REVIEW ARTICLE

New methodological approaches for assessing thrombus formation in cardiovascular disease

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ABSTRACT
Antiplatelet therapy is the mainstay preventive strategy for cardiovascular diseases, and dual antiplatelet therapy comprising aspirin and a P2Y12 inhibitor is the standard treatment for patients who underwent percutaneous coronary intervention. The Total Thrombus-Formation Analysis System (T-TAS) is a microchip flow-chamber system developed to evaluate overall thrombus formation under flow conditions, which is reportedly able to assess single and combined antithrombotic therapy. Here, we focus on this new system, T-TAS, and review its characteristics together with those of the conventional systems available for evaluation of antithrombotic therapies for cardiovascular diseases.

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Introduction Atherothrombosis, which causes acute coronary syndrome and ischemic stroke, is the leading cause of morbidity and mortality in developed countries. Various antithrombotic agents targeting distinct activating pathways of platelets and the coagulation system have been developed, and their treatment and prophylactic efficacy regarding cardiovascular events have been evaluated in numerous randomized controlled trials. Antiplatelet therapy is the mainstay strategy in the treatment of cardiovascular disease, because platelets play a crucial role in arterial thrombus formation.[1,2] Aspirin and P2Y12 inhibitors are widely used to treat cardiovascular thrombosis. Further, their combined use in the form of dual antiplatelet therapy (DAPT), is the standard antithrombotic strategy for patients who underwent percutaneous coronary intervention (PCI).[3-5] In contrast, anticoagulant agents have mainly been used for the treatment of venous thromboembolism,[6-8] and direct oral anticoagulants (DOACs), having anticoagulant effects that are more predictable than those of vitamin K antagonists, are widely used in patients with atrial fibrillation (AF) to reduce cardioembolic cerebrovascular events.[9-11] Recent studies have suggested the potential usefulness of DOACs for managing cardiovascular events. The safety and efficacy of the direct factor Xa inhibitor, rivaroxaban, in combination with single and dual antiplatelet therapy, have been confirmed and shown to reduce ischemic events in patients with AF after PCI and in those with stable atherosclerotic vascular disease.[12,13]

Intensive antithrombotic therapy and bleeding complications represent 2 sides of the same coin, with physicians seeking to optimize antithrombotic regimens to maximize the net clinical benefit of avoiding ischemic events versus bleeding complications for individual patients with various forms of cardiovascular disease and associated conditions.

Diagnostic methods for platelet function testing Several diagnostic devices have been developed to evaluate the efficacy of anticoagulant and antiplatelet treatments. For platelet function, light transmission aggregometry is still widely used and regarded as the gold standard. However, this method requires preparation of platelet-poor plasma and is quite laborious.[14] Therefore, platelet function tests

TABLE 1 Comparison of coagulation and platelet function tests in whole blood

Criterion	Total Thrombus-Formation Analysis System		VerifyNow	PFA 100/200	Multiplate or ROTEM Platelet	TEG / ROTEM	
Method	PL chip	AR chip	3 different assays: GPIIb / IIIa or P2Y12 antagonists and aspirin	3 different assays: Col / Epi, Col / ADP, P2Y	Different assays: aspirin, P2Y12 inhibitors, GPIIb / IIIa antagonists, general platelet function	Different assays	TEG platelet mapping
Sample	BAPA--anticoagulated whole blood	Citrate-anticoagulated whole blood	Citrate--anticoagulated whole blood	Citrate--anticoagulated whole blood	Hirudin--anticoagulated whole blood diluted 1:2 Some studies also used citrate--anticoagulated blood.	Citrated whole blood	Various anticoagulants
Principle	Blood is perfused over collagen in 26 microchannels in a disposable chip. A sensor detects changes in pressure during thrombus formation. Arterial shear forces (1500 s^{-1}).	Blood is perfused over collagen and tissue factor in a single microcapillary in a disposable chip in the presence of CTI. A sensor detects changes in pressure during thrombus formation. Arterial shear forces (600 s^{-1}).	Fibrinogen--coated beads adhere when platelets are activated by agonists. Detection by turbidimetry.	Measurement of closure time when blood is aspirated in a capillary and comes into contact with a bioactive surface. High shear system.	Impedance aggregometry: binding of platelets to electrodes immersed into the magnetically stirred diluted sample during aggregation induces change of impedance.	Viscoelastic detection of clot formation and dissolution, triggered by various reagents. No shear forces involved.	Viscoelastic detection of clot formation by reptilase and FXIIIa, triggered by different agonists. No shear forces involved.
Influenced by	Platelet number and quality, VWF, HCT, and antiplatelet drugs	DOACs, VKAs, antiplatelet drugs, coagulation factors and inhibitors, platelet number and quality, VWF, and HCT	Platelet number and quality, HCT, and antiplatelet drugs	Platelet number and quality, hematocrit, VWF, and drug effects (cartridge--dependent)	Platelet number and quality, VWF, HCT, and antiplatelet drugs	Platelet count and quality, coagulation factors, hyperfibrinolysis, and heparin (assay-dependent). No or very weak influence of aspirin or P2Y12 antagonists	Platelet count, quality, antagonists, coagulation factors
Technical aspects	Requires manual pipetting	Requires manual pipetting	Fully automated	Fully automated, no exact pipetting required. Narrow measurement range for abnormal samples.	Requires manual reagent preparation and handling	Requires exact pipetting or fully automated (different systems)	Requires exact pipetting or fully automated (different systems)
Main application	Measurement of individual or combined effects of antiplatelet drugs General platelet function	Anticoagulants alone and in combination with antiplatelet drugs (additive effects)	Antiplatelet drug monitoring	Cartridge--specific: general platelet function, preoperative screening, VWF diagnosis and monitoring, antiplatelet drug monitoring	Antiplatelet therapy monitoring General platelet function (eg, during surgery)	Detection of general platelet function, clotting, and fibrinolysis, often during surgery	Detection of antiplatelet drug effects on clot formation

Abbreviations: ADP, adenosine triphosphate; Col, collagen; CTI, corn trypsin inhibitor; DOACs, direct oral anticoagulants; Epi, epinephrine; HCT, hematocrit; VKAs, vitamin K antagonists; VWF, von Willebrand factor

(PFTs) performed on whole blood (TABLE 1), such as the Multiplate (Roche Diagnostics) and VerifyNow (Werfen) systems, are frequently used to monitor the efficacy of antiplatelet therapies.[15,16] Multiple electrode aggregometry (MEA, Multiplate), a modification of the well-known impedance aggregometry, assesses platelet aggregation in hirudin-anticoagulated whole blood in disposable cuvettes with 2 independent impedance sensor units under stirring.[17] As in light transmission aggregometry, several reagents allow for differentiation of various antiplatelet drug effects.

The VerifyNow system assesses platelet aggregation in whole blood by optical detection in cartridges containing fibrinogen-coated beads and platelet agonists.[18] During the assay, platelets activated by specific agonists bind to the fibrinogen-coated beads in proportion to the number of GPIIb / IIIa receptors and induce a change in turbidity. Various cartridges with specific reagents for important groups of antiplatelet drugs are available.

Under physiological conditions, thrombus formation takes place under physiological laminar shear forces that vary strongly depending on the diameter of the blood vessel. The first diagnostic device that implemented laminar shear forces was the platelet function analyzer (PFA-100/200).[19] The analyzer utilizes disposable test cartridges containing a membrane coated with collagen and other platelet-activating substances. A blood sample is aspirated under constant vacuum through a capillary and contacts with a microscopic aperture cut into the membrane. Very high shear rates generated under these flow conditions cause platelet adhesion and aggregation, resulting in the formation of a platelet plug at the aperture. The time required to obtain full occlusion of the aperture is reported as the "closure time."

The device is quite sensitive for von Willebrand factor (VWF).[20] Using 2 types of cartridges, various antiplatelet drugs can be evaluated. Thus, PFA-100/200 has been utilized for analyzing both acquired (including antiplatelet drug effects) and congenital platelet disorders (von Willebrand disease).

The correlation between the different methods in studies on patients with PCI and on DAPT is limited and clinical data are therefore dependent on the specific method used, including potential variation in the type of the blood sample (anticoagulant). The whole-blood devices mentioned above have been used in various applications, including assessment of antiplatelet drug effects and adjustment of antiplatelet therapy.[21,22]

Thromboelastography (TEG, Haemonetics) and rotational thromboelastometry (ROTEM, Werfen) are useful for analyzing viscoelastic changes in whole blood caused by fibrin formation and platelet activation. These devices are sensitive for anticoagulant and thrombolytic agents and potentially useful for evaluating their therapeutic efficacy. In contrast, these viscoelastic assays, in which thrombin is generated, are generally insensitive to antiplatelet agents.[23-25] To make up for this lack of sensitivity, a specific modification of TEG, called "platelet mapping",[26] and an extension of the ROTEM system, ROTEM Platelet, which is based on impedance aggregometry, have been developed to evaluate antiplatelet therapy with these tools.

Recently, the Total Thrombus-Formation Analysis System (T-TAS, Fujimori Kogyo Co., Ltd.) was developed to evaluate thrombogenicity of whole blood under shear stress conditions. This system enables quantitative analysis of platelet and fibrin-rich platelet thrombus formation under flow conditions using 2 distinct types of microchips.[27,28]

Measurement principles of the Total Thrombus-Formation Analysis System

The Total Thrombus-Formation Analysis System is a microchip flow-chamber system developed for the quantitative assessment of thrombus formation in whole blood under flow conditions. With this system, microchips coated with collagen (PL chip) or collagen and tissue thromboplastin (AR chip) are used to analyze platelet thrombogenicity in the absence or presence of an active blood coagulation system, respectively.[27,28] Accordingly, measurement using the PL chip is specific for primary hemostatic (ie, platelet and VWF) function, while the AR chip is used to assess the combination of both platelet and coagulation function. Thrombus formation inside the microchip is analyzed according to changes in flow pressure that arise as a result of capillary occlusion following thrombus formation. Total thrombogenicity is subsequently quantified by calculating the area under the flow pressure curve (AUC).

Both devices are commercially available for research use only (T-TAS plus) and in vitro diagnosis (T-TAS 01). The PL chip assay with T-TAS 01 is approved by the Food and Drug Administration and has received the CE mark for the analysis of the platelet thrombus formation process in patients with impaired primary hemostatic function or receiving antiplatelet therapy.

Analysis of platelet thrombus formation using the PL chip The PL chip flow path contains 26 microcapillary channels attached to a type 1 collagen surface (Supplementary material, *Figure S1A*). Whole blood anticoagulated with hirudin or BAPA (dual inhibitor of factors Xa and IIa) is perfused into the flow path at a flow rate of 18 µl/min, which corresponds to an initial wall shear rate of 1500 s^{-1}.[28] Platelets adhere to and aggregate on the surface of the collagen, and

small platelet aggregates form within the microcapillary channels. Endogenous adenosine diphosphate (ADP) and thromboxane A_2 (TXA_2) released by activated platelets generate autocrine and paracrine signals to recruit and further activate additional platelets, which increases the size and stability of the platelet thrombi. Through continued formation and breakdown, the platelet thrombi become solid and eventually occlude the microcapillaries. As a result, an increase and a decrease in flow pressure through the microcapillaries reflect the formation and breakdown of thrombi, respectively, and overall platelet thrombogenicity is quantified by calculating the AUC value (PL-AUC).[28]

Analysis of fibrin-rich platelet thrombus formation using the AR chip The AR chip flow path consists of a single microcapillary attached to a coating of collagen and tissue thromboplastin (Supplementary material, *Figure S2A*). Recalcified whole blood containing corn trypsin inhibitor (CTI, FXIIa inhibitor), which eliminates any effect of the intrinsic coagulation pathway in the assay, is perfused into the flow path at a flow rate of 10 µl/min, which corresponds to an initial wall shear rate of 600 s^{-1}.[27] On the surface of the collagen and tissue thromboplastin, platelets and the extrinsic pathway of the coagulation system are simultaneously activated, leading to the formation of white thrombi comprising activated platelets and fibrin fibers. Through the mutual activation of platelets and blood coagulation, fibrin-rich platelet thrombi gradually increase in size and consequently occlude the capillary. As fibrin-rich platelet thrombus formation can be cooperatively achieved in the AR chip via the activation of platelets and coagulation, both antiplatelet and anticoagulant agents can reduce the AUC values obtained using the AR chip (AR-AUC), and a combination of these agents more potently inhibits thrombus formation (Supplementary material, *Figure S2B*).[27]

Comparison of existing platelet function tests, coagulation tests, and the Total Thrombus-Formation Analysis System VerifyNow, Multiplate, and PFA-100/200 have been widely used to evaluate antiplatelet therapies. VerifyNow and Multiplate were designed to analyze platelet aggregation and agglutination in response to exogenous agonists.[15,16] PFA-100/200 measures platelet aggregate formation on the surface of collagen containing a soluble agonist (epinephrine or ADP) under arterial shear flow. As the data obtained from these devices are primarily affected by the agonists tested, it is necessary to choose the agonist specific for the platelet activation pathway targeted by the drug. For example, collagen or arachidonic acid are used to evaluate the effect of aspirin, and ADP is generally used to evaluate the effect of P2Y12 inhibitors.[14,15] In contrast, platelet thrombus formation in the PL chip occurs directly on a collagen surface, with the involvement of VWF-mediated platelet adhesion, release of endogenous agonists, and stabilization and growth of platelet thrombi.[28] Thus, it is possible to assess single and combined inhibitory effects of antiplatelet agents with different modes of action on the platelet thrombus formation process using the PL chip. However, it is not feasible to specifically evaluate individual platelet activation pathways using the PL chip. In this regard, the PL chip and agonist-specific assays are complimentary and combining the results of these analyses can provide a more comprehensive understanding of the efficacy of antiplatelet therapies in individual patients.[29]

Although TEG and ROTEM have been utilized to evaluate anticoagulant therapy, they are reported to be insensitive to antiplatelet therapy with aspirin and/or P2Y12 inhibitors.[23-25] In contrast, as the AR chip is designed to measure fibrin-rich platelet thrombus formation under flow conditions, it can be used to evaluate single and combined effects of anticoagulant and/or antiplatelet agents.[27] However, it is not feasible to analyze the separate effects of anticoagulant and antiplatelet agents. The AR chip may therefore provide a comprehensive simulation of the atherothrombotic process, with activation of both platelets and the coagulation system, whereas TEG and ROTEM rather reflect the process of venous thromboembolism. In addition, specific assay reagents are available for TEG and ROTEM to evaluate individual pathways, as well as the functions of the intrinsic and extrinsic coagulation pathways, fibrinolysis and fibrinogen, and the elimination of heparin effects. Combining the results of analysis with the AR chip, TEG, and ROTEM may therefore aid in the evaluation and understanding of the pharmacological efficacy of these drugs in arterial and venous circulation.[27]

Previous clinical research involving the Total Thrombus-Formation Analysis System in cardiovascular diseases The PL chip has been used to examine antiplatelet therapies and bleeding complications (TABLE 2). In a comparative study to evaluate the antiplatelet effects of aspirin and clopidogrel in patients with cardiovascular disease using the PL chip and VerifyNow,[30] the PL-AUC decreased in patients who received aspirin monotherapy compared with nonantiplatelet therapy and further decreased in those who received DAPT compared with aspirin monotherapy, thereby demonstrating the escalating effect of the P2Y12 inhibitor. In contrast, while VerifyNow-PRU decreased in patients receiving DAPT, no significant difference was observed between patients who received aspirin monotherapy and nonantiplatelet therapy.

TABLE 2 Studies evaluating antiplatelet therapies and bleeding complications with the Total Thrombus-Formation Analysis System

Criterion	Arima et al[30]	Oimatsu et al[31]	Ito et al[36]	Ichikawa et al[37]	Mitsuse et al[38]
Patients and shear rates tested	274 patients with suspected CAD, receiving aspirin or DAPT PL chip; 2000 s^{-1}	313 patients with CAD undergoing PCI, receiving DAPT PL chip; 2000 s^{-1}	128 patients with AF undergoing CA, receiving OACs PL chip; 2000 s^{-1} AR chip; 600 s^{-1}	145 patients with CAD received OACs plus antiplatelet agents (SAPT or DAPT) PL chip; 1500 s^{-1} AR chip; 240 s^{-1}	561 CAD patients with CAG receiving OACs and / or antiplatelet agents (SAPT or DAPT) PL chip; 2000 s^{-1}, AR chip; 600 s^{-1}
Results	The PL-AUC decreased in patients receiving aspirin alone compared with patients receiving nonantiplatelet therapy (mean [SD], 256 [108] vs 358 [111]; P <0.001). Patients receiving DAPT showed a further decreased PL-AUC compared with those receiving aspirin monotherapy (mean [SD], 113 [90] vs 256 [108]; P <0.001). The VerifyNow-PRU decreased in patients receiving DAPT compared with those receiving nonantiplatelet therapy (mean [SD], 213 [67] vs 264 [50]; P <0.01) and aspirin (278 [57]; P <0.01), but no significant difference was observed between these patient groups. PM patients with the *CYP2C19* polymorphism receiving DAPT had a higher PL-AUC than non-PMs (mean [SD], 213 [67] vs 152 [112]; P = 0.001), and combined analysis of PL-AUC and VerifyNOW-PRU improved discrimination of PMs.	The PL-AUC was lower in patients with periprocedural bleeding during PCI compared with those without bleeding events (median [IQR], 48.9 [18.2–114.8] vs 92.1 [50.2–164.7]; P = 0.002), but no significant difference was detected in AR-AUC or VerifyNow-PRU values between bleeding and nonbleeding patients (median [IQR], 242 [179–264] vs 232 [179–277]; P = 0.6).	The AR-AUC was lower in patients with periprocedural bleeding after CA compared with nonbleeding patients (median [IQR], 1525 [1447–1713] vs 1805 [1702–1861]; P <0.001, cutoff level, 1648), but no significant difference was detected in PL-AUC or VerifyNow-PRU values between bleeding and nonbleeding patients.	The AR-AUC, but not PL-AUC, decreased in patients with bleeding events during 22 months of follow-up compared with patients without bleeding events (median [IQR], 584 [96–993] vs 1028 [756–1252]; P <0.001).	The AR-AUC, but not PL-AUC, decreased in patients with bleeding events during 12 months of follow-up compared with patients without bleeding events (median [IQR], 1590 [1442–1734] vs 1687 [1546–1797]; P = 0.04).

Abbreviations: AUC, area under the flow pressure curve; CA, catheter ablation; CAD, cardiovascular disease; CAG, coronary angiography; DAPT, dual antiplatelet therapy; OAC, oral anticoagulant; PCI, percutaneous coronary intervention; PM, poor metabolizer; PRU, P2Y12 reaction unit; SAPT, single antiplatelet therapy

These data suggest that the PL-AUC values reflect the overall efficacy of DAPT, but that it is not feasible to evaluate each effect separately. In contrast, the VerifyNow-PRU and -ARU values specifically reflect the effects of a P2Y12 inhibitor and aspirin, respectively, but not their combined effect.

Further, Oimatsu et al[31] reported that the PL-AUC was lower in patients experiencing periprocedural bleeding complications during PCI who were receiving DAPT. Periprocedural bleeding is a frequent complication during PCI and is associated with an increased risk of readmission for treatment of recurrent bleeding, major adverse cardiovascular events, and all-cause mortality.[32] Therefore, PL chip measurements may be useful for predicting bleeding events.

Studies have also examined patients with high residual platelet thrombogenicity after receiving DAPT. Hosokawa et al[33] reported that high residual platelet thrombogenicity in cardiac patients receiving aspirin monotherapy and DAPT is associated with both elevated levels of agonist-induced platelet aggregability and platelet-monocyte aggregate (PMA) formation, as evaluated by flow cytometry. Similarly, Yamazaki et al[34] noted that high residual platelet thrombogenicity measured using the PL chip in patients with cerebrovascular disease receiving clopidogrel monotherapy was associated with significant elevations in both VerifyNow-PRU values and PMA formation.

Furthermore, patients with carotid and intracranial stenosis also show significantly elevated PL-AUC values. However, no significant association was observed between VerifyNow and flow cytometry parameters in these patients, indicating that these tests use a technology that is insensitive to certain aspects of platelet function, namely, platelet reactivity to certain stimuli and levels of activated platelets in circulation. Both high residual platelet aggregability and elevated PMA formation are associated with an increased risk of ischemic vascular events,[35] and evaluation of platelet thrombogenicity using the PL chip comprehensively reflects this.[33]

The use of VerifyNow and Multiplate for evaluating antiplatelet therapy is supported by an abundance of clinical study data.[15,16] In contrast, clinical studies using the PL chip are limited. However, as analysis using agonist-specific PFTs and the PL chip shed light on different aspects of the platelet activation, the combined data from these analyses may increase the understanding of the efficacy of individual antiplatelet treatments for improving patient outcomes.

The AR chip is used to evaluate the efficacy of DOACs in the presence or absence of antiplatelet therapy in patients with cardiovascular diseases. Ito et al[36] reported that treatment with DOACs significantly decreases AR-AUC values in patients with AF after catheter ablation. Low AR-AUC values among these patients are associated with a high incidence of periprocedural bleeding after catheter ablation, although there is no significant association between plasma clotting tests (prothrombin time and activated partial thromboplastin time) and bleeding events.

In addition, studies have shown that, unlike most other methods, the AR chip is useful for evaluating the overall antithrombotic efficacy of DOACs plus antiplatelet agents. Ichikawa et al[37] reported that low AR-AUC values are associated with bleeding complications in patients with stable coronary artery disease receiving oral anticoagulants plus single- or dual-antiplatelet therapy. Mitsuse et al[38] also noted that AR-AUC values were associated with bleeding risk in patients receiving antiplatelet and/or anticoagulant agents who underwent coronary angiography. In these studies, the PL chip failed to show a significant association with bleeding events, probably because platelet thrombogenicity measured using the PL chip is primarily affected by antiplatelet therapy, especially by DAPT.

These data suggest that the AR chip might be useful for evaluating the overall combined efficacy of anticoagulant and antiplatelet therapy, which is not feasible using other existing devices. Therefore, the AR chip may be useful for predicting the risk of bleeding complications in patients receiving multiple antithrombotic therapies. At this point, it is too early to speculate whether the results obtained with the AR chip may be used to define a "therapeutic range," that is, a degree of antithrombotic effects that is protective against atherothrombosis and bleeding.

Potential usefulness of the Total Thrombus-Formation Analysis System and future perspectives Antithrombotic regimens for the management of cardiovascular diseases have become more complex, with antiplatelet and anticoagulant agents used alone or in combination in different timeframes.[39,40] The strategy of previous devices for measuring the effectiveness of antithrombotic drugs was to evaluate the pharmacological effects of each drug separately. The most important feature of the T-TAS is its ability to evaluate the overall combined antithrombotic effects of multiple drugs using PL and AR chips [27, 28].

Dual antiplatelet therapy is the standard treatment for preventing stent thrombosis after PCI, and several studies have demonstrated its potent efficacy for reducing ischemic events, albeit with an increased risk of bleeding complications.[3-5] Recent improvements in cardiac stents have reduced the incidence of stent thrombosis, making bleeding complications an increasingly relevant issue.[41] To balance the antithrombotic benefits and bleeding complications linked to DAPT, it is crucial to determine the safety and efficacy of short-term DAPT (<6 months) in post-PCI patients. In addition, individualized treatment by PFT-guided de-escalation of DAPT may be a potential effective approach for improving antithrombotic therapy after PCI.[42] The PL chip enables evaluation of the overall combined antiplatelet effects of DAPT, which may contribute to improving the management of antiplatelet therapies through assessment of residual thrombogenicity and bleeding risk.

Anticoagulant agents have been mainly used for the treatment and prophylaxis of venous thromboembolism and cardioembolic stroke, which occur under relatively mild or static blood flow. However, recent clinical studies have demonstrated the safety and efficacy of DOACs in combination with antiplatelet therapy for the treatment of several types of cardiovascular disease.[12,13] Direct oral anticoagulants with predicable effects and specific reversal agents, alone or in combination with antiplatelet agents, are expected to be increasingly utilized for the treatment of various cardiovascular diseases.

Previous clinical studies have shown that the AR chip may be useful for predicting bleeding complications in patients receiving anticoagulant and antiplatelet agents, although the numbers of patients included in those studies were relatively small.[37,38] Thus, further studies in larger populations are needed to determine whether and how the PL or AR chip can reflect bleeding or thrombotic events in patients with various cardiovascular diseases.

Limitations Some of the previous clinical studies performed PL and AR analyses under different shear conditions and the threshold level of PL-AUC or AR-AUC for thrombotic or bleeding events has not yet been conclusively established. In addition, PL and AR assays give only overall information on platelet and/or plasma coagulation and additional studies are needed to evaluate their use in dosing of anticoagulants and antiplatelet drugs, which might be complemented by combined analysis using specific PFTs and coagulation tests.

Therefore, additional clinical studies including diverse subject populations should be conducted to confirm the threshold level for adverse events and clinical usefulness of T-TAS in cardiovascular disease.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/kardiologiapolska.

ARTICLE INFORMATION

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