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SHORT COMMUNICATION

Detection of acquired von Willebrand syndrome after ventricular assist device by total thrombus-formation analysis system

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Abstract

Aims Bleeding is a serious complication in patients with continuous-flow left ventricular assist device (CF-LVAD). Acquired von Willebrand syndrome (AVWS; type 2A) develops because of high shear stress inside the pumps and is a cause of bleeding complication. Although von Willebrand factor (vWF) multimer analysis is useful for diagnosing AVWS, it is only performed in specialized research institutes. A novel microchip flow chamber system, the total thrombus-formation analysis system (T-TAS), is a point-of-care system to evaluate the thrombus-formation process and useful for monitoring platelet thrombus-formation capacity in patients receiving antiplatelet therapy and the diagnosis and evaluation of the clinical severity of von Willebrand disease type 1. However, little is known about the association between AVWS and platelet thrombus-formation capacity evaluated by T-TAS in patients with CF-LVAD. We aimed to evaluate the utility of T-TAS for easy detection of AVWS in patients with CF-LVAD.

Methods and results We simultaneously evaluated the vWF large multimers and T-TAS parameters in four consecutive patients with axial-type CF-LVAD and eight control patients treated with aspirin and warfarin. vWF large multimer index was defined as the proportion of large multimers in total vWF derived from a normal control plasma. T-TAS analyses different thrombus-formation processes using two microchips with different thrombogenic surfaces. PL_{24}-AUC_{10} levels in the platelet (PL) chip are highly sensitive for platelet functions, while AR_{10}-AUC_{30} levels in the atheroma (AR) chip allow the assessment of the overall haemostatic ability. vWF large multimer index and T-TAS parameters were decreased in all patients with CF-LVAD. The mean PL_{24}-AUC_{10} level (5.4 ± 2.9 vs. 219 ± 67; $P < 0.01$), AR_{10}-AUC_{30} level (338 ± 460 vs. 1604 ± 160; $P < 0.01$) and vWF large multimer index (49 ± 11% vs. 112 ± 27%; $P < 0.01$) were significantly lower in the patients with CF-LVAD than in control patients. One patient showed changes in T-TAS levels before and after implantation of CF-LVAD. PL_{24}-AUC_{10} and AR_{10}-AUC_{30} levels decreased from 438.1 to 5.0 and from 1667.9 to 1134.3, respectively.

Conclusions In patients with CF-LVAD, the platelet thrombus-formation capacity was extremely impaired because of AVWS, and T-TAS parameters could detect the presence of AVWS. T-TAS can be used for easy detection of AVWS as a point-of-care testing. Further studies with a large sample size are needed to validate our results in several LVAD models and evaluate the prognostic value of bleeding complications and thromboembolism in patients with LVAD.

Keywords Total thrombus-formation analysis system; Acquired von Willebrand syndrome; Ventricular assist device; Bleeding; Coagulation

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Background

Left ventricular assist device (LVAD) is an established treatment for patients with advanced heart failure. However, bleeding complication and thromboembolism remain common and occasionally result in poor outcomes. In most patients with continuous-flow LVAD (CF-LVAD), acquired von Willebrand syndrome (AVWS; type 2A) develop and is considered a cause of bleeding complication.[1,2] AVWS was caused by high shear forces resulting in the destruction of von Willebrand factor (vWF) large multimers, which are cleaved by the serine protease ADAMTS13. A quantitative analysis of vWF large multimers is useful to diagnose AVWS and evaluate the risk of gastrointestinal bleeding[3]; however, this analysis is only performed in specialized research institutes.

A novel microchip flow chamber system, the total thrombus-formation analysis system [T-TAS® (Fujimori, Co., Tokyo)], was recently developed for easy and rapid quantitative analysis of the thrombus-formation process under flow conditions.[4] This and other previous studies have reported the usefulness of T-TAS for monitoring platelet thrombus-formation capacity and predicting bleeding events in patients receiving antiplatelet therapy and for the diagnosis and evaluation of the clinical severity of von Willebrand disease type 1.[5–7] However, limited information is available on the association between AVWS and platelet thrombus-formation capacity evaluated by T-TAS in patients with CF-LVAD.

Aims

This study aimed to evaluate the utility of T-TAS system for easy detection of AVWS in patients with CF-LVAD.

Methods

We evaluated four consecutive patients with end-stage heart failure who underwent axial-type CF-LVAD implantation (Heart Mate II, Abbott, Lake Bluff, IL, USA) as bridge to transplantation and eight control patients treated with aspirin and warfarin. All control patients had history of atrial fibrillation and coronary artery or peripheral artery disease and had no comorbidity associated with AVWS such as aortic stenosis and bleeding or thromboembolic complication. The study protocol was approved by the human ethics committee of Kumamoto University, and a written informed consent was obtained from each patient. Blood samples were collected to analyse the vWF large multimers and T-TAS simultaneously. Blood samples were collected from the antecubital vein using a 21-gauge butterfly needle into a hirudin-containing blood sampling tube [MP0600 (Verum Diagnostia); final concentration of hirudin: 25 μg/mL], blood collection tubes (VP-CA050K70, Venoject II; Terumo) and a syringe containing 0.11 mL of 3.8% sodium citrate solution. T-TAS was analysed 1 to 4 h after sample collection. The plasma samples were kept frozen at −80°C until vWF large multimer analysis.

Quantitative vWF large multimer analysis was performed to determine the following two parameters: 'large vWF multimer ratio' and 'large vWF multimer index'. vWF multimers were divided into three parts (large, medium, and small). The large vWF multimer ratio was calculated as the ratio of the large vWF multimer area to the total vWFs. The ratio was calculated based on the densitometric analysis of the western blot of patients' plasma. vWF large multimer index (%) was defined as the proportion of large multimers in total vWF derived from a normal control plasma (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, Lot No. 503258).[2,8]

Table 1 Patients' characteristics and results of vWF multimer analysis

Patient	1	2	3	4 (Pre LVAD)	4 (Post LVAD)
Age	46	58	36	21	
Gender	F	F	M	M	
Aetiology	DCM	DCM	DCM	DCM	
Device	HM II	HM II	HM II	—	HM II
Rotation speed (rpm)	8400	8600	9200	—	9200
Period from LVAD implantation to analysis (month)	25	22	12	—	6
PL_{24}-AUC_{10}	2.9	3.5	10.2	438.1	5.0
AR_{10}-AUC_{10}	54.6	61.2	103.6	1667.9	1134.3
Platelet count ($\times 10^3/\mu L$)	188	173	225	282	248
PT-INR	2.54	2.20	2.35	1.87	2.30
LDH (U/L)	263	325	214	184	259
vWF large multimer index (%)	64.7	37.5	48.9	nd	45.2
Antiplatelet therapy	aspirin	aspirin	aspirin	aspirin	aspirin clopidogrel
Anticoagulant therapy	warfarin	warfarin	warfarin	warfarin	warfarin

AR, atheroma; AUC, area under the flow-pressure curve; DCM, dilated cardiomyopathy; HM II, Heart Mate II; LDH, lactase dehydrogenase; nd, not done; PT-INR, prothrombin time-international normalised ratio; PL, platelet; rpm, revolutions per minute; vWF, von Willebrand factor

T-TAS analyses different thrombus-formation processes using two microchips with different thrombogenic surfaces. Inside the platelet (PL) chip, which is coated with Type I collagen, platelets adhere and aggregate onto the surface of the collagen, thus occluding the microchip capillaries. Inside the atheroma (AR) chip, which is coated with Type I collagen plus tissue thromboplastin, platelets and the coagulation system are activated simultaneously by collagen and tissue thromboplastin, respectively. The area under the flow-pressure curve (AUC) was computed to assess the platelet thrombogenicity inside the microchips (PL_{24}-AUC_{10}; AUC at a flow rate of 24 μL/min for the first 10 min in PL chip and AR_{10}-AUC_{30}; AUC at a flow rate of 10 μL/min for the first 30 min in AR chip).

Results

Patient characteristics are shown in *Table 1*. The four patients with CF-LVAD were treated with both aspirin and warfarin. Patient 4 was additionally treated with clopidogrel because of pump thrombosis that required pump exchange in the early postoperative period. In the T-TAS analysis, the mean PL_{24}-AUC_{10} and AR_{10}-AUC_{30} levels in four patients with CF-LVAD were 5.4 ± 2.9 and 338 ± 460, respectively. Patient 4 showed changes in T-TAS levels before and after implantation of CF-LVAD (PL_{24}-AUC_{10}: 438.1 and 5.0, AR_{10}-AUC_{30}: 1667.9 and 1134.3, respectively; *Table* 1). In eight control patients, the mean PL_{24}-AUC_{10} and AR_{10}-AUC_{30} levels were 219 ± 67 and 1604 ± 160, respectively. The PL_{24}-AUC_{10} and AR_{10}-AUC_{30} levels were significantly lower in patients with CF-LVAD than in control patients ($P < 0.01$, *Figure 1A*; and $P < 0.01$, *Figure 1B*). *Figure 2* shows the western blot results of the vWF large multimer analysis of LVAD patients. The vWF large multimer index was significantly lower in patients with CF-LVAD than in control patients (49 ± 11% vs. 112 ± 27%; $P < 0.001$; *Figure 1C*). *Figure 3* presents a scatter plot showing the correlation between the T-TAS parameters and vWF large multimer index. Both T-TAS parameters and vWF large

Figure 2 Results of western blotting of vWF large multimer analysis in patients with CF-LVAD. C, control; CF-LVAD, continuous-flow left ventricular assist device; vWF, von Willebrand factor.

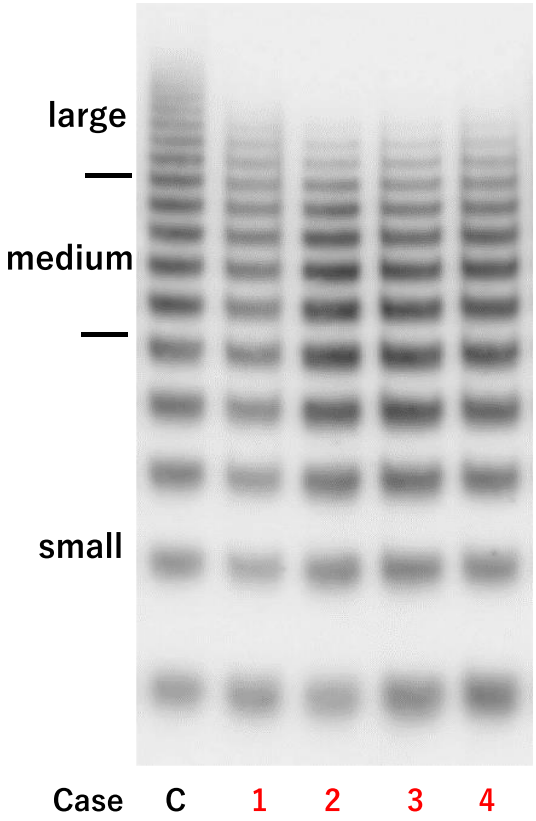

Figure 1 Comparison of (A) PL_{24}-AUC_{10}, (B) AR_{10}-AUC_{30}, and (C) von Willebrand factor (vWF) large multimer index between patients with continuous-flow left ventricular assist device (CF-LVAD) and control patients treated with aspirin and warfarin.

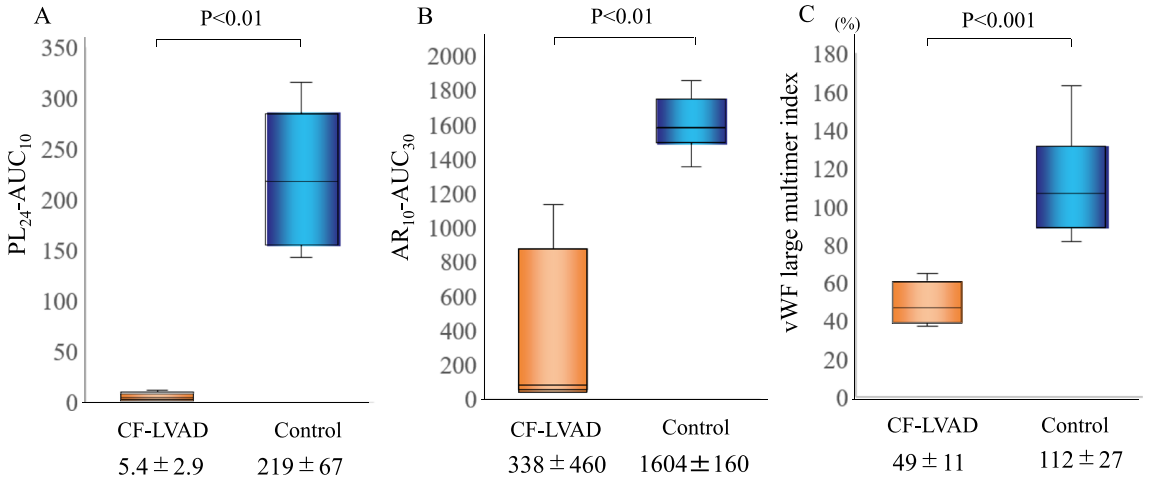

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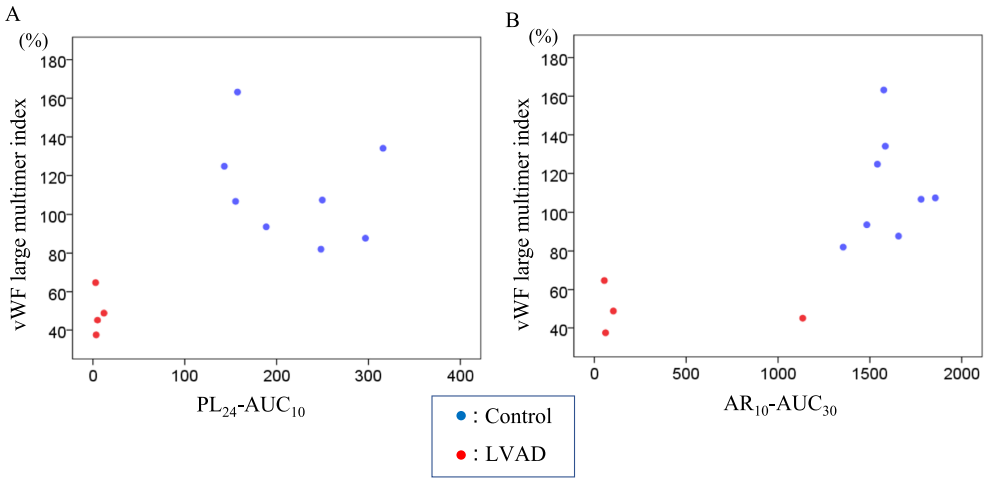
Figure 3 Scatter plot showing the correlation between the T-TAS parameters and vWF large multimer index. (A) PL_{24}-AUC_{10}, (B) AR_{10}-AUC_{30}. vWF, von Willebrand factor, T-TAS, total thrombus-formation analysis system.



multimer index were lower in LVAD patients than in control patients.

Conclusions

This is the first study to report that the platelet thrombus-formation capacity in patients with CF-LVAD was extremely impaired due to AVWS compared with that in control patients treated with aspirin and warfarin. These results suggested that T-TAS could detect the presence of AVWS in patients with CF-LVAD. PL_{24}-AUC_{10} levels in the PL chip are highly sensitive for platelet functions, while AR_{10}-AUC_{30} levels in the AR chip allow the assessment of the overall haemostatic ability in various cardiovascular diseases. The PL chip may be more useful for the detection of AVWS than the AR chip. In the present study, the AR_{10}-AUC_{30} level in Patient 4, who had pump thrombosis, was extremely high. This result suggests that high AR_{10}-AUC_{30} levels might indicate high risk of thrombosis. Furthermore, we have previously reported that low AR_{10}-AUC_{30} level was a significant predictor for periprocedural and 1 year bleeding events in patients who underwent catheter ablation for atrial fibrillation and coronary artery disease, respectively.[9,10] Based on these observations, AR_{10}-AUC_{30} levels in the AR chip may be useful for risk stratification of bleeding or thromboembolism. However, further studies are warranted to clarify this point.

This study has the following limitations: it had a small sample size, it was conducted in a single centre, and the changes in T-TAS parameters before and after LVAD implantation were limited. However, this study is the first to evaluate the utility of T-TAS for AWVS in patients with LVAD. We believe that T-TAS is convenient tool to detect the presence of AVWS as the point-of-care testing. Further studies with a large sample size are needed to validate our results in several LVAD models, to clarify the factors affecting the T-TAS values (e.g. rotation speed) and the association between T-TAS parameters and vWF large multimer index, and to evaluate the prognostic value of bleeding complications and thromboembolism in patients with LVAD.

Conflict of interest

None declared.

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