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Reduction in thrombogenic activity and thrombocytopenia after transcatheter aortic valve implantation — The ATTRACTIVE-TTAS study

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ABSTRACT

Introduction: Bleeding complications after transcatheter aortic valve implantation (TAVI) is a major problem in clinical practice. However, there is few information on thrombogenicity after TAVI. The aim of this study was to establish a monitoring of total thrombogenicity in perioperative TAVI using the Total Thrombus-formation Analysis System (T-TAS), a microchip-based flow chamber system for analysis of thrombus formation under flow condition.

Methods: Twenty-three patients with severe aortic stenosis who underwent TAVI between August 2017 and March 2018 at Kumamoto university hospital were enrolled. After exclusion, data of 21 patients were analyzed. Blood samples were obtained before, 2, 7, and 30 days after TAVI. Thrombogenicity were assessed by the T-TAS to compute the area under the curve (AUC) (AR_{10}-AUC_{30}) in the AR chip. We also measured platelet count, high-molecular-weight von Willebrand factor (HMW-vWF) multimers, and plasma thrombopoietin. Computational fluid dynamics (CFD) analysis was performed to calculate the wall shear stress (WSS).

Results: The AR_{10}-AUC_{30} levels and platelet counts were significantly lower at 2 days post-TAVI, and then increased gradually. HMW-vWF multimers, and plasma thrombopoietin, were significantly higher at 2 days post-TAVI, compared with before TAVI. CFD analysis showed that WSS of the aortic valve and posterior ascending aortic wall were significantly lower after TAVI than before-TAVI. Multivariate analysis identified max velocity measured by echocardiography, platelet count, and D-dimer as significant determinants of AR_{10}-AUC_{30}, representing total thrombogenicity.

Conclusions: Although HMW-vWF multimers improved earlier after TAVI, total thrombogenic activity evaluated by T-TAS remained relatively low followed by improvement in thrombogenic activity at 30 days after TAVI.

Clinical Trial Registration: https://clinicaltrials.gov. Unique identifiers: NCT03248232.

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1. Introduction

Transcatheter aortic valve implantation (TAVI) is a well-established technique and can improve clinical outcome of patients with severe aortic valve stenosis (AS) who are inoperable or at high surgical risk [1–3]. Although the rate of periprocedural bleeding events may be relatively lower in TAVI compared to that in surgical aortic valve replacement (11.3%, 22.7%, respectively) [4], it itself remains absolutely high at present (10.6–14.0%) [1]. In addition, the current guidelines recommend dual antiplatelet therapy (DAPT) with aspirin plus clopidogrel, for a 3- to 6-month period after TAVI, though it is not evidence-based [5]. In fact, two recent reports concluded that aspirin monotherapy alone tended to reduce the risk of bleeding without increasing the rates of total death, acute coronary syndrome (ACS), and stroke in AS patients who underwent TAVI [6,7]. These results emphasize the need for a standardized antithrombotic regimen in patients undergoing TAVI. Another issue in the management of AS is related to acquired von Willebrand syndrome in some patients with AS, which is due to the deficiency of von Willebrand factor (vWF), a high-molecular-weight (HMW) multimer [8,9], indicating potentially high risk of bleeding. Based on these observations, it is important to evaluate the risk of bleeding complications before, during and after TAVI. Unfortunately, there is currently no standardized monitoring system for antithrombotic therapy in TAVI.

The Total Thrombus-Formation Analysis System (T-TAS), a microchip-based flow chamber system, can provide an accurate quantitative estimate of whole blood thrombogenicity in patients on various

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anti-thrombotic agents, such as anti-platelet drugs, warfarin, or direct oral anticoagulants (DOAC) [10–13]. In two recent studies, our group reported the usefulness of T-TAS in predicting the risk of periprocedural bleeding complications in coronary artery disease patients who underwent percutaneous coronary intervention (PCI), and those with atrial fibrillation (AF) who underwent catheter ablation [14,15].

The ATTRACTIVE-TTAS (AssessmenTs of ThRombogenicity for trAns-Catheter aorTIc valVE implantation by Total Thrombus-formation Analysis System; NCT03248232) Study is a prospective observational study designed to evaluate the total thrombogenicity monitored by T-TAS in AS patients during the TAVI perioperative period. In addition, wall shear stress (WSS) plays an important role in regulation of unfolding of vWF-HMW multimer, indicating that the WSS is one of the components of thrombogenic activity in severe AS patients. Therefore, the WSS is also analyzed by using computational fluid dynamics (CFD), which can visualize blood flow condition and can quantify WSS, in the present study.

2. Methods

2.1. Study design and population

All procedures were conducted in accordance with the Declaration of Helsinki and its amendments. The ATTRACTIVE-TTAS study is a prospective, observational study approved by the Human Ethics Review Committee of Kumamoto University Hospital. A written informed consent was obtained from each participating subject. This study was supported financially by the Bayer Scholarship for Cardiovascular Research from the Japan Cardiovascular Research Foundation.

The study enrolled 23 consecutive patients with severe AS who underwent TAVI between August 2017 and March 2018 at Kumamoto University Hospital. The patients fulfilled the following inclusion criteria: 1) severe symptomatic aortic valve stenosis, 2) scheduled to undergo TAVI, and 3) trans-femoral approach was used in TAVI. The following exclusion criteria were also applied: 1) patients with non-transfemoral approach (e.g., trans-apical approach), 2) patients who were later switched to the surgical aortic valve replacement, and 3) patients with critical illness, such as severe infectious disease, cancer, and severe bleeding disorder. After exclusion of 2 patients because of the insufficient blood sampling for AR_{10}-AUC_{30} measurement after enrollment, 21 patients were analyzed finally.

The multidisciplinary heart team that performed TAVI evaluated patient eligibility for the procedure, the transcatheter heart valve selection [Sapien 3 (Edwards Lifesciences, Irvine, CA) or Evolut R (Medtronic, Minneapolis, MN)], and the appropriate access site. In the transfemoral approach, the surgical cut-down approach and closure were performed by a cardiovascular surgeon. Otherwise, vascular closure in patients who underwent percutaneous femoral artery access was performed using Perclose ProGlide (Abbott Vascular Co, Abbott Park, IL). At the delivery of transcatheter heart valve, 14Fr eSheath (Edwards Lifesciences) for Sapien 3 and 14 Fr InLine sheath (Medtronic) for Evolut R were used.

2.2. Sampling points and antithrombotic regimen

Blood samples were collected from the antecubital vein from each patient on admission day (baseline, before TAVI), and at 2, 7, and 30 days post-TAVI. These samples were used for T-TAS measurement, vWF-HMW multimer analysis, and measurement of plasma thrombopoietin. A pre-TAVI CT was performed 1 to 3 months before TAVI in all patients in order to assess procedural feasibility, access route, severity of aortic valve calcification, and anatomical features. To assess shear stress by CFD analysis, a post-TAVI CT was performed in 5 patients with normal renal function and considered suitable for contrast-enhanced CT.

Patients on DAPT for PCI continued to use these medications throughout the TAVI procedure. AF patients who required anticoagulation therapy continued to use warfarin or DOACs, unless they developed bleeding severe enough to warrant discontinuation. The patient was added with 100 mg/day aspirin on these two medications after the procedure. For all other patients, 100 mg/day aspirin was administered at least 1 week before TAVI, combined with 75 mg/day clopidogrel after the procedure.

2.3. T-TAS measurement

Thrombogenic activity was measured by calculating the area under the pressure curve (AUC) of the atheroma chip (AR-chip) in the T-TAS, which is an automated microchip-based flow chamber system and is an easy-to-use device, for analysis of thrombus formation under flow condition, as described previously [10,12,13,16,17]. Briefly, the AR-chip was used to assess the fibrin-rich platelet thrombus formation processes. Whole blood sample collected into a 3.2% sodium citrate-containing tube was mixed with corn trypsin inhibitor and $CaCl_2$ immediately before the assay. The sample was then applied into the AR-chip, which included a single capillary channel coated with type I collagen plus tissue thromboplastin, set at a flow rate of 10 μL/min, which is equivalent to an initial wall shear rate of 600 s^{-1}. After the supply, both the platelets and coagulation system were activated inside the AR-chip by collagen and tissue thromboplastin, respectively. Change of flow pressure within the AR-chip was monitored from initiation of the fibrin-rich thrombus formation process to occlusion of the microchip. The AUC was computed by continuous monitoring of the pressure within the single capillary channel. We evaluated changes in the AR_{10}-AUC_{30}, which was defined as the AUC for the first 30 min for the AR-chip tested at flow rate of 10 μL/min at each sampling point. The T-TAS instrument was purchased from Fujimori Kogyo (Fujimori Kogyo Co., Yokohama, Kanagawa, Japan).

2.4. vWF-HMW multimer analysis

To assess the change of vWF-HMW multimer during peri-procedural period, we performed vWF multimeric analysis. A plasma sample from each patient was used for vWF multimeric analysis (based on the established method of sodium dodecyl sulfate agarose gel electrophoresis [18]) outsourced to SRL, Inc. (Tokyo, Japan). The value of vWF-HMW multimers was estimated using Image J software and represented the relative amount of the largest multimers in the sample compared with those of the normal pooled plasma in the next lane of the same gel, and expressed as vWF-HMW multimer ratio, as described previously [19].

2.5. Measurement of plasma thrombopoietin

Activity of platelet production was evaluated by measuring the concentration of plasma thrombopoietin, which reflects platelet productivity, using human thrombopoietin enzyme-linked immunosorbent assay kit (#ab219632) and the protocol recommended by the manufacturer (Abcam plc, Cambridge, UK).

2.6. Computational fluid dynamics (CFD) analysis

CFD analysis is widely used to analyze blood flow condition. CFD can visualize blood flow and can quantify the flow streamline, flow velocity, WSS, oscillatory index, and other parameters, using a patient-specific 3D model based on the individual CT images. Using contrast-enhanced CT at baseline and at 7 days post-TAVI, CFD analysis for measuring WSS was conducted by Cardio Flow Design, Inc. (Tokyo, Japan), according to the method established previously [20–22]. Briefly, 3-dimensional patient-specific geometries of the area extending from the aortic valve to the thoracic descending aorta was reconstructed from the individual CT images. Computational meshes were generated by the ANSYS-ICEM 16.0 software (ANSYS Inc., Tokyo), and the finite volume solver package ANSYS Fluent 18.0 (ANSYS Inc.) was used to solve the Navier-Stokes equation of incompressible transient Newtonian fluid the in setting, using the individual's blood pressure and blood flow/cardiac outputs,

measured by transthoracic echocardiography. WSS was calculated at the aortic valve and the posterior wall of the thoracic ascending aorta before and after TAVI.

2.7. Outcomes

The primary outcome was changes in the thrombogenic activity evaluated by T-TAS at 2, 7, and 30 days after TAVI, relative to the baseline (before TAVI). The secondary outcomes were the extent of decrease in the levels of vWF-HMW multimer and plasma thrombopoietin, the incidence of periprocedural complications (at ≤7 and 30 days post-TAVI), defined by VARC-2 criteria [23], and changes in shear stress, which was estimated by CFD analysis of contrast-enhanced computed tomography (CT).

2.8. Sample size calculation

Before the start of the study, we calculated the required sample size using IBM SPSS SamplePower (IBM Corporation, Armonk, NY). In a preliminary study involving 13 patients with severe AS admitted to our hospital (M:F = 4:9, mean 82.2 years), we found that the mean ± SD level of AR_{10}-AUC_{30} was 1485 ± 263. We hypothesized that a change of 200 in AR_{10}-AUC_{30} level would be a clinically meaningful thrombogenic change after TAVI. Thus, AR_{10}-AUC_{30} level for severe AS patients before TAVI was assumed to be 1485 ± 263, while AR_{10}-AUC_{30} level for the same patients was assumed to change to 1685 ± l63 after TAVI. The sample size of the participants was determined by 2-tailed paired Student's *t*-test with significance level set at 0.050, power of 0.897, with a mean difference before and after TAVI of 200, and standard deviation of 263. The required sample size based on these assumptions was 20. Considering potential drop out cases, we set the required sample number to 25.

2.9. Statistical analysis

Data were expressed as median (IQR: interquartile range) for continuous variables, or numbers (percentages) for categorical variables. For the sequential observational data before and after TAVI, a generalized linear mixed model followed by a sequential Bonferroni correction, Wilcoxon singed-rank test or Friedman test followed by Bonferroni multiple comparison adjustment were used, as appropriate. To investigate the factors that correlated with AR_{10}-AUC_{30} levels, i.e., total thrombogenicity, multivariate generalized linear mixed models with random effect were performed. Patient characteristics, known coagulability markers, platelet count, vWF-HMW multimer ratio, echocardiographic parameters and plasma thrombopoietin were entered into the model as the explanatory variables. A two-tailed *P* value of <0.05 denoted the presence of a statistically significant difference. All statistical analyses were performed by using The Statistical Package for Social Sciences software version 23.0 (IBM Corporation, Armonk, NY).

3. Results

3.1. Patient characteristics

After exclusion of two patients due to the lack of sampling data, the data of 21 patients were finally analyzed, representing 23 consecutive patients with AS who underwent TAVI. Table 1 lists the clinical characteristics of the participants at baseline. The median age was 87.0 years (IQR: 84.0–90.5) and the proportion of females was 57.1%. The median aortic valve mean pressure gradient (PG) before TAVI was 48.2 mm Hg (IQR: 37.8–65.4), with an aortic valve area of 0.53 cm^2 (IQR: 0.45–0.66) and EuroSCORE II of 3.72% (IQR: 2.67–4.63). Of all, 28.6% of the patients had the severe symptomatic heart failure (NYHA class III or IV), and median level of plasma brain natriuretic peptide was 357.4 pg/mL (IQR: 126.5–534.1), with preserved ejection fraction (median 61.4%, IQR: 50.5–65.3). Oral anticoagulants were used by 28.6% of the patients (warfarin in 14.3% and DOACs in 14.3%) for the treatment of AF, while DAPT was used by 33.3% of the patients for PCI.

With regard to the TAVI procedure, the trans-femoral approach was applied in all patients (Table S1). The distribution of transcatheter heart valve was 6 (28.6%), 6 (28.6%), 3 (14.3%), 2 (9.5%), and 4 (19.0%) for Sapien 3 23 mm, Sapien 3 26 mm, Evolut R 23 mm, Evolut R 26 mm, and Evolut R 29 mm, respectively.

3.2. Serial changes in T-TAS parameters, vWF-HMW multimers, and plasma thrombopoietin

Various parameters related to the thrombogenic activity were measured at baseline, 2, 7 and 30 days post-TAVI (Fig. 1). The AR_{10}-AUC_{30} levels were significantly lower at 2 and 7 days after TAVI, compared to the baseline ($p < 0.01$, $p < 0.05$, respectively). The AR_{10}-AUC_{30} levels were accordingly significantly higher at 30 days after TAVI, compared to 2 and 7 days post-TAVI ($p < 0.01$, $p < 0.05$, respectively). The vWF-HMW multimer ratio was significantly higher at 2 days post-TAVI, compared to the baseline ($p < 0.01$), and tended to be higher at 7 and 30 days post-TAVI than the baseline, albeit insignificantly. The level of plasma thrombopoietin was significantly higher at 2 days post-TAVI compared to baseline ($p < 0.01$) while the levels at 7 and 30 days post-TAVI tended to be higher than those of baseline TAVI, similar to the trend noted for the vWF-HMW multimer ratio. The platelet count was lowest at 2 days post-TAVI but increased later at 7 and 30 days after TAVI, though it was still significantly lower at 30 days relative to the baseline.

Fig. S1 shows scatter plots for the relation between baseline AR_{10}-AUC_{30} levels, vWF-HMW multimer ratio, platelet count, and various echocardiographic parameters, including mean and max aortic valve PG. AR_{10}-AUC_{30} level correlated moderately and negatively with PG (mean PG: $r = -0.47$, max PG: $r = -0.48$), but there was no relation between vWF-HMW multimer ratio, platelet count, and PG.

3.3. Serial changes in WSS analyzed by CFD

Next, we performed contrast-enhanced CT at 7 days post- TAVI in 5 patients with preserved renal function in order to determine the changes in WSS analyzed by CFD. As shown in Fig. 2, WSS was high at the posterior wall of the thoracic ascending aorta and aortic valve leaflets at baseline. CFD analysis demonstrated that TAVI improved the WSS on the aortic valve and posterior wall of the thoracic ascending aorta in patients with severe AS (Fig. S2).

3.4. Factors related to total thrombogenicity

To clarify the factors that correlate periprocedurally with changes in AR_{10}-AUC_{30}, we used multivariate analysis with the generalized linear mixed models (Table 2). All models identified max velocity (measured by echocardiography), platelet count, and D-dimer, but not vWF-HMW multimer ratio or plasma thrombopoietin, as significant determinants of total thrombogenicity, as represented by the AR_{10}-AUC_{30} level.

3.5. Clinical outcome

Fig. S3 shows serial changes in aortic valve velocity and PG. Both parameters were significantly lower after TAVI compared to the baseline values ($p < 0.01$, each). During the 7-day follow-up period, TAVI-related complications were noted in 13 patients (major vascular complications: $n = 2$, bleeding complications: $n = 1$, new pacemaker implantation: $n = 1$, conduction disorders: $n = 8$, cardiac tamponade: $n = 1$). During the 30-day follow-up period, clinical adverse events were recorded in 7 patients, including cardiovascular death ($n = 1$), non-cardiovascular death ($n = 1$), stroke ($n = 1$), new pacemaker implantation ($n = 1$), and deterioration of heart failure ($n = 3$). In the present study, one patient with periprocedural bleeding complication that was

Table 1
Baseline characteristics of AS patients who underwent TAVI.

	Overall ($n = 21$)		Overall (n = 21)
Patient characteristics		Anti-thrombotic regimen before TAVI	
Age, years	87.0 [84.0–90.5]	Aspirin	17 (81.0)
Females	12 (57.1)	Clopidogrel	7 (33.3)
Body surface area, m^2	1.43 [1.31–1.57]	Prasugrel	1 (4.8)
Body mass index, Kg/m^2	23.2 [19.9–24.9]	DAPT	7 (33.3)
NYHA class III or IV	6 (28.6)	Warfarin	3 (14.3)
Diabetes	5 (23.8)	DOACs	3 (14.3)
Hypertension	18 (85.7)	Echocardiographic parameters	
Dyslipidemia	13 (61.9)	LVEF, %	61.4 [50.5–65.3]
Coronary artery disease	12 (57.1)	Aortic valve area, cm^2	0.53 [0.45–0.66]
Previous MI	3 (14.3)	Mean PG, mmHg	48.2 [37.8–65.4]
Previous stroke	3 (14.3)	Max velocity, m/s	4.57 [4.12–5.20]
Previous PCI	9 (42.9)	Mean velocity, m/s	3.20 [2.81–3.96]
Previous CABG	0 (0)	TRPG, mmHg	26 [23–36]
Peripheral artery disease	2 (9.5)	AR ≥ mild	14 (66.7)
Atrial fibrillation	6 (28.6)	MR ≥ mild	11 (52.4)
COPD	3 (14.3)	Laboratory data	
Pulmonary hypertension	3 (14.3)	eGFR, mL/min/1.73m^2	41 [28.5–55.0]
		PT-INR	1.09 [1.01–1.39]
Risk scores		APTT, sec	30.6 [28.4–36.3]
STS score, %	5.021 [3.817–6.832]	D-dimer, μg/mL	1.70 [0.98–3.20]
Logistic EuroSCORE, %	13.57 [9.09–23.20]	Platelet count, $x10^3/10$	170 [139–212]
EuroSCORE II, %	3.72 [2.67–4.63]	BNP, pg/mL	357.4 [126.5–534.1]

Data are median [25%–75%], or n (%).
AS = aortic stenosis, TAVI = transcatheter aortic valve implantation, MI = myocardial infarction, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft surgery, COPD = chronic obstructive pulmonary disease, STS = Society of Thoracic Surgeons, DAPT = dual antiplatelet therapy, DOAC = direct oral anticoagulant, LVEF = left ventricular ejection fraction, PG = pressure gradient, TRPG = tricuspid regurgitation pressure gradient, AR = aortic regurgitation, MR = mitral regurgitation, eGFR = estimate glomerular filtration rate, PT-INR = prothrombin time-international normalized ratio, APTT = activated partial thromboplastin time, Plt = platelet count, BNP = brain natriuretic peptide.

hematoma 1 day after TAVI at the puncture site without surgical intervention was observed. Although statistical analysis was not performed because of small sample size, the level of AR_{10}-AUC_{30} in the patients with bleeding complication was extremely low among study participants (Table S2).

4. Discussion

In the present study, we evaluated the thrombogenic activity before and after TAVI by measuring T-TAS, vWF-HMW multimers, plasma thrombopoietin, and platelet count, and analyzed the effects of TAVI on WSS using the CFD model.

In patients with severe AS, vWF-HMW multimers are often reduced due to high shear stress at the narrowed valve, which may cause bleeding complications [8,9]. Previous studies reported that of the low levels of vWF-HMW multimers and platelet dysfunction, as measured by the closure time with adenosine diphosphate, return to normal levels immediately after TAVI [19,24]. The incidence of major bleeding complications was lower at 30-day post-TAVI (conducted by the trans-femoral approach), compared with surgical aortic valve placement (11.3% vs. 22.7%) [4], although the incidence after TAVI was higher than that after elective PCI for stable coronary artery disease [14]. In other words, the incidence of bleeding complications after TAVI remains relatively high in spite of the fact that the correction of AS by TAVI can improve platelet dysfunction. These findings suggest that certain factors other than vWF-HMW multimers are responsible for the low thrombogenic activity after TAVI. In this regard, our group reported previously that the level of AR_{10}-AUC_{30} was a useful predictor of periprocedural bleeding events in AF patients who underwent catheter ablation [15]. Consistent with this finding [15], the levels of AR_{10}-AUC_{30} at 2 and 7 days post-TAVI were significantly lower than those before and 30 days post-TAVI in the present study, which may explain the high risk of bleeding complication after TAVI associated with the low thrombogenic activity. Unfortunately, we could not assess the relationship between AR_{10}-AUC_{30} levels and bleeding complications due to the small sample size and low rate of these complications.

Several recent studies on thrombocytopenia after TAVI have described a fall in platelet count at 2–4 days after TAVI, to levels as low as 50,000–100,000/μL in severe cases [25–27]. Other studies reported that severe thrombocytopenia was an independent predictor of major vascular complications, bleeding complications, and one-year all-cause death after TAVI [26,27]. In the present study, the platelet count was significantly lower at 2 days post-TAVI (median 94,000/μL), compared with the baseline value of 170,000/μL. Although the count increased to 144,000/μL at 30 days after TAVI, it was still significantly lower than that before TAVI. The most common causes of thrombocytopenia are enhanced platelet turnover, low platelet production, and significant hemodilution by frequent red blood cell transfusion [27]. However, the exact reason for the development of thrombocytopenia after TAVI remains to be elucidated. This is perplexing even when the frequency of the fall in platelet count after TAVI is higher than that after other endovascular treatments, such as PCI. To evaluate platelet production, we measured the levels of plasma thrombopoietin, which is involved in the differentiation and proliferation of progenitor cells of platelets. Our results showed significant increase in plasma thrombopoietin level after TAVI, relative to the baseline, suggesting preserved platelet production. However, since platelet count was still low after TAVI, this finding suggests that platelet production in patients with severe AS might be relatively reduced, or that platelet turnover exceeds platelet production. Further studies are needed to clarify the mechanism of platelet reduction after TAVI.

We applied CFD analysis to evaluate shear stress before and after TAVI. In this analysis, a three-dimensional individual structure was reconstructed from contrast-enhanced CT for each patient, and the condition of blood flow in the model was reproduced by fluid simulation using patient-specific physiological parameters, such as blood pressure, cardiac output, and area of the valve orifice measured by echocardiography. Thus, the CFD simulation model resembled to a large extent the in vivo situation [21,22]. The CFD analysis demonstrated that the correction of valve stenosis by TAVI improved the high WSS in the aortic valve, and the posterior wall of the ascending aorta. Although improvement in shear stress by TAVI, demonstrated in previous studies [19,24] and the present investigation, can contribute to the normalization of vWF-HMW multimers in patients with severe AS, such change in shear stress could affect other thrombus formation factors related to shear stress. Vascular endothelial cells regulate the cascade of platelet aggregation and anticoagulation by

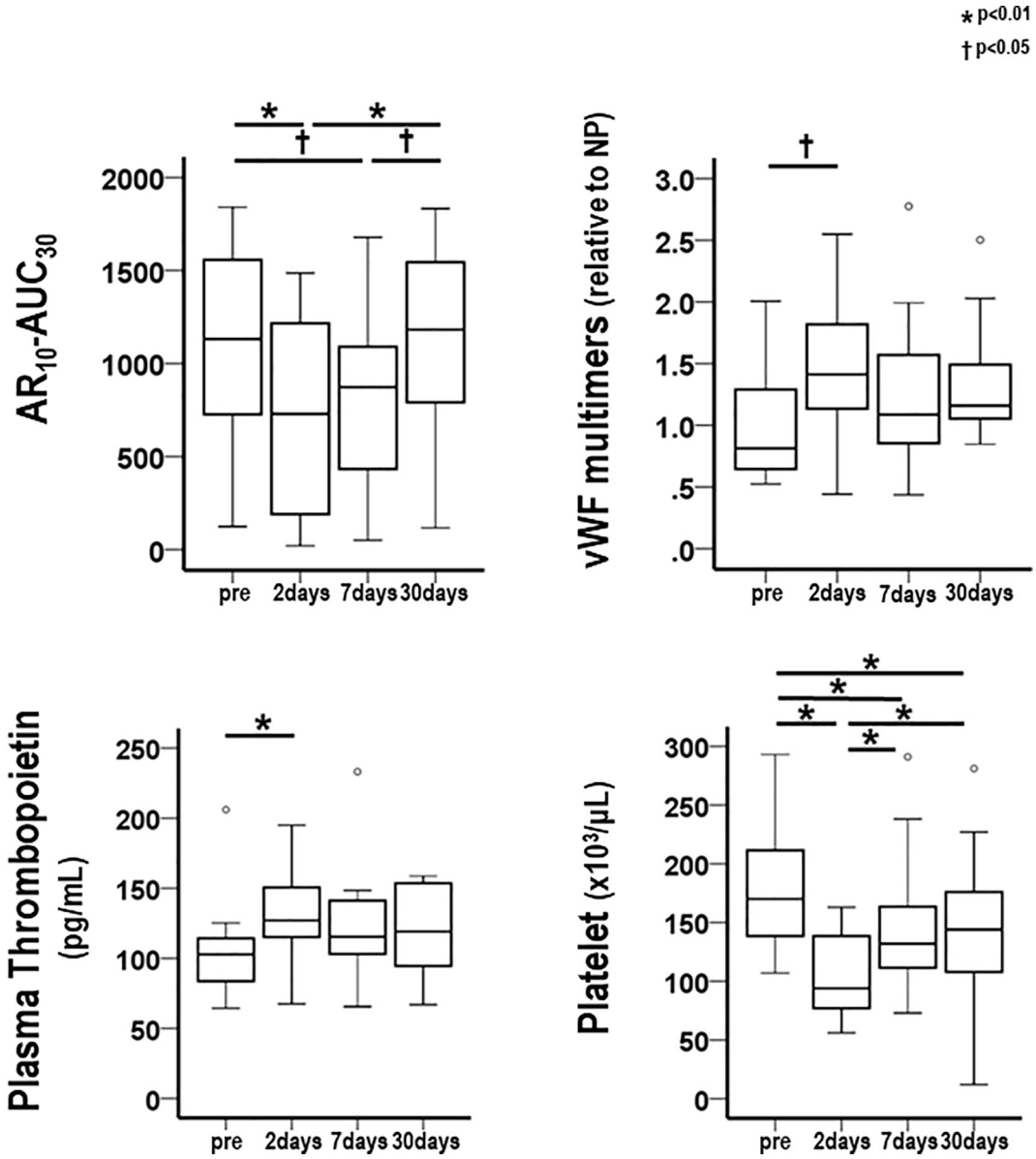

Fig. 1. Effects of TAVI on AR_{10}-AUC_{30}, vWF HMW multimers, plasma thrombopoietin, and platelet count. In these box-and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 75th and 25th percentiles, respectively; and the upper and lower bars outside the boxes represent maximum and minimum values within 1.5 times the interquartile range from the 75th and 25th percentile, respectively. *$p < 0.01$, Bonferroni's multiple comparison test. †$p < 0.05$, Bonferroni's multiple comparison test. AR_{10}-AUC_{30} = area under the curve for the first 30 min for the atheroma chip tested at flow rate of 10 μL/min, vWF = von Willebrand factor, HMW = high molecular weight, TAVI = transcatheter aortic valve implantation.

expressing the related molecules on the cell membrane surface, or by producing various molecules, such as thrombomodulin (TM), nitric oxide (NO), prostacyclin, tissue plasminogen activator (t-PA), tissue factor pathway inhibitor, or antithrombin [28–31]. Shear stress involves endothelial expression of these molecules. In this regard, Takada et al. [28] reported increases in TM protein and mRNA expression in response to fluid shear stress in human umbilical vein endothelial cells. Furthermore, Ishibazawa et al. [29] also demonstrated significant shear stress-dependent increases in the expression of eNOS and TM mRNAs, but significant decreases in of the mRNA expression of endothelin-1. On the other hand, Malek et al. [30] indicated that shear stress decreased TM mRNA expression in bovine aortic endothelial cells in the late-phase although the expression was transiently elevated in the early phase under higher shear rate conditions. Furthermore, the downregulated expression of TM mRNA increased following a change from moderate shear rate to static condition [30]. Based on these findings, we speculate that severe AS-induced high shear stress is associated with poor production of anti-thrombotic factors from vascular endothelial cells, and that normalization of shear stress by TAVI might enhance the production of anti-thrombotic factors from vascular endothelia cells, resulting in excessive transient drop in thrombotic activity after TAVI.

The present study has certain limitations. First, we cannot rule out selection bias of the participants because this is a single-center observational study and the participants were limited to patients with the trans-femoral approach. The aim of this study was to assess whether the T-TAS could monitor change of thrombus formation during periprocedural TAVI. Difference in approach site such as trans-femoral and trans-apical approach may lead to variations in the magnitude of the intervention, which affects hemostasis and coagulation system. As a result, it would be difficult to assess the performance of the T-TAS on measurement of the thrombus formation. Therefore, in order to increase internal validity, we restricted the subjects to patients with the trans-femoral approach. However, to obtain external validity, future study that does not limit the approach site should be conducted. Second,

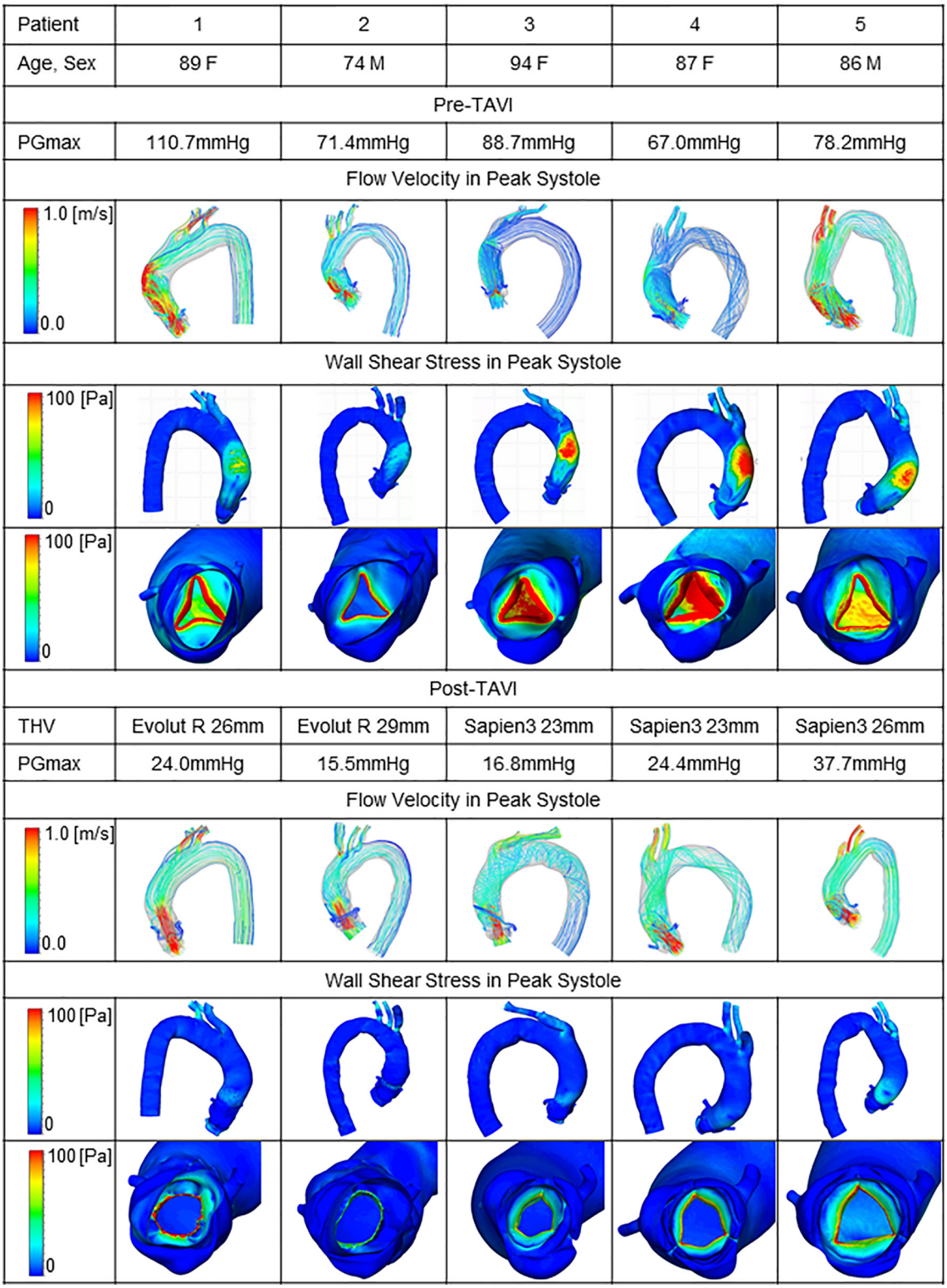

Fig. 2. Effects of TAVI on results of CFD analysis in patients with severe AS. Clinical characteristics (age, sex), pressure gradient measured by echocardiography, and transcatheter heart valve (THV). CFD analysis showed flow velocity on the view from the front of the ascending aorta, and wall shear stress on the view from the back of the ascending aorta and on the view of aortic base from the left ventricular in pre- and post-TAVI. CFD = computational fluid dynamics, AS = aortic valve stenosis, TAVI = transcatheter aortic valve implantation.

we could not evaluate the association of total thrombogenic activity, measured by T-TAS, with clinical outcome after TAVI due to the small sample size. The sample size was calculated based on the power of detection of the primary outcome. In addition, we could not evaluate another important clinical relevance such as hospitalization period and peri-procedural management of femoral access, which would reduce the bleeding complication and thrombogenic activity after TAVI in short-term. Third, CFD analysis was conducted only in patients with preserved renal function after TAVI since such analysis requires reconstruction of the patient-specific 3-D structure by contrast-enhanced CT. External validity of the results of CFD analysis would be limited for that reason.

Table 2
Multivariate generalized linear mixed model for factors related to total thrombogenicity.

Variables	Coefficient	SE	95%CI	t	*p* value
Model 1					
Intercept	7.777	0.738	6.269, 9.286	10.532	<0.001
Max velocity, m/s	−0.159	0.077	−0.315, −0.002	−2.071	0.047
Platelet count, x10^3/10	0.005	0.002	0.001, 0.009	2.716	0.012
D-dimer, μg/mL	−0.113	0.041	−0.196, −0.030	−2.790	0.009
APTT, *sec*	−0.018	0.014	−0.047, 0.010	−1.295	0.205
PT-INR	−0.222	0.227	−0.685, 0.242	−0.976	0.337
vWF-HMW multimer ratio	−0.017	0.212	−0.449, 0.416	−0.079	0.938
Thrombopoietin	0.000	0.001	−0.003,0.002	−0.148	0.884
Model 2					
Intercept	5.015	2.423	−0.262, 10.29	2.070	0.061
Max velocity, m/s	−0.173	0.073	−0.323, −0.024	−2.365	0.025
Platelet count, x10^3/μL	0.006	0.002	0.002, 0.009	3.187	0.004
D-dimer, μg/mL	−0.123	0.039	−0.203, −0.043	−3.126	0.004
Age, years	0.018	0.029	−0.046, 0.082	0.629	0.542
Female	0.341	0.333	−0.378, 1.060	1.024	0.324
Model 3					
Intercept	6.690	0.338	−6.004, 7.376	19.769	<0.001
Max velocity, m/s	−0.146	0.072	−0.292, −0.000	−2.038	0.050
Platelet count, x10^3/μL	0.005	0.002	0.002, 0.009	3.004	0.005
D-dimer, μg/mL	−0.099	0.038	−0.177, −0.021	−2.569	0.014

SE = standard error, CI = confidence interval, Plt = platelet, vWF-HMW = von Willebrand factor high molecular weight, APTT = activated partial thromboplastin time, PT-INR = prothrombin time-international normalized ratio.

5. Conclusions

The present study demonstrated significant decreases in total thrombogenic activity measured by T-TAS and platelet count after TAVI despite the improvement in vWF-HMW multimers. This phenomenon might explain the high risk of bleeding complication after TAVI. Future studies are needed to confirm the usefulness of T-TAS in the assessment of bleeding complications after TAVI.

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Disclosure

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