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Effect of preoperative P_2Y_{12} and thrombin platelet receptor inhibition on bleeding after cardiac surgery

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Editor's key points

- The association between preoperative platelet function and postoperative bleeding was assessed in patients treated with P_2Y_{12} receptor antagonists.
- Multiplate electrode aggregometry platelet function testing predicted postoperative bleeding with high negative and low positive predictive values.
- P_2Y_{12} receptor inhibition by the ADPtest can be compensated by normal protease-activated receptor (PAR) function by the TRAPtest.

Background. Drugs that act on the platelet P_2Y_{12} receptor are responsible for postoperative bleeding in cardiac surgery. However, protease-activated receptor (PAR) that reacts to thrombin stimulation might still be active in patients treated with P_2Y_{12} inhibitors. Preoperative platelet function testing could possibly guide the timing of surgery. We investigated the association between P_2Y_{12} receptor and PAR inhibition and bleeding after cardiac surgery.

Methods. A retrospective cohort study of 361 patients undergoing cardiac surgery and treated with P_2Y_{12} anti-platelet agents was undertaken. All patients received a preoperative multiplate electrode aggregometry testing of platelet P_2Y_{12} receptor activity (ADPtest) and PAR reactivity with thrombin receptor-activating peptide (TRAP) stimulation. ADPtest and TRAPtest data measured before surgery were analysed for association with postoperative bleeding (ml per 12 h) and severe postoperative bleeding.

Results. Both the ADPtest and the TRAPtest were significantly ($P=0.001$) associated with postoperative bleeding. A threshold of 22 U for the ADPtest yielded a negative predictive value (NPV) of 94% and a positive predictive value (PPV) of 20%, and a threshold of 75 U for the TRAPtest yielded an NPV of 95% and a PPV of 23%. In the subgroup of patients with ADPtest <22 U, TRAPtest ≥ 75 U was not associated with severe bleeding (NPV of 100% and PPV of 37%).

Conclusions. In patients taking P_2Y_{12} receptor inhibitors, residual platelet reactivity to thrombin stimulation limits the risk of severe postoperative bleeding.

Keywords: blood, loss; blood, platelets; surgery, cardiovascular

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Patients treated with platelet P_2Y_{12} receptor inhibitors are at risk of severe postoperative bleeding after heart operations.[1 2] Existing guidelines suggest that drug discontinuation at least 5 days before surgery,[3 4] but some patients can be safely operated after only 3 days of drug discontinuation.[5] A recent update of Society of Thoracic Surgeons guidelines[6] states, with a level of evidence IIa, that the use of platelet function tests (PFTs) is reasonable to settle timing for cardiac and non-cardiac surgery.

In previous studies, we have used multi-electrode aggregometry (MEA) as a preoperative PFT in patients undergoing cardiac surgery who are treated with thienopyridines (ticlopidine, clopidogrel, and prasugrel) or ticagrelor, anti-platelet agents that inhibit the adenosine-diphosphate (ADP)-dependent P_2Y_{12} platelet receptor. We identified a cut-off value for the ADPtest of 31 U for avoidance of excessive postoperative bleeding,[7] and we confirmed that recovery of platelet function after drug discontinuation is highly individualized, being only partially dependent on the timing of discontinuation.[8]

During cardiac surgery with cardiopulmonary bypass (CPB) thrombin is extensively generated.[9] Thrombin is a powerful platelet activator, acting on the protease-activated human platelet receptors (PARs) PAR-1 and PAR-4. PAR activation is partially dependent on P_2Y_{12} inhibition,[10] and therefore it is possible that platelet aggregation remains within an acceptable range even in the presence of strong drug-induced inhibition of P_2Y_{12} receptors. However, evidence supporting the use of PFTs before surgery is based on assessment of P_2Y_{12} activity only.[7 8 11 12]

The present study assessed the association between P_2Y_{12} receptor and PAR reactivity with postoperative bleeding in patients under dual anti-platelet therapy undergoing cardiac operations with CPB. The primary endpoint was to verify the hypothesis that preoperative thrombin-dependent platelet

aggregation testing allows better determination of the correct timing of surgery, avoiding the risk of excessive bleeding and also unnecessary delay.

Methods

Study design

Since 2009 patients undergoing heart operations and treated with dual anti-platelet therapy with P_2Y_{12} inhibitors not discontinued at least 1 week before operation were usually screened with a point-of-care PFT (Multiplate™, Verum Diagnostica GmbH, Munchen, Germany) at our institution. We therefore designed a retrospective cohort study based on data stored in our institutional database and in the Multiplate database. The study was approved by our Local Ethics Committee (ASL Milano 2 Melegnano), and the need for an informed consent was waived.

Patient population and platelet function testing

For the period of January 2009–March 2012 we retrieved data on 435 patients who received PFTs before cardiac surgery. The PFT was performed point-of-care within the operating theatre, the day before the operation, or the morning of the operation for afternoon operations. All patients were treated with thienopyridines before surgery, with discontinuation <7 days.

PFT was performed as previously described.[7 8] Briefly, whole blood was collected into 3.0 ml tubes containing hirudin as an anti-coagulant. Three hundred microlitres of blood was added to 300 μl of 37°C saline solution, and platelet aggregation was analysed after activation with ADP (ADPtest, 6.5 μM final concentration) or thrombin receptor-activating peptide (TRAP-6, TRAPtest, 32 μM final concentration). ADPtest is sensitive to ADP receptor (P_2Y_1 and P_2Y_{12}) inhibition induced by direct ADP receptor antagonists like thienopyridines, whereas TRAP-6 is a potent platelet activator via the thrombin-dependent PAR-1 and PAR-4 receptors.

Increasing electric impedance was electronically measured for 6 min and expressed as area under the aggregation curve plotted over time [AUC, (U)] by an integrated software. Reference ranges indicated by the manufacturer were AUCs of 53 122 U for ADPtest and 94 156 U for TRAPtest. Blood collection tubes, test cells, reagents, and analysing software were all provided by the manufacturer as a part of the standard supply to our hospital and without support linked to the study.

Before 2009, it was our standard practice to interrupt thienopyridine therapy at least 5 days before operation. However, patients might be operated even without discontinuing thienopyridines if considered urgent. Starting in 2011 patients were admitted to surgery even in case of discontinuation <5 days, in case of acceptable (>31 U) platelet function by the ADPtest.[7] If the operation was considered urgent, the patient was always admitted to surgery regardless of platelet function.

TRAPtest was performed routinely until 2011, but was selectively performed according to operator choice from 2011. Operators include trained staff cardiac anaesthesiologists and a laboratory technician in charge of the point-of-care coagulation laboratory of our institution. Seventy-four patients received only an ADPtest and were excluded. The final patient population (361 subjects) received both ADPtest and TRAPtest on the same day before surgery. In case of multiple tests, the last value immediately before surgery was considered. This corresponds to a test always done within 24 h before surgery.

All patients were treated with aspirin that was not discontinued before surgery.

Data collection and definitions

All patients received tranexamic acid intra-operatively (15 mg kg^{-1} after induction, followed by 15 mg kg^{-1} after protamine administration). From our institutional database we retrieved the following data for each patient: general characteristics; left ventricular ejection fraction (%); recent (30 days) myocardial infarction; congestive heart failure; active endocarditis; chronic obstructive pulmonary disease; diabetes mellitus on medication; previous cerebrovascular accident; previous heart operation; haematocrit (%); serum creatinine (mg dl^{-1}); serum bilirubin (mg dl^{-1}); platelet count (cells μl^{-1}); type of thienopyridine used (ticlopidine, clopidogrel, prasugrel) and date of last intake; result and date of ADPtest and TRAPtest; type of surgery; CPB duration (min); postoperative bleeding (ml in 12 postoperative h); and allogeneic blood products transfusions.

Severe bleeding was defined according to the Universal Definition of Perioperative Bleeding (UDPB) in adult cardiac surgery[13] as the presence of at least one of the following: chest drain fluid loss >1 litre in the first 12 postoperative hours, need for surgical re-exploration, and need for >5 units of red blood cells or fresh frozen plasma. The UDPB criteria were partially applied because of absent retrospective data like delayed sternal closure; massive bleeding (defined according to the UDPB) was included in the definition of severe bleeding.

Statistical analysis

Data are presented as mean (SD) for continuous variables, and as number and percentage for categorical variables. The univariate association between continuous variables and postoperative bleeding (after testing for normality of distribution) was explored using linear or non-linear regression analyses. The association between binary categorical variables and postoperative bleeding was tested with Student's *t*-test. Multivariable models were based on linear regression models (stepwise forward), with inclusion of the factors that demonstrated an association with postoperative bleeding at a *P* value <0.1 by univariate analysis. The diagnostic properties of PFTs for the association with severe postoperative bleeding were investigated using Receiver Operating Characteristics (ROC) analysis with *c*-statistics. The coordinates of the ROC curve were explored in order to identify adequate threshold for severe bleeding, using a combination of sensitivity and specificity (Youden's index, sensitivity+specificity−1). For each identified threshold, statistical analysis for the association with severe bleeding was applied using Pearson's χ^2 and assessing negative and positive predictive power. Analysis was done

Table 1 Patient characteristics, perioperative variables, and association with postoperative bleeding ($n=361$). *Normal range: 53–122 U; **94–156 U; ADP, adenosine diphosphate; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; CVA, cerebrovascular accident; MEA, multi-electrode aggregometry; SD, standard deviation; TRAP, thrombin receptor-activating peptide

Variable	Mean (SD) or number (%)	*P* value for association with postoperative bleeding
Anti-platelet drugs		0.734
Ticlopidine	53 (14.7)	
Clopidogrel	298 (82.5)	
Prasugrel	10 (2.8)	
Age (yr)	67.8 (10.7)	0.738
Gender male	287 (79.5)	0.279
Weight (kg)	75.0 (14.3)	0.465
Ejection fraction (%)	51.5 (11.6)	0.204
Recent myocardial infarction	39 (10.8)	0.068
Congestive heart failure	19 (5.3)	0.082
Active endocarditis	2 (0.6)	0.532
Serum creatinine (mg dl^{-1})	1.13 (0.84)	0.122
Serum bilirubin (mg dl^{-1})	0.65 (0.48)	0.356
COPD	25 (6.9)	0.668
Previous CVA	9 (2.5)	0.733
Diabetes mellitus		
on medication	73 (20.2)	0.050
Previous heart surgery	14 (3.9)	0.061
Haematocrit (%)	38.0 (4.7)	0.857
Platelet count ($\times 1000$ μl^{-1})	209 (75)	0.269
Surgery		0.022
Isolated CABG	247 (68.4)	
Isolated valve	28 (7.8)	
CABG+valve	59 (16.3)	
Others	27 (7.5)	
CPB duration (min)	78 (37)	0.083
Days from drug discontinuation	4 (3.2)	0.481
MEA-ADPtest (U)*	49.7 (24.2)	0.001
MEA-TRAPtest (U)**	97.5 (26.7)	0.001
Total bleeding (ml in 12 postoperative h)	495 (364)	–
Severe bleeding	27 (7.5)	–
Surgical revision	10 (2.8)	0.001
Transfusion (number of patients and %)		
Red blood cells	190 (52.6)	0.001
Fresh frozen plasma	37 (10.2)	0.001
Platelets	58 (16.1)	0.001

using SPSS 20.0 (IBM, Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Results

General characteristics of the patient population are depicted in Table 1. Platelet count was always $>80\,000$ cells μl^{-1}. There was a positive association between platelet count and ADPtest ($P=0.043$), and positive association between platelet count and TRAPtest ($P=0.006$).

Both preoperative ADPtest and TRAPtest were negatively associated with postoperative bleeding ($P<0.001$). Other factors associated ($P<0.1$) with postoperative bleeding were congestive heart failure, recent myocardial infarction, diabetes mellitus (lower bleeding), type of operation (larger bleeding in all surgeries other than isolated coronary surgery), previous heart surgery (higher bleeding), and longer CPB duration (higher bleeding). Included in a multivariable model, both ADPtest ($P=0.002$) and TRAPtest ($P=0.001$) remained independently associated with postoperative bleeding.

The association between ADPtest, TRAPtest, and postoperative bleeding was explored using different models of linear and non-linear regression analyses. The best fit for both tests was logarithmic (Figs 1 and 2), demonstrating that for both tests postoperative bleeding remains substantially unchanged for even large changes in aggregometry when the test yielded results from moderately decreased to normal values. Conversely, postoperative bleeding rapidly climbs when low values of aggregometry are reached.

Severe bleeding was found in 27 (7.5%) patients.

Cut-off values for the association with severe bleeding were searched by testing general accuracy of ADPtest and TRAPtest as diagnostic tests for severe bleeding using ROC analysis. For both tests general accuracy was poor (c-statistics for ADPtest: 0.62; for TRAPtest: 0.65), reflecting the nature of the relationship between PFT and bleeding, with a large zone corresponding to normal or moderately depressed platelet function where postoperative bleeding does not depend on preoperative platelet function. Based on the coordinates of the ROC curve, sensitivity and specificity, and negative and positive predictive power of different cut-off values were tested for both ADPtest and TRAPtest. The best combination of negative and positive predictive power was found for ADPtest <22 U and TRAPtest <75 U (Table 2). Both tests yielded a very good NPV (94 and 95%, respectively), with a low positive predictive value (20 and 23%). After correction for potential confounders, both threshold values identified for ADPtest and TRAPtest remained independently associated with severe bleeding (Table 3). ADPtest and TRAPtest were tested in separate models because of their intercorrelation, with potential confounders identified in Table 1.

The association between ADPtest and TRAPtest was explored using linear and non-linear regression analyses. The best fit was a logarithmic function (Fig. 3). From the analysis of this relationship and based on the identified cut-off values

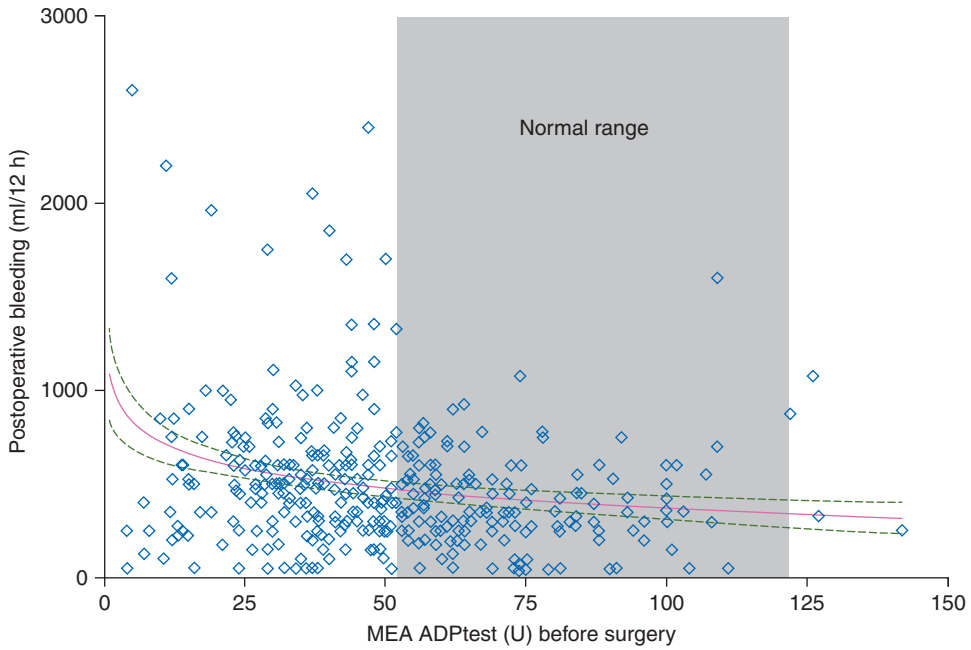

Fig 1 Logarithmic association between preoperative MEA-ADPtest and postoperative bleeding. Dashed lines are 95% confidence interval. ADP: adenosine diphosphate; MEA, multiple electrode aggregometry.

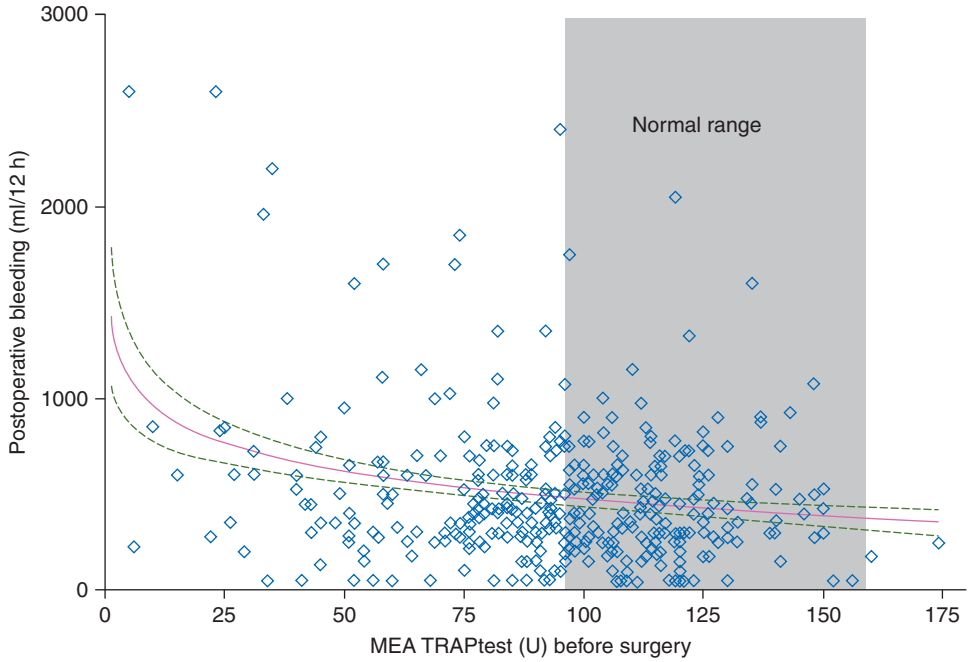

Fig 2 Logarithmic association between preoperative MEA-TRAPtest and postoperative bleeding. Dashed lines are 95% confidence interval. MEA, multiple electrode aggregometry; TRAP, thrombin receptor-activating peptide.

for ADPtest and TRAPtest, patients with an ADPtest value of <22 U did not demonstrate severe bleeding if TRAPtest was >75 U. A combined ADPtest–TRAPtest in patients with an ADPtest <22 U demonstrated further improvement in both negative (100%) and positive (37%) predictive values for severe bleeding (Table 2).

Table 2 Contingency table of ADPtest and TRAPtest thresholds for association with severe bleeding. ADP, adenosine diphosphate; NPV, negative predictive value; PPV, positive predictive value; SB, severe bleeding; TRAP, thrombin receptor-activating peptide

Test and threshold		No SB	SB	Total	*P*	NPV (%)	PPV (%)
Total population (n=361)							
ADPtest	≥22 U	306	20	326	0.003	94	20
	<22 U	28	7	35			
	Total	334	27	361			
TRAPtest	≥75 U	291	14	305	0.001	95	23
	<75 U	43	13	56			
	Total	334	27	361			
Patients with ADPtest <22 U (n=35)							
TRAPtest	≥75 U	16	0	16	0.007	100	37
	<75 U	12	7	19			
	Total	28	7	35			

Table 3 Multivariable logistic regression analysis for independent association with severe bleeding. Adjustment factors considered: congestive heart failure, recent myocardial infarction, diabetes mellitus, type of surgery, previous cardiac surgery, cardiopulmonary bypass duration

Factor	Regression coefficient	Odds ratio (95% CI)	*P*-value
ADPtest <22 U	1.02	2.77 (1.01–7.71)	0.048
Isolated CABG	−1.11	0.33 (0.15–0.75)	0.008
Constant	−1.98		
TRAPtest <75 U	1.59	4.91 (2.08–11.6)	0.001
Isolated CABG	−0.96	0.38 (0.17–0.89)	0.026
Constant	−2.36		
ADPtest <22 U and			
TRAPtest <75 U	1.75	5.74 (1.17–17.6)	0.002
Isolated CABG	−0.97	0.38 (0.16–0.87)	0.024
Constant	−2.10		

Discussion

The main results of this study are that (i) patients with moderately decreased to normal values of platelet reactivity by preoperative PFT have a very low risk of severe postoperative bleeding, and (ii) low values of platelet aggregation by the ADPtest can be compensated by acceptable values of platelet aggregation by the TRAPtest. Although anti-platelet agents commonly used on top of aspirin inhibit the P_2Y_{12} receptor, our data demonstrate that their action exerts partial inhibition even PARs. TRAPtest was below the normal range in many of our patients, and there was a logarithmic relationship between P_2Y_{12} and PAR inhibition. This relationship was observed by Badr Eslam and colleagues[14] in a smaller series of patients using the same PFT technology, but these authors suggest a linear relationship in their relatively small patient population. Other studies could demonstrate that P_2Y_{12} inhibition exerts a degree of PAR-1 inhibition.[15 16] The interpretation of this phenomenon is based on the dynamic concept of platelet activation: thrombin initiates the process activating the PAR-1 and PAR-4, the activated platelets release ADP, which in turn further activates platelets through P_2Y_{12} receptors. Thrombin and ADP act synergistically in the process of platelet activation, and P_2Y_{12} receptor inhibition partially attenuates the effect of thrombin receptors activation.

The existence of a sort of 'cross-reactivity' between the P_2Y_{12} receptors and PARs receptors stresses the potential role of PAR reactivity as a factor associated with postoperative bleeding. Our data demonstrate that abnormalities in the ADPtest and the TRAPtest are associated with postoperative bleeding, and that both tests have very good negative predictive power as diagnostic tests for severe bleeding.

With respect to our previous study,[7] we found a lower threshold for the ADPtest association with severe bleeding. This could be because of two factors: the much larger patient population and the outcome (severe bleeding) had a different definition. The UDPB was not available when we performed the first study; this led to a more restrictive definition in the previous study (where excessive bleeding was defined as >0.8 litre over 12 h^{-1}). Using the new definition, the ADPtest threshold decreased from 31 to 22 U. This value is close to the one of 19 U previously identified as the threshold for major bleeding events in patients undergoing percutaneous coronary intervention.[17]

Another important finding is that PAR-related platelet function seems able to compensate for poor P_2Y_{12}-related function. TRAPtest ≥75 U in patients with ADPtest <22 U has a 100% negative predictive power for severe postoperative bleeding. This information is new and clinically relevant, because application of TRAPtest in this subset of patients avoids unnecessary delay in surgical timing. In our previous study,[7] because of the relatively low number of patients, we did not investigate the combination of ADP and TRAP test for association with severe bleeding, and we could not identify the predictive power of combined ADP–TRAP test. In the present study, ~45% of patients with ADPtest <22 U have a TRAPtest ≥75 U and thus can be operated without risk of severe bleeding. Thrombin-

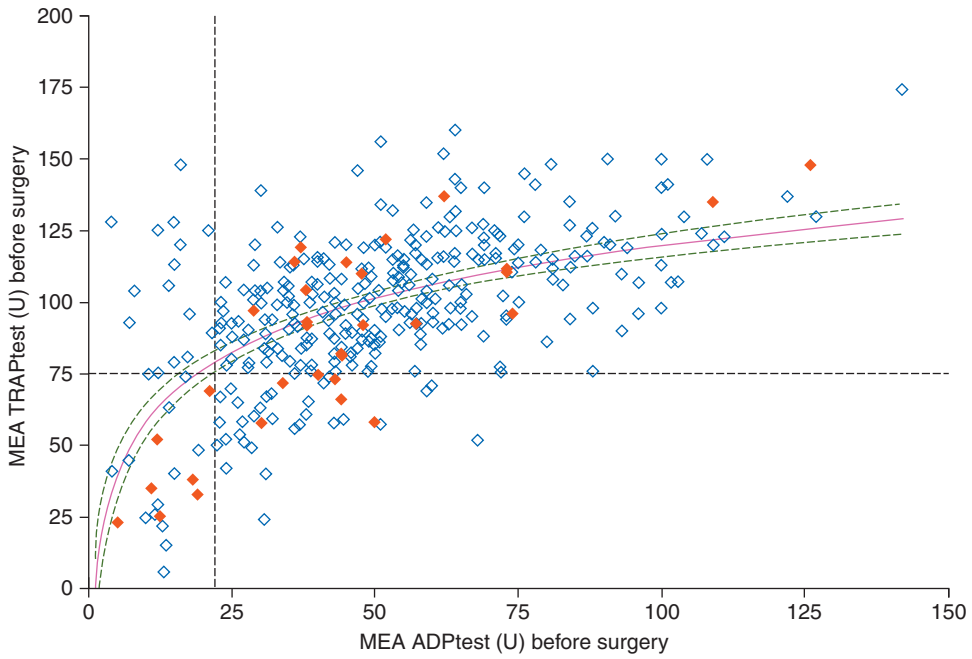

Fig 3 Logarithmic association between preoperative MEA-ADPtest and MEA-TRAPtest. Dashed lines are 95% confidence interval. Orange diamonds are patients with severe bleeding. ADP, adenosine diphosphate; MEA, multiple electrode aggregometry; TRAP, thrombin receptor-activating peptide.

associated platelet activation is particularly important in the setting of cardiac operations with CPB, where extensive thrombin generation is the rule. Combined ADPtest/TRAPtest analysis was based on a small subset of 35 patients. These data should be confirmed in larger patient series. However, in other clinical scenarios, like percutaneous coronary intervention, it has already been demonstrated that in patients treated with P_2Y_{12} inhibitors thrombin-mediated PAR activation persists,[18] therefore suggesting the usefulness of specific PFT tests addressing PAR function in patients receiving standard dual anti-platelet therapy.

The results of our study highlight the high negative predictive power of preoperative platelet function testing. Conversely, the positive predictive power remains remarkably low. This depends on the multifactorial nature of postoperative bleeding, where preoperative platelet function seems to underlie only a limited part of the problem. Other factors play a well-recognized role: residual heparin, consumption of coagulation factors including fibrinogen, and surgical sources. Postoperative platelet dysfunction is also an important component of the bleeding process. We are lacking PFT performed after operation, and therefore cannot address the potential role of postoperative PFTs. However, the purpose of the present study was to assess the usefulness of preoperative tests in order to determine the adequate time between P_2Y_{12} discontinuation and surgery, a 'modifiable' risk factor for postoperative bleeding. Conversely, post-surgery tests are more related to the choice of adequate treatment of bleeding.

A strength of our study is the definition of severe bleeding according to the UDPB, which can be applied to different institutions. There are, however, limitations in our study: the most important of which is the retrospective nature. We cannot exclude that some potential confounders were not included in our data collection; moreover, we are lacking data linking postoperative bleeding to postoperative PFTs and to other pre- and postoperative measures of haemostatic balance, like viscoelastic tests.

There are practical implications of this study. PFTs exploring P_2Y_{12} receptor function should be routinely used to assess platelet function before cardiac surgery in patients receiving dual anti-platelet therapy. Conversely, routine use of tests exploring PAR activity like the TRAPtest is likely to result in redundant information and increased costs, as already suggested in other studies.[15] Our suggestion is that only the presence of a very low level of P_2Y_{12} reactivity (by the ADPtest) justifies a second PFT test based on PAR function (like the TRAPtest). This 'sequential' combined strategy avoids unnecessary delays in surgery and allows cost-containment.

PFTs are of increased interest in surgery for patients treated with P_2Y_{12} inhibitors, and their usefulness in the setting of platelet transfusions has been recently highlighted.[19 20] Additionally, new anti-platelet drugs inhibiting PARs (vorapaxar and atopaxar) will soon become available, and the TRAP test has already been used to test the platelet inhibition induced by these drugs.[21] Further studies are needed to clarify the potential role of PFTs before and after cardiac surgery as tools to

establish the correct time for surgery and to guide platelet transfusion therapy.

Authors' contributions

M.R. helped design the study, analyse the data, and write the manuscript. He has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files. D.C. helped design the study and conduct the study. He has seen the original study data, reviewed the analysis of the data, and approved the final manuscript. E.B. helped conduct the study and write the manuscript. She has seen the original study data, reviewed the analysis of the data, and approved the final manuscript. U.D.D. helped conduct the study and analyse the data. He has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

Declaration of interest

M.R. has received honoraria from Verum Diagnostica (Roche), CSL Behring, Medtronic, and Grifols.

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