ORIGINAL RESEARCH

Platelet Secretion Defects and Acquired von Willebrand Syndrome in Patients With Ventricular Assist Devices

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***Background*—**The number of implanted ventricular assist devices (VADs) has increased significantly recently. Bleeding, the most frequent complication, cannot be solely attributed to anticoagulation therapy. Acquired von Willebrand syndrome (AVWS) caused by increased shear stress is frequent in VAD patients and can increase the bleeding risk. The HeartMate III (HM III) is a novel left VAD featuring potential improvements over the HeartMate II.

***Methods and Results*—**In this study, we investigated the prevalence and onset of AVWS in 198 VAD patients. To our knowledge, this is the largest cohort of VAD patients whose longitudinal data on AVWS have been collected. We also analyzed whether AVWS is less severe in HM III patients than in HeartMate II patients. Because platelet dysfunction can raise the bleeding risk, we investigated platelet function in a subset of patients. In total, 198 VAD patients and 60 patients with heart transplants as controls were included in this study. The ratio of von Willebrand factor collagen binding capacity to von Willebrand factor:antigen, multimer analyses, and platelet function (especially secretion of α- and δ-granules) were investigated. All 198 VAD patients developed AVWS. As soon as the VAD was explanted, the AVWS disappeared within hours. AVWS was less severe in the HM III patients than in the HeartMate II patients. The HM III patients had fewer bleeding symptoms. In addition, VAD patients exhibited a platelet α- and δ-granule secretion defect.

***Conclusions*—**AVWS develops in VAD patients and may increase the bleeding risk. The HM III device causes less severe AVWS. Platelet secretion defects should be investigated in VAD patients because they also raise the bleeding risk.

***Clinical Trial Registration*—**https://www.drks.de/drks_web. Unique identifier: DRKS00000649. **(*J Am Heart Assoc*. 2018;7: e006519. DOI: 10.1161/JAHA.117.006519.)**

Key Words: platelet • ventricular assist device • von Willebrand factor

Implantation of a ventricular assist device (VAD) is a life-saving therapeutic option for patients with end-stage heart failure. Within the past 15 years, the number of patients awaiting heart transplantation (HTX) doubled, whereas the number of available donor organs dropped. According to the Eurotransplant Yearly Statistics Overview, 703 patients were on the active waiting list in Germany for HTX in 2016, but only 287 hearts were transplanted. Consequently, the demand for VADs as bridge to transplant has risen dramatically. The number of patients with a VAD as destination therapy has also risen substantially because in recent years, more patients needed either HTX or VAD support; however, the number of donors had not increased. In addition, VAD implantation was performed more often than HTX in older patients because of patient age. Moreover, the newer devices' advantages of smaller size and fewer complications made them more attractive for implantation.

A major complication in patients with VAD is bleeding events, which are often life-threatening and occur more frequently than thromboembolic events.[1,2] Anticoagulation is necessary; however, nonsurgical hemorrhages cannot be attributed to anticoagulation alone. Pathological flow conditions and elevated shear stress occur in VAD patients

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Clinical Perspective

What Is New?

- In this large ventricular assist device cohort, all patients developed acquired von Willebrand syndrome.
- Acquired von Willebrand syndrome is less severe in patients with HeartMate III support compared with patients with the HeartMate II device.
- To date, this study is the largest comparing HeartMate III and HeartMate II regarding the von Willebrand factor parameter.
- Platelet function analyses in a subset of patients with a ventricular assist device revealed, for the first time, that these patients present with a secretion defect of platelet α- and δ-granules.

What Are the Clinical Implications?

- Patients with a HeartMate III device who developed less distinct acquired von Willebrand syndrome than patients with the HeartMate II had fewer bleeding symptoms than patients with the HeartMate II.
- The knowledge of a platelet secretion defect is very important for ventricular assist device patients with bleeding symptoms and normal platelet counts.

because of changes in hemodynamics.[3] Therefore, our hypothesis on initiating this prospective observational study (2006–2016) was that acquired von Willebrand syndrome (AVWS) is present in all VAD patients. AVWS may be one cause of the exacerbated bleeding symptoms, as has been described previously.[4–6] AVWS is characterized by the loss of high-molecular-weight (HMW) multimers of von Willebrand factor (VWF) due to enhanced shear stress, which results in impaired interaction between VWF and collagen and platelets[7,8] that is identifiable by decreased VWF collagen binding capacity (VWF:CB) and VWF ristocetin cofactor values. The VWF:CB/VWF antigen (VWF:Ag) and VWF ristocetin cofactor/VWF:Ag ratios are decreased in these patients. In studies with small cohorts, we then demonstrated that AVWS occurs in VAD patients and seems to contribute to their bleeding predisposition.[9,10]

The degree of hemodynamic changes that a VAD incurs depends on the VAD design.[11] Centrifugal-flow left VADs (LVADs) seem to induce less VWF degradation and hemolysis than axial-flow LVADs and biventricular assist devices (BVADs).[11,12] The HeartMate II (HM II) is an axial continuous-flow LVAD implanted into 15 000 patients worldwide.[13] The HeartMate III (HM III) is a novel LVAD featuring several modifications and potential improvements compared with its predecessor.[14] HM III contains a centrifugal-flow pump with wide blood-flow paths, a magnetic levitation rotor to reduce friction, and surfaces textured with titanium microspheres to stimulate formation of an endothelial coating. In addition, an artificial pulse mode has been implemented. These design improvements are expected to result in lower shear stress and greater hemocompatibility. We hypothesized that patients on HM III support might have less severe AVWS, lower bleeding tendency, and better outcome.

We also hypothesized that the hemodynamic changes in VAD patients could lead to impaired platelet function. We demonstrated previously that platelet aggregometry is impaired in VAD patients.[15] In this study, we analyzed platelet function using flow cytometry.

Our aim in this study was to investigate the development of AVWS and platelet function in a large cohort of VAD patients and to compare the effects of different VAD devices on VWF parameters. In addition, we focused on the impact of the HM III's novel design on VWF and platelet parameters.

Methods

Data and methods will be made available to other researchers on reasonable request for the purposes of reproducing the results or replicating the procedure.

Patients

In total, 198 patients who received a VAD at the University Hospital in Freiburg (between 2006 and 2016) were included in this study. All patients were white. Overall, 126 patients had only HM II device (Thoratec Corp), and 27 patients had an HM III device (Thoratec Corp). These patients had the support of just 1 device (LVAD). We also investigated VWF parameters in 23 patients who had an HM II and additional temporary right VAD (RVAD). A cohort of 22 patients with a BVAD was also examined. We included 60 patients with HTX as controls. In addition, 24 HTX patients had had an LVAD as a bridge to transplant before transplantation. They were excluded as controls but included in longitudinal data presentation. These data were obtained within the scope of our institutional monitoring program on hemostaseological changes during support via VAD, approved by the ethics committee of the University of Freiburg and supported by the German Research Foundation. All patients provided their informed consent. Data from patients with additional extracorporeal life support or extracorporeal membrane oxygenation support were excluded. Baseline characteristics and longitudinal recruitment of the cohorts are listed in Table 1 and Figure 1.

Surgical Procedures

VADs were implanted according to techniques described previously.[9,11] The inflow graft was inserted into the left

Table 1. Baseline Data of All Included Patients With HM II, HM III, HTX, HM II+RVAD, or BVAD and Number of Patients With Respective Diagnosis and INTERMACS Scores

	HM II	HM III	HTX	HM II+RVAD	BVAD
Male/female, n/n	104/22	25/2	49/11	17/6	11/11
Age, y, mean±SD	51.1±16.7	56.5±10.0	51.9±13.2	56.4±9.7	39.8±15.0
Diagnosis, n (%)					
DCMP	79 (62.7)	15 (55.6)	31 (51.7)	13 (56.5)	9 (41.0)
DCMP/ICMP	17 (13.5)	2 (7.4)	2 (3.3)	6 (26.1)	0 (0.0)
CHD	13 (10.3)	2 (7.4)	12 (20.0)	3 (13.0)	1 (4.5)
ICMP	11 (8.7)	6 (22.2)	1 (1.7)	1 (4.4)	1 (4.5)
Myocarditis	0 (0.0)	1 (3.7)	0 (0.0)	0 (0.0)	8 (36.5)
Other*	6 (3.2)	1 (3.7)	14 (23.3)	0 (0.0)	3 (13.5)
INTERMACS score, n (%)					
1. Critical cardiogenic shock (patients)	18 (14.3)	4 (14.8)	1 (1.7)	6 (26.1)	13 (59.2)
2. Progressive decline (patients)	25 (19.8)	5 (18.6)	2 (3.3)	10 (43.5)	3 (13.6)
3. Stable but inotrope dependent (patients)	51 (40.5)	4 (14.8)	38 (63.3)	4 (17.4)	5 (22.7)
4. Resting symptoms (patients)	30 (23.8)	12 (44.4)	10 (16.7)	3 (13.0)	1 (4.5)
5. Exertion intolerant (patients)	1 (0.8)	2 (7.4)	9 (15.0)	0 (0.0)	0 (0.0)
6. Exertion limited (patients)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

BVAD indicates biventricular assist device; CHD, coronary heart disease; DCMP, dilated cardiomyopathy; HM II, HeartMate II; HM III, HeartMate III; HTX, heart transplantation; ICMP, ischemic cardiomyopathy; INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; RVAD, right ventricular assist device.
*For example, congenital heart disease, valvular heart disease, amyloidosis, muscle dystrophy, or Marfan syndrome.

ventricular apex, and the outflow graft anastomosed to the ascending aorta.[16,17] The HM II was typically operating at 8600 to 10400 rpm, and the HM III operated at 5000 to 6000 rpm. Pump speed for initial operation during the implant procedure was determined by transesophageal echocardiography to minimize septal shifting. This procedure was used routinely in both HM II and HM III pumps and was also used for pump speed adaptation during the first postoperative days. To achieve these optimal settings regarding ventricular geometry, different ranges of pump speed in HM II versus HM III pumps are required, caused by the different technology of the pumps.

Paracorporeal Thoratec VAD implantation was also performed, as previously described.[9,11] The RVAD's inflow cannula was implanted in the right ventricular apex while the right ventricular outflow graft was anastomosed to the main pulmonary artery. Left ventricular support was achieved by cannulating the left ventricular apex for the inflow cannula and an anastomosis in the ascending aorta for the outflow graft. The BVAD's pumping rate was adjusted to each patient's physiological range, and we aimed for a systolic ejection time of 300 ms.

In case of right ventricular failure during or shortly after LVAD implantation, a temporary RVAD was put in place. Venous drainage was achieved via cannulation of the vena cava or the right atrium while blood was returned into to the pulmonary artery.

Anticoagulation was usually started with heparin after 48 hours in the majority of VAD patients with a target activated partial thromboplastin time of 60 to 80 seconds. In addition, heparin effect was measured by anti-Xa (target 0.2 IU/mL). Phenprocoumon was initiated after removal of the chest drains and sufficient oral ingestion.[18] The target International Normalized Ratio was 2.0 to 3.0. Acetylsalicylic acid 100 mg/d was also used to inhibit platelet aggregation when the International Normalized Ratio was stable at the target level. The effect of acetylsalicylic acid was monitored by aggregometry analyses.

HTX was performed with biatrial or bicaval anastomosis of the donor heart. Patients received low-dose heparin and acetylsalicylic acid 100 mg/d postoperatively.

Laboratory Analyses

Blood samples were taken on days 1, 3, 7, 30, and 90 after implantation. Whenever possible, blood samples were also collected before VAD implantation. VWF:Ag (normal 0.6–1.5 U/L), VWF:CB (normal 0.6–1.5 U/L), and VWF multimers were determined, as described previously.[10] Briefly, VWF:Ag was measured in sodium citrate plasma using an in-house

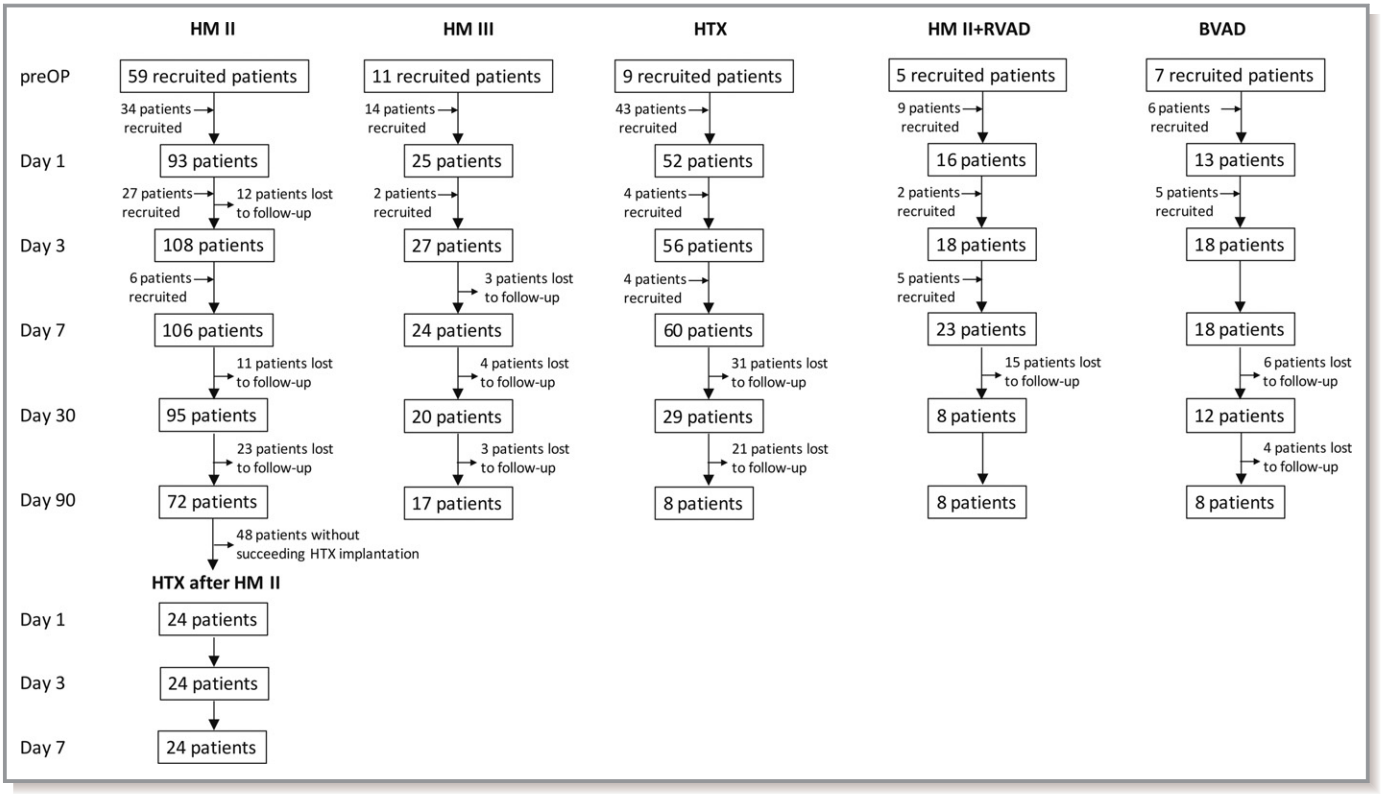

Figure 1. Study diagram of the investigated patients. BVAD indicates biventricular assist device; HM II, HeartMate II; HM III, HeartMate III; HTX, heart transplantation; preOP, preoperative; RVAD, right ventricular assist device.

ELISA.[19] Collagen type I was immobilized on a microtiter plate. Collagen binding capacity in plasma was measured photometrically via the ELISA technique. We calculated ratios of VWF:CB/VWF:Ag (normal ≥0.7). They reflect the biological capacity of the available VWF to bind to collagen. VWF multimers were separated on sodium dodecyl sulfate–agarose gel and blotted on a polyvinylidene fluoride membrane to assess the HMW multimers. VWF was determined using appropriate primary and secondary antibodies and 3.30-diaminobenzidin/cobalt chloride. Standard human plasma was used as control. AVWS was diagnosed if HMW multimers were missing and if the VWF:CB/VWF:Ag ratio was reduced.

Light transmission aggregometry of platelets was performed using the APACT 4.0 aggregometer (BioMedical Technologies) and APACT software, as described.[15] Platelet counts were determined using standard laboratory techniques.

For the flow-cytometric quantification of platelet granule secretion, platelets in platelet-rich plasma were stimulated using increasing concentrations of thrombin (0, 0.05, 0.1, 0.2, 0.5, and 1.0 U/mL).[20] After fixation, cells were washed and incubated with FITC (fluorescein isothiocyanate)–conjugated anti-CD62 or FITC-conjugated anti-CD63. The expression of CD41, CD42a, and CD42b as well as ristocetin-induced VWF binding and ADP-induced fibrinogen binding was tested using the appropriate FITC-conjugated antibodies. Surface fluorescence was analyzed with a flow cytometer (FACSCalibur; Becton Dickinson). Data are expressed as linear arbitrary units. Analyses were performed with patients' platelets in pairs with platelets from healthy controls.

Statistical Analysis

Values for age and platelet aggregation are given as mean±SD. Distributions of VWF:CB/VWF:Ag ratios as well as hemoglobin and creatinine levels are depicted in box-and-whisker plots. Differences between various VAD systems were analyzed using ANOVA. Corrections for multiple comparisons were made via the Holm-Šídák method (α=0.05), and multiplicity adjusted P values were calculated for each comparison. Differences between the HM II and HM III groups regarding HMW multimer loss and bleeding were evaluated using the Fisher exact test. Calculations were done using GraphPad Prism 6.0 (GraphPad Software) and IBM SPSS Statistics version 23 (IBM Corp).

Results

Rapid Onset and Reversibility of AVWS in LVAD Patients

All patients with VAD developed decreased VWF:CB/VWF:Ag ratios during the time of measurements (P<0.001; Figure 2A).

Case–control data from a large cohort of VAD patients (n=198) and HTX patients (n=60) revealed pathological VWF:CB/VWF:Ag ratios in all VAD patients and normal levels in 95% of all HTX patients ($P<0.001$; Figure 2A). Some patients were preoperatively (for VAD implantation) in such bad conditions that we could not ask them to take part at this study; therefore, it was not possible to draw blood from these patients preoperatively to perform these analyses. Nevertheless, the preoperative data of the other patients were included because they provided values before VAD implantation and after VAD explantation. All HM II patients presented reduced VWF:CB/VWF:Ag ratios within 24 hours (Figure 2B). The VWF:CB/VWF:Ag ratio remained pathological as long as patients were on VAD support. After VAD explantation, the VWF:CB/VWF:Ag ratio recovered within 24 hours after HTX. Longitudinal data from a subset of patients (n=24) who received an HM II implant as bridge-to-transplant therapy displayed a rapid decline in the VWF:CB/VWF:Ag ratio within 24 hours after implantation.

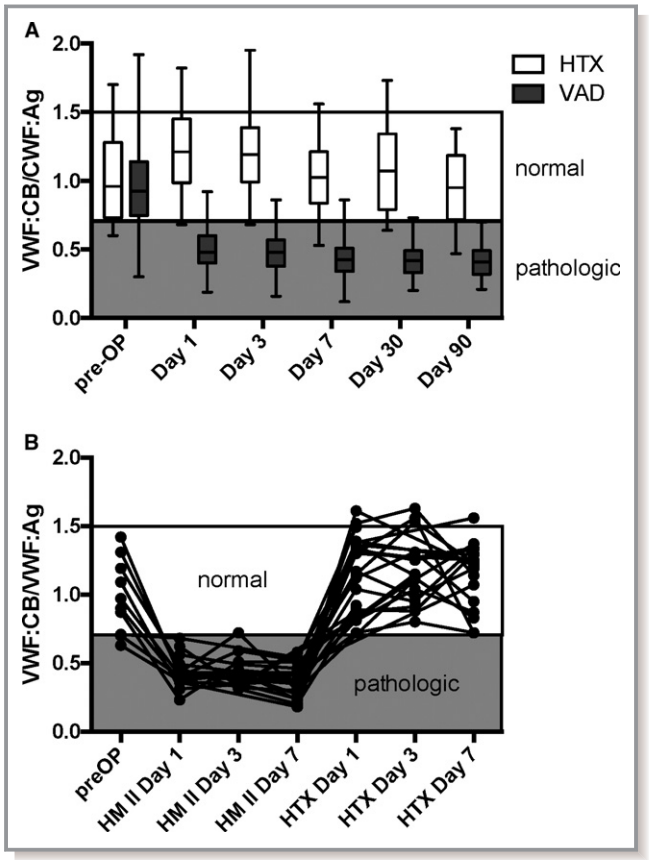

Figure 2. A, VWF:CB/VWF:Ag ratio of patients with VAD (n=198) and of HTX patients (n=60). B, Longitudinal progression of VWF:CB/VWF:Ag ratios in patients (n=24) before HM II implantation, with HM II, and after HTX. HM II indicates HeartMate II; HTX, heart transplantation; pre-OP, preoperative; VWF:Ag indicates VWF antigen; VWF:CB, VWF collagen binding capacity.

Differences in the Severity of AVWS

We documented a drop in collagen-binding capacity after VAD implantation in all patients with VAD. VWF:Ag was not reduced in these patients; therefore, the VWF:CB/VWF:Ag ratios of all VAD patients were decreased, implying AVWS. Furthermore, most of these patients (91%) revealed a loss of VWF HMW multimers, confirming the AVWS diagnosis. Interestingly, the VWF:CB/VWF:Ag ratios were significantly lower in the patients with an HM II device than in those with an HM III during our entire observation period (90 days). Although the VWF:CB/VWF:Ag ratio after implantation in the HM II group reached its minimum soon after implantation, the ratio decreased gradually in the HM III group (Figure 3A).

Patients with an HM II and an RVAD exhibited an even lower VWF:CB/VWF:Ag ratio than those with just an HM II (significant differences on days 3 and 7). The BVAD patients' VWF parameters resembled those of the HM II cohort.

We obtained similar results analyzing HMW multimers. The percentage of HM III patients presenting a loss of HMW multimers was lower than that of the patients with an HM II during the entire observation period. On day 1, LVAD implantation resulted in a loss of HMW multimers in 83% of the HM II patients but just 58% of the HM III patients ($P<0.05$). Loss of HMW multimers was observed within 24 hours after LVAD implantation in patients on HM II support, with significant differences on days 1, 3, and 7 after implantation ($P<0.05$) compared with HM III patients. A trend toward a lower incidence of HMW multimer loss in HM III patients was apparent between days 30 and 90 after implantation (85% versus 93%; Figure 3B). The loss of HMW multimers in the HM II patients, the HM II patients with RVAD, and the BVAD patients was so pronounced that the severity of loss in these patients was indistinguishable.

Impaired Platelet Function and Platelet Secretion in Patients With VAD

Platelet aggregometry analyses could not be performed because of low platelet counts (<100 000/μL) in 27% of all samples. Aggregometry was performed in VAD patients whose platelet counts were >100 000/μL, demonstrating impaired platelet function. We observed hypoaggregability after stimulation with ADP, collagen, and epinephrine. This platelet dysfunction was independent of VAD type (Table 2).

Interestingly, further flow-cytometric platelet analyses in 22 VAD patients revealed severely reduced expression of CD62 (impaired α-granule secretion) in 91% (20/22 patients; Figure 4) and CD63 (impaired δ-granule secretion) in 91% (20/22 patients, $P<0.001$; Figure 5). Only 2 HM III patients revealed no secretion defects. Expression of CD41, CD42a, and CD42b as well as ristocetin-induced VWF binding and ADP-induced fibrinogen binding were normal in all tested patients (n=16).

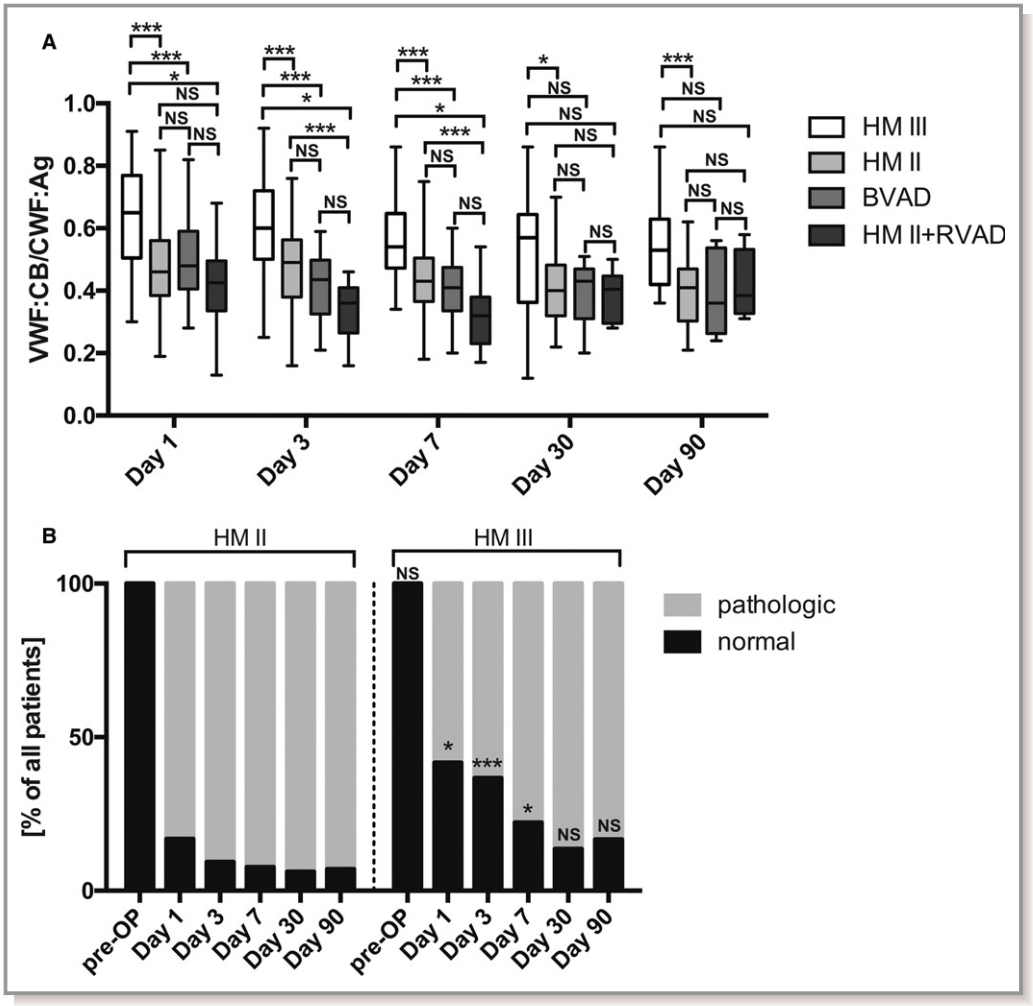

Figure 3. A, VWF:CB/VWF:Ag ratios in patients with HM II, HM III, BVAD, and HM II and RVAD (HM II+RVAD). Significance symbols are based on multiplicity corrected *P* values. B, Percentage of patients with pathological and normal VWF multimers in HM II and HM III patients pre-OP and on days 1, 3, 7, 30, and 90 after implantation. Significance markings relate to differences between the HM II and HM III groups. **P*<0.05, ****P*<0.001. BVAD indicates biventricular assist device; HM II, HeartMate II; HM III, HeartMate III; HTX, heart transplantation; NS indicates not significant; pre-OP, preoperative; RVAD, right ventricular assist device; VWF:Ag, VWF antigen; VWF:CB, VWF collagen binding capacity.

Differences in Bleeding Events

There was no severe episode of epistaxis requiring embolization of the facial arteries. Two patients with the HM II device died because of cranial bleeding, 1 patient with a BVAD died because of lung bleeding, and 1 patient with an HM II and an RVAD died because of hematothorax. Bleeding events were less frequent in the HM III patients (19%) than in the HM II

Table 2. Platelet Aggregometry Analyses in Patients With HM II (n=107) and HM III (n=25)

	Day 1		Day 3		Day 7		Day 30	
	HM II	HM III	HM II	HM III	HM II	HM III	HM II	HM III
ADP	48±16	42±17	43±18	41±14	44±16	53±16	52±14	49±10
Collagen	53±16	59±10	46±17	37±9	40±19	44±15	52±16	43±25
Epinephrin	45±18	46±17	43±23	43±15	44±17	54±11	52±19	45±20
Ristocetin%	60±17	75±12	59±21	64±17	59±21	70±17	62±20	64±25

Platelet aggregometry data are depicted in percentages of platelet-poor plasma transmission (mean±SD). HM II indicates HeartMate II; HM III, HeartMate III.

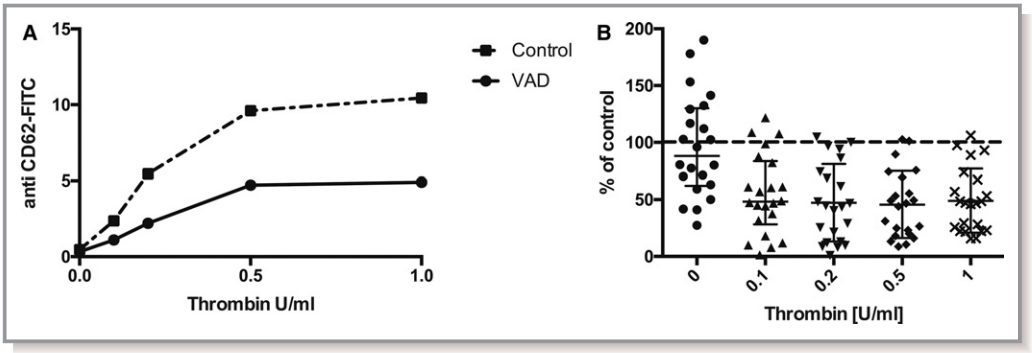

Figure 4. CD62 expression (α-granule secretion) in VAD patients. A, Representative IgG-corrected CD62 expression on platelets from a VAD patient and a healthy control at increasing thrombin concentrations. B, CD62 expression on platelets of VAD patients (n=22) in relation to paired healthy controls at increasing thrombin concentrations (data depicted in percentage of respective fluorescence from a healthy control). FITC indicates fluorescein isothiocyanate; VAD, ventricular assist device.

patients (41%; 5/27 HM III patients versus 52/126 HM II patients experienced bleeding events within 90 days after implantation, $P<0.05$; Table 3). Interestingly, no HM III patients had gastrointestinal bleeding. The HTX patients who received similar anticoagulation regimens had less bleeding than patients with a VAD.

Median hemoglobin levels were lower than the reference range in all cohorts (Figure 6A). Hemoglobin levels <8 g/dL were observed in 39 HM II patients, 11 HM III patients, 17 HTX patients, 10 BVAD patients, and 15 patients with an HM II and an RVAD. Three HM II patients exhibited hemoglobin levels <7.0 g/dL.

Median creatinine levels did not differ significantly between the cohorts (Figure 6B). Creatinine levels >3.9 mg/dL were observed in 11 HM II patients, 2 HM III patients, 3 HTX patients, 1 BVAD patient, and 3 patients with an HM II and an RVAD. Only 1 HM II patient had creatinine levels >6 mg/dL.

No patients with low hemoglobin or high creatinine levels exhibited increased bleeding compared with other patients in their respective cohorts.

Discussion

Bleeding has been reported as the most frequent complication with a VAD, occurring in 29.5% of all VAD patients.[13,21,22]

This study is the first with such a large cohort of VAD patients for whom VWF and platelet-function parameters were determined. Our data demonstrate that AVWS developed in all VAD patients in this study and that AVWS persisted as long as the device was implanted. Longitudinal progression of the VWF:CB/VWF:Ag ratios in HM II patients showed that AVWS manifested in all cases within 24 hours after implantation. Once patients underwent HTX, AVWS disappeared within 24 hours. This may explain recent study results showing that HTX patients with a prior VAD needed more intraoperative

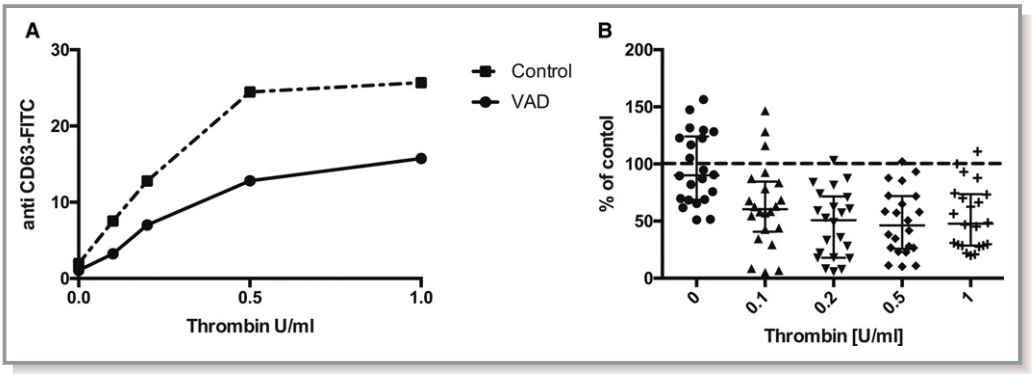

Figure 5. CD63-expression (δ-granule secretion) in VAD-patients. A, Representative IgG corrected CD63-expression on platelets of a VAD-patient and of a healthy control at increasing thrombin concentrations. B, CD63-expression on platelets of VAD patients (N=22) in relation to paired healthy controls at increasing thrombin concentrations (data depicted in % of respective fluorescence from a healthy control). FITC indicates fluorescein isothiocyanate; VAD, ventricular assist device.

Table 3. Number and Type of Bleeding Complications and Number of Patients With Respective Bleeding Complications (parenthesized) Within 90 Days After Surgery in BVAD, HM II+RVAD, HM II, HM III, and HTX Cohorts

	BVAD (n=22)	HM II+RVAD (n=23)	HM II (n=126)	HM III (n=27)	HTX (n=60)
Surgical site	32 (13)	13 (10)	45 (28)	6 (5)	5 (5)
Gastrointestinal	2 (2)	1 (1)	18 (13)	0	1 (1)
Epistaxis*	3 (3)	1 (1)	20 (18)	1 (1)	0
Pulmonary	2 (2)	2 (2)	2 (2)	1 (1)	0
Intracerebral	1 (1)	2 (2)	4 (4)	0	0
Thoracic	0	3 (2)	5 (4)	0	0
Total	40 (19)	22 (16)	94 (52)	8 (5)	6
Event rate (per patient-months)	0.606	0.319	0.249	0.099	0.033

Event rates were calculated by dividing the number of episodes observed by the total amount of follow-up time the patients were being observed. BVAD indicates biventricular assist device; HM II, HeartMate II; HM III, HeartMate III; HTX, heart transplantation; RVAD, right ventricular assist device.
*Did not require embolization of the facial arteries.

blood than HTX patients without a prior VAD.[23] HM II patients who also received an RVAD implant exhibited even more severe AVWS.

In addition, this is the first large-scale study comparing VWF parameters for patients with an HM III versus an HM II device. Several technical improvements in the HM III, particularly its centrifugal flow design and lower shear stress, promise to lessen its impact on blood constituents. The VWF parameters we documented were less affected in HM III patients, and this may explain the fewer bleeding events. AVWS, as reflected by the VWF:CB/VWF:Ag ratio, was less severe in HM III patients than in HM II patients throughout our observation period (90 days after implantation). Likewise, the percentage of patients with intact HMW multimers remained higher for those with the HM III. These effects may contribute to less bleeding diathesis and better clinical outcome compared with patients with an HM II. AVWS was less pronounced in patients on HM III support, particularly during the initial postoperative period, when most bleeding events occur. Our results concur with a recent multicenter study demonstrating excellent survival and low adverse event rates in HM III patients and with the study of Netuka et al, who showed greater preservation of VWF HMW multimers in the HM III group (n=15) compared with the HM II group (n=11).[24,25] Nevertheless, they could not detect a difference regarding the VWF ristocetin cofactor/VWF:Ag ratio in these patients. This phenomenon may be due to the higher sensibility of the in-house VWF:CB test used in our study compared with the VWF ristocetin cofactor test.[26]

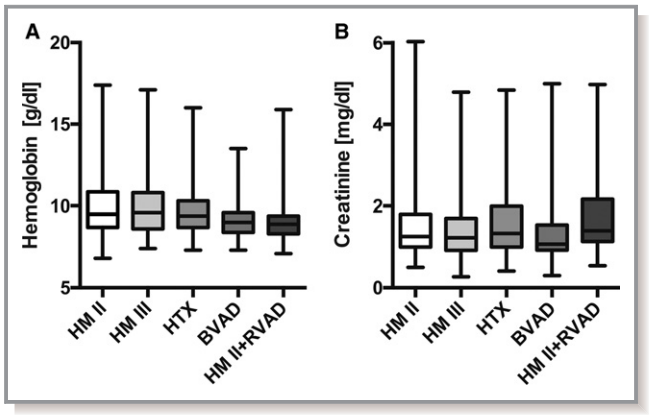

Figure 6. Distribution of (A) hemoglobin levels and (B) creatinine levels in patients with HM II, HM III, HTX, BVAD, and HM II and RVAD (HM II+RVAD). BVAD indicates biventricular assist device; HM II, HeartMate II; HM III, HeartMate III; HTX, heart transplantation; RVAD, right ventricular assist device.

AVWS may raise the bleeding risk in VAD patients; however, the bleeding incidence in a particular patient depends on the occurrence and severity of the challenge (ie, surgery, trauma).[9,27–30] If patients suffer from AVWS, the risk of bleeding may be increased by surgery, trauma, or injury, especially in mucocutaneous areas.[27,29] Uchida et al noted that intracranial bleeding can sometimes occur, and we also observed intracranial bleeding in 2 HM II patients.[28] Di Sabatino et al published that patients with AVWS may suffer from late onset of bleeding symptoms.[31]

This issue should be considered, particularly because many VAD patients bleed postoperatively and have gastrointestinal bleeding.[32–35] According to Randi et al, patients with AVWS can develop gastrointestinal bleeding because more angiodysplasia occurs in these patients.[36] Blackshear et al noted that acquired abnormalities of VWF multimers are associated with aortic and mitral prosthesis dysfunction, with occasional gastrointestinal bleeding and gastrointestinal angiodysplasia.[37] Interestingly, in our study, gastrointestinal bleeding occurred more often in HM II patients compared with HM III patients

(18/0; Table 3). Nevertheless, because gastrointestinal bleeding can become very serious, all patients with VAD should be observed for gastrointestinal bleeding on a regular basis. If patients with AVWS bleed, the bleeding may be stopped by substitution of VWF-containing factor VIII, as has been described by other authors.[4–6] We were also able to stop bleeding in individual patients with VAD and AVWS and gastrointestinal bleeding.

Our study demonstrates, for the first time, that 91% of the 22 investigated VAD patients exhibited secretion defects of platelet α- and δ-granules. The small number of VAD patients is a limitation of this study; however, the flow cytometry analyses of the platelets showed statistically significant results. These platelet-secretion defects may be triggered by platelet activation due to the artificial VAD surface and pathological blood flow.[7,38] Baghai et al reported platelet hypoaggregability using aggregometry. Using flow cytometry, we demonstrated that patients with VAD support have a platelet-secretion defect that may cause the hypoaggregability. Platelet dysfunction exacerbates the already heightened bleeding risk with AVWS. In particular, platelet-secretion defects can lead to increased bleeding symptoms. This is very important to consider in patients with VAD and bleeding symptoms but normal platelet count. In patients with VAD and bleeding symptoms who do not respond to a therapy with VWF-containing factor VIII, the application of platelet concentrates may be an option.

Heart failure is a severe medical condition affecting >5 million people in the United States, and it is a leading cause of death there. It is essential that better devices be developed with fewer and less serious associated complications.[39] Because >4000 patients are currently on the waiting list for HTX (http://www.unos.org/data/transplant-trends) and only 2000 to 2500 transplants are performed each year because of the shortage of organs, novel VADs need to be developed that incur less shear stress so as to reduce the severity of AVWS and its impact on platelet function, thus reducing bleeding symptoms.

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Disclosures

None.

References

1. Eckman PM, John R. Bleeding and thrombosis in patients with continuous-flow ventricular assist devices. *Circulation*. 2012;125:3038–3047.
2. Susen S, Rauch A, Van Belle E, Vincentelli A, Lenting PJ. Circulatory support devices: fundamental aspects and clinical management of bleeding and thrombosis. *J Thromb Haemost*. 2015;13:1757–1767.
3. Benk C, Lorenz R, Beyersdorf F, Bock J, Klemm R, Korvink JG, Markl M. Three-dimensional flow characteristics in ventricular assist devices: impact of valve design and operating conditions. *J Thorac Cardiovasc Surg*. 2011;142:1019–1026.
4. Tiede A. Diagnosis and treatment of acquired von Willebrand syndrome. *Thromb Res*. 2012;130(suppl 2):S2–S6.
5. Tiede A, Rand JH, Budde U, Ganser A, Federici AB. How I treat the acquired von Willebrand syndrome. *Blood*. 2011;117:6777–6785.
6. Budde U, Scheppenheim S, Dittmer R. Treatment of the acquired von Willebrand syndrome. *Expert Rev Hematol*. 2015;8:799–818.
7. Nascimbene A, Neelamegham S, Frazier OH, Moake JL, Dong J-F. Acquired von Willebrand syndrome associated with left ventricular assist device. *Blood*. 2016;127:3133–3141.
8. Rauch A, Legendre P, Christophe OD, Goudemand J, van Belle E, Vincentelli A, Denis CV, Susen S, Lenting PJ. Antibody-based prevention of von Willebrand factor degradation mediated by circulatory assist devices. *Thromb Haemost*. 2014;112:1014–1023.
9. Geisen U, Heilmann C, Beyersdorf F, Benk C, Berchtold-Herz M, Schlensak C, Budde U, Zieger B. Non-surgical bleeding in patients with ventricular assist devices could be explained by acquired von Willebrand disease. *Eur J Cardiothorac Surg*. 2008;33:679–684.
10. Heilmann C, Geisen U, Beyersdorf F, Nakamura L, Benk C, Berchtold-Herz M, Trummer G, Schlensak C, Zieger B. Acquired von Willebrand syndrome in patients with ventricular assist device or total artificial heart. *Thromb Haemost*. 2010;103:962–967.
11. Heilmann C, Geisen U, Benk C, Berchtold-Herz M, Trummer G, Schlensak C, Zieger B, Beyersdorf F. Haemolysis in patients with ventricular assist devices: major differences between systems. *Eur J Cardiothorac Surg*. 2009;36:580–584.
12. Bartoli CR, Kang J, Zhang D, Howard J, Acker M, Atluri P, Motomura T. Left ventricular assist device design reduces von Willebrand factor degradation: a comparative study between the HeartMate II and the EVAHEART Left Ventricular Assist System. *Ann Thorac Surg*. 2017;103:1239–1244.
13. Kirklin JK, Naftel DC, Pagani FD, Kormos RL, Stevenson LW, Blume ED, Myers SL, Miller MA, Baldwin JT, Young JB. Seventh INTERMACS annual report: 15,000 patients and counting. *J Heart Lung Transplant*. 2015;34:1495–1504.
14. Bourque K, Gernes DB, Loree HM, Richardson JS, Poirier VL, Barletta N, Fleischli A, Foiera G, Gempp TM, Schoeb R, Litwak KN, Akimoto T, Watach MJ, Litwak P. HeartMate III: pump design for a centrifugal LVAD with a magnetically levitated rotor. *ASAIO J*. 2001;47:401–405.
15. Baghai M, Heilmann C, Beyersdorf F, Nakamura L, Geisen U, Olschewski M, Zieger B. Platelet dysfunction and acquired von Willebrand syndrome in patients with left ventricular assist devices. *Eur J Cardiothorac Surg*. 2015;48:421–427.
16. Siegenthaler MP, Westaby S, Frazier OH. Advanced heart failure: feasibility study of long-term continuous axial flow pump support. *ACC Curr J Rev*. 2005;14:52.
17. Martin J, Siegenthaler MP, Friesewinkel O, Fader T, van de Loo A, Trummer G, Berchtold-Herz M, Beyersdorf F. Implantable left ventricular assist device for treatment of pulmonary hypertension in candidates for orthotopic heart transplantation-a preliminary study. *Eur J Cardiothorac Surg*. 2004;25:971–977.
18. Brehm K, Krumnau O, Heilmann C, Beyersdorf F. Genetic variations of phenprocoumon metabolism in patients with ventricular assist devices. *Eur J Cardiothorac Surg*. 2016;50:275–280.
19. Sutor AH, Thomas KB, Prüfer FH, Grohmann A, Brandis M, Zimmerhackl LB. Function of von Willebrand factor in children with diarrhea-associated hemolytic-uremic syndrome (D+ HUS). *Semin Thromb Hemost*. 2001;27:287–292.
20. Lahav J, Jurk K, Hess O, Barnes MJ, Farndale RW, Luboshitz J, Kehrel BE. Sustained integrin ligation involves extracellular free sulfhydryls and enzymatically catalyzed disulfide exchange. *Blood*. 2002;100:2472–2478.

21. Lahpor J, Khaghani A, Hetzer R, Pavie A, Friedrich I, Sander K, Strüber M. European results with a continuous-flow ventricular assist device for advanced heart-failure patients. *Eur J Cardiothorac Surg*. 2010;37:357–361.
22. Tsiouris A, Paone G, Nemeh HW, Brewer RJ, Morgan JA. Factors determining post-operative readmissions after left ventricular assist device implantation. *J Heart Lung Transplant*. 2014;33:1041–1047.
23. Awad M, Czer LSC, De Robertis MA, Mirocha J, Ruzza A, Rafiei M, Reich H, Trento A, Moriguchi J, Kobashigawa J, Esmailian F, Arabia F, Ramzy D. Adult heart transplantation following ventricular assist device implantation: early and late outcomes. *Transplant Proc*. 2016;48:158–166.
24. Zimpfer D, Netuka I, Schmitto JD, Pya Y, Garbade J, Morshuis M, Beyersdorf F, Marasco S, Rao V, Damme L, Sood P, Krabatsch T. Multicentre clinical trial experience with the HeartMate 3 left ventricular assist device: 30-day outcomes. *Eur J Cardiothorac Surg*. 2016;50:548–554.
25. Netuka I, Kvasnička T, Kvasnička J, Hrachovinová I, Ivák P, Mareček F, Bílková J, Malíková I, Jančová M, Malý J, Sood P, Sundareswaran KS, Connors JM, Mehra MR. Evaluation of von Willebrand factor with a fully magnetically levitated centrifugal continuous-flow left ventricular assist device in advanced heart failure. *J Heart Lung Transplant*. 2016;35:860–867.
26. Geisen U, Zieger B, Nakamura L, Weis A, Heinz J, Michiels JJ, Heilmann C. Comparison of von Willebrand factor (VWF) activity VWF: Ac with VWF ristocetin cofactor activity VWF:RCo. *Thromb Res*. 2014;134:246–250.
27. Patel SR, Madan S, Saeed O, Algodi M, Luke A, Gibber M, Goldstein DJ, Jorde UP. Association of nasal mucosal vascular alterations, gastrointestinal arteriovenous malformations, and bleeding in patients with continuous-flow left ventricular assist devices. *JACC Heart Fail*. 2016;4:962–970.
28. Uchida T, Hamasaki A, Ohba E, Yamashita A, Hayashi J, Sadahiro M. Life-threatening subdural hematoma after aortic valve replacement in a patient with Heyde syndrome: a case report. *J Cardiothorac Surg*. 2017;12:65.
29. Leebeek FWG, Eikenboom JCJ. Von Willebrand's disease. *N Engl J Med*. 2016;375:2067–2080.
30. Sadler JE. Low von Willebrand factor: sometimes a risk factor and sometimes a disease. *Hematology Am Soc Hematol Educ Program*. 2009;2009:106–112.
31. Di Sabatino A, Ambaglio C, Aronico N, Ghidelli N, Lenti MV, Gamba G, Corazza GR. Acquired von Willebrand syndrome in inflammatory bowel disease. *Haemophilia*. 2017;23:e231–e233.
32. Makris M, Federici AB, Mannucci PM, Bolton-Maggs PHB, Yee TT, Abshire T, Berntorp E. The natural history of occult or angiodysplastic gastrointestinal bleeding in von Willebrand disease. *Haemophilia*. 2015;21:338–342.
33. Harvey L, Holley CT, John R. Gastrointestinal bleed after left ventricular assist device implantation: incidence, management, and prevention. *Ann Cardiothorac Surg*. 2014;3:475–479.
34. Hudzik B, Kaczmarski J, Pacholewicz J, Zakliczynski M, Gasior M, Zembala M. Von Willebrand factor in patients on mechanical circulatory support—a double-edged sword between bleeding and thrombosis. *Kardiochir Torakochirurgia Pol*. 2015;12:233–237.
35. Grosman-Rimon L, Tumiati LC, Fuks A, Jacobs I, Lalonde SD, Cherney DZI, Rao V. Increased cyclic guanosine monophosphate levels and continuous-flow left-ventricular assist devices: implications for gastrointestinal bleeding. *J Thorac Cardiovasc Surg*. 2016;151:219–227.
36. Randi AM, Laffan MA, Starke RD. Von Willebrand factor, angiodysplasia and angiogenesis. *Mediterr J Hematol Infect Dis*. 2013;5:e2013060.
37. Blackshear JL, McRee CW, Safford RE, Pollak PM, Stark ME, Thomas CS, Rivera CE, Wysokinska EM, Chen D. von Willebrand factor abnormalities and Heyde Syndrome in dysfunctional heart valve prostheses. *JAMA Cardiol*. 2016;1:198–204.
38. Zhang W, Deng W, Zhou L, Xu Y, Yang W, Liang X, Wang Y, Kulman JD, Zhang XF, Li R. Identification of a juxtamembrane mechanosensitive domain in the platelet mechanosensor glycoprotein Ib-IX complex. *Blood*. 2015;125:562–569.
39. WRITING GROUP MEMBERS, Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Rosamond W, Sacco R, Sorlie P, Roger VL, Stafford R, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2010 update: a report from the American Heart Association. *Circulation*. 2010;121:e46–e215.