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Is platelet function testing at the acute phase under P2Y₁₂ inhibitors helpful in predicting bleeding in real-life patients with acute coronary syndrome?

The AVALANCHE study

Abbreviated title: P2Y₁₂ inhibitors and VASP index

Tweet: Bleeding is no predicted by platelet function testing at the acute phase of acute coronary syndrome

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Summary

Background. – In patients with acute coronary syndrome (ACS), current international guidelines recommend newer potent and predictable P2Y₁₂ inhibitors as first-line treatment despite a greater bleeding risk compared with clopidogrel.

Aim. – To determine if platelet function testing can predict bleeding in real-life patients with ACS treated with newer P2Y₁₂ inhibitors.

Methods. – In this retrospective study, all consecutive adults admitted to the Lariboisière University Hospital for ACS, whatever the P2Y₁₂ inhibitor prescribed, who had platelet function testing (vasodilator-stimulated phosphoprotein phosphorylation [VASP] index and aggregation tests) during the initial hospital stay were included. Follow-up was performed to record bleeding events according to the Bleeding Academic Research Consortium (BARC) classification.

Results. – 364 patients were included, treated with ticagrelor ($n=123$), prasugrel ($n=105$) or clopidogrel ($n=136$); 42.3% after an ST-segment elevation myocardial infarction, 27.1% after a non-ST-segment elevation myocardial infarction and 30.6% with unstable angina. Mean age was 64 ± 11 years. Median VASP index was significantly lower with the newer P2Y₁₂ inhibitors (14% under ticagrelor, 14% under prasugrel) than with clopidogrel (42%). Despite these differences in the degree of platelet inhibition, the occurrence of bleeding (BARC 2–5) during follow-up was 7.7% overall, and was similar for all P2Y₁₂ inhibitors (ticagrelor 8.9%; prasugrel 6.6%; clopidogrel 7.4%). For each P2Y₁₂ inhibitor, it was impossible to determine a VASP index threshold under which bleeding was significantly greater during follow-up. Similarly, ADP-induced aggregation was more profoundly inhibited by ticagrelor and prasugrel than by clopidogrel, but this did not allow a threshold to be set for increased haemorrhagic risk.

Conclusions. – Despite the substantial occurrence of bleeding in patients with ACS during follow-up, neither the VASP index nor platelet aggregation test results measured at the acute phase were helpful in predicting bleeding risk. Whether platelet function testing could be helpful later in the course of treatment remains to be evaluated.

Résumé

Contexte. – Chez les patients atteints d'un syndrome coronarien aigu (SCA), les recommandations internationales actuelles préconisent l'emploi de nouveaux inhibiteurs P2Y₁₂ comme traitement de première intention malgré un risque de saignement plus élevé par rapport au clopidogrel.

Objectif. – L'étude AVALANCHE avait pour objectif de tester si les fonctions plaquettaires (VASP, agrégation à l'ADP) à la phase aiguë chez les patients avec SCA traités par P2Y₁₂ pouvaient prédire le saignement (classification BARC 2–5) durant le suivi.

Méthodes. – Dans cette étude rétrospective, tous les patients adultes consécutifs admis au CHU Lariboisière pour un SCA, quel que soit l'inhibiteur P2Y₁₂ et ayant bénéficié d'un test de la fonction plaquettaire (VASP et agrégométrie optique) au cours de l'hospitalisation initiale, ont été inclus. Le suivi a été réalisé afin de recueillir les événements hémorragiques selon la classification BARC.

Résultats. – 364 patients ont été inclus, dont 123 traités avec ticagrelor, 105 par le prasugrel et 136 par le clopidogrel. Parmi eux, 42,3 % après un STEMI, 27,1 % après un NSTEMI et 30,6 % présentant un angor instable. L'âge moyen était de 64 ± 11 ans. L'indice VASP médian était significativement plus bas avec les nouveaux inhibiteurs de P2Y₁₂ (14 % sous ticagrelor, 14 % sous prasugrel) par rapport à 42 % sous clopidogrel. Malgré ces différences du degré d'inhibition plaquettaire, la fréquence des saignements (BARC 2–5) au cours du suivi était de 7,7 % au total et était similaire pour tous les inhibiteurs de P2Y₁₂ (8,9 % sous ticagrelor, 6,6 % sous prasugrel et 7,4 % sous clopidogrel). En effectuant les courbes ROC, il n'a pas été possible pour chacun des inhibiteurs P2Y₁₂ de déterminer un seuil d'indice VASP prédictif du risque hémorragique durant la première année de traitement. De même, l'agrégation plaquettaire induite par l'ADP était plus profondément inhibée (chez les patients sous ticagrelor ou prasugrel que sous clopidogrel sans qu'il soit possible de déterminer un seuil pour prédire le risque hémorragique pendant le suivi.

Conclusions. - Malgré la survenue d'un nombre significatif de saignements pendant le suivi, l'évaluation des fonctions plaquettaires (indice VASP et agrégation à l'ADP) mesurées à la phase aiguë n'est pas prédictive des saignements. L'intérêt de l'évaluation des fonctions plaquettaires après la phase aiguë pour prédire le risque hémorragique au cours du suivi reste à démontrer.

KEYWORDS

Ticagrelor;
Prasugrel;
VASP index;
Platelets;
Bleeding

MOTS CLÉS

Ticagrelor ;
Prasugrel ;
Index VASP ;
Plaquettes ;
Hémorragies.

Abbreviations: AA, arachidonic acid; ACS, acute coronary syndrome; ADP, adenosine diphosphate; BARC, Bleeding Academic Research Consortium; DAPT, dual antiplatelet therapy; IQR, interquartile range; LTA, light transmission aggregometry; MFI, median fluorescence intensity; PCI, percutaneous coronary intervention; PGE1, prostaglandin E1; PLATO, PLATelet inhibition and patient Outcomes; ROC, receiver operating characteristic; TIMI, Thrombolysis in Myocardial Infarction; VASP, vasodilator-stimulated phosphoprotein phosphorylation.

Background

Antiplatelet drugs are a cornerstone therapy for the prevention or treatment of arterial thrombosis, an acute complication of atherosclerosis. After an acute coronary syndrome (ACS), all patients systematically receive dual antiplatelet therapy (DAPT), consisting of a low dose of aspirin combined with an oral inhibitor of the platelet P2Y₁₂ receptor. The only matters for discussion are DAPT duration and the type of P2Y₁₂ inhibitor. Current international guidelines recommend more constant, more potent and more predictable P2Y₁₂ inhibitors (i.e. prasugrel, a new-generation thienopyridine; or ticagrelor, a cyclopentyl-triazolo-pyrimidine reversible P2Y₁₂ antagonist) as first-line treatment, because they provide faster and stronger platelet inhibition compared with clopidogrel [1,2]. However, the benefit of these drugs is counterbalanced by a greater bleeding risk than clopidogrel [3,4]. Thus, clopidogrel is now recommended for patients who cannot receive ticagrelor or prasugrel or have an increased bleeding risk, such as those requiring oral anticoagulation. Consequently, clopidogrel is still widely used in current practice [5,6].

The clinical relevance of platelet function testing in the acute phase of ACS under aspirin or P2Y₁₂ inhibitors to identify patients at high risk of ischaemic events has been demonstrated in various clinical settings [7-9]. A positive predictive value for high on-treatment platelet reactivity and occurrence of ischaemic events has been largely explored. Conversely, but more recently, clinical studies have shown an association between low on-treatment platelet reactivity with clopidogrel and prasugrel and the occurrence of bleeding complications (low on-treatment platelet reactivity being defined mostly using the vasodilator-stimulated phosphoprotein phosphorylation [VASP] index, and different values of the VASP index have been proven predictive, such as VASP < 16%) [9]. Among the P2Y₁₂ antagonists, clopidogrel and prasugrel decrease the VASP index only by inhibition of the P2Y₁₂ receptor pathway. Consequently, for these thienopyridines, there is a direct correlation between the degree of inhibition of the P2Y₁₂ receptor pathway and the decrease in the VASP index. Besides, ticagrelor potentially decreases the VASP index by two different pathways: one is certain (i.e. inhibition of the P2Y₁₂ pathway similarly to thienopyridines), but the other (through stimulation by adenosine of the platelet A_{2b} pathway) is under discussion [10]. The increase in plasma adenosine level is caused by the inhibition by ticagrelor of the “erythrocyte equilibrative nucleoside transporter”, the active transporter of adenosine incorporation in red blood cells [11]. This could elevate local adenosine levels at sites of ischaemia and tissue damage,

although this latter effect has been challenged recently; it is unclear whether ticagrelor via this mechanism can impact the *ex vivo* platelet functions (VASP index, adenosine diphosphate [ADP]-induced platelet aggregation). As adenosine has a very short half-life in whole blood (a few seconds), because of its very rapid cell uptake and metabolism, adenosine present *in vivo* is not likely to be present *ex vivo* during the tests, but platelets challenged *in vivo* by the increased adenosine plasma level could produce the effect. Therefore, if besides P2Y₁₂ receptor inhibition, ticagrelor induced additional adenosine-related VASP phosphorylation, a similar decrease in the VASP index should not confer the same clinical haemorrhagic risk when it is achieved by a thienopyridine or by ticagrelor. Several studies have shown that ticagrelor is associated with higher platelet inhibition and a higher incidence of low on-treatment platelet reactivity than prasugrel after ACS, without an increased incidence of bleeding [12-14]. These results suggest that the VASP index should not have the same predictive value for haemorrhagic risk in patients treated with a thienopyridine compared with ticagrelor.

Considering results of preliminary trials, such as TOPIC [15] and TROPICAL-ACS [16], a new concept of de-escalation of P2Y₁₂ inhibitor treatment has been introduced and implemented in the latest European Society of Cardiology recommendations: based on platelet function testing in the chronic phase, it proposes a switch from prasugrel or ticagrelor to clopidogrel for patients in whom low on-treatment platelet reactivity under a newer P2Y₁₂ inhibitor indicates a higher haemorrhagic risk and, consequently, to consider clopidogrel as an alternative strategy, especially for patients with ACS deemed unsuitable for 12 months of potent platelet inhibition [17].

The aim of the AVALANCHE (Analysis of VASp index after ticagrelor or thienopyridines Loading And mainteNanCe doses to predict HEmorrhages) study was to determine whether the VASP index or platelet aggregation results measured at the acute phase could predict bleeding in patients with ACS according to P2Y₁₂ inhibitor.

Methods

Study design

This was a retrospective study on outcome events in patients with ACS treated with DAPT (aspirin and a P2Y₁₂ inhibitor). All consecutive adult patients admitted to the Lariboisière University Hospital (AP-HP,

Paris, France) from January 2012 to December 2016 for ST-segment elevation or non-ST-segment elevation myocardial infarction, and who had platelet aggregation tests because of at least one additional atherothrombotic risk factor (diabetes, smoking, age \geq 65 years, chronic renal failure or multivessel coronary disease) were selected for the study. Patients were included if they were followed by our structure and they (or their relatives) had agreed to participate in a telephone interview. The exclusion criteria were cardiogenic shock, concomitant anticoagulation therapy, use of fibrinolytic drug or glycoprotein IIb/IIIa inhibitors, known serious haemostatic disorder, neoplasia, pregnancy or lactation, participation in another investigational drug study within 30 days, patient under curatorship and patient without social security affiliation.

All patients' clinical and biological baseline characteristics were obtained from the hospital database. Follow-up was performed using the hospital database (computerized medical file [MiddleCare®. Evolucare, Villers Bretonneux, France] along with telephone interview of the patient and their referring doctor if necessary. In case of a significant event during follow-up, all medical records available (hospitalization report, biological data, electrocardiogram, etc.) were collected and reviewed by two blinded investigators to determine the classification of the event. Bleeding events were categorized according to the Bleeding Academic Research Consortium (BARC) classification [18] and also the Thrombolysis in Myocardial Infarction (TIMI) [19] and PLATelet inhibition and patient Outcomes (PLATO) [20] scales (see [Table A.1](#), [Table A.2](#) and [Table A.3](#)). If DAPT was modified or interrupted during follow-up without any event, the patient's follow-up was considered completed and events were not recorded after DAPT modification. The study was approved by the institutional review board in accordance with national guidelines (IDRCB 2017-A01159-44). All patients provided informed consent.

Antiplatelet drugs

The patients were treated initially with an aspirin loading dose of 250–500 mg intravenously, followed by a daily morning dose of 75 mg of non-enteric coated lysine acetylsalicylate (Kardegic®; Sanofi-Aventis, Paris, France). A loading dose of P2Y₁₂ inhibitor (600 mg clopidogrel, 60 mg prasugrel or 180 mg ticagrelor) was given in the ambulance or just after coronary angiography. A maintenance dose was administered in the morning for clopidogrel (75 mg) or prasugrel (10 mg) and twice a day for ticagrelor

(180 mg/day). DAPT was planned for 1 year according to European Society of Cardiology guidelines [1,21]. The choice of P2Y₁₂ inhibitor and the duration of DAPT was left to the discretion of the practitioner in charge of the patient, and was independent of the present study, which was retrospective.

Platelet function testing

Platelet function assays were assessed during the hospitalization for ACS before discharge as previously described (i.e. between day 2 and day 4) [7,21]. Compliance was systematically assessed before each blood sampling. All blood samples were taken under fasting conditions, and were performed in the morning at trough level (between 8:00 am and 9:00 am) before ingestion of the morning dose of aspirin (i.e. 24 hours after the last aspirin intake) and P2Y₁₂ inhibitor (i.e. 24 hours after the last intake of clopidogrel or prasugrel, and 12 hours after the last intake of ticagrelor). Blood samples were delivered to the hospital's clinical laboratory within 1 hour.

Flow cytometry for VASP index and platelet activation characterization [22]

Samples of peripheral blood were collected in citrated anticoagulants tubes and processed within the 4 hours after sampling. Flow cytometry measurements were performed on a FACSCanto™ II flow cytometer, using FACSDiva™ software (Becton Dickinson, San Jose, CA, USA) for data acquisition and analysis. VASP phosphorylation was measured by quantitative flow cytometry using a commercially available kit PLT VASP/P2Y₁₂ (ByoCytex, Marseille, France). The VASP index was calculated from the median fluorescence intensity (MFI) of samples incubated with prostaglandin E1 (PGE1) and ADP, according to the formula: $VASP\ index = [(MFI(PGE1) - MFI(PGE1+ADP)) / MFI(PGE1)] \times 100$

In addition, platelet-rich plasma diluted 1:40 in phosphate-buffered saline was incubated with anti-P-selectin phycoerythrin-conjugated monoclonal antibody (Clone AC1.2; Becton Dickinson, San Jose, CA, USA). Platelet activation markers were measured in unstimulated platelets and after stimulation of platelets with ADP (50 μmol/L final concentration) and thrombin receptor-activating peptide (TRAP; (70 μmol/L final concentration).

Light transmission aggregometry

Briefly, platelet-rich plasma was obtained by centrifugation of citrated whole blood at 160 g for 12 minutes at 20 °C, and platelet-poor plasma was obtained by further centrifugation at 2300 g for 10 minutes at 20 °C. Ex vivo platelet aggregation was measured at 37 °C within 1 hour following sampling, using an aggregometer (Model 490-4D; CHRONO-LOG Corporation, Kordia, the Netherlands) following the optical aggregometry method of Born [23]. To assess the biological response of the P2Y₁₂ inhibitor, light transmission aggregometry (LTA) was induced by the addition of ADP at final concentrations of 5 µM, 10 µM (not shown) and 20 µM. Aggregation was expressed as the maximum aggregation intensity as well as the final aggregation extent (measured 6 minutes after ADP activation) using the autologous platelet-poor plasma as a reference [7,24].

To assess the biological response to aspirin, platelet-rich plasma LTA triggered by arachidonic acid (AA) at concentrations of 0.8 µM (0.25 mg/mL) and 1.6 µM (0.5 mg/mL) was performed [25].

Follow-up of bleeding and ischaemic outcomes

Bleeding events were recorded chronologically starting from the ACS hospitalization, and followed throughout the time that the patient was on the DAPT regimen under which the platelet function studies were performed. Bleeding events were classified according to BARC, TIMI and PLATO scales [18-20].

Statistical methods

The aim of the study was to determine for each P2Y₁₂ inhibitor the optimal VASP index cut-off to predict bleeding (BARC score 2–5) in patients with ACS, using a receiver operating characteristic (ROC) curve model and calculation of Youden's J statistic [26]. The optimal cut-off level was calculated by determining the smallest distance between the ROC curve and the upper left corner of the graph. ROC curves were also performed for other bleeding scales (PLATO, TIMI). Continuous variables were analysed for normal distribution with the D'Agostino-Pearson test. Continuous variables are presented as means and standard deviations if normally distributed and were compared with Student's unpaired *t* test; if not normally distributed, they are presented as median (interquartile range [IQR]), and were compared with the Mann-Whitney rank-sum test. Categorical variables are presented as counts and percentages and were

compared by means of the χ^2 test or Fisher's exact test. Comparison of continuous variables between P2Y₁₂ inhibitor groups was performed with a one-way analysis of variance. A *P* value < 0.05 was considered statistically significant. Statistical analyses were performed with the use of MedCalc Statistical Software (MedCalc Software bv, Ostend, Belgium).

Results

Cohort characteristics

The present study included 364 patients with ACS from January 2012 to December 2016. Among them, 123 were treated with ticagrelor, 105 with prasugrel and 136 with clopidogrel. An invasive approach was used in all patients. Patients with an ST-segment elevation myocardial infarction were admitted directly to the catheterization laboratory, and all patients with a non-ST-segment elevation myocardial infarction underwent a coronary angiography within 48 hours and most often within 24 hours after admission. Platelet function testing (VASP index and aggregation tests) was performed on day 3 ± 1 day. The median follow up was 2.6 years (IQR 1.9–3.3 years). The clinical characteristics are presented in [Table 1](#). Briefly, 297 patients (81.6%) were male, the mean age was 64 ± 11 years and 40.9% had diabetes. Seventeen patients were excluded because DAPT was modified during follow-up: 11 were no longer on clopidogrel at the time of the event (four were on aspirin only, three switched to prasugrel and four switched to ticagrelor); four patients on ticagrelor were switched to another treatment (one to clopidogrel, two to anticoagulant and one to aspirin only); finally, two patients on prasugrel were switched to another treatment (one to clopidogrel and one to aspirin only).

Platelet function tests

Spontaneous platelet activation

Spontaneous platelet activation measured by platelet P-selectin expression was similar in the three groups: 6% (IQR 4–9%) in the clopidogrel group; 5% (IQR 3–8%) in the prasugrel group; and 5% (IQR 3–7%) in the ticagrelor group (*P* = 0.38).

VASP index

Overall, the median VASP index was 22% (IQR 10.0–39.4%). The VASP index was lower in patients on ticagrelor (14.0%, IQR 7.8–23.0%) or prasugrel (13.9%, IQR 7.0–25.0%) than in those on clopidogrel (42.0%, IQR 25.0–64.0%) ($P < 0.001$) (Table 2 and Fig. 1). Of note, a greater interindividual variability of response was observed in patients on clopidogrel compared with in those on prasugrel or ticagrelor.

Platelet aggregation measured by LTA induced by ADP

Overall, the median maximal intensity of platelet aggregation measured by LTA induced by ADP 20 μM was 50% (IQR 34–70%); it was lower in patients on ticagrelor (37%, IQR 29–56%) or prasugrel (45%, IQR 30–60%) than in those on clopidogrel (69%, IQR 55–81%) ($P < 0.001$). The median residual intensity of platelet aggregation induced by ADP 20 μM at 6 minutes was 39% (IQR 15–55%); it was lower in patients on ticagrelor (20%, IQR 10–44%) or prasugrel (25%, IQR 5–47%) than in those on clopidogrel (50%, 36–65%) ($P < 0.001$). Consistent results were obtained for LTA induced by ADP 5 μM (Table 2).

Platelet aggregation measured by LTA induced by AA

No significant differences were observed between the biological responses to aspirin in the three subgroups, whatever the AA concentration used to induce aggregation. For LTA triggered by AA 0.8 μM tests, the median maximal intensity of platelet aggregation was 5% (IQR 0–10%) on ticagrelor, 5% (IQR 0–7%) on prasugrel and 4% (IQR 0–8%) on clopidogrel. For LTA triggered by AA 1.6 μM , the median maximal intensity of platelet aggregation was 12% (IQR 5–16%) on ticagrelor, 9% (IQR 6–16%) on prasugrel and 10% (IQR 4–17%) on clopidogrel (Table 2).

Follow-up

Seventy-seven haemorrhagic events were recorded: 28 concerning patients on ticagrelor, 18 on prasugrel and 31 on clopidogrel (Table 3). On the BARC scale, 36.3% of these events were rated equal to or greater than 2 out of 5, which corresponds to 7.7% of the total population. The delay between platelet function testing and the occurrence of the event was 10 months on average.

Platelet function tests and bleeding

Whatever the definition of bleeding that we explored (BARC score ≥ 2 , TIMI and PLATO scales), there was no significant relationship between inhibition of platelet function (VASP index and ADP-induced platelet aggregation) and the subsequent occurrence of bleeding events (Fig. 2).

Discussion

Baseline cohort characteristics were similar to those in previous ACS studies [3,4]. It should be noted that the proportion of patients with diabetes was relatively high (40.9 %). VASP index and LTA triggered by ADP 20 μM have been widely used by our team and others for platelet function testing under P2Y₁₂ inhibitors [7,13,27]. Median VASP indexes as well as ADP-induced maximal and residual intensities of platelet aggregation were significantly decreased in patients on ticagrelor or prasugrel compared with in those on clopidogrel, and the median values were in accordance with results from previous studies [11,13,14].

During the follow-up, the occurrence of bleeding was frequent: approximately 20% of patients reported haemorrhage on DAPT; overall, the occurrence of BARC 2–5 bleeding was 7.7%. Using TIMI major or minor score, the bleeding rate in our study lies between those in the TRITON and PLATO studies [3,4]. So, despite the retrospective study design, the rate of events collected during the follow-up was in accordance with those in the literature.

Using ROC curves, the accuracy of the VASP index or platelet aggregation intensities at the acute phase to predict bleeding was poor, whatever the P2Y₁₂ inhibitor used. These results conflict with previous studies showing that the lower the VASP index, the more bleeding occurs [9]. Bonello et al. demonstrated that, in patients treated with thienopyridines, the prevalence of bleeding was 15% in those with VASP $\leq 15\%$ vs 3–4% in patients with VASP $> 15\%$ [28]. According to Cuisset et al., the prevalence of bleeding in patients on thienopyridines was 33% when VASP was $\leq 10\%$ vs 8% when VASP was $> 10\%$ [29]. Thus, the bleeding odds ratio was multiplied by 4.7 (95% confidence interval: 2.7–8.3) when the VASP index showed low on-treatment platelet reactivity.

However, adaptation of DAPT according to aggregation test results does not modify patient prognosis. In the GRAVITAS study, bad responders had a worse prognosis than those who had an adapted pharmacodynamic response to clopidogrel. Nonetheless, the use of high-dose clopidogrel among patients with high on-treatment platelet reactivity after percutaneous coronary intervention (PCI) did not reduce the incidence of death from cardiovascular causes, non-fatal myocardial infarction or stent thrombosis [30]. Similarly, the ANTARCTIC study did not demonstrate that adjusting antiplatelet therapy according to platelet function monitoring improved clinical outcomes in patients undergoing PCI [31]. In the TRIGGER PCI trial, switching from clopidogrel to prasugrel in stable patients with high on-treatment platelet reactivity afforded effective platelet inhibition without a difference in the outcomes rate. However, the study was stopped prematurely for futility because of the low rate of adverse ischaemic events after PCI with contemporary drug-eluting stents in stable coronary artery disease [32]. Other studies have aimed to adjust the therapeutics to the genetic profile of patients or to perform de-escalation of antiplatelet therapy, without proving any benefit [33].

In the present retrospective study, we sought to determine a VASP index cut-off that could predict increased bleeding risk in patients treated with a P2Y₁₂ inhibitor and, more specifically, to establish whether the threshold was similar for ticagrelor compared with thienopyridines. The rationale is that ticagrelor is known to induce a more profound decrease in the VASP index than thienopyridines, even a more profound decrease than prasugrel, without evidence of comparatively increased bleeding risk. The two main conclusions of this retrospective study are: (1) that the bleeding rate was similar with the three P2Y₁₂ inhibitors, even though the VASP index was lower with prasugrel compared with clopidogrel and lower with ticagrelor compared with prasugrel; and (2) that in the overall cohort, the occurrence of bleeding during DAPT was not significantly related to the VASP index measured at the initial phase. Moreover, for each of P2Y₁₂ inhibitor individually, no relationship between ex vivo platelet inhibition and subsequent bleeding incidence was observed.

To explain the first point, we can hypothesize that patients treated with clopidogrel are those at higher bleeding risk; so, they are more likely to bleed with less platelet inhibition. Additionally, we put forward the hypothesis that ticagrelor could decrease the VASP index by two alternative pathways: the VASP index of patients treated with ticagrelor is lower without meaning more profound platelet reactivity inhibition,

explaining why patients treated with ticagrelor do not experience more bleeding complications than patients treated with prasugrel, even if their VASP index is lower, but we admit that this hypothesis is controversial [11].

To explain the absence of association between the VASP index and bleeding complications, even for P2Y₁₂ inhibitors taken individually, the mean delay in occurrence of bleeding was 10 months in the overall cohort, which is a very late occurrence. The VASP index was assessed in the ACS acute phase. At this time, the degree of platelet inhibition could be more related to the efficacy of the platelet inhibitor (prevention of ischaemic events), and the platelet reactivity is modified both by platelet stimulation as a result of the clinical situation in the acute phase and by the effect of the antiplatelet treatment. Later in the evolution, the patient's platelet reactivity returns to baseline, and the effect of the inhibitor is probably more related to haemorrhagic risk. As in many other trials evaluating the association between platelet function monitoring and clinical events, we did not pay enough attention to this point, and our lack of association between clinical and laboratory data probably reflects this major point. We should consider early platelet testing as a test for efficacy and late platelet testing (e.g. at 1 month) as a test for safety. At present, platelet function tests cannot be proposed routinely as they are not cost-effective, even using the tests that are considered the gold standard, which are time consuming.

Study limitations

Our study has several limitations. First, the main limitation is the retrospective design. Secondly, it is possible that we were faced with a lack of statistical power, especially in the clopidogrel group. However, the bleeding rate during follow-up appeared to be what we expected. Further studies are needed to definitively determine VASP index cut-off values to predict bleeding in patients with coronary artery disease, including both early and late evaluations of platelet function.

Conclusions

This study shows that platelet function testing in the acute phase failed to predict bleeding events in patients with ACS and treated with P2Y₁₂ inhibitors, whatever the drug. We could consider early platelet function testing as a test for efficacy and late platelet function testing as a test for safety.

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Disclosure of interest

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The other authors declare no conflicts of interest.

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Figure legends

Figure 1. Vasodilator-stimulated phosphoprotein (VASP) index (%) distribution according to P2Y₁₂ inhibitor.

Figure 2. Receiver operating characteristic curves to predict bleeding (PLATElet inhibition and patient Outcomes [PLATO] minor/major score) according to the vasodilator-stimulated phosphoprotein (VASP) index. AUC: area under the curve.

Table 1 Baseline clinical characteristics of patients, overall and according to P2Y₁₂ inhibitor.

	All patients (<i>n</i> = 364)	Ticagrelor (<i>n</i> = 123)	Prasugrel (<i>n</i> = 105)	Clopidogrel (<i>n</i> = 136)	<i>P</i>
Age (years)	64.0 (55.8–72.6)	63.1 (55.9–72.4)	59.4 (53.1–66.0)	68.4 (61.4–78.0)	< 0.001
Men (%)	81.6	82.1	81.9	80.9	0.94
Weight (kg)	78.2 (68.5–87.0)	78.3 (69.5–86.5)	80.0 (70.0–89.5)	74.2 (68.0–85.0)	0.92
BMI (kg/m ²)	22.9 (20.3–25.3)	22.8 (20.3–25.2)	23.3 (20.6–25.9)	22.6 (20.2–24.9)	0.29
Cardiovascular risk factor (%)					
Current smoker	34.8	36.0	46.6	24.8	< 0.01
Diabetes mellitus	40.9	39.8	36.1	44.1	0.53
Dyslipidaemia	38.1	34.4	31.3	46.6	0.14
Hypertension	42.3	32.2	41.7	51.9	0.01
History of MI	12.5	13.8	13.6	10.4	0.64
Previous CABG	2.5	0.8	2.9	3.7	0.29
History of angioplasty	25.3	22.8	20.2	31.6	0.10
Previous stroke	3.0	1.6	0.0	6.7	< 0.01
LVEF					
LVEF ≥ 50%	79.1	77.2	81.9	78.7	0.85
LVEF 30–50%	16.2	21.9	14.3	14.7	

LVEF < 30%	3.6	0.8	3.8	3.7	
Indication for DAPT, clinical setting (%)					
STEMI	42.3	46.2	62.9	22.9	0.03
NSTEMI	27.1	34.7	27.6 %	20.0	
Unstable angina	30.6	19.1	9.5	57.1	
Angiography and intervention (<i>n</i>)					
Radial artery access	352 ^a	120	102	130	0.36
Left main coronary artery	44	17	10	17	0.63
Left anterior coronary artery	266	94	75	97	0.74
Circumflex coronary artery	47	11	20	16	0.12
Right coronary artery	203	76	52	75	0.24
Triple vessel CAD	48	17	9	22	< 0.01
Number of angioplasty	320	115	115	90	< 0.01
Number of stents	324	125	112	87	< 0.01
Number of drug-eluting stents	281	107	101	73	< 0.01

Data are expressed as median (interquartile range) unless otherwise indicated. BMI: body mass index; CABG: coronary artery bypass graft; CAD: coronary artery disease; DAPT: dual antiplatelet therapy; LVEF: left ventricular ejection fraction; MI: myocardial infarction; NSTEMI: non-ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction.

^a96.7%.

Table 2 Baseline laboratory data of patients, overall and according to P2Y₁₂ inhibitor.

	All patients (<i>n</i> = 364)	Ticagrelor (<i>n</i> = 123)	Prasugrel (<i>n</i> = 105)	Clopidogrel (<i>n</i> = 136)	<i>P</i>
Complete blood count					
Haemoglobin (g/dL)	13.7 (12.6–14.5)	13.9 (13.0–14.7)	13.4 (12.4–14.7)	13.6 (12.6–14.4)	0.54
Haematocrit (%)	40.8 (37.8–43.8)	41.8 (38.9–44.2)	40.2 (37.4–44.5)	40.6 (37.5–42.8)	0.38
White blood cells (G/L)	7.4 (6.0–9.2)	7.6 (6.0–8.9)	7.3 (5.9–9.3)	7.3 (5.9–9.4)	0.90
Platelets (G/L)	236 (195–289)	242 (202–242)	242 (192–306)	227 (191–277)	0.64
Mean platelet volume (fL)	7.8 (7.0–8.5)	7.8 (7.1–8.5)	7.8 (6.9–8.7)	7.8 (7.2–8.5)	0.94
Biochemical analysis					
Creatinine (μmol/L)	83 (71–95)	81 (71–96)	83 (71–93)	86 (74–97)	0.12
Creatinine clearance (mL/min)	84 (66–102)	85 (66–102)	94 (76–111)	76 (60–95)	0.04
Glycated haemoglobin (%)	6.1 (5.6–7.0)	5.9 (5.6–6.7)	6.0 (5.6–6.7)	6.4 (5.8–7.5)	0.02
Fibrinogen (g/L)	4.2 (3.5–5.3)	4.4 (3.6–5.7)	4.5 (3.8–5.5)	3.8 (3.4–4.8)	0.05
Prothrombin ratio (%)	98 (88–109)	97 (90–107)	98 (89–112)	96 (85–109)	0.21
Aggregation tests (%)					
VASP index	22.0 (10.0–39.4)	14.0 (7.8–23.0)	13.9 (7.0–25.0)	42.0 (25.0–64.0)	< 0.001
LTA – maximal intensity, 5 μM ADP	37 (23–55)	25 (21–41)	31 (22–45)	50 (37–63)	< 0.001
LTA – residual activity, 5 μM ADP	10 (0–30)	4 (0–15)	6 (0–23)	24 (8–41)	< 0.001

LTA – maximal intensity, 20 μ M ADP	50 (34–70)	37 (29–56)	45 (30–60)	69 (55–81)	< 0.001
LTA – residual activity, 20 μ M ADP	39 (15–55)	20 (10–44)	25 (5–47)	50 (36–65)	< 0.001
LTA – maximal intensity, 0.8 μ M AA	5 (0–9)	5 (0–10)	5 (0–7)	4 (0–8)	0.42
LTA – maximal intensity, 1.6 μ M AA	10 (5–17)	12 (5–16)	9 (6–16)	10 (4–17)	0.14

Data are expressed as median (interquartile range). AA: arachidonic acid; ADP: adenosine diphosphate; LTA: light transmission aggregometry; VASP: vasodilator-stimulated phosphoprotein phosphorylation.

Table 3 Bleeding events according to the Bleeding Academic Research Consortium (BARC), PLATelet inhibition and patient Outcomes (PLATO) and Thrombolysis in Myocardial Infarction (TIMI) scales.

	Overall			Ticagrelor			Prasugrel			Clopidogrel		
	Total	VASP <	VASP ≥	Total	VASP <	VASP ≥	Total	VASP <	VASP ≥	Total	VASP <	VASP ≥
		16%	16%		16%	16%		16%	16%		16%	16%
	(n = 364)	(n = 150)	(n = 214)	(n = 123)	(n = 76)	(n = 47)	(n = 105)	(n = 58)	(n = 47)	(n = 136)	(n = 16)	(n = 120)
BARC ≥ 1	77 (20.1)	23 (15.3)	54 (25.2)	28 (22.8)	10 (13.2)	18 (38.3)	18 (17.1)	10 (17.2)	8 (17.0)	31 (22.8)	3 (18.8)	28 (23.3)
BARC ≥ 2	28 (7.7)	7 (4.7)	21 (9.8)	11 (8.9)	3 (3.9)	8 (17.0)	7 (6.7)	3 (5.2)	4 (8.5)	10 (7.4)	1 (6.3)	9 (7.5)
BARC ≥ 3	16 (4.4)	3 (2.0)	13 (6.1)	7 (5.7)	0 (0.0)	7 (14.9)	2 (1.9)	2 (3.4)	0 (0.0)	7 (5.1)	1 (6.3)	6 (5.0)
BARC ≥ 4	2 (0.05)	-	-	2 (1.6)	-	-	0 (0)	-	-	0 (0)	-	-
BARC = 5	1 (0.02)	-	-	1 (0.8)	-	-	0 (0)	-	-	0 (0)	-	-
PLATO	77 (20.1)	23 (15.3)	54 (25.2)	28 (22.8)	10 (13.2)	18 (38.3)	18 (17.1)	10 (17.2)	8 (17.0)	31 (22.8)	3 (18.8)	28 (23.3)
Minor or major	27 (7.4)	7 (4.7)	20 (9.3)	10 (8.1)	3 (3.9)	7 (14.9)	7 (6.7)	3 (5.2)	4 (8.5)	10 (7.4)	1 (6.3)	9 (7.5)
PLATO												
TIMI	77 (20.1)	23 (15.3)	54 (25.2)	28 (22.8)	10 (13.2)	18 (38.3)	18 (17.1)	10 (17.2)	8 (17.0)	31 (22.8)	3 (18.8)	28 (23.3)
Minor or major	27 (7.4)	7 (4.7)	20 (9.3)	10 (8.1)	3 (3.9)	7 (14.9)	7 (6.7)	3 (5.2)	4 (8.5)	10 (7.4)	1 (6.3)	9 (7.5)
TIMI												

Data are expressed as number (%). VASP: vasodilator-stimulated phosphoprotein phosphorylation.

Figure 1.

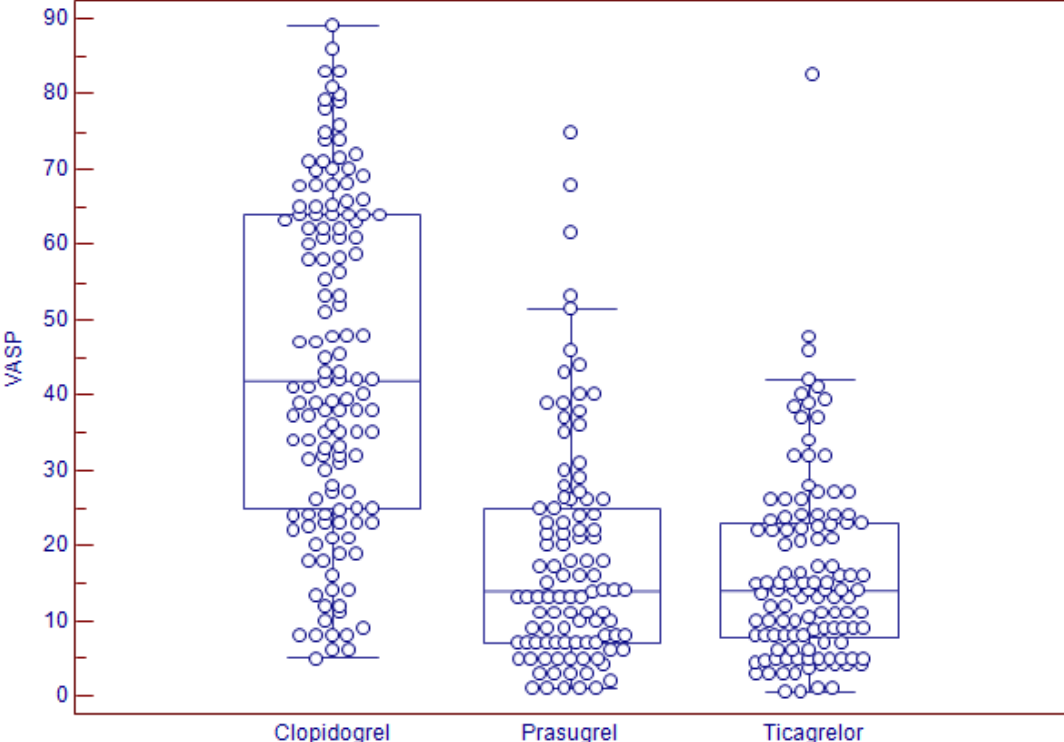


Figure 2.

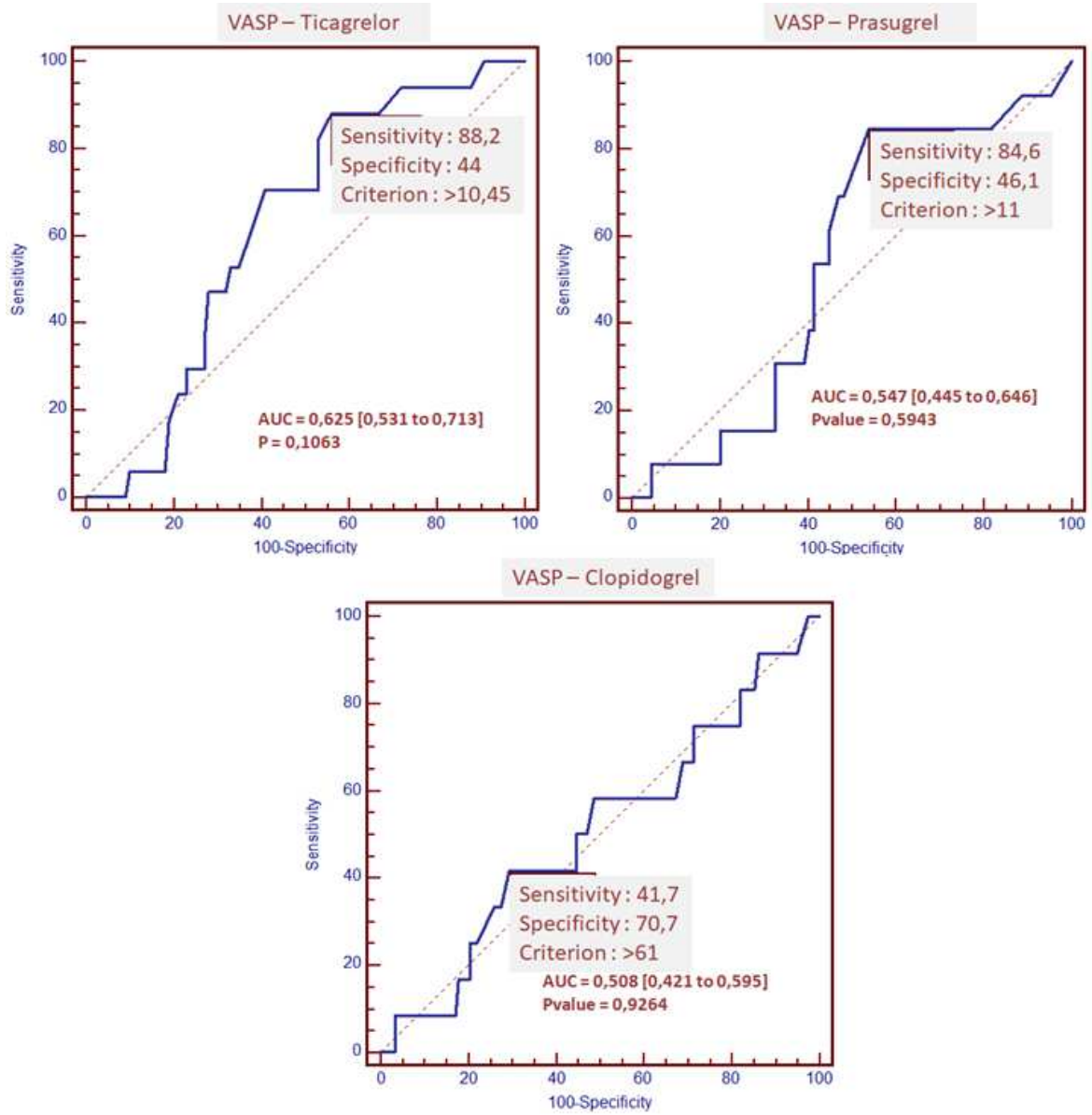


Table A.1 Bleeding Academic Research Consortium (BARC) scale [18].

Type 0	No bleeding
Type 1	Bleeding that is not actionable and does not cause the patient to seek treatment
Type 2	Any clinically overt sign of haemorrhage that “is actionable” and requires diagnostic studies, hospitalization or treatment by a healthcare professional
Type 3	a. Overt bleeding plus haemoglobin drop of 3 to < 5 g/dL (provided haemoglobin drop is related to bleed); transfusion with overt bleeding b. Overt bleeding plus haemoglobin drop of < 5 g/dL (provided haemoglobin drop is related to bleed); cardiac tamponade; bleeding requiring surgical intervention for control; bleeding requiring IV vasoactive agents c. Intracranial haemorrhage confirmed by autopsy, imaging or lumbar puncture; intraocular bleed compromising vision
Type 4	CABG-related bleeding within 48 hours
Type 5	a. Probable fatal bleeding b. Definite fatal bleeding (overt or autopsy or imaging confirmation)

CABG: coronary artery bypass graft; IV: intravenous.

Table A.2 PLATelet inhibition and patient Outcomes (PLATO) scale [20].

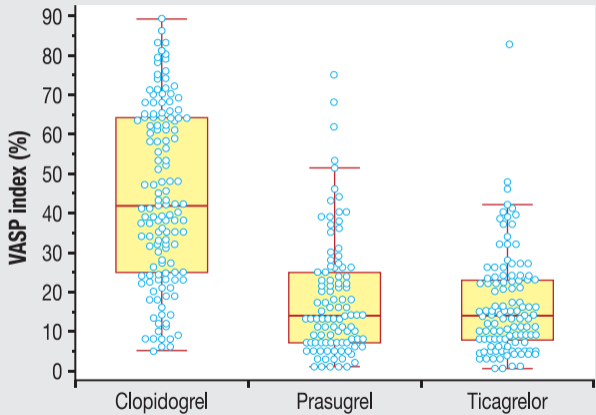
Major life-threatening	Fatal
	Intracranial
	Intrapericardial with cardiac tamponade
	Resulting in hypovolemic shock or severe hypotension that requires pressors or surgery
	Clinically overt or apparent bleeding associated with decrease in haemoglobin > 5 g/dL
	Requiring transfusion of ≥ 4 units of whole blood or PRBCs
Other major	Significantly disabling (e.g. intraocular with permanent vision loss)
	Associated drop in haemoglobin of 3–5 g/dL
	Requiring transfusion of 2–3 units whole blood or PRBCs
Minor	Requiring medical intervention to stop or treat bleeding (e.g. epistaxis requiring visit to medical facility for packing)
Minimal	All others (e.g. bruising, bleeding gums, oozing from injection sites) not requiring intervention or treatment

PRBC: packed red blood cell.

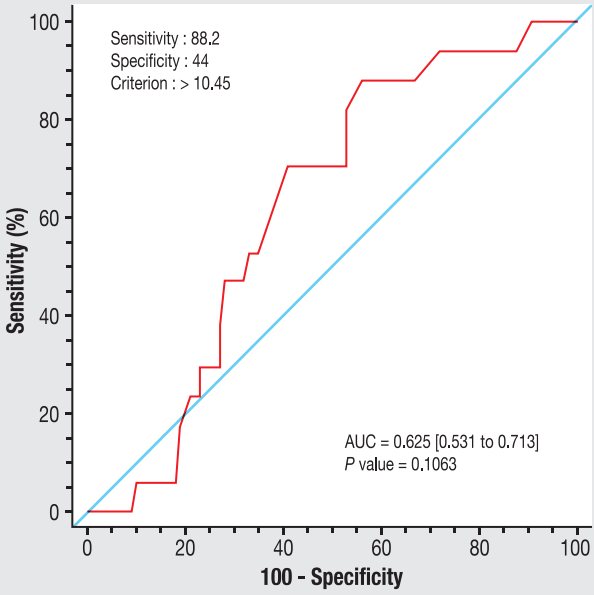
Table A.3 Thrombolysis in Myocardial Infarction (TIMI) bleeding scale [19].

Major	Any intracranial bleeding (excluding microhaemorrhages < 10 mm evident only on gradient-echo MRI) Clinically overt signs of haemorrhage associated with a drop in haemoglobin of ≥ 5 g/dL or a $\geq 15\%$ absolute decrease in haematocrit Fatal bleeding (bleeding that directly results in death within 7 days)
Minor	Clinically overt (including imaging), resulting in haemoglobin drop of 3 to < 5 g/dL or a $\geq 10\%$ decrease in haematocrit No observed blood loss: ≥ 4 g/dL decrease in the haemoglobin concentration or $\geq 12\%$ decrease in haematocrit Any overt sign of haemorrhage that meets one of the following criteria and does not meet criteria for a major or minor bleeding event, as defined above: a. Requiring intervention (medical practitioner-guided medical or surgical treatment to stop or treat bleeding, including temporarily or permanently discontinuing or changing the dose of a medication or study drug) b. Leading to or prolonging hospitalization c. Prompting evaluation (leading to an unscheduled visit to a healthcare professional and diagnostic testing, either laboratory or imaging)
Minimal	Any overt bleeding event that does not meet the criteria above Any clinically overt sign of haemorrhage (including imaging) associated with a < 3 g/dL decrease in haemoglobin concentration or < 9% decrease in haematocrit

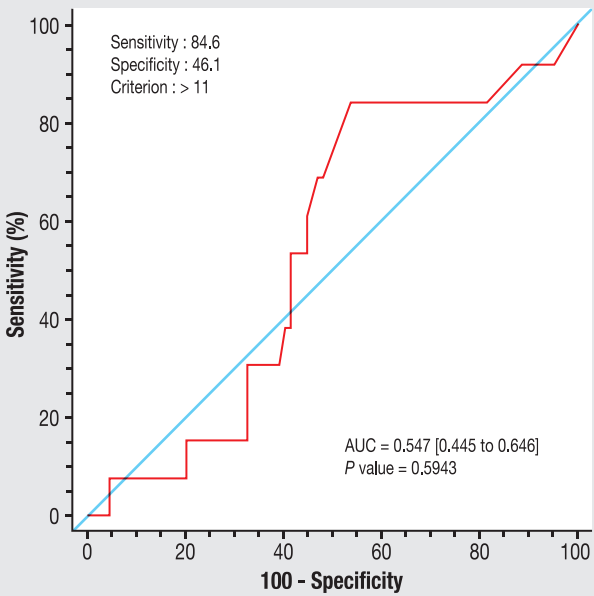
MRI: magnetic resonance imaging.



VASP - Ticagrelor



VASP - Prasugrel



VASP - Clopidogrel

