin IgMκ antibody served as the negative control for PAC-1 (IgMκ-FITC clone G155–228) (BD Biosciences). The expression level of the GPIbα receptor on the platelet surface was detected using anti-human CD42b antibody conjugated with FITC (CD42b-FITC, IgG1κ, clone HIP1) (BD Biosciences). FITC-labeled immunoglobulin IgG1κ antibody served as the negative control for CD42b (IgG1κ-FITC clone MOPC-21). The expression of GPVI receptor on the platelet surface were detected using anti-human GPVI antibody conjugated with Phycoerythrin (GPVI-PE, IgG1κ, clone HY101) (BD Biosciences). PE-labeled IgG1κ (IgG1κ-PE clone MOPC-21) (BD Biosciences) was used as the negative control for the expression level GPVI receptor.

To prepare the platelet samples for flow cytometry, 50μL of Tyrode's containing 10mM HEPES and 0.35% BSA was added to each 5mL tube followed by 5μL of blood sample. Samples were run in 2-color panels, CD41a-APC with CD42b-FITC, GPVI-PE, or PAC-1-FITC. Antibodies were then added to each specific tube: 1μL of anti-CD41, 10μL of anti-CD42b, 2.5μL of anti-GPVI, and 20μL of anti-PAC-1. For positive controls, blood was activated for 2 minutes by adding 2μL of adenosine diphosphate (1nM) to 100μL of blood. Negative isotype controls included 1μL of anti-CD41 and 0.5μL of each isotype. Samples were then incubated in the dark at room temperature for 20 mins. The cells were then fixed using 500μL of 1% PFA in 0.9% NaCl. Compensations were performed using Anti-Mouse IgG1κ/Negative Control Compensation Particles Set (No. 552843, BD Biosciences). The data was collected by either FACSVerse or FACSCalibur (BD Biosciences) and was analyzed with FCS Express software (De Novo Software, Glendale, CA, USA). The platelet population was identified using CD41a gating (gating on CD41a-APC and side scatter [SSC]). Negative isotype controls were used for quadrant gating for PAC-1-FITC, GPVI-PE, and CD42b-FITC. The gating strategy is shown in supplemental figure 1.

Analysis of Multimeric Profile of vWF with Western blotting

Western blotting was performed to examine the loss of HMWM of vWF in the samples collected at baseline, 2 weeks and 4 weeks post-implantation. This analysis was conducted on a subgroup of 46 patients (31 non-bleeders and 15 bleeders). The details of the procedure for the vWF analysis can be found in the previously published article that derived the methods from Krizek and Rick.[11,20] Briefly, 2 mL of blood samples were centrifuged at 160xg for 15 minutes at 20°C, without brake, followed by a second centrifugation on the supernatant at 14000 rpm for 15 minutes at 4°C, without brake, to obtain plasma samples for western blotting. Plasma samples were mixed with laemmli samples buffer (1:20) (Bio-Rad, Hercules, CA, USA). vWF multimers were separated using electrophoresis with SDS-agarose gel (0.6%) at 30 mA for 30 minutes and then 50 mA until tracking dye reached bottom of gel (~3.5 hours). Transfer to a 0.45 μm nitrocellulose membrane was performed overnight at 70 mA in 4°C. Polyclonal rabbit anti-human vWF-horseradish peroxidase antibody (Dako, Glostrup, Denmark) was used to detect the multimers of vWF. Blots were developed using Clarity Western ECL Blotting Substrates (Bio-Rad, Hercules, CA, USA) and scanned with a ChemiDoc Imaging System (Bio-Rad). The optical density

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of each band was quantitated using UN-SCAN-IT Gel 6.1 analysis software (Silk Scientific, Orem, UT, USA). Western blot bands above 10 were classified as HMWM. The HMWM portion of vWF was calculated as the densitometric area of those HMWM bands. The HMWM vWF index was defined as the ratio of the HMWM portion to the densitometric area of all vWF bands.

Statistical Analysis

The data is presented as mean ± SE for each group at the respective blood collection timepoint unless otherwise noted. The Shapiro-Wilk test was used to test for the normality of the data. Student's t-test was used to determine the significance between the bleeder and non-bleeder groups for those with normal distribution. Mann-Whitney test was used as the nonparametric alternative. Chi-square tests were used for categorical data. Statistical significance was set at $P<0.05$. Logistic regression was performed to examine the significance of the measured parameters for platelet defects and HMWM of vWF on the probability of bleeding. NSB events were considered a binary variable. Receiver operating characteristic (ROC) curves were created and the area under curve (AUC) was calculated. The analyses were done using Minitab (Minitab, LLC, State College, PA).

Results

NSB in LVAD Patients

Twenty-three patients experienced at least one postoperative NSB incident within the two-year follow-up period. Over half of these patients experienced more than one NSB event. The majority of NSB events were GI bleeds (56.5%), followed by mediastinal bleeds (30.4%) and mucosal bleeds (26.1%). In some patients, NSB events occurred at more than one site. Cerebral (13%), intra-abdominal (13%), and respiratory (8.7%) bleeds were also observed. Over 90% of bleeder patients experienced their first NSB event within 6-months of implantation, all events occurred within the two years. Each patient's anticoagulation regimen was clinically optimized at the time of bleeding. Comparative examination of patient demographics and clinical characteristics of the groups are summarized in Table 1. No statistical significance in the patient demographics and clinical characteristics was found between the groups, except for age where the bleeder patients were older. Patients were implanted with four different LVADs. The majority (77%) of the patients received the HVAD pump. Additionally, no differences were seen in hemodynamics among the patients, such as flow rate as well as blood pressure before and after implantation. Left ventricular external end-diastolic diameter and left ventricular ejection fraction showed no difference between the groups before or after LVAD implantation. The outcomes of the 39 Non-bleeder patients were as follows: 8 died while on LVAD support, 17 were still on LVAD support at the two-year mark, and 14 had heart transplants. Out of the 23 bleeder patients, 12 died while on LVAD support, 6 were still on LVAD support at the two-year mark, 5 received a heart transplant.

Laboratory Hematology and Blood Chemistry

Routine laboratory and blood chemistry tests were analyzed between the groups at baseline and the 4-post implantation timepoints (Fig. 1). White blood cells (WBC) at 3- and

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4-weeks post-implantation were significantly higher for the bleeder group. WBC counts for bleeders were significantly elevated at all post implantation timepoints compared to their baseline measurements. Non-bleeders had significantly higher WBC counts at 1- and 2-weeks compared to their baseline. Platelets (2, 3, and 4 weeks) and RBC, hemoglobin, and hematocrit (3 and 4 weeks) levels significantly decreased for the bleeder group compared to the non-bleeders. Non-bleeders had significantly higher platelet levels at all post implantation points compared to baseline, while bleeders showed no significant difference. Compared to their baseline bleeders had significantly lower RBC (1-, 3-, and 4-weeks), hemoglobin (1–4 weeks), and hematocrit (1–4 weeks). Non-bleeders had lower RBC (1- and 2-weeks), hemoglobin (1–3 weeks), and hematocrit (1–2 weeks) compared to baseline. Blood urea nitrogen (BUN) levels at all post-implantation timepoints were significantly higher for the bleeder group. No significant differences in all other tests including creatine, AST, ALT, LDH, blood chemistry (end-organ function and metabolic panels), and coagulation function were found. LDH results are shown in supplemental figure 2. The levels of INR and PTT for the two groups were within the therapeutic range for CF-LVADs and similar.

Platelet Monitoring

PFA 100 and TEG were performed for all samples. Out of the various parameters rendered by these tests, the ones that showed significance between the groups were PFA-100 Epinephrine clot time, TEG K time, and TEG fibrinogen activity α-angle degree (Fig. 2). The bleeder group had significantly lower PFA-100 EPI-Ct at 4-weeks post-implantation. Non-bleeders have significantly higher PFA-100 EPI-Ct values at 2–4 weeks compared to baseline. For the TEG test, the bleeder group had significantly higher TEG K-time values at 3- and 4-weeks. Regarding the fibrinogen activity, the α degree was significantly lower for the bleeder group at 2, 3, and 4-weeks. Non-bleeders have significantly higher PFA-100 EPI-Ct and α degree values, and lower TEG K-time values at 2–4 weeks compared to baseline. There was no difference in all other PFA-100 and TEG measurements between the two groups.

Reduced Expression Levels of Platelet GPIbα and GPVI Receptors and Activation of Platelet GPIIb/IIIa

The percentages of the platelets with positive GPIbα or GPVI expression in the total platelet population in the blood samples from the bleeder and non-bleeder groups are shown in Fig. 3. The levels of GPIbα or GPVI expression on the platelet surface in the flow cytometry data are directly related to the numbers of GPIbα or GPVI receptors available for respective antibody binding. The positive GPIbα or GPVI expression on the platelet surface indicates the platelets that had sufficient GPIbα or GPVI receptors above the designated thresholds determined from the negative control samples. The positive GPIbα or GPVI expression separates the platelets with functioning GPIbα or GPVI binding from those with dysfunctional GPIbα or GPVI binding due to the loss of those receptors. Representative flow cytometry histograms are shown in Fig. 3a and 3d. The percentages of the platelets with positive GPIbα or GPVI expression at the baseline time point for both groups were similar. Over the 4 weeks, the percentage of platelets with positive GPIbα or GPVI expression in the non-bleeder group remained stable after LVAD implantation. In contrast,

the percentage of platelets with positive GPIbα or GPVI expression decreased after LVAD implantation in the bleeder group. At all the postoperative time points the bleeder group had a significantly lower percentage of platelets with positive GPIbα expression compared with the non-bleeder group (Fig. 3b). Bleeders also had significantly lower GPIbα expression at 2, 3, and 4-weeks compared to their baseline. Although a similar trend was observed for the percentages of platelets with positive GPVI expression, the difference in the percentage of the platelets with positive GPVI expression became significant between the bleeder and non-bleeder groups at 4-weeks (Fig. 3e). At 4-weeks the bleeder group GPVI expression was significantly less than its baseline.

The data at all post-implantation timepoints were averaged and compared to the baseline measurements to have a summarized view of the data (Fig. 3c and 3f). Overall, the averaged percentage of platelets with positive GPIbα expression in the bleeder group at the post LVAD implantation was significantly lower compared with that at the baseline. The percentage of platelets with positive GPVI expression in the bleeder patients was also lower at post-implantation compared with that at the baseline ($p=0.06$). Between the groups, the bleeder group had a significantly lower percentage of platelets with positive GPIbα expression post LVAD implantation than the non-bleeder group. The lower percentage of platelets with positive GPVI expression was also observed in the bleeder group post-LVAD implantation, but the significance level was lower ($p=0.09$).

The levels of platelet activation in the samples from the two groups were compared (Fig. 4a). The samples from the bleeder group had a higher number of activated platelets as indicated by PAC-1 expression. In particular, the increased platelet activation was significantly evident at 1-week time point. Collectively, the averaged level of platelet activation over the four post-operative weeks was also significantly higher in the bleeder group compared to that in the non-bleeder group (Fig 4b). The averaged levels of platelet activation post LVAD implantation were significantly higher in the bleeder and non-bleeder groups than those at the baseline.

Loss of HMWM of vWF in LVAD Patients

Representative western blots of vWF multimeric profiles from a non-bleeder patient and a bleeder patient are shown in Figure 5a. The blots clearly show the loss of HMWM of vWF in both the bleeder and non-bleeder patients at two and four weeks after LVAD implantation compared with those at the baseline. The calculated HMWM vWF indices of the blood samples collected at the baseline, two, and four weeks from the groups were shown in Fig. 5b. Both the bleeder and non-bleeder groups had significantly lower vWF HMWM indices at the two postoperative time points compared to the baseline indices. No significant difference was found between the groups at any timepoints. The averaged HMWM vWF indices of the two groups post-LVAD implantation were very close (Fig. 5c). They both were significantly lower post LVAD implantation compared with their baseline level. No differences were seen between the two groups.

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Logistic Regression and ROC Analysis

The multivariable logistic regression models were created to establish predictive relationships between the bleeding occurrence (dependent variable) and acquired platelet defects or the loss of HMWM of vWF (independent variables). Four models were generated based on the measured parameters for PAC-1, GPIbα, GPVI, and vWF. Wald chi-square tests were performed to determine each model's significance when compared to the random model. The models were created using two indicators that showed predictive power for NSB. One indicator was the averaged post-implantation measurements for platelet activation (PAC-1), GPIbα expression, GPVI expression, and HMWM vWF index. The other was the difference between the post-implantation indicator and its respective baseline. The Wald chi-square statistics for PAC-1, GPIbα, GPVI were 3.59, 9.09, 4.15 with P values of 0.06, 0.01, and 0.13, respectively. In contrast, the Wald chi-square statistic for the HMWM vWF index was 0.45 with a P-value of 0.78. The AUC values of the constructed ROC curves (Fig. 6) for PAC-1, GPIbα, GPVI, and vWF were 0.62, 0.72, 0.68, and 0.57, respectively.

Discussion

This study examined platelet integrity in 62 HF patients who underwent LVAD implantation in order to see if acquired platelet defects induced by an implanted CF-LVAD were linked to postoperative NSB. Over 35% of patients experienced at least one NSB event. This overall bleeding rate is on par with other studies which have reported bleeding rates in LVAD patients between 20–40%.[4,21] In the present study, the bleeder group had significantly severe platelet defects after LVAD implantation when compared to the non-bleeder group. This was seen through reduced expression levels of key platelet adhesion receptors (GPIbα and GPVI) and increased platelet activation (PAC-1 binding). Logistic regression analysis suggested that these three biomarkers had significant predictive power for NSB, with AUCs of 0.72, 0.68, and 0.62 for GPIbα, GPVI, and PAC-1, respectively. These acquired platelet defects appeared to be associated with platelet dysfunction that is manifested through compromised hemostatic capacity that leads to NSB. This suggests that the severity of platelet defects induced by LVADs has a direct link to NSB risk.

In our study, the bleeder group seemed to be more susceptible to the adverse consequences associated with LVAD implantation. They had significantly more WBCs than the non-bleeder group. During the four-week post-operative period, WBC count was over 12 K/mcL, out of the healthy range of 5–10 K/mcL. This elevation cannot be entirely attributed to implant surgery because the non-bleeder group's WBC count returned to the healthy range, suggesting that the bleeders might be in a continued inflammatory state after implantation. RBC and platelet counts for the bleeders were lower than those for the non-bleeders at various timepoints, possibly due to LVADs' destruction of blood cells and bleeding.[22] Non-physiological shear stress generated by CF-LVADs can induce partial or complete cell membrane destruction as well as microparticle (MP) generation. MP generation had been previously confirmed as an LVAD induced platelet defect that can be indicative of cell activation and is associated with adverse complications.[23–25] Abnormal hematological and laboratory blood test results for LVAD bleeding patients, including increased WBC count, decreased RBC, lower platelet counts, and lower hemoglobin level, have been reported.[15,26]

Platelet function in 62 LVAD patients was evaluated using PFA-100 and TEG. The normal ranges of PFA-100 EPI-ct are 98–185 seconds for healthy individuals. All the CF-LVAD patients (bleeder and non-bleeder) in our study had higher closure times at all postoperative time points. The bleeder group had lower closure times at 4 weeks. These closure times were in line with the observed value of around 250 sec that has been previously reported for LVAD patients.[25,27] Prolonged closure times might be caused by anticoagulation/antiplatelet treatments. However, some studies suggested that they were attributed to AVWS.[28,29] Our study data showed that AVWS developed in all the LVAD patients, not just bleeders. They both had similar closure times. The TEG K time and α degree are associated with fibrinogen activity and platelet count. The TEG K time trends for the two groups were similar, except that the bleeder group had a higher TEG K time at 3 and 4 weeks. Despite the difference, all TEG K times appeared to be in the normal range of 1–3 minutes. Most of the α degree measurements were within the normal range of 53–72 degrees. Nevertheless, the bleeder group had a significantly lower α angle at 2 to 4 weeks post-LVAD implantation. The combination of the prolonged K time and the reduced α angle in the bleeder group at 3- and 4-weeks might signify a decreased overall fibrinogen activity. Nonetheless, all the bleeder patient measurements were within the normal range.

Consistent with the lower RBC and platelet counts, more pronounced platelet defects were confirmed in the bleeder group in terms of surface expression of adhesion receptors and platelet activation. The percentage of the platelets with positive GPVI and GPIbα receptors in the bleeder patients declined over the four weeks, indicating that the platelets became defective after LVAD implantation. The reduced expression of platelet receptors due to high mechanical shear stress had been reported.[11,30] The mechanism behind the reduced expression of these receptors is not fully understood. ADAM proteolysis, direct shear stress damage, and microvesiculation may be possible mechanisms. Shear-induced ADAM (metalloproteinase)-dependent ectodomain shedding of GPIbα and GPVI has been reported.[31] However, others reported that GPVI shedding is independent of the ADAM10 pathway.[32] In another study our group looked at ADAM proteolysis and direct shear stress damage as pathways for shear-induced-receptor shedding.[33] This study found that ADAM inhibition could lessen the shear stress-induced reduced expression of platelet adhesion receptors. However, the reduced expression could not be fully prevented, indicating that direct shear stress damage contributes to platelet receptor shedding. Microvesiculation has recently been suggested as an alternative mechanism of platelet receptor loss. Downregulation of platelet surface adhesion receptors was shown to be attributed to the generation of receptor-enriched platelet microparticles.[30] ADAM proteolysis, direct shear stress damage, and microvesiculation are considered viable mechanisms for receptor reduction in LVAD patients. Further research may be needed to confirm if any of these mechanisms is dominant in LVAD patients. The loss of these receptors the platelet surface inhibits the platelet's ability to effectively adhere to areas of exposed subendothelium during vascular injury. Our early in-vitro studies have shown reduced adhesion capacities of platelets on vWF and collagen as well as reduced aggregation induced by collagen and ristocetin after exposed to shear stress as the level of shear stress increased.[11,34] In the present study, the platelets in the bleeder patients also had a higher level of PAC-1 binding, indicating that GPIIb/IIIa became activated[35] and available for fibrinogen and vWF binding.

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Activated platelets are more likely to form aggregates, possibly thrombi, leading to platelet consumption. Previous studies have shown that shear stress can cause both activation of GPIIb/IIIa and loss of GPIIb/IIIa surface receptors simultaneously.[36]

Decreased expression levels of platelet adhesion receptors may reduce the platelets' ability to maintain hemostasis while the platelet activation could simultaneously increase thrombotic risk by amplifying platelet adhesion and aggregation.[37] This paradoxical phenomenon has been previously seen in in-vitro studies.[11] GPIb receptor reduction has been linked to an increased risk in NSB and re-occurrent bleeds.[15,16] GPVI receptor reduction has also been associated with an increased risk of NSB.[16,38] Both platelet activation and receptor reduction can increase platelet consumption and clearance in the circulation. Our data show that platelets in the bleeder patients on LVAD support had the reduced expression levels of GPIbα and GPVI receptors and an increased activated GPIIbIIIa, suggesting a direct link between NSB and reduced expression of platelet adhesion receptors and platelet activation.

In the last decade, the loss of HMWM of vWF after CF-LVAD implantation has gained increased attention as a presumable cause for NSB in patients and is considered as the most significant parameter in identifying patients at higher risks of bleeding.[5,39] Our data are consistent with the reported observation of the loss of HMWM of vWF in various articles. However, there was no difference between the bleeder and non-bleeder groups. The loss of HMWM of vWF occurred in all our LVAD patients, agreeing with previously published clinical reports. Though, the majority of those reports couldn't link the loss of HMWM of vWF to patients who experienced bleeding.[5,39] One study showed a difference between bleeders and non-bleeders in terms of vWF fragmentation but only for those with angiodysplasia. Non-bleeders and bleeders without angiodysplasia showed no difference.[40] Another study of 41 patients stated that bleeders have a more severe loss of HMWM of vWF compared to non-bleeders. However, the samples were collected and evaluated for vWF multimers at very different postoperative time points, ranging from day 3 to 1254.[41] In our study we compared the levels at the baseline and two postoperative time points (2 and 4-weeks); no difference was seen in the loss of HMWM of vWF between the groups at any timepoint. The logistic regression analysis indicated that the predictive powers of the biomarkers for GPIIb/IIIa activation (PAC-1) and expressions of GPIbα and GPVI were stronger than the loss of HMWM of vWF according to the AUC values for the ROC curves. GPIbα shows the most predictability, while GPVI and PAC-1 show modest predictability. The Wald chi-square tests indicated that the PAC-1, GPIbα, and GPVI models were better at estimating bleeders than the loss of HMWM of vWF. Therefore, the biomarkers for GPIIb/IIIa, GPIbα, and GPVI may predict the probability of postoperative NSB more accurately than vWF. Our data suggest that the acquired platelet defects seem to have a direct link to NSB and can potentially be used to identify CF-LVAD patients who are at greater risk.

Study Limitations

We acknowledge that there are limitations in this prospective observational study. The sample size was relatively small, not all CF-LVAD patients in our center were enrolled in this study. Most logistic regression models use larger sample sizes therefore these findings

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are exploratory. A larger cohort of CF-LVAD patients with bleeding complications is needed to further confirm our findings by enhancing the predictability of our models to make them more clinically relevant. Various methods are used throughout literature to examine the loss of platelet receptors, however, this study exclusively used flow cytometry for platelet evaluation. Longer post-operative timepoints might offer additional insights on acquired platelet defects in CF-LVAD patients. This study began in 2013, therefore various LVADs were used throughout the study including Jarvik200, HVAD, HMII, and HMIII. Other studies that showed similar results also reported their patients being implanted with these LVADs.[15,16] More importantly, contemporary devices like the HeartMate 3 still show a 43.7% in overall bleeding and specifically in 24.5% GI bleeds.[42] Acquired platelet defects cannot be linked to specific devices until further research is conducted.

Conclusion

The study demonstrated that a subclass of CF-LVAD patients are more vulnerable to device-induced platelet defects. These patients' platelets may be more prone to dysfunction resulting in loss of normal hemostatic ability, which is shown through their propensity for NSB. This vulnerability increases their risk of developing an adverse complication such as bleeding due to the impairment of hemostasis. The loss of HMWM of vWF may not be the dominating factor in predicting NSB but it may further exacerbate the risk of bleeding. Bleeding events can lead to increased morbidity and mortality, longer hospital stays, loss of quality of life, and increased cost. The study shows that acquired platelet defects may be the underlying mechanism of NSB in CF-LVAD patients. This knowledge can be leveraged to manage the risk of bleeding and therefore reduce its reoccurrence and devastating effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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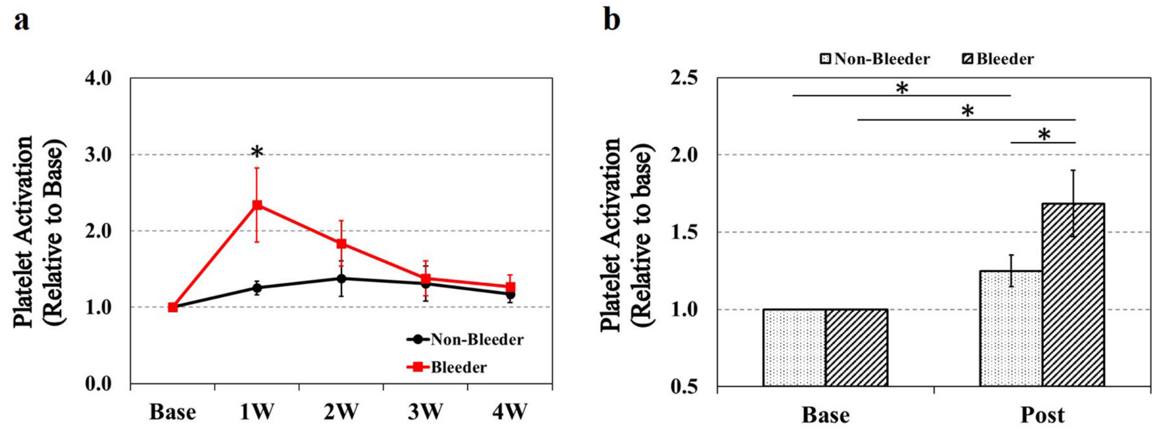

Figure 1:

Laboratory hematology and blood chemistry test results over the first four postoperative weeks between the bleeder and non-bleeder groups.

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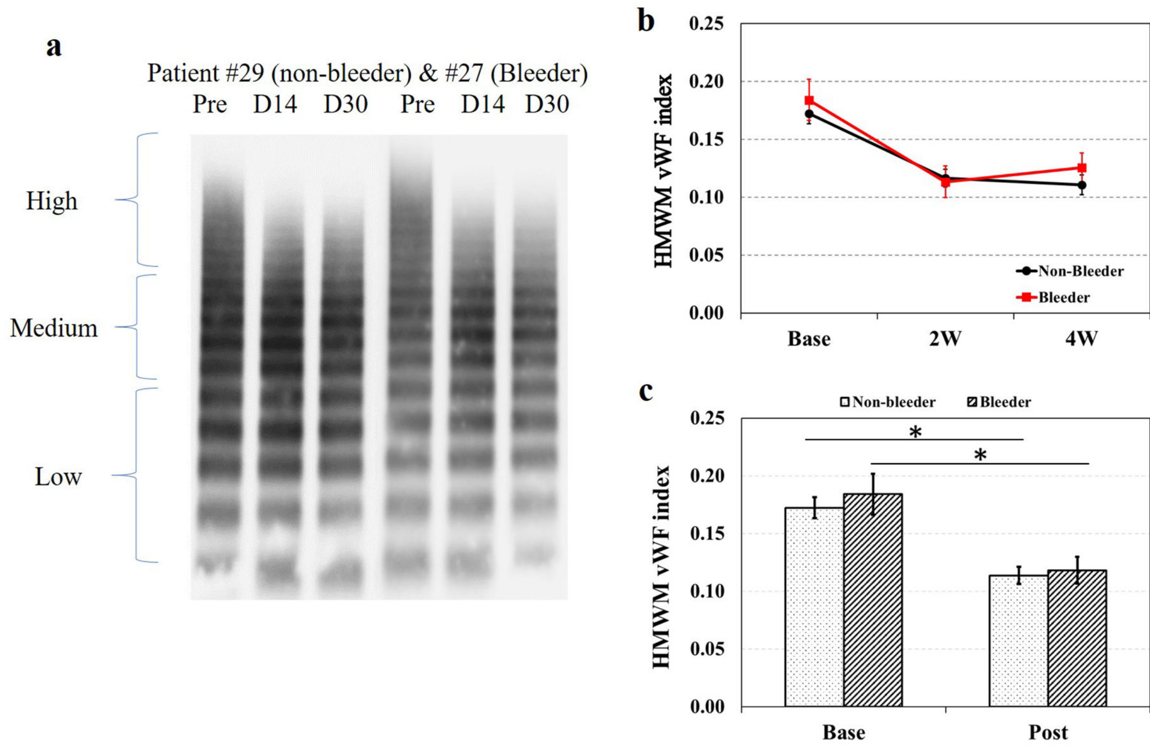

Figure 2:
Coagulation monitoring tests over the first four postoperative weeks between the bleeder and non-bleeder groups.

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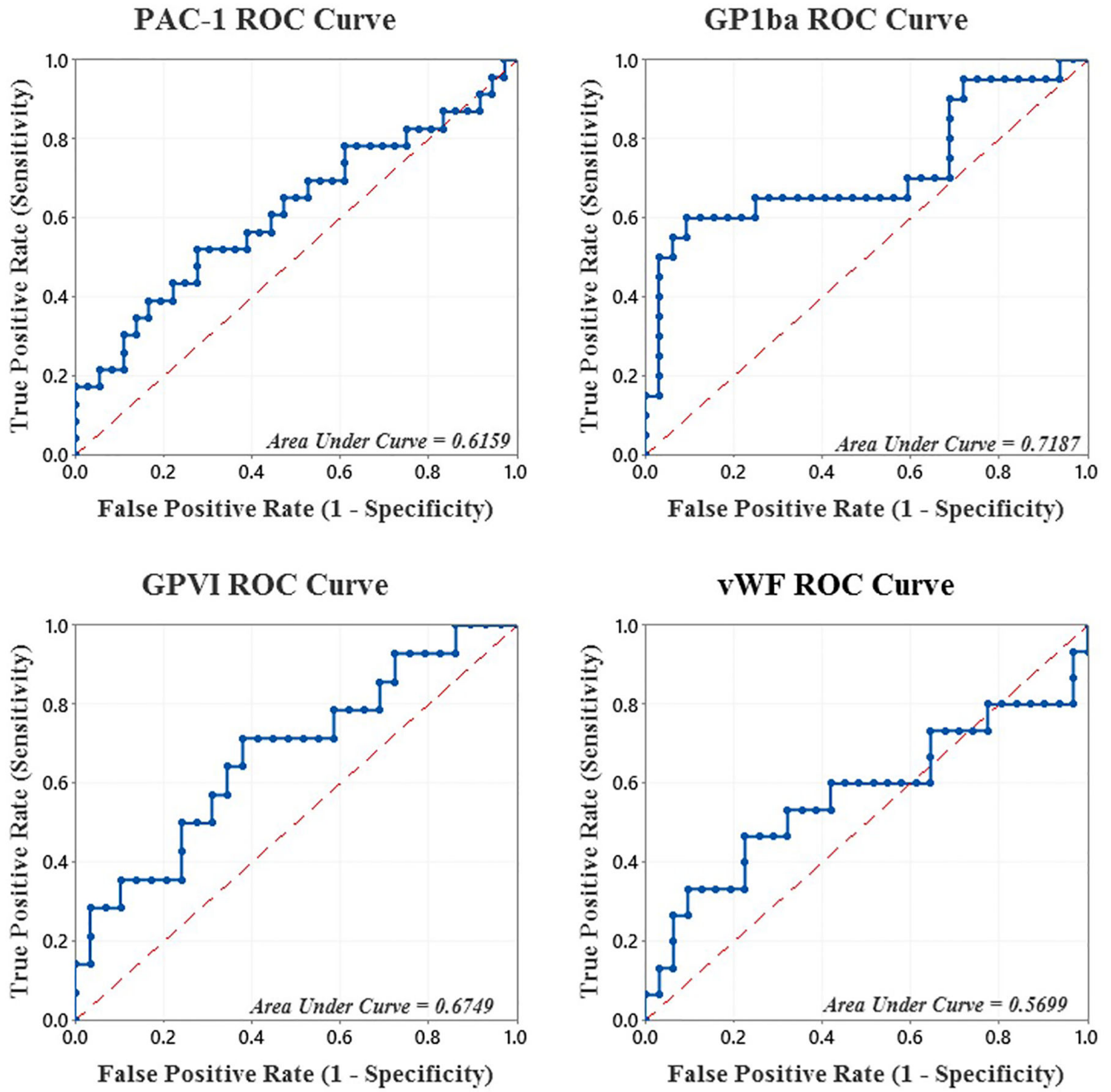

Figure 3:

Percentages of platelets with positive surface GPIbα (3a) or GPVI (3c) over the first four postoperative weeks between the bleeder and non-bleeder groups. The averaged values of the percentage of platelets with positive GPIbα (3b) or GPVI (3d) over the four postoperative weeks between the two groups. *P<0.05 compared between groups and #P<0.05 compared to baseline.

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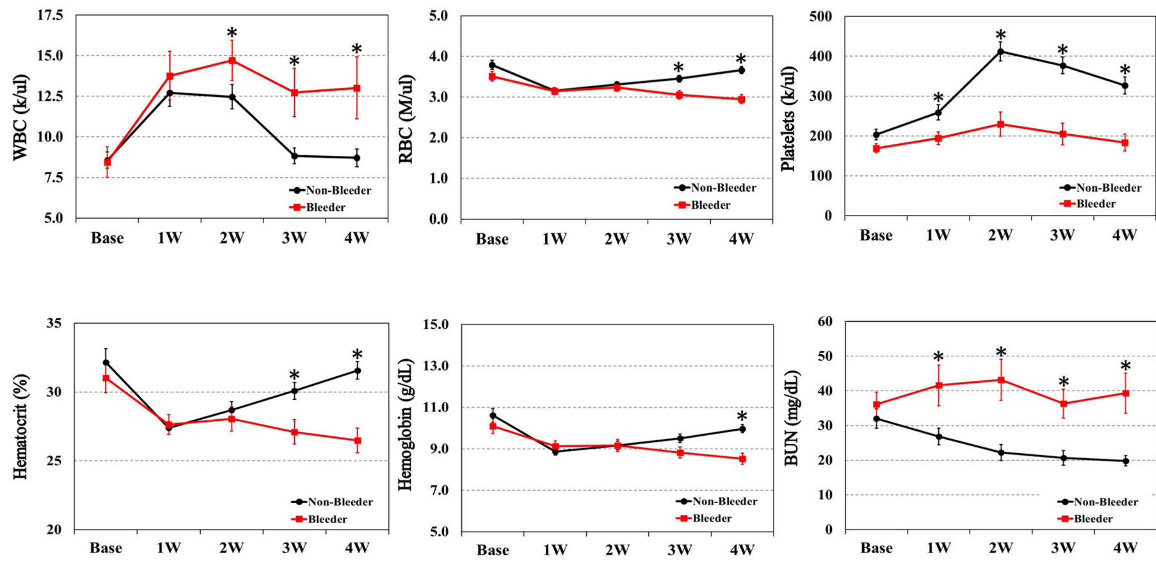

Figure 4:

Fold increase of platelet activation (PAC-1 positive) over the first four postoperative weeks between the bleeder and non-bleeder groups (4a). The averaged values of the fold increase of platelet activation over the four postoperative weeks between the two groups (4b). *P<0.05 compared between groups and #P<0.05 compared to baseline.

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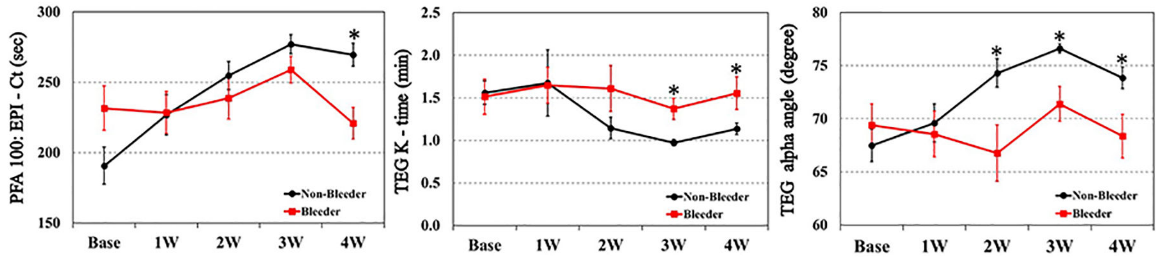

Figure 5:

Representative western blots of multimeric profiles of vWF of one non-bleed patient (#29) and one bleeder patient (#27) (5a). HMWM vWF indices over the first postoperative month between bleeder and non-bleeder (5b) and comparison of calculated HMWM vWF indices between groups at baseline (pre-LVAD) and post-LVAD implantation (5c). #P<0.05 compared to baseline.

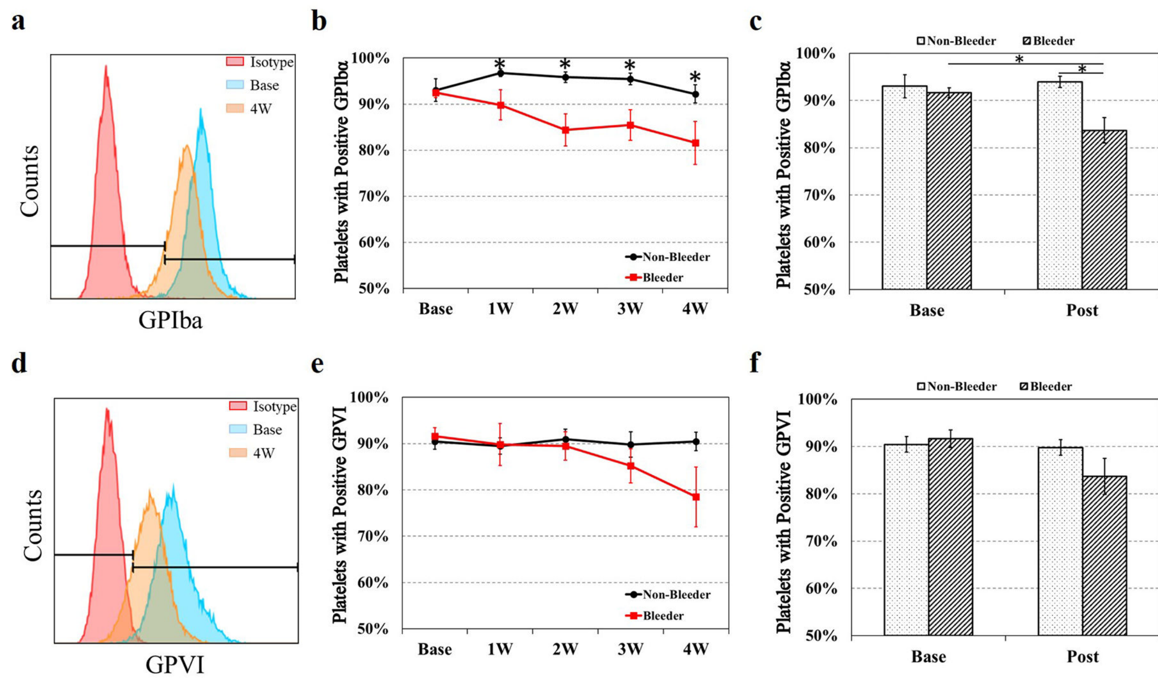

Figure 6:

Receiver operating characteristic (ROC) curves for predicting NSB in CF-LVAD patients based on the measures for acquired platelet defects (platelet activation: PAC-1, and receptor shedding: GPIbα and GPVI) and the loss of HMWM of vWF.

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Table 1.

Demographics and clinical characteristics of LVAD recipients with a comparison between non-bleeders and bleeders.

Characteristics	Non-bleeder group (n=39)	Bleeder group (n=23)	P-value
Age in years, mean ± SD	53.4 ± 12.4	60.5 ± 9.4	P = 0.02
Sex, n (% male)	36 (92.3%)	21 (91.3%)	P = 0.89
Race			P = 0.83
White, n (%)	18 (46.2%)	11 (47.8%)	
Black, n (%)	19 (48.7%)	10 (43.5%)	
Other, n (%)	2 (5.1%)	2 (8.7%)	
Height in meters, mean ± SD	1.79 ± 0.09	1.78 ± 0.07	P = 0.59
Weight in kilograms, mean ± SD	92.1 ± 21.7	87.4 ± 21.5	P = 0.42
Body mass index, mean ± SD	28.5 ± 5.97	27.4 ± 6.02	P = 0.50
LVAD type			P = 0.11
HVAD, n (%)	34 (87.2%)	14 (60.1%)	
HM II, n (%)	2 (5.1%)	3 (13%)	
HM III, n (%)	1 (2.6%)	1 (4.3%)	
Jarvik, n (%)	2 (5.1%)	5 (21.7%)	

Statistical significance was measured using Student's T-test for all except sex, race, and LVAD type where a Chi-square test was performed. P<0.05 was considered significant.