Thrombosis Research 241 (2024) 109094

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Investigation of von Willebrand factor multimer abnormalities before and after aortic valve replacement using the Hydragel-5 assay

Marwan Hamiko, MD [a], Lena Gerdes [a], Miriam Silaschi, MD [a], Holger Seidel, MD [b], Philipp Westhofen, PhD [b], Johannes Kruppenbacher, MD [b], Hans-Joerg Hertfelder, MD, PhD [b,c], Johannes Oldenburg, MD [c], Farhad Bakhtiary, MD [a], Markus Velten, MD [d], Mehmet Oezkur, MD [e], Georg Daniel Duerr, MD [e,*]

[a] Department of Cardiac Surgery, University Hospital Bonn, Bonn, Germany
[b] Center for Bleeding Disorders and Transfusion Medicine, (CBT), Bonn, Germany
[c] Institute of Experimental Hematology and Transfusion Medicine, University Hospital Bonn, Bonn, Germany
[d] Department of Anesthesiology and Pain Management, The University of Texas Southwestern Medical Center, Dallas, TX, USA
[e] Department of Cardiovascular Surgery, University Medical Center, Johannes-Gutenberg University, Mainz, Germany

ARTICLE INFO

Keywords:
Aortic valve stenosis
Acquired von Willebrand disease
Surgical aortic valve replacement
Echocardiography
Postoperative bleeding

ABSTRACT

Background: Severe aortic stenosis (sAS) is associated with acquired von Willebrand syndrome (AVWS) by loss of high-molecular-weight multimers (HMWM) of von Willebrand factor (VWF), potentially resulting in perioperative bleeding. Analysis of VWF multimers remains challenging. Recently, the new, rapid Hydragel 5 assay has been developed, using electrophoretic protein separation for dividing VWF-multimers into low (LMWM), intermediate (IMWM), and HMWM, the hemostatically active part of VWF. Here, we evaluated its impact on predicting blood loss in presence of AVWS after surgical aortic valve replacement (SAVR).

Methods: We prospectively examined 52 patients (age: 68 ± 7 years; 54 % male) admitted to SAVR. They were divided in two groups (A: normal VWF, $n = 28$; B: abnormal VWF, $n = 24$, defined as VWF-activity/antigen (VWF:Ac/Ag)-ratio < 0.7 and/or HMWM loss). Blood samples and echocardiographic data were collected before, seven days and three months after SAVR. Blood loss and transfusions were recorded.

Results: Baseline characteristics and clinical data were similar in both groups. HMWM loss was present in 38.5 % of all patients. HMWM, the VWF:Ac/Ag- and HMWM/(IMWM+LMWM)-ratios were significantly decreased preoperatively in group B but normalized after SAVR. Bleeding, re-thoracotomy and transfusion rates were comparable. HMWM loss was inversely correlated with the peak aortic gradient (Pmax) and positively with the aortic valve area (AVA), while HMWM/(IMWM+LMWM)-ratio negatively correlated with the mean aortic gradient (Pmean).

Conclusion: HMWM and HMWM/(IMWM+LMWM)-ratio inversely correlate with severity of AS and normalize after SAVR. The Hydragel-5 assay's might be valuable for routine diagnostics to assess bleeding risk and postoperative normalization of AS and VWF abnormalities in SAVR patients.

1. Introduction

Aortic valve stenosis (AS) is the most common valvular heart disease [1]. In symptomatic patients, surgical aortic valve replacement (SAVR) and transcatheter aortic valve replacement (TAVR) have become standard procedures with low perioperative risk [1,2]. Severe AS (sAS) can be complicated by peri- or postoperative bleeding, which, among other causes like platelet activation due to cytokine-triggered inflammation following the use of a cardiopulmonary bypass (CPB) (= heart-lung machine), can be caused by impaired function of von Willebrand factor (VWF) [3–7]. VWF is a glycoprotein of multimers that plays an important role in the cascade of primary hemostasis: VWF initially adheres to the site of vascular damage, undergoes a structural transformation into a linear or filamentous form bound to structures like collagen, followed by adhesion and activation of platelets bound to the VWF [8]. Acquired von Willebrand syndrome (AVWS) is characterized

* Corresponding author at: Department of Cardiovascular Surgery, University Medical Center, Johannes-Gutenberg University Mainz, Langenbeckstr. 1, 55131 Mainz, Germany.

https://doi.org/10.1016/j.thromres.2024.109094
Received 8 February 2024; Received in revised form 24 June 2024; Accepted 5 July 2024
Available online 6 July 2024

by the loss of high molecular weight multimers of VWF (HMWM) and has been reported to occur in patients with sAS due to unfolding of the globular multimeric VWF structure by high shear stress [9]. HMWM is the hemostatic most active form of the VWF protein complex [10–13], and its loss results for example in gastrointestinal bleeding due to submucosal angiodysplasias as described by Heyde in 1958, thus known as "Heyde syndrome" [14]. Loss of HMWM is found in sAS patients with AVWS with a prevalence up to 80 %, recognized as 'Von Willebrand Disease (VWD) type 2A like'. [15] The structural change in the VWF molecule is attributable to increased shear stress. This stress is caused by the rapid movement of blood cells and plasma through a severely calcified aortic valve [4,6,16].. This conformational change enhances the proteolytic enzymatic cleavage of VWF by the metalloprotease ADAMTS-13 (A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) with subsequent degradation of HMWM to multimers of intermediate (IMWM) and low molecular weight multimers (LMWM) [11,14,17–22]. Although VWF multimer structure and platelet function show an early recovery after SAVR or TAVR [4,11,23,24], there is an association between gastrointestinal hemorrhage and complex surgical or interventional procedures [4,25–28]. While there is limited data from the literature that proves an increased perioperative bleeding risk after TAVR or SAVR, the latter cannot be ruled out. Thus, bleeding remains a serious complication associated with unfavorable postoperative outcome with high morbidity and mortality after SAVR. Postoperative bleeding requires a higher amount of packed red blood cells (RBC), which is associated with higher incidence of acute renal failure, sepsis, acute respiratory distress syndrome, neurological impairment like postoperative delirium (POD) and death [29–34].

Although a correlation between perioperative bleeding and impaired VWF function has been suggested in previous studies [35,36], there is a lack of information about the association between altered VWF multimer structures on peri- or postoperative bleeding after SAVR and transfusion requirement. In this regard, it is of importance that dysfunction of VWF often remains undiagnosed, suggesting that their impact on bleeding complications might probably be underestimated. To date only a few studies investigated the correlation between echocardiographic parameters and the loss of HMWM, providing information on severity of stenosis and HMWM loss [37]. The identification of a potential VWF dysfunction is initially performed by the comparison of VWF activity (VWF:Ac) and VWF antigen concentration (VWF:Ag) by calculating the "VWF-ratio" of VWF:Ac/VWF:Ag. A VWF dysfunction is defined by a ratio < 0.7, as described [38]. Laboratory diagnosis of AVWS depends on specialized assays, determining VWF:Ag and VWF:Ac as first diagnostic tests. VWF:Ag levels are measured by enzyme-linked immunosorbent assay (ELISA) or by Latex-Immunoturbidimetric Assay (LIA) technique. VWF activity examine the binding of VWF to platelet glycoprotein Ib (GPIb) and collagen. Binding of VWF to platelet GPIb can be assessed by Ristocetin cofactor (VWF:RCo) or by VWF activity assays based on monoclonal antibodies directed against the VWF binding structures of the platelet VWF receptor GPIb (VWF:Ab). While most experts do not consider the VWF:Ab assay to measure VWF activity directly, it correlates with other assays that do measure VWF activity [39]. As a second diagnostic step in case of a decreased VWF-ratio, the multimer distribution is analyzed [40,41]. Standard VWF multimer analysis is technically difficult and not available as commercially standardized routine assay, but requires a complex in-house developed electrophoresis and immunoblot technique which is only provided by few expert laboratories, making it complex and time-consuming. Thus, multimer analysis remains challenging and is not part of a routine laboratory diagnostic. Recently, a new semi-automated multimer assay, namely Hydragel 5 von Willebrand multimers (H5VWM assay; Sebia, Evry, France), has become commercially available as a rapid, sensitive assay for screening VWF multimers in Von Willebrand disease (VWD) [41–44]. In contrast to standard multimer analysis, H5VWM facilitates the additional quantification of VWF multimer (VWF:MM) band patterns of LMWM, IMWM and HMWM fractions. Qualitatively, defects can be detected by visually observing densitometry results. Additionally, LWMW, IMWM, and HMWM are calculated automatically and expressed as a relative percentage of the area under the curve (AUC). This integrated AUC enables the calculation of the HMWM/(IMWM+LMWM)-ratio. This ratio may be a promising parameter for assessing the shear-induced degradation of HMWM in the light of sAS, potentially helping in the clinical identification of patients with bleeding complications. Like the VWF ratio, it provides indirect information about the qualitative damage of HMWM seen in sAS. However, it is important to note that data on the clinical impact of the HMWM/(IMWM+LMWM)-ratio are currently limited, and its role as an indicator is part of an ongoing investigation and not yet established [41].

Given that AVWS is linked to less blood clot formation, which can increase the risk of bleeding after surgery, and that the loss of HMWM is tied to the severity of AS, our study aimed to examine HMWM levels before and after SAVR, and how they relate to AS severity and bleeding during and after surgery. For these investigations, the new Sebia H5VWM assay was utilized.

2. Material and methods

2.1. Study population

After approval by the ethics committee of the University of Bonn (ethical approval No. 389/17) and written informed consent from all participating patients, we prospectively enrolled 55 consecutive patients in the study. All patients with sAS were admitted to our department of cardiac surgery for elective SAVR. Indication for SAVR was defined by a calculated effective orifice area (EOA) <1.0 cm^2; or a mean aortic gradient (Pmean) >40 mmHg, or maximum jet velocity (Vmax) >4.0 m/s. Patients were not included if they were under 18 years of age or not willing to participate. Three patients were excluded from the study postoperatively, as they underwent additional coronary artery bypass grafting (CABG). 52 patients could be analyzed. Acetylsalicylate acid (ASA; 100 mg per day) was administered if it was part of a daily medication. Patients on oral anticoagulation were switched to low molecular weight heparin at least three days before SAVR.

A comprehensive data set of pre-, intra- and postoperative parameters was analyzed by review of patient charts and IT based data sets. Clinical and demographic data, including cardiovascular risk factors, former cerebrovascular disease, presenting symptoms by classifying in NYHA and CCS-classification and pre-operative risk score systems e.g., the Society Thoracic of Surgeons (STS)-Score, and the European System for Cardiac Operative Risk Evaluation (EuroScore) II were calculated and recorded. To evaluate the pre-operative risk for bleeding and perioperative risk for transfusion, the HAS-BLED-score, the PBRS-Score (Papworth Bleeding Risk Score) and the ACTA-PORT (Association of Cardiothoracic Anesthetists (ACTA) perioperative risk of blood transfusion score) were calculated as described [45,46].

2.2. Blood collection and laboratory assays

Venous peripheral blood samples were collected at three time points: day before SAVR, seven days after SAVR, and three months after SAVR. VWF:Ag was measured with the "HemosIL VWF Antigen" kit (catalogue number 0020002300), and VWF:Ac with the "HemosIL VWF Activity" kit, which is a monoclonal antibody binding-based assay (catalogue number 0020004700) on ACL Top CTS (Werfen, Barcelona, Spain) using the manufacturer's protocol. We also determined Ristocetin-dependent VWF cofactor activity (VWF:RCo) by using "HemosIL Von Willebrand Factor Ristocetin Cofactor Activity (catalogue number 0020300900). For the analysis of blood clotting parameters including all VWF parameters, peripheral venous blood samples were drawn into trisodium citrate tubes (Sarstedt Monovette Systems, Nümbrecht, Germany). Samples were frozen and stored at −35 °C for a maximum of three

months.

2.3. VWF multimer analysis

VWF multimers were analyzed with a semi-automated system using the Hydragel 5 VWF multimer kit with the Hydrasys 2 Scan Instrumentation (H5VMK assay). Agarose gel electrophoresis using preformed 2 % agarose gels, direct immunofixation, visualization with peroxidase-labelled antibody and densitometry was performed. According to the manufacturers' instructions, samples were initially tested at a 1:6 dilution of plasma with sample diluent. Individuals with VWF:Ag levels >150 IU/dL were also tested at 1:10 dilution of plasma, as described [41]. The Sebia-method is described in detail by Oliver et al. [47] Additionally, a graphical summary is provided in the supplemental Fig. S1 for better understanding. Densitometry was performed by Hydrasys 2 Scan, as described [41]. The percentage of low molecular weight multimers (LMWM), intermediate molecular weight multimers (IMWM) and high molecular weight multimers (HMWM) were assessed using Phoresis software (Sebia) with peaks 1–3 designated as LMWM, peaks 4–7 as IMWM and peaks >7 as HMWM, as shown in the manufacturer's protocol.

2.4. Study groups

We categorized patients with abnormalities in the multimer structure before SAVR as group B. Abnormalities of VWF multimers were defined by either VWF:Ac/VWF:Ag-ratio < 0.7, and/or loss of HMWM [38]. To clarify the etiological or pathological reasons for the pre-surgery abnormal VWF status in group B, it is essential to highlight that these patients have a smaller aortic valve annulus size and higher Pmean and peak aortic gradient (Pmax) values compared to group A. These factors contribute to shear stress related VWF multimer loss, as extensively documented in the literature [4,22,48]. The relevant echocardiographic data of our patients is provided in Table 1.

2.5. Surgical procedure and postoperative care

A standard anesthetic protocol was performed using sufentanil, etomidate, and rocuronium for induction of anesthesia followed by endotracheal intubation. Anesthesia was maintained using sufentanil and sevoflurane. During CPB, sevoflurane was administered continuously. Surgical procedure was similar in all patients: full median or superior median mini sternotomy was performed. To achieve an activating clotting time of >450 s, anticoagulation with 400–500 U/kg sodium heparin was initiated. After cannulation of the ascending aorta and the right atrium, CPB was established using a Terumo Advanced Perfusion System-1 (Terumo Cardiovascular Systems, Ann Arbor, Michigan, USA). The non-heparin coated tube system was primed with crystalloid solution (1.000 ml Jonosteril, Fresenius, Bad Homburg, Germany) and 10.000 U of heparin. In all patients, retrograde autologous blood priming (RAP) was performed before beginning of heart-lung-machine (HLM). Cardioprotection and myocardial arrest were achieved using Bretschneider HTK-cardioplegia (Custodiol, Dr. Franz Kohler Chemie GmbH, Bensheim, Germany). A non-pulsatile pump flow of 2.2–2.6 l/min/m^2 was conducted to maintain a mean arterial pressure (MAP) of 50–60 mmHg during CPB.

After the surgical procedure, all patients were transferred to the intensive care unit (ICU), where sedation was achieved using propofol (Fresenius) and sufentanil (Piramal Critical Care GmbH, Hallbergmoos, Germany). Early extubation and transfer to the intermediate care (IMC) ward were attempted in all cases. Further analgesic regimen followed piritramide bolus injection (3–5 mg intravenous, Piramal).

Blood loss from chest tube drainages after arrival on the ICU was monitored after 1, 2, 3, 6 and 24 h after surgery. In addition, transfusion of RBC, fresh frozen plasma (FFP) and platelet concentrates (PC) during surgery, ICU stay, and further hospital stay as well as adverse events

Table 1
Patients baseline characteristics and preexisting conditions. Baseline characteristics, symptoms, underlying health conditions, and laboratory parameters in patients received SAVR with normal multimer (A) compared to patients with abnormal multimer analysis (B). For metric data, Mann-Whitney-*U* test was used, for categorial variables, Chi2- or Fisher Exact Test was performed. Mean and SD are shown. Significant *p*-values are shown in bold italics. *Abbreviations: ACTA-PORT Score: Association of Cardiothoracic Anesthetists (ACTA) perioperative risk of blood transfusion score; APTT: activated partial thromboplastin time; ASA: Acetylsalicylate acid; AVA: Aortic Valve Area; EuroScore II: European System for Cardiac Operative Risk Evaluation II; INR: international normalized ratio; LVEF: left ventricular ejection fraction; NOAC: non-vitamin K oral anticoagulant; NYHA: New York Heart Association; PBRP-Score: Papworth Bleeding Risk Score; Pmax: peak aortic gradient; Pmean: mean aortic gradient; STS-Score: The Society Thoracic of Surgeons-Score; Vmax: maximum jet velocity; VKA: vitamin K antagonist.*

	Total n = 52	Group A n = 28	Group B n = 24	p-value
Baseline data				
Age	67.9 ± 7.2	68.2 ± 8.0	67.5 ± 6.3	0.736
Male	28 (53.8 %)	14 (50.0 %)	14 (58.3 %)	0.548
BMI	28.5 ± 5.5	28.9 ± 5.2	28.0 ± 5.9	0.602
Blood groups				
0	24 (46.4 %)	13 (46.4 %)	11(45.8 %)	>0.999
A	22 (42.3 %)	13 (46.4 %)	9 (37.5 %)	0.581
B	4 (7.7 %)	1 (3.6 %)	3 (12.5 %)	0.324
AB	2 (3.8 %)	1 (3.6 %)	1 (4.2 %)	>0.999
Scores				
EuroScore II in %	2.0 ± 0.6	1.9 ± 1.0	2.0 ± 1.2	0.869
STS-Score	1.2 ± 0.6	1.2 ± 0.7	1.2 ± 0.6	0.582
PBRS-Score	1.5 ± 0.6	1.5 ± 0.7	1.5 ± 0.6	0.785
HAS-Bled-Score	2.6 ± 0.9	2.6 ± 0.9	2.5 ± 0.9	0.645
PORTScore in %	33.0 ± 14.1	33.6 ± 13.5	32.3 ± 15.1	0.555
Previous medical history				
Diabetes mellitus	14 (26.9 %)	10 (35.7 %)	4 (16.7 %)	0.209
Hypertension	43 (82.7 %)	26 (92.9 %)	17 (70.8 %)	***0.036***
Hyperlipidaemia	38 (73.1 %)	20 (71.4 %)	18 (75.0 %)	0.772
Nicotine abuse	17 (32.7 %)	7 (25.0 %)	10 (41.7 %)	0.245
Peripheral arterial vascular disease	5 (9.6 %)	1 (3.6 %)	4 (16.7 %)	0.169
Cerebrovascular disease	10 (19.2 %)	4 (14.3 %)	6 (25.0 %)	0.483
Previous stroke	6 (11.5 %)	2 (7.1 %)	4 (16.7 %)	0.397
Atrial fibrillation	12 (23.1 %)	5 (17.9 %)	7 (29.2 %)	0.510
Chronic kidney failure	6 (11.5 %)	3 (10.7 %)	3 (12.5 %)	>0.999
Chronic obstructive pulmonary disease	14 (26.9 %)	9 (32.1 %)	5 (20.4 %)	0.532
Coronary artery disease	7 (13.5 %)	4 (14.3 %)	3 (12.5 %)	>0.999
Prior PCI/Stent-therapy	2 (3.8 %)	1 (3.6 %)	2 (4.2 %)	>0.999
Angiodysplasia	4 (7.7 %)	3 (10.7 %)	1 (4.2 %)	0.615
Anemia	12 (23.1 %)	8 (28.6 %)	4 (16.7 %)	0.346
Clinical presentation				
NYHA I	3 (5.8 %)	1 (3.6 %)	2 (8.3 %)	0.590
NYHA II	5 (9.6 %)	4 (14.3 %)	1 (4.2 %)	0.358
NYHA III	42 (80.8 %)	21 (75.0 %)	21 (87.5 %)	0.309
NYHA IV	2 (3.8 %)	2 (7.1 %)	0 (0.0 %)	0.493
Angina pectoris	30 (57.7 %)	19 (67.9 %)	11 (45.8 %)	0.109

(*continued on next page*)

Table 1 (*continued*)

	Total n = 52	Group A n = 28	Group B n = 24	p-value
Syncope	5 (9.6 %)	3 (10.7 %)	2 (8.3 %)	>0.999
Preoperative anticoagulation therapy				
ASA	28 (53.8 %)	16 (57.1 %)	12 (50.0 %)	0.606
Dual platelet aggregation therapy	1 (1.9 %)	1 (3.6 %)	0 (0.0 %)	>0.999
VKA	3 (5.8 %)	1 (3.5 %)	2 (8.3 %)	0.590
NOAC	8 (15.4 %)	5 (17.9 %)	3 (12.5 %)	0.190
Preoperative echocardiographic data				
AVA (cm^2)	0.74 ± 0.19	0.79 ± 0.21	0.69 ± 0.15	***0.043***
Pmax (mmHg)	76.2 ± 26.5	64.4 ± 20.3	87.9 ± 27.3	***<0.001***
Pmean (mmHg)	47.6 ± 15.4	43.2 ± 11.8	53.1 ± 17.7	***0.032***
Vmax (m/s)	4.2 ± 0.6	4.1 ± 0.5	4.5 ± 0.6	***0.044***
LVEF (%)	61.0 ± 8.2	60.4 ± 7.8	61.6 ± 8.9	0.589
Preoperative laboratory data				
Platelet count (10^9/L)	214.6 ± 39.4	219.7 ± 38.5	208.6 ± 40.6	0.338
INR	1.01 ± 0.06	1.01 ± 0.06	1.00 ± 0.07	0.750
APTT (s)	25.6 ± 6.0	26.5 ± 7.6	24.5 ± 3.2	0.250
Hemoglobin (g/dL)	13.2 ± 1.6	13.2 ± 1.7	13.2 ± 1.5	0.931
White blood cells (10^9/L)	7.2 ± 1.6	7.3 ± 1.5	7.1 ± 1.7	0.355
Creatinine (mg/dL)	0.97 ± 0.68	0.87 ± 0.19	1.09 ± 0.97	0.538
Lactate (mmol/L)	1.07 ± 0.29	1.09 ± 0.32	1.04 ± 0.24	0.859

during the hospital stay were documented. Bleeding was defined as a mean blood loss exceeding 400 ml/h measured between arrival on ICU and the earliest of the following events: the elapse of 3 h or re-thoracotomy due to major blood loss, as generally accepted.

Duration of surgery, CPB and aortic cross-clamping time, parameters of organ function, and other routine postoperative laboratory parameters were recorded. Outcome data included: pneumonia, need for reintubation or tracheostomy, renal failure with need for continuous veno-venous hemofiltration or -dialysis (CVVH/HD), postoperative stroke or myocardial infarction, length of ICU, IMC and hospital stay, nurse workload score ("Therapeutic Intervention Scoring System" [TISS]-10), and monitoring of the 'Simplified Acute Physiology Score II' (SAPS II), as well as 30-day mortality.

Transthoracic echocardiography (TTE) was performed at the same day of blood sample collection. The mean and peak transvalvular aortic pressure gradients were calculated using the modified Bernoulli equation, and the effective orifice area (EOA) was calculated with the continuity equation and the left ventricular ejection fraction (LVEF) was calculated using the Simpson's equation.

2.6. Statistical analysis

Statistical analysis was performed using the IBM SPSS statistics version 25 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 8.4.3 (La Jolla, CA, USA). Data were tested for normal distribution by using Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed demographic and clinical data were analyzed using the students *t*-test. Not normally distributed data were compared using the Mann-Whitney *U* test. Categorial variables were evaluated with the Pearson chi-square-test or Fisher's exact test, as indicated. The Wilcoxon signed-rank test was used to compare significant differences between each timepoints. Correlation between two continuous variables was measured with the bivariate Pearson correlation. For the tables, continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were given as absolute values and percentages. For the diagrams, violine plots were generated to visualize the median (line), first and third quartiles (dotted lines) and individual values as well as their distribution are shown. A *p*-value of <0.05 was considered statistically significant.

3. Results

Baseline characteristics, comorbidities, symptoms at admission and preoperative echocardiographic parameters are shown in Table 1. No differences in baseline demographic data were found between both groups. According to the surgical risk scores, the patients were on low risk for surgery, e.g., EuroScore II < 2.0 % and STS-Score < 1.2 %. There were no significant differences with respect to the documented bleeding scores.

Patients of group A suffered more frequently from hypertension. About 13 % of all patients had a medical history of coronary artery disease (CAD), explaining the frequent anti-platelet drug therapy with ASA. No difference was observed with respect to anti-platelet drug therapy and oral anticoagulation in both groups. Nearly one quarter of all patients suffered from anemia. Most of the patients presented with dyspnea according to NYHA class III and angina pectoris. The preoperative echocardiographic data revealed normal LVEF in both groups. Notably, parameters revealing AS, e.g., Pmean, Pmax and Vmax, were enhanced, while aortic valve area (AVA) was reduced in group B compared to A.

Fig. 1 shows the course of the VWF:Ag (Fig. 1A), VWF:Ac (Fig. 1B) and the VWF:Ac/VWF:Ag-ratio (Fig. 1C). In both groups, VWF:Ag and VWF:Ac levels significantly increased seven days after SAVR compared to preoperative values, decreasing again after three months. VWF:RCo showed a similar course (supplemental Fig. S2A). While VWF:Ac, VWF:RCo and VWF:Ag were significantly higher in group B compared to A before SAVR only VWF:Ac and VWF:Ag were significantly elevated seven days after SAVR. Both groups showed a significant increase of VWF:Ac/VWF:Ag-ratio as well as WWF:RCo/VWF:Ag-ratio (supplemental Fig. S2B) seven days after SAVR, whereas after three months a further significant increase is only observed in VWF:Ac/VWF:Ag-ratio for group B.

Fig. 2 shows the temporal alteration of HMWM, IMWM and LMWM, as detected by the H5VWM assay. A loss of HMWM was detected in 20 of all 52 patients before surgery (38.5 %). It was significantly more profound in group B before surgery compared to group A. This could be recognized as a plausibility check, as patients were categorized as groups A and B based on VWF:Ac/VWF:Ag-ratio < 0.7 and/or HMWM loss. This means that there are patients who do not have HMWM loss but still end up in group B due to VWF:Ac/VWF:Ag-ratio < 0.7, but their number was quite low ($n = 4$). Group B showed a significant increase of the HMWM levels seven days and three months after SAVR, catching up with group A (Fig. 2A). At the same time, a significant decrease of IMWM levels was seen in group B after SAVR over time, which was also observed in group A, but to a lesser extent (Fig. 2B). LMWM levels only somewhat decreased in group B three months after SAVR (Fig. 2C). The ratio of HMWM/(LMWM+IMWM) showed a significant increase after SAVR in group B in contrast to group A. This reflects the data of VWF:Ac/VWF:Ag-ratio, however, it shows an even more distinct difference between both groups. Notably, values were significantly lower in B compared to A before and seven days after SAVR but reached levels of group A after three months (Fig. 2D).

Valve function was assessed by evaluation of the Pmax and the AVA (Fig. 3A–B). Fig. 3A reveals a significant drop of Pmax in both groups after SAVR (day 7 and 3 months) compared to preoperative values. However, the Pmax was significantly higher in group B compared to A. Fig. 3B shows significantly smaller preoperative AVA in group B

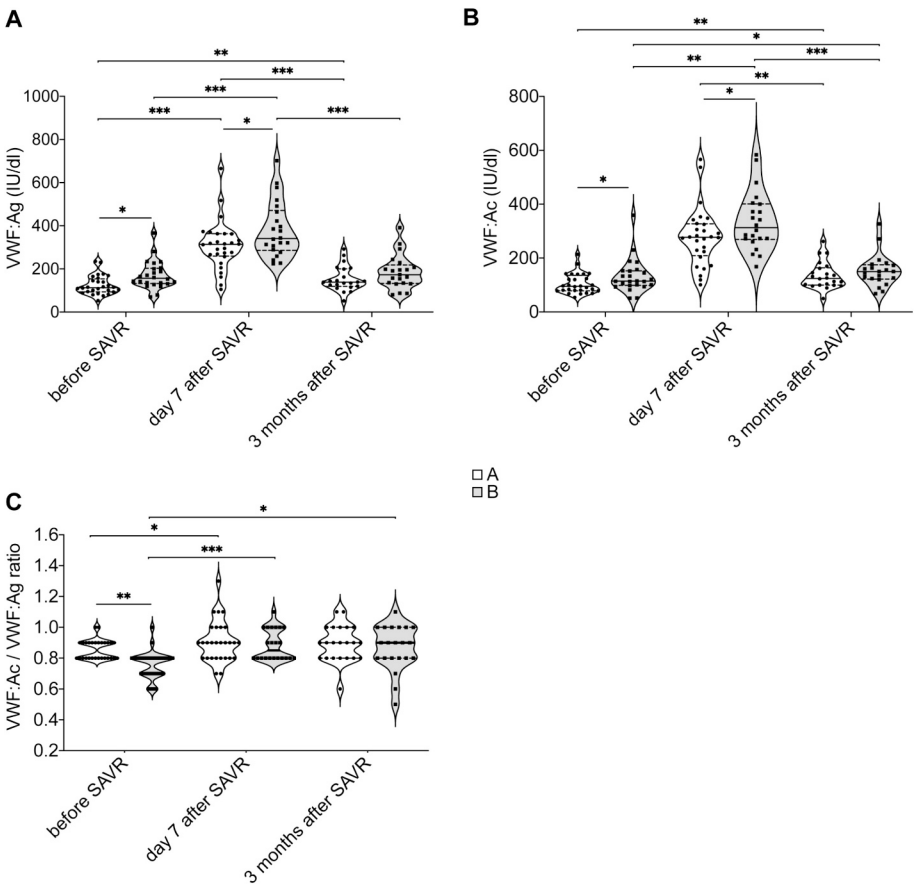

Fig. 1. Violine plots show the median (line), first and third quartiles (dotted lines) and individual values as well as their distribution. Von Willebrand factor antigen (VWF:Ag) (A), von Willebrand factor activity (VWF:Ac) (B) and von Willebrand activity-to-antigen ratio (VWF:Ac/VWF:Ag ratio) (C) before, 7 days and 3 months after SAVR. $p < 0.05$ was considered statistically significant (*). **, $p < 0.01$; ***, $p < 0.001$. *Abbreviations: SAVR: Surgical Aortic Valve Replacement.*

compared to A.

The correlation between HMWM and HMWM/(IMWM+LMWM)-ratio with echocardiographic parameters is demonstrated in Fig. 4. HMWM was negatively correlated with Pmax (Fig. 4A) and positively correlated with the AVA (Fig. 4B), while HMWM/(IMWM+LMWM)-ratio was negatively correlated with Pmean (Fig. 4C). Furthermore, VWF:Ac/VWF:Ag ratio was negatively correlated with Pmean (Fig. 4D).

3.1. Intraoperative data and postoperative outcome

Table 2 summarizes intra-operative and post-operative data. Surgery- and cardiopulmonary bypass (CPB)-related times, as well as body temperature and fluid balance did not differ between both groups and mean implanted valve size was similar. Thirty-day and one-year mortality were comparable between both groups. As shown in Table 2, no further outcome parameters differed between both groups. No significant difference could be detected regarding ventilation time, ICU-, IMC- and total hospital stay as well as TISS10 and SAPS-II scores 24 h after SAVR between both groups. Postoperative bleeding was not followed by the need for intra- and postoperative transfusion products, or the need for re-thoracotomy in both groups.

Intraoperative and postoperative bleeding within 24 h after SAVR as well as the need of RBC transfusion and hemostatic products (FFP and PC) were comparable between both groups (Fig. 5A and B). The hemoglobin values significantly decreased until seven days after SAVR in both groups, but no difference was observed between both groups (Fig. 5C). One week after SAVR, a significant increase of the platelet count could be observed in group B. After three months, both groups showed a significant increase of platelet count compared to the respective baselines (Fig. 5D).

4. Discussion

Our study investigated the presence of HMWM before and after SAVR, its relationship with aortic stenosis severity and the impact of AVWS on postoperative bleeding in patients suffering from sAS. Moreover, we aimed to explore a potential correlation between echocardiographic parameters for sAS and the alteration of VWF multimers, e.g., a lowering of HMWM, as AVWS is linked to high aortic gradient [49]., For this evaluation, we used the new H5VWM assay.

In our study, significantly reduced HMWM levels were shown in 38.5 % of all patients with sAS undergoing SAVR (group B), going along with a significantly reduced VWF:Ac/VWF:Ag-ratio before surgery as indicator for a decrease in the functional activity of VWF. This suggested an impaired ability to form platelet hemostatic plugs and subsequently blood clots properly [50]. In stenotic aortic valves, high shear forces unravel VWF, exposing binding sites for the VWF protease ADAMTS13, leading to inactivation of VWF. Of course, the role of other pathologies, such as hypertension, in AVWS is debated due to its potential to increase shear stress. Elevated blood pressure can exacerbate turbulent blood flow, particularly in those with preexisting aortic valve abnormalities [51]. However, our data did not support this association, as patients in group B suffered less frequently from hypertension. After SAVR, diminished shear stress stimuli enable VWF recovery, reflected by significantly increased HMWM and decreased IMWM and LMWM, which is supported by a recent study showing longer-term (>1 week)

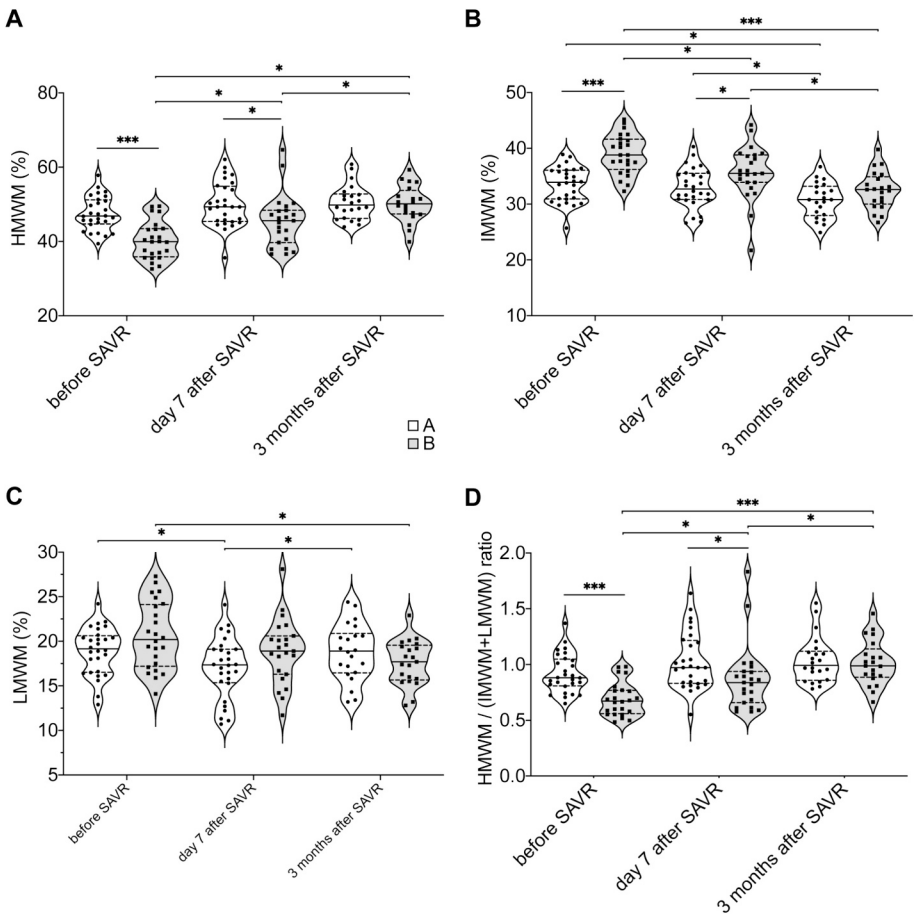

Fig. 2. Violine plots show the median (line), first and third quartiles (dotted lines) and individual values as well as their distribution. Serum levels of high (A), intermediate (B), low (C) molecular weight multimers and the ratio of HMWM and the sum of LMWM and IMWM (D) before, seven days after and three months after SAVR. $p < 0.05$ was considered statistically significant (*). **, $p < 0.01$; ***, $p < 0.001$. *Abbreviations: HMWM: high molecular weight multimers; IMWM: intermediate molecular weight multimers; LMWM: low molecular weight multimers; SAVR: surgical aortic valve replacement.*

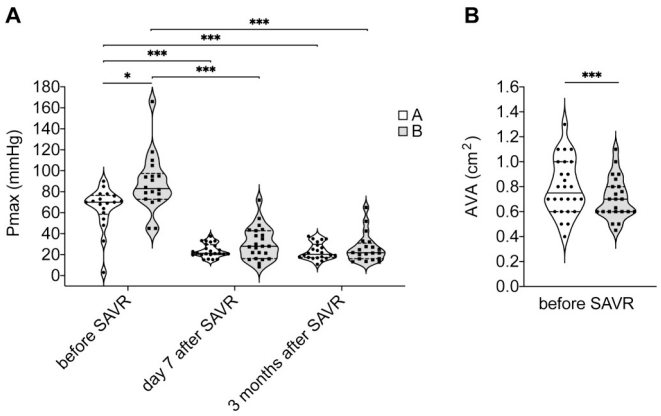

Fig. 3. Violine plots show the median (line), first and third quartiles (dotted lines) and individual values as well as their distribution. Pmax (A) before, seven days and three months after SAVR (A), and AVA before SAVR (B). $p < 0.05$ was considered statistically significant (*). **, $p < 0.01$; ***, $p < 0.001$. *Abbreviations: AVA: aortic valve area; Pmax: peak aortic gradient; SAVR: surgical aortic valve replacement.*

improvements in HMWM after TAVR in patients with sAS [52]. Accordingly, group B, with pronounced preoperative VWF shredding due to high pressure gradient and smaller AVA, benefits most from SAVR, further evidenced by the normalization of the HMWM/(IMWM+LMWM-ratio). Importantly, no significant changes were observed in group A after SAVR, where no preoperative disturbance in HMWM or the HMWM/(IMWM+LMWM)-ratio were noted. This is underlined by a significant postoperative increase of the VWF:Ac/VWF:Ag-ratio only in group B, likely due to the significantly lower VWF:Ac/VWF:Ag-ratio before SAVR in group B compared to A, again resulting from higher preoperative shear stress in patients with sAS.

Moreover, Egger et al. had shown a close correlation between the VWF:RCo/VWF:Ag-ratio and HMWM loss [50]. Now, we can confirm this for the H5VWM assay as well. These results are in accordance with other studies showing HMWM loss and reduced VWF:Ac/VWF:Ag-ratio before TAVR, using standard multimer analyses [37,28,53,54]. In line, we could show a restoration of HMWM in patients with preoperatively reduced HMWM to normal levels after SAVR (group B), being accompanied a significant increase of preoperatively reduced VWF:Ac/VWF:Ag- and HMWM/(IMWM+LMWM)-ratio, as described above. In contrast, we used the H5VWM assay.

The reported prevalence of AVWS in patients with sAS varies from 33 % to over 80 % in the literature and remains a topic of ongoing debate [6,55,56]. Diagnosing AVWS remains challenging and depends on the method and diagnostic criteria employed [4,57–59]. The gold standard for detecting structural abnormities of VWF is the standard multimer assay [60]. As previously reported, we used the H5VWM gel-based assay to assess VWF multimer patterns and characterized fractions with the "Sebia" analysis software. This rapid, semi-automated method, employing an almost ready-to-use kit system, holds potential for future laboratory diagnostics of AVWS. It has the advantage of reducing the

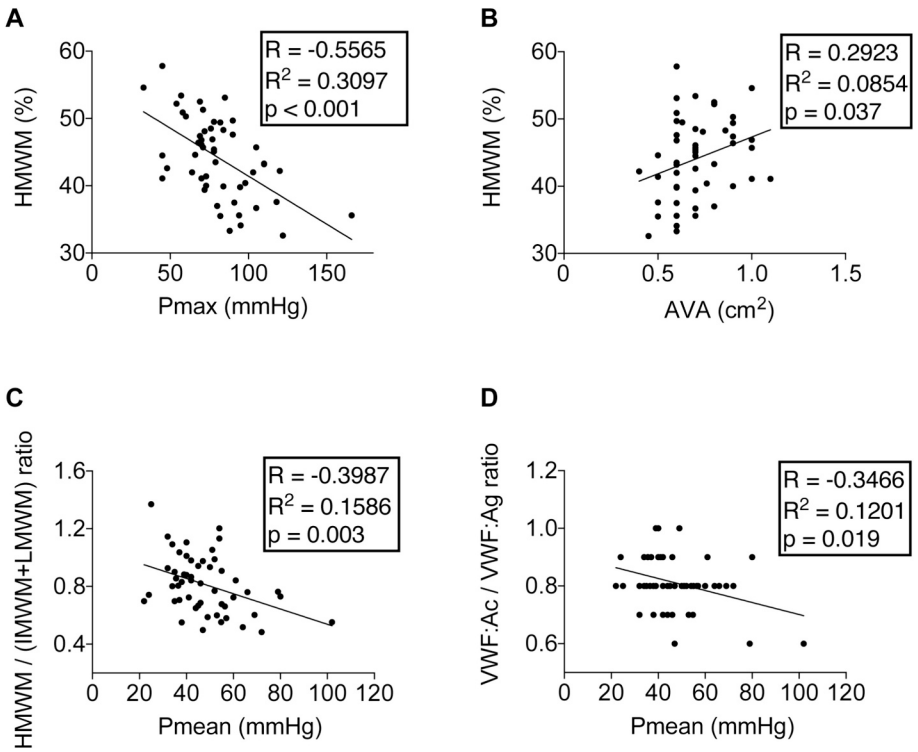

Fig. 4. Correlation analysis before SAVR; Scatter plot shows negative correlation between the high molecular weight multimers (HMWM) and the Pmax (A), positive correlation between HMWM and the AVA (B), negative correlation between HMWM/(IMWM+LMWM)-ratio and Pmean (C), and negative correlation between VWF:Ac/VWF:Ag-ratio and Pmean (D). Correlation curves are shown in a straight line. *Abbreviations: AVA: aortic valve area; HMWM: high molecular weight multimers; IMWM: intermediate molecular weight multimers; LMWM: low molecular weight multimers; Pmax: peak aortic gradient; Pmean: mean aortic gradient.*

workload compared to traditional multimer methods and – in contrast to standard multimer analysis – provides same-day results. However, the H5VWM gel-based assay may not detect the highest molecular weight multimers and lacks the resolution to visualize VWF multimer triplets, potentially impeding precise VWD diagnosis [43].

Thus, the novelty of our study lies in the revelation that, in addition to the VWF:Ac/VWF:Ag-ratio, the HMWM/(IMWM+LMWM)-ratio highlights the disparity between abnormal VWF multimers and their degradation. Hence, this ratio can be used for detection of abnormal VWF molecules in patients with sAS before surgery, using the H5VWM assay. To our knowledge, the H5VWM assay as well as the HMWM/(IMWM+LMWM)-ratio have not been described yet with regard to detection of a AVWS in patients with sAS and could be useful as a sophisticated screening tool with similar expressiveness as the VWF:Ac/VWF:Ag-ratio and less expensiveness than standard multimer analysis. Besides, this new diagnostic method carries the advantage that the measurement of AVWS multimers is significantly simpler and less time-consuming compared to standard multimer analysis, allowing for a quicker assessment of bleeding probability in patients with sAS before SAVR. A comprehensive data set with this information is provided in Table 3.

Nevertheless, it is noteworthy that these diagnostic markers are reduced only in patients of group B and normalize to levels observed in patients without pre-existing abnormal VWF multimers after correcting sAS through SAVR. In this context, it has been demonstrated that preoperatively reduced HMWM multimers are associated with an elevated transvalvular gradient in AS [37]. As to this, we were able to confirm a correlation between the severity of AS and the loss of HMWM, as described [37]. In our study, HMWM was negatively correlated with the Pmax. Furthermore, we could show an influence of aortic valve size on HMWM multimers: The AVA positively correlated with HMWM. Noteworthy, the authors of the above-described studies used a large multimer index, where the results of the VWF-ratio were normalized to values of a reference patient. In contrast, we correlated the loss of HMWM with the Pmax and the AVA. This has the advantage that the evaluation is less confusing, and results are more robust and comparable. Most importantly, analogous to the HMWM, it correlated with functional parameters in sAS, e.g., the Pmean. While it must be acknowledged that there are currently no reference values for the HMWM/(IMWM+LMWM)-ratio, it is worth mentioning that this is the subject of ongoing investigations [61].

In line with previous studies [4,11,56,62], our data suggests that Pmax is a major cause for HMWM loss and reduced VWF:Ac/VWF:Ag-ratio in patients with preoperatively detectable AVWS. Most importantly, our data, for the first time, reveal an association between Pmax and AVWS in patients undergoing SAVR, without a concurrent association with bleeding complications. In this regard, in case of postoperative bleeding complications, most hemostatic products are transfused early after surgery. Therefore, a potential influence of abnormal preoperative VWF multimers on early postoperative bleeding seems reasonable. Notably, while our report does not actually examine the multimer structure, we could show that the preoperative loss of HMWM in group B did not affect postoperative bleeding in the first 24 h after SAVR. This could be explained by the observed rapid postoperative increase of HMWM multimers and therefore normalization of VWF functionality, which is likely attributed to the reduction of shear stress by correcting the pathologically elevated Pmax by SAVR. [28,63,64] Our results are consistent with recent research on HMWM loss and blood loss after SAVR, suggesting no correlation between preoperative multimer pattern changes and cumulative blood loss within the initial 24 h [56,65]. Accordingly, we found no difference with respect to the amount of transfusion products in our work. On the other hand, other studies described that VWF abnormalities, e.g., development of an AVWS, are predictive of major bleedings after surgery, e.g. in SAVR patients and after systemic-to-pulmonary shunt implantation in infants, advocating the advantages of a more rapid assay for assessment of the perioperative

Table 2
Summary of data on intra- and postoperative outcome and adverse events in patients with normal (A) and abnormal (B) VWF multimer analysis. Values are expressed as mean ± SD, or as number and percentage (in brackets). For metric data, Mann-Whitney-U test was used. For categorial variables, Chi^2- or Fisher Exact Test was performed. Significant *p*-values are shown in bold italics. *Abbreviations: ACT: activated clotting time; CABG: coronary arterial bypass grafting, CPB, cardiopulmonary bypass, ECMO extracorporeal membrane oxygenation; CPR: Cardiopulmonary Resuscitation; FFP: fresh frozen plasma; ICU, intensive care unit; IMC: intermediate care unit; LVEF: Left ventricular ejection fraction; MIS: minimally invasive surgery; PC: platelet concentrates; postop: postoperative; PM: pacemaker; Pmax: peak aortic gradient; Pmean: mean aortic gradient; RBC: red blood cell concentrate; SAPS II, Simplified Acute Physiology Score II; TAA: tachyarrhythmia absoluta; TISS-10: Therapeutic Intervention Scoring System; Vmax: maximum jet velocity.*

	Total n = 52	Group A n = 28	Group B n = 24	p-Value
Intraoperative data				
MIS	25 (48.1 %)	11 (39.3 %)	14 (58.3 %)	0.266
Valve size in mm	22.7 ± 1.8	22.9 ± 1.9	22.7 ± 1.6	0.778
Time to skin closure	231.4 ± 28.9	228.7 ± 38.6	234.7 ± 39.8	0.586
CPB time in min	122.8 ± 24.5	120.4 ± 24.5	125.7 ± 24.7	0.438
Aortic X-clamp time in min	91.4 ± 18.2	89.9 ± 16.9	93.2 ± 19.9	0.524
ACT after antagonisation in s	122.7 ± 12.1	121.6 ± 11.4	123.9 ± 13.1	0.512
CPB fluid balance in ml	669.9 ± 938.1	596.1 ± 954.2	756.0 ± 931.8	0.438
Min. body temperature during CPB in °C	33.6 ± 1.6	33.9 ± 0.7	33.3 ± 2.3	0.220
Postoperative outcome				
Myocardial infarction	1 (1.9 %)	1 (3.6 %)	0 (0.0 %)	>0.999
Re-CPB necessary	2 (3.8 %)	2 (7.1 %)	0 (0.0 %)	0.493
CABG necessary	1 (1.9 %)	1 (3.6 %)	0 (0.0 %)	>0.999
ECMO	1 (1.9 %)	1 (3.6 %)	0 (0.0 %)	>0.999
Bleeding	8 (15.4 %)	4 (14.3 %)	4 (16.7 %)	>0.999
Re-thoracotomy	5 (9.6 %)	3 (10.7 %)	2 (8.3 %)	>0.999
pneumonia	8 (15.4 %)	6 (21.4 %)	2 (8.3 %)	0.262
Re-intubation	2 (3.8 %)	1 (3.6 %)	1 (4.2 %)	>0.999
TAA	28 (53.8 %)	12 (42.9 %)	16 (66.7 %)	0.102
PM implantation	1 (1.9 %)	1 (3.6 %)	0 (0.0 %)	>0.999
CPR	2 (3.8 %)	2 (7.1 %)	0 (0.0 %)	0.493
Stroke	4 (7.7 %)	2 (7.1 %)	2 (8.3 %)	>0.999
Delirium	13 (25.0 %)	9 (32.1 %)	4 (16.7 %)	0.336
Mortality				
30-day mortality	1 (1.9 %)	1 (3.6 %)	0 (0.0 %)	0.240
1-year mortality	3 (5.8 %)	3 (10.7 %)	0 (0.0 %)	0.240
wound infection	2 (3.8 %)	1 (3.6 %)	1 (4.2 %)	>0.999
Transfusion (therapeutic units)				
RBC intraoperative	1.0 ± 1.5	1.2 ± 1.6	0.9 ± 1.3	0.545
FFP intraoperative	0.4 ± 1.2	0.5 ± 1.2	0.4 ± 1.3	0.599
PC intraoperative	0.3 ± 0.8	0.3 ± 1.0	0.3 ± 0.7	0.361
RBC postoperative	0.9 ± 1.3	1.1 ± 1.4	0.7 ± 1.3	0.265
FFPs postoperative	1.1 ± 2.7	0.9 ± 1.6	0.7 ± 1.5	0.446
PC postoperative	0.2 ± 0.9	0.0 ± 0.0	0.1 ± 0.4	0.496
Ventilation time in min	22.2 ± 35.6	17.8 ± 10.1	17.0 ± 6.5	0.811
ICU time in h	67.3 ± 68.2	71.3 ± 77.7	54.8 ± 41.2	0.679
Length of hospital stay in d	15.6 ± 6.6	17.1 ± 7.7	14.2 ± 4.9	0.103
Postoperative scores				
TISS-10 24 h	16.2 ± 4.4	17.9 ± 4.1	16.4 ± 3.9	0.070
SAPS II 24 h	37.7 ± 7.1	34.9 ± 6.5	43.8 ± 3.5	0.414
Postoperative echocardiographic data				
Day 7				
Pmax (mmHg)	23.2 ± 6.4	21.8 ± 4.4	24.7 ± 8.1	0.871
Pmean (mmHg)	11.2 ± 5.0	11.1 ± 4.6	11.3 ± 5.5	0.894
Vmax (m/s)	2.6 ± 0.5	2.4 ± 0.3	2.7 ± 0.6	0.157
LVEF (%)	56.1 ± 10.8	53.2 ± 11.8	59.5 ± 8.6	***0.022***
3 months				
Pmax (mmHg)	22.3 ± 8.5	21.2 ± 5.3	22.3 ± 6.8	0.993
Pmean (mmHg)	11.2 ± 5.9	11.5 ± 5.2	11.0 ± 6.6	0.356
Vmax (m/s)	2.4 ± 0.5	2.4 ± 0.4	2.5 ± 0.6	0.837
LVEF (%)	60.6 ± 6.7	57.7 ± 6.3	64.0 ± 5.7	***0.003***

bleeding risk [66,67]. Notably, unlike in our study, shear stress from turbulent blood flow over the systemic-to-pulmonary shunt persists postoperatively.

Seven days after SAVR, we observed a significant increase in platelet counts in patients with preoperatively altered VWF multimers (group B). As this is not seen in patients with normal VWF multimers (group A) at that time, the observed increase of platelet counts does not seem to be exclusively related to the postoperative acute phase reaction but also due to less shear stress and hence less platelet activation and VWF unfolding. Thus, normalization of the VWF distribution pattern increases and corrects HMW VWF, leading to less clearance of platelets [68,69]. Platelet dysfunction is frequently observed in sAS patients [70], exacerbated not only by shear stress but also by the loss of HMWM in the VWF molecule [11]. On the other hand, platelet count increased 3 months after SAVR in both groups, which – along with comparable platelet counts before SAVR – indicates some long-term recovery in both groups and possibly also demonstrates the similarly low bleeding risk in our study. Taken together, the easy assessment of VWF multimers (e.g., HMWM) using the H5VWM assay and Sebia-analysis software and its correlation with echocardiographic parameters could serve as a valuable tool for the early identification and hemostyptic management of SAVR patients with possible AVWS. Although we did not observe excessive bleeding in AVWS patients, anticipating potential bleeding complications by early identification of AVWS might help to improve the primary VWF-dependent hemostatic function to reduce transfusion of blood components. This is important, although conflicting evidence exists regarding the relationship between altered VWF multimer structure and periprocedural bleeding: Bolliger et al. found no link between preoperative alterations in VWF multimer structure and increased bleeding after SAVR replacement, whereas Grodecki et al. reported that although VWF abnormalities are corrected after TAVR and SAVR, they predict major or life-threatening bleeding only in surgical patients [56,66]. While this data is in opposition with our findings, it is noteworthy that patients who underwent re-thoracotomy due to bleeding after any cardiac surgery had significantly higher unadjusted mortality at 30 and over 90 days, and that re-thoracotomy was linked to longer ICU stays, organ dysfunction and mechanical circulatory support [71]. However, the mentioned study did not examine pre-existing AVWS.

With respect to perioperative coagulation therapy after aortic valve replacement, an older RCT showed that tranexamic acid reduces postoperative bleeding and transfusion needs after SAVR [72]. A meta-analysis identified thrombocytopenia-related adverse events primarily in TAVR compared to SAVR patients, while this could be, at least in part, due to a more stringent pro-hemostatic therapy, such as platelet transfusion, after SAVR compared to TAVR [73]. However, an observational study revealed a more pro-thrombotic state in TAVR patients than SAVR

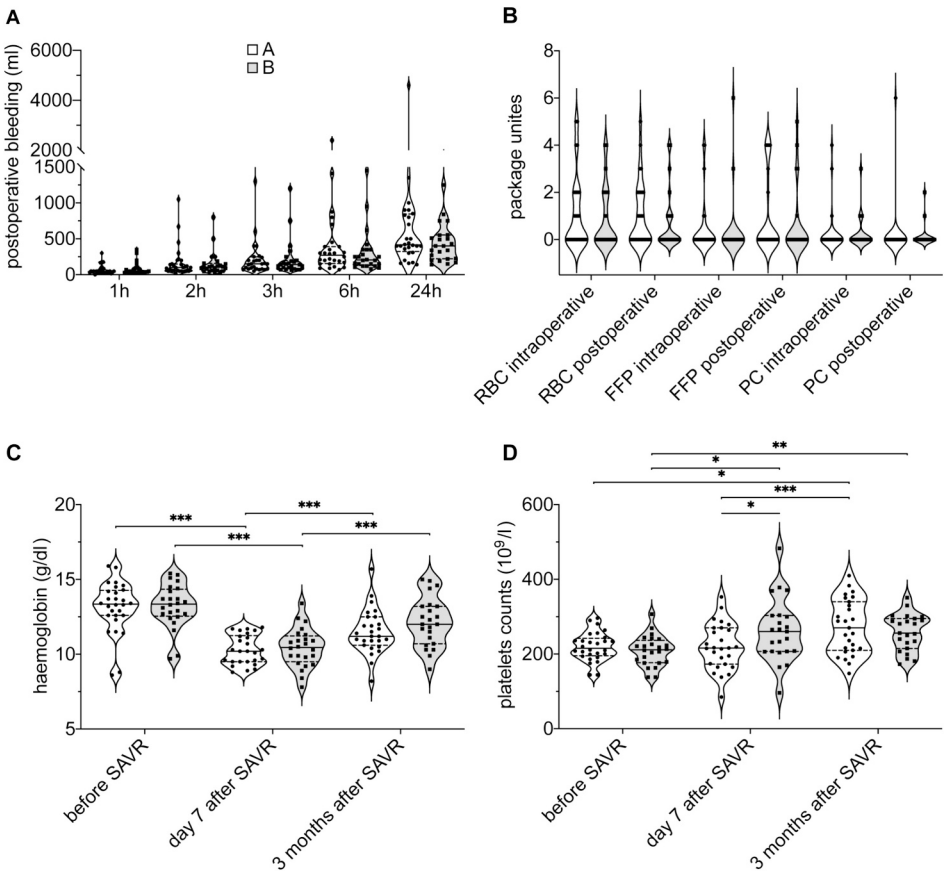

Fig. 5. Violine plots show the median (line), first and third quartiles (dotted lines) and individual values as well as their distribution. Postoperative bleeding amount in the first 24 h (A), intra- and postoperative amount of transfused package unites of red blood cells (RBC), fresh frozen plasma (FFP) and platelet concentrates (PC) (B), relative changes of the hemoglobin (C) and the platelets counts (D) levels before, on day seven after and three months after surgery. $p < 0.05$ was considered statistically significant (*). **, $p < 0.01$; ***, $p < 0.001$. *Abbreviations:* SAVR: surgical aortic valve replacement. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and PCI cohorts [74]. Interestingly, one randomized controlled trial found that prolonged collagen adenosine diphosphate closure time (CADP-CT) indicates platelet dysfunction in sAS and guides effective desmopressin use [75]. In the light of patients with AVWS undergoing SAVR, it could be shown that administration of desmopressin reduced the risk of bleeding and the perioperative need of transfusion [76]. This was demonstrated in patients undergoing cardiac surgery who are at high risk of excessive bleeding [76]. Specifically, patients with sAS and impaired platelet function benefit from preoperative desmopressin infusion [75]. Besides, Desmopressin and FVIII/VWF concentrate temporarily improved bleeding time and factor levels in AVWS patients undergoing surgeries like dental extractions and abdominal procedures [77]. The main effects of desmopressin lie in the substantial stimulation of the endothelium, leading to the release of VWF into the blood plasma. This results in the release of intact HMW-VWF into circulation, enhancing the adhesion of intact platelets [78]. This could therefore help avoid excessive use of other coagulation and transfusion products, along with the associated risks. Of note, the use of Desmopressin in cardiovascular surgery has several restrictions, e.g., volume restriction and hyponatremia, which can lead to increased risk of heart failure due to fluid overload and neurological problems such as seizures or brain swelling due to severely low sodium levels [79]. Hence, in our opinion, a risk-benefit calculation should be made before administering Desmopressin to patients after SAVR.

Of course, other factors not associated with AVWS also contribute to postoperative bleeding after SAVR, such as cytokine-triggered post-CPB platelet-leukocyte crosstalk leading to platelet activation or exhaustion [80]. Otherwise, platelets seem not to be sufficiently pro-aggregatory to increase thrombotic events due to the release of pro-inflammatory substances from platelets during CPB supporting postoperative inflammation [7]. The authors of the latter study hypothesized that this likely results from effective hemostasis interventions and the use of protamine to reverse anticoagulation post-CPB, preventing pro-aggregatory but not pro-inflammatory platelet functions. However, we could nor demonstrate any differences with respect to postoperative bleeding or platelet transfusion in our study. Furthermore, since the above-described conditions apply to both groups in our study, it is unlikely that any of these effects – especially with the same platelet count in both groups – specifically impact group B.

Moreover, the correlation between VWF multimers and the aortic valve gradients and AVA may help identify patients at risk for AS. For instance, in cases where echocardiographic findings are not sufficiently clear, the decrease of HMWM proportion of the VWF multimer pattern could strengthen the suspicion of valve degeneration. These patients would benefit from early presentation to a heart center as well as to a hemostasis specialist to evaluate an intervention and anticipation of bleeding complications in a team approach. Therefore, we advocate the analysis of the VWF multimers (e.g., HMWM) and its correlation with echocardiographic parameters as a sophisticated tool system for early detection and confirmation of sAS. Furthermore, shear forces are also important in paravalvular leakage. In this regard, a study focusing on mild postoperative paravalvular regurgitation showed that nearly a third of TAVR patients had impaired VWF functionality, which was not observed in SAVR patients [81]. Van Belle et al. showed that the presence of HMWM defects is predictive of the presence of paravalvular aortic regurgitation after TAVR and was even associated with higher

Table 3
Laboratory assays and their requirements for diagnosing AVWS before SAVR. Patients with known AVWS-associated disorder (like sAS) are suggested to undergo testing before surgery. Diagnosing of AVWS is challenging and clinical features (e.g. personal and family history of bleeding) as well as further laboratory results (e.g. PFA test, FVIII activity, platelet count) must be considered. *Abbreviations: HMWM: high molecular weight multimers; IMWM: intermediate molecular weight multimers; LMWM: low molecular weight multimers; Pmean: mean aortic gradient; sAS: severe aortic stenosis; VWF:Ac: Von Willebrand factor activity; VWF:Ag: Von Willebrand factor antigen; VWS: Von Willebrand Syndrome.*

Method	Device requirements	Costs	Handling / Time	Availability	Sensitivity / standardization	Impact of the methods for AVWS detection in SAVR
VWF:Ac/VWFAg ratio (VWF ratio)	- applicable to various laboratory equipment	→/↓ - compared to Sebia-method (reagents and staff)	*Easy* / ↓ - no special training for medical technologists required	↑ - widely available in most laboratories investigating hemostasis	↓ / ↓ [35] - high variability: results strongly depend on the VWF activity assay and VWF ratio used [82,83]	1. Determining VWF ratio (e.g. VWF:Ac/VWF:Ag) and multimer analyses are not competing assays, and usually applied as a two-stage procedure for diagnosing AVWS [84]. This might increase the sensitivity of diagnosing AVWS in patients with sAS.
HMWM/ (IMWM+LMWM) ratio (Sebia) [47]	- hard- and software restricted to manufacturer Sebia (Hydrasys 2 Scan using Phoresis software)	→/↑ - compared to VWF ratio (reagents and staff)	Moderate / ↓↓ - personnel must be trained for H5VWM. - once established, its results are available within hours	↓ - restricted to specialized laboratories	↑ / ↑↑ - low variability - well standardized assay - inability to detect the highest MWM - lack of resolution to visualize VWF multimer triplet	2. VWF ratio and H5VWM based HMWM/ (IMWM+LMWM) ratio negatively correlates with the Pmean (Fig. 4C, D).
Standard multimer analysis	- various electrophoresis and blot transfer methods including VWF multimer visualization [85–87] - in house method	↑ - compared to VWF ratio (reagents and staff)	Difficult / ↑↑ - specially trained staff with sufficient expert knowledge necessary - time-consuming	↓↓ - restricted to specialized laboratories with high expert level needed - not standardized	↑↑ / ↑↑ - highest sensitivity in diagnosing AVWS - diagnostic gold standard	3. Due to the strong correlation with transvalvular gradients, the ratios can be useful for the identification of patients with sAS and/or predicting patient/ prosthesis mismatch after SAVR.

mortality one year after the procedure [64]. We are therefore convinced that diagnosing of AVWS should be implemented in all patients before and after TAVR and SAVR to address possible development of post-operative Heydes' syndrome with all potential clinical risks. The key findings of or work are summarized in the graphical abstract (Fig. 6).

5. Conclusion

In summary, diagnosing AVWS preoperatively in routine clinical practice is challenging due to the absence of standardized diagnostic algorithms, and the standard diagnostic process can be time-consuming. However, we found that patients with abnormal VWF multimer analysis did not exhibit an increased bleeding tendency after SAVR. Loss of HMWM before surgery disappeared within 7d after SAVR. We showed a strong and positive correlation between HMWMM and AVA, and negative correlation between HMWM and Pmax as well as HMWM/ (IMWM+LMWM)-ratio and Pmean, suggesting that patients with abnormal echocardiographic parameters should undergo VWF analysis including the examination of VWF multimer pattern to detect AVWS in clinical practice. Additionally, for patients with sAS, early detection of valve degeneration can be facilitated. Finally, the H5VWM assay offers a simple and rapid alternative to the time-consuming standard VWF multimer analysis and could therefore become a sophisticated new tool in the future for pre- and postoperative AVWS diagnostics.

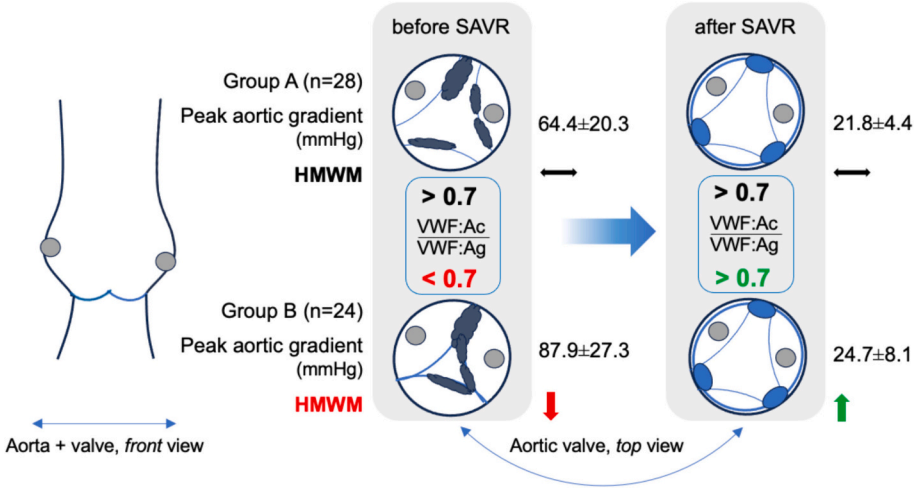

Fig. 6. Graphical abstract, summarizing the key findings of our work. *Abbreviations: HMWM: high-molecular-weight multimers; VWF: von Willebrand factor; VWF:Ag: von Willebrand factor antigen; SAVR: surgical aortic valve replacement.*

CRediT authorship contribution statement

Marwan Hamiko: Writing – review & editing, Validation, Software, Resources, Investigation, Formal analysis, Data curation. **Lena Gerdes:** Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Miriam Silaschi:** Writing – review & editing, Visualization, Validation, Software, Resources. **Holger Seidel:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Philipp Westhofen:** Formal analysis, Data curation. **Johannes Kruppenbacher:** Formal analysis, Data curation. **Hans-Joerg Hertfelder:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Johannes Oldenburg:** Writing – review & editing, Validation, Supervision. **Farhad Bakhtiary:** Writing – review & editing, Conceptualization. **Markus Velten:** Writing – review & editing, Visualization, Resources. **Mehmet Oezkur:** Writing – review & editing, Validation, Methodology. **Georg Daniel Duerr:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

There is no conflict of interest to declare.

Acknowledgement

Preliminary results of the study have been presented as poster abstract at the Scientific Sessions 2021 of the American Heart Association (DOI: https://doi.org/10.1055/s-0042-1742798). The authors received no funding for research, authorship, and/or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2024.109094.

References

[1] Joint Task Force on the Management of Valvular Heart Disease of the European Society of C, European Association for Cardio-Thoracic S, Vahanian A, Alfieri O, Andreotti F, Antunes MJ, Baron-Esquivias G, Baumgartner H, Borger MA, Carrel TP, De Bonis M, Evangelista A, Falk V, Iung B, Lancellotti P, Pierard L, Price S, Schafers HJ, Schuler G, Stepinska J, Swedberg K, Takkenberg J, Von Oppell UO, Windecker S, Zamorano JL and Zembala M. Guidelines on the management of valvular heart disease (version 2012). Eur. Heart J. 2012;33:2451–96.

[2] D.H. Adams, J.J. Popma, M.J. Reardon, S.J. Yakubov, J.S. Coselli, G.M. Deeb, T. G. Gleason, M. Buchbinder, J. Hermiller Jr., N.S. Kleiman, S. Chetcuti, J. Heiser, W. Merhi, G. Zorn, P. Tadros, N. Robinson, G. Petrossian, G.C. Hughes, J. K. Harrison, J. Conte, B. Maini, M. Mumtaz, S. Chenoweth, J.K. Oh, U.S.C. C. Investigators, Transcatheter aortic-valve replacement with a self-expanding prosthesis, N. Engl. J. Med. 370 (2014) 1790–1798.

[3] M. Weinstein, J.A. Ware, J. Troll, E. Salzman, Changes in von Willebrand factor during cardiac surgery: effect of desmopressin acetate, Blood 71 (1988) 1648–1655.

[4] A. Vincentelli, S. Susen, T. Le Tourneau, I. Six, O. Fabre, F. Juthier, A. Bauters, C. Decoene, J. Goudemand, A. Prat, B. Jude, Acquired von Willebrand syndrome in aortic stenosis, N. Engl. J. Med. 349 (2003) 343–349.

[5] C. Solomon, U. Budde, S. Schneppenheim, E. Czaja, C. Hagl, H. Schoechl, M. von Depka, N. Rahe-Meyer, Acquired type 2A von Willebrand syndrome caused by aortic valve disease corrects during valve surgery, Br. J. Anaesth. 106 (2011) 494–500.

[6] A. Casonato, S. Sponga, E. Pontara, M.G. Cattini, C. Basso, G. Thiene, G. Cella, V. Daidone, G. Gerosa, A. Pagnan, von Willebrand factor abnormalities in aortic valve stenosis: pathophysiology and impact on bleeding, Thromb. Haemost. 106 (2011) 58–66.

[7] E. Hosseini, J. Ahmadi, F. Kargar, M. Ghasemzadeh, Coronary artery bypass grafting (CABG) induces pro-inflammatory and immunomodulatory phenotype of platelets in the absence of a pro-aggregatory state, Microvasc. Res. 153 (2024) 104669.

[8] S. Shiozaki, S. Takagi, S. Goto, Prediction of molecular interaction between platelet glycoprotein Ibalpha and von Willebrand factor using molecular dynamics simulations, J. Atheroscler. Thromb. 23 (2016) 455–464.

[9] J.C. Gill, A.D. Wilson, J. Endres-Brooks, R.R. Montgomery, Loss of the largest von Willebrand factor multimers from the plasma of patients with congenital cardiac defects, Blood 67 (1986) 758–761.

[10] W.L. Nichols, M.B. Hultin, A.H. James, M.J. Manco-Johnson, R.R. Montgomery, T. L. Ortel, M.E. Rick, J.E. Sadler, M. Weinstein, B.P. Yawn, von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) expert panel report (USA), Haemophilia 14 (2008) 171–232.

[11] S. Panzer, R. Badr Eslam, A. Schneller, A. Kaider, D. Koren, B. Eichelberger, R. Rosenhek, U. Budde, I.M. Lang, Loss of high-molecular-weight von Willebrand factor multimers mainly affects platelet aggregation in patients with aortic stenosis, Thromb. Haemost. 103 (2010) 408–414.

[12] M. Sugimoto, H. Matsui, T. Mizuno, S. Tsuji, S. Miyata, M. Matsumoto, M. Matsuda, Y. Fujimura, A. Yoshioka, Mural thrombus generation in type 2A and 2B von Willebrand disease under flow conditions, Blood 101 (2003) 915–920.

[13] W. Gola, M. Lelonek, Clinical implication of gastrointestinal bleeding in degenerative aortic stenosis: an update, Cardiol. J. 17 (2010) 330–334.

[14] Loscalzo J. From clinical observation to mechanism–Heyde's syndrome. N. Engl. J. Med. 2012;367:1954–6.

[15] J. Natorska, P. Mazur, A. Undas, Increased bleeding risk in patients with aortic valvular stenosis: from new mechanisms to new therapies, Thromb. Res. 139 (2016) 85–89.

[16] F. Loeffelbein, D. Funk, L. Nakamura, B. Zieger, J. Grohmann, M. Siepe, J. Kroll, B. Stiller, Shear-stress induced acquired von Willebrand syndrome in children with congenital heart disease, Interact. Cardiovasc. Thorac. Surg. 19 (2014) 926–932.

[17] X. Zheng, D. Chung, T.K. Takayama, E.M. Majerus, J.E. Sadler, K. Fujikawa, Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura, J. Biol. Chem. 276 (2001) 41059–41063.

[18] J.A. Dent, S.D. Berkowitz, J. Ware, C.K. Kasper, Z.M. Ruggeri, Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIA von Willebrand factor, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 6306–6310.

[19] M. Furlan, R. Robles, M. Solenthaler, M. Wassmer, P. Sandoz, B. Lammle, Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura, Blood 89 (1997) 3097–3103.

[20] S.E. Lyons, M.E. Bruck, E.J. Bowie, D. Ginsburg, Impaired intracellular transport produced by a subset of type IIA von Willebrand disease mutations, J. Biol. Chem. 267 (1992) 4424–4430.

[21] J.E. Sadler, Aortic stenosis, von Willebrand factor, and bleeding, N. Engl. J. Med. 349 (2003) 323–325.

[22] H.M. Tsai, I.I. Sussman, R.L. Nagel, Shear stress enhances the proteolysis of von Willebrand factor in normal plasma, Blood 83 (1994) 2171–2179.

[23] M. Yashige, K. Inoue, K. Zen, R. Kobayashi, S. Nakamura, T. Fujimoto, K. Takamatsu, S. Sugino, M. Yamano, T. Yamano, T. Nakamura, T. Doman, S. Teramukai, H. Horiuchi, Y. Itoh, S. Matoba, Gastrointestinal Angiodysplasia before and after treatment of severe aortic stenosis, N. Engl. J. Med. 389 (2023) 1530–1532.

[24] L. Goltstein, M.J.P. Rooijakkers, N.C.C. Gortjes, R.P. Akkermans, E.S. Zegers, R. Pisters, M.H. van Wely, K. van der Wulp, J.P.H. Drenth, E.J.M. van Geenen, N. van Royen, Reduction of gastrointestinal bleeding in patients with Heyde syndrome undergoing Transcatheter aortic valve implantation, Circ. Cardiovasc. Interv. 15 (2022) e011848.

[25] R. Abi-Akar, I. El-Rassi, N. Karam, Y. Jassar, R. Slim, V. Jebara, Treatment of Heyde's syndrome by aortic valve replacement, Curr. Cardiol. Rev. 7 (2011) 47–49.

[26] R.D. Frank, R. Lanzmich, P.K. Haager, U. Budde, Severe aortic valve stenosis, Clin. Appl. Thromb. Hemost. 23 (2017) 229–234.

[27] J. Bander, S. Elmariah, L.M. Aledort, J. Dlott, P. Stelzer, J.L. Halperin, A.S. Kini, S. K. Sharma, Changes in von Willebrand factor-cleaving protease (ADAMTS-13) in patients with aortic stenosis undergoing valve replacement or balloon valvuloplasty, Thromb. Haemost. 108 (2012) 86–93.

[28] T. Spangenberg, U. Budde, D. Schewel, C. Frerker, T. Thielsen, K.H. Kuck, U. Schafer, Treatment of acquired von Willebrand syndrome in aortic stenosis with transcatheter aortic valve replacement, JACC Cardiovasc. Interv. 8 (2015) 692–700.

[29] A. Vivacqua, C.G. Koch, A.M. Yousuf, E.R. Nowicki, P.L. Houghtaling, E. H. Blackstone, J.F. Sabik 3rd., Morbidity of bleeding after cardiac surgery: is it blood transfusion, reoperation for bleeding, or both? Ann. Thorac. Surg. 91 (2011) 1780–1790.

[30] B.H. Scott, F.C. Seifert, R. Grimson, Blood transfusion is associated with increased resource utilisation, morbidity and mortality in cardiac surgery, Ann. Card. Anaesth. 11 (2008) 15–19.

[31] G.J. Murphy, B.C. Reeves, C.A. Rogers, S.I. Rizvi, L. Culliford, G.D. Angelini, Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery, Circulation 116 (2007) 2544–2552.

[32] V.A. Ferraris, M. Hochstetler, J.T. Martin, A. Mahan, S.P. Saha, Blood transfusion and adverse surgical outcomes: the good and the bad, Surgery 158 (2015) 608–617.

[33] K. Karkouti, D.N. Wijeysundera, T.M. Yau, W.S. Beattie, E. Abdelnaem, S. A. McCluskey, M. Ghannam, E. Yeo, G. Djaiani, J. Karski, The independent association of massive blood loss with mortality in cardiac surgery, Transfusion 44 (2004) 1453–1462.

[34] U. Guenther, N. Theuerkauf, I. Frommann, K. Brimmers, R. Malik, S. Stori, M. Scheidemann, C. Putensen, J. Popp, Predisposing and precipitating factors of

delirium after cardiac surgery: a prospective observational cohort study, Ann. Surg. 257 (2013) 1160–1167.
[35] V. Icheva, M. Nowak-Machen, U. Budde, K. Jaschonek, F. Neunhoeffer, M. Kumpf, M. Hofbeck, C. Schlensak, G. Wiegand, Acquired von Willebrand syndrome in congenital heart disease surgery: results from an observational case-series, J. Thromb. Haemost. 16 (2018) 2150–2158.
[36] M. Stockschlaeder, R. Schneppenheim, U. Budde, Update on von Willebrand factor multimers: focus on high-molecular-weight multimers and their role in hemostasis, Blood Coagul. Fibrinolysis 25 (2014) 206–216.
[37] A. Sedaghat, H. Kulka, J.M. Sinning, N. Falkenberg, J. Driesen, B. Preisler, C. Hammerstingl, G. Nickenig, B. Potzsch, J. Oldenburg, H.J. Hertfelder, N. Werner, Transcatheter aortic valve implantation leads to a restoration of von Willebrand factor (VWF) abnormalities in patients with severe aortic stenosis - incidence and relevance of clinical and subclinical VWF dysfunction in patients undergoing transfemoral TAVI, Thromb. Res. 151 (2017) 23–28.
[38] N.T. Connell, V.H. Flood, R. Brignardello-Petersen, R. Abdul-Kadir, A. Arapshian, S. Couper, J.M. Grow, P. Kouides, M. Laffan, M. Lavin, F.W.G. Leebeek, S. H. O'Brien, M.C. Ozelo, A. Tosetto, A.C. Weyand, P.D. James, M.A. Kalot, N. Husainat, R.A. Mustafa, ASH ISTH NHF WFH 2021 guidelines on the management of von Willebrand disease, Blood Adv. 5 (2021) 301–325.
[39] Bodo I, Eikenboom J, Montgomery R, Patzke J, Schneppenheim R, Di Paola J, von Willebrand factor Subcommittee of the S, Scientific Committee of the International Society for T and Haemostasis. Platelet-dependent von Willebrand factor activity. Nomenclature and methodology: communication from the SSC of the ISTH, J. Thromb. Haemost. 13 (2015) 1345–1350.
[40] Z.M. Ruggeri, T.S. Zimmerman, von Willebrand factor and von Willebrand disease, Blood 70 (1987) 895–904.
[41] A.E. Bowyer, K.J. Goodfellow, H. Seidel, P. Westhofen, F. Stufano, A. Goodeve, S. Kitchen, M. Makris, Evaluation of a semi-automated von Willebrand factor multimer assay, the Hydragel 5 von Willebrand multimer, by two European centers, Res Pract Thromb Haemost. 2 (2018) 790–799.
[42] E.J. Favaloro, S. Oliver, Evaluation of a new commercial von Willebrand factor multimer assay, Haemophilia 23 (2017) e373–e377.
[43] M. Pikta, G. Zemtsovskaja, H. Bautista, G. Nouadje, T. Szanto, M. Viigimaa, V. Banys, Preclinical evaluation of a semi-automated and rapid commercial electrophoresis assay for von Willebrand factor multimers, J. Clin. Lab. Anal. 32 (2018) e22416.
[44] R.A. Crist, N.M. Heikal, G.M. Rodgers, D.G. Grenache, K.J. Smock, Evaluation of a new commercial method for von Willebrand factor multimeric analysis, Int. J. Lab. Hematol. 40 (2018) 586–591.
[45] A. Vuylsteke, C. Pagel, C. Gerrard, B. Reddy, S. Nashef, P. Aldam, M. Utley, The Papworth bleeding risk score: a stratification scheme for identifying cardiac surgery patients at risk of excessive early postoperative bleeding, Eur. J. Cardiothorac. Surg. 39 (2011) 924–930.
[46] E.A. Vlot, L.M. Vernooij, S.A. Loer, E.P.A. van Dongen, P.G. Noordzij, External validation of the ACTA-PORT transfusion risk score in older cardiac surgery patients at risk of frailty, J. Cardiothorac. Vasc. Anesth. 36 (2022) 2983–2990.
[47] S. Oliver, K.K.E. Lau, K. Chapman, E.J. Favaloro, Laboratory testing for Von Willebrand factor Multimers, Methods Mol. Biol. 1646 (2017) 495–511.
[48] H.M. Tsai, Shear stress and von Willebrand factor in health and disease, Semin. Thromb. Hemost. 29 (2003) 479–488.
[49] N. Roth, C. Heidel, C. Xu, U. Hubauer, S. Wallner, C. Meindl, A. Holzamer, M. Hilker, M. Creutzenberg, S. Sossalla, L. Maier, C. Jungbauer, K. Debl, The impact of bicuspid aortic valve morphology on von Willebrand factor function in patients with severe aortic stenosis and its change after TAVI, Clin. Res. Cardiol. 111 (2022) 1348–1357.
[50] C. Egger, J. Maas, T. Hufen, T. Schmitz-Rode, U. Steinseifer, Establishing a method for in vitro investigation of mechanical parameters causing acquired von Willebrand syndrome in ventricular assist devices, Artif. Organs 37 (2013) 833–839.
[51] H. Horiuchi, A hemostatic disorder caused by high shear stress: acquired von Willebrand syndrome, Rinsho Ketsueki 59 (2018) 2233–2237.
[52] S.S. Varghese, M.F. Hetu, M. Bowman, J. Herr, M. Al-Turki, Z. Jaff, P. James, P. Malik, D. Payne, A.M. Johri, Impact of transcatheter aortic valve implantation on circulating von Willebrand factor in patients with severe aortic stenosis, Haemophilia 29 (2023) 1306–1312.
[53] J.L. Blackshear, E.M. Wysokinska, R.E. Safford, C.S. Thomas, M.E. Stark, B. P. Shapiro, S. Ung, G.S. Johns, D. Chen, Indexes of von Willebrand factor as biomarkers of aortic stenosis severity (from the biomarkers of aortic stenosis severity [BASS] study), Am. J. Cardiol. 111 (2013) 374–381.
[54] O. Marggraf, S. Schneppenheim, A. Daubmann, U. Budde, M. Seiffert, H. Reichenspurner, H. Treede, S. Blankenberg, P. Diemert, Correction of acquired von Willebrand syndrome by transcatheter aortic valve implantation, J. Invasive Cardiol. 26 (2014) 654–658.
[55] M.M. Mikkelsen, C. Fenger-Eriksen, S.P. Johnsen, T.D. Christensen, B. Sorensen, No excess surgical blood loss in patients with acquired type 2A von Willebrand disease, Scand. Cardiovasc. J. 45 (2011) 120–126.
[56] D. Bolliger, S. Dell-Kuster, M.D. Seeberger, K.A. Tanaka, M. Gregor, U. Zenklusen, D.A. Tsakiris, M. Filipovic, Impact of loss of high-molecular-weight von Willebrand factor multimers on blood loss after aortic valve replacement, Br. J. Anaesth. 108 (2012) 754–762.
[57] C. Velik-Salchner, T. Haas, P. Innerhofer, W. Streif, W. Nussbaumer, A. Klingler, G. Klima, U. Martinowitz, D. Fries, The effect of fibrinogen concentrate on thrombocytopenia, J. Thromb. Haemost. 5 (2007) 1019–1025.
[58] C. Velik-Salchner, S. Eschertzhuber, W. Streif, H. Hangler, U. Budde, D. Fries, Acquired von Willebrand syndrome in cardiac patients, J. Cardiothorac. Vasc. Anesth. 22 (2008) 719–724.
[59] C. Velik-Salchner, S. Maier, P. Innerhofer, C. Kolbitsch, W. Streif, M. Mittermayr, M. Praxmarer, D. Fries, An assessment of cardiopulmonary bypass-induced changes in platelet function using whole blood and classical light transmission aggregometry: the results of a pilot study, Anesth. Analg. 108 (2009) 1747–1754.
[60] U. Budde, A. Pieconka, K. Will, R. Schneppenheim, Laboratory testing for von Willebrand disease: contribution of multimer analysis to diagnosis and classification, Semin. Thromb. Hemost. 32 (2006) 514–521.
[61] M. Pikta, M. Vasse, K.J. Smock, K.A. Moser, van DM, Lejniece S, Szanto T, Bautista H, Nouadje G and Banys V., Establishing reference intervals for von Willebrand factor multimers, J Med Biochem. 41 (2022) 115–121.
[62] J. Kellermair, H.W. Ott, H. Baumgartner, D. Kiblboeck, H. Blessberger, J. Kammler, C. Reiter, T. Lambert, M. Grund, C. Steinwender, High-molecular-weight von Willebrand factor Multimer ratio: a novel biomarker for low-flow, low-gradient aortic stenosis subclassification, J. Am. Coll. Cardiol. 72 (2018) 1982–1984.
[63] E. Van Belle, A. Rauch, A. Vincentelli, E. Jeanpierre, P. Legendre, F. Juthier, C. Hurt, C. Banfi, N. Rousse, A. Godier, C. Caron, A. Elkalioubie, D. Corseaux, A. Dupont, C. Zawadzki, C. Delhaye, F. Mouquet, G. Schurtz, D. Deplanque, G. Chinetti, B. Staels, J. Goudemand, B. Jude, P.J. Lenting, S. Susen, Von Willebrand factor as a biological sensor of blood flow to monitor percutaneous aortic valve interventions, Circ. Res. 116 (2015) 1193–1201.
[64] E. Van Belle, A. Rauch, F. Vincent, E. Robin, M. Kibler, J. Labreuche, E. Jeanpierre, M. Levade, C. Hurt, N. Rousse, J.B. Dally, N. Debry, J. Dallongeville, A. Vincentelli, C. Delhaye, J.L. Auffray, F. Juthier, G. Schurtz, G. Lemesle, T. Caspar, O. Morel, N. Dumonteil, A. Duhamel, C. Paris, A. Dupont-Prado, P. Legendre, F. Mouquet, B. Marchant, S. Hermoire, D. Corseaux, K. Moussa, A. Manchuelle, J.J. Bauchart, V. Loobuyck, C. Caron, C. Zawadzki, F. Leroy, J.C. Bodart, B. Staels, J. Goudemand, P.J. Lenting, S. Susen, Von Willebrand factor Multimers during Transcatheter aortic-valve replacement, N. Engl. J. Med. 375 (2016) 335–344.
[65] C. Sucker, P. Feindt, R.B. Zotz, J. Litmathe, No evidence for increased intraoperative bleeding in aortic-valve stenosis: a comparative analysis of haemotherapy in 136 patients undergoing aortic-valve replacement, Acta Cardiol. 65 (2010) 675–679.
[66] K. Grodecki, K. Zbronski, E. Przybyszewska-Kazulak, A. Olasinska-Wisniewska, R. Wilimski, B. Rymuza, P. Scislo, P. Czub, D. Koper, J. Kochman, K. Pawlak, O. Ciepiela, M. Grygier, M. Jemielity, M. Lesiak, K.J. Filipiak, G. Opolski, Z. Huczek, Pre-procedural abnormal function of von Willebrand factor is predictive of bleeding after surgical but not transcatheter aortic valve replacement, J. Thromb. Thrombolysis 48 (2019) 610–618.
[67] V. Icheva, U. Budde, H. Magunia, K. Jaschonek, C. Hinterleitner, F. Neunhoeffer, C. Schlensak, M. Hofbeck, G. Wiegand, Acquired von Willebrand syndrome is common in infants with systemic-to-pulmonary shunts: retrospective case-series, Front. Pediatr. 10 (2022) 1040128.
[68] B.H. Foy, T.M. Sundt, J.C.T. Carlson, A.D. Aguirre, J.M. Higgins, Human acute inflammatory recovery is defined by co-regulatory dynamics of white blood cell and platelet populations, Nat. Commun. 13 (2022) 4705.
[69] J. Natorska, K. Bykowska, M. Hlawaty, G. Marek, J. Sadowski, A. Undas, Increased thrombin generation and platelet activation are associated with deficiency in high molecular weight multimers of von Willebrand factor in patients with moderate-to-severe aortic stenosis, Heart 97 (2011) 2023–2028.
[70] J.R. O'Brien, M.D. Etherington, J. Brant, J. Watkins, Decreased platelet function in aortic valve stenosis: high shear platelet activation then inactivation, Br. Heart J. 74 (1995) 641–644.
[71] V. Frojd, A. Jeppsson, Reexploration for bleeding and its association with mortality after cardiac surgery, Ann. Thorac. Surg. 102 (2016) 109–117.
[72] G. Armellin, S. Casella, S. Guzzinati, L. Pasini, A. Marcassa, G. Giron, Tranexamic acid in aortic valve replacement, J. Cardiothorac. Vasc. Anesth. 15 (2001) 331–335.
[73] F. Jiritano, G. Santarpino, G.F. Serraino, H. Ten Cate, M. Matteucci, D. Fina, P. Mastroroberto, R. Lorusso, Peri-procedural thrombocytopenia after aortic bioprosthesis implant: a systematic review and meta-analysis comparison among conventional, stentless, rapid-deployment, and transcatheter valves, Int. J. Cardiol. 296 (2019) 43–50.
[74] J.P. Fanning, S. Roberts, C. Anstey, S. Yerkovich, L.Y. Lu, K. Poon, A. Incani, S. Natani, J. McCullough, J. Winearls, J.F. Fraser, Hemostatic profiles of patients who underwent Transcatheter versus surgical aortic valve replacement versus percutaneous coronary intervention, Am. J. Cardiol. 207 (2023) 260–270.
[75] B. Steinlechner, P. Zeidler, E. Base, B. Birkenberg, H.J. Ankersmit, M. Spannagl, P. Quehenberger, M. Hiesmayr, B. Jilma, Patients with severe aortic valve stenosis and impaired platelet function benefit from preoperative desmopressin infusion, Ann. Thorac. Surg. 91 (2011) 1420–1426.
[76] G.J. Despotis, V. Levine, R. Saleem, E. Spitznagel, J.H. Joist, Use of point-of-care test in identification of patients who can benefit from desmopressin during cardiac surgery: a randomised controlled trial, Lancet 354 (1999) 106–110.
[77] A.B. Federici, F. Stabile, G. Castaman, M.T. Canciani, P.M. Mannucci, Treatment of acquired von Willebrand syndrome in patients with monoclonal gammopathy of uncertain significance: comparison of three different therapeutic approaches, Blood 92 (1998) 2707–2711.
[78] S. Lethagen, Desmopressin (DDAVP) and hemostasis, Ann. Hematol. 69 (1994) 173–180.
[79] P.D. Mongan, M.P. Hosking, The role of desmopressin acetate in patients undergoing coronary artery bypass surgery. A controlled clinical trial with thromboelastographic risk stratification, Anesthesiology 77 (1992) 38–46.

[80] M. Ghasemzadeh, J. Ahmadi, E. Hosseini, Platelet-leukocyte crosstalk in COVID-19: how might the reciprocal links between thrombotic events and inflammatory state affect treatment strategies and disease prognosis? Thromb. Res. 213 (2022) 179–194.
[81] F. Pawlitschek, C. Keyl, B. Zieger, U. Budde, F. Beyersdorf, F.J. Neumann, C. Stratz, T.G. Nuhrenberg, D. Trenk, Alteration of von Willebrand factor after Transcatheter aortic valve replacement in the absence of paravalvular regurgitation, Thromb. Haemost. 118 (2018) 103–111.
[82] E.J. Favaloro, Collagen binding assay for von Willebrand factor (VWF:CBA): detection of von Willebrands disease (VWD), and discrimination of VWD subtypes, depends on collagen source, Thromb. Haemost. 83 (2000) 127–135.
[83] R. Paczuski, Determination of von Willebrand factor activity with collagen-binding assay and diagnosis of von Willebrand disease: effect of collagen source and coating conditions, J. Lab. Clin. Med. 140 (2002) 250–254.
[84] James PD, Connell NT, Ameer B, Di Paola J, Eikenboom J, Giraud N, Haberichter S, Jacobs-Pratt V, Konkle B, McLintock C, McRae S, R RM, O'Donnell JS, Scappe N, Sidonio R, Flood VH, Husainat N, Kalot MA and Mustafa RA. ASH ISTH NHF WFH 2021 Guidelines on the diagnosis of von Willebrand disease. Blood Adv. 2021;5: 280–300.
[85] D.R. Krizek, M.E. Rick, A rapid method to visualize von willebrand factor multimers by using agarose gel electrophoresis, immunolocalization and luminographic detection, Thromb. Res. 97 (2000) 457–462.
[86] H.W. Ott, A. Griesmacher, M. Schnapka-Koepf, G. Golderer, A. Sieberer, M. Spannagl, B. Scheibe, S. Perkhofer, K. Will, U. Budde, Analysis of von Willebrand factor multimers by simultaneous high- and low-resolution vertical SDS-agarose gel electrophoresis and Cy5-labeled antibody high-sensitivity fluorescence detection, Am. J. Clin. Pathol. 133 (2010) 322–330.
[87] R.K. Pruthi, T.M. Daniels, J.A. Heit, D. Chen, W.G. Owen, W.L. Nichols, Plasma von Willebrand factor multimer quantitative analysis by in-gel immunostaining and infrared fluorescent imaging, Thromb. Res. 126 (2010) 543–549.