Acquired von Willebrand Syndrome in Patients with an Axial Flow Left Ventricular Assist Device

Running Title: Meyer et al: Von Willebrand Syndrome in patients with LVADs

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Abstract

Background—Rotary blood pumps used as left ventricular assist devices (LVADs) allow for long term support and may become suitable alternatives to heart transplantation. Effects of this technology on the coagulation system are not completely understood leading to controversial anticoagulation protocols. Thus, we investigated the primary hemostasis in patients with chronic LVAD therapy.

Methods and Results—Twenty-six out-patients received axial flow LVAD (HeartMate II, Thoratec) for a median support time of 4.5 months. In a cross-sectional protocol, platelet aggregation in response to adenosine diphosphate and epinephrine, von Willebrand antigen (vWF:AG) and collagen binding capacity (vWF:CB) were obtained. Von Willebrand factor (vWF) multimer analyses were performed and patients were screened for bleeding events. This analysis was repeated after removal of the device for transplantation or recovery (n=12) and after 15.5 months (median) in ongoing patients (n=11). In all patients on devices, severe impairment of platelet aggregation, as well as a loss of large vWF multimers was found. In 10 patients, a decreased vWF:CB/vWF:AG ratio was observed. Bleeding episodes occurred with an incidence of 0.17 per patient year. After removal of the device, normal patterns of platelet aggregation, multimer analysis and vWF:CB/vWF:AG ratio were recorded. In the second analysis of ongoing patients, impairment of platelet aggregation and loss of large vWF multimers were verified.

Conclusions—A diagnosis of von Willebrand syndrome type 2 was established in all patients after LVAD implantation, and bleeding events confirmed this finding. Reversibility of this condition was found after removal of the device.

Key Words: Acquired von Willebrand syndrome, LVAD, HeartMate II
LVADs have been successfully used as a bridge to heart transplantation in patients with high pulmonary vascular resistance [1] and in deteriorating candidates waiting for a donor heart. Due to the scarcity of resources in cardiac transplantation, these devices have also been used as a chronic therapy in heart failure patients unsuitable for transplantation. Initially, this approach was limited by the mechanical stability of first generation pulsatile devices. However, the clinical introduction of second and third generation nonpulsatile devices suggests that these devices can be used as a long term approach [2]. Despite this, bleeding complications and thromboembolic events still remain the most serious adverse events of this therapy. Early experience with continuous flow-device-related thrombosis and thromboembolic events, led to a maximum tolerable regimen of antiplatelet and anticoagulation therapies. With further improvements in the devices, less aggressive regimens were advised.

Nevertheless minor and major bleeding episodes are the leading adverse events. Although this axial flow pump is engineered to provide long term stability and full cardiac support without hemolysis, it is not known whether this technology has an impact on the coagulation system itself. Fifty years ago, an association between gastrointestinal bleeding and aortic stenosis was reported [3]. In 1992 was postulated that acquired von Willebrand syndrome (vWS) plays a key role in the pathogenesis of bleeding complications in patients with severe aortic stenosis [4].

The vWF circulates in the blood as the largest soluble multimeric protein. It is cleaved by a metalloprotease (ADAMTS 13), particularly under conditions of high shear stress [5]. Specifically, structural changes can be induced by high shear stress in the von Willebrand molecule exposing the bond between amino acids 842 and 843 [6] to the specific von Willebrand protease (ADAMTS 13) [7]. This results in acquired type 2 vWS, which is characterized by the loss of the largest von Willebrand multimers, which are most effective in
platelet-mediated hemostasis [8, 9]. The incidence of bleeding events in patients with aortic stenosis within six months before valve replacement was 21% [10]. It was also shown that the severity of the stenosis, indicated by higher mean transvalvular gradients, as well as higher wall shear stress was negatively correlated with the percentages of the highest molecular weight multimers.

It has been recently speculated that this mechanism may also impair patients with ventricular assist devices (VADs). The typical laboratory findings of an acquired vWS have been demonstrated in patients with different types of VAD (Thoratec biventricular assist device, HeartMate II and other rotary blood pumps) [11, 12, 13]. To evaluate the prevalence of vWS with the use of a modern device, we analyzed outpatients on chronic support with the HeartMate II assist device and reported the haemostatic abnormalities and clinical consequences for this patient cohort. The analysis was repeated after removal of the device for transplantation or recovery, as well as in the majority of still ongoing patients.

Methods

Patients

Within the last 5 years, 70 patients received a LVAD implantation using the HeartMate II LVAD (Thoratec, Pleasanton, California USA) at Hannover Medical School [14]. The study was designed as a cross-sectional study. Included were all patients of our outpatient clinic after LVAD implantation at the point of data evaluation in summer 2008. This cohort included 2 female and 24 male patients (Table 1). The mean age was 43 years, ranging from 16 to 64 years. All of these patients were seen on a regular basis and complete follow up was required to be included into this study. Informed consent was obtained from each patient and the study was approved by the local ethics committee. Indication for LVAD implantation was heart failure, despite maximum medical treatment including catecholamine
support. However, median support time at the investigation was 4.5 months ranging from 1 to 24 months. The cardiac diagnosis, histologically confirmed by the specimen at the time of implantation, was ischemic heart disease in 13 and dilated cardiomyopathy in 12 cases. One patient suffered from acute myocarditis. All patients had an additional implantable cardioverter defibrillator (ICD) implanted and were on heart failure medication after implantation of the device. Echocardiographic assessment was carried out as routine outpatient care in order to titrate the optimal pump speed for each patient. Major parameters for the pump speed were prevention of left ventricular enlargement and suction events of the device, and if possible, to allow opening of the aortic valve. Pump speed was set between 9,000 and 11,000 rpm according to these factors in a patient dependent fashion.

The analysis was repeated in 23 patients of the original cohort. Meanwhile, in 12 patients the device had been removed for transplantation (n=10) or recovery (n=2) (Table 2). Twelve patients continued with the device, one of them was unavailable for further follow up, and two patients died, Figure 1.

Anticoagulation regimen

After implantation of the LVAD, continuous infusion of heparin was initiated within 24 hours when the chest tube drainage was less than 50 ml/hour. After removal of drains and implantation of an ICD, Phenprocoumon (Roche, Grenzach – Wyhlen, Germany) was given orally with a target INR of 2.5±0.5. For platelet inhibition acetylsalicylic acid (ASS, 100 mg/d) was added to the medication on postoperative day 3. When a diagnosis of vWS was made, ASS medication was discontinued.

Blood collection and laboratory assays
In patients with LVAD support, blood samples were collected during an outpatient visit. Platelet related hemostasis was tested with a platelet function analyzer (PFA-100, Dade International) by determining closure times of epinephrine and adenosine diphosphate cartridges. Based on the work of Kratzer and Born [15], the analyzer is a high shear stress system for in vitro testing of platelet function that simulates primary hemostasis after injury to a small vessel. Platelet inhibition by Aspirin can be distinguished from arachidonic acid-independent processes. Furthermore this technique represents a highly sensitive way to screen patients for a von Willebrand defect (vWD) [16]. The closure time (CT) is measured by a capillary and a biologically active membrane coated with either collagen and epinephrine (C-EPI) or collagen and adenosine diphosphate (C-ADP), as an aspirin independent test. The normal value is less than 160 sec for the test with C-EPI and 121 sec for C-ADP.

On the day of platelet function analysis, blood was also sampled for plasma vWF:AG, for the functional analysis of vWF by measuring vWF:CB ratio, and for electrophoresis to determine the multimeric structure of plasma vWF. All samples were obtained from citrated blood and centrifuged at 4,000 rpm for 5 minutes. The vWF:AG was measured using the vWF Antigen Assay (Gradipore, Frenchs Forest NSW, Australia) following the method described by Mazurier [17] (normal value 50 - 160%). The vWF:CB was measured by an enzyme linked immunosorbent assay (ELISA) using a collagen binding assay, as previously described by Brown and Bosak [18]. The ratio of vWF:CB and vWF:AG was calculated. A decreased ratio of \( \leq 0.8 \) was considered a surrogate marker of acquired vWS. SDS-agarose discontinuous gel electrophoresis was carried out essentially as described (Figure 2) Briefly, medium (1.6%) and low resolution (1.2%) gels (LGT agarose type VII, Sigma, Munich, Germany) were prepared. Electrophoresis was performed for 16 hr at 55 volts. VWF multimers were transferred to nitrocellulose filters by electroblotting using transfer buffer...
(0.05M phosphate, pH7.4 with 0.04M SDS, without methanol). Visualization of the multimers was performed by videodensitometry. The filters were incubated twice for approximately 30 sec in buffer (20 mM Tris/HCl, 500 mM NaCl, pH 7.5) and thereafter placed into the video-detection system (Fluorchem™, Alpha Innotech Corp. San Leandro, CA) consisting of a dark housing, a sensitive, cooled (-30°C) CCD camera and software generating 12-bit computer graphics. The filters were overlaid with 5 ml solution containing 0.4 mg/ml luminol (Sigma-Aldrich Chemie, Steinheim, Germany), 0.01 mg/ml 4-iodophenol (Sigma-Aldrich Chemie, Steinheim, Germany) and 2.5 μl/ml 30% H2O2 (Perhydrol, Merck, Darmstadt, Germany) in Tris buffer (20 mM Tris/HCl, 500 mM NaCl, pH 7.5). Typical exposure times were between 30 and 60 sec [19].

In subsamples of 6 patients, the activity of vWF cleaving protease ADAMTS13 and the antigen level were analysed by a modified fluorescence resonance energy transfer assay (FRETS-test, Technozym ADAMTS-13, Technoclone GmbH, Vienna, Austria) [20].

**Screening for bleeding events**

Each patient’s bleeding history was evaluated at every outpatient visit using a standard screening questionnaire. All bleeding episodes during the outpatient follow-up were summarized.

**Statistics**

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, Ill). Distribution of relevant variables was summarized using frequencies, medians and range, as appropriate. Bivariate correlation (Spearman-Rho) was carried out to define correlations between time on the device and levels of vWF:AG and vWF:CB. All probability values were
obtained using two-sided analyses. A probability value of 0.05 or less was considered statistically significant for all analyses.

**Results**

In the first analysis with all patients being on the LVAD, the PFA 100 revealed pathologic high closure times (>250 s) in all samples using both epinephrine and adenosine diphosphate cartridges, independent of any patient dependent factor such as gender, diagnosis and time on the LVAD (Table 1). The vWF:CB ranged from 64 - 372% with a median value of 134%. There also was a significant decrease in vWF:CB with respect to time on the device (correlation coefficient – 0.51; p=0.007). In 10 patients, a decreased ratio of the vWF:CB/vWF:AG was determined. In all samples, a loss of the large multimers was found. A quantitative examination by densitometry was done in 54% (Table 1). Normal values of vWF cleaving protease ADAMTS13 activity (median 89%, range 65 - 121%) and antigen (median 0.98 µg/ml, range 0.61 - 1.67 µg/ml) were reported.

The total follow up period was 48 patient-years, ranging from 4 to 53 months, with a median of 28 months. During this time, there were no single hemorrhagic or ischemic cerebrovascular events. One patient died due to mesenteric ischemia four months after implantation of the LVAD and one died due to sepsis after 40 months. In one patient, a pump thrombosis occurred after Phenprocoumon was terminated for gastrointestinal bleeding. The patient was referred to our center and received a heart transplant due to the high urgency of the case. In a second patient, a pump thrombosis was suspected due to signs of hemolysis. This condition resolved after temporary anticoagulation therapy with Lepirudin (Pharmion, Hamburg, Germany).
All bleeding events occurring after discharge of the patient from LVAD implantation are summarized in Table 3. The median follow-up time of bleeding onset in outpatients was 6 months with a range of 1 to 19 months. At the time of any bleeding event, no patient was thrombocytopenic. In all but one patient, INR was within range. For patient No 1 with gastrointestinal bleeding the last INR before signs of bleeding was 2.90. At emergency admission a lower INR of 1.53 was noted. In total, 8 bleeding episodes were recorded within 48 patient-years leading to a calculated incidence of 0.17 per patient year (95% confidence interval 0.03 – 0.31). These bleeding events ranged from epistaxis to life threatening gastrointestinal bleeding. In the three patients with gastrointestinal bleeding, endoscopic examination revealed the source to be distal of the duodenum in the small bowel.

To date, 10 successful heart transplantations were performed in 10 patients of this cohort. In two patients, the device was explanted for myocardial recovery. Blood samples were taken in median 14.5 months (range 2 - 34 months) after device removal (Table 2). Gel electrophoresis displayed all fractions of multimers of the vWF in all patients. Quantitative analysis of multimers was done in 9 patients. In these patients, the percentage of high molecular weight multimers was normal (median value 27.8%, range 20.1% to 34.1% vs 26.63%, range 22.1% to 33.2% in normal pool plasma). The ratio of vWF:CB/vWF:AG was within the normal range in 10 patients, but reduced in one patient and borderline in another patient.

Platelet function testing was done in 11 patients. The test with adenosine diphosphate was within normal range in all but one patient, who suffered from a severe pulmonary infection at the time of investigation.

Except for 2, the remaining patients (n=12) are still receiving therapy with the LVAD. A second analysis of platelet function and vWF diagnostics were carried out in 11 of these
Discussion

An acquired loss of large vWF monomers leads by definition to the diagnosis of vWS. These monomers were missing in all samples taken from our patients on LVAD support. Since data prior to implantation of the device are missing, it is an inherent limitation of this study, that the acquisition of vWS is not directly provable. However, after the removal of the device in all 12 patients restoration of normal vWF monomer pattern was found. This indirect evidence supports our hypothesis that not only a vWS is present in all patients being supported with the HeartMate II axial flow device, but also that it is an acquired form which resolves after removal of the device. The ratio of vWF:CB/vWF:AG used as a surrogate marker for acquired vWS was pathological in 10 out of 26 patients in the first analysis and in 10 out of 11 patients in a repeat analysis 15.5 months later. We chose a threshold of <0.8 for the ratio because it showed a sensitivity of 80% for the detection of vWS. At a threshold of <0.7, used by other investigators, sensitivity was only 46% [21]. The vWF:CB/vWF:AG ratio has been shown to perform at least equal to the measurement of ristocetin cofactor activity, as long as suitable collagens are used [22]. The vWF:CB/vWF:AG ratio used here, was the preferred method due to a higher level of precision. However, a limitation of this approach is that other abnormalities in addition to the presence or absence of large multimers were not detected in our analysis.

The clinical events of spontaneous bleeding were also similar to the findings in patients with acquired vWS due to aortic stenosis [10]. Mucosal bleeding predominates;
however, in some patients more severe gastrointestinal bleeding occurs. The similarity to patients with acquired vWS and aortic stenosis may be explained by the fact that blood is exposed to high shear stress, either by the aortic stenosis or the LVAD. Cessation of life threatening gastrointestinal bleeding episodes have been found after removal of the source of high shear stress, the aortic stenosis [23] or the LVAD [24], respectively. Geisen et al [11] report non-surgical bleeding in patients with VADs in association with acquired vWS and Steinlechner et al reported the loss of large vWF multimers in 12 outpatients with rotary blood pumps except HeartMate II [12]. These findings indicate that acquired vWS is present in patients with differently designed rotary blood pumps and not related to one particular design. Platelet dysfunction unrelated to the vWF was also described in this study. Other studies emphasize the potential role of leukocyte-platelet interaction [25].

Impaired platelet aggregation was also described by Klovaite et al [13] in a cohort of 12 patients being supported by the HeartMate II assist device. This group also found the absence of large vWF monomers in patients on support and restitution after heart transplantation. The authors also concluded that implantation of a HeartMate II LVAD is associated with impaired platelet aggregation and induction of an acquired vWS type 2.

The effects on primary hemostasis increase the risk for bleeding events, especially in combination with anticoagulation and platelet inhibitors. Individual confounding patient factors may be an underlying cause of the actual bleeding, for example mucosal defects, angiodysplasias, injuries and infection. To determine these factors, large longitudinal studies are required. Despite this, the findings of this investigation may have implications for the patient’s treatment and care management: Dental procedures or other surgical interventions should be performed in the knowledge that a coagulation disorder is present, in addition to anticoagulation treatment. Therefore, special care in terms of local bleeding control, antifibrinolytic therapy and substitution of coagulation factor concentrate are recommended.
[26]. As a consequence, we consider our outpatients as a high-risk group, with respect to potential bleeding complications when all surgical interventions are performed.

In life threatening bleeding episodes or planned surgical (dental) interventions, supplementation with FVIII/vWF concentrate may be necessary [27]. With the improvements made in designing LVADs mechanically stable enough to provide support for many years and the demographic changes in the western population, it can be expected that these devices will not only be used as a bridge to transplantation, but also as an alternative to heart transplantation [28]. While hemolysis avoidance has already been achieved in the design of modern rotary blood pumps, future efforts must address the challenges of long term support including the impact of the devices on inflammation, platelet function and the coagulation system.

As pump thromboses still occur in some patients after LVAD implantation, a result of the withdrawal of anticoagulation medication and/or the activation of platelets [29] in patients with various types of VADs, most centers choose an anticoagulation regime consisting of Coumadin (Bristol-Myers Squibb, Munich, Germany) or Phenprocoumon and a platelet inhibitor. However, a more adapted anticoagulation in LVAD therapy is warranted due to results and changes of the coagulation system.
Disclosures

Martin Strueber was a primary investigator in the HeartMate II Pilot trial and member of the European Advisory board of Thoratec.

Martin Strueber was a primary investigator in the HeartWare HVAD trial.

Mark Slaughter: research/grant support Thoratec Corporation.

Anna L. Meyer, Doris Malehsa, Christoph Bara, Ulrich Budde and Axel Haverich have none disclosure.
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Table 1: Patient characteristics and results of the von Willebrand diagnostics

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<th>vWF:CB (%)</th>
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<th>Loss of largest vWF MM (%) vs.pool</th>
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Abbreviations from Table 1 and Table 2:

1. von Willebrand factor antigen
2. von Willebrand factor collagen binding capacity
3. ratio of von Willebrand factor collagen binding capacity and von Willebrand factor antigen
4. von Willebrand factor multimers
5. multimers
6. platelet function analysis with collagen and epinephrine
7. platelet function analysis with collagen and adenosine diphosphate
8. hematocrit
9. C-reactive protein
10. Coronary heart disease
11. Bridge to Transplant
12. Dilated cardiomyopathy
13. Destination Therapie

* selected patients for Table 2
Table 2: Results of von Willebrand diagnostics after LVAD explantation / heart transplantation

<table>
<thead>
<tr>
<th>Nr</th>
<th>Time after Ex\textsuperscript{14}/Tx\textsuperscript{15} (months)</th>
<th>vWF:AG\textsuperscript{1} (%)</th>
<th>vWF:CB\textsuperscript{2} (%)</th>
<th>Loss of largest vWF MM\textsuperscript{4}</th>
<th>vWF:CB/ vWF:AG\textsuperscript{3}</th>
<th>vWF MM\textsuperscript{4} (% vs.pool)</th>
<th>PFA C-EPI\textsuperscript{6} (sec)</th>
<th>PFA C-ADP\textsuperscript{7} (sec)</th>
<th>Platelet Hct\textsuperscript{8} (1,000/μl)</th>
<th>CRP\textsuperscript{9} (mg/l)</th>
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Abbreviations from Table 2:

14. explant
15. transplant
16. not done
Table 3: Bleeding complications and thromboembolic events after LVAD implantation

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<th>Thromboembolic event</th>
<th>INR</th>
<th>Platelet (1,000/µl)</th>
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Figure Legends

**Figure 1:** Time and study flowchart of patients inclusion, follow up and diagnostic studies.

**Figure 2:** Normal plasma (#2 and #3) and plasma from a patient on LVAD (#1) in a discontinuous gel of medium (1.6% LGT-agarose) resolution. The dye front is at the bottom of the gel and the large multimers are found in the upper part of the gel. Lane 2 = normal plasma (pool of 30), lane 3 = plasma sample from a patient without vWD, lane 1 = patient on LVAD with a loss of the large multimers and slightly increased proteolytic sub-bands. The arrow shows the border between the intermediate (6 - 10) and large (>10) multimers.
LVAD implantation (n=26)

PFA and von Willebrand diagnostic (n=26) median 4.5 mts (1-24) post implantation

LVAD ongoing (n=11) median 15.5 mts (7-25) post first sampling

n=2 died

n=1 not available for follow up

LVAD explantation for HTx (n=10) or recovery (n=2) median 16.5 mts (8-41) post implantation

PFA (n=11) and von Willebrand diagnostic (n=12) median 14.5 mts (2-34) post explantation
Acquired von Willebrand Syndrome in Patients with an Axial Flow Left Ventricular Assist Device
Anna L. Meyer, Doris Malehsa, Christoph Bara, Ulrich Budde, Mark S. Slaughter, Axel Haverich and Martin Strueber

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