Acquired Von Willebrand syndrome is an early-onset problem in ventricular assist device patients

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Abstract

Objective: Acquired Von Willebrand syndrome (AVWS) can contribute to bleeding complications in patients with ventricular assist devices (VADs). AVWS results from shear stress, which causes unfolding of the high-molecular-weight (HMW) multimers of Von Willebrand factor (VWF) with subsequent cleavage. Loss of the HMW multimers of VWF is the leading finding in AVWS. In consequence, binding of VWF to collagen and to platelets is impaired. The onset of AVWS after VAD implantation is not yet determined. We examined VAD patients for presence of an AVWS in the early, intermediate, and late phase after VAD implantation.

Methods: Patients with a biventricular Thoratec-PVAD® (BVAD, n = 6) or a left-ventricular HeartMateII® (HMII, n = 11) were analyzed prior to VAD implantation and after 1, 3, 14, 30, and 60 days. Diagnosis of AVWS based on VWF:ristocetin cofactor activity/VWF:VWF antigen (VWF:RCa/VWF:Ag), collagen-binding capacity:VWF antigen (VWF:CB/VWF:Ag), and multimeric analysis. In addition, we analyzed the number of bleeding episodes, which required surgical intervention.

Results: No patient had an AVWS prior to VAD implantation. An AVWS was identified already in the very early postoperative period, that is, in almost all patients on the first day and in all patients on the third day. The AVWS was also detected in the majority of patients in the further course. Nine of all 17 patients suffered bleeding complications and required a total of 25 interventions due to hemorrhages. Forty percent of re-interventions were carried out within the first 10 days after implantation; five of these were necessary within the first 24 h.

Conclusion: The AVWS is present already in the early postoperative phase after VAD implantation. Therefore, reduced shear stress has to be an important feature of newly developed assist devices in the future.

Keywords: Ventricular assist devices; Hemostasiology; Acquired Von Willebrand disease

1. Introduction

Acquired Von Willebrand syndrome (AVWS) can contribute to bleeding complications in patients with ventricular assist devices (VADs) [1–4]. AVWS results from shear stress in the pump itself and in the tubes that connect the pump to the heart and the great vessels. This shear stress causes unfolding of the high-molecular weight (HMW) multimers of Von Willebrand factor (VWF) with exposure of the D2 domains of the VWF monomers and subsequent cleavage by the protease ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin motif) [5]. Loss of the high-molecular weight (HMW) multimers of VWF is the leading finding in AVWS. In consequence, binding of VWF to collagen of injured vessel walls and to the GPIb/IX receptor of the platelets is impaired. Recently, the reversal of AVWS after explantation of the VAD has been described [2,4]. However, the onset of AVWS after VAD implantation is not yet determined. We examined patients with a biventricular Thoratec-PVAD® (BVAD) or a left-ventricular HeartMateII® (HMII) for presence of an AVWS in the early, intermediate, and late phase after VAD implantation.

2. Materials and methods

2.1. Patients

Patients with a biventricular PVAD® (paracorporeal ventricular assist device, Thoratec Corporation, Pleasonton,
CA, USA; VAD, \( n = 6 \) or a left-ventricular HeartMate II \(^{\text{TM}}\) (Thoratec Corporation; HMII, \( n = 11 \)) were analyzed prior to VAD implantation and after 1, 3, 14, 30, and 60 days for laboratory data. Patients with HMII support combined with a temporary right-ventricular assist device, and all patients with incomplete laboratory data per time point or with any indication for AVWS prior to VAD implantation were excluded (Table 1). In addition, bleeding episodes requiring surgical intervention were evaluated for all 17 patients.

The retrospective use of data was approved by the institutional ethics committee; all prospectively enclosed patients provided informed consent.

### 2.2. Laboratory analyses

Sodium citrate plasma was obtained by centrifugation at \( 1500 \times g \) for 15 min at \( 20^\circ \text{C} \) and analyzed within 4 h after blood drawing or stored at \(-80^\circ \text{C} \). VWF antigen (VWF:Ag; Siemens Healthcare Diagnostics) and ristocetin cofactor activity (VWF:RCo; Siemens Healthcare Diagnostics) were measured using the Analyser Behring Coagulation System (BCS), according to standard procedures. Collagen type I (Nycoderm Pharma, Unterschleissheim, Germany) was immobilized on a microtiter plate, and collagen-binding capacity (VWF:CB) in plasma was determined photometrically using the enzyme-linked immunosorbent assay (ELISA) technique. We calculated the ratios of VWF:RCo and VWF:CB, respectively, to VWF:Ag (VWF:RCo/VWF:Ag, normal: \( >0.65 \), VWF:CB/VWF:Ag, normal: \( >0.7 \)). These values reflect the biological activity of the available VWF with regard to binding to platelets and to collagen.

VWF multimers were separated on sodium dodecylsulfate (SDS)-agarose low-resolution gels (1.0% agarose) and blotted on a poly(vinylidene fluoride) (PVDF) membrane to assess the HMII multimers. VWF was detected using the appropriate primary and secondary antibodies (DAKO, Hamburg, Germany) and stained with 3,3’-diaminobenzidin/cobalt chloride (Bio-Rad, Munich, Germany). Standard human plasma (Siemens Healthcare Diagnostics, Eschborn, Germany) was used for controls. An AVWS was diagnosed if HMII multimers were missing and at least one functional VWF ratio was below the normal range [6–9].

INR (International normalized ratio, Innovin\(^{\text{TM}}\) Siemens Healthcare Diagnostics, Eschborn, Germany), activated partial thromboplastin time (aPTT) (Pathromtin SL\(^{\text{TM}}\), Siemens Healthcare Diagnostics, Eschborn, Germany), hemoglobin (Hb), hematocrit (Hk), platelet counts, C-reactive protein (CRP), and factor VIII (FVIII) activity were determined employing laboratory standard protocols.

### 2.3. Surgical procedures

VADs were implanted according to usual surgical techniques, as previously described [1,10]. The HMII is operated at nearly 9000 rpm. Pumping frequency of the BVAD is adjusted to the patient’s physiological range, and a systolic ejection time of 300 ms is aimed at. Anticoagulation is started for both systems with heparin (or hirudin in patients with heparin-induced thrombocytopenia) with a target aPTT of 60–80 s, and is changed to phenprocoumon after removal of the chest drains and sufficient oral ingestion. Target INR for patients with HMII is 2.0–3.0, and for the BVAD 3.0–3.5. Platelet aggregation is inhibited by acetylsalicylic acid (ASA) 100 mg day\(^{-1}\).

### 2.4. Statistics

The program PASW (Predictive Analytics SoftWare) Statistics 18 (SPSS Inc., Chicago, IL, USA) was used for all calculations. Non-parametric tests were employed due to the small size of the groups. For comparison of paired data, the Friedman test was executed first. The Wilcoxon signed ranks test was applied to the separate combinations of time points within the groups. Corrections for multiple testing in post hoc comparisons of time points were not applied. The Kruskal–Wallis test and the Mann–Whitney U test were employed for comparison of unpaired data, accordingly.

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**Table 1. Number of patients with VAD (on device) and with complete laboratory analysis (analyzed) at the different time points.**

<table>
<thead>
<tr>
<th></th>
<th>BVAD On device</th>
<th>BVAD Analyzed</th>
<th>HMII On device</th>
<th>HMII Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preop</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Day 1</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Day 3</td>
<td>6</td>
<td>4</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Day 14</td>
<td>5</td>
<td>5</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Day 30</td>
<td>3</td>
<td>2*</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Day 60</td>
<td>3</td>
<td>2*</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

* One of the two patients is the same at both time points, the other data set is obtained from two different patients.

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**Fig. 1. Outcome of VAD patients. Black triangles, BVAD, white rhombs, HMII.**
3. Results

The outcome of the patients is presented in Fig. 1. Diagnosis of AVWS was based on VWF:RCo/VWF:Ag, VWF:CB/VWF:Ag, and multimeric analysis.

No patients had an AVWS prior to VAD implantation.

Early on, on the first day after implantation, five of six patients with a BVAD and 10 of 11 with a HMII exhibited AVWS. At day 3, all tested patients (BVAD, n = 4, HMII, n = 8) were affected. In the intermediate phase, after 2 weeks, four of five BVAD patients and all seven analyzed HMII patients had AVWS. Later, an AVWS was diagnosed in both BVAD patients, and in all nine HMII patients after 30 days, and in both BVAD and all four HMII patients after 60 days. Distributions of functional properties of VWF are shown quantitatively in Fig. 2(A) and (B) and qualitatively with regard to patient numbers in Table 2. Of note, all patients with normal values for both VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag had normal HMW multimers.

VWF:RCo/VWF:Ag reflects VWF binding to platelets. It did not differ between the time points (BVAD, p = 0.119, HMII, p = 0.51) and between the devices at the most. Only on day 3 after implantation, BVAD patients had lower values than those with HMII (p = 0.033) (Fig. 2(A)). For VWF:CB/VWF:Ag, which mirrors VWF binding to collagen, preoperative values in HMII patients were higher than values on days 1, 3, 14, and 30 (p = 0.022, Fig. 2(B)). There were no differences within the BVAD group (p = 0.107) and between both VAD groups at all time points (Fig. 2(B)). Fig. 3(A) displays the counts for presence or absence of HMW VWF multimers, Fig. 3(B) shows an example of a VWF multimer blot of a BVAD patient prior to and on the first day after VAD implantation.

Accompanying laboratory data are shown in Table 3.

All of the six BVAD patients suffered bleeding complications and required a total of 18 interventions. Seven re-explorations due to bleedings were necessary in three of the 11 HMII patients. Ten of 25 (40%) of the interventions were carried out within the first 10 days after implantation; five of these were necessary within the first 24 h (Table 4).

4. Discussion

Bleeding events are still a typical complication in VAD patients [11,12] and can occur due to an AVWS [1—4]. We analyzed patients with a biventricular Thoratec-PVAD® (BVAD) or a left-ventricular HeartMateII® (HMII) for the presence of AVWS and for bleeding complications from the first day to 2 months after implantation. An AVWS was

![Fig. 2. Ratios of ristocetin-cofactor activity (VWF:RCo, A), and collagen-binding capacity (VWF:CB, B), respectively, to VWF antigen (VWF:Ag). White bars, BVAD; grey bars, HMII. Circles and asterisks mark outliers and extreme values, respectively. Double-crosses # indicate p < 0.05.](image-url)
Table 3. Laboratory data prior to and after VAD implantation (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Parameter normal</th>
<th>Device</th>
<th>Prior</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 14</th>
<th>Day 30</th>
<th>Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>BVAD</td>
<td>2.3</td>
<td>1.2</td>
<td>1.6</td>
<td>0.5</td>
<td>2.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.6–1.5 U/l</td>
<td>HMII</td>
<td>2.2</td>
<td>0.5</td>
<td>2.2</td>
<td>0.6</td>
<td>2.6</td>
<td>0.8</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>BVAD</td>
<td>2.3</td>
<td>1.3</td>
<td>1.4</td>
<td>0.6</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>0.5–1.8 U/l</td>
<td>HMII</td>
<td>2.2</td>
<td>0.5</td>
<td>1.7</td>
<td>0.3</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>BVAD</td>
<td>2.1</td>
<td>1.3</td>
<td>1.1</td>
<td>0.6</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>0.6–1.5 U/l</td>
<td>HMII</td>
<td>2.3</td>
<td>0.6</td>
<td>1.3</td>
<td>1.1</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>FVIII:C</td>
<td>BVAD</td>
<td>0.8</td>
<td>0.4</td>
<td>1.1</td>
<td>0.2</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>0.7–1.6 U/l</td>
<td>HMII</td>
<td>1.9</td>
<td>0.4</td>
<td>1.5</td>
<td>0.3</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Platelets</td>
<td>BVAD</td>
<td>87.5</td>
<td>43.7</td>
<td>85.2</td>
<td>66.8</td>
<td>82.3</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>HMII</td>
<td>190.5</td>
<td>86.2</td>
<td>124.2</td>
<td>32.3</td>
<td>94.6</td>
<td>36.6</td>
</tr>
<tr>
<td>140–400 × 1000/μl</td>
<td>HMII</td>
<td>35.7</td>
<td>6.0</td>
<td>27.5</td>
<td>2.5</td>
<td>29.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

VWF:Ag, Von Willebrand factor antigen; VWF:RCo, ristocetin cofactor activity of VWF; VWF:CB, collagen binding capacity of VWF; FVIII:C, factor VIII activity; aPTT, activated thromboplastin time; CRP, C-reactive protein; HB, hemoglobin; HKT, hematocrit

Table 4. Surgical interventions due to bleeding events within first 10 days (d1–d10) and two months (d11–d60) after VAD implantation.

<table>
<thead>
<tr>
<th>Number of bleeding patients</th>
<th>Total number of bleeding events</th>
<th>Events per bleeding patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/11</td>
<td>3/2</td>
<td>3 ± 2 (1–6)</td>
</tr>
</tbody>
</table>

identified already in the very early postoperative period, that is, in almost all patients on the first day and in all patients on the third day. The AVWS was also detected in the majority of patients in the further course. These observations are in accordance with our previous analysis in a small cohort [2].

Bleeding events that require surgical revisions are especially worrying for VAD patients, even when they can be treated using endoscopic techniques in some cases [13]. Recently, Genovese et al. [14] analyzed the adverse events of 195 patients with VAD support within 60 days after implantation. Bleeding events occurred in almost 50% of the patients, and nearly 25% of the patients required a re-operation due to bleeding. The majority of all bleedings, that is in nearly 40% of all patients, was observed within 10 days. John et al. [15] reported surgical revisions due to bleeding in 16% of their HMII patients but omitted events on the day of surgery, which are included in our analysis, and Klovais et al. [4] observed bleedings in 75% of their HMII patients. Our observations of bleeding events requiring re-interventions are in the same range. Nine out of 17 patients suffered such bleedings within the first 2 months, and 40% of bleedings occurred within the first 10 days after VAD implantation.

The pneumatic VAD system with pulsatile flow and the axial HMII with non-pulsatile flow do not seem to differ significantly in the severity of the AVWS, according to the laboratory data. We consider this a relevant finding of the study. It indicates that AVWS is one but not the only reason for bleeding complications. We observed bleeding episodes more frequently in BVAD than in HMII patients, both in the early and in the later course. This clinical effect could be explained by the facts that BVAD patients suffer from more severe heart failure, have a more severely compromised liver function, undergo a more extended surgical procedure with higher consumption of coagulation factors, and receive a more aggressive anticoagulation therapy.

Whereas investigators [16] compared patients with the left-ventricular pulsatile-flow HeartMate XVE with HMII patients and observed an AVWS only in the latter, the debate of the overall advantages and disadvantages of pulsatile and non-pulsatile devices is still ongoing [17–21].

In patients with hereditary Von Willebrand syndrome type 1, the most prevalent form of VWS, ristocetin cofactor activity and collagen-binding capacity correlate to VWF:Ag measurements due to reduced VWF. In AVWS, however, the diagnostic value of the single parameters is limited. VWF:Ag is an acute-phase protein and is therefore often enhanced in VAD patients, which was also observed in our study population. Of note, VWF:RCo/VWF:Ag has been shown to vary in a larger extent than VWF:CB/VWF:Ag [22]. This is in accordance with our results. We found pathologic VWF:RCo/VWF:Ag ratios in 18 out of 55 cumulative tests with missing HMW multimers of VWF, but pathologic VWF:CB/VWF:Ag values in all of them. This high sensitivity of the collagen-binding capacity test at our institution is probably due to the employment of an ELISA with collagen type I. Collagen type I has been shown to have a higher affinity for HMW multimers.
of VWF compared with collagen type III [23], and is therefore more discriminative for AVWS. Multimeric analysis can prove the AVWS but it is a time-consuming task and requires an experienced investigator for evaluation of the results.

In conclusion, AVWS is present already in the early postoperative phase after VAD implantation. Therefore, reduced shear stress has to be an important feature of newly developed assist devices in the future.

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References


Fig. 3. Presence of HMW multimers of VWF. White bars, normal; grey bars, HMW multimers missing (A). Typical VWF multimer blot of a HMW patient prior to (pre) and on the first day after VAD implantation (B). Note the loss of HMW multimers at VAD support (arrow). SHP, standard human plasma.


Appendix A. Conference discussion

Dr M. Stalder (Bern, Switzerland): The authors present a series of 17 patients who received a ventricular assist device. In six patients a biventricular extracorporeal assist device, and in the other 11 patients an intracorporeal left ventricular assist device, was implanted. They analyzed the onset of an acquired von Willebrand syndrome after implantation which was not present before implantation in any of the patients. They could show that all patients developed an acquired von Willebrand syndrome at a very early stage, between days 1 and 3 postoperatively, and they interpreted this as the consequence of shear stress which occurs in the assist device pumps. This is an important finding, as it is well known that an acquired von Willebrand syndrome plays a major role in bleeding complications after cardiac surgery. However, the authors did not find any differences between types of pump, axial continuous flow pump versus pulsatile pump, in relation to severity of the syndrome. In contrast, bleeding events were more frequent in BiVAD patients. This shows that the acquired von Willebrand syndrome is only one factor out of many others, as you stated before, contributing to bleeding disorders after VAD implantation. It would be interesting to know whether systematic use of desmopressin would reduce bleeding rates after VAD implantation. Did you use desmopressin during your study?

Dr Heilmann: We do not use desmopressin regularly because the von Willebrand factor is an acute-phase protein and we assume that the stores of the patients are already depleted, so it wouldn’t have as much of an effect as we would like. In patients other than those with VAD implantation, we use it if they present with bleeding for any reason prior to operation.

Dr Stalder: The second question would be at what time was heparin started after surgery, as this may also have an impact on postoperative bleeding.

Dr Heilmann: We start the heparin on the first day because we are afraid of thromboembolic events. The margin between coagulation and bleeding is really narrow but, in our experience, the heparin does not influence acquired von Willebrand syndrome.

Dr Stalder: Third, it seems obvious that shear stress has negative effect on different blood components. However, your conclusion that shear stress is caused only by the pumps cannot be reached from your data since you did not show any differences between two kinds of completely different pumps. Can you comment on this?

Dr Heilmann: We are aware of that fact. But you have the different devices: you have the suction in the BVAD system, and you have really high rotational speed in the axial system. In our opinion, the cannulas also play a role, because we found that patients with a total artificial heart did not develop acquired von Willebrand syndrome. We were astonished at that. Moreover, the patients who need a BVAD are much sicker, they have more liver Insufficiency and a lot of other problems. Maybe that is the reason for the higher bleeding rate.

Dr T. Krabatsch (Berlin, Germany): Was pump speed measured? Do you have any experience? Is it a critical value, that the incidence goes up or down?

Dr Heilmann: We couldn’t test that because we have about the same pump speed due to using only two rotational systems. We found that the VentAssist patients had less acquired von Willebrand syndrome. So probably the speed plays a role, but it is not the only reason for the shear stress. I’m a bit cautious about this trend toward miniaturization of pumps. I think the shear stress of a pump should be calculated or tested before it goes into practice.

Dr G. Wieseltiether (Vienna, Austria): I think the conclusion that you draw is probably not absolutely correct. Due to the fact that we see a lot of bleeding postoperatively due to various factors, and actually you mentioned that, I think acquired von Willebrand factor disease in the long run plays that kind of role, that we see right now. That means spontaneous onset of bleeding in patients who have been on the pump for, let’s say, 200, 300, 400 days probably is what is of most interest for the majority of us. Bleeding in the early postop period can be quite natural. So the question is always, is it really the diminished multimers of von Willebrand in the long run that causes bleeding? We all know that probably you have to have AV malformations in order to bleed. You have been doing testing over a long period of time, patients who have been on the pump for a long period of time. My question is, in terms of quantity of the reduction or diminished von Willebrand multimers, is it always diminished to a certain extent, or are there some days when they really drop down to nearly no multimers at all? This could then mean that on those days, they are at an extremely high risk of bleeding, whereas otherwise, if there are just a little diminished numbers of multimers, they don’t bleed. We have no clue at the moment, at least to my knowledge, why people start to bleed.

Dr Heilmann: Of course, to comment on the early period, there are many reasons, but we think that the acquired von Willebrand syndrome is really one of the reasons in the early period. It comes with a large wound area, it comes with liver Insufficiency, it comes with a lot of problems, but it is really a problem early on. Humate and platelets help, as they are supposed to do. In the long run, we have a lot of data and we have done numerous Western blots of the multimers. We have the loss of the large high-molecular weight multimers throughout the course. As long as we test, it is always the same pattern. The smaller or intermediate size multimers of the von Willebrand factor are not lost, they are always there. Sometimes in the later period, we have a little less loss of the multimers. The von Willebrand factor is an acute-phase protein, but the ADAMTS13 is regulated by inflammatory processes, and maybe you get a balance between the molecules, but you cannot speculate on this. We don’t know what patients will achieve that balance, or if the cannula positions itself in a benign way. We still don’t know. We look for that.

Dr Wieseltiether: Unfortunately, I think this is the truth. Nobody knows it at the moment.