Platelet dysfunction and acquired von Willebrand syndrome in patients with left ventricular assist devices

Maral Baghai, Claudia Heilmann, Friedhelm Beyersdorf, Lea Nakamura, Ulrich Geisen, Manfred Olschewski and Barbara Zieger

OBJECTIVES: Unexplained bleeding events are a severe complication in patients with left ventricular assist devices (LVADs). Platelet dysfunction and acquired von Willebrand syndrome (AVWS) may contribute to bleeding tendencies. Yet, comprehensive data with respect to platelet function and AVWS in LVAD patients in terms of bleeding events are scarce.

METHODS: Thirty-nine HeartMate II patients were included in this study. Data of at least two time points were available for each patient. Platelet function was analysed via light transmission aggregometry in 19 patients without LVAD, 28 in early (≤14 days) and 30 in late post-implantation states (≥30 days). Von Willebrand factor (VWF) antigen, VWF collagen binding capacity and VWF multimeric analyses were performed in 26 patients without LVAD, 39 in early and 33 in late postimplantation states to diagnose AVWS. Bleeding complications were recorded for 39 patients in the early and 33 in the late postoperative period.

RESULTS: Platelet dysfunction was detectable in 18 of 19 without LVAD and in all patients following LVAD implantation. Platelet aggregation values did not change over time (without-early, \( P = 0.27, n = 14 \); early-late, \( P = 0.17, n = 21 \)). AVWS was not diagnosed in patients without LVAD, except for one. On LVAD, 33 of 39 patients had AVWS in the early and all in the late period \( (n = 33) \). Bleeding events occurred in 44% of patients in the early and in 64% of patients in the late period.

CONCLUSIONS: According to our data, platelet aggregation is often impaired in LVAD patients even without an implanted LVAD. Additionally, appearance of AVWS seems to be closely linked to LVAD implantation.

Keywords: Circulatory assist devices • Platelets • Bleeding • Acquired von Willebrand syndrome

INTRODUCTION

Mechanical circulatory support is a therapeutic option for patients with end-stage heart failure. However, thromboembolism and bleeding events are still the most common complications after implantation of a left ventricular assist device (LVAD) [1, 2]. Thromboembolic episodes occur due to overactivation of haemostasis. Their prevention requires adequate anticoagulation. Uncontrolled bleeding events are a common side effect of this treatment. However, not all bleeding symptoms can be expounded by the individual anticoagulation regimen alone. Previous studies indicated that bleeding events could be partly explained by impaired platelet function in patients with LVAD [3, 4]. Interaction of platelets with artificial material as well as pathological blood flow conditions are thought to be causes of platelet dysfunction [1]. However, comprehensive data of platelet function in LVAD patients with regard to bleeding events are scarce. In particular, the comparison of platelet function in LVAD patients both with and without implanted LVAD is only poorly investigated. In addition, an acquired von Willebrand syndrome (AVWS) might contribute to bleeding tendencies in LVAD patients [5, 6]. AVWS is a bleeding disorder which is characterized by loss of high molecular weight (HMW) multimers of von Willebrand factor (VWF) due to enhanced shear stress and implies impaired interaction of VWF with platelets [7, 8]. The purpose of this study is to investigate platelet function and AVWS in patients with and without LVAD.
METHODS

Patients

Thirty-nine patients with a left ventricular HeartMate II® (Thoratec Corporation, Pleasanton, CA, USA; HM II) were included in this study. All patients underwent LVAD implantation between March 2008 and November 2012 with a minimal postoperative observation period of 2 weeks. The analysis was performed without LVAD, in an early (<14 days) and in a late (≥30 days) postoperative state after LVAD implantation. Platelet function of patients was analysed via light transmission aggregometry in 19 patients without LVAD (prior to LVAD implantation, n = 11; after LVAD explantation, n = 8), as well as within 14 days (n = 28) and after at least 30 days following LVAD implantation (n = 30, Table 1). The analysis was rendered impossible by severe thrombocytopenia in 1 patient without LVAD, in 3 patients in the early and in 1 patient in the late postoperative state.

VWF function of patients was analysed in 26 patients without LVAD (before implantation, n = 18; after explantation, n = 8), in 39 patients in an early postoperative state and in 33 in a late postoperative state following LVAD implantation (Table 1).

Data of at least two time points were available for each patient. Bleeding complications were recorded for all 39 patients within the early (n = 39) and the late postoperative period (n = 33, Table 1). Bleeding complications were defined as any local or diffuse blood loss that led to haemoglobin levels lower than 8 g/dl requiring blood transfusions or reoperation. Patients with temporary right ventricular assist devices were excluded.

The data were obtained within the scope of our institutional monitoring programme on haemostatic changes during support by mechanical ventricular assist devices, which is approved by the ethics committee of the University of Freiburg. All patients provided informed consent before any data were used for research purposes.

Surgical procedures

HeartMate II (HM II) is a left ventricular intracorporeal assist device with continuous axial flow. Implantation was performed by standard techniques, as previously described [5, 9]. The HM II was typically operating at 9000 rpm. Anticoagulation was usually started with heparin after 48 h in the majority of LVAD patients with a target activated partial thromboplastin time (aPTT) of 60–80 s and was changed to phenprocoumon after removal of the chest drains and sufficient oral ingestion. The target international normalized ratio (INR) was 2.0–3.0. Acetylsalicylic acid (ASA) 100 mg/day was used in addition for inhibition of platelet aggregation when INR was stable at the target level. It was started earlier when prompted by concomitant diseases or was omitted when bleeding events occurred. Four of 26 patients without LVAD, 6 of 39 in the early and 10 of 33 patients in the late postimplantation phase took ASA.

Laboratory analysis

Light transmission aggregometry of platelets was performed using the APACT 4.0 aggregometer (BioMedical Technologies, Ahrensburg, Germany) and the APACT software. Sodium citrate plasma was centrifuged for 15 min at 111 g and room temperature. The supernatant was used as platelet-rich plasma (PRP). The remaining blood was again centrifuged for 10 min at 2773 g. The resulting supernatant was used as platelet-poor plasma (PPP). To achieve platelet counts of 250 000/µl, PRP and PPP were mixed in the desired ratio. Aggregation-inducing agents were collagen at a final concentration of 2 µg/ml (Nycomed), ristocetin 1.2 mg/ml (abp Limited, Surrey, UK), ADP 4 µmol/l (Sigma-Aldrich, St Louis, USA) and epinephrine 8 µmol/l (Sanofi Aventis, Paris, France). After transfer of the adjusted PRP in cuvettes, transmission of light was registered under permanent agitation of the samples. Results were obtained as a percent of the maximally possible aggregation (normal ≥70%). Since light transmission through PPP samples is the highest, these samples served as reference for the maximally possible aggregation. The mean value of the four resulting percentages was calculated for each measuring point per patient and classified in four categories (normal, ≥70%; mild, 50–69%; medium, 30–49%; severe, ≤29%). The categories follow no standard classification, but are arbitrarily chosen to make comparisons easier.

Platelet counts were determined by employing standard laboratory techniques (Sysmex). VWF antigen (VWF:Ag), collagen binding capacity (VWF:CB), ristocetin co-factor activity (VWF:RCo) and VWF multimers were determined as described previously [10]. Briefly, VWF antigen (Siemens Healthcare Diagnostics, Eschborn, Germany) and ristocetin co-factor activity (Siemens Healthcare Diagnostics) were measured in sodium citrate plasma using the Behring Coagulation System (BCS, Dade Behring, Marburg, Germany) according to standard protocols. Collagen type I (Nycomed Pharma, Unterschleissheim, Germany) was fixed on a microtitre plate. Collagen binding capacity (VWF:CB)

<table>
<thead>
<tr>
<th>Without LVAD</th>
<th>Early</th>
<th>Late</th>
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<tr>
<td>Prior to LVAD</td>
<td>After LVAD explantation</td>
<td>≤14 days postoperatively</td>
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<tr>
<td>Mean (days)</td>
<td>2</td>
<td>47</td>
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<tr>
<td>SD</td>
<td>1</td>
<td>39</td>
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<td>Minimum</td>
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<tr>
<td>Maximum</td>
<td>5</td>
<td>93</td>
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<tr>
<td>Median</td>
<td>2</td>
<td>31</td>
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<tr>
<td>PA, n</td>
<td>11</td>
<td>8</td>
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<tr>
<td>B/VWF, n</td>
<td>18</td>
<td>8</td>
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LVAD: left ventricular assist device; SD: standard deviation; n: number of patients for PA, platelet aggregation; B/VWF: assessment of bleedings and von Willebrand factor function.
in plasma was detected photometrically by the ELISA technique. We calculated ratios of VWF:CB/VWF:Ag (normal ≥0.7) and VWF:RCo/VWF:Ag (normal ≥0.6). They reflect the biological capacity of the available VWF to bind to collagen and platelets, respectively. VWF multimers were isolated on sodium dodecyl sulfate-agarose gel and blotted on a PVDF membrane. VWF was determined using appropriate primary and secondary antibodies (DAKO, Hamburg, Germany) and 3,3’-diaminobenzidin/cobalt chloride (Bio-Rad, Munich, Germany). Standard human plasma (Siemens Healthcare Diagnostics) was used as control. Presence or lack of HMW multimers was determined visually by an experienced haemostaseologist in comparison with the control plasma. AVWS was diagnosed if HMW multimers were missing and at least one functional VWF ratio (VWF:CB/VWF:Ag or VWF:RCo/VWF:Ag) was reduced. INR and aPTT were determined according to standard protocol.

Statistics

All calculations were done using the SPSS 19 software (SPSS, Inc., Chicago, IL, USA). Values are given as mean ± standard deviation. The paired Student’s t-test for normally distributed data and the Wilcoxon signed ranks test for not normally distributed or ordinal data were applied to analyse changes in parameters. Kendall’s tau coefficient and the chi-squared test were calculated to analyse correlations between the different parameters. A probability level of $P < 0.05$ was considered significant in all calculations. Boxes of the box plots contain the middle 50% of the values. The upper and the lower quartile represent the 25th and 75th percentile, respectively. The horizontal line inside the box marks the median. Whiskers indicate the largest and smallest values, excluding extreme outliers.

RESULTS

Patients

Thirty-nine adult LVAD patients with a mean age of 57 ± 14 years (range 19–77 years, median 61 years) were analysed. Main reasons for cardiac failure were dilated cardiomyopathy (DCMP, n = 23) and ischaemic cardiomyopathy (n = 14). Other causes were restrictive cardiomyopathy (n = 1) and congenital heart defect (corrected transposition of the great vessels and ventricular septal defect, n = 1). Table 2 summarizes indications for and outcome of LVAD implantation. Patients with LVAD were analysed at three time points: without LVAD, within 14 days and ±30 days following LVAD implantation.

Platelet-related parameters

We analysed the platelet-related parameters platelet aggregation and platelet count. Almost all patients displayed pathological overall platelet aggregation even without implanted LVAD. Only 1 patient had normal platelet aggregation values. This was also the only patient who suffered from heart failure due to a congenital heart defect. After LVAD implantation, he developed platelet dysfunction with medium severity in both postoperative states. All other patients had platelet dysfunction in both postoperative time periods after LVAD implantation (Fig. 1). We could not find a significant difference between the three time periods in overall platelet aggregation values (without-early, $P = 0.27$, n = 14; early-late, $P = 0.17$, n = 21, Fig. 1) or for the single agonists (Fig. 2). Of note, overall platelet aggregation improved in 8 of 21 patients in the late period compared with the early period, 11 patients remained at the same level and 2 patients had deteriorated overall platelet aggregation values. Platelet counts did not differ significantly in blood samples without LVAD and blood samples in the early time period ($P = 0.33$). However, we could find a significant increase in platelet counts in the late time period of LVAD support compared with values without LVAD support ($P < 0.001$, Fig. 3).

Von Willebrand factor-related parameters

Ratios of VWF:CB and VWF:RCo to VWF:Ag in conjunction with multimeric analysis were used to diagnose AVWS. Missing HMW multimers without implanted LVAD were detected in only 1 patient without LVAD with DCMP after acute myocarditis. All other patients had normal multimer patterns. Within the first 2 weeks after LVAD implantation, 33 of the 39 patients with ventricular assist devices developed AVWS. A typical VWF analysis with missing HMW multimers is shown in Fig. 4. AVWS diagnosis was not possible in 1 patient because multimer analysis was not available. Five patients

<table>
<thead>
<tr>
<th>Table 2: Indication for and outcome of LVAD implantation</th>
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<tr>
<td>Indication</td>
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<td>------------</td>
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<tr>
<td>Living on device</td>
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<td>Died</td>
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<td>Heart transplantation</td>
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<td>Weaned</td>
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<td>Total</td>
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Differences to 100% occur due to rounding.

Postoperative observation time ranges from March 2008 to February 2013.

DCMP: dilative cardiomyopathy; ICMP: ischaemic cardiomyopathy.
had normal multimer patterns. However, the ratios for VWF:CB/VWF:Ag or VWF:RCo/VWF:Ag were reduced in 3 of them. In the late time period, all patients with LV AD were afflicted by AVWS. We found a significant decrease in VWF:CB/VWF:Ag ratio between all three time periods (without-early, $P \leq 0.001$; early-late, $P = 0.01$, Fig. 4). Furthermore, the VWF:RCo/VWF:Ag ratios differed significantly between values without LV AD and early postoperative values ($P = 0.01$, Fig. 4). Platelet aggregation values and AVWS did not correlate ($P = 0.84$).

Bleeding complications

Within the first 2 postoperative weeks (early time period), 17 of 39 patients (44%) experienced bleeding complications (Table 3). Nine of them (23%) needed re-explorations due to bleeding. In total, 36 patients (92%) received blood transfusions. Platelet concentrates were required in 30 patients (77%).

Twenty-five of 33 patients (64%) suffered from bleeding complications in the late period (Table 3). Surgical re-explorations were necessary in 9 of them (36%). Eight patients (21%) needed blood transfusions; platelet concentrates were administered in 3 patients (8%). INR and aPTT were in the target range during all bleeding events.

DISCUSSION

Coagulation disorders and bleeding events are common complications in patients with LVAD [1, 2]. However, the underlying mechanisms have not been fully investigated. We analysed platelet function and VWF function in 39 LVAD patients. Additionally, we recorded occurrence of bleeding events. Values without LVAD from the same patients served as internal comparison. Mechanical circulatory support has recently been associated with platelet dysfunction [3, 4]. In the present study, platelet aggregation was impaired in patients with LVAD in varying degrees compared with standard values. These findings are in congruence with the results of Steinlechner et al., who analysed 12 patients after LVAD implantation and showed that platelet aggregation was significantly reduced [4]. Accordingly, Klovaite et al. demonstrated that platelet
aggregation was impaired in 16 patients after LVAD implantation. Moreover, the group studied 5 LVAD patients after heart transplantation and showed normal aggregation values [3]. However, both studies used blood samples of healthy volunteers or of patients with congestive heart failure (CHF) as a control. In contrast, we obtained internal comparisons of the same LVAD patients without LVAD. Hence, we analysed platelet function with as well as without an implanted LVAD. Interestingly, both cohorts showed platelet dysfunction. We did not find a difference in platelet aggregometry between values without LVAD and values following LVAD implantation. Our data suggest that platelet function is impaired even without LVAD in our patient population. Several explanations are possible: Firstly, heart failure patients are often in need of several drugs. Some of these common heart failure drugs like diuretics, β-blockers, ACE-blocking agents, nitrates, calcium channel blockers, catecholamines and aspirin affect platelet function [11–13]. Secondly, patients with LVAD experience severe haemodynamic decompensation. Reduced blood perfusion leads to liver and kidney dysfunction, which can influence platelet function [14–17]. Thirdly, increased vascular resistance and stasis of blood flow in CHF patients supposedly contribute to the development of hypersensitive platelets, which can be activated spontaneously [12, 18, 19]. Subsequently, granule proteins are released and platelets affiliate to loose aggregates. Disaggregation of these conglomerates leads to recirculation of exhausted and degranulated platelets, which is typical for a secondary storage pool defect with diminished platelet aggregation [18]. Our data do not explain whether the detectable platelet dysfunction with and without LVAD is caused by the same pathological mechanism and whether platelet dysfunction after LVAD implantation is the result of terminal heart failure conditions or due to specific characteristics of the LVAD. Notably, platelet counts increased to normal values in the late period, which is in congruence with findings of Dewald et al., who revealed a recovery of platelet count 2 weeks after LVAD implantation [20]. However, an improved platelet aggregation was only observed in 8 of 21 patients in the late period. Our data did not show a consistent pattern of recovery of platelet function. It seems possible that platelet function is more vulnerable towards external factors than the overall platelet count.

In addition to platelet dysfunction, AVWS has been described as a possible cause of bleeding disorders in patients with LVAD [5, 6]. Our results are consistent with previous findings, which demonstrated that AVWS is already present in the early postoperative state and disappears quickly after LVAD explantation [3, 21]. The decrease in ratios of VWF:CB/VWF:Ag and VWF:RCo/VWF in nearly all patients between the time period without LVAD and the early time period indicates a qualitative defect of VWF which affects the interaction both with platelets and with collagen. AVWS achieves probably even more importance in these patients because it further contributes to ineffective functioning of platelets.

Bleeding events occurred in 44% of the patients in the early and in 64% of the patients in the late time period. These results are in congruence with previous findings, which reported of 20–81% of the LVAD patients suffering from bleeding complications [1]. An explanation for the increase of bleeding events in the late time period could be the longer observation period (early, 14 days; late, up to
1430 days), with more time for bleeding events to occur. The analysed patients showed a decreased need of blood transfusions during the late time period, which is most likely attributed to the lower number of major bleeding episodes like major thoracic haemorrhage and increased number of minor bleedings, e.g. epistaxis.

There are several limitations to our study. The number of included patients is relatively small and takes only patients with a left ventricular HeartMate II into account. Data were not available for every time point. In particular, comparison with values without LVAD was not possible in every patient due to short preparation times before LVAD implantation. Specifically, platelet aggregation can only be analysed in very fresh samples and is not available any time. Reduced patient numbers in the late time period were due to 2 patients undergoing heart transplantation and 3 patients dying before reaching the late time period. Since laboratory data were not taken at the exact time of bleeding, we refrained from statistical correlation of platelet data and bleeding events in order to avoid any misinterpretation of the results. However, the lack of an obvious correlation between the occurrence of bleeding events, platelet dysfunction and AVWS underlines the clinical observation that haemostasis is a multifactorial process. It is influenced by medication, platelet number and function, renal and hepatic function, blood product utilisation, surgical procedures, mucosal injuries, inflammatory response, sepsis, genetic preconditions and many other factors. Disturbances of the haemostatic equilibrium can be compensated quite extensively and bleeding events occur only when too many factors are out of normal range. Especially the large wound after LVAD implantation presents a major challenge to the coagulation system. We are also aware of the fact that platelet function cannot be fully evaluated by platelet aggregometry alone. Therefore, a comprehensive statement about platelet function in LVAD patients is only partially possible.

In conclusion, platelet function is most often impaired in our patient cohort also without implanted LVAD. Platelet dysfunction was also observed in these patients after LVAD implantation. Analysing platelet aggregometry, we could not show statistical differences without LVAD and after LVAD implantation. The bleeding events in LVAD patients most probably result from a combination of deteriorated platelet function, platelet-inhibiting medication, AVWS, impaired function of liver and kidney and perhaps other, thus far, unexplored factors. The degree to which each parameter contributes to bleeding events, as well as detailed analysis of the interactions between LVAD and platelets, are subjects of future research. The fact that platelet function is impaired in LVAD patients even without an implanted device should draw our attention to pre-existing—and often persisting—coagulation disorders in the context of LVAD support. Further investigations on this topic could facilitate optimization of anticoagulation treatment in LVAD patients, resulting in less bleeding events and better outcome.

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Conflict of interest: none declared.

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