Published in final edited form as:

ASAIO J. 2019 July ; 65(5): 481–488. doi:10.1097/MAT.0000000000000849.

Benchtop von Willebrand Factor Testing: Comparison of Commercially Available VADs and Evaluation of Variables for a Standardized Test Method

Phillip A. Coghill*, Suren Kanchi*, Zheila J. Azartash-Namin*, James W. Long*[†], and Trevor A. Snyder*[†]

*VADovations, Inc

[†]INTEGRIS Advanced Cardiac Care, INTEGRIS Baptist Medical Center

Abstract

Gastrointestinal bleeding occurs in 20–30% of patients receiving ventricular assist devices (VADs) due, in part, to acquired von Willebrand syndrome (aVWS). We examined factors to optimize a benchtop method to quantify changes in von Willebrand Factor (VWF) multimer distribution and function in VADs, then applied them to evaluate commercially available devices.

Human plasma was circulated through flow loops with VADs. Several experimental conditions were examined, including temperature, viscosity, and enzyme inhibition. Samples were analyzed for VWF collagen binding activity (VWF:CB) and VWF antigen level (VWF:Ag). VWF multimer profiles were quantified using gel electrophoresis, near-infrared in-gel visualization, and densitometric analysis.

The VWF:CB/Ag ratio in the HeartMate II, CentriMag, and HVAD exhibited average decreases of 46%, 44%, and 36% from baseline after 360 minutes of operation. High molecular weight (hVWF) multimer loss occurred within 30 minutes, while the Levacor and control loop profiles were unchanged. Varying temperature and viscosity altered hVWF degradation rate, but not the final results. Inhibition of ADAMTS13 can potentially distinguish mechano-enzymatic cleavage of VWF from mechanical degradation.

We developed a repeatable benchtop method to evaluate VWF-compatibility of VADs similar to hemolysis testing that can be adopted for pre-clinical VAD evaluation.

Keywords

von Willebrand Factor; hemocompatibility; shear; ventricular assist device; blood pump

Continuous flow ventricular assist devices (VADs) improve survival and quality of life in end-stage heart failure patients^{1, 2}. However, cost³, complexity^{4, 5}, and complications^{4, 6, 7} limit their use. Bleeding is the most frequent adverse event with 7.79 events per 100 patient

Corresponding Author: Trevor A. Snyder, PhD, 1333 Cornell Pkwy, Oklahoma City, OK 73108, PH: 405-601-5520, FAX: 405-601-5538, trevor.snyder@vadovations.com.

Conflicts of Interest: Phillip Coghill, Suren Kanchi, Zheila J. Azartash-Namin, and Trevor Snyder are employees of VADovations.

months⁷. The increased risk of non-surgical bleeding has been linked, in part, to an acquired von Willebrand Factor (VWF) functional deficiency, similar to Heyde Syndrome in aortic valve stenosis^{8–10}. VAD patients immediately lose high molecular weight VWF (hVWF) post-implant, which returns following device explant for heart transplantation^{11, 12}. Acquired VWF syndrome has been reported in many VADs including the Jarvik 2000 Flowmaker (Jarvik Heart, New York, NY), HeartMate II (HMII), HeartMate III, and CentriMag (Abbott, Abbott Park, IL), HVAD and Circulite Synergy (HeartWare-Medtronic, Miami Lakes, FL), and the pediatric Excor (Berlin Heart, Berlin, Germany)^{12–17}. The EVAHEART (Sun Medical Technology Research Corp, Nagano, Japan) preserves VWF multimer distribution in benchtop^{18, 19} and clinical studies²⁰ with very low clinical bleeding rates²¹. For future VAD development, using benchtop testing to investigate VWF-compatibility, rather than measurements during clinical trials, would be beneficial.

Elevated shear in VADs increases VWF proteolysis by mechanical and mechano-enzymatic degradation²². In vitro circulation of blood through rotary blood pumps has previously been shown to replicate clinical findings^{23, 24}. Herein, we examined several experimental factors for impact on VWF function in VADs *in vitro* toward developing a standardized VWF compatibility test, similar to the well-established standard for hemolysis.

Methods

FFP characterization

Human fresh frozen plasma (FFP) units, obtained from a blood bank, were warmed to 37°C and aliquoted into silicone-coated vacutainer tubes (BD). Aliquots supplemented with 1 or 4µg/mL recombinant human ADAMTS13 (rhADAMTS13, R&D Systems, Minneapolis, MN), or 10mM ethylenediaminetetraacetic acid (EDTA) were rocked on a VariMix platform rocker (Model M79735, Thermo Scientific) at 1Hz, with samples collected into low protein retention microcentrifuge (LPRM) tubes (Fisher Scientific, Pittsburgh, PA) at 30 and 60 minutes, then hourly until 6h. VWF and rhADAMTS13 antigen and activity levels were determined using ELISA-based kits: Quantikine ADAMTS13 antigen kits (ADAMTS13:Ag, R&D Systems), Technozym ADAMTS13 activity kits (ADAMTS13:Ac, DiaPharma, West Chester, OH), Technozym VWF collagen type I binding (VWF:CB), and Technozym VWF Antigen (VWF:Ag, DiaPharma).

In vitro recirculation loop

FFP units were pooled in a polycarbonate carboy. In some tests, pooled FFP was supplemented with 25% w/v dextran sulfate/phosphate buffered saline (PBS) and sodium heparin (Fisher Scientific, FFP+dextran). Viscosity was measured with a rotary viscometer (Fungilab USA, Hauppauge, NY). VADs were connected to 4L polycarbonate reservoirs using 42cm of blood-compatible Tygon® tubing (ND100–65 formulation, Saint Gobain, Malvern, PA). Standard polyvinyl chloride (PVC) bags exhibited high VWF adsorption, creating excessive artefact (data not shown). A control loop including a 42cm tubing section was rocked at 1Hz for the duration of the test. Each loop was primed with 1% bovine serum albumin in saline, rinsed with saline, 2L of FFP was added, and the pump and loop de-aired.

VADs were adjusted to generate standard hemolysis (5L/min, 100mmHg afterload), nominal clinical (4L/min, 75mmHg), or pediatric (2L/min, 80mmHg) conditions. Flow was measured with a TS410 flow meter and 9PXL flow probe (Transonic, Ithaca, NY). Outlet pressure was measured with a fluid-filled pressure transducer (Medex/Smiths Medical, Dublin, OH) and patient monitor (M1204A, Hewlett-Packard) 2.5cm downstream of the VAD outlet. Baseline samples for VWF:CB/Ag were collected into LPRM tubes containing EDTA before turning on the VADs, then at 30 and 60 minutes and hourly to 6h.

Multimer analysis samples were collected into LPRM tubes containing Halt protease inhibitor cocktail (Thermo Scientific). Multimer profiles were visualized using gel electrophoresis and near-infrared in-gel fluorescence techniques, as previously described²⁵. Briefly, 10µL samples (1:25 plasma:sample buffer) were loaded onto 1.5% agarose gels and electrophoresed at 50V for 30 minutes followed by 90V for 2h. Following electrophoresis, gels were stained with rabbit anti-VWF antibody (Dako, Carpinteria, CA), then IRDYE 680RD-conjugated goat anti-rabbit IgG (Licor, Lincoln, NE), scanned at 700nm on an Odyssey imager and analyzed with Image Studio Lite (LiCor). Densitometric tracings and multimer band analysis were performed using NIH ImageJ. The multimer ratio was calculated for each lane by dividing hVWF region intensity by the cumulative intensities of the six lowest molecular weight bands.

Devices Tested

The VADs used in this study were the HeartMate II (HMII), HVAD, CentriMag, and Levacor (WorldHeart, now Medtronic-HeartWare, Miami Lakes, FL). Pumps were recovered post-transplantation from patients with no history of thromboembolic complications at explant and cleaned with tergazyme,alconox, and simple green solutions. Normal function with expected power and flow ranges were confirmed prior to use. The same pumps were used throughout the study.

Statistical Methods

Data are reported as mean $\pm$ one standard deviation. Statistically significant differences were determined using an analysis of variance (ANOVA) with post-hoc Tukey's tests. Statistical significance was defined as $p < 0.05$.

Results

Characterization of FFP

To determine whether FFP was suitable for VWF-compatibility testing of VADs, we characterized ADAMTS13:Ac, ADAMTS13:Ag, VWF:CB and VWF:Ag in FFP, then supplemented aliquots with rhADAMTS13. Figure 1A shows ADAMTS13:Ag in FFP was approximately 50% of normal value specified by the kit manufacturer; however, ADAMTS13:Ac fell within the normal range. In FFP supplemented with 1µg/mL rhADAMTS13, ADAMTS13:Ag and ADAMTS13:Ac increased to 140% and 220% of normal value (Figures 1A&B). Additional supplementation further elevated ADAMTS13:Ag (331%) and ADAMTS13:Ac (443%). EDTA-supplementation of FFP (FFP+EDTA) did not affect ADAMTS13:Ag but inhibited ADAMTS13:Ac, as expected.

FFP and FFP+EDTA rocked for 360 minutes, retaining collagen binding activity, Figure 2A. After 360 minutes, VWF:CB decreased to 89% and 74% of the baseline in rhADAMTS13-supplemented FFP (1 and 4 µg/mL, respectively). VWF:Ag increased in supplemented FFP by 36% and 47% for 1 and 4 µg/mL rhADAMTS13, respectively, while VWF:Ag slightly decreased in FFP+EDTA (Figure 2B).

VWF:CB/Ag remained unchanged for FFP+EDTA, but steadily decreased in rhADAMTS13-supplemented FFP, Figure 2C. After 360 minutes VWF:CB/Ag had decreased by 34% and 50% in rhADAMTS13-supplemented FFP (1 and 4 µg/mL, respectively). Regression analysis, performed on all four data series, showed a significant difference in 1 µg/mL vs 4 µg/mL rhADAMTS13-supplemented FFP. Based on these findings, we used FFP as a test fluid without VWF or ADAMTS13 supplementation.

VWF Functional Decreases during Circulation through VADs

Figure 3A shows VWF:CB remained unchanged in FFP+dextran (viscosity = 3 cP) in control and Levacor loops; however, VWF:CB significantly decreased in the HVAD, HMII, and CentriMag to 75%, 60%, and 55% of baseline, respectively. Figure 3B shows VWF:CB/Ag had decreased by 33%, 41%, and 42% in the HVAD, HMII, and CentriMag, respectively. Regression analysis of VWF:CB/Ag decreases showed that the HMII and CentriMag displayed similar rates of VWF:CB/Ag decrease, which were significantly faster than the HVAD.

We observed a complete loss of hVWF multimers with the HVAD, HMII, and CentriMag after 360 minutes (Figure 4A). At all timepoints, hVWF multimers were present in the control and Levacor loops. The horizontal position of the five highest molecular weight VWF multimer bands present were determined (Figure 4B). We quantified hVWF loss using two methods: first, by calculating the multimer ratio for baseline and 360 minute samples in three separate gels (Figure 4C) and second, by averaging positions of the five highest molecular weight bands (Figure 4D). The HMII, HVAD, and CentriMag multimer ratios significantly decreased, while the Levacor and control loop multimer ratios were relatively unchanged. Similarly, the positions of the five highest weight molecular bands shifted significantly with the HMII, HVAD, and CentriMag, but not in the Levacor or control loops.

The Effects of Flow Conditions, Plasma Viscosity and Temperature on VWF Function in VADs

The HMII, HVAD, and Levacor were adjusted to generate pediatric (2L/min against 80mmHg) or standard hemolysis (5L/min against 100mmHg) conditions with FFP+dextran for 360 minutes. Figure 5A shows the HMII lost 16% and 27% VWF:CB/Ag at standard hemolysis and pediatric conditions, respectively, while the HVAD lost 18% and 30% VWF:CB/Ag (Figure 5B). The rate of VWF:CB/Ag loss in the HMII was significantly different for each flow condition. However, the rate of VWF:CB/Ag loss in the HVAD was significantly lower at standard hemolysis conditions compared to 4L/min against 75mmHg. VWF functionality was preserved with the Levacor at all three conditions tested (Figure 5C). VWF:CB/Ag decreased significantly with the HMII and HVAD at the flow conditions tested compared to Levacor and control loops (Figure 6).

Figure 7A shows that FFP (viscosity = 1.5 cP) circulated through the HMII displayed a 24% decrease of VWF:CB/Ag after 30 minutes vs. 10% for FFP+dextran (3.0 cP). After 360 minutes of circulation the results were similar, showing a 45% and 41% VWF:CB/Ag decline for FFP vs. FFP+dextran, respectively. The CentriMag showed VWF:CB/Ag losses of 15% and 38% in FFP at 30 and 360 minutes compared to losses of 6% and 42% at the same time points for FFP+dextran (Figure 7B). Given the observed higher rate of early decrease in VWF:CB/Ag, separate regressions were performed for 0–60 and 60–360 minute data. There was a significant difference between the VWF:CB/Ag losses over the first hour in the HMII but not the final five hours. Figure 7C shows the HVAD differed from the other VADs in that VWF:CB/Ag loss was unaffected by FFP viscosity. Loss of VWF functionality in the HVAD was similar for FFP and FFP+dextran (30% and 34% decrease at 360 minutes, respectively).

FFP+dextran was circulated through an HVAD for 360 minutes at 37° C. Figure 8 displays a faster loss of VWF functionality at body temperature (37°C) vs. room temperature (22°C). After 360 minutes of circulation, we observed a 51% decrease in VWF:CB/Ag at 37°C compared to 34% at 22°C. These results demonstrate that flow conditions, test fluid viscosity (and consequently shear stress magnitude), and temperature affect VWF functionality changes during VAD circulation.

ADAMTS13-Mediated Proteolysis in VADs

To examine ADAMTS13-mediated VWF mechano-enzymatic proteolysis vs. mechanical degradation, we added EDTA to FFP+dextran and circulated it through the HMII, HVAD, and Levacor. EDTA inhibits metalloproteinases including ADAMTS13²⁶. We observed VWF:CB decreased in the HMII, but not the HVAD, Levacor, or control loops (Figure 9). There were no significant differences in VWF:CB changes for FFP+dextran+EDTA compared to FFP+dextran. VWF:Ag non-significantly increased in FFP+dextran+EDTA in the HMII and HVAD; however, VWF:CB/Ag decreased to 68% in the HMII and 67% in the HVAD. These decreases were not statistically different from those in FFP+dextran alone, suggesting the primary mechanism of VWF cleavage is not enzymatic.

Discussion

In 1994, Naito and colleagues demonstrated the need to standardize hemolysis testing and reporting²⁷. ASTM standards F1841, “Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps” and F1830 “Standard Practice for Selection of Blood for *in vitro* Evaluation of Blood Pumps” provide device developers, regulators, and users a standardized method for hemolysis assessment. In parallel, computational fluid dynamic analysis models were developed to predict hemolysis and guide device design. Similar efforts will be required to address VWF-compatibility issues of current and future VADs. Unfortunately, the recognized standard for biocompatibility testing, International Standards Organization (ISO)-10993, does not mention VWF.

Egger and colleagues described *in vitro* testing of blood pumps to examine VWF compatibility, demonstrating the tests required human blood or plasma due to available assay kits and that using a ratio of VWF activity (equivalent to VWF:CB in this study) to VWF:Ag

level provided similar information to VWF multimer analysis²⁴. Using FFP alone avoids potential artefacts: including 1) Hemolysis: free hemoglobin inhibits ADAMTS13 proteolysis of VWF²⁸ and 2) Platelets: an endogenous source of VWF upon activation and a site for increased VWF proteolysis^{29, 30}. Prohibitive challenges to using human blood include obtaining multi-liter volumes of freshly collected human blood on a repeated basis, the need for typing and cross-matching, and blood type dependence of VWF antigen levels³¹. FFP can be obtained from blood banks in significant volumes by blood type and can be readily pooled. Thus, we first characterized FFP as a test fluid for VWF compatibility testing.

FFP can have diminished levels of some blood proteins, including VWF and ADAMTS13^{32, 33}; however, VWF:Ag and VWF:CB levels in FFP were within normal range. ADAMTS13:Ag was ~50% of normal, but ADAMTS13:Ac was within the normal range and sufficient to observe VWF multimer distribution changes in VAD-circulated FFP. Supplementation increased ADAMTS13:Ac and led to a decrease in hVWF multimers in uncirculated plasma, an undesired effect. These results indicated that FFP was an acceptable test fluid to examine VWF-compatibility in VADs without ADAMTS13 supplementation. Additionally, supplementing FFP with EDTA to inhibit ADAMTS13 did not alter the degree of VWF function loss. This finding parallels recent reports from Houston that ADAMTS13 cleavage of VWF is relatively unchanged following VAD implant^{34, 35}.

Supplementing FFP with dextran to approximate whole blood viscosity resulted in a change in the rate of hVWF loss and VWF:CB/Ag decrease, but not a change in the final results at 360 minutes. Temperature, independent of viscosity, influenced the rate of VWF function loss, requiring it to be controlled as an experimental parameter. The extent of VWF function loss also varied based on hemodynamic conditions, indicating hemodynamic parameters of the intended treatment population (adult vs. pediatric, and hypo- or hypertensive) be included in protocol development.

Our results confirm that quantifying VWF:CB/Ag is sufficient for VWF-compatibility assessment, and VWF multimer analysis did not provide additional information. Even when applying different densitometric analyses, no new or different conclusions emerged for the devices and conditions tested. Simple and cost-effective methods to quantify hemolysis helped enable widespread adoption. ELISA-based assay kits employed herein permit VWF-compatibility quantitation with widely-available microplate reader devices at a reasonable cost²⁴ within a few hours, whereas, multimer blotting requires up to three days to perform.

A recent comparison of 734 VAD recipients found no difference in gastrointestinal bleeding rates for the HMII and HVAD⁶, replicating a previous report of 175 patients¹⁴. Thus, the significant differences observed during these *in vitro* VWF-compatibility tests of the HMII and HVAD suggest VWF multimer and functional changes may be a more sensitive indicator of shear-related blood trauma than clinical non-surgical bleeding rates. The results of VWF-compatibility testing of the EVAHEART VAD supports the proposition that benchtop testing^{18, 19} reflected clinical results^{20, 21}. We observed that the Levacor preserved VWF function and multimer distribution; however, this device is no longer in use and clinical VWF-compatibility and bleeding rates have not been published. The “wide-gap”,

magnetically levitated, CentriMag produced similar VWF functional changes as the HMII, which suggests that shear stress alone does not determine VWF-compatibility and that time exposure is likely a critical parameter as well.

In this study, we confirmed previous findings that *in vitro* VWF-compatibility testing of VADs is feasible and explored several variables of concern. We especially focused efforts on identifying a readily available, reasonable-cost test fluid compatible with commercially-available VWF assay kits. We applied these methods to 4 commercially-available VADs, producing the 1st head-to-head comparisons of the HMII, HVAD, and CentriMag devices, with the HVAD producing the smallest loss of VWF function. We also identified a second VAD, the Levacor, that does not appear to cause VWF degradation, along with the EVAHEART, indicating VWF degradation is not inherent to continuous flow VADs, as has been posited previously¹⁰. It is our hope that these results, building upon previous efforts, provide a foundation for a consensus approach to develop standardized VWF-compatibility test methods for VAD design improvement and performance evaluation.

Acknowledgments

Sources of Funding: This work was supported in part by NIH grants R44HL114246, R44HL118907, and R21HL115601.

Research reported in this publication was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award numbers R44HL114246, R44HL118907, and R21HL115601. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

References

1. Rogers JG, Aaronson KD, Boyle AJ, et al. Continuous flow left ventricular assist device improves functional capacity and quality of life of advanced heart failure patients. *J Am Coll Cardiol*. 2010; 55(17):1826–1834. [PubMed: 20413033]
2. Jorde UP, Kushwaha SS, Tatroles AJ, et al. Results of the destination therapy post-food and drug administration approval study with a continuous flow left ventricular assist device: a prospective study using the INTERMACS registry (Interagency Registry for Mechanically Assisted Circulatory Support). *J Am Coll Cardiol*. 2014; 63(17):1751–1757. [PubMed: 24613333]
3. Long EF, Swain GW, Mangi AA. Comparative survival and cost-effectiveness of advanced therapies for end-stage heart failure. *Circ Heart Fail*. 2014; 7(3):470–478. [PubMed: 24563450]
4. Toda K, Sawa Y. Clinical management for complications related to implantable LVAD use. *General thoracic and cardiovascular surgery*. 2015; 63(1):1–7. [PubMed: 25370679]
5. Baumann Kreuziger LM. Management of anticoagulation and antiplatelet therapy in patients with left ventricular assist devices. *J Thromb Thrombolysis*. 2015; 39(3):337–344. [PubMed: 25549823]
6. Stulak JM, Davis ME, Haglund N, et al. Adverse events in contemporary continuous-flow left ventricular assist devices: A multi-institutional comparison shows significant differences. *J Thorac Cardiovasc Surg*. 2016; 151(1):177–189. [PubMed: 26545967]
7. Kirklin JK, Naftel DC, Pagani FD, et al. Seventh INTERMACS annual report: 15,000 patients and counting. *J Heart Lung Transplant*. 2015; 34(12):1495–1504. [PubMed: 26520247]
8. Warkentin TE, Moore JC, Anand SS, Lonn EM, Morgan DG. Gastrointestinal bleeding, angiodysplasia, cardiovascular disease, and acquired von Willebrand syndrome. *Transfus Med Rev*. 2003; 17(4):272–286. [PubMed: 14571395]
9. Klovaitte J, Gustafsson F, Mortensen SA, Sander K, Nielsen LB. Severely impaired von Willebrand factor-dependent platelet aggregation in patients with a continuous-flow left ventricular assist device (HeartMate II). *J Am Coll Cardiol*. 2009; 53(23):2162–2167. [PubMed: 19497443]

10. Tabit CE, Chen P, Kim GH, et al. Elevated Angiopoietin-2 Level in Patients With Continuous-Flow Left Ventricular Assist Devices Leads to Altered Angiogenesis and Is Associated With Higher Nonsurgical Bleeding. *Circulation*. 2016; 134(2):141–152. [PubMed: 27354285]
11. Davis ME, Haglund NA, Tricarico NM, Matafonov A, Gailani D, Maltais S. Immediate recovery of acquired von Willebrand syndrome after left ventricular assist device explantation: implications for heart transplantation. *ASAIO J*. 2015; 61(1):e1–4. [PubMed: 25251586]
12. Goda M, Jacobs S, Rega F, et al. Time course of acquired von Willebrand disease associated with two types of continuous-flow left ventricular assist devices: HeartMate II and CircuLite Synergy Pocket Micro-pump. *J Heart Lung Transplant*. 2013; 32(5):539–545. [PubMed: 23570742]
13. Letsou GV, Shah N, Gregoric ID, Myers TJ, Delgado R, Frazier OH. Gastrointestinal bleeding from arteriovenous malformations in patients supported by the Jarvik 2000 axial-flow left ventricular assist device. *J Heart Lung Transplant*. 2005; 24(1):105–109. [PubMed: 15653390]
14. Meyer AL, Malehsa D, Budde U, Bara C, Haverich A, Strueber M. Acquired von Willebrand syndrome in patients with a centrifugal or axial continuous flow left ventricular assist device. *JACC Heart failure*. 2014; 2(2):141–145. [PubMed: 24720921]
15. Dranishnikov N, Stepanenko A, Frumkin A, et al. Course of von Willebrand factor in recipients of axial-flow and centrifugal blood pumps in up to 12 months of follow-up. *Thorac Cardiovasc Surg*. 2012; 60(S 01):1. [PubMed: 22374687]
16. Netuka IKT, Kvasni ka J, Hrachovinová I, Ivák P, Mare ek F, Bílková J, Malíková I, Jan ová 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. *The Journal of Heart and Lung Transplantation*. 2016
17. Gossai N, Brown NM, Ameduri R, Zantek ND, Louis JS, Steiner ME. Pediatric Acquired von Willebrand Disease With Berlin Heart Excor Ventricular Assist Device Support. *World journal for pediatric & congenital heart surgery*. 2016; 7(5):614–618. [PubMed: 27587498]
18. Bartoli CR, Kang J, Zhang D, et al. 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*. 2016
19. Naito N, Mizuno T, Nishimura T, et al. Influence of a Rotational Speed Modulation System Used With an Implantable Continuous-Flow Left Ventricular Assist Device on von Willebrand Factor Dynamics. *Artif Organs*. 2016
20. Ichihara Y, Nishinaka T, Yamada Y, et al. Impact of vWF Activity in the Long-term Management of Centrifugal Type Continuous-flow LVAD Patients. *The Journal of Heart and Lung Transplantation*. 2014; 33(4):S87.
21. Saito S, Yamazaki K, Nishinaka T, et al. Post-approval study of a highly pulsed, low-shear-rate, continuous-flow, left ventricular assist device, EVAHEART: a Japanese multicenter study using J-MACS. *J Heart Lung Transplant*. 2014; 33(6):599–608. [PubMed: 24746637]
22. Dassanayaka S, Slaughter MS, Bartoli CR. Mechanistic pathway(s) of acquired von willebrand syndrome with a continuous-flow ventricular assist device: in vitro findings. *ASAIO J*. 2013; 59(2):123–129. [PubMed: 23438773]
23. Restle DJ, Zhang DM, Hung G, et al. Preclinical Models for Translational Investigations of Left Ventricular Assist Device-Associated von Willebrand Factor Degradation. *Artif Organs*. 2015; 39(7):569–575. [PubMed: 25810063]
24. Egger C, Maas J, Hufen T, Schmitz-Rode T, Steinseifer U. Establishing a method for in vitro investigation of mechanical parameters causing acquired von Willebrand syndrome in ventricular assist devices. *Artif Organs*. 2013; 37(9):833–839. [PubMed: 23834027]
25. Pruthi RK, Daniels TM, Heit JA, Chen D, Owen WG, Nichols WL. Plasma von Willebrand factor multimer quantitative analysis by in-gel immunostaining and infrared fluorescent imaging. *Thromb Res*. 2010; 126(6):543–549. [PubMed: 20889192]
26. Kato S, Matsumoto M, Matsuyama T, Isonishi A, Hiura H, Fujimura Y. Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity. *Transfusion*. 2006; 46(8):1444–1452. [PubMed: 16934083]
27. Naito K, Mizuguchi K, Nose Y. The need for standardizing the index of hemolysis. *Artif Organs*. 1994; 18(1):7–10. [PubMed: 8141660]

28. Zhou Z, Han H, Cruz MA, Lopez JA, Dong JF, Guchhait P. Haemoglobin blocks von Willebrand factor proteolysis by ADAMTS-13: a mechanism associated with sickle cell disease. *Thromb Haemost.* 2009; 101(6):1070–1077. [PubMed: 19492149]
29. Shim K, Anderson PJ, Tuley EA, Wiswall E, Sadler JE. Platelet-VWF complexes are preferred substrates of ADAMTS13 under fluid shear stress. *Blood.* 2008; 111(2):651–657. [PubMed: 17901248]
30. Kraus E, Kraus K, Obser T, et al. Platelet-free shear flow assay facilitates analysis of shear-dependent functions of VWF and ADAMTS13. *Thromb Res.* 2014; 134(6):1285–1291. [PubMed: 25201004]
31. Franchini M, Lippi G. Relative Risks of Thrombosis and Bleeding in Different ABO Blood Groups. *Semin Thromb Hemost.* 2016; 42(2):112–117. [PubMed: 26716497]
32. Woodhams B, Girardot O, Blanco MJ, Colesse G, Gourmelin Y. Stability of coagulation proteins in frozen plasma. *Blood Coagul Fibrinolysis.* 2001; 12(4):229–236. [PubMed: 11460005]
33. Scott EA, Puca KE, Pietz BC, Duchateau BK, Friedman KD. Comparison and stability of ADAMTS13 activity in therapeutic plasma products. *Transfusion.* 2007; 47(1):120–125. [PubMed: 17207240]
34. Nascimbene A, Hilton T, Konkle BA, Moake JL, Frazier OH, Dong J-f. Von willebrand factor proteolysis by adamts-13 in patients on left ventricular assist device support. *The Journal of Heart and Lung Transplantation.* 2017
35. Zhou Y, Qin S, Hilton T, et al. Quantification of Von Willebrand Factor Cleavage by adamts-13 in Patients Supported by Left Ventricular Assist Devices. *ASAIO J.* 2017; 63(6):849–853. [PubMed: 28682993]

Figure 1.

Normal FFP, FFP+rhADAMTS13 (1 or $4\mu\text{g/mL}$) and FFP+EDTA (10mM) were assayed for ADAMTS13 antigen level(A) and activity(B). The double solid line (=) denotes normal levels. Error bars are $\pm$ one standard deviation.

Figure 2.

Normal FFP, FFP+rhADAMTS13 (1 or 4 μg/mL) and FFP+EDTA (10mM) were assayed for (A)VWF:CB and (B)VWF:Ag. (C)VWF:CB/Ag was calculated at each time point.

Regression analysis showed a significant difference in FFP+rhADAMTS13 and control FFP.

Figure 3.

Samples of VAD-circulated FFP+dextran were assayed for (A)VWF:CB and (B)VWF:Ag and (C)VWF:CB/Ag was calculated. Regression analysis showed a significant difference between the HVAD, the HeartMate II and the control loop.

Figure 4.

Baseline and 360 minutes samples of FFP+dextran were analyzed by electrophoresis +blotting for VWF multimers. (A)The HMII, HVAD and CentriMag all depleted hVWF multimers. (B)The five largest hVWF bands were identified using densitometric tracing for baseline (▲) and 360 minute (△) samples. (C)The multimer ratio was calculated for each pump and control loop at baseline and 360 minutes. (D)The average position of the five hVWF bands was calculated for each baseline and 360 minute sample, showing significant differences for the HMII, HVAD and CentriMag.

Figure 5.

FFP+dextran was circulated through the A)HMII, B)HVAD and C)Levacor at 5L/min against 100mmHg and 2L/min against 80mmHg. The rate of VWF:CB/Ag decrease was significantly dependent on flow and pressure conditions in the HMII and HVAD, but not the Levacor.

Figure 6.

VWF:CB/Ag significantly decreased for all flow conditions at 360 minutes in the HMII and HVAD, but not the Levacor.

Figure 7.

Normal FFP (viscosity = 1.5cP) circulated through VADs was assayed for VWF:CB/Ag. These results were compared to those for FFP+dextran (3cP) in the A)HMII, B)CentriMag and C)HVAD. Regression analysis showed a statistically significant difference for the first hour of circulation by the HMII, but not the CentriMag or HVAD.

Figure 8.

There was a significant difference in VWF:CB/Ag decrease in FFP+dextran (3.0cP) circulated with an HVAD at 37° vs. 22°C by regression analysis.

Figure 9.

There were no statistically significant changes in VWF parameters between FFP+dextran and FFP+dextran+EDTA after 360 minutes of VAD circulation.