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ORIGINAL ARTICLE

ADAMTS13 inhibition to treat acquired von Willebrand syndrome during mechanical circulatory support device implantation

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Abstract

Background: Acquired von Willebrand syndrome (aVWS) is common in patients with mechanical circulatory support (MCS) devices. In these patients, the high shear stress in the device leads to increased shear-induced proteolysis of von Willebrand factor (VWF) by A Disintegrin And Metalloprotease with Thrombospondin type 1 repeats, number 13 (ADAMTS13). As a result, the high molecular weight (HMW) VWF multimers are lost, leading to a decreased VWF function and impaired hemostasis that could explain the bleeding complications that are frequently observed in these patients. To counteract this abnormal VWF degradation by ADAMTS13, we developed a novel targeted therapy, using an anti-ADAMTS13 monoclonal antibody (mAb) that inhibits the shear-induced proteolysis of VWF by ADAMTS13.

Methods: Human or bovine blood was circulated through *in vitro* MCS device systems with either inhibitory anti-ADAMTS13 mAb 3H9 or 17C7 (20 μg/ml) or control anti-ADAMTS13 mAb 5C11 or phosphate buffered saline (PBS). VWF multimers and function (collagen binding activity) were determined at different time points. Next, Impella pumps were implanted in calves and the calves were injected with PBS and

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subsequently treated with mAb 17C7. VWF, ADAMTS13, and blood parameters were determined.

Results: We demonstrated that blocking ADAMTS13 could prevent the loss of HMW VWF multimers in *in vitro* MCS device systems. Importantly, our antibody could reverse aVWS in a preclinical Impella-induced aVWS calf model.

Conclusion: Hence, inhibition of ADAMTS13 could become a novel therapeutic strategy to manage aVWS in MCS device patients.

KEYWORDS
acquired von Willebrand syndrome, ADAMTS13, bleeding, mechanical circulatory support, von Willebrand factor

1 | INTRODUCTION

It is estimated that more than 30 million people suffer from heart failure worldwide.[1] The advanced stage of heart failure occurs in 6% to 25% of the heart failure patients and is associated with an unacceptably high mortality when treated with medical therapy alone.[2] A small number of the patients with advanced heart failure can be referred for cardiac transplantation. Unfortunately, only around 5000 transplantations are annually performed worldwide, limited by the availability of donor organs.[3] Hence, this has led to an advancement in the field of mechanical circulatory support (MCS) devices. MCS devices to treat advanced stage heart failure can either be long-term or short-term depending on the clinical needs and potential of cardiac recovery.[4] Left ventricular assist devices (LVADs) are long-term support devices (e.g., Heartmate II or III) that pump blood from the left ventricle to the aorta.[5,6] Devices providing urgent/emergent MCS to treat patients with cardiogenic shock (e.g., Impella or extracorporeal membrane oxygenation [ECMO]) are classified as short-term support devices, whose primary goal is to restore normal hemodynamics.[7,8]

Although MCS devices tremendously improve the survival of advanced-stage heart failure patients, thrombotic and bleeding complications are serious side effects.[9] Importantly, gastrointestinal bleeding is one of the most common severe adverse events of LVAD therapy with an incidence of 20%–30%.[9–11] Gastrointestinal bleeding in LVAD patients was initially linked to the use of anticoagulation following device implantation. Bleeding rates are, however, higher in LVAD patients compared with patients treated with anticoagulation for other indications.[12] Hence, the use of anticoagulation only partially explains the hemorrhagic complications in these patients. Recently, it was suggested that bleeding in MCS device patients might be caused by a loss of function of the hemostatic protein von Willebrand factor (VWF).[13] VWF is a multimeric protein and its high molecular weight multimers (HMW) are crucial for its hemostatic activity. However, HMW VWF multimers disappear in LVAD patients within minutes after device implantation,[13–16] leading to decreased VWF function and increased risk for bleeding, known as acquired von Willebrand syndrome (aVWS). aVWS is therefore hypothesized to be a major contributing factor for the bleeding episodes observed in these patients. Likewise, aVWS is also observed in patients implanted with an Impella or with an ECMO device and also in those patients the loss of HMW VWF multimers might contribute to the frequently observed bleedings in these patients.[17–20] Remarkably, removal of the device and subsequent heart transplantation results in a restoration of the HMW VWF multimers and VWF function and corrects the bleeding.[14]

Essentials

- Patients with a heart pump suffer from the acquired von Willebrand syndrome (aVWS).
- A targeted therapy to treat heart pump-induced aVWS is currently unavailable.
- Blocking ADAMTS13 prevents aVWS in *in vitro* heart pump systems and reverses aVWS in a calf model.
- Blocking ADAMTS13 could become a novel treatment to manage aVWS in patients with a heart pump.

Current treatment of LVAD-induced gastrointestinal bleeding consists of supportive care (iron supplementation, blood transfusion), endoscopic therapy (argon plasma coagulation, placement of endoscopic hemoclips, or contact thermal therapy) or a reduction in antithrombotic treatment.[10] However, identifying and accessing the lesions in the gastrointestinal tract is not straightforward and recurrent bleeding occurs in 50% of the patients despite endoscopic procedures.[11] Moreover, a downshift in antithrombotic therapy poses the patients at high thrombotic risk, again increasing morbidity and mortality.[21,22] Hence, there is a need for a targeted therapy to treat the underlying cause of MCS device-induced bleeding, which is thought to be the acquired VWF defect.

During the past few years, efforts have been made to determine the mechanism by which HMW VWF multimers are lost in MCS device patients. First, it was hypothesized that VWF degradation was mainly caused by mechanical destruction of VWF but novel insights into the role of ADAMTS13 in the shear-induced VWF degradation were provided by *in vitro* LVAD studies.[23,24] Because of the high shear stress, caused by MCS devices, HMW VWF unfolds, exposing

the cleavage site for the VWF-cleaving protease A Disintegrin And Metalloprotease with Thrombospondin type 1 repeats, number 13 (ADAMTS13) (Figures S1 and S2).[9,13] As a consequence, these excessively cleaved VWF multimers have a reduced potential for binding to collagen and platelets and thus a diminished hemostatic activity, thereby rendering MCS device patients more prone to bleeding.

We therefore aimed to consolidate the role of ADAMTS13 in the shear-induced proteolysis of VWF *in vitro* by using a specific ADAMTS13 inhibitor and to determine whether blocking ADAMTS13 using this specific inhibitor can rescue the loss of HMW VWF multimers in a preclinical MCS device animal model.

2 | METHODS

2.1 | Monoclonal anti-human ADAMTS13 antibodies

Murine anti-human ADAMTS13 monoclonal antibodies (mAbs) 3H9 and 5C11 are in-house generated mAbs.[25] mAb 3H9 inhibits ADAMTS13 activity and is directed against the metalloprotease (M) domain of ADAMTS13, mAb 5C11 does not inhibit ADAMTS13 and has an epitope in the second thrombospondin type 1 repeat (T2) of ADAMTS13.[25] Both mAbs have been extensively characterized both *in vitro* and *in vivo*.[25] Murine anti-human ADAMTS13 mAb 17C7 has been recently generated and is also directed against the M domain of ADAMTS13.[26] mAb 17C7 is known to inhibit ADAMTS13 activity but was not further characterized. Further characterization of mAb 17C7 was done in the current study. Methods used for characterization of the murine mAb 17C7 are provided in Supplemental Methods.

2.2 | Human and bovine blood

Human citrated blood was collected from the "Etablissement Français du Sang" blood bank (Lille, FR) and from the Belgian Red Cross-Flanders Blood Establishment. Citrate (CPDA 12.5%, Cantaert Medical) and heparin (5000 IU/L, VWR) anticoagulated bovine blood was provided by the animal facility of Gasthuisberg. Blood collections were approved by the institutional ethics committee; human blood donors consented to the use of their blood donation in scientific research and the study was in accordance with the Declaration of Helsinki.

2.3 | *In vitro* Impella CP and 5.5 system

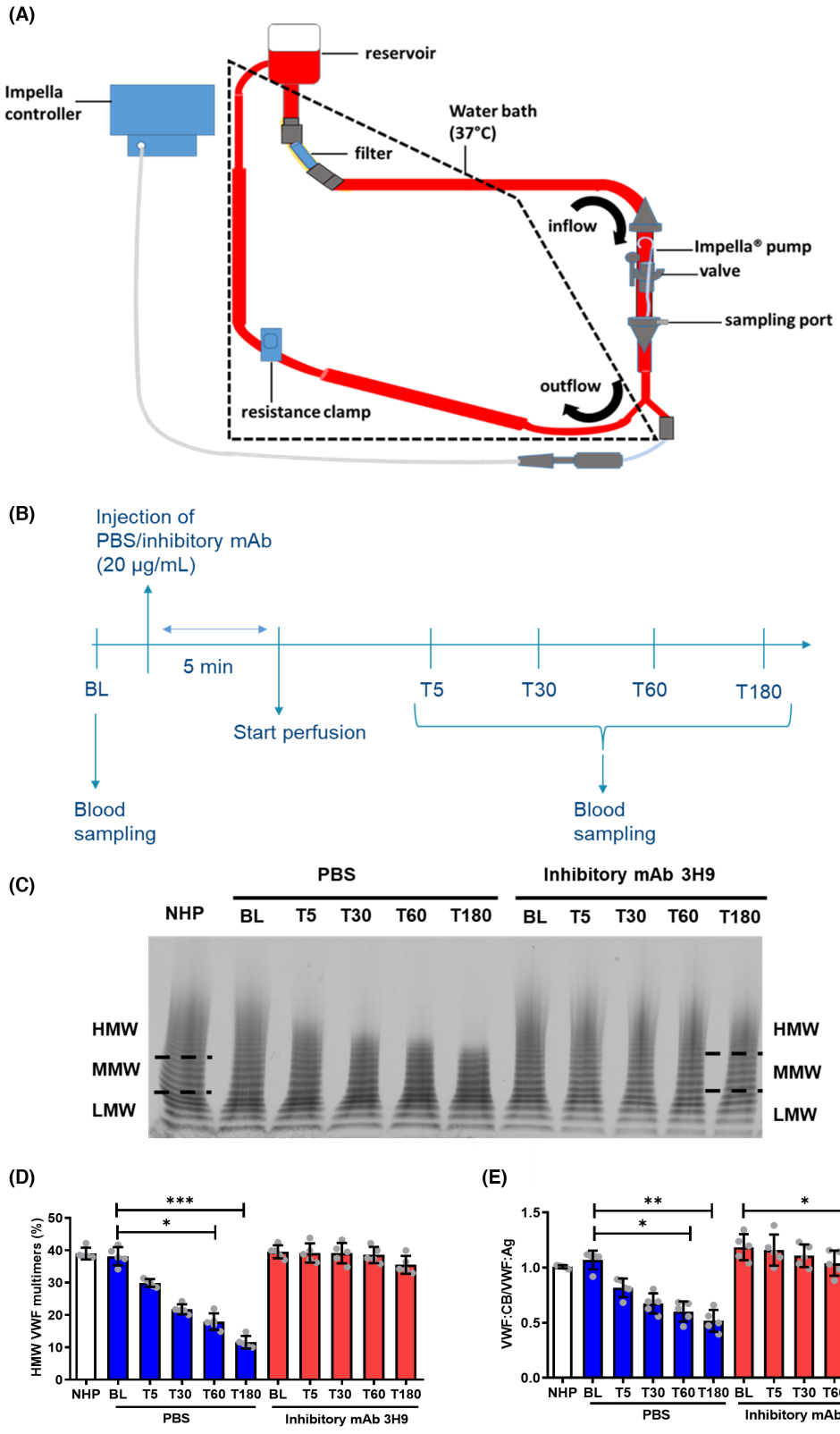
The Impella (Abiomed, Aachen, DE) pump, a microaxial continuous-flow (CF) device commonly used for short-term LVAD support (Figure S2), was localized inside a closed tubing system containing a valve, resistance clamp, and sampling device (Figure 1A). The system was filled with 750 ml citrate anticoagulated human blood (Red Cross) or bovine blood (animal facility Gasthuisberg). Where needed, blood was supplemented with 20 μg/ml of the inhibitory anti-ADAMTS13 mAb 3H9 or mAb 17C7 or the control anti-ADAMTS13 mAb 5C11 or with an equal volume of phosphate buffered saline (PBS). The pump rotor was set at 44 000 (Impella CP) or 30 000 (Impella 5.5) rotations per minute (rpm); speeds applied in patients with cardiogenic shock.[7] Blood was sampled 5 min before the onset of perfusion (BL) and 5 (T5), 30 (T30), 60 (60), and 180 (T180) (and 300 [T300] minutes for bovine blood) minutes after the onset of perfusion (Figure 1B). Plasma was obtained through centrifugation (2000*g*, 15 min) of the whole blood samples. Human quality CP and 5.5 pumps were manufactured by Abiomed Europe GmbH and underwent manufacturing verification. Technical parameters such as pump performance level (pump flow) and motor current were carefully monitored and displayed in real time using the clinically approved Automated Impella Controller (interface for the Impella platform).

2.4 | *In vitro* Heartmate II system using human blood

To investigate the degradation of VWF in a device used for long-term LVAD support, an axial CF Heartmate II (manufactured and provided by Thoratec Corp.) device was used[27] (Figure S1) and *in vitro* perfusion of human blood through the device was performed as described.[24] The inlet and outlet ducts of the HeartMate II device were connected to two tubings to make a closed system. In the closed system, a sampling device was included (Figure S3A). The system was filled with 250 ml citrate anticoagulated whole human blood (blood bank "Etablissement Français du Sang"). Where needed, human blood was supplemented with 20 μg/ml of the inhibitory anti-ADAMTS13 mAb 3H9 or control anti-ADAMTS13 mAb 5C11. The pump rotor was set at 9000 rpm; a speed applied in patients with heart failure.[28] Blood was sampled 5 min before the onset of perfusion (T0) and 5 (T5), 30 (T30) and 180 (T180) min after the onset of perfusion (Figure S3B). Plasma was obtained through centrifugation (2000*g*, 15 min) of the whole blood samples. Clinical pump parameters (e.g., pump flow, speed, and power) were displayed in real time by the Heartmate II system monitor.

2.5 | VWF multimer analysis

VWF multimer analysis was performed as described before.[29,30] Briefly, VWF multimers present in the plasma samples were separated on a sodium dodecyl sulphate (SDS) isoelectric focusing 1.2% agarose gel. After electrophoresis, the gel, bound to a Gelbond (Cambrex Bio Science Rockland Inc.), was dried and the VWF multimer pattern was visualized with anti-human VWF immunoglobulins labeled with alkaline phosphatase (AP) and the AP-conjugate-substrate kit (BioRad). Densitometric analysis was performed to determine the percentage of HMW VWF multimers



using ImageJ software (version 1.47, NIH, Maryland, USA). The low molecular weight (LMW, band 1–5), the medium molecular weight (MMW, band 6–10), and high molecular weight (HMW, band >10) multimers were selected and the density of the HMW multimers relative to the total multimer density was calculated as a percentage.

2.6 | VWF antigen

VWF antigen (VWF:Ag) levels in the plasma samples obtained from the *in vitro* LVAD systems, were quantified by ELISA as described.[30,31] Briefly, a 96-well microtiter plate was coated with 4.1 µg/ml of polyclonal anti-human VWF antibodies (Dako), blocked with a 3% milk

FIGURE 1 Direct blocking of ADAMTS13 activity in an *in vitro* Impella CP system with human blood prevents the loss of HMW VWF multimers and preserves VWF collagen binding activity. (A) Schematic representation of the *in vitro* Impella CP system. (B) A blood sample was taken before the onset of perfusion (BL), after which the human blood was supplemented with either PBS or 20 μg/ml of the inhibitory anti-ADAMTS13 mAb 3H9. After a 5-min incubation period, the blood was added to the *in vitro* Impella CP system and perfusion of the blood through the pump was started. Next, blood was sampled 5 (T5), 30 (T30), 60 (T60), and 180 (T180) min after the start of perfusion. (C) Representative VWF multimeric pattern was obtained by separating VWF on SDS-agarose gels resulting in the separation of the low molecular weight (LMW), medium molecular weight (MMW), and high molecular weight (HMW) VWF multimers. (D) The percentage of HMW VWF multimers was determined via densitometry. (E) VWF collagen binding activity (VWF:CB) and VWF antigen (VWF:Ag) was determined using ELISA and the ratio of VWF:CB/VWF:Ag is depicted. Data are represented as mean ± standard deviation ($n = 5$ independent biological replicates). NHP, normal human pooled plasma. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Friedmann test with corrections for multiple comparisons (Dunn's multiple comparisons test). Images of the other gels that support the findings of this study are available from the corresponding author upon request.

powder solution and a 1/80 dilution of human or bovine plasma was added and the samples were further diluted by one half in PBS containing 0.3% milk. Next, bound VWF was detected using polyclonal anti-human VWF antibodies labeled with horseradish peroxidase (Dako). Coloring reaction was performed with orthophenylene diamine and H_2O_2. The reaction was stopped with 4 M sulfuric acid. Absorbance was measured at 490 nm. A normal human plasma pool from 20 individuals (NHP) or a normal bovine plasma pool from six calves (NBP) was used to set up a calibration curve and undiluted plasma was set at 100%. The anti-VWF antibodies are known to crossreact with bovine VWF.

2.7 | VWF collagen binding activity

VWF collagen binding activity (VWF:CB) was determined using ELISA as described.[32,33] Briefly, 25 μg/ml of human collagen type III (Sigma-Aldrich) for binding of human VWF or 25 μg/ml of human collagen type I (Sigma-Aldrich) for binding of bovine VWF was coated onto a 96-well microtiter plate and the plate was blocked with a 3% milk powder solution. Next, a 1/80 dilution of plasma was added and the samples were further diluted by half in PBS containing 0.3% milk. Bound VWF was detected with horseradish peroxidase-labeled rabbit anti-human VWF antibodies (Dako) and visualized as described previously. The VWF:CB was calculated using NHP as a reference of which the VWF:CB was set at 100%.

2.8 | Surgical procedure

New Jersey calves (Leurs GbR, Kerken, GE weighing 85–150 kg [one male, three females], 211–236 days old with baseline ADAMTS13 activity between 50% and 150% and baseline platelet counts between $100–800 \times 10^9/L$[34]) were premedicated with Ketamin (Nimatek 15 mg/kg intramuscular, Dechra) and Xylazine (XYL-M 2%, Inovet (V.M.D. nv) and general anesthesia was induced with Isoflurane (5% in O_2 100%, Dechra). The animals were intubated, and general anesthesia was maintained using isoflurane (2%, Dechra). An arterial line was placed in the right ear and a central venous line in the left jugular vein. The skin was disinfected with povidone-iodine and antibiotic prophylaxis was given (penicillin [40 000 IU/kg, Dechra]) and gentamycin (6.6 mg/kg intravenous, Inovet [V.M.D. nv]). Next, a left-sided thoracotomy was made through the fourth intercostal space. The descending aorta was exposed, and a side-biting vascular clamp was applied on the aorta after administration of heparin (200 IU/kg, Leo Pharma nv). An 8-mm Dacron graft was put end-to-side on the aorta with Prolène 5-0. Through this graft, the Impella 5.5 pump (manufactured by Abiomed Europe GmbH) was inserted using the Seldinger technique and under fluoroscopic guidance placed in the left ventricle.[35] Once a proper placement was achieved, the Impella pump was fixed in its position by fixation of the driveline in the 8-mm Dacron graft. The driveline was tunneled to the back of the animal. A thorax drain was left in the left pleural space and the thoracic wall was closed in layers. Metacam (1.5 ml, Boehringer Ingelheim) was administered postoperatively and the chest wound was inspected daily. Impella 5.5 pumps ran at a speed that is applied in patients with cardiogenic shock (30 000 rpm). All animal experiments were performed by the rules as set by the animal ethical committee of the KU Leuven (P218-2015) and according to the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources (National Institutes of Health). Injections and blood sampling were performed by venipuncture of the jugular vein.

2.9 | Study design

Calves were implanted with an Impella, which was expected to reduce the size of the VWF multimers, as has been observed in patients.[19,36] Next, after 168 h (7 days) of Impella implantation, one bolus of PBS (volume based on the volume of the administered mAb 17C7) was injected and blood was sampled 2 (T2), 24 (T24), 48 (T48), 72 (T72), and 168 (T168) h after PBS injection. As PBS injection was not expected to have an effect on VWF multimers, the same calf was treated with the inhibitory anti-ADAMTS13 mAb 17C7 because this allowed us to reduce the number of animals needed for this study. Subsequently, 168 h after PBS injection, one bolus of 600 μg/kg of the inhibitory mAb 17C7 was given intravenously and blood was sampled, 2 (T2), 24 (T24), 48 (T48), 72 (T72), and 168 (T168) h after mAb 17C7 injection (Figure 4A). Blood was collected on

citrate, heparin and EDTA collection tubes (BD) right before Impella implantation (BL) and at 2 (T2), 24 (T24), 48 (T48), 72 (T72), and 168 (T168) hours after Impella implantation and PBS/mAb 17C7 injection (Figure 3A).

2.10 | Blood analysis

Plasma was obtained through centrifugation (2000*g*, 15 min) of the whole blood samples. Total blood cell counts, lactate dehydrogenase (LDH), hemoglobin, and creatinine were determined blinded using automated and standardized methods (XE-5000 analyzer, Sysmex for full blood count and hemoglobin and Cobas 8000 modular analyzer series, Roche Diagnostics for LDH and creatinine) in the Medicine laboratory at Gasthuisberg. Analyses of ADAMTS13 and VWF parameters and mAb load were performed as described in Supplemental Methods.

2.11 | Statistical analysis

Statistical significance between T0 and all other time points (T5, T30, T60, T180, T300) within one group (within control [PBS or mAb 5C11] or within treated [mAb 3H9 and 17C7] groups) was assessed with the Friedmann test with corrections for multiple comparisons (Dunn's multiple comparisons test) for the *in vitro* MCS device experiments. This test was also used to determine statistical significance between baseline (BL) and all time points (T2, T24, T48, T72, and T168) of the different conditions (Impella implantation, PBS, and mAb 17C7 injections) for the preclinical Impella-induced aVWS animal study. All statistical analysis was performed using GraphPad Prism version 8.0.1 (GraphPad Software, Inc.). All values are expressed as mean ± standard deviation and p values were calculated using $p < .05$ as a cutoff for significance.

3 | RESULTS

3.1 | Inhibition of human ADAMTS13 prevents the loss of HMW VWF multimers in *in vitro* MCS device systems

To test whether inhibition of ADAMTS13 can prevent cleavage of HMW VWF multimers during MCS device implantation, we first performed an *in vitro* study using an Impella CP device (Figure 1A). This micro-axial device was selected for our *in vitro* studies as it can be easily implanted in large animals, a prerequisite for our subsequent *in vivo* experiments (see the following section).[37] Our previously developed and well-characterized mAb 3H9 was used to block ADAMTS13 activity.[25] Human blood was preincubated with either placebo (PBS) or mAb 3H9 and was next perfused through an Impella CP pump for 180 min at 37°C at a speed that is used in the clinical setting (44 000 rpm). Blood was sampled at 5, 30, 60, and 180 min (Figure 1B); VWF multimers and VWF function were determined as described in the Methods section.

As expected, a time-dependent decrease in HMW VWF multimers was observed when human blood, supplemented with PBS, was perfused through the system (38.13 ± 2.83% HMW VWF multimers before versus 11.56 ± 1.92% at 180 min after perfusion, $p = .0003$) (Figure 1C,D). This loss in HMW VWF multimers was reflected by a loss in VWF function as the VWF multimers had a significantly lower affinity for collagen. The VWF collagen binding activity over VWF antigen (VWF:CB/VWF:Ag) ratio decreased from 1.07 ± 0.08 to 0.52 ± 0.10 at 180 min after perfusion ($p = .001$) (Figure 1E). Interestingly, blocking ADAMTS13 activity using mAb 3H9 at 20 μg/ml prevented the loss of HMW VWF multimers (39.51 ± 2.03% HMW VWF multimers before versus 35.49 ± 2.78% at 180 min after perfusion) (Figure 1C,D). When using mAb 3H9, VWF function was mainly preserved except for a slight decrease in VWF:CB/VWF:Ag after 180 min of perfusion (Figure 1E).

To assess whether blocking ADAMTS13 can also prevent aVWS using the Heartmate II device, a device that is US Food and Drug Administration-approved for both destination therapy and bridge-to-transplantation,[38] we next studied the efficacy of the mAb 3H9 in preventing the loss of HMW VWF multimers and VWF function *in vitro* using our previously described *in vitro* Heartmate II system (Figure S3A).[24] Similar as with the Impella CP device, blocking ADAMTS13 activity with mAb 3H9 prevented the loss of HMW VWF multimers (36.27 ± 1.79% HMW VWF multimers before versus 30.99 ± 6.50% at 180 min after perfusion) (Figure S3C,D) and of VWF function (VWF:CB/VWF:Ag ratio was 0.93 ± 0.09 before perfusion versus 0.92 ± 0.16 at 180 min) (Figure S3E). Taken together, these *in vitro* experiments show that pharmacological inhibition of ADAMTS13 prevents the loss of HMW VWF multimers and safeguards VWF function.

3.2 | mAb 17C7 prevents the loss of HMW VWF multimers in an *in vitro* Impella 5.5 system perfused with bovine blood

Based on our promising *in vitro* findings, we next wanted to expand our study by testing the efficacy of ADAMTS13 inhibition to treat aVWS after MCS device implantation in a clinically relevant large animal model. Because mAb 3H9 could not inhibit ADAMTS13 from large animal species that are frequently used for LVAD testing (e.g., sheep, pigs, calves),[39] we developed a novel inhibitory anti-ADAMTS13 mAb, mAb 17C7. mAb 17C7 is one of a novel panel of in-house generated mAbs against ADAMTS13[40] and was further characterized in depth in this study. The inhibitory mAb 17C7 has an apparent dissociation constant (Kd) for binding to human ADAMTS13 of 0.51 ± 0.06 μg/ml (Figure S4A) and a half-maximal inhibitory concentration (IC50) of 0.48 ± 0.06 μg/ml (Figure S4B). As shown in Figure S5, mAb 17C7 not only inhibits human ADAMTS13, but also efficiently blocks the activity of bovine, ovine, cynomolgus monkey and baboon ADAMTS13. Hence, mAb 17C7 could be used

to test the efficacy of ADAMTS13 inhibition to treat aVWS after MCS device implantation in a calf model. We selected the calf as a preclinical animal model because these animals are suitable for long-term fixation in the stable, which is necessary to prevent damage or tangling of the pump cable that is connected to the nonwearable automated Impella controller.

Before testing the efficacy of our novel therapy in the calves, we first evaluated *in vitro* whether mAb 17C7 could prevent the loss of HMW VWF multimers in bovine blood using the Impella 5.5 device. We here used the recently developed Impella 5.5 device instead of the Impella CP, as the Impella 5.5 was specifically designed for longer duration of support (i.e., 30 days compared with 6 days with the Impella CP), which is necessary for the later *in vivo* study in the calves. For the *in vitro* experiments, the same *in vitro* setup was used as with the Impella CP device (Figure 1A) and bovine blood was perfused for 300 min in the presence of PBS or mAb 17C7 (Figure 2A). Similar as with the experiments with human blood, perfusion of bovine blood supplemented with PBS resulted in a time-dependent loss of HMW VWF multimers (54.19 ± 3.44% HMW VWF multimers before versus 29.83 ± 4.01% at 300 min after perfusion, $p = .005$) (Figure 2B,C). This loss of HMW VWF multimers resulted in a loss of VWF function as indicated by a decrease of the VWF:CB/VWF:Ag ratio (from 0.98 ± 0.13 before perfusion to 0.42 ± 0.18 at 300 min after perfusion, $p = .01$) (Figure 2D). Addition of 20 μg/ml mAb 17C7 prevented VWF proteolysis by ADAMTS13 (HMW VWF multimers were 54.98 ± 4.00% before versus 50.47 ± 8.69% at 300 min after perfusion) (Figure 2B,C) and prevented the loss of VWF function (VWF:CB/VWF:Ag ratio was 0.89 ± 0.09 before perfusion and remained 0.87 ± 0.15 at 300 min after perfusion) (Figure 2D). A similar protective effect of mAb 17C7 was observed in human blood using the in vitro Impella CP system (Figure S6).

3.3 | Blocking ADAMTS13 in a preclinical Impella 5.5 calf model rescues the loss of HMW VWF multimers

To further validate our approach *in vivo*, Impella 5.5 pumps were implanted in calves ($n = 4$). To assess the specific effect of mAb 17C7, calves were injected at 168 h (7 days) after pump implantation with PBS, and subsequently (168 h after PBS injection) with mAb 17C7 (Figure 3A). In line with patient observations and as shown in Figure 3B,C, Impella 5.5 implantation in the calves resulted in a significant decrease of HMW VWF multimers at 72 h (51.42 ± 4.37% before versus 26.04 ± 7.61% at 72 h after Impella implantation, $p = .003$), which persisted for the full observation period of 168 h after Impella implantation (with 31.00 ± 3.66% HMW VWF multimers at 168 h after Impella implantation, $p = .007$). Subsequent injection of PBS did not alter the VWF multimeric profiles because decreased percentages were maintained 2, 24 ,and 72 h after PBS injection (respectively, 28.57 ± 4.66% [$p = .002$], 32.54 ± 5.17% [$p = .03$], and 31.89 ± 2.94% [$p = .03$] HMW VWF multimers) (Figure 3B,C). The decrease in HMW VWF multimers after Impella implantation and after PBS injection resulted in a decreased VWF function indicated by a reduced VWF:CB/VWF:Ag ratio at 72 h after Impella implantation (0.80 ± 0.09 compared with 1.03 ± 0.03 before implantation, $p = .04$) and at 48 and 72 h after PBS injection (0.75 ± 0.12 [$p = .01$] and 0.83 ± 0.06 [$p = .04$], respectively) (Figure 3D).

Interestingly, however, injection of one bolus of 600 μg/kg (dose predetermined with an *in vitro* ADAMTS13 activity assay[41]) of mAb 17C7 resulted in a rescue of HMW VWF multimers as the decrease in HMW VWF multimers was restored after 2, 24, 48, 72, and 168 h (42.13 ± 4.96%; 43.69 ± 1.31%; 44.10 ± 2.78%; 34.55 ± 13.62%, and 42.62 ± 4.16% HMW VWF multimers at 2, 24, 48, 72, and 168 h after mAb injection respectively compared with before Impella implantation [51.42 ± 4.37%]) (Figure 3B,C), demonstrating the therapeutic potential to rescue aVWS after device implantation. Accordingly, VWF function was restored after blocking ADAMTS13 activity with mAb 17C7 (VWF:CB/VWF:Ag ratio was 0.93 ± 0.09 at 2 h, 0.97 ± 0.07 at 24 h, 0.93 ± 0.16 at 48 h, 0.86 ± 0.22 at 72 h, and 0.94 ± 0.14 at 168 h [7 days] after mAb injection) (Figure 3D).

Besides the analysis of VWF, we also assessed other critical parameters. First, we followed ADAMTS13 activity throughout the experiment and we confirmed full inhibition of ADAMTS13 activity until the end of the experiment (168 h) after mAb 17C7 injection (Figure S7A). However, it is noteworthy to mention that despite full ADAMTS13 inhibition, HMW VWF multimers did not restore to baseline values after mAb injection (51.42 ± 4.37% HMW VWF multimers), indicating that possibly an additional mechanism (besides ADAMTS13 proteolysis) might play a minor role in the residual loss of HMW VWF multimers. Inhibition of ADAMTS13 activity was in line with the detection of mAb 17C7 in plasma, which was still 3.70 ± 0.87 μg/ml at 168 h after mAb injection (Figure S7B). ADAMTS13 antigen levels were not affected after the injection of mAb 17C7, indicating that the antibody did not accelerate clearance of ADAMTS13 from circulation (Figure S7C).

Finally, an important concern when blocking ADAMTS13 is the risk of inducing thrombotic thrombocytopenic purpura (TTP) symptoms that include severe thrombocytopenia, hemolytic anemia, and organ failure (e.g., increased creatinine and LDH). No severe thrombocytopenia (Figure 4A), hemolytic anemia (Figure 4B,C), nor organ damage (Figure 4D,E) was observed in the 7-day time frame post-injection of mAb 17C7 during which the calves were monitored. Of note, 2 h after device implantation, creatinine levels were slightly elevated (Figure 4E), whereas LDH levels (Figure 4D) and WBC count (Figure 4F) were increased 24 h postimplantation, reflecting temporary changes that were most likely surgery related.

Patients with MCS device-associated gastrointestinal bleeding often present with melena or rectoral bleeding and laboratory analysis may reveal anemia.[42,43] However, no signs of bleeding were observed in the calves as confirmed by normal red blood cell counts and hemoglobin levels (Figure 4B,C) and the absence of melena. That no active gastrointestinal bleeding was observed in our calf

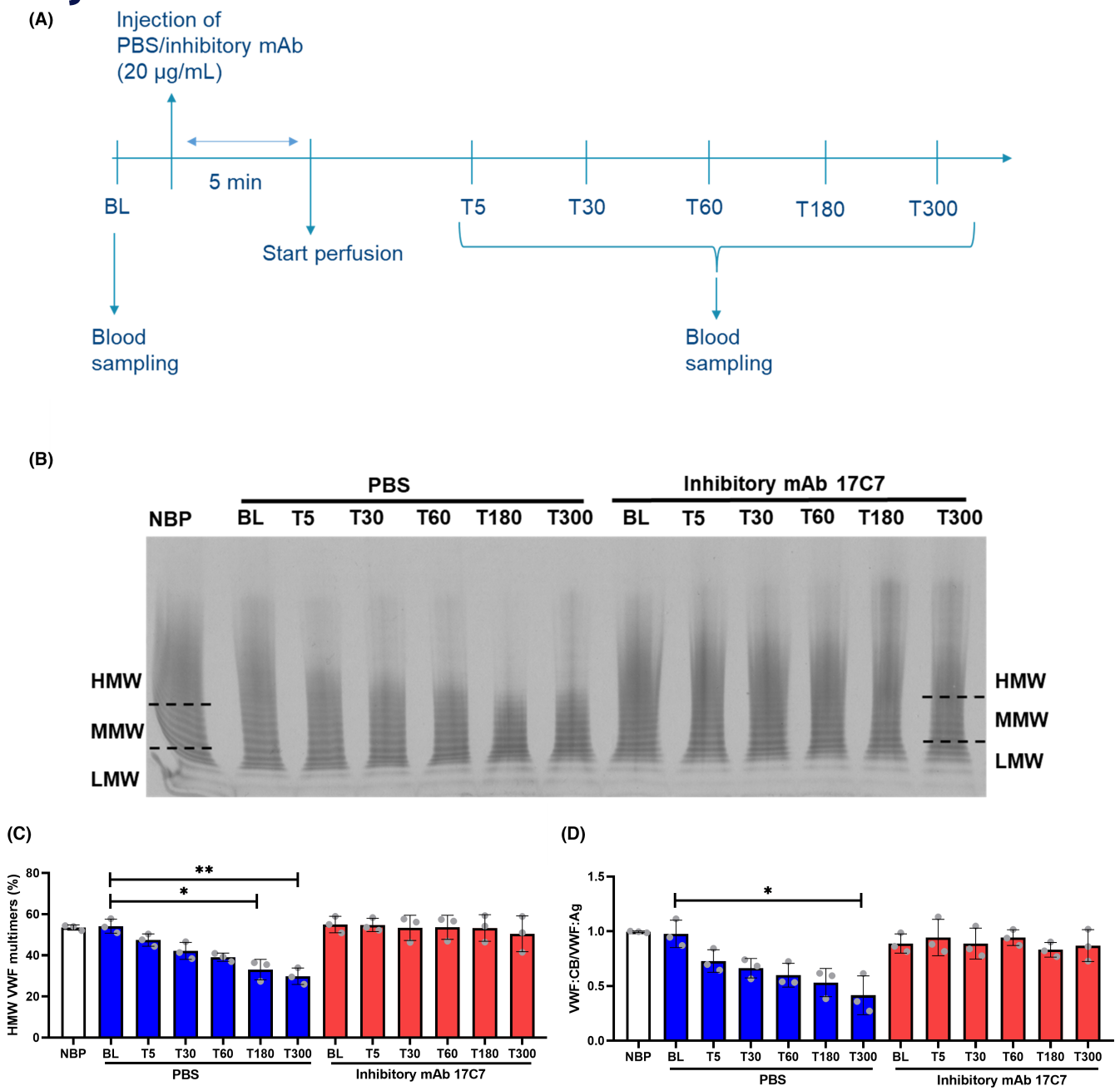


FIGURE 2 Direct blocking of ADAMTS13 activity in an *in vitro* Impella 5.5 system with bovine blood prevents the loss of HMW VWF multimers and preserves VWF collagen binding activity. (A) A blood sample was taken before the onset of perfusion (BL), after which the bovine blood was supplemented with either PBS or 20 µg/ml of the inhibitory anti-ADAMTS13 mAb 17C7. After a 5-min incubation period, the blood was added to the *in vitro* Impella 5.5 system and perfusion of the blood through the pump was started. Next, blood was sampled 5 (T5), 30 (T30), 60 (T60), 180 (T180), and 300 (T300) min after the start of perfusion. (B) Representative VWF multimeric pattern was obtained by separating VWF on SDS-agarose gels resulting in the separation of the low molecular weight (LMW), medium molecular weight (MMW), and high molecular weight (HMW) VWF multimers. (C) The percentage of HMW VWF multimers was determined via densitometry. (D) VWF collagen binding activity (VWF:CB) and VWF antigen (VWF:Ag) was determined using ELISA and the ratio of VWF:CB/VWF:Ag is depicted. Data are represented as mean ± standard deviation ($n = 3$ independent biological replicates). NBP, normal bovine pooled plasma. $^{*}p < .05$, $^{**}p < .01$. Friedmann test with corrections for multiple comparisons (Dunn's multiple comparisons test). Images of the other gels that support the findings of this study are available from the corresponding author upon request.

model, might be explained by the absence of an underlying mechanism (e.g., angiodysplasia) because gastrointestinal bleeding occurs most often from arteriovenous malformations in the gastrointestinal tract.[10,44,45]

4 | DISCUSSION

Nowadays, treatment of gastrointestinal bleedings in LVAD patients relies on endoscopic intervention or downshifting of antithrombotic

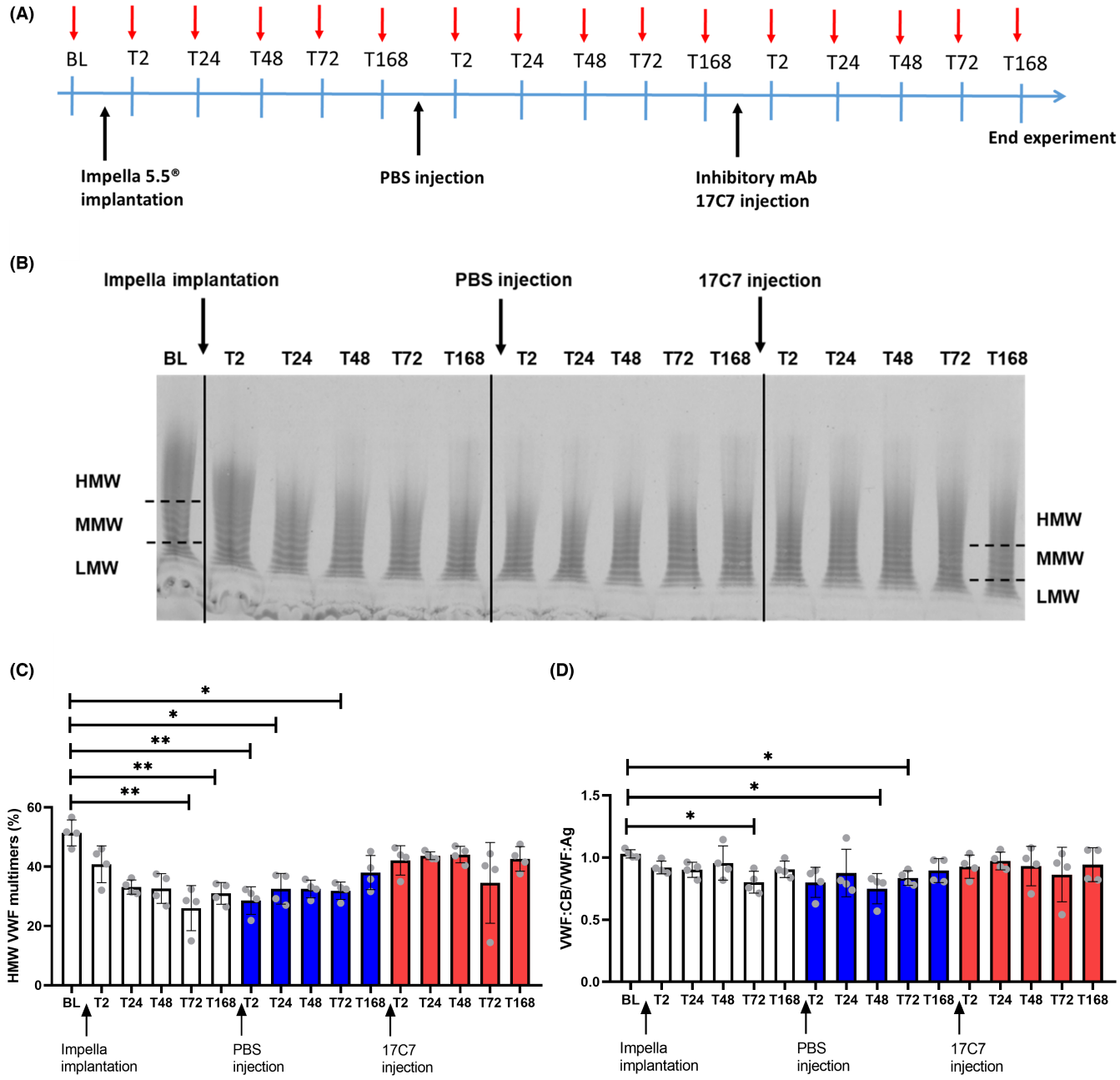


FIGURE 3 Blocking ADAMTS13 in the LVAD-induced aVWS calf model rescues the loss of HMW VWF multimers. (A) Impella 5.5 pumps were implanted in calves (black arrow) and 168 h (T168) after pump implantation, the calves were injected with one bolus of PBS intravenously (black arrow). Next, 168 h (T168) after injection with PBS, one bolus (600 μg/kg) of inhibitory mAb 17C7 was injected (black arrow). Plasma samples were obtained from calves before Impella 5.5 implantation (BL) and at 2 (T2), 24 (T24), 48 (T48), 72 (T72), and 168 (T168) hours after Impella 5.5 implantation and subsequent PBS and inhibitory mAb 17C7 injection. (B) Representative VWF multimeric pattern was obtained by separating VWF on SDS-agarose gels resulting in the separation of the low molecular weight (LMW), medium molecular weight (MMW), and high molecular weight (HMW) VWF multimers. (B) The percentage of HMW VWF multimers was determined via densitometry. (D) VWF collagen binding activity (VWF:CB) and VWF antigen (VWF:Ag) was determined using ELISA and the ratio of VWF:CB/VWF:Ag was determined. Data are represented as mean ± standard deviation ($n = 4$ independent biological replicates). *$p < .05$, **$p < .01$. Friedmann test with corrections for multiple comparisons (Dunn's multiple comparisons test). Images of the other gels that support the findings of this study are available from the corresponding author upon request.

therapy or a reduction in pump speed.[10,11] However, none of these conventional treatment strategies target the molecular mechanism (i.e., excessive degradation of VWF by ADAMTS13) that could be responsible for the bleeding.[46] Our data show that targeting ADAMTS13 using an inhibitory anti-ADAMTS13 mAb is a successful strategy to prevent aVWS in *in vitro* MCS devices and reverse aVWS in a preclinical Impella-induced aVWS calf model. These data further underline the idea that the main cause of VWF degradation in LVADs

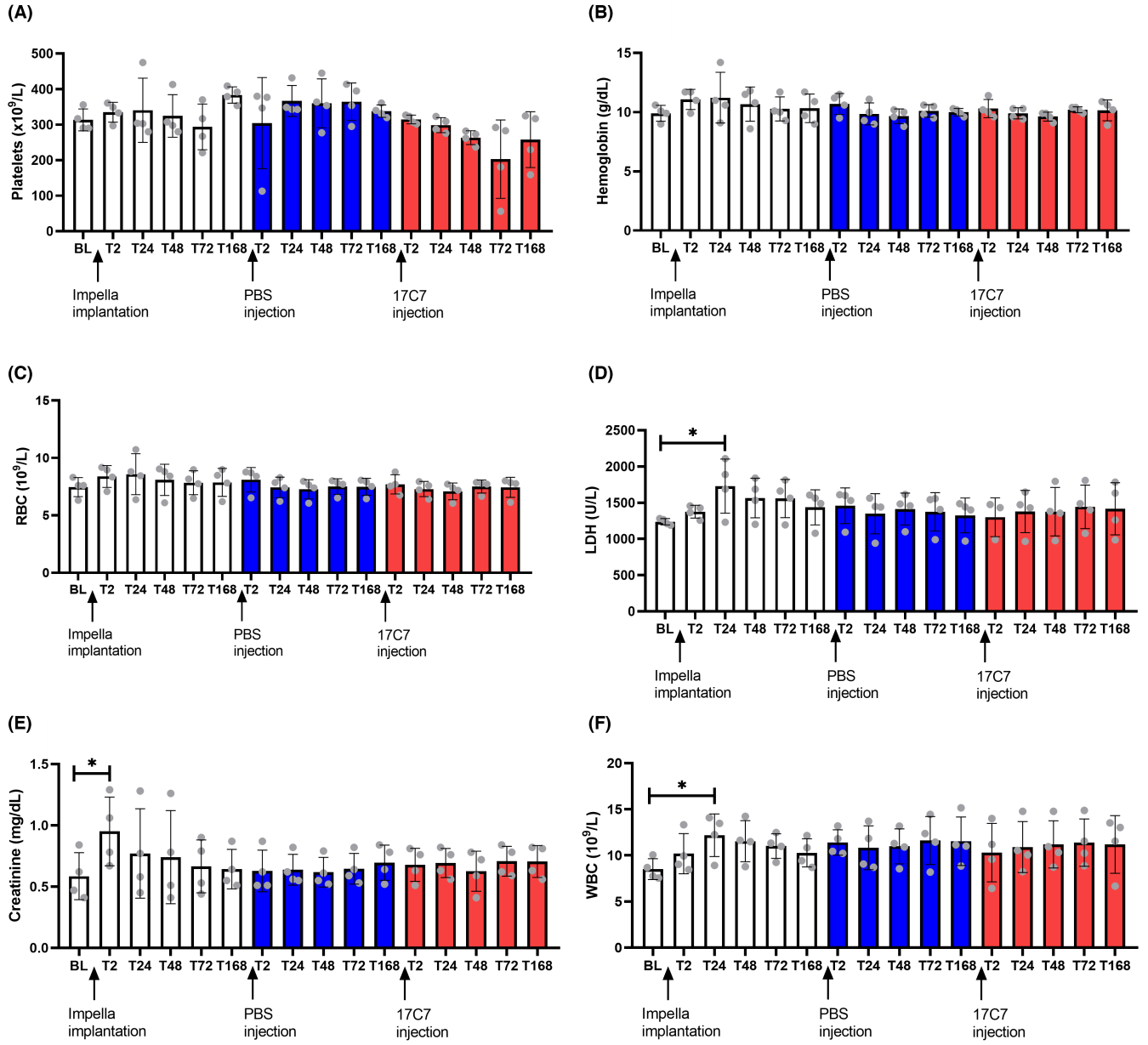


FIGURE 4 Inhibiting ADAMTS13 does not cause thrombocytopenia, hemolytic anemia, nor organ damage in the LVAD-induced aVWS calf model. (A) Platelet counts, (B) hemoglobin levels, (C) red blood cell (RBC) counts, (D) Lactate dehydrogenase (LDH) levels, (E) creatinine levels, and (F) white blood cell (WBC) counts were measured in blood or plasma samples of calves before Impella 5.5 implantation (BL) and at 2 (T2), 24 (T24), 48 (T48), 72 (T72), and 168 (T168) h after Impella 5.5 implantation and subsequent PBS and inhibitory mAb 17C7 (600 μg/kg) injection, using automated or standardized methods. Data are represented as mean ± standard deviation ($n = 4$ independent biological replicates). *$p < .05$. Friedmann test with corrections for multiple comparisons (Dunn's multiple comparisons test).

is increased VWF proteolysis of VWF by ADAMTS13 rather than shear-induced mechanical destruction of VWF or clearance of VWF-platelet complexes, as previously suggested.[47,48] Nevertheless, mechanical destruction of VWF or platelet binding to VWF and subsequent clearance cannot be excluded and could possibly account for the observation that there was no 100% reversal of HMW VWF multimers loss upon ADAMTS13 inhibition (Figure 3B,C). In addition, our study corroborates previous *in vitro* work showing that an anti-VWF mAb inhibiting the binding of VWF to ADAMTS13 was also able to prevent shear-induced degradation of VWF using an in vitro Heartmate II system.[24] Similarly, no loss of HMW VWF multimers was observed when perfusing blood of patients with a congenital deficiency in ADAMTS13 through an *in vitro* Heartware HVAD system.[23]

The main concern when inhibiting ADAMTS13 is the induction of TTP-like symptoms. However, when blocking ADAMTS13 *in vivo*, we did not observe signs and symptoms of TTP during a 7-day follow-up. These results fit with the requirement of a secondary trigger that is needed to induce full-blown TTP.[49] Indeed, ADAMTS13-deficient mice or rats in which ADAMTS13 is inhibited

only develop TTP when a secondary trigger (injection of Shigatoxin or rVWF) is used.[50–52] Similarly, patients with deficient ADAMTS13 can live for years without the development of TTP signs and symptoms.[53] However, because infections and multiple adverse events leading to the activation of inflammatory pathways are common complications in MCS device patients, it should be considered that these conditions may potentially evoke an acute TTP episode.[54] On the other hand, inhibition of ADAMTS13 function in baboons led to an early and mild stage of TTP including thrombocytopenia and hemolytic anemia but no life-threatening complications.[25] The reasons for these differences between species remain unclear. Of note, mild TTP signs and symptoms in baboons only appeared after 48 h.[25] As the anti-ADAMTS13 therapy would be used to treat acute bleedings, a short-term treatment is warranted (24–48 h), thereby minimizing the potential risk for full-blown TTP development. Although our preclinical study in the calves demonstrated no TTP-like signs and symptoms, the safety profile of the drug warrants further investigation and will be mapped during future studies. However, it is important to acknowledge that a small risk for early stage TTP remains a concern when inhibiting ADAMTS13. Caplacizumab, which is currently approved in the European Union and United States for the treatment of acquired TTP could be used as a counteracting agent, in case that TTP might set in in the initial clinical studies in humans.[55]

Although the aim of our current study was to demonstrate proof of concept to reverse aVWS, the observation that calves on Impella support do not show signs of active bleeding as confirmed by the absence of melena and anemia (no drop in red blood cell count and hemoglobin) is a limitation of this study. Hence, the current model could not be used to evaluate if aVWS reversal was sufficient to treat bleedings and thus plays a major contributing factor in the development of gastrointestinal bleedings. To our knowledge, no clinically significant LVAD-induced bleeding model exists so future clinical studies will need to point out if targeted inhibition of ADAMTS13 can treat the bleedings in the acute setting.

In conclusion, inhibiting ADAMTS13 function could become a promising therapeutic strategy to rescue aVWS-induced bleeding, not only in LVAD patients, but in any disorder where increased VWF proteolysis leads to the loss of HMW VWF multimers as in aortic stenosis, ECMO patients, and VWD type 2A patients. Especially because there currently exist no targeted pharmacotherapeutics to treat bleedings in these patients, this novel drug answers the unmet medical need. Although our initial results demonstrate no major safety issues, the potential risks that are linked to ADAMTS13 inhibition should be carefully evaluated in future clinical trials.

AUTHOR CONTRIBUTIONS

Shannen J Deconinck designed research, performed the experiments, had full access to all data, analyzed data, and wrote the manuscript. Elien Roose performed experiments and analyzed data. An-Sofie Schelpe and Hendrik B. Feys produced the monoclonal antibodies 17C7 and 3H9. Joshua Muia provided essential reagents. Christoph Nix, Svenja Barth, and Eveline Bennek-Schöpping provided essential materials and assisted with the animal studies. Antoine Rauch and Sophie Susen provided essential materials and performed experiments. Steven Jacobs performed the animal studies. Inge Pareyn and Aline Vandenbulcke performed experiments. Christoph Nix, Svenja Barth, Eveline Bennek-Schöpping, Antoine Rauch, Sophie Susen, Steven Jacobs, Christophe Vandenbriele, Claudia Tersteeg, Simon F. De Meyer, and Bart Meyns provided helpful discussions. Karen Vanhoorelbeke supervised, designed research, had full access to all data, analyzed the data, and wrote and reviewed the manuscript. All authors critically reviewed and approved the final manuscript.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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