# 学位論文

**Increased thrombogenicity is associated with revascularization outcomes in patients with chronic limb-threatening ischemia**
**（包括的高度慢性下肢虚血患者において亢進した血栓形成能は再血行再建イベントと関連する）**

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# Increased thrombogenicity is associated with revascularization outcomes in patients with chronic limb-threatening ischemia

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## ABSTRACT

**Objectives:** Clinically driven target lesion revascularization (CD-TLR) frequently occurs after endovascular therapy (EVT) in patients with chronic limb-threatening ischemia (CLTI). The total thrombus-formation analysis system (T-TAS) can quantitatively evaluate thrombogenicity. Therefore, we aimed to elucidate the association of the T-TAS parameters with CD-TLR.

**Methods:** We analyzed 34 patients with CLTI and 62 patients without CLTI who had undergone EVT. Blood samples collected on the day of EVT were used in the T-TAS to compute the thrombus formation area under the curve for the first 10 minutes for the platelet chip tested at a flow rate of 24 μL/min ($PL_{24}$-$AUC_{10}$) and area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min ($AR_{10}$-$AUC_{30}$). After EVT, clinical follow-up was performed, and the presence of CD-TLR was assessed.

**Results:** During the follow-up period (median, 574 days), 10 patients (29%) in the CLTI group and 11 (18%) in the non-CLTI group had required CD-TLR. In the CLTI group, the patients with CD-TLR had had a higher $AR_{10}$-$AUC_{30}$ vs those without (median, 1694 [interquartile range, 1657-1799] vs median, 1561 [interquartile range, 1412-1697]; $P = .01$). In contrast, the $PL_{24}$-$AUC_{10}$ showed no significant differences when stratified by CD-TLR in either group. For the CLTI patients, multivariable Cox regression analysis using propensity score matching revealed that the $AR_{10}$-$AUC_{30}$ was an independent predictor of CD-TLR even after adjusting for baseline demographics, lesion characteristics, and anticoagulant use (hazard ratio, 2.04; 95% confidence interval, 1.18-3.88; $P = .01$; per 100-unit increase). In contrast, for those without CLTI, neither the $AR_{10}$-$AUC_{30}$ nor the $PL_{24}$-$AUC_{10}$ was significantly associated with CD-TLR. Receiver operating characteristics curve analysis identified an $AR_{10}$-$AUC_{30}$ level of 1646 as an optimal cutoff value to predict for CD-TLR (AUC, 0.85; sensitivity, 0.93; specificity, 0.56).

**Conclusions:** For patients with CLTI, but not for those without CLTI, the $AR_{10}$-$AUC_{30}$ showed potential to predict for CD-TLR. This finding suggests that hypercoagulability might play a predominant role in the progression of CLTI and that anticoagulant therapy might be useful in preventing revascularization. (J Vasc Surg 2022;76:513-22.)

**Keywords:** $AR_{10}$-$AUC_{30}$; Chronic limb-threatening ischemia; T-TAS; Target lesion revascularization

Chronic limb-threatening ischemia (CLTI) is considered the most severe manifestation of peripheral artery disease (PAD), and effective revascularization is the cornerstone of limb salvage. In the past decade, endovascular therapy (EVT) has evolved into the mainstream treatment of revascularization in patients with CLTI by the advancements in devices and techniques.[1,2] However, the long-term patency after intervention for

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below-the-knee (BTK) lesions, often present in patients with CLTI,[3] has remained low.[4,5]

Although previous reports have identified some lesion characteristics[6-10] and patient comorbidities[8,11,12] as predictors of restenosis after EVT, few biomarkers have been established to predict for restenosis or reocclusion. A recent pathologic study histologically characterized the lower leg vessels of patients with CLTI and found the presence of thrombotic luminal occlusion without significant atherosclerosis, suggesting the possibility that thromboembolic phenomena might play an important role in reocclusion after EVT, especially in patients with CLTI. These observations suggest the presence of distinct pathologic mechanisms underlying CLTI and non-CLTI cases and highlight the importance of thrombogenicity assessments of patients with PAD undergoing EVT.

The total thrombus-formation analysis system (T-TAS), a novel system developed to quantitatively analyze thrombus formation using microchips with thrombogenic surfaces, has been validated in previous studies and can be used to evaluate total thrombogenicity.[13-15] This system can analyze different thrombosis formation processes. The platelet (PL) chip reflects the platelet activation pathway, and the atheroma (AR) chip evaluates platelet activation and subsequent coagulation cascade. These two distinct properties of each T-TAS parameter have the potential to clarify the underlying mechanism of thrombotic disease.

Considering these characteristics of the T-TAS, we investigated the association between the thrombogenicity evaluated using the T-TAS and the clinical outcomes after EVT.

## ARTICLE HIGHLIGHTS

- **Type of Research:** A single-center, observational study to examine whether thrombogenicity, evaluated using the total thrombus-formation analysis system, would predict the occurrence of adverse clinical events, including clinically driven target lesion revascularization (CD-TLR) in chronic limb-threatening ischemia (CLTI)
- **Key Findings:** Of the CLTI and non-CLTI groups, 29% and 18% had required CD-TLR, respectively. We found that a higher $AR_{10}$-$AUC_{30}$ (area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min) level, a measure that suggests that the entire coagulation cascade has been activated, was associated with CD-TLR in the CLTI group but not in the non-CLTI group. Multivariable Cox regression also revealed a higher $AR_{10}$-$AUC_{30}$ level as an independent predictor of CD-TLR in those with CLTI.
- **Take Home Message:** Hypercoagulability, indicated by a higher $AR_{10}$-$AUC_{30}$ level, might play an important role in the progression of CLTI, and anticoagulant therapy might be useful in preventing revascularization, especially for patients with CLTI and a higher $AR_{10}$-$AUC_{30}$ level.

## METHODS

**Study population and protocol.** We conducted a single-center, retrospective observational study using the Kumamoto EVT registry database (UMIN clinical trial registration no. 000034702). The flow chart for patient recruitment for the present study is shown in Fig 1. We enrolled 130 consecutive patients who had been admitted for the diagnosis of PAD at Kumamoto University Hospital and had undergone elective peripheral angiography between January 2018 and April 2021. Of these 130 patients, 34 were excluded from our analysis for the following reasons: EVT failure (n = 5), did not undergo EVT (only angiography; n = 7), received hemodialysis (n = 21), or had undergone EVT of an upper limb artery (n = 1). The hemodialysis patients were excluded because hemodialysis has been reported to affect the T-TAS parameters.[16] Thus, 96 consecutive patients were included in the present analysis. The patients were divided into two groups: those with CLTI (CLTI group; n = 34) and those without CLTI (non-CLTI group; n = 62). CLTI was defined as chronic rest pain and/or the presence of tissue loss (ulcer or gangrene) in the lower extremities secondary to ischemia with a duration of >2 weeks.[1] Additionally, each group was divided into two subgroups: those who had required clinically driven target lesion revascularization (CD-TLR) during the follow-up period and those who had not required CD-TLR. CD-TLR was defined as any repeat percutaneous intervention or bypass surgery performed on the target lesion with deterioration of the Rutherford category and/or an increase in the size of preexisting wounds and/or the occurrence of new wounds.[17]

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and its amendments. The human ethics committee of Kumamoto University approved the study protocol (approval no. 1655). The requirement for patient informed consent was waived because of the low-risk nature of our retrospective study and the inability to obtain consent directly from all participants. We extensively promoted the study protocol at Kumamoto University Hospital and on our website (available at: http://www.kumadai-junnai.com) and gave the patients the opportunity to withdraw from the study.

**T-TAS measurement.** Thrombogenic activity was measured using the T-TAS (Fujimori Kogyo Co, Tokyo, Japan), which is an automated microchip-based flow chamber system for the analysis of thrombus formation under different flow conditions, as described previously.[13,15,18,19] The T-TAS has been used as a medical

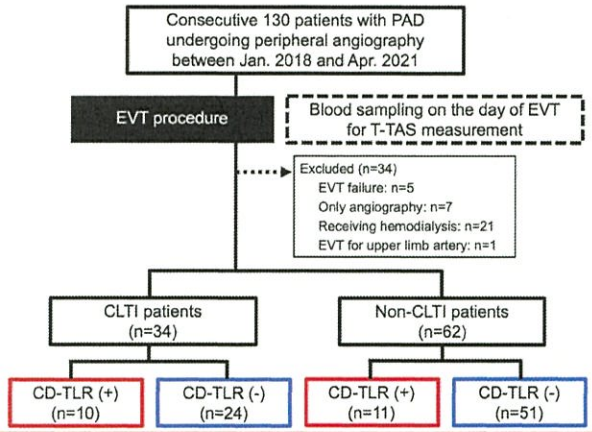



**Fig 1.** Study flow chart. *CD-TLR,* Clinically driven target lesion revascularization; *CLTI,* chronic limb-threatening ischemia; *EVT,* endovascular therapy; *PAD,* peripheral artery disease; *T-TAS,* total thrombus-formation analysis system.

instrument in the field of thrombosis and hemostasis in 17 European countries since March 2019 and in the United States since February 2020. In brief, this system analyzes different thrombus formation processes with a simple procedure using two microchips coated with different thrombogenic surfaces. The PL chip is specifically designed for the quantitative analysis of the platelet thrombus formation process involving platelet adhesion and aggregation, granule secretion, and thrombus growth under arterial flow conditions. The PL chip is coated with type I collagen. Inside the microchip, platelets adhere and aggregate on the surface of the collagen, and the microchip capillaries are occluded. The AR chip is specifically designed for the quantitative analysis of the white thrombus formation process mediated by the combined activation of platelets and the coagulation system under venous flow conditions. The AR chip is covered with type I collagen and tissue thromboplastin. Inside the microchip, platelets are activated by triggering of the coagulation system by collagen and tissue thromboplastin. The process of thrombus formation inside the two chips is analyzed by monitoring the changes in flow pressure. The area under the curve (AUC) for flow pressure was computed to assess the platelet thrombogenicity inside the microchips. The $PL_{24}$-$AUC_{10}$ parameter represents the AUC for the first 10 minutes for the PL chip at a flow rate of 24 μL/min (ie, under arterial flow conditions). The $AR_{10}$-$AUC_{30}$ represents the AUC for the first 30 minutes for the AR chip at a flow rate of 10 μL/min (ie, under venous flow conditions).

The T-TAS parameters were measured on the day of EVT. During the EVT procedure, blood samples for the T-TAS were obtained from a sheath inserted into the arteries before injection of unfractionated heparin just before peripheral angiography.

**EVT procedure and follow-up assessment.** The patients with CLTI had undergone EVT for relief of pain, wound healing, and limb preservation. The patients without CLTI had undergone EVT because of intermittent claudication. The devices and approach site used for EVT and postprocedural management, including antithrombotic agents, were left to the discretion of each physician. The characteristics of the lesions evaluated using peripheral angiography were recorded and classified. For the CLTI patients, in accordance with expert guidelines,[20] the femoropopliteal and infrapopliteal lesions were categorized using the Global Limb Anatomic Staging System to evaluate anatomic complexity. Poor distal runoff was defined as one vessel or no vessels with infrapopliteal runoff as assessed by angiography after the procedure.[21] Clinical follow-up was performed after EVT. During the follow-up period, the incidence of adverse events, including all-cause death, amputation, major adverse cardiovascular events (MACE), and CD-TLR, was assessed. MACE were defined as a composite of cardiovascular death, nonfatal myocardial infarction, and stroke.

**Statistical analysis.** Continuous variables are presented as the mean ± standard deviation and were compared using the *t* test if the data were normally distributed. Non-normally distributed continuous data are presented as the median and interquartile range (IQR) and were compared using the Wilcoxon signed rank test. Categorical data are presented as frequencies and proportions and were compared using the $\chi^2$ test. Cox proportional hazards regression analysis was used to compute the hazard ratios (HRs) and 95% confidence intervals (CIs) as estimates of the requirement for CD-TLR and was conducted to elucidate the association between each T-TAS parameter and the requirement for CD-TLR in patients with PAD. In addition, owing to the limited number of events, we used a propensity score-adjusted model to reduce the covariate differences between the patients who had and had not required CD-TLR. Propensity scores were calculated using a binary logistic regression analysis with the following baseline covariates or lesion characteristics previously reported to be associated with CD-TLR or the T-TAS parameters: for CD-TLR, age,[7] sex,[7] BTK lesions,[8] occlusion lesions at baseline,[9] restenotic lesions,[9] lesion length,[6,7] poor runoff,[22] and treatment with plain old balloon angioplasty (POBA) alone[23]; for T-TAS parameters, prothrombin time–international normalized ratio (PT-INR),[19] activated partial thromboplastin time (APTT),[19] and antithrombotic agents ($AR_{10}$-$AUC_{30}$: anticoagulant agents[15]; $PL_{24}$-$AUC_{10}$, DAPT[13]). A receiver operating characteristic (ROC) curve analysis was performed to assess the ability of the $AR_{10}$-$AUC_{30}$ level to predict for CD-TLR and to identify the optimal cutoff $AR_{10}$-$AUC_{30}$ level for the prediction of CD-TLR. According to the cutoff $AR_{10}$-$AUC_{30}$ level calculated by ROC curve analysis, we divided

**Table I.** Comparison of baseline clinical demographics stratified by chronic limb-threatening ischemia (*CLTI*) and clinically driven target lesion revascularization (*CD-TLR*)

| | CLTI group (n = 34) | | | Non-CLTI group (n = 62) | | |
|---|---|---|---|---|---|---|
| Variable | CD-TLR (n = 10) | No CD-TLR (n = 24) | *P* value | CD-TLR (n = 11) | No CD-TLR (n = 51) | *P* value |
| Age, years | 78.3 ± 9.9 | 74.2 ± 10.8 | .31 | 68.2 ± 10.1 | 73.0 ± 9.1 | .12 |
| Female sex | 8 (80) | 9 (38) | .02 | 3 (27) | 14 (27) | .99 |
| BMI, kg/m$^2$ | 20.9 ± 3.5 | 21.2 ± 3.2 | .82 | 22.4 ± 2.9 | 23.0 ± 3.1 | .55 |
| Hypertension | 8 (80) | 19 (79) | .96 | 9 (82) | 43 (84) | .84 |
| Diabetes mellitus | 5 (50) | 13 (54) | .82 | 6 (55) | 26 (51) | .83 |
| Dyslipidemia | 7 (70) | 17 (71) | .96 | 11 (100) | 41 (80) | .04 |
| Atrial fibrillation | 2 (20) | 1 (4) | .16 | 1 (9) | 7 (14) | .67 |
| CAD | 6 (60) | 11 (46) | .45 | 10 (91) | 30 (59) | .03 |
| Biochemistry data | | | | | | |
| eGFR, mL/min/1.73 m$^2$ | 62.1 ± 14.6 | 63.7 ± 22.5 | .84 | 62.6 ± 30.2 | 60.0 ± 17.9 | .70 |
| CRP, mg/dL | 0.34 (0.16-1.05) | 1.68 (0.37-2.62) | .04 | 0.14 (0.08-0.36) | 0.12 (0.05-0.30) | .34 |
| HbA1c, % | 6.3 ± 0.5 | 6.5 ± 1.4 | .62 | 6.6 ± 1.0 | 6.6 ± 1.0 | 1.00 |
| LDL-C, mg/dL | 93 ± 45 | 90 ± 32 | .82 | 90 ± 34 | 92 ± 27 | .84 |
| Hemoglobin, g/dL | 11.9 ± 1.6 | 11.0 ± 2.1 | .26 | 12.6 ± 1.8 | 13.0 ± 2.0 | .56 |
| Platelet count, 10$^4$/μL | 24.0 ± 9.3 | 26.3 ± 9.6 | .54 | 21.3 ± 4.3 | 23.8 ± 7.1 | .27 |
| PT-INR | 1.26 ± 0.55 | 1.10 ± 0.35 | .36 | 1.13 ± 0.38 | 1.02 ± 0.19 | .17 |
| APTT, seconds | 34.4 ± 8.7 | 32.7 ± 5.1 | .50 | 32.6 ± 6.6 | 29.3 ± 4.1 | .03 |
| D-dimer, μg/mL | 2.56 ± 2.66 | 2.11 ± 2.19 | .64 | 1.55 ± 0.98 | 1.13 ± 0.61 | .19 |
| T-TAS parameter | | | | | | |
| $PL_{24}$-$AUC_{10}$ | 169 (52-347) | 257 (102-394) | .37 | 193 (159-366) | 218 (86-349) | .96 |
| $AR_{10}$-$AUC_{30}$ | 1694 (1657-1799) | 1561 (1412-1697) | .01 | 1528 (1353-1829) | 1683 (1579-1820) | .08 |
| Medication use at EVT | | | | | | |
| Aspirin | 6 (60) | 12 (50) | .59 | 7 (64) | 28 (55) | .59 |
| Clopidogrel | 5 (50) | 10 (42) | .66 | 5 (45) | 25 (49) | .83 |
| DAPT[a] | 4 (40) | 7 (29) | .54 | 5 (45) | 16 (31) | .37 |
| Anticoagulant[b] | 2 (20) | 4 (17) | .82 | 3 (27) | 7 (14) | .29 |
| Statin | 7 (70) | 13 (54) | .39 | 10 (91) | 35 (69) | .10 |

*APTT*, Activated partial thromboplastin time; *$AR_{10}$-$AUC_{30}$*, area under the curve for the first 30 minutes for the atheroma chip tested at flow rate of 10 μL/min; *BMI*, body mass index; *CAD*, coronary artery disease; *CRP*, C-reactive protein; *DAPT*, dual antiplatelet therapy; *eGFR*, estimated glomerular filtration rate; *HbA1c*, hemoglobin A1c; *LDL-C*, low-density lipoprotein cholesterol; *$PL_{24}$-$AUC_{10}$*, area under the curve for the first 10 minutes for the platelet chip tested at flow rate of 24 μL/min; *PT-INR*, prothrombin time–international normalized ratio; *T-TAS*, total thrombus-formation analysis system.

Data presented as mean ± standard deviation, number (%), or median (interquartile range).

[a]Defined as the combination of aspirin plus a P2Y12 inhibitor.

[b]Defined as the use of warfarin or a direct oral anticoagulant.

the CLTI patients into two groups, and Kaplan-Meier curve analysis of CD-TLR for each group was conducted. $P < .05$ was considered statistically significant. All analyses were performed using IBM SPSS Statistics, version 26 (IBM Corp, Armonk, NY).

## RESULTS

**Baseline clinical demographics and lesion characteristics.** During a median follow-up period of 574 days (IQR, 282-863 days), 10 patients (29%) in the CLTI group and 11 patients (18%) in the non-CLTI group had required CD-TLR. First, we compared the baseline clinical demographics between the patients with and without CLTI (Supplementary Table I, online only). The CLTI patients were more often women and had a lower body mass index and hemoglobin level and a higher C-reactive protein level compared with those without CLTI. The T-TAS parameters, including the $PL_{24}$-$AUC_{10}$ level (median, 232 [IQR, 88-373]; vs median, 206 [IQR, 91-350]; $P = .73$) and $AR_{10}$-$AUC_{30}$ level (median, 1641 [IQR, 1458-1708]; vs median, 1671 [IQR, 1560-1822]; $P = .14$), among patients with and without CLTI were comparable. Two thirds of the patients with CLTI had received antiplatelet therapy, and 18% of those with CLTI had received an anticoagulant, comparable to the rates for those without CLTI, as a preprocedural antithrombotic regimen. Next, we compared the baseline clinical demographics between the patients who had and had not required CD-TLR in

**Table II.** Comparison of target lesion and procedural characteristics stratified by chronic limb-threatening ischemia (*CLTI*) and clinically driven target lesion revascularization (*CD-TLR*)

| | CLTI group (n = 34) | | | Non-CLTI group (n = 62) | | |
|---|---|---|---|---|---|---|
| **Variable** | **CD-TLR (n = 10)** | **No CD-TLR (n = 24)** | ***P* value** | **CD-TLR (n = 11)** | **No CD-TLR (n = 51)** | ***P* value** |
| Rutherford class | 4.9 ± 0.6 | 4.9 ± 0.4 | .92 | 2.5 ± 0.5 | 2.5 ± 0.5 | .60 |
| ABI at enrollment | 0.79 ± 0.21 | 0.72 ± 0.23 | .54 | 0.63 ± 0.09 | 0.65 ± 0.14 | .69 |
| Stenosis | 3 (30) | 10 (42) | .52 | 7 (64) | 22 (43) | .22 |
| Lesion length, cm | 11 (5-20) | 8 (4-20) | .74 | 4 (1-9) | 5 (3-11) | .29 |
| Total occlusion | 7 (70) | 14 (58) | .52 | 4 (36) | 29 (57) | .22 |
| Lesion length, cm | 11 (6-21) | 18 (11-21) | .30 | 30 (24-38) | 10 (5-19) | .003 |
| GLASS grade III[a] | 5 (56) | 8 (35) | .28 | NA | NA | NA |
| Recurrent lesion | 3 (30) | 2 (8) | .12 | 3 (27) | 4 (8) | .10 |
| Poor distal runoff | 8 (80) | 14 (58) | .21 | 5 (45) | 11 (22) | .10 |
| Lesion location | | | | | | NA |
| Above the knee | 5 (50) | 15 (63) | .50 | 11 (100) | 51 (100) | |
| Iliac artery | 3 (30) | 4 (17) | | 3 (27) | 22 (43) | |
| Common femoral artery | 0 (0) | 0 (0) | | 0 (0) | 1 (2) | |
| Superficial femoral artery | 1 (10) | 11 (46) | | 7 (64) | 25 (49) | |
| Popliteal artery | 1 (10) | 0 (0) | | 1 (9) | 3 (6) | |
| Below the knee | 5 (50) | 9 (37) | .50 | 0 (0) | 0 (0) | |
| Anterior tibial artery | 4 (40) | 6 (25) | | 0 (0) | 0 (0) | |
| Posterior tibial artery | 1 (10) | 1 (4) | | 0 (0) | 0 (0) | |
| Peroneal artery | 0 (0) | 2 (8) | | 0 (0) | 0 (0) | |
| Procedure | | | | | | |
| Stenting | 2 (20) | 12 (50) | .09 | 5 (45) | 32 (63) | .29 |
| Total stent length, cm | 7 (4-10) | 19 (10-27) | .12 | 6 (6-17) | 12 (6-16) | .41 |
| POBA alone | 8 (80) | 9 (38) | .02 | 5 (45) | 3 (6) | .002 |
| DCB | 0 (0) | 2 (8) | .23 | 0 (0) | 17 (33) | .005 |
| IVUS usage | 7 (70) | 22 (92) | .12 | 9 (82) | 51 (100) | .007 |
| Medication use after EVT | | | | | | |
| Aspirin | 6 (60) | 15 (63) | .89 | 8 (73) | 36 (71) | .89 |
| Clopidogrel | 4 (40) | 14 (58) | .33 | 7 (64) | 37 (73) | .56 |
| DAPT | 4 (40) | 9 (38) | .89 | 8 (73) | 35 (69) | .79 |
| Anticoagulant | 3 (30) | 3 (13) | .24 | 4 (36) | 8 (16) | .14 |

*ABI*, Ankle brachial index; *DAPT*, dual antiplatelet therapy; *DCB*, drug-coated balloon; *EVT*, endovascular therapy; *GLASS*, Global Limb Anatomic Staging System; *IVUS*, intravascular ultrasound; *NA*, not applicable; *POBA*, plain old balloon angioplasty.
Data presented as mean ± standard deviation, number (%), or median (interquartile range).
[a]Data available for 32 patients with CLTI.

both the CLTI and the non-CLTI groups (Table I). In the CLTI group, those who had required CD-TLR were more often women and had had a lower C-reactive protein level than those who had not required CD-TLR. However, no significant differences were found in age, renal function, platelet count, PT-INR, or APTT between the two subgroups. In the non-CLTI group, those who had required CD-TLR had had a greater prevalence of dyslipidemia and coronary artery disease and a higher APTT level than those who had not required CD-TLR. However, no significant differences in age, sex, and renal function were observed between the two subgroups. Also, no significant differences were found in the preprocedural antithrombotic regimens between the CLTI and non-CLTI groups regardless of whether they had required CD-TLR.

The baseline lesion and procedural characteristics are shown in Table II. In the CLTI patients, approximately one half of the target lesions had been located below the knee. In contrast, all target lesions in the non-CLTI patients had been located above the knee. As expected, in the CLTI patients, CD-TLR had frequently been required for total occlusive lesions or poor distal runoff lesions. In addition, treatment with POBA alone was significantly associated with the requirement for CD-TLR in both groups. Longer occlusive lesions had a significant

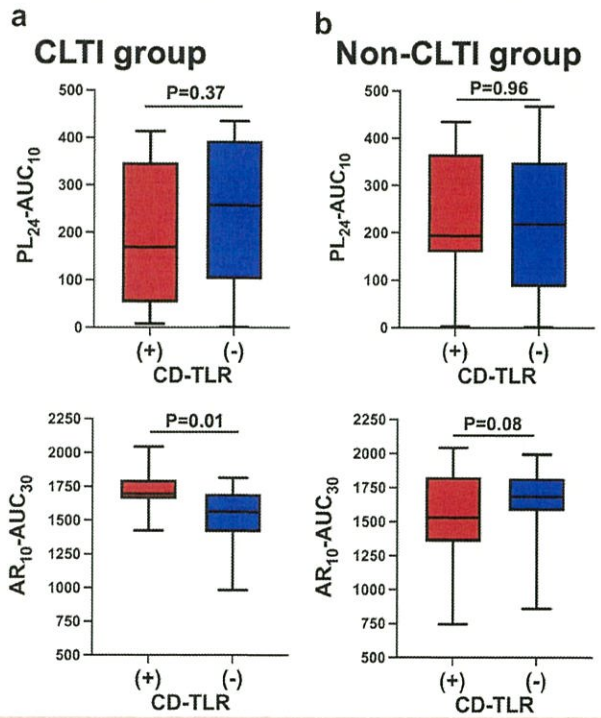



**Fig 2.** Comparison of total thrombus-formation analysis system (T-TAS) parameters between patients who had required clinically driven target lesion revascularization (*CD-TLR*) and those who had not in the chronic limb-threatening ischemia (*CLTI*) group **(a)** and non-CLTI group **(b)**. *$AR_{10}$-$AUC_{30}$*, Area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min; *$PL_{24}$-$AUC_{10}$*, area under the curve for the first 10 minutes for the platelet chip tested at a flow rate of 24 μL/min.

association with CD-TLR in the non-CLTI group. The frequency of Global Limb Anatomic Staging System grade III was comparable between the CLTI patients with and without CD-TLR. The postprocedural antithrombotic regimens used in the CLTI and non-CLTI groups were also comparable between the patients with and without CD-TLR.

**T-TAS parameters and clinical events.** In the non-CLTI group, neither the $PL_{24}$-$AUC_{10}$ level (median, 193 [IQR, 159-366]; vs median, 218 [IQR, 86-349]; $P = .96$) nor the $AR_{10}$-$AUC_{30}$ level (median, 1528 [IQR, 1353-1829]; vs median, 1683 [IQR, 1579-1820; $P = .08$) was significantly different. In contrast, in the CLTI group, the $AR_{10}$-$AUC_{30}$ level was significantly higher for the patients who had required CD-TLR than for those who had not (median, 1694 [IQR, 1657-1799]; vs median, 1561 [IQR, 1412-1697]; $P = .01$). However, no significant difference was found in the $PL_{24}$-$AUC_{10}$ level between the two subgroups (median, 169 [IQR, 52-347]; vs median, 257 [IQR, 102-394]; $P = .37$; Fig 2).

Adverse events other than CD-TLR are listed in Supplementary Table II (online only). Of the 34 patients with CLTI, 5 (15%) had died, 6 (18%) had required an amputation, and 4 (12%) had experienced MACE during the follow-up period. The $AR_{10}$-$AUC_{30}$ level was significantly higher in the CLTI patients with MACE than in those without MACE (median, 1749 [IQR, 1697-1792]; vs median, 1597 [IQR, 1423-1700]; $P = .03$). Obvious thrombosis without major plaques on intravascular ultrasound was present in the diseased segments in one CLTI patient and eight non-CLTI patients. In the non-CLTI patients, no significant difference was found in the $PL_{24}$-$AUC_{10}$ level (median, 295 [IQR, 137-412]; vs median, 164 [IQR, 79-342]; $P = .20$) or $AR_{10}$-$AUC_{30}$ level (median, 1665 [IQR, 1259-1811]; vs median, 1687 [IQR, 1590-1782]; $P = .57$) between those with and without thrombosis found on intravascular ultrasound.

**Association between T-TAS parameters and CD-TLR.** To elucidate the association between the T-TAS parameters and the requirement for CD-TLR, we performed a Cox proportional hazards regression analysis (Table III). In the CLTI group, an unadjusted model revealed that a higher $AR_{10}$-$AUC_{30}$ level (HR, 1.65; 95% CI, 1.04-2.68; $P = .03$; per 100-unit increase) but not a higher $PL_{24}$-$AUC_{10}$ level was significantly associated with the requirement for CD-TLR. In contrast, in the non-CLTI group, neither the $AR_{10}$-$AUC_{30}$ level nor the $PL_{24}$-$AUC_{10}$ level was significantly associated with the requirement for CD-TLR.

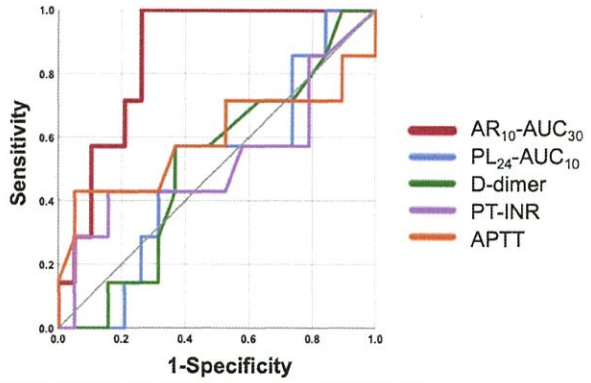
In the multivariable model using propensity score matching, for the CLTI group, the $AR_{10}$-$AUC_{30}$ level was still an independent predictor of CD-TLR even after adjustment for age, sex, PT-INR, APTT, BTK lesions, occlusion lesions at baseline, restenotic lesions, lesion length, poor distal runoff, treatment with POBA alone, and anticoagulant use (HR, 2.04; 95% CI, 1.18-3.88; $P = .01$; per 100 increase). However, the $PL_{24}$-$AUC_{10}$ level was not. In contrast, in non-CLTI patients, neither the $AR_{10}$-$AUC_{30}$ nor the $PL_{24}$-$AUC_{10}$ levels were significantly related to the requirement for CD-TLR in the propensity score-adjusted model.

We performed a ROC curve analysis to assess the ability of the T-TAS parameters, PT-INR, and APTT to predict for CD-TLR in the CLTI group (Fig 3). The AUC for the $AR_{10}$-$AUC_{30}$ level for the prediction of CD-TLR was 0.85 (95% CI, 0.72-0.99; $P < .001$). In contrast, the AUCs for the $PL_{24}$-$AUC_{10}$ level, D-dimer, PT-INR, and APTT for the prediction of CD-TLR were 0.51 (95% CI, 0.27-0.70; $P = .92$), 0.52 (95% CI, 0.27-0.76; $P = .90$), 0.53 (95% CI, 0.27-0.78; $P = .83$), and 0.59 (95% CI, 0.31-0.86; $P = .54$), respectively. Furthermore, the ROC curve analysis elucidated the optimal cutoff $AR_{10}$-$AUC_{30}$ level to predict for CD-TLR was 1646 (sensitivity, 93%; specificity, 56%). Using the cutoff $AR_{10}$-$AUC_{30}$ level for the prediction of CD-TLR, we divided the CLTI group into the two subgroups: high $AR_{10}$-$AUC_{30}$ group ($AR_{10}$-$AUC_{30}$ ≥1646) and low $AR_{10}$-$AUC_{30}$ group ($AR_{10}$-$AUC_{30}$ <1646). The Kaplan-Meier curve analysis demonstrated that the high $AR_{10}$-$AUC_{30}$

**Table III.** Cox proportional hazards regression analysis for clinically driven target lesion revascularization (CD-TLR) stratified by chronic limb-threatening ischemia (*CLTI*)

| | Unadjusted model | | | Propensity score-adjusted model[a] | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* value | HR | 95% CI | *P* value |
| CLTI group | | | | | | |
| $AR_{10}$-$AUC_{30}$ (per 100 units) | 1.65 | 1.04-2.68 | .03 | 2.04 | 1.18-3.88 | .01 |
| $PL_{24}$-$AUC_{10}$ (per 100 units) | 0.76 | 0.49-1.15 | .18 | 0.78 | 0.44-1.40 | .40 |
| Non-CLTI group | | | | | | |
| $AR_{10}$-$AUC_{30}$ (per 100 units) | 0.85 | 0.71-1.03 | .09 | 0.90 | 0.75-1.12 | .31 |
| $PL_{24}$-$AUC_{10}$ (per 100 units) | 0.92 | 0.59-1.43 | .71 | 0.88 | 0.54-1.44 | .60 |

*$AR_{10}$-$AUC_{30}$*, Area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min; *CI*, confidence interval; *HR*, hazard ratio; *$PL_{24}$-$AUC_{10}$*, area under the curve for the first 10 minutes for the platelet chip tested at a flow rate of 24 μL/min.
[a]Adjusted by age, sex, prothrombin time–international normalized ratio, activated partial thromboplastin time, below-the-knee lesions (vs above-the-knee lesions), occlusion lesions at baseline (vs stenosis), restenotic lesions (vs de novo lesions), lesion length, poor distal runoff, treated with plain old balloon angioplasty alone, and antithrombotic agents ($AR_{10}$-$AUC_{30}$, anticoagulants; $PL_{24}$-$AUC_{10}$, dual antiplatelet therapy).




| Predictors | AUC (95% CI) |
|---|---|
| $AR_{10}$-$AUC_{30}$ | 0.85 (0.72 – 0.99) |
| $PL_{24}$-$AUC_{10}$ | 0.51 (0.27 – 0.70) |
| D-dimer | 0.52 (0.27 – 0.76) |
| PT-INR | 0.53 (0.27 – 0.78) |
| APTT | 0.59 (0.31 – 0.86) |

**Fig 3.** Receiver operating characteristics (ROC) curve analysis for area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min (*$AR_{10}$-$AUC_{30}$*), area under the curve for the first 10 minutes for the platelet chip tested at flow rate of 24 μL/min (*$PL_{24}$-$AUC_{10}$*), prothrombin time–international normalized ratio (*PT-INR*), and activated partial thromboplastin time (*APTT*) to predict clinically driven target lesion revascularization (CD-TLR) in chronic limb-threatening ischemia (CLTI) group.

group had had a significantly greater incidence of CD-TLR during the follow-up period compared with the low $AR_{10}$-$AUC_{30}$ group ($P$ = .02, log-rank test; Supplementary Fig, online only).

## DISCUSSION

In the present study, we investigated the clinical characteristics associated with CD-TLR by dividing patients with PAD into CLTI and non-CLTI groups. An assessment of thrombogenicity using the T-TAS, a novel microchip-based flow chamber system designed to evaluate total thrombogenicity in the whole blood, revealed an elevated $AR_{10}$-$AUC_{30}$ level, but not an elevated $PL_{24}$-$AUC_{10}$ level, as a significant predictor of CD-TLR for CLTI patients but not for non-CLTI patients. To the best of our knowledge, the present study is the first to describe the association between thrombogenicity evaluated by the T-TAS and disease progression of CLTI, which might account for the pathologic disparities causing restenosis or reocclusion between patients with and without CLTI.

A recent pathologic study examined amputation specimens from CLTI patients and revealed that thrombotic luminal occlusion with insignificant atherosclerosis was often observed, suggesting the possibility that thromboembolic phenomena might play an important role in the development of CLTI.[24] Given that a large proportion of CLTI patients will have infrapopliteal arterial occlusive lesions, blood flow will be more often and more severely impaired owing to the presence of concomitant inflow/outflow disease and a narrowed blood vessel, which could activate the coagulation cascade. In particular, in patients who require EVT, the endovascular procedure will disrupt the endothelium and atherosclerotic plaques, resulting in platelet activation through complex biologic pathways.[25,26] Platelet activation will subsequently activate the coagulation cascade at the platelet surface, resulting in the production of thrombi. In addition to the derangement in the coagulation cascade, several detrimental properties often observed in CLTI patients, including the presence of infection, loss of mobility, and noncompliant vessels with medial calcification, could also contribute to the production of luminal thrombi.[27] Therefore, CLTI patients might possess higher thrombogenic potential. The association of a hypercoagulable state evaluated by coagulation biomarkers with restenosis or intimal hyperplasia has been reported in patients with PAD after intervention.[28,29] However, few systems are available to evaluate the thrombogenicity of patients with CLTI.

The T-TAS, a novel microchip-based flow chamber apparatus, can quantitatively monitor the degree of thrombogenesis and analyze different thrombus formation processes using two microchips with different thrombogenic surfaces: the PL and AR chips. The PL chip reflects the whole platelet activation pathway, not including subsequent coagulation cascade activation, and the AR chip reflects activation of, not only platelets, but also the coagulation cascade. Thus, elevated $AR_{10}$-$AUC_{30}$ levels represent increased total thrombogenicity involving activation of both platelets and the coagulation cascade. In the present study, an elevated $AR_{10}$-$AUC_{30}$ level, but not an elevated $PL_{24}$-$AUC_{10}$ level, was significantly associated with the need for CD-TLR for CLTI patients. Therefore, the results of the present study and the distinct features of each T-TAS parameter support the finding that activation of the coagulation cascade and subsequent thrombus formation might play important roles in the progression of CLTI. In addition, the $AR_{10}$-$AUC_{30}$ measurement is performed with low-to mid-shear stress (600 $s^{-1}$), which stimulates arterial blood flow in small to medium-size arteries.[30] Since the BTK artery frequently involved in CLTI patients is small and narrow and its blood flow is impaired owing to concomitant inflow disease, the shear rate in the BTK artery can be expected to be low. Therefore, the T-TAS parameter $AR_{10}$-$AUC_{30}$ might be suitable for assessing focal coagulability in the distal peripheral arteries.

We had previously reported that the $AR_{10}$-$AUC_{30}$ level will be lowered by anticoagulant agents, but not by antiplatelet agents, suggesting the possibility that anticoagulant agents might have the potential to prevent revascularization after EVT in patients with CLTI.[14,15] The current guidelines regarding PAD recommend the long-term use of antiplatelet therapy for all patients with symptomatic PAD in general.[1,2,20] However, this recommendation is not specific to CLTI patients. However, because CLTI and non-CLTI patients have distinct pathologic properties causing restenosis after EVT, postinterventional antithrombotic regimens should be customized to each individual. Some recent large randomized clinical trials have demonstrated the clinical efficacy of the addition of direct oral anticoagulant agents to antiplatelet drugs for preventing cardiovascular or limb events in patients with PAD.[31,32] Additionally, the combination therapy consistently reduced overall and cardiovascular mortality compared with antiplatelet therapy alone for patients with PAD.[33] These results are consistent with the findings of previous studies demonstrating the efficacy of the combination of antiplatelet and anticoagulant therapy after EVT for CLTI.[34,35] Our findings demonstrating the relationship between increased total thrombogenicity and the development of CLTI are consistent with the clinical benefit of anticoagulant agents for patients with CLTI. Our findings also suggest that the T-TAS measurements might allow us to identify patients with CLTI with a high risk of requiring CD-TLR before EVT and support the usefulness of anticoagulant agents, especially for CLTI patients with a high $AR_{10}$-$AUC_{30}$ level. However, further largescale, prospective studies to validate this approach are strongly warranted.

Several caveats should be noted. First, we performed a retrospective, single-center study, and the number of patients and events examined was relatively small. Thus, the results should be carefully interpreted. Also, we performed a pilot study to emphasize the need for further largescale studies validating these findings. Moreover, all study participants were Japanese; thus, the results might not be generalizable to populations of other countries and ethnicities. Some clinical demographics of the CLTI patients in the present study, including sex and body mass index, differed from those previously reported.[36,37] However, these factors do not tend to affect the $AR_{10}$-$AUC_{30}$ value.[16,19] Second, the devices used in EVT and postprocedural management, including antithrombotic agents, were at each physician's discretion, which could have introduced biases. Third, because we did not monitor the serial changes in the T-TAS parameters, we could not investigate the association between changes in the T-TAS parameters before and after EVT and the clinical outcomes. In addition, we did not have a biochemical measure of blood thrombogenicity apart from the T-TAS results. Therefore, the findings from the present study highlight the importance of further investigations to identify the underlying mechanism of the elevated $AR_{10}$-$AUC_{30}$ level in the CLTI patients who had required CD-TLR. Fourth, no significant efficacy of the use of anticoagulant agents on the outcomes was observed in the present study, potentially because of the small sample size. Fifth, given that all the patients enrolled in the present study had undergone elective EVT, those with acute thrombotic events requiring emergent revascularization were excluded from the present study.

## CONCLUSIONS

The $AR_{10}$-$AUC_{30}$ level measured by the T-TAS before EVT could predict for CD-TLR after EVT only in CLTI patients. This could allow us to identify patients at high risk of requiring CD-TLR after EVT. In addition, this finding indicates the presence of hypercoagulability in CLTI patients and suggests that anticoagulant agents might be useful in preventing revascularization in CLTI patients after EVT.

## AUTHOR CONTRIBUTIONS

Conception and design: NK, KK, TM, KT
Analysis and interpretation: NK, MI, SH, DS, ST, SA, EY, KM, KT
Data collection: NK, TM, NN, KF, YO
Writing the article: NK, KK

Critical revision of the article: KK, MI, TM, NN, KF, YO, SH, DS, ST, SA, EY, KM, KT
Final approval of the article: NK, KK, MI, TM, NN, KF, YO, SH, DS, ST, SA, EY, KM, KT
Statistical analysis: NK, MI
Obtained funding: Not applicable
Overall responsibility: KK

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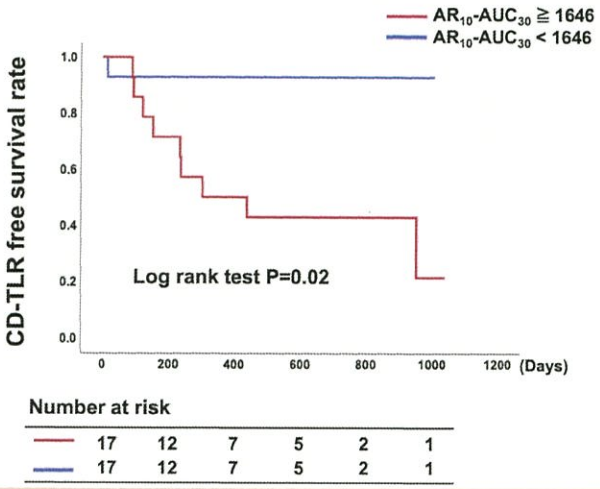


**Supplementary Fig (online only).** Kaplan-Meier curve analysis for clinically driven target lesion revascularization (CD-TLR) among those with an area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min ($AR_{10}$-$AUC_{30}$) of ≥1646 and those with an $AR_{10}$-$AUC_{30}$ of <1646.

**Supplementary Table I (online only).** Comparison of baseline clinical demographics stratified by chronic limb-threatening ischemia (*CLTI*)

| Variable | CLTI group (n = 34) | Non-CLTI group (n = 62) | *P* value |
|---|---|---|---|
| Age, years | 75.4 ± 10.5 | 72.1 ± 9.4 | .12 |
| Female sex | 17 (50) | 17 (27) | .03 |
| BMI, kg/m$^2$ | 21.1 ± 3.3 | 22.9 ± 3.0 | .007 |
| Hypertension | 27 (79) | 52 (84) | .58 |
| DM | 18 (53) | 32 (52) | .90 |
| IDDM | 8 (24) | 13 (21) | .80 |
| Dyslipidemia | 24 (71) | 52 (84) | .13 |
| Smoking | 21 (64) | 48 (77) | .15 |
| Atrial fibrillation | 3 (9) | 8 (13) | .54 |
| CAD | 17 (50) | 40 (65) | .17 |
| Biochemistry data | | | |
| eGFR, mL/min/1.73 m$^2$ | 63.2 ± 20.3 | 60.4 ± 20.3 | .52 |
| eGFR group | | | |
| 30-60 mL/min/1.73 m$^2$ | 12 (35) | 31 (50) | .17 |
| <30 mL/min/1.73 m$^2$ | 1 (3) | 2 (3) | .94 |
| C-reactive protein, mg/dL | 1.05 (0.23-2.12) | 0.13 (0.07-0.30) | <.001 |
| HbA1c, % | 6.5 ± 1.2 | 6.6 ± 1.0 | .53 |
| LDL-C, mg/dL | 90 ± 35 | 92 ± 28 | .85 |
| Hemoglobin, g/dL | 11.3 ± 2.0 | 12.9 ± 2.0 | <.001 |
| Platelet count, $10^4$/μL | 25.6 ± 9.5 | 23.3 ± 6.7 | .17 |
| PT-INR | 1.15 ± 0.42 | 1.04 ± 0.23 | .10 |
| APTT, seconds | 33.2 ± 6.2 | 29.9 ± 4.7 | .006 |
| D-dimer, μg/mL | 2.24 ± 2.29 | 1.21 ± 0.70 | .02 |
| T-TAS parameter | | | |
| $PL_{24}$-$AUC_{10}$ | 232 (88-373) | 206 (91-350) | .73 |
| $AR_{10}$-$AUC_{30}$ | 1641 (1458-1708) | 1671 (1560-822) | .14 |
| Medication use at EVT | | | |
| Aspirin | 18 (53) | 35 (56) | .74 |
| Clopidogrel | 15 (44) | 30 (48) | .69 |
| DAPT | 11 (32) | 21 (34) | .88 |
| Anticoagulant | 6 (18) | 10 (16) | .85 |
| Statin | 20 (59) | 45 (73) | .17 |

*APTT*, Activated partial thromboplastin time; *$AR_{10}$-$AUC_{30}$*, area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min; *BMI*, body mass index; *CAD*, coronary artery disease; *DAPT*, dual antiplatelet therapy; *DM*, diabetes mellitus; *eGFR*, estimated glomerular filtration rate; *HbA1c*, hemoglobin A1c; *LDL-C*, low-density lipoprotein cholesterol; *$PL_{24}$-$AUC_{10}$*, area under the curve for the first 10 minutes for the platelet chip tested at a flow rate of 24 μL/min; *PT-INR*, prothrombin time–international normalized ratio; *T-TAS*, total thrombus-formation analysis system.

Data presented as mean ± standard deviation, number (%), or median (interquartile range).

**Supplementary Table II (online only).** Comparison of total thrombus-formation analysis system (T-TAS) parameters between chronic limb-threatening ischemia (*CLTI*) patients with and without adverse clinical events

| | CLTI patients (n = 34) | | |
|---|---|---|---|
| **Event** | **Events** | **No events** | ***P* value** |
| All-cause death | 5 (15) | 29 (85) | |
| $PL_{24}$-$AUC_{10}$ | 228 (149-384) | 232 (59-378) | .75 |
| $AR_{10}$-$AUC_{30}$ | 1698 (1528-1918) | 1636 (1422-1707) | .22 |
| Amputation | 6 (18) | 28 (82) | |
| $PL_{24}$-$AUC_{10}$ | 286 (83-361) | 229 (72-382) | .91 |
| $AR_{10}$-$AUC_{30}$ | 1700 (1607-1731) | 1597 (1421-1705) | .21 |
| MACE | 4 (12) | 30 (88) | |
| $PL_{24}$-$AUC_{10}$ | 99 (35-322) | 257 (99-373) | .34 |
| $AR_{10}$-$AUC_{30}$ | 1749 (1697-1792) | 1597 (1423-1700) | .03 |

*$AR_{10}$-$AUC_{30}$*, Area under the curve for the first 30 minutes for the atheroma chip tested at a flow rate of 10 μL/min; *MACE*, major adverse cardiovascular events; *$PL_{24}$-$AUC_{10}$*, area under the curve for the first 10 minutes for the platelet chip tested at a flow rate of 24 μL/min.
Data presented as number (%) or median (interquartile range).