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VON WILLEBRAND FACTOR PROTEOLYSIS BY ADAMTS-13 IN PATIENTS ON LEFT VENTRICULAR ASSIST DEVICE SUPPORT†

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Left ventricle assist devices (LVADs) have significantly improved the survival of patients with end-stage heart failure, but device-related hemostatic complications remain common and are associated with poor outcomes. The bleeding diathesis, primarily found in gastrointestinal (GI) tract at the site of an arteriovenous malformation (1, 2), is believed to be caused by the loss of large von Willebrand factor (VWF) in plasma, leading to the term of acquired von Willebrand syndrome (AVWS). LVAD-associated AVWS is believed to be caused by the excessive cleavage of large VWF multimers by the metalloprotease ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) in a high shear stress environment of the LVAD-driven blood flow (3). However, AVWS is observed in nearly all patients, but only 11–30% of them bleed (3, 4) and the excessive VWF cleavage has not been directly demonstrated.

Here, we report findings from a longitudinal study that directly measured VWF cleavage by ADAMTS-13 in 23 patients before and after LVAD implantation and 39 healthy controls (25–59 yrs, 36% female). The patient data were further complemented by examining the impacts of pathologically high shear stress on VWF binding to platelets and its cleavage by

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Conflict of Interests

The authors claim no conflict of interests related to this manuscript

Author contributions

Angelo Nascimbene formulated the hypotheses tested in the study, designed the study, recruited the patients, performed clinical analyses, and wrote the manuscript

Tristan Hilton performed experiments and analyzed data

Barbara A. Konkle analyzed data and wrote the manuscript

Joel L. Moake analyzed data and wrote the manuscript

O.H. Frazier directed patient recruitments and wrote the manuscript

Jing-fei Dong formulated hypotheses tested in the study, designed the study, analyzed data, and wrote the manuscript

ADAMTS-13 in vitro. All patients received HeartMateII LVAD (Thoratec, Pleasanton, CA) and, after implantation, aspirin (81 mg/day), warfarin with a targeted INR of 2–3, and dipyridamole (75 mg 3 times daily). Patients with malignancy, autoimmune disease, or hypercoagulable state were excluded to minimize confounding factors associated with thrombosis and bleeding independent of LVAD.

Among these patients, we observed 10 adverse events: ventricular tachycardia storm ($n = 1$), non-ST-segment elevation MI ($n = 1$), arterial thrombosis ($n = 3$), stroke ($n = 3$), and gastrointestinal bleeding ($n = 2$). This cohort intentionally included more patients with thrombotic complications to evaluate the impacts of AVWS on these patients. LVAD function and the clinical parameters of patients at times of sample collections were comparable (Table S1), with the exception of INR as a result of post-LVAD anticoagulation.

Plasma VWF (VWF: Ag) was significantly elevated in patients before LVAD implantation. While progressively reduced after LVAD implantation, it remained higher than controls (Figure S1). In contrast, VWF:pp were elevated at levels comparable before and after LVAD implantation. The VWF:pp to VWF:Ag ratio was significantly higher in patients with thrombotic complications compared to those with bleeding or no complications during the first three months post-LVAD.

The loss of large VWF multimers was not detected in samples collected before, but it was found in 21/23 patients (91.3%) after LVAD implantation (Figure S1), including 7/8 patients with post-LVAD thrombosis. This VWF deficiency has been widely attributed to excessive cleavage by ADAMTS-13, but actual rates of VWF cleavage in patients on LVAD have not been reported. Using an antibody that specifically recognizes cleaved VWF (Figure S2), we determined that the amounts of cleaved VWF varied significantly among patients and were proportional to VWF:Ag ($R^2 = 0.734$, $p < 0.001$). VWF cleavage was significantly increased in three patients (20%, Table 1) drastically reduced in three (20%), and not changed in the rest (60%) as compared to controls, suggesting that, despite the loss of large VWF multimers in 91.3% of patients, only 20% of patients had increased VWF cleavage.

The discrepancy between the persistent loss of large VWF multimers and variable rates of VWF cleavage among the patients led us to measure ristocetin-induced platelet aggregation (VWF:Rco). VWF:Rco was significantly reduced in post-LVAD samples compared to baseline, but it remained higher than controls (Figure S3). The VWF:Rco to VWF:Ag ratio, which measures VWF reactivity after adjustment for high VWF:Ag was significantly lower in post-LVAD samples from patients with bleeding. These data suggest that the loss of large VWF multimers reduced the activity of plasma VWF, but the defect was largely compensated by elevated VWF:Ag in patients on LVAD supports.

This discrepancy also raises the possibility that high shear stress induces VWF-binding to platelets, leading a selective removal of large multimers from the circulation. This notion is supported by several lines of evidence. First, platelet-bound VWF was progressively increased in post-LVAD samples (Figure 1A). Second, VWF:Rco, reduced after LVAD implantation, remained higher than that of normal subjects (Figure S3). Third, a pathological high shear stress of 100 dynes/cm² induced platelet aggregation in a force-dependent

manner (Figure 1B) and platelet activation measured by expressions of CD62p (Figure 1C) and phosphatidylserine (Figure 1D). Among 10 normal subjects examined, high shear stress induced the loss of large VWF multimers in 7 (an example in Figure 1E), but it did not increase VWF cleavage by ADAMTS-13 (Figure 1F).

In summary, the loss of large VWF multimers was observed in 91.3% patients on LVAD supports in this case series, including patients who developed post-LVAD thrombosis, but excessive VWF cleavage was only found in 20% of them (Table 1). To identify attributes to this discrepancy, we made several novel observations that suggest shear-induced VWF binding to platelets as an alternative cause of LVAD-associated AVWS (Figure 1 and Figure S3). These observations are consistent with well-established roles of high shear stress/tensile forces in inducing VWF cleavage by ADAMTS-13 and in promoting VWF binding to platelets (2, 5) as two mechanisms for LVAD-associated AWAS (Figure 1G). These two mechanisms resemble the loss-of-function and gain-of-function bleeding phenotypes respectively found in patients with type 2A and type 2B von Willebrand disease. More important, the gain-of-function phenotype will not only selectively remove large VWF multimers from the circulation, but also promote platelet aggregation to potentially occlude cerebral and GI microvessels, as often found in patients on LVAD supports. These findings require further validation through large clinical studies. If validated, the two mechanisms need to be distinguished as they may require very different treatments: blocking ADAMTS-13 activity in the case of excessive VWF cleavage and reducing VWF reactivity in the case of shear stress-induced VWF binding to platelets. LVADs of future generations may reduce hemostatic complications, but altered fluid hydrodynamics likely remains as a key feature of the LVAD-driven blood flow. Therefore, studying VWF reactivity in this altered hydrodynamic environment remains critical for improving clinical outcomes of patients after LVAD implantation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Shear induced VWF interaction with platelets

(A) Platelet-bound VWF was detected by apoclonal antibody using flow cytometry (n = 8, ANOVA, *p < 0.01 vs. healthy subjects, # p < 0.01 vs. BL). (B) Platelet aggregation of PRP was induced by venous (5 dynes/cm²), arterial (10 and 50 dynes/cm²), and pathologically high (100 dynes/cm²) shear stresses (n = 26). Shear-induced CD62p expression (C) and annexin V binding (D) to platelets before and after PRP from healthy subjects was exposed to 100 dynes/cm² of shear stress for 5 min at 37°C (n = 10, paired t test). (E) An example of VWF multimers patterns before and after normal PRP subjected to 100 dynes/cm² of shear stress (representative of 10 subjects). (F) VWF cleavage in normal PRP before and after the shear exposure was detected by two antibodies that recognize uncleaved (top) and cleaved (bottom) VWF, respectively. (G) Schematic illustration of two potential mechanisms of LVAD-induced loss of large VWF multimers. The high shear stress developed in a LVAD-driven blood flow induces conformational changes of VWF multimers (bottom of the panel G) that could trigger ADAMTS-13 cleavage and promote VWF binding to platelets (top of the panel G). The former will reduce VWF multimer sizes and adhesive activity, resulting in bleeding (loss-of-function), whereas the latter will activate/aggregate platelets, leading to thrombosis or consumptive bleeding (gain-of-function).

Table 1Rates of VWF cleavage by ADATMS-13 in plasma from patient on LVAD^{*}

Patient code	Baseline	Discharge	Outcome
1	106.7	72.7	Arterial thrombosis/GI bleeding
2	134.7	108.2	GI bleeding
4	64.9	151.6	Ventricular tachycardia storm
6	118.8	131.7	No complication
7	159.5	156.1	No complication
8	92.7	36.7	No complication
9	136.2	110.7	No complication
10	122.1	159.9	Aortic thrombosis/NSTEMI
11	150.7	22.7	No complication
12	119.2	128.5	No complication
15	24.3	140.3	No complication
17	71.2	63.8	Stroke
19	118.2	98.7	Stroke
22	78.2	69.1	No complication
23	108.0	112.7	No complication

^{*} The values are the ratio of VWF densitometry measurements of cleaved to uncleaved VWF bands in immunoblots. The value for plasma pooled from 39 healthy subjects was set at 100. This method had a variation coefficient of 18.7%.