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Total Thrombus-Formation Analysis System can Predict 1-Year Bleeding Events in Patients with Coronary Artery Disease

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Aims: The assessment of bleeding risk in patients with coronary artery disease (CAD) is clinically important. We recently developed the Total Thrombus-Formation Analysis System (T-TAS) for the quantitative analysis of thrombus formation using microchips with thrombogenic surfaces. Here, we assessed the utility of T-TAS parameters in predicting 1-year bleeding events in patients with CAD.

Methods: The study subjects were 561 consecutive patients who underwent coronary angiography (CAG) between August 2013 and September 2016 for suspected CAD. Blood samples collected at the time of CAG were used for T-TAS to compute the area under the curve (AUC) (AR_{10}-AUC_{30}) in the AR chip. Patients were divided into three groups according to AR_{10}-AUC_{30} (low: ≤ 1603, intermediate, and high: >1765, n=187 each). One-year bleeding events were defined by the Platelet Inhibition and Patient Outcomes criteria.

Results: Bleeding occurred in 21 (3.7%) patients and was classified as major (8 [1.4%]) and minor (13 [2.3%]). The AR_{10}-AUC_{30} levels were significantly lower in the bleeding group than the non-bleeding group (median [interquartile range] 1590 [1442–1734] vs. 1687 [1546–1797], p=0.04). Univariate Cox regression analysis demonstrated that low AR_{10}-AUC_{30} , high prothrombin time-international normalized ratio levels, and diabetes correlated with bleeding events. Multivariate Cox regression analysis identified low AR_{10}-AUC_{30} levels as a significant determinant of bleeding events. Kaplan-Meier survival curves showed a higher rate of bleeding events in the low than the high AR_{10}-AUC_{30} group (p=0.007).

Conclusions: The results highlight the potential usefulness of the AR_{10}-AUC_{30} levels in the prediction of 1-year bleeding events in patients with CAD treated with various antithrombotic therapies.

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Key words: T-TAS, AR_{10}-AUC_{30}, Bleeding, CAD, Antithrombotic therapy

Introduction

Antithrombotic drugs are widely used in the field of cardiovascular diseases to prevent or treat thrombosis. Dual antiplatelet therapy (DAPT) is applied in acute coronary syndrome (ACS) or after percutaneous coronary intervention (PCI). Recently, the use of direct oral anticoagulants (DOAC) has spread as a useful strategy for the prevention or treatment of thromboembolism in patients with atrial fibrillation (AF) and venous thromboembolism. However, there is no adequate monitoring system that can evaluate antithrombotic drugs. Bleeding events are one of the major concerns in patients treated with these drugs. Bleeding events after PCI are associated with early and late mortality[1-4]. The reported incidence of major bleeding events in patients with stable coronary artery disease (CAD) is 0.6%/year, and major bleeding events are significantly associated with mortality[5]. Furthermore, approximately 5%–8% of patients who

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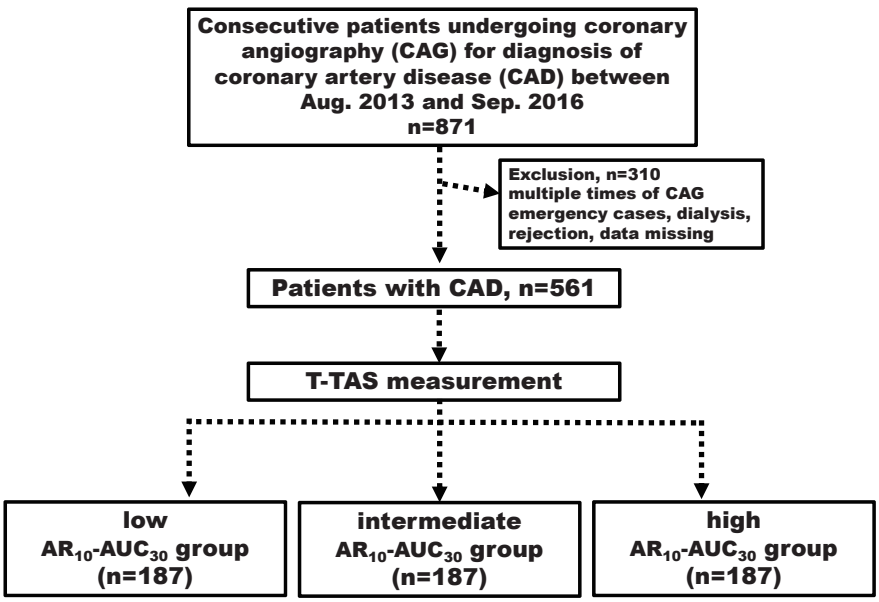

Fig. 1. Patient selection process and study flow chart
CAG: coronary angiography, CAD: coronary artery disease

underwent PCI developed AF[6-8], and the reported incidence of major bleeding events in those who received the combination of DAPT plus anticoagulants was 2.2% within the first month and 4%–12% within the first year of treatment[9].

Previous studies showed that the DAPT score allowed the successful stratification of bleeding risks[10], and the European Society of Cardiology guidelines recommended the use of this score to stratify stroke risk, bleeding risk, and clinical setting regarding the use of antithrombotic therapy in patients with CAD and AF[11]. However, it is difficult to evaluate the efficacy of different types of antithrombotic drugs. In this regard, the Total Thrombus-Formation Analysis System (T-TAS [Fujimori Kogyo Co., Tokyo, Japan]), a microchip-based flow chamber system used for the evaluation of whole blood thrombogenicity, was developed as an easy-to-use system for the quantitative analysis of thrombus formation[12, 13]. We previously reported the usefulness of the T-TAS in the assessment of the pharmacological effects of edoxaban in patients who underwent total knee arthroplasty[14] and also recommended the use of the T-TAS parameter as a significant predictor of procedural bleeding events in patients undergoing catheter ablation for AF[15]. In another series of clinical studies, we provided evidence for the potential suitability of the T-TAS parameter as an index for the assessment of antiplatelet therapy in patients with CAD[16] and of periprocedural bleeding events in patients who underwent PCI[17]. However, the usefulness of the T-TAS parameter in predicting medium-to-long-term bleeding risk in patients with CAD treated with antithrombotic drugs, including anticoagulants, remains to be investigated. The present study is an extension of the above study on the applicability of the T-TAS in the field of whole blood thrombogenicity. Specifically, we investigated the association between the inhibition of thrombus formation, measured quantitatively by T-TAS, and the incidence of bleeding events within one year of treatment.

Methods

Study Population and Protocol

A total of 871 consecutive patients who were admitted for diagnosis or treatment of ischemic heart disease (IHD) at Kumamoto University Hospital and underwent coronary angiography (CAG) between August 2013 and September 2016 were screened in this study. After exclusion of 310 ACS patients who underwent urgent CAG and PCI, required more than one CAG, received hemodialysis, or refused signing the consent form, the remaining 561 patients who underwent elective CAG or PCI were enrolled in the present study (**Fig. 1**).

The study protocol was approved by the human ethics committee of Kumamoto University, and informed consent was obtained from each patient or family of the subject.

Antiplatelet Therapy

All patients were being treated with 100 mg/day

oral aspirin long before PCI, and then this was combined with clopidogrel or prasugrel before the scheduled PCI. For patients treated with clopidogrel, the maintenance dose before admission was 75 mg/day. If no such treatment had been administered previously, then the patient was treated with a loading dose 300 mg of clopidogrel the night before the first CAG or PCI.

The maintenance dose of prasugrel used in Japan is 3.75 mg/day, which was used by some of the patients before hospitalization. For prasugrel-naïve patients, they received 20 mg loading dose the night before the first CAG or PCI.

Thrombogenicity Measured by T-TAS

Blood samples were obtained using a 6 Fr sheath inserted into the femoral vein before treatment with unfractionated heparin just before the first CAG or at the time of PCI. All blood samples of patients treated with DOACs were obtained 2–4 h after taking DOACs, and those of patients treated with warfarin were obtained after the doses were fixed in the steady state.

T-TAS is an automated microchip-based flow chamber system developed for the easy and rapid assessment of platelet thrombus formation under certain flow conditions. Briefly, this system analyzes different thrombus formation processes with a simple procedure using two microchips coated with different thrombogenic surfaces. One chip, the platelet chip (PL), is coated with type I collagen. Inside the microchip, platelets adhere and aggregate on the surface of the collagen, and microchip capillaries are occluded. The other chip, the atheroma chip (AR), is covered with type I collagen and tissue thromboplastin. Inside the microchips, the platelets are simultaneously activated with the triggering of the coagulation system by collagen and tissue thromboplastin The process of thrombus formation inside the two chips was analyzed by monitoring the flow pressure change. The area under the curve (AUC) for the flow pressure was computed to assess platelet thrombogenicity inside the microchips. The PL_{24}-AUC_{10} parameter represents the AUC for the first 10 minutes for the PL tested at a flow rate of 24 μL/min, and AR_{10}-AUC_{30} is the parameter representing the AUC for the first 30 minutes for the AR tested at a flow rate of 10 μL/min.

The prothrombin time-international normalized ratio (PT-INR) and activated partial thromboplastin time (APTT) were measured using commercially available thromboplastin reagents (Coagupia PT-N and Coagupia APTT-N, respectively; Sekisui Medical, Tokyo) in accordance with the instructions provided by the manufacturer. PT-INR and APTT are well-known markers of anticoagulation effects.

Clinical Outcome

We collected 1-year follow-up data from the medical records, patients, and their families. The primary endpoint was bleeding events, and the secondary endpoint was the composite endpoint including bleeding, all-cause death, and major adverse cardiac events (MACE) during the follow-up period that began from the date of the first CAG or PCI to that of the first event or until September 2017. We excluded periprocedural bleeding events in patients who underwent PCI. We defined MACE as cardiac death, hospitalization for acute myocardial infarction unstable angina, and stroke. We defined bleeding events through the Platelet Inhibition and Patient Outcomes (PLATO) criteria. Major life-threatening bleeding was defined as fatal bleeding, intracranial bleeding, intrapericardial bleeding with cardiac tamponade, hypovolemic shock or severe hypotension due to bleeding and requiring the use of pressors or surgery, a decline in the hemoglobin level of 5.0 g per deciliter or more, or the need for transfusion of at least 4 units of red cells. Other major bleeding events were defined as bleeding that led to clinically significant disability (e.g., intraocular bleeding with permanent vision loss) or bleeding either associated with a drop in the hemoglobin level of at least 3.0 g/dL but less than 5.0 g/dL or requiring transfusion of 2 to 3 units of erythrocytes. Minor bleeding was defined as any bleeding that required medical intervention but not meeting the criteria for major bleeding[18].

Statistical Analysis

Data of normally distributed continuous variables are expressed as mean ± SD, whereas those with skewed distribution are described as median values (interquartile range, IQR) and categorical variables as frequencies and percentages. Group comparisons were analyzed by the unpaired *t* test or Mann-Whitney *U* test for continuous variables between two groups and by one-way analysis of variance or Kruskal–Wallis test for continuous variables followed by multiple comparison with the Bonferroni method among the three groups and the chi-square test or Fisher's exact test for categorical variables as appropriate. A log-rank test for MACE-free survival curves was performed. Cox proportional hazards regression analysis was used to compute hazards ratios (HRs) and 95% confidential interval (CI) as estimates of bleeding events. Logistic regression analysis was performed to compute odds ratios (ORs) and 95% CI as estimates of lowering of the AR_{10}-AUC_{30} levels. Traditional coagulation markers, namely, platelet count and T-TAS parameter, were

Table 1. Clinical characteristics of the entire cohort and comparison of baseline demographics, clinical parameters among the three groups

Variables	Total ($n=561$)	low AR_{10}-AUC_{30} ($n=187$)	Intermediate AR_{10}-AUC_{30} ($n=187$)	High AR_{10}-AUC_{30} ($n=187$)	*P* value
Age, years	70.7 ± 10.7	72.9 ± 9.5	70.7 ± 11.5	68.4 ± 10.7	< 0.001
Male (%)	390 (69.5)	135 (71.8)	127 (68.3)	128 (68.4)	0.70
BMI, kg/m^2	23.9 ± 3.8	23.8 ± 4.0	24.2 ± 3.8	23.9 ± 3.7	0.53
Hypertension, *n* (%)	467 (83.2)	166 (88.3)	156 (84.3)	145 (77.5)	0.02
Dyslipidemia, *n* (%)	444 (79.1)	149 (79.7)	147 (79.5)	148 (79.6)	1.00
Diabetes, *n* (%)	277 (49.4)	90 (48.1)	97 (52.4)	90 (48.1)	0.63
CKD, *n* (%)	204 (36.4)	86 (45.7)	67 (36.2)	51 (27.3)	0.001
Current smoking, *n* (%)	74 (13.4)	25 (13.3)	18 (9.8)	31 (16.8)	0.14
Family history of IHD, *n* (%)	126 (23.0)	42 (22.7)	42 (22.7)	42 (22.7)	1.00
OMI, *n* (%)	184 (33.6)	71 (38.4)	60 (32.6)	53 (29.0)	0.15
History of PCI, *n* (%)	285 (50.8)	94 (50.8)	99 (53.8)	92 (50.3)	0.77
CCB, *n* (%)	325 (57.9)	117 (63.9)	107 (58.8)	101 (55.8)	0.28
β-Blocker, *n* (%)	315 (58.0)	110 (60.1)	110 (60.1)	95 (52.5)	0.22
ARB/ACE-I, *n* (%)	339 (60.4)	121 (66.1)	117 (64.3)	101 (55.8)	0.10
Statins, *n* (%)	436 (77.7)	147 (80.3)	148 (81.3)	141 (77.9)	0.71
Aspirin, *n* (%)	520 (92.7)	177 (94.1)	174 (93.5)	169 (90.4)	0.32
Clopidogrel, *n* (%)	398 (71.5)	134 (71.3)	134 (71.3)	130 (69.5)	0.86
Prasugrel, *n* (%)	90 (16.0)	32 (17.1)	34 (18.3)	24 (12.9)	0.33
Other antiplatelet agents, *n* (%)	26 (4.7)	11 (5.9)	7 (3.8)	8 (4.3)	0.61
DOAC, *n* (%)	14 (2.5)	10 (5.3)	1 (0.5)	3 (1.6)	0.008
Warfarin, *n*(%)	43 (7.7)	23 (12.2)	15 (8.1)	5 (2.7)	0.002
EF (%)	60.1 ± 9.4	59.4 ± 10.5	60.7 ± 8.8	60.0 ± 8.8	0.43
Hb (g/dL)	13.0 ± 1.90	12.7 ± 1.82	12.9 ± 1.78	13.5 ± 2.01	< 0.001
Platelet count (10^3μL)	203 ± 57.4	176 ± 52.0	202 ± 49.2	232 ± 57.1	< 0.001
PT-INR	1.1 ± 0.30	1.19 ± 0.43	1.06 ± 0.19	1.02 ± 0.16	< 0.001
APTT (sec)	32.5 ± 6.0	33.6 ± 6.2	32.4 ± 5.9	31.7 ± 5.7	0.008

Data are mean ± SD, or *n* (%). Data for this parameter were measured at admission.
BMI; body mass index, CKD; chronic kidney disease, ACE-I; angiotensin-converting enzyme inhibitor, ARB; angiotensin II receptor blocker, CCB; calcium channel blocker, PPI; proton pomp inhibitor, DOAC; direct oral anticoagulant, OMI; old myocardial infarction, EF; left ventricular ejection fraction, Hb; hemoglobin, Hct; hematocrit, PT; prothrombin time, INR; international normalized ratio, APTT; activated partial thrombin time, IHD; ischemic heart disease, PCI; percutaneous coronary intervention, SD; standard deviation.

entered through the forced entry method in the multivariate model. A two-tailed *P* value of <0.05 denoted a statistically significant difference. All statistical analyses were performed with the Statistical Package for the Social Sciences software version 23 (IBM Corporation, Armonk, NY).

Results

T-TAS Parameters and Baseline Characteristics

The AR_{10}-AUC_{30} levels ranged from 24.8 to 2004, with a median value of 1686, and the 25th to 75th percentiles were 1541 to 1796. The respective values for the PL_{24}-AUC_{10} levels were 0.7–466, 99.3, and 43.2 to 173.6. The baseline characteristics are shown in **Table 1**. We categorized the 561 patients into three groups according to the AR_{10}-AUC_{30} levels: the low AR_{10}-AUC_{30} ($n=187$, AR_{10}-$AUC_{30} \leq 1603$), the intermediate AR_{10}-AUC_{30} ($n=187$, $1603 < AR_{10}$-$AUC_{30} \leq 1765$), and the high AR_{10}-AUC_{30} ($n=187$, $1765 < AR_{10}$-AUC_{30}) groups. Significant difference was observed among the three groups in terms of age, hypertension, chronic kidney disease (CKD), defined as estimated glomerular filtration rate <60 mL/min per 1.73 m^2, oral administration of DOAC or warfarin, hemoglobin level, platelet count, PT-INR, and APTT. Patients of the low AR_{10}-AUC_{30} group were more likely to be hypertensive, have CKD, and on anticoagulation treatments and had lower hemoglobin, lower platelet counts, higher APTT, and higher PT-INR among the three groups. Multiple logistic regression analysis identified platelet count and PT-INR to be associated with low AR_{10}-AUC_{30} levels (**Table 2**).

Table 2. Results of logistic regression analysis for low AR_{10}-AUC_{30} levels

	Simple regression analysis		Multiple regression analysis			
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age	1.03 (1.01-1.05)	0.001	1.00 (0.97-1.03)	0.84	1.00 (0.97-1.03)	0.82
Male	1.18 (0.80-1.73)	0.40				
Obesity (BMI >25 kg/m^2)	0.89 (0.62-1.30)	0.56				
Hypertension	1.78 (1.06-2.98)	0.028	1.75 (0.76-4.04)	0.19	1.81 (0.77-4.22)	0.17
Dyslipidemia	1.01 (0.65-1.56)	0.96				
Diabetes	0.92 (0.65-1.31)	0.63				
CKD	1.82 (1.27-2.60)	0.001	1.38 (0.77-2.49)	0.29	1.46 (0.81-2.64)	0.21
Use of aspirin	1.41 (0.69-2.88)	0.35				
Use of clopidogrel	1.03 (0.70-1.51)	0.90				
Use of prasugrel	1.12 (0.70-1.79)	0.64				
Use of DOAC	5.20 (1.60-16.8)	0.006	3.83 (0.46-32.2)	0.22	2.22 (0.26-19.4)	0.47
Use of Warfarin	2.46 (1.31-4.61)	0.005	2.49 (0.92-6.71)	0.71		
Hb	0.86 (0.78-0.94)	0.002	0.89 (0.75-1.06)	0.20	0.89 (0.74-1.06)	0.19
Platelet count	0.99 (0.98-0.99)	<0.001	0.98 (0.97-0.99)	<0.001	0.98 (0.97-0.99)	<0.001
APTT	1.04 (1.01-1.08)	0.006	1.01 (0.97-1.06)	0.58	1.00 (0.95-1.05)	0.87
PT-INR	7.12 (3.28-15.5)	<0.001			4.56 (1.49-13.9)	0.008

Data are mean ± SD, or *n* (%). See Table 1 for abbreviations.

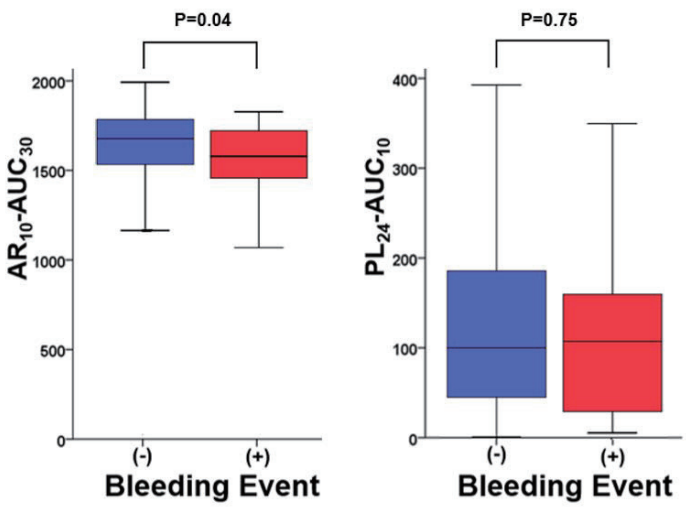

Fig. 2. T-TAS parameters in patients with and without bleeding

(A) AR_{10}-AUC_{30}; (B) PL_{24}-AUC_{10}. In the box-and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively.

Primary and Secondary Endpoints

We identified 21 patients (21/561, 3.7%) who developed bleeding events. Eight (1.4%) patients developed major bleeding complications (5 gastrointestinal bleeding, 2 intracranial bleeding, and 1 retinal bleeding). Three of the patients with gastrointestinal bleedings required >2 units of blood transfusion. Minor bleeding was noted in 13 (2.3%) patients (2 nasal bleeding, 2 hemoptysis, 2 frank hematuria, 3 minor gastrointestinal bleeding, 2 retinal hemorrhage, 1 stomatorrhagia, and 1 rupture of pseudoaneurysm).

The baseline clinical characteristics of the bleeding and non-bleeding groups are shown in **Supplemental Table 1**. The analysis of the factors that influenced bleeding events showed higher prevalence of diabetes and oral administration of DOAC in the bleeding group compared with the non-bleeding group. However, there were no differences in other variables between the two groups.

We also examined the PL_{24}-AUC_{10} and AR_{10}-AUC_{30} levels in patients with and without bleeding events. As shown in **Fig. 2**, the AR_{10}-AUC_{30} levels

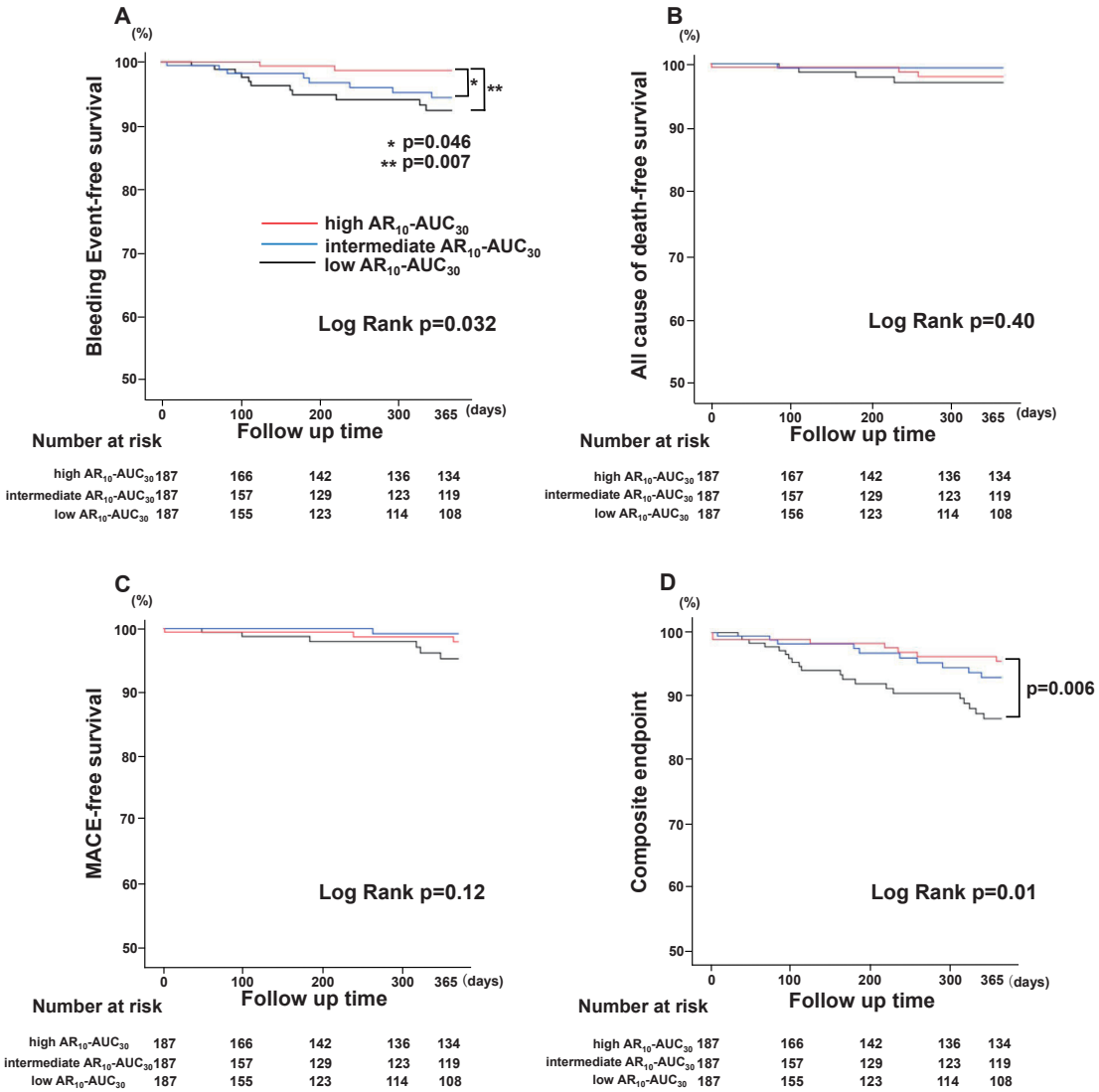

Fig. 3. Kaplan-Meier curves for (A) bleeding event-free survival; (B) all-cause death; (C) MACE; (D) composite endpoint for the three AR_{10}-AUC_{30} groups

were significantly lower in the bleeding group compared with the non-bleeding group (1590 [1442–1734] vs. 1687 [1546–1797], $p=0.04$), whereas the PL_{24}-AUC_{10} levels were almost identical in the two groups (107 [20.9–162] vs. 98.8 [43.6–174], $p=0.75$).

The Kaplan-Meier survival curves showed a higher rate of bleeding events in the low AR_{10}-AUC_{30} group compared with the high AR_{10}-AUC_{30} group ($p=0.007$, **Fig. 3A**). We also examined the composite endpoint, including bleeding, all-cause death, and MACE, during a median follow-up period of 16 months (IQR: 5.7–25.4 months). The numbers of bleeding events, MACE, and all-cause deaths were 21, 9, and 10, respectively, with a composite endpoint of 40. There were no significant differences among the three AR_{10}-AUC_{30} groups in terms of all-cause deaths ($p=0.40$) and MACE ($p=0.12$) (**Fig. 3B, C**). The Kaplan-Meier survival curve demonstrated a lower rate of the composite endpoint in the low AR_{10}-AUC_{30} group compared with the high AR_{10}-AUC_{30} group ($p=0.006$, **Fig. 3D**). We used Cox regression analysis to examine the factors that can predict bleeding events. Univariate Cox regression analysis of the entire patients group showed that diabetes, high PT-INR (higher than the median value), combinations of anticoagulants and antiplatelet therapy, and low AR_{10}-AUC_{30} correlated with 1-year bleeding events (**Table 3**). Multivariate Cox regression analysis (Models 1–4) indicated that low AR_{10}-AUC_{30} levels and combinations of anticoagulants and antiplatelet therapy were associated with 1-year bleeding events (**Table 4**).

Table 3. Results of univariate Cox proportional analysis for risk factors of bleeding events

	Univariate analysis	
	HR (95% CI)	*p* value
Old age (>75)	0.60 (0.23-1.55)	0.29
Male	1.82 (0.61-5.41)	0.28
Obesity (BMI >25 kg/m^2)	1.73 (0.74-4.08)	0.21
Hypertension	1.07 (0.31-3.62)	0.92
Dyslipidemia	1.47 (0.43-5.00)	0.53
Diabetes	3.83 (1.29-11.39)	0.02
CKD	1.50 (0.64-3.53)	0.35
Family history of IHD	0.55 (0.16-1.86)	0.33
OMI	1.78 (0.76-4.19)	0.19
History of PCI	2.41 (0.94-6.22)	0.07
Low Hb (<median)	1.65 (0.68-3.98)	0.26
Low platelet count (<median)	0.90 (0.38-2.11)	0.80
High APTT (>median)	1.11 (0.47-2.61)	0.81
High PT-INR (>median)	2.91 (1.13-7.49)	0.03
AR_{10}-AUC_{30}		
High	Ref	
Intermediate	4.30 (0.91-20.27)	0.07
Low	6.08 (1.35-27.45)	0.02
Single antiplatelet therapy	1.18 (0.27-5.05)	0.82
Dual antiplatelet therapy	1.04 (0.31-3.54)	0.95
Anticoagulants and antiplatelet therapy	3.82 (1.49-9.86)	0.005

Table 4. Results of multivariate Cox proportional hazard analysis for risk factors of bleeding events

	HR (95%CI)			
	Model 1	Model 2	Model 3	Model 4
AR_{10}-AUC_{30}				
high	Ref	Ref	Ref	Ref
intermediate	3.76 (0.79-17.83)	4.27 (0.91-20.11)	4.72 (0.99-22.46)	4.05 (0.86-19.11)
low	5.01 (1.10-22.95)	6.19 (1.36-28.12)	7.32 (1.57-34.10)	4.87 (1.05-22.53)
High PT-INR	2.35 (0.90-6.12)			
High APTT		0.96 (0.40-2.26)		
Low platelet count			0.61 (0.25-1.47)	
Anticoagulants and antiplatelet therapy				2.94 (1.11-7.80)

Discussion

In the present study, we investigated the association among 1-year bleeding events, MACE, all-cause deaths, composite endpoints, and T-TAS parameters in patients with CAD. Multivariate Cox hazard models showed that a low AR_{10}-AUC_{30} level, but not traditional coagulation markers and platelet count, was associated with a high risk of 1-year bleeding events in patients with CAD. Multiple logistic regression analysis showed that platelet count and PT-INR were associated with the lowering of AR_{10}-AUC_{30} levels. To the best of our knowledge, this is the first report to describe the usefulness of T-TAS as a tool for the prediction of 1-year bleeding events in patients with CAD. Although the results of the present study are somewhat similar in terms of the study concept to our previous report[17], which examined the association of short-term PCI-related periprocedural bleeding events with thrombogenicity measured by T-TAS, the present study analyzed the relationship between 1-year bleeding events and AR_{10}-AUC_{30} levels measured by T-TAS in patients with CAD on various antithrombotic therapies, including antiplatelet and anticoagulation therapies.

With regard to the primary endpoint, the present study showed major bleeding in 1.36% of the patients, with more than half being gastrointestinal bleeding, a finding consistent with that of Hamon *et al.*[5], who reported 0.6% BARC type ≥ 3 bleeding events (major bleeding events) per patient-year in patients with stable CAD, with more than half being gastrointestinal bleeding[5]. However, other major bleeding events were more frequent in the present study than the above study. Previous studies identified old age and diabetes as independent risk factors for major bleeding events[5, 19, 20]. The present study showed a significantly high prevalence of diabetes in the bleeding group (**Supplemental Table 1**). Furthermore, our patients were older and were more likely to be diabetics compared with the above study[5]. Thus, the two factors related to the study subjects might have influenced the high bleeding events noted in the present study.

Hamon *et al.* reported that long-term oral anticoagulation is the strongest risk factor for bleeding in patients with stable CAD[5]. As the use of anticoagulants, including warfarin and DOACs, is associated with a significant decrease in AR_{10}-AUC_{30} levels[15], this could be one of the reasons for the usefulness of AR_{10}-AUC_{30} levels in detecting bleeding events in patients with stable CAD treated with various antithrombotic agents, compared with PL_{24}-AUC_{10} levels. In addition, in the present study, the use of anticoagulation agents (warfarin and DOACs), old age, high prevalence of hypertension, CKD, low hemoglobin, low platelet counts, high APTT, and high PT-INR correlated with low AR_{10}-AUC_{30} levels. These results suggest that the AR_{10}-AUC_{30} is a comprehensive marker and potentially useful for the prediction of bleeding events in patients on and without anticoagulants.

In terms of the secondary endpoint, our results showed significant differences among the three AR_{10}-AUC_{30} groups in the composite endpoint but not in all-cause deaths and MACE, the latter being probably related to the low number of all-cause deaths (n=9, 1.6%) and MACE (n=10, 1.8%). The significant differences in composite endpoints were due to significant differences in bleeding events among the three groups. Accordingly, further large-population studies are needed to estimate the significance of antithrombotic agents in the development of these cardiovascular events.

Due to the difficulty in evaluating total thrombogenicity, which is influenced by various types of pharmacological effects, there are no studies that have investigated the association between total antithrombotic effects of various antithrombotic drugs and long-term bleeding events, excluding periprocedural bleeding. In this study, low levels of AR_{10}-AUC_{30} at the time of CAG were associated with 1-year bleeding events in patients with CAD. Further prospective studies of large number of patients are needed to establish the usefulness of AR_{10}-AUC_{30} measured by T-TAS as a predictor of long-term bleeding events in patients with CAD.

The present study has several limitations. First, the study was performed in a single center with a relatively small number of patients and clinical events. Further large-population studies are needed to estimate the association between AR_{10}-AUC_{30} levels measured by T-TAS and bleeding events. Second, because bleeding events were classified using only the PLATO criteria, further studies using other bleeding criteria are needed to evaluate the association between bleeding events and the use of antithrombotic agents. Third, we could not adequately assess changes in antithrombotic therapies after discharge from the hospital. The changes in the antithrombotic therapy after discharge might affect the bleeding events during the follow-up period. In the present study population (n=561) treated with the various antithrombotic therapies, the changes in antiplatelet therapies were observed in 303 cases during the 1-year follow-up period (data not shown). In detail, 294 patients changed DAPT to single antiplatelet therapy, 9 patients changed single antiplatelet therapy to DAPT, and the remaining 258 patients continued the same antiplatelet therapies during the 1-year follow-up period. These findings might be one of the reasons why T-TAS PL_{24}-AUC_{10} levels failed to predict the long-term bleeding events in the present study population treated with various antithrombotic therapies. By contrast, only 1 out of the 57 patients treated with antiplatelet and anticoagulants discontinued anticoagulant therapy with rivaroxaban because of a successful catheter ablation for AF, and the remaining 56 patients continued the same anticoagulant therapies during the 1-year follow-up period. It is possible that the T-TAS AR_{10}-AUC_{30} levels may be useful for predicting the long-term bleeding events in the real-world patient population with CAD.

Conclusion

The present study demonstrated that the AR_{10}-AUC_{30} levels measured by T-TAS can be a significant predictor of 1-year bleeding events in patients with CAD treated with various antithrombotic therapies.

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Disclosure

The authors declare no conflict of interest.

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Supplemental Table 1. Comparison of baseline demographics and clinical parameters between the bleeding and non-bleeding groups

Total = 561	Bleeding group (*n* = 21)	Non-bleeding group (*n* = 540)	*P* Value
Age, years	68.8 ± 7.8	70.8 ± 10.8	0.41
Male (%)	17 (81.0)	371 (69.2)	0.25
BMI, kg/m^2	25.2 ± 3.5	23.9 ± 3.8	0.12
Hypertension, *n* (%)	18 (85.7)	446 (83.4)	0.53
Dyslipidemia, *n* (%)	18 (85.7)	423 (79.4)	0.35
Diabetes, *n* (%)	17 (81.0)	259 (48.5)	0.003
CKD, *n* (%)	10 (47.6)	193 (36.1)	0.28
Current smoking, *n* (%)	4 (19.0)	70 (13.2)	0.31
Family history of IHD, *n* (%)	3 (14.3)	123 (23.3)	0.24
OMI, *n* (%)	10 (47.6)	174 (33.0)	0.25
CCB, *n* (%)	11 (52.4)	312 (59.8)	0.50
Use of β-Blocker, *n* (%)	16 (76.2)	299 (57.3)	0.09
Use of ARB/ACE-I, *n* (%)	15 (71.4)	322 (61.7)	0.37
Use of statins, *n* (%)	18 (85.7)	416 (79.7)	0.36
Use of aspirin, *n* (%)	19 (90.5)	497 (92.7)	0.47
Use of clopidogrel, *n* (%)	13 (61.9)	385 (71.8)	0.32
Use of prasugrel, *n* (%)	4 (19.0)	84 (15.7)	0.43
Other antiplatelet agents, *n* (%)	2 (9.5)	24 (4.5)	0.26
DOAC, *n* (%)	3 (14.3)	11 (2.1)	0.01
Warfarin, *n* (%)	3 (14.3)	40 (7.5)	0.22
EF (%)	59.2 ± 9.3	60.1 ± 9.4	0.66
Hb (g/dL)	12.6 ± 1.7	13.1 ± 1.9	0.27
Platelet count (10^{3}μL)	208 ± 64.0	203.0 ± 57.3	0.73
PT-INR	1.25 ± 0.43	1.08 ± 0.29	0.10
APTT(sec)	34.6 ± 8.5	32.5 ± 9.4	0.11

Data are mean ± SD, or *n* (%).
See Table 1 for abbreviations.