In vitro comparison of the hemocompatibility of two centrifugal left ventricular assist devices

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ABSTRACT

Objectives: Shear stress from left ventricular assist devices induces von Willebrand factor degradation and platelet dysfunction, leading to nonsurgical bleeding. We characterized the hemostatic changes induced by 2 centrifugal left ventricular assist devices, the HeartMate 3 (Abbott Inc, Chicago, Ill) and the EVAHEART (Evaheart Inc, Houston, Tex), for comparison.

Methods: Whole blood from 8 healthy volunteers was used ex vivo. Blood from the same donor was used for 6 hours of circulation in a miniature mock-loop system consisting of 2 identical extracorporeal circuits to compare the following experimental settings: (1) optimal revolutions per minute (rpm) for the HeartMate 3 ($n = 4$; 5000 rpm) and the EVAHEART ($n = 4$; 2500 rpm) and (2) equal rpm (3000 rpm for the HeartMate 3 and EVAHEART, $n = 4$ vs $n = 4$). For both settings, blood flow was adjusted to 1 mock-loop filling volume per minute (HeartMate 3 = 82 mL/min, EVAHEART = 100 mL/min). A panel of coagulation markers was analyzed to investigate hemostatic changes.

Results: The free plasma hemoglobin concentration was significantly lower in the EVAHEART compared with the HeartMate 3 after 6 hours of mock-loop circulation under both settings (optimal: 37 ± 31 vs 503 ± 173 mg/dL, $P < .0001$; equal: 27 ± 4 vs 139 ± 135 mg/dL, $P = .024$). Loss of von Willebrand factor high-molecular-weight multimers occurred in both left ventricular assist devices and settings, but the von Willebrand factor:activity/von Willebrand factor:antigen ratio after 6 hours was significantly lower in optimal settings for the HeartMate 3 ($P = .009$). The thrombin-antithrombin complex level was significantly lower with the EVAHEART for both settings ($P < .0001$).

Conclusions: The EVAHEART left ventricular assist device caused less hemolysis, resulted in lower coagulation activation, and provided better preservation of von Willebrand factor functional activity compared with the HeartMate 3 device. These findings prove that left ventricular assist device design plays a major role in minimizing blood damage during left ventricular assist device support. (J Thorac Cardiovasc Surg 2019;157:591-9)

In this graphical abstract, the schematic illustrations demonstrate the full magnetically levitated (Full MagLev) rotor of HeartMate 3 and the hydrodynamically levitated rotor of EVAHEART. The two LVADs, the EVAHEART and HeartMate 3, are shown as they are integrated in the twin-mock-loop. Two main findings are also presented: The concentration of free plasma haemoglobin (fHb) was significantly lower over time in the EVAHEART group representing a lower haemolysis. The concentration of the Thrombin-Antithrombin-Complexes (TAT) was significantly higher in HeartMate 3 group, indicating an increased activation of the procoagulant pathway.

Central Message

In a circulatory mock-loop, the EVAHEART LVAD caused less hemolysis, resulted in lower coagulation activation, and provided better preservation of vWF functional activity compared with the HM3.

Perspective

It has been proposed that different LVAD designs may result in better biocompatibility. In circulatory mock-loop with whole human blood, we showed that the EVAHEART was associated with reduced hemolysis and provided significantly greater preservation of vWF functional activity compared with the HM3. These findings imply that the design of LVADs plays a major role in providing better hemocompatibility.

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Abbreviations and Acronyms

CF-LVAD	= continuous-flow left ventricular assist device
EQU	= equal setting
fHb	= free plasma hemoglobin
HM3	= HeartMate3
HMII	= HeartMate II
HMWM	= high-molecular-weight multimers
OPT	= optimal setting
rpm	= revolutions per minute
TAT	= thrombin-antithrombin III complex
vWF	= von Willebrand factor
vWF:Ac/vWF:Ag	= vWF activity and vWF antigen

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INTRODUCTION

Nonsurgical bleeding is a major complication during continuous-flow left ventricular assist device (CF-LVAD) support, and its incidence has been reported to be up to 30% to 75%.¹⁻⁴ Several factors, including acquired von Willebrand syndrome and impaired platelet function under high shear stress settings, contribute to the high bleeding incidence during CF-LVAD support.^{1,2,5} New CF-LVAD devices have been designed to provide better hemocompatibility, including the centrifugal CF-LVAD HeartMate 3 (HM3) (Abbott Inc, Chicago, Ill), which has a fully magnetically levitated rotor, wide blood flow gaps, and an intrinsic pulse designed to avert stasis within the pump,^{6,7} and the EVAHEART (Evaheart Inc, Houston, Tex), a centrifugal CF-LVAD designed to minimize shear stress with a low operational revolutions per minute (rpm) range, large blood flow gaps, and low shear stress with a hydrodynamically levitated bearing rotating in a noncontact manner^{8,9} (Figure 1 and Table E1). Because of the pressure-flow characteristics of the EVAHEART, it provides a significant flow difference between systole and diastole, resulting in highly pulsed flow⁹ (Figure E1). The effects of LVAD design on hemostatic changes, platelet function, and von Willebrand factor (vWF) degradation are not yet characterized. Previous data suggest that each LVAD has a unique hematologic profile and that centrifugal LVADs provide better preservation of vWF than axial-flow LVADs.^{10,11} In this study, we assessed induced hemostatic changes using 2

centrifugal CF-LVADs with different designs. The EVAHEART and the HM3 devices were tested using a miniature mock-loop system consisting of 2 identical extracorporeal circuits filled with the same blood from 1 donor, enabling systematic head-to-head comparisons. A summary of the whole project including the main results can be found in Video 1.

MATERIALS AND METHODS**Design of the Twin Mock-Loop**

Both loops were identical consisting of an HM3 or EVAHEART LVAD (Figure 2) connected via polyvinyl chloride tubing (3/8", 1/4", 1/16"; RAUMEDIC AG, Münchberg, Germany) with exactly the same length to a blood reservoir for extracorporeal lung assist devices with a maximum blood volume of 30 mL (ECMO R-14 Assist Reservoir; Medtronic Inc, Minneapolis, Minn). The in-loop blood pressure, flow, and temperature were measured via suitable monitoring systems connected to the mock-loop as shown in Figure 1. Both loops were positioned under a heating hood (Certomat HK; Labexcahnge.com, Burladingen, Deutschland). The HM3 loop had a filling volume of 82 mL, and the EVAHEART loop was filled completely with 100 mL of blood. The different filling volumes were related to the different sizes of the 2 LVAD devices. This setting allowed up to 5 analytic samples of 4.5 mL each without significant decreases in pressure or flow.

Blood Collection From Healthy Human Volunteers and Mock-Loop Preparation

After receiving ethical approval from the University Hospital of the RWTH, Aachen, Germany (File No: EK355/16) and obtaining informed consent, our experiments were performed in accordance with the study protocol and Good Clinical Practice guidelines. A total of 150 mL of venous blood was collected from 8 healthy volunteers. Blood was collected in three 50-mL syringes primed with 3.75 IU/mL of B. BRAUN heparin (5000 IU/mL; B.BRAUN, Melsungen, Germany). In each comparison, blood was used from the same donor in twin mock-loops. The HM3 loops were primed with 35 mL of isotonic saline (B.BRAUN), and the EVAHEART loops required a priming volume of 42 mL of isotonic saline before whole blood was added to the loops. Therefore, we achieved a clinically relevant hematocrit value of 25% to 30%.

Experimental Groups

The operational speed ranges of the HM3 and EVAHEART are 3000 to 9000 rpm and 800 to 3000 rpm, respectively. The experiments were performed in 2 experimental groups. For the first group (OPT), the optimal rpm was selected, which represents the normal clinical operational range, recommended by the manufacturer: 5000 rpm for the HM3 and 2500 rpm for the EVAHEART. For the second group (EQU), an equal rpm was selected; therefore, the HM3 had to be operated at its minimum speed (3000 rpm), and the EVAHEART had to run at its maximum speed (3000 rpm). For both groups and settings, the blood flow was adjusted to 1 mock-loop filling volume, reflecting 1 cardiac output per minute, which represents a realistic cardiac output for an LVAD recipient. This equals 82 mL/min for the HM3 and 100 mL for the EVAHEART. Figure 3 illustrates the experimental setting.

Blood Sampling and Analysis

Detailed methodology is provided in the Appendix. vWF studies, metalloprotease ADAMTS-13, free plasma hemoglobin (fHb), platelet activation studies (P-selectin [CD62P]), and thrombin-antithrombin III complex (TAT) were assessed, among other things.

Statistical Analysis

Continuous data are presented as the mean $\pm$ standard deviation. Categorical variables are described in absolute numbers and percentages.

FIGURE 1. The EVAHEART device (Evaheart Inc, Houston, Tex) and HM3 device (Abbott Inc, Chicago, Ill). A and B, External views of the EVAHEART device and impeller. C, Cross-section of the EVAHEART demonstrating the wide blood gaps and the hydrodynamically levitated bearing rotates in a noncontact manner. D, Cross-section of the HM3 shows the full magnetically levitated (Full MagLev) rotor. D, Reprinted from.⁶ CS, cool seal; CSU, cool seal fluid-unit. With permission of Elsevier.

FIGURE 2. Schematic illustration of the mock-loop design. One loop was set up for the EVAHEART, and an identical loop was set up for the HM3 device (1). The loops consist of the following: 2: 3/8" tubing; 3: a 3/8" to 1/4" polycarbonate connector; 4: a pressure transducer (Pressure Probe Xtrans, CODAN pvb Critical Care Inc, Forstinning, Germany); 5: 1/16" tubing; 6: a hose clip; 7: a 1/16" to 1/4" polycarbonate connector; 8: a temperature probe (Physitemp TCAT-2LV controller; Physitemp Instruments Inc, Clifton, NJ); 9: an ECMO reservoir; 10: 1/4" tubing; 11: a clamp on the flow probe (BioProTTM Clamp-On™ Transducer; em-tec Inc, Finning/Germany); 12: a blood withdrawal site; and 13: a 3-way stop cock. We positioned pressure sensors via Luer connectors before and behind the blood pumps (Pressure Probe Xtrans, CODAN pvb Critical Care Inc) to monitor the positive and negative blood pressures within the system. To monitor and regulate blood flow, a flow probe (BioProTT Clamp-On Transducer; em-tec Inc) was connected to the circuit. Temperature was monitored by a digital thermometer (physitemp TCAT-2LV controller; Physitemp Instruments Inc) and was maintained at 37°C using a heating-hood (Certomat HK; Labexcahnge.com, Burladingen, Deutschland) that covered both complete mock-loops. To regulate blood pressure and flow, we used a custom-made hose clip positioned shortly behind the outflow of the blood pumps on the 1/16" tubing part. *HCT*, Hematocrit.

A multivariate analysis for repeated measurements with contrast analysis for consecutive time points was performed, followed by post hoc Tukey testing to prove the effect of time or time and group on the observed parameters using SPSS software (SPSS statistics version 20; IBM Inc, Ehningen, Germany). A 2-way analysis of variance was performed to assess the significance of differences between the groups at single time points using GraphPad Prism (Prism 6.04; GraphPad Software, La Jolla, Calif). For the graph design, GraphPad Prism software was used.

RESULTS

Hemodynamics

For the OPT and EQU settings, flow (mL/min) did not differ between the groups (Table E2). The pressure with the OPT settings did not differ between the EVAHEART

and the HM3, but with the EQU settings, when the EVAHEART operated at its maximum rpm (3000 rpm) and the HM3 at its minimum rpm (3000 rpm), the pressure was significantly higher with the EVAHEART ($P < .0001$) (Table E2).

The pump power (Watt) was significantly higher with the EVAHEART for both the OPT and EQU settings compared with that for the HM3 ($P = .043$ and $P < .0001$, respectively) (Table E2).

Hemogram

An overview of all hemogram results is presented in Figure 4. The platelet count with the OPT settings was significantly lower in the EVAHEART group compared with that in the HM3 group after 360 minutes (108 ± 26.4 vs $281 \pm 153.9 \times 10^3/\mu\text{L}$, $P = .001$), whereas the platelet count with the EQU setting was significantly lower in the HM3 group compared with that in the EVAHEART group at the same time point.

Hemolysis

The fHb was significantly higher in the HM3 group at 360 minutes with both settings compared with that in the EVAHEART group (OPT: 503.1 ± 173.9 vs 37.2 ± 1.1 mg/dL, $P < .0001$; EQU: 139.3 ± 153.8 vs 27.3 ± 24.1 mg/dL, $P = .029$) (Figure 5).

Platelet Activation

In both groups, the EVAHEART and HM3, and with both settings (OPT and EQU), CD62P was expressed on less than 4% of unstimulated platelets. However, CD62P/CD61+ at 360 minutes was significantly higher in the EVAHEART group with both the OPT and EQU settings compared with that in the HM3 group (1.75 ± 0.98 vs 0.92 ± 0.37 , $P = .003$ and 3.2 ± 2.3 vs 2.9 ± 0.9 , $P = .024$) but remained within the normal range of 1.21 (interquartile range, 0.69-2.17)¹² (Figure 6). Regarding changes within each group, with the OPT settings, no significant increase in CD62P expression was observed between baseline and 360 minutes in either group (Figure 6). In contrast, under EQU settings, CD62P expression increased significantly between baseline and 360 minutes in both groups (EVAHEART: $0.50\% \pm 0.34\%$ vs $3.23\% \pm 2.39\%$, $P = .006$; HM3: $0.40\% \pm 0.69\%$ vs $2.93\% \pm 0.92\%$, $P = .012$).

Procoagulant Marker Thrombin-Antithrombin III Complex

TAT levels were significantly elevated after 5 minutes of mock-loop circulation and remained elevated until the end of the experiment in both groups and with both settings compared with the normal cutoff level (<4 $\mu\text{g/L}$). After 2 hours of circulation, the TAT concentration increased significantly in both groups compared with that at baseline, with no difference between the groups. In the

FIGURE 3. Flow chart of the experiments. HM3, HeartMate 3; EVA, EVAHEART; OPT, optimal setting; EQU, Equal setting.

HM3 group, TAT levels were significantly higher for both settings at 4 and 6 hours (Figure 6) compared with those in the EVAHEART group (OPT: 11.02 ± 0.82 vs 8.42 ± 1.14 mg/L; $P = .0178$; EQU: 9.35 ± 0.84 vs 5.6 ± 1.17 mg/L, $P = .0179$).

von Willebrand Factor Analysis

Loss of vWF high-molecular-weight multimers (HMWM) was observed in both groups and under both settings (Figure E2). The EVAHEART group had a significantly higher vWF activity and vWF antigen (vWF:Ac/vWF:Ag) ratio than the HM3 group with the OPT settings (0.69 ± 0.08 vs 0.40 ± 0.15 , $P = .009$) (Figure 7, A). Under the EQU setting, the vWF:Ac/vWF:Ag ratio was significantly lower in the EVAHEART group after 1 and 2 hours of mock-loop circulation (Figure 7, B), but this significant difference diminished after 6 hours. ADAMTS-13 activity did not differ between the EVAHEART and HM3 groups at any time points under both settings (Figure 7, C and D).

DISCUSSION

This study is the first to investigate the hemostatic changes and hemocompatibility of 2 centrifugal CF-LVADs in a miniature mock-loop system. We showed that the EVAHEART was associated with reduced hemolysis, provided significantly greater preservation of vWF functional activity, and resulted in lower coagulation activation. These findings imply that the design of LVADs and their flow characteristics play major roles in providing better hemocompatibility and causing less blood damage and may provide important information for the design of future LVADs.

We selected 2 different experimental settings, one to investigate the normal clinical standard (optimal pump speed according to the manufacturer) and another to allow for a direct comparison between the studied devices (equal

pump speed). Because we used identical extracorporeal circuits and the same donor blood for each device and setting, we determined that the differences in vWF degradation, erythrocyte damage, and platelet activation are most likely due to the unique design of each device.

Hemolysis

Hemolysis has been proven to correlate with a higher incidence of thromboembolic events.¹³ A widely accepted and sensitive marker for detecting hemolysis in patients with LVAD is fHb.¹⁴ In our study, we detected up to 500 mg/dL of fHb in the HM3 group, whereas the fHb in the EVAHEART group was significantly lower, and after the experiments under both settings, fHb was clearly below the defined limit of 40 mg/dL.¹⁴ Although the EVAHEART-LVAD operated at its maximum rpm and the HM3 operated at its minimum rpm under the EQU setting, the fHb concentration remained approximately 112 mg/dL lower in the EVAHEART group compared with that in the HM3 group. Although these findings were derived from an in vitro setting, they are consistent with those of Matsumoto and colleagues,¹⁵ who collected in vivo data from patients who received the HeartMate II (HMII) or the EVAHEART and detected a significantly lower rate of hemolysis in the EVAHEART recipients. Both the HM3 and the EVAHEART were designed with wide blood flow gaps to minimize shear stress and blood damage.^{6,9} However, the EVAHEART was associated with markedly reduced hemolysis. The mechanical seal system in the EVAHEART-LVAD with a recirculating purge system (Cool-Seal) may contribute to the low hemolysis rate. In this seal system, the seal temperature is maintained at less than 40°C to prevent heat denaturation of blood proteins.¹⁶

Platelet Activation

We used flow cytometry to determine the level of P-selectin (CD62P) expression on platelet surfaces as an activation marker as described previously.¹⁷ According to the strong

FIGURE 4. Overview of the hemogram results comparing the HM3 and the EVAHEART. pH value decreased significantly over time in both settings (OPT, EQU) and independent from the experimental groups

hemolysis in the HM3 group, which is related to higher shear stress, we expected stronger platelet activation in this group. In contrast, CD62P expression was significantly higher in the EVAHEART group after 360 minutes compared with that in the HM3 group; however, CD62P was expressed on less than 4% of unstimulated platelets in both groups, which is within the normal range of platelet activation and is consistent with the findings of Slaughter and colleagues,¹⁸ who did not detect increased platelet activation during CF-LVAD support with the HMII.

Procoagulant Marker

TAT levels represent a sensitive indicator of procoagulant pathway activation.¹⁹ Data from our previous study²⁰ and from Spanier and colleagues²¹ indicate that patients with LVADs exhibit increased coagulation activation as indicated by higher TAT concentrations. In our present in vitro study, TAT values at 240 minutes and 360 minutes were significantly higher in the HM3 group compared with those in the EVAHEART group under both OPT and EQU settings, indicating a higher activation level of plasmatic coagulation. Nevertheless, we also detected time-dependent activation of coagulation in the EVAHEART group because TAT levels increased significantly from baseline to the end of the experiment. Activation of the procoagulant pathway may increase the risk for thrombus formation.¹⁹

Because the normal TAT cutoff value is less than 4 $\mu\text{g/L}$, we found that both LVAD groups under both experimental flow settings showed significantly elevated TAT values ($>769 \mu\text{g/L}$) only 5 minutes after initiating extracorporeal blood circulation in the miniature mock-loop. This early elevation of TAT concentrations may be related to the design of the miniature mock-loop, leading to prompt activation of coagulation due to contact activation by foreign materials, which initiates intrinsic coagulation factors and triggers prothrombin conversion into thrombin.²²

Von Willebrand Factor Analysis

In this study, we demonstrated that both the HM3 and the EVAHEART caused similar degrees of vWF HMWM degradation under both OPT and EQU settings, confirming that the differences in vWF HMWM degradation are not dependent on the absolute or average speeds, which is consistent with Meyer and colleagues,²³ who found that

(A and B). Hemoglobin and hematocrit remained stable over time without any differences between the groups or the setting (C-F). The lactate concentration increased significantly over time in both settings and was significantly higher after 360 minutes in the HM3 group during the OPT experiments (G and H). The glucose concentration decreased over time without differences between the groups and independent from the setting (I and J). *** $P < .001$ effect of time. # $P < .05$ versus EVAHEART. OPT, optimal setting; EQU, Equal setting; HGB, hemoglobin; HCT, hematocrit; HM3, HeartMate 3; EVA, EVAHEART.

FIGURE 5. Time course of fHb in the HM3 and EVAHEART with the optimal rpm setting (A) and the equal rpm setting (B). * $P < .05$ effect of time. # $P < .05$ versus EVAHEART. ### $P < .001$. HM3, HeartMate 3; EVA, EVAHEART.

the lower-speed HVAD-LVAD caused similar vWF HMWM degradation compared with that caused by the higher-speed HMII. In contrast to our findings, Bartoli and colleagues¹⁰ compared the EVAHEART with the HMII in a mock-circulatory loop and detected significantly better vWF HMWM preservation with the EVAHEART. Netuka and colleagues²⁴ compared HM3 and HMII recipients and found significantly better vWF HMWM preservation in the HM3 recipients. Despite similar degrees of vWF HMWM degradation with the EVAHEART and the HM3, we detected a

significant difference in functional assays of vWF activity between the EVAHEART and the HM3. Under OPT settings, the vWF:Ac/vWF:Ag ratio was significantly lower in the HM3 group, indicating downstream functional activity of vWF. Clinically, both devices have demonstrated promising results in terms of bleeding events. Of note, clinical studies with the EVAHEART have shown very low bleeding rates. In the first 96 patients who received the EVAHEART, no gastrointestinal bleeding was noted,⁹ and the Japanese Registry for Mechanically Assisted Circulatory Support

FIGURE 6. Time course of CD62P expression and TAT under the optimal (A and C) and equal rpm settings (B and D). * $P < .05$ effect of time. ## $P < .01$ versus HM3. TAT, Thrombin-antithrombin III complex.

FIGURE 7. Time course of vWF activity and the vWF antigen ratio (vWF:Ac/vWF:Ag) under the optimal (A) and equal rpm settings (B). Metalloprotease concentration (ADAMTS13) under the OPT (C) and EQU flow settings (D). * $P < .05$ effect of time. # $P < .05$ versus EVAHEART. ## $P < .01$ versus EVAHEART. HM3, HeartMate 3; EVA, EVAHEART.

reports a 3-year postoperative bleeding rate of only 6.2%.²⁵ In contrast, the rate of bleeding events in HM3 recipients reached 15%.^{26,27} Preservation of vWF functional activity may contribute to the lower bleeding rate observed with the EVAHEART.

Our results demonstrate the potential benefits of developing designs and techniques that lower interactions between blood and pump surfaces to achieve better hemocompatibility.

Study Limitations

Our study has several limitations. As in all ex vivo models, the physiologic regulations seen in patients with chronic heart failure could not be reproduced in our mock-loop. Our avascular mock-loop was not able to reproduce certain blood-flow and pressure conditions of the human circulatory system. Consequently, our findings should be interpreted with caution when extrapolated to patients on LVAD support.

The most relevant limitation of this study is that a mock-loop system is an artificial in vitro system lacking organs and cells as sources of procoagulant factors or cytokines. Nevertheless, we set up a mock-loop system enabling physiologic pressure, blood flow, and temperature conditions that is compatible with human blood instead of

animal blood. Therefore, we only observed the effects of contact between human blood and an LVAD and artificial surfaces without a persistent supply of coagulation factors and proinflammatory mediators. The artificial surface of our miniature mock-loop system may have influenced our results because blood contact with an artificial surface activates many pathways that affect the coagulation cascade

VIDEO 1. Significance of findings. The principal investigator and senior author Dr Christian Bleilevens and first author Dr Rashad Zayat explain the relevance and importance of the study results. Video available at: [https://www.jtcvs.org/article/S0022-5223\(18\)32078-6/fulltext](https://www.jtcvs.org/article/S0022-5223(18)32078-6/fulltext).

and platelet function. On the other hand, the tubing and reservoir bags that we used were developed for clinical use in patients with extracorporeal lung assist and feature the gold standard of biocompatibility used in clinical practice. Finally, we performed extracorporeal circulation for only 6 hours, restricting our findings to an acute-phase scenario.

CONCLUSIONS

The EVAHEART LVAD caused less hemolysis, resulted in lower coagulation activation, and provided better preservation of vWF functional activity compared with the HM3. These findings prove that LVAD design plays a major role in minimizing blood damage during LVAD support and may add useful information to the design of future LVADs.

Conflict of Interest Statement

T.M. and C.S.-M. are employees of Evaheart Inc. Evaheart Inc provided the EVAHEART LVAD that was studied in this investigation. Evaheart employees were not involved in the experiments, collection of data, or analysis of data. All other authors have nothing to disclose with regard to commercial support.

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Key Words: Left ventricular assist device, circulatory mock-loop, HeartMate 3, EVAHEART, haemolysis, hemocompatibility

APPENDIX

Blood Sampling and Analysis

Blood was collected at 5 minutes, 1 hour, 2 hours, 4 hours, and 6 hours in standard citrate collection tubes (S-Monovette; Sarstedt Inc, Nümbrecht, Germany) and centrifuged at 2000g for 10 minutes at room temperature. The blood plasma was then used for vWF activity and multimer analysis and detection of CD62P-positive platelets via flow cytometry (fluorescence-activated cell sorting). Furthermore, fHb was measured as an indicator of hemolysis; 1 mL of plasma was collected in standard serum gel collection tubes containing coagulation activator gel (S-Monovette; Sarstedt Inc). The blood was allowed to clot for 30 minutes at room temperature before centrifugation at 2000g for 10 minutes at 4°C. Blood serum was stored at -80°C for subsequent measurement of the TAT and metalloproteinase (ADAMST13). A total of 50 µL of citrated whole blood was used to measure the activated clotting time using a hemochron junior device with appropriate ready-to-use cuvettes (Keller Medical Inc, Bad Soden, Germany). A hemogram was measured on an automated cell counter using 20 µL of citrated whole blood (MEK-6550K; Nihon Khoden Inc, Rosbach, Germany). Finally, blood gas analysis was performed using 430 µL of citrated whole blood on an ABL900-flex analyzer (Radiometer Inc, Fichtenhain, Germany).

Von Willebrand Factor Diagnostic

Approximately 1.2 mL of the citrated blood plasma supernatant was stored at -80°C after centrifugation; 200 µL was used for the measurement of vWF-activity (vWF:Ac) and vWF-antigen (vWF:Ag) on a semiautomated ACL-TOP700 analyzer (Werfen Inc, Munich, Germany). On the basis of these results, the vWF-multimer analysis was performed in an external reference German laboratory (MVZ Labor Dr Reising Ackermann und Kollegen, Leipzig, Germany) using Western blot with sodium dodecylsulfate agarose gel electrophoresis and densitometry-based software IMAGE J.

Flow Cytometry

Enhanced coagulation activity is indicated by increasing levels of P-selectin (CD62P) positive platelets. We used 20 µL of the citrated blood for flow cytometry to detect cell surface expression of P-selectin using fluorescein isothiocyanate- and phycoerythrin-conjugated CD61 (MCA2263F; BioRad Inc, Munich, Germany) and CD62P (MCA2418PE; BioRad Inc) antibodies. After incubation for 15 minutes at room temperature, as described previously,^{28,29} platelets were fixed for analysis (Cell Fix; BD Biosciences Inc, Heidelberg, Germany) before the fluorescence-activated cell sorting analysis on a FACSCanto-II analyzer (BD Biosciences Inc).

Hemolysis Assay

A total of 25 µL of the stored blood plasma was mixed with 85 µL of the hemolysis reagent (hemoglobin FS; Diasys Inc, Holzheim, Germany). The mixture was pipetted in double into a 96-well plate for absorption measurement on a microplate reader (ELx800; BioTek Inc, Bad Friedrichshall, Germany) at 540 (A1) and 680 nm (A2) wavelength. The concentration of free hemoglobin was calculated according to the formula (A1-A2)*733 in mg dL⁻¹.

Enzyme-Linked Immunosorbent Assay

TAT and ADAMST13 concentrations were detected from blood serum via enzyme-linked immunosorbent assay (ELISA) technology. For the TAT ELISA, samples were diluted 1:50 with appropriate diluent according to the manufacturer's protocol (#OWMG Enzygnost; Dade Behring Inc, Marburg, Germany), transferred to a 96-well plate, and the concentration was detected via absorption measurement at 492-nm wavelength on a microplate reader (ELx800; BioTek Inc). For the ADAMST13 ELISA (DADT130; R&D Systems by Bio-Techne Inc, Wiesbaden-Nordenstadt, Germany), the serum samples were not diluted and were measured according to the manufacturer's protocol on a microplate reader at 450 nm and 540 nm wavelength as reference.

FIGURE E1. EVAHEART pressure-flow curve. At low pressure, the pump flow reaches a high flow rate of 15 to 20 liters. Pump speed is steady, yet aortic pulsatility is preserved by high peak flow with the unique impeller design. Example of separation of plasma vWF multimers with 1.6% sodium dodecylsulfate agarose gel electrophoresis and computer-assisted development of curves with comparative calculation of number of peaks at different time points in the HMWM domain. A, Separation of plasma vWF multimers with 1.6% sodium dodecylsulfate agarose gel electrophoresis: (1) baseline probe, (2) same probe from the donor of probe 1 after 6 hours of circulation in the mock-loop with EVAHEART, (3) normal plasma for comparison, and (4) same probe from the donor of probe 1 after 6 hours circulation in the mock-loop with the HM3. B to D, Computer-assisted development of curves with comparative calculation of number of peaks at different time points in the HMWM domain. AOP, aortic pressure; LV-AO, left ventricular-aortic pressure.

FIGURE E2. Exemplary results from von Willebrand Multimeren analysis with western blot. Demonstration of the loss of high-molecular-weight multimers of von Willebrand factor in both groups HeartMate 3 and EVAHEART. Number 3 is a normal reference probe. Number 1 is the preoperative Probe from the healthy volunteer, number 2 the same probe after 6h circulation in the mock-loop with HeartMate 3 and probe number 4 after 6h circulation in the mock-loop with EVAHEART.

TABLE E1. Comparison between the design of the EVAHEART and HeartMate 3 left ventricular assist devices

	HeartMate 3 (Abbott Inc, Chicago, Ill)	EVAHEART (EVAHEART Inc, Houston, Tex)
Design	Cf-LVAD centrifugal	Cf-LVAD centrifugal
Impeller	Magnetically levitated	Hydraulically levitated
Pump speed range	3000-9000 rpm	1400-3000 rpm
Inflow diameter	19 mm	19 mm
Outflow diameter	14 mm (polyester)	16 mm (ePTFE) 14 mm (polyester)
Pump diameter	50 mm	51 mm

Cf-LVAD, Continuous-flow left ventricular assist device; rpm, revolutions per minute; ePTFE, expandable polytetrafluoroethylene.

TABLE E2. Blood flow and pressure in left ventricular assist device mock circulatory loops

Parameter	EVAHEART	HeartMate 3	P value
Optimal rpm settings			
Probe flow mL/min	101.4 ± 7.4	99.2 ± 12.4	.445
Power watt	3.9 ± 0.1	2.8 ± 0.05	<.001
Pressure mm Hg	84.9 ± 50.6	55.6 ± 38.7	.359
Equal rpm settings			
Probe flow mL/min	90.2 ± 11.7	89.7 ± 4.5	.999
Power watt	8.1 ± 4.5	1.8 ± .5	<.0001
Pressure mm Hg	152.2 ± 71	78.2 ± 73.1	.0177

rpm, Revolutions per minute.