

# von Willebrand Factor as a Biological Sensor of Blood Flow to Monitor Percutaneous Aortic Valve Interventions

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**Rationale:** Percutaneous aortic valve procedures are a major breakthrough in the management of patients with aortic stenosis. Residual gradient and residual aortic regurgitation are major predictors of midterm and long-term outcome after percutaneous aortic valve procedures. We hypothesized that (1) induction/recovery of high molecular weight (HMW) multimers of von Willebrand factor defect could be instantaneous after acute changes in blood flow, (2) a bedside point-of-care assay (platelet function analyzer-closure time adenine DI-phosphate [PFA-CADP]), reflecting HMW multimers changes, could be used to monitor in real-time percutaneous aortic valve procedures.

**Objective:** To investigate the time course of HMW multimers changes in models and patients with instantaneous induction/reversal of pathological high shear and its related bedside assessment.

**Methods and Results:** We investigated the time course of the induction/recovery of HMW multimers defects under instantaneous changes in shear stress in an aortic stenosis rabbit model and in patients undergoing implantation of a continuous flow left ventricular assist device. We further investigated the recovery of HMW multimers and monitored these changes with PFA-CADP in aortic stenosis patients undergoing transcatheter aortic valve implantation or balloon valvuloplasty. Experiments in the aortic stenosis rabbit model and in left ventricular assist device patients demonstrated that induction/recovery of HMW multimers occurs within 5 minutes. Transcatheter aortic valve implantation patients experienced an acute decrease in shear stress and a recovery of HMW multimers within minutes of implantation which was sustained overtime. In patients with residual high shear or with residual aortic regurgitation, no recovery of HMW multimers was observed. PFA-CADP profiles mimicked HMW multimers recovery both in transcatheter aortic valve implantation patients without aortic regurgitation (correction) and transcatheter aortic valve implantation patients with aortic regurgitation or balloon valvuloplasty patients (no correction).

**Conclusions:** These results demonstrate that variations in von Willebrand factor multimeric pattern are highly dynamic, occurring within minutes after changes in blood flow. It also demonstrates that PFA-CADP can evaluate in real time the results of transcatheter aortic valve procedures. (*Circ Res.* 2015;116:1193-1201. DOI: 10.1161/CIRCRESAHA.116.305046.)

**Key Words:** aortic valve stenosis ■ blood flow velocity ■ von Willebrand factor

Percutaneous aortic valve procedures, including transcatheter aortic valve implantation (TAVI) and balloon aortic valvuloplasty (BAV), are recent major breakthrough in the management of patients with aortic stenosis (AS).<sup>1,2</sup> In some circumstances their results can still be inadequate, whereas

their evaluation in real-time may remain difficult with current techniques.<sup>3</sup> Among examples are the cases of balloon valvuloplasty procedures and valve-in-valve TAVI procedures, where an insufficient opening of the valve and a high residual gradient can still be observed or the cases of periprocedural

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**Nonstandard Abbreviations and Acronyms**

<b>AR</b>	aortic regurgitation
<b>AS</b>	aortic stenosis
<b>BAV</b>	balloon aortic valvuloplasty
<b>HMW</b>	high molecular weight
<b>IMW</b>	intermediate molecular weight
<b>LMW</b>	low molecular weight
<b>LVAD</b>	left ventricular assist device
<b>PFA-CADP</b>	platelet function analyzer-closure time adenine DI-phosphate
<b>TAVI</b>	transcatheter aortic valve implantation
<b>VWF</b>	von Willebrand factor
<b>VWF:Ag</b>	VWF antigen
<b>VWFpp</b>	VWF propeptide

aortic regurgitation (AR) observed in 10% to 30% of TAVI procedures with current techniques.<sup>1,2</sup>

Acquired deficiency of von Willebrand factor (VWF), characterized by a loss of high molecular weight (HMW) multimers, is associated with cardiovascular disorders in which the entire blood volume is exposed to high shear stress.<sup>4-9</sup> It has been demonstrated that acquired VWF deficiency can be detected within days after implantation of an axial continuous flow left ventricular assist device (LVAD).<sup>10</sup> We and others<sup>11,12</sup> also demonstrated that the VWF deficiency present in patients with AS is corrected within days after its surgical treatment. Based on in vitro studies, it was inferred that unfolding and cleavage of the VWF A2 domain in vivo could occur within 200 seconds in response to acute changes in shear conditions.<sup>13</sup> However, the initial time course of loss/recovery of VWF HMW multimers after acute changes in blood flow in vivo has not yet been studied.

PFA-closure time ADP (CADP) is a highly sensitive way to screen for HMW multimers defects<sup>14</sup> and has been shown to be prolonged in patients with high shear-cardiovascular disorders including those with AS.<sup>6,11,15</sup> As PFA-CADP can be assessed by a small whole blood analyzer (PFA-100) it has the potential to be used as a bedside monitor of HMW multimers changes.

We hypothesized that induction/recovery of HMW multimers defect could occur within minutes of acute changes in blood flow induced by cardiac interventions and we further investigated the potential underlying mechanisms. We also hypothesized that HMW multimers recovery, as assessed by PFA-CADP, could be used to monitor in real time the results of transcatheter aortic valve procedures, including the presence of a high postprocedural aortic gradient and the presence of a significant postprocedural AR. To evaluate these hypotheses in vivo, we investigated the time course of HMW multimers loss/recovery in an animal model of reversible AS specifically developed for that purpose. We further investigated the time course of HMW multimers loss/recovery and its related bedside whole blood assessment (PFA-100 analyzer) in 38 patients included in a prospective registry and undergoing (1) implantation of an axial continuous flow LVAD (HeartMate-II, n=8) for heart failure and (2) transcatheter aortic valve procedures, either BAV (n=10) or TAVI (n=20), to treat AS.

**Methods****Instantaneous Induction and Reversion of High Shear Stress in a Rabbit Model of Reversible AS**

We developed a new rabbit model of instantaneous, reversible, calibrated supra-AS, adapted from Assad et al<sup>16</sup> and Godier et al<sup>17</sup> (see Methods in the Online Data Supplement). This model allowed the evaluation in the same rabbit, of the dynamic time course of loss and recovery of HMW multimers. In each rabbit (n=17), blood was sampled, before (T0) and after the induction of AS (5 and 30 minutes). Then the stenosis was reversed 30 minutes after its induction and blood was sampled 5 and 30 minutes after reversion (35 and 60 minutes).

**Prospective Patients Registry**

After approval from the local ethics committee, we performed a prospective registry of patients undergoing HeartMate-II implantation or percutaneous aortic valve intervention, including clinical data collection and blood sampling during the procedure. All patients provided informed written consent and were included in the Willebrand TAVI registry.

**Induction of High Shear Stress in Patients Undergoing Implantation of HeartMate-II LVAD**

HeartMate-II (Thoratec Corp, Pleasanton, CA) is an axial continuous flow LVAD. A time course of VWF multimeric analysis was performed in vivo in 8 consecutive patients at the time of initiation of HeartMate-II support. Samples were collected before (T0) and after initiation of HeartMate-II support at 9000 rpm (5, 30, and 180 minutes).

**Reversion of High Shear Stress in Patients With AS Undergoing Transcatheter Aortic Valve Procedure**

A time course of HMW multimers analysis and its related whole blood assessment (PFA-CADP) was performed in vivo in 30 patients with severe aortic valve stenosis in stable clinical condition, with a clinical need for either TAVI (n=20) or BAV (n=10) procedures. Both procedures were performed through a percutaneous transfemoral approach according to standard practice, whereas TAVI was performed with the Edwards-Sapien XT device.<sup>2,3,18</sup> Samples were collected before (0) and after the procedure (5, 30, and 180 minutes and week 4).

Evaluation of shear stress conditions, including aortic velocity and gradient and postprocedural AR, was performed by a transthoracic echocardiography performed before and 24 hours after the procedure. The presence of a significant postprocedural AR was defined as the presence of an AR moderate or greater according to the VARC2 (valve academic research consortium) classification.<sup>19</sup>

**Induction of High Shear Stress In Vitro Using a HeartMate-II Assist Device Model**

Using an in vitro HeartMate-II model, we first investigated the kinetics of HMW multimers loss and recovery in the absence of endothelium. For each experiment, human blood (either heparinized or citrated) from healthy donors was perfused in a tubing system using a circulatory flowing pump device in which the HeartMate-II was the pump. Because the results of the experiments performed using heparinized or citrated blood were similar, they are presented together. The HeartMate-II rotor was set to high shear (9000 rpm), as achieved in patients implanted with HeartMate-II or to low shear (3000 rpm).

We further assessed the role of VWF proteolysis as a mechanism underlying the loss of HMW multimers in this model (see Methods in the Online Data Supplement).

**Laboratory Assessment**

VWF antigen (VWF:Ag; Sta Liatest, Diagnostica Stago, Inc) and VWF propeptide (VWFpp; Lifecodes VWF & Propeptide Assay, Gen-probe) levels were measured by ELISA. VWF activity was assessed by a latex immunoturbidimetric assay (Innovance VWF Ac; Siemens Healthcare Diagnostics, Marburg, Germany). As our aim was to detect changes in HMW multimers, we chose to perform

experiments using gels with low agarose concentrations.<sup>20,21</sup> VWF multimeric analysis was performed as previously described.<sup>11</sup> The results are expressed as a ratio to normal pooled plasma (standard human plasma Siemens Healthcare Diagnostics, Marburg, Germany). Immunoprecipitation/Western blot analysis was performed to measure VWF proteolysis fragments (176 and 140 kDa; see Methods in the Online Data Supplement).

PFA-CADP was assessed by platelet-function analyzer PFA-100, (Siemens Healthcare Diagnostics, Marburg, Germany) using ADP cartridges (PFA-CADP, normal range, 68–121 seconds) as previously described.<sup>11,14</sup>

VWF:Ag and VWF multimeric analysis were newly developed for rabbits. Loading of the electrophoretic gels was normalized for VWF:Ag content. The results are expressed as relative to baseline values determined for each animal.

**Statistical Analysis**

Data were expressed as mean (±SD), unless indicated otherwise. Multiple time comparisons were performed using repeated measures of 1-way ANOVA. When appropriate, time points were compared with a Wilcoxon rank test for paired or Mann–Whitney for unpaired groups. *P* values <0.05 were considered statistically significant.

**Results**

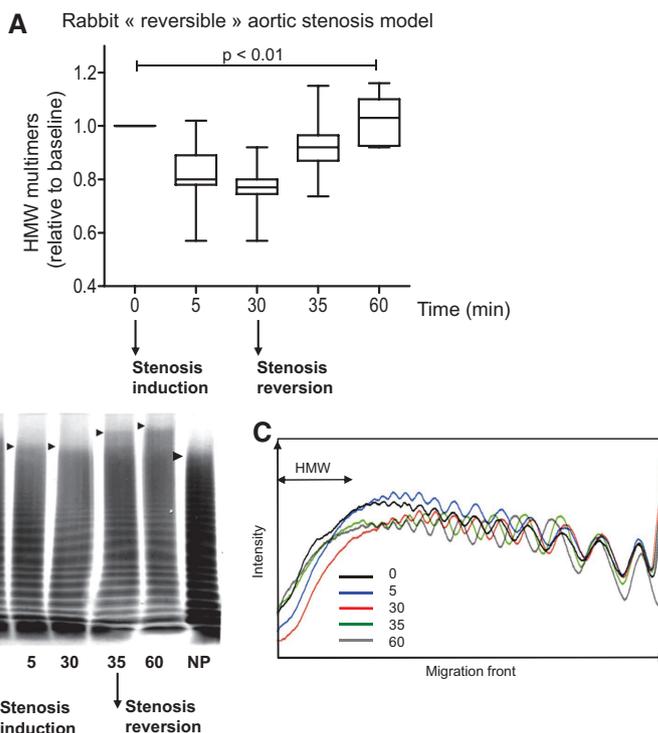
**Instantaneous Induction and Reversion of High Shear Stress in a Rabbit Model of Reversible AS**

In the AS-rabbit model, a significant decrease in HMW multimers was observed 5 minutes (0.76±0.13; *P*<0.01) and further 30 minutes (0.74±0.07; *P*<0.01) after stenosis induction when compared with baseline values (Figure 1). Conversely, a significant increase in HMW multimers was already observed 5 minutes after reversion of the stenosis (0.89±0.12; *P*<0.01). Thirty minutes after the reversion, a complete recovery of HMW multimers was observed (0.98±0.10; Figure 1).

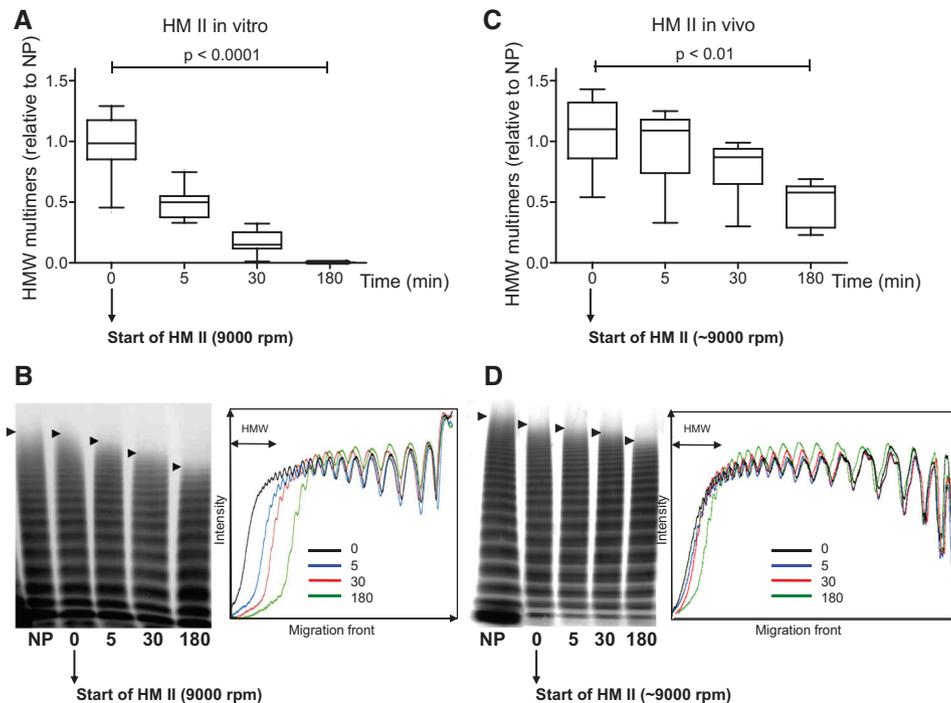
**Rapid Loss of HMW Multimers After Induction of High Shear Stress in Patients Undergoing HeartMate-II Implantation**

The kinetics of HMW multimers loss in human blood was studied at the time of HeartMate-II implantation in 8 consecutive patients (6 men and 2 women, aged 59±12 years). A significant time-dependent loss of HMW multimers was observed after initiating the pump (rotor set ≈9000 rpm) reaching 0.86±0.37, 0.69±0.32, and 0.48±0.18 at 5, 30, and 180 minutes, respectively (*P*<0.01; Figure 2C and 2D). A significant time-dependent increase in intermediate (I) plus low (L) MW mirroring the loss of HMW multimers was observed reaching 1.11±0.11 at 180 minutes compared with 1.01±0.08 at baseline (*P*<0.05). Consistent with the loss of HMW multimers, a time-dependent decrease in VWF collagen-binding activity/VWF:Ag ratio was also observed reaching 0.75±0.22 at 180 mm versus 0.88±0.18 at baseline (*P*<0.05).

These findings were further investigated in the in vitro HeartMate-II model. In the in vitro HeartMate-II-model, when whole human blood was submitted to high shear stress (rotor set at 9000 rpm), a progressive and time-dependent loss of HMW multimers was also observed. The loss of HMW multimers was more pronounced after 5 minutes than in LVAD patients and was complete after 180 minutes (*P*<0.0001; Figure 2A and 2B). The role of VWF proteolysis was verified by (1) a time-dependent increase in specific VWF proteolytic fragments (140 and 176 kDa) in patients (Online Figure IA) and (2) an absence of time-dependent loss of HMW multimers when spiking EDTA before pump initiation in vitro (Online Figure IB). The shear dependency of HMW multimers loss was also verified by setting the rotor of HeartMate-II at 3000 rpm (Online Figure IC).



**Figure 1. Dynamic loss and recovery of high molecular weight (HMW) multimers in a rabbit reversible aortic stenosis model.** **A**, Quantitative analysis of HMW multimers (relative to baseline) after induction and reversion of aortic stenosis (repeated ANOVA, *P*<0.01 overall; n=17). Significant loss of HMW multimers 5 and 30 minutes after induction of stenosis (*P*<0.01 vs baseline) and immediate recovery 5 minutes and 30 minutes after reversion of stenosis (*P*<0.01 vs 30 minutes after induction) were observed. **B**, Representative profile of von Willebrand factor multimeric patterns at the different time points after induction and reversion of stenosis in 1 rabbit. **C**, Densitometric analysis of electrophoretic gel image (black arrows indicate the front of migration). NP indicates normal human pooled plasma.



**Figure 2. Immediate loss of high molecular weight (HMW) multimers on high shear stress at initiation of axial continuous flow HeartMate-II (HM II) device.** **A**, Quantitative analysis of HMW multimers (relative to normal human pooled plasma) after perfusion of human whole blood under high shear conditions (9000 rpm) in the HM II in vitro model (repeated ANOVA,  $P < 0.0001$  overall;  $n = 10$ ). A significant loss of HMW multimers occurred overtime, already significant 5 minutes after HM II start ( $P < 0.01$  vs baseline) and complete at 180 minutes. **B**, Representative time course of HMW multimers loss (with densitometric analysis) after initiating the HM II in vitro. **C**, Quantitative analysis of HMW multimers (relative to normal human pooled plasma) in patients undergoing initiation of HM II support (repeated ANOVA,  $P < 0.01$  overall;  $n = 8$ ). A significant loss of HMW multimers occurred overtime, already significant 30 minutes after initiating the HM II support ( $P < 0.01$  vs baseline). **D**, Representative time course of HMW multimers loss (with densitometric analysis) after initiating the HM II support in vivo (**B** and **D**, black arrows indicate the front migration). NP indicates normal human pooled plasma.

In patients undergoing HeartMate-II implantation, a time-dependent increase in VWFpp was observed. This VWFpp increase, already significant 5 minutes after initiating the pump ( $528 \pm 184$  versus  $259 \pm 139$  UI/dL at baseline;  $P = 0.01$ ), was still apparent after 30 minutes ( $538 \pm 139$  UI/dL) and 180 minutes ( $560 \pm 140$  UI/dL). In vitro, no change in VWFpp was observed overtime ( $89 \pm 27$  at 180 minutes versus  $89 \pm 32$  at baseline, ns).

### HMW Multimers Increase Rapidly After Reversion of Pathological High Shear Stress in Patients Undergoing TAVI Procedure

The effect of the reversion of high shear on the VWF multimeric pattern was studied in 30 patients with AS requiring to undergo either BAV ( $n = 10$ ; 5 men and 5 women; aged  $82 \pm 6$  years;  $LVEF = 53\% \pm 10\%$ ) or TAVI ( $n = 20$ ; 9 men and 11 women; aged  $82 \pm 6$  years,  $LVEF = 53\% \pm 10\%$ ). All patients had New York Heart Association class 3 or 4 and no patient had decompensated heart failure.

As expected, in patients with AS a HMW multimers defect was observed at baseline ( $0.50 \pm 0.19$  compared with normal pooled plasma), whereas increased levels of IMW+LMW multimers ( $1.07 \pm 0.04$ ) were present.

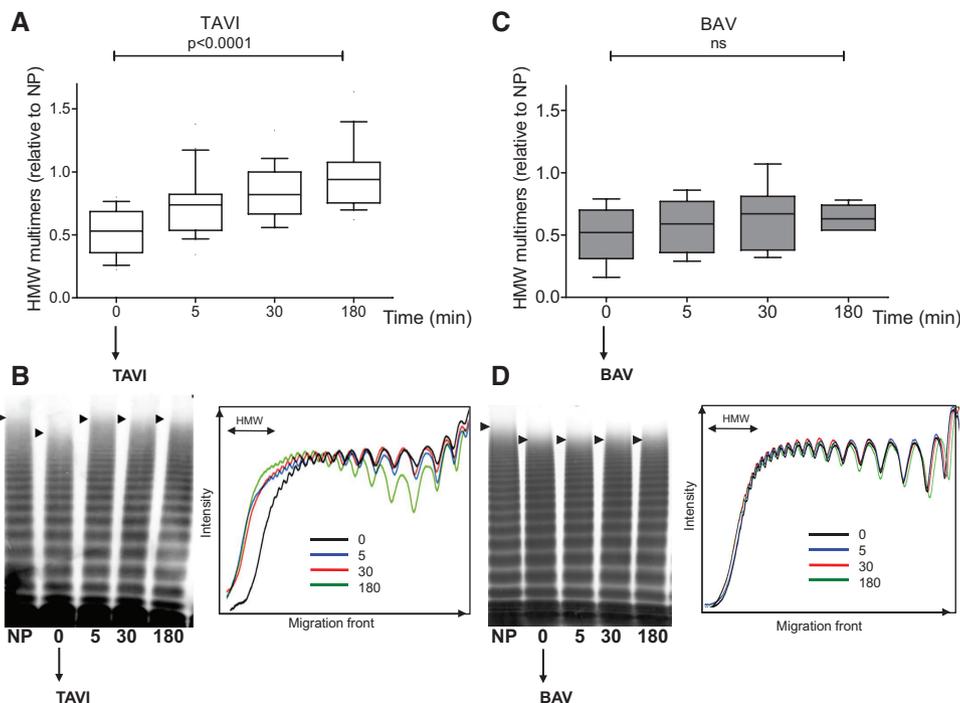
In patients treated with TAVI, the procedure resulted in a near normalization of maximal transvalvular velocity (from  $4.44 \pm 0.47$  m/s at baseline to  $2 \pm 0.56$  m/s after valve replacement;  $P < 0.0001$ ) inducing a marked reduction in mean transvalvular gradient ( $50.6 \pm 12.5$  to  $9.6 \pm 5.1$  mmHg;  $P < 0.0001$ ), whereas in 4 of them (20%) a postprocedural AR moderate or greater was

observed. Those treated with BAV experienced a modest improvement in shear conditions (max transvalvular velocity from  $4.47 \pm 0.25$  m/s at baseline to  $3.88 \pm 0.65$  m/s after BAV;  $P < 0.05$ ) and, as a consequence, a modest decrease in mean transvalvular gradient ( $49.6 \pm 3.8$  to  $35.6 \pm 13.4$  mmHg;  $P < 0.05$ ).

In the 20 patients undergoing TAVI, the amount of HMW multimers dramatically increased 5 minutes after valve implantation (from  $0.51 \pm 0.18$  at baseline to  $0.75 \pm 0.24$ ;  $P < 0.001$ ). An almost complete recovery was observed after 180 minutes ( $0.97 \pm 0.25$ ;  $P < 0.0001$ ; Figure 3A and 3B) which was sustained by 4 weeks ( $0.91 \pm 0.15$ ). Together with the HMW multimers recovery, a significant time-dependent decrease of IMW+LMW multimers already significant at 5 minutes ( $1.03 \pm 0.05$ ) and peaking at 180 minutes ( $0.99 \pm 0.04$ ) was observed ( $P < 0.01$ ). A time-dependent correction of VWF collagen-binding activity/VWF:Ag ratio was also observed (from  $0.76 \pm 0.14$  at baseline to  $0.94 \pm 0.30$  at 180 minutes;  $P < 0.01$ ).

BAV procedures did not increase significantly the amount of HMW multimers ( $0.58 \pm 0.2$ ,  $0.66 \pm 0.25$ ,  $0.64 \pm 0.15$ , and  $0.65 \pm 0.21$  at 5, 30, 180 minutes, and 4 weeks after BAV, respectively;  $P = 0.59$ ; Figure 3C and 3D). No significant time-dependent changes in IMW+LMW multimers nor in VWF:Act/VWF:Ag ratio were observed.

Of note, as part of the TAVI procedure a balloon predilatation was performed before valve implantation. This predilatation had no significant impact on HMW multimers ( $0.54 \pm 0.11$  at 5 minutes;  $P = 0.61$  versus baseline).



**Figure 3. Time course of high molecular weight (HMW) multimers recovery in patients with severe aortic stenosis undergoing transcatheter aortic valve interventions.** **A**, Quantitative analysis of HMW multimers in patients undergoing correction of aortic stenosis by transcatheter aortic valve implantation (TAVI; repeated ANOVA,  $P < 0.0001$  overall;  $n = 20$ ). HMW multimers recovery was significant 5 minutes after valve implantation ( $P < 0.05$ ) and completed at 180 minutes ( $P < 0.01$ ). **B**, Representative time course of von Willebrand factor (VWF) multimeric pattern (with densitometric analysis) in a patient undergoing TAVI procedure. **C**, Quantitative analysis of HMW multimers in patients undergoing balloon valvuloplasty (BAV) procedure (repeated ANOVA,  $P = 0.21$  overall;  $n = 10$ ). No significant changes in VWF multimeric pattern occurred after valve dilatation. **D**, Representative time course of VWF multimeric pattern (with densitometric analysis) in a patient undergoing BAV procedure. NP indicates normal human pooled plasma.

When all TAVI and BAV patients were analyzed together ( $n = 30$ ), a significant and inverse relation between postprocedural mean transvalvular gradient and postprocedural HMW multimers was observed ( $r = -0.68$ ;  $P < 0.0001$ ; Figure 4).

After TAVI, despite a consistently low residual gradient ( $9.6 \pm 5.1$  mm Hg), a relatively large standard deviation in HMW multimer values was observed. This was mainly related to the occurrence of a significant postprocedural AR in 4 patients in whom the HMW multimers increased to a lesser extent and in whom HMW multimer at 180 minutes was significantly lower than in the 16 TAVI patients without postprocedural AR ( $0.74 \pm 0.10$  versus  $1.02 \pm 0.25$ ;  $P = 0.04$ ).

### Acute Endothelial Release of VWF in Patients Undergoing TAVI Procedures

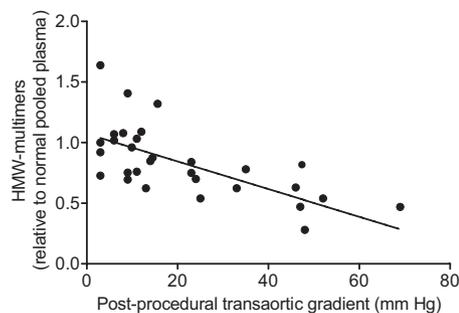
A potential role of the vascular endothelium in the HMW multimers recovery was investigated by evaluating the secretion of VWF by the endothelium after reversion of high shear in TAVI and BAV procedures. It was further investigated by studying the recovery of HMW multimers after reversion of high shear in a model free of endothelium (in vitro HeartMate-II).

In TAVI procedures, VWFpp significantly increased 5 minutes after valve implantation ( $190 \pm 85$  UI/dL), and further after 30 ( $240 \pm 111$  UI/dL) and 180 minutes ( $394 \pm 191$  UI/dL) when compared with baseline ( $171 \pm 84$  UI/dL;  $P < 0.01$ ). In BAV procedures, VWFpp did not increase significantly overtime ( $275 \pm 136$  UI/dL at 180 minutes versus  $199 \pm 107$  UI/dL at baseline, ns).

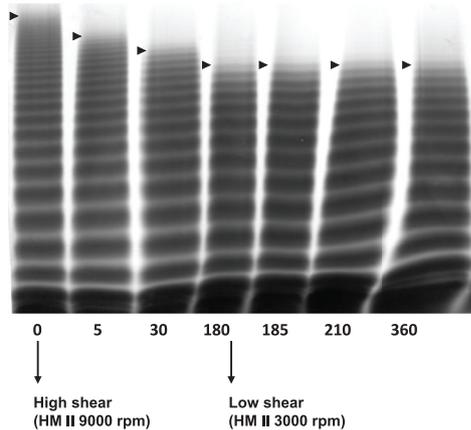
In the in vitro HeartMate-II model, high shear was induced for 3 hours (9000 rpm), then the blood flow was submitted to low shear (by switching the speed from 9000 to 3000 rpm) for the next 3 hours, mimicking reversal of pathological high shear. In the absence of endothelium, no recovery of HMW multimers was observed in this model (Figure 5).

### Real-Time Monitoring of Percutaneous Aortic Valve Procedures by PFA-CADP Closure Time

As expected and mimicking the VWF multimeric profile, characterized by reduced HMW multimers, PFA-CADP was prolonged in AS patients ( $243 \pm 65$  seconds). In TAVI patients, a time-dependent correction of PFA-CADP was observed ( $195 \pm 74$ ,  $165 \pm 75$ ,  $139 \pm 73$ ,  $141 \pm 73$  seconds at 5, 30, 180 minutes, and 4



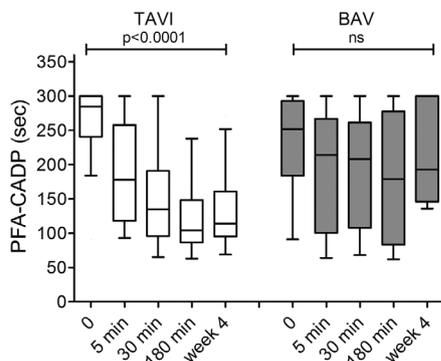
**Figure 4. Relation between postprocedural gradient and postprocedural high molecular weight (HMW) multimers.** The mean postprocedural gradient is plotted against the postprocedural HMW multimers values (180 minutes,  $r = -0.68$ ;  $P < 0.0001$ ).



**Figure 5. Absence of high molecular weight (HMW) multimers recovery after reversion of pathological high shear in the HeartMate-II (HM II) assist device model.** Whole human blood was submitted to high shear (9000 rpm) during 3 hours and then to low shear for the next 3 hours (3000 rpm) by switching the pump. The von Willebrand factor multimeric profile remained unchanged after the offset of high shear stress.

weeks respectively,  $P < 0.0001$ ; Figure 6). By contrast, in BAV patients no significant change in PFA-CADP was observed overtime ( $212 \pm 61$ ,  $204 \pm 71$ ,  $219 \pm 76$ ,  $221 \pm 75$  seconds at 5, 30, 180 minutes, and 4 weeks;  $P = 0.82$ ; Figure 6). Mirroring the observation made with HMW multimers, patients with a prolonged PFA-CADP value had a higher final residual gradient than patients with a normal PFA-CADP value ( $29.2 \pm 5.1$  versus  $7.85 \pm 1.12$ ;  $P < 0.001$ ). Importantly all patients with a normal final PFA-CADP had final residual gradient  $< 15$  mm Hg.

After TAVI, and similar to the heterogeneity observed with HMW multimer values, a relatively large SD in PFA-CADP measurement was observed. This was mainly related to the occurrence of a significant postprocedural AR in 4 patients in whom PFA-CADP measurements were significantly higher than in the 16 patients without AR ( $225 \pm 41$  versus  $100 \pm 23$  seconds;  $P < 0.01$ ; Figure 7). In all patients with a residual AR the PFA-CADP at the



**Figure 6. Real-time assessment of changes in von Willebrand factor (VWF) multimeric pattern with platelet function analyzer-closure time adenine DI-phosphate (PFA-CADP) in patients undergoing transcatheter aortic valve interventions.** In transcatheter aortic valve implantation (TAVI) patients, a time-dependent correction of PFA-CADP was observed (repeated ANOVA,  $P < 0.0001$ ). In balloon valvuloplasty (BAV) patients, no significant time-dependent change of PFA-CADP was observed. In TAVI patients this correction was still present 4 weeks after the procedure and conversely PFA-CADP remained prolonged at this time point in BAV patients.

end of the procedure was  $> 180$  seconds, whereas of those without any residual AR the final PFA-CADP was  $< 140$  seconds.

Conversely, in patients undergoing implantation of a HeartMate-II device, a sudden increase in PFA-CADP was observed as soon as 5 minutes after initiation of the support ( $246 \pm 63$  versus  $106 \pm 40$  seconds;  $P = 0.01$ ).

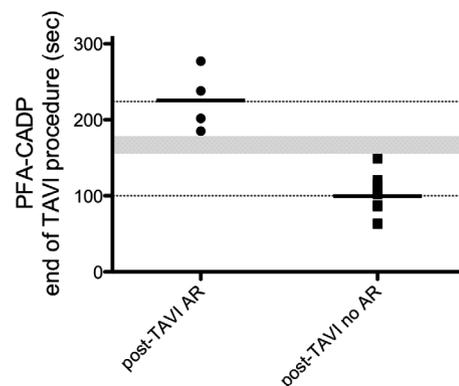
## Discussion

The present study, performed in 3 clinical conditions and 1 animal model in which the entire blood volume is exposed to high shear stress, demonstrates that acute changes in blood flow are associated with highly dynamic consequences on the VWF multimeric profile, occurring within minutes and then remaining steady overtime. It demonstrates the key roles of HMW multimers proteolysis and VWF multimers release by the vascular endothelium in those acute changes of VWF multimeric profile. It further demonstrates that bedside whole blood assessment (PFA-CADP), reflecting HMW multimers changes, could be used in clinical practice to monitor in real time the quality of the results of percutaneous aortic valve procedures, in particular, to detect the occurrence of postprocedural AR. Altogether these results provide the first integrated demonstration that VWF can be considered as a biological sensor of blood flow in vivo.

### Dynamic Variations in HMW Multimers in Response to Acute Changes in Blood Flow

The present study is the first one to demonstrate that variations in VWF multimeric profile in response to acute changes in blood flow in vivo are highly dynamic.

Although it has been demonstrated that the loss of HMW multimers could be observed the day after the initiation of LVAD support,<sup>10</sup> the initial response of VWF multimers after induction of high shear in vivo was unknown. The dynamic onset of shear-induced proteolysis of HMW multimers has been extensively described in vitro.<sup>13,20,22</sup> Hence, when subjecting VWF to high shear forces, unfolding of large VWF multimers has been shown to occur in  $< 1$  second in vitro and VWF cleavage was inferred to be effective within 200 seconds in vivo.<sup>13</sup> The present study confirms that the loss of HMW



**Figure 7. Impact of a significant postprocedural aortic regurgitation (AR) on platelet function analyzer-closure time adenine DI-phosphate (PFA-CADP) at the end of the transcatheter aortic valve implantation (TAVI) procedure.** In patients with a significant postprocedural AR (n=4) the postprocedural PFA-CADP was significantly higher than in patients without AR (n=16,  $P < 0.01$ ) and remained as prolonged as in patients with aortic stenosis.

multimers follows a similar time frame in vivo and occurs almost immediately after the induction of high shear stress. Indeed, a significant decrease in HMW multimers was observed 5 minutes after induction of high shear, both in rabbits submitted to an acute AS and after initiation of HeartMate-II support at high speed (9000 rpm). Additional experiments performed in the HeartMate-II LVAD model further confirmed the shear dependency of HMW multimers loss; a rapid loss of HMW multimers was observed at high speed (9000 rpm), whereas no loss was observed at low speed (3000 rpm).

Although HMW multimers recovery has been observed within days after aortic valve surgical replacement in AS patients,<sup>11,12</sup> no information was available on the initial phase of correction of AS. A major finding of this study is to demonstrate a nearly immediate recovery of the HMW multimers on reversion of the high-shear conditions, whereas no recovery was observed in the absence of correction. In the rabbit model and in AS patients undergoing TAVI, HMW multimers recovery was observed 5 minutes after correction of AS and was sustained overtime. In AS patients undergoing a percutaneous procedure but in whom only a weak reduction in shear forces was achieved, as those undergoing BAV or those undergoing TAVI with a significant postprocedural AR, no consistent HMW multimers recovery was observed.

#### **HMW Multimers Proteolysis as a Shear-Dependent Process**

VWF shear-induced proteolysis is considered the main mechanism underlying the acquired HMW multimers defect observed in high-shear cardiovascular conditions, such as AS or continuous axial flow LVAD support.<sup>4,23</sup> The present study provides new experimental evidence that proteolysis links the induction of high shear to the nearly immediate loss of HMW multimers. First, in the HeartMate-II LVAD patients, the loss of HMW multimers was associated with an increase in VWF proteolytic fragments. Second, the loss of HMW multimers at initiation of high shear conditions was blunted when a protease inhibitor (EDTA) was added to the in vitro device model. Finally, the increase in IMW and LMW multimers as seen in HeartMate-II LVAD patients and the decrease of IMW and LMW multimers seen in TAVI patients are also consistent with this hypothesis. Altogether these results further re-enforce that shear-induced proteolysis is the major mechanism underlying the acquired HMW multimers loss observed in high-shear cardiovascular disorders.

#### **Vascular Endothelium and Recovery of HMW Multimers Defect**

The inhibition of the proteolysis of HMW multimers is not sufficient to explain alone their sudden rise in TAVI patients, unless newly secreted VWF circulate in the blood. This question was investigated by measuring VWFpp in patients undergoing TAVI. In these patients the increase of VWFpp was indicative of an acute release of VWF by the vascular endothelium.<sup>24</sup> This demonstrates that in combination with the acute inhibition of HMW multimers proteolysis, an acute release of VWF multimers by the endothelium is requested for the acute recovery of the HMW multimers defect. The absence of recovery of the HMW multimers in a model of acute shear recovery but without endothelium is also consistent with this hypothesis.

Recent studies have demonstrated that an increase in the arterial luminal pressure is able to induce an acute release of VWF by the vascular endothelium.<sup>25</sup> In our study, the observations of a sudden rise in VWFpp in situations where an increase of arterial luminal pressure is observed (such as TAVI or HeartMate-II LVAD patients), and the lack of VWFpp increase in a model without endothelium, is consistent with this hypothesis.

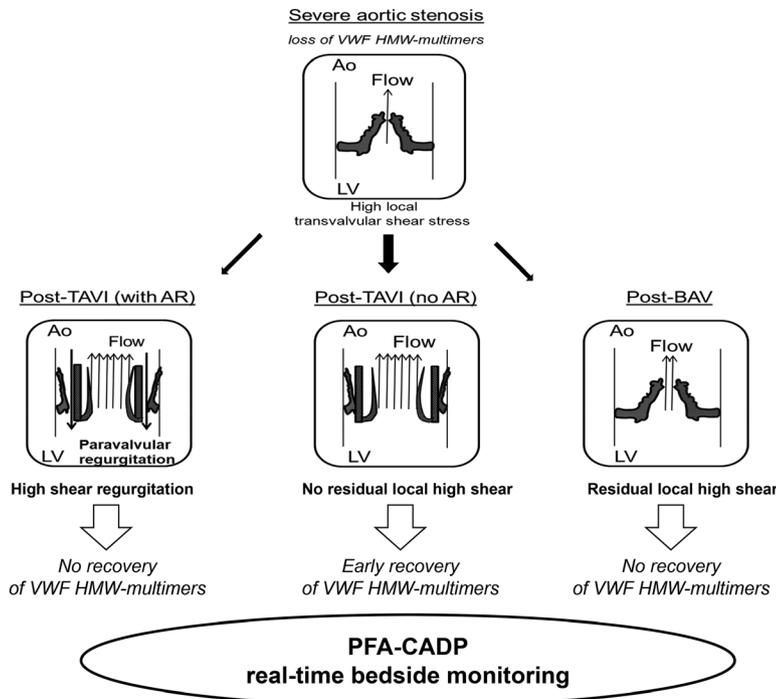
Altogether this suggests that in TAVI patients, the multimers newly provided by the endothelium in response to the increased arterial luminal pressure are no longer submitted to local abnormal high shear and proteolysis when passing through the valve, thus resulting in an ultimate increase in the proportion of HMW multimers (Figure 8).

#### **PFA-CADP to Monitor in Real Time the Result of Aortic Percutaneous Interventions**

Periprocedural evaluation of the result of percutaneous aortic interventions, while important because corrective measures can be undertaken at that time, remains a challenging issue. In particular, the occurrence of postprocedural AR after TAVI is a vexing clinical problem observed in 10% to 30% of cases. Although it has been associated with an increased long-term mortality,<sup>2</sup> its detection and accurate evaluation in the catheterization laboratory remain difficult.<sup>19</sup> There is therefore a critical need for a quick and reliable method of evaluation of the results of these interventions.

PFA-100, which is a whole blood functional test of primary hemostasis, has been shown to be highly sensitive to HMW multimers defects.<sup>14</sup> A major finding is that a rapid correction in PFA-CADP, reflecting HMW multimers recovery, was observed in patients undergoing TAVI, whereas no significant change was observed in those undergoing BAV. Furthermore in TAVI patients with a clinically significant AR, an incomplete correction of PFA-CADP was also observed and PFA-CAP values were able to segregate perfectly patients with (<180 seconds) or without (<140 seconds) residual AR. This demonstrates that PFA-100 can reflect in real-time acute shear modification and evaluate the quality of the results of transcatheter aortic valve procedures.

PFA-CADP could therefore be used to monitor TAVI procedures in some critical patients such as those with a high risk of mortality in case of AR, for example, patients with atrial fibrillation, renal failure, or pure AS without AR,<sup>2</sup> and those with conflicting results about the significance of postprocedural AR by other investigatory means (angiography, echocardiography, etc). In such circumstances, it has been shown that balloon postdilatation could decrease the magnitude of AR but at the price of an increased risk of stroke or bioprosthesis damages.<sup>26</sup> The lack of improvement of PFA-CADP measured in real time could provide additional informations and be integrated in the decision process. Similarly, although TAVI is often performed in patients with a degenerated biological prosthesis in the so-called valve-in-valve procedure, the result can be hampered by the high residual transvalvular gradient because of a prosthesis/prosthesis mismatch. The development of a broader size choice and fully retrievable devices will provide the opportunity to adapt the initial choice during the procedure pending that prosthesis/prosthesis mismatch can be accurately and quickly recognized. In this situation also, the lack of improvement



**Figure 8. von Willebrand factor (VWF) as a biomarker of transcatheter aortic valve interventions.** A loss of VWF high molecular weight (HMW) multimers is observed in severe aortic stenosis consecutive to an increase in shear-induced proteolysis of VWF through the pathological aortic valve. A recovery of VWF HMW multimers is observed early after a transcatheter aortic valve implantation (TAVI)-mediated new aortic valve implantation excepted in case of postprocedural aortic regurgitation (AR), whereas VWF multimeric profile remains unchanged after a balloon valvuloplasty (BAV)-mediated aortic valve dilatation associated with high residual mean transaortic gradient. We proposed that the time course of HMW multimers recovery after TAVI procedure is mediated by 2 mechanisms dependent on the correction of aortic stenosis: (1) a normalization in local aortic transvalvular shear stress conditions preventing the proteolysis of circulating HMW multimers through the implanted aortic valve. (2) An increase of HMW multimers release by the vascular endothelium secondary to an increase in arterial luminal pressure. Finally platelet function analyzer (PFA)-bedside assessment reflecting HMW multimers changes could be used clinically to monitor in real time the results of transcatheter aortic valve interventions. Ao indicates aorta; CADP, closure time adenine Diphosphate; and LV, left ventricle.

of PFA-CADP could help the medical decision while the patient is still in the catheterization laboratory.

Such approach has also the potential to be helpful in tuning a ventricular assist system.

**Study Limitations**

The number of patients included in this study could be considered as limited. This was largely a consequence of the translational approach of our study and of our goal to provide a real-time assessment of the processes involved. We think that such an approach favoring multiple clinical situations and the assessment of multiple time points in each clinical situation rather than a high number of subjects in each clinical situation was more adapted to our research. It did not preclude the detection of significant differences, while the findings obtained in one situation allowed further validation of the findings from another.

Although the rabbit model allowed us to investigate onset/offset of loss of HMW multimers, the underlying mechanisms could not be investigated in the same model because of the lack of specific reagents for rabbits. However, these mechanisms were investigated using the HeartMate-II LVAD model and in patients undergoing transcatheter aortic valve procedures.

The use of multimeric analysis of VWF as a biomarker of blood flow is potentially limited by the fact that it is a time-consuming technique. This issue was offset, however, by the use of a point-of-care PFA-CADP assay, which renders our observation clinically relevant.

Finally, we have to acknowledge that the mechanistic arguments for rapid recovery of VWF multimers, involving pressure-related endothelium-release of VWpp, while consistent with our study findings, are partly speculative. Similarly, our study does not allow drawing any definite conclusion on the impact of pulsatile versus continuous blood flow on the release of VWpp. These 2 important issues will require further and dedicated investigations.

**Conclusions: VWF as a Biological Sensor of Blood Flow**

Although this was previously speculated based on in vitro findings,<sup>13</sup> our results provide the first integrated demonstration that circulating VWF acts as a biological mechanosensor and a dynamic marker of changes in blood flow in vivo. This observation, together with the recently described<sup>27</sup> pleiotropic function of VWF, suggests a key role of VWF as a biological transducer of changes in blood flow (Figure 8).

In addition, the mechanosensor property of VWF, as assessed by a point-of-care assay, could be useful in clinical practice to monitor in real-time TAVI procedures, detect key procedural complications with a deleterious impact on clinical outcome (high residual gradient, postprocedural AR), and assist the clinical decision.

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**Disclosures**

None.

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## Novelty and Significance

### What Is Known?

- Acquired defect of von Willebrand factor (VWF) has been reported in various cardiovascular disorders associated with high shear, in particular, with aortic valve stenosis.
- Correction of the pathological condition has been associated with reversion of the VWF defect.
- In vitro data suggest that changes in VWF multimeric could be highly dynamic in response to changes in shear.

### What New Information Does This Article Contribute?

- In response to in vivo changes in shear, the VWF multimer can change within minutes.
- VWF can be used as a biomarker of change in blood flow to evaluate percutaneous aortic valve interventions.
- Point-of-care assay could be implemented in the catheterization laboratory as part of a real-time monitoring strategy of the result of percutaneous aortic valve interventions.

Based on in vitro findings, it has been previously speculated that the multimeric pattern of VWF could change dynamically in response to high shear. Our results show that circulating VWF acts as a biological mechanosensor and a dynamic marker of changes in blood flow in vivo. We describe a highly dynamic recovery of HMW multimers along with the sudden changes in blood flow after a complete correction of aortic stenosis during percutaneous aortic valve procedures. We document that the failure of percutaneous aortic valve procedures, because of a high residual gradient and a post-procedural aortic regurgitation, is detected by a point-of-care assay sensitive to HMW multimers defect. These results provide the basis for a per-procedural evaluation of percutaneous aortic valve interventions, using VWF as a biomarker of complete aortic stenosis reversion/acute change in blood flow. We suggest that such point-of-care assay could be implemented in the catheterization laboratory as part of a real-time monitoring strategy for the early detection of transcatheter aortic valve implantation procedural failure.

## von Willebrand Factor as a Biological Sensor of Blood Flow to Monitor Percutaneous Aortic Valve Interventions

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**Online data supplement**

**Von Willebrand Factor As A Biological Sensor Of Blood Flow To Monitor**

**Percutaneous Aortic Valve Interventions: Insights From The Witavi Registry**

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## **Supplemental methods**

### **Rabbit model of reversible aortic stenosis**

#### *Animals*

All experiments were conducted in syngeneic male New Zealand rabbits weighing 2.7–3 kg, of the same blood group. Animals were obtained from the CEGAV Breeding Colony (Les Hautes Noës, St Mars d'Egrenne, France). Rabbits were housed in individual cages under standard conditions of temperature (14–20°C) and light (12 h per day) with food and water provided ad libitum. All experiments were conducted in accordance with the European Communities Council directive (86/609/EEC) after approval was obtained from the local Ethics Review Board of Lille University, and conformed to the US National Institutes of Health guidelines for the care and use of laboratory animals. Anaesthesia, ventilation and monitoring were performed as previously described <sup>1</sup>

#### *Aortic stenosis and reversion*

An adjustable silicone vascular clamp, consisting of a banding ring, a connecting tube and an inflation reservoir (Harvard Apparatus, Holliston, Kent, UK), was placed on the ascending aorta to induce a controlled circumferential stenosis. Non-inflated, the device was adjusted to the outside diameter of the aorta while inflation of the vascular clamp with a predefined volume allowed a reproducible stenosis. The volume was defined to obtain a reduction in cross sectional area >75% as observed in patients with severe aortic stenosis <sup>2</sup>. Inflation and deflation using this device were performed in less than 5 seconds thus stenosis and its reversion were immediate.

### *Blood sampling*

Blood samples were collected through a carotid artery catheter. Blood samples were obtained before stenosis (T0), after induction of stenosis (T5, T30) and 5 and 30 minutes after reversion of the stenosis (T35, T60). Blood loss secondary to blood sampling was compensated by continuous isotonic NaCl infusion via the carotid catheter. At the end of the experiment, once the time course was completed, rabbits were sacrificed by injection of a 2mL of T-61 (Tanax ®). VWF antigen and multimeric profile were analyzed.

### **VWF antigen and VWF multimeric analysis in rabbits**

All samples were collected in 0.129 M trisodium citrate tubes (9NC BD Vacutainer, Plymouth, UK) and centrifugated at 2500 g for 15 minutes. Poor-platelet plasma (PPP) was frozen and stored at -80°C until analysis.

Rabbits PPP-samples were tested for VWF antigen (VWF:Ag) by Elisa as recommended by the manufacturer (Cedarlane® CL20403K). Results were expressed relative to baseline values observed for each animal.

Rabbits PPP-samples were subsequently tested for VWF multimeric patterns via 1.4% SDS-agarose electrophoresis as previously described for human samples<sup>3</sup>, excepting the revelation step. Loading of SDS-agarose gels was normalized for VWF:Ag content. The revelation step was adapted for rabbit species using a polyclonal anti-human VWF antibody (Cedarlane® CL20403K), cross-reacting with rabbit VWF, that was conjugated to alkaline phosphatase enzyme (Lynx rapid alkaline phosphatase antibody conjugation kit, AbD serotec®). VWF multimeric pattern was detected via alkaline phosphatase-mediated NBT/BCIP (Nitroblue Tetrazolium / 5-Bromo- 4-Chloro-3-Indolyl Phosphate) hydrolysis. HMW-multimers were

determined using densitometric scanning. Results were expressed relative to baseline values determined for each rabbit.

### **HeartMate-II® assist device model**

Anticoagulated whole human blood (250 mL) was perfused in a circulatory perfusion system incorporating a HeartMate-II® pump (Thoratec Corp., Pleasanton, California). Two cylindrical tubings (1x2 x 3/32 xs; Sorin Group Implant®) were used to connect the device. The inlet and outlet ducts of the HeartMate-II® were connected with these two tubings to obtain a closed circuit. HeartMate-II® rotation was set to 9,000rpm. Samples were taken 5 min before the onset of perfusion (T0) and after 5, 30 and 180 minutes.

### **Assessment of VWF proteolysis as underlying mechanism of HMW-multimers loss induced by HeartMate-II® support**

#### *Spiking experiments in the presence of EDTA*

Ethylenediaminetetraacetic acid (EDTA 10mM -Sigma®) was spiked in whole human blood before the initiation of the HeartMate-II® pump. Subsequently blood was perfused under high shear conditions (9,000rpm). Blood was sampled before the onset of perfusion (T0) and after 5 min (T5), 30 min (T30) or 180 min (T180) for VWF multimeric analysis by SDS-agarose gel electrophoresis.

#### *VWF proteolysis fragments analysis by immunoprecipitation/western-blot*

VWF proteolysis fragments were assessed in 3 HeartMate-II®-patients via western-blot analysis of immuno-precipitated VWF. VWF was immuno-precipitated using rabbit polyclonal anti-VWF antibodies (50 µg/ml beads; Dako, Glostrup, Danmark) adsorbed onto

Protein-G coated magnetic beads (Dynabeads Protein G, Invitrogen, Saint Aubin, France) for 2 h at room temperature. After extensive washing in PBS/0.1 % Tween-20, immunoprecipitated VWF was released from the beads via a 5 min incubation at 100°C in 30 µL PBS/10 µL NuPAGE-LDS 4×sample buffer (Life Technologies, Saint Aubin, France) in the presence of 2 mM dithiothreitol. Electrophoresis was performed using discontinuous 4-12% SDS-page (Invitrogen). After transfer to an Immobilon P membrane (Millipore, Molsheim, France), the presence of VWF proteolysis fragments was revealed via incubation with a pool of 10 distinct monoclonal antibodies recognizing distinct epitopes of VWF (10 µg/ml). Bound antibodies were probed using peroxidase-labeled goat anti-mouse antibodies (dilution 1:500; Santa Cruz, Heidelberg, Germany) and visualized with SuperSignal West-Pico Enhanced Chemiluminescence Substrate (Thermo-Fischer Scientific, Villebon-sur-Yvette, France). Blots were analyzed via ImageJ-1.44 software (<http://rsbweb.nih.gov/ij/index.html>) in order to quantify uncleaved VWF (225 kDa bands) and VWF proteolysis fragments (140 kDa and 176 kDa bands).

## Supplemental references

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## Supplemental legends to figures

### **Online Figure I. Shear-induced proteolysis as underlying mechanism of VWF HMW-multimers loss after initiation of HeartMate-II® support**

A: VWF proteolysis was assessed via Western blot analysis. Representative time course of immuno-precipitated VWF proteolytic bands (140 and 176 kDa bands) under high shear conditions (9,000rpm) and densitometric integration of 3 HeartMate-II®-patients. Results (mean±SD) are expressed as a ratio versus baseline. A time-dependant increase in immuno-precipitated VWF proteolytic bands was observed.

**B, C:** *In-vitro*, no loss of HMW-multimers was observed under high shear conditions (9,000 rpm) when spiking a protease inhibitor (EDTA 10mM) before pump initiation (**B**) nor at low shear (3,000 rpm) (**C**).

# Online Figure I

