Original Article

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Relationship between thromboelastography and long-term ischemic events as gauged by the response to clopidogrel in patients undergoing elective percutaneous coronary intervention

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Summary **Ischemic events after percutaneous coronary intervention (PCI) remain a major concern for patients with coronary heart disease (CHD). The aim of the current study was to investigate whether thromboelastography (TEG) was a satisfactory technique to measure platelet function *in vitro* in order to improve risk stratification and the individual response to antiplatelet therapy. The diagnostic and prognostic utility of the maximum amplitude of adenosine diphosphate induced platelet-fibrin clots (MA_{ADP}) was measured with TEG in 759 patients undergoing elective PCI. A 600-mg dose of clopidogrel was taken more than 12 h before surgery in addition to a maintenance dose of aspirin 100 mg/day and clopidogrel 75 mg/day for 2 y. Platelet-fibrin clot strength was also measured in this study. An MA_{ADP} > 34 mm significantly predicted ischemic events after PCI, as indicated by an area under the curve (AUC) of 0.79 (95% CI: 0.72-0.87, $P < 0.05$) according to receiver operating characteristic (ROC) curve analysis. The multivariate Cox proportional hazards model identified MA_{ADP} > 34 mm and an FBG level > 7.0 mmol/L as significant independent predictors of first ischemic events at the 2-year time point ($P < 0.05$). With adequate clopidogrel pretreatment, patients who underwent elective PCI and who experienced ischemic events could be diagnosed with a certain MA_{ADP} according to TEG. TEG could be a good tool to measure platelet function.**

Keywords: Antiplatelet, clopidogrel, thromboelastography (TEG), percutaneous coronary intervention (PCI)

1. Introduction

Ischemic events usually occur in patients who undergo percutaneous coronary intervention (PCI). Whether those events occur mainly depends on the activation of platelets and the generation of thrombin, and both processes are mediated by a variety of agonists (*1,2*). Dual antiplatelet treatment with clopidogrel and aspirin to suppress platelet reactivity (PR) has proven to be an efficacious therapy and can be used to prevent ischemic events in patients with coronary heart disease (CHD) after PCI (*1,3,4*). Thromboelastography (TEG) has been performed in order to monitor and alleviate ischemic events, and TEG platelet mapping can adeptly assess the response of circulating platelets to both aspirin and clopidogrel in whole blood, as a previous study by the current authors indicated (*5*). A TEG analysis can provide a large amount of information on the overall likelihood of thrombosis and the reaction to antiplatelet therapy. In TEG, an oscillating cup and a rotating pin suspended in a blood sample are connected by blood clots. Blood clot strength is measured using the proportional amplitude of the rotating pin. The maximum amplitude (MA) is the maximum clot strength, which defined as the parameter MA (*2,6*). Thus far, TEG is the only point-of-care testing (POCT) of platelet function that has been approved by China's Food and Drug Administration (FDA). However, the relationship between TEG and ischemic events in patients undergoing PCI has yet to be determined.

A previous study by the current authors indicated a definite relationship between gender and clopidogrel

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resistance in patients after PCI (*5*). The aim of the current study was to investigate a potential cut-off value for the maximum amplitude of adenosine diphosphate-induced platelet-fibrin clots (MA_{ADP}) measured with TEG and to examine its predictive and prognostic value with regard to the occurrence of long-term ischemic events in Chinese patients.

2. Materials and Methods

2.1. *Patients*

Seven hundred and fifty-nine consecutive patients who underwent elective PCI from January 2014 to February 2015were enrolled in a prospective observational study. All patients over the age of 18 had been administered aspirin in a dosage of 100 mg/day for at least 7 days. Exclusion criteria included a history of bleeding, an acute myocardial infarct (AMI) up to 48 h before enrolment, and use of glycoprotein (GP) IIb/IIIa inhibitors prior to the procedure. All of the patients were pre-treated with aspirin and a 600-mg dose of clopidogrel more than 12 h prior to the procedure, followed by a maintenance dose of aspirin 100 mg/day and clopidogrel 75 mg/day for 2 y. This study was approved by the Institutional Review Board of Shanghai Chest Hospital (7563893).

2.2. *Blood sampling and measurement of platelet-fibrin clot strength*

Blood samples were obtained 18 to 24 hours after PCI. After the procedure, blood was drawn by venipuncture into vacutainer tubes (Becton-Dickinson, NJ, USA) with 3.8% trisodium citrate and USP lithium heparin (for TEG assay). The vacutainer blood tube was filled with blood and gently inverted three to five times in order to ensure that the sample was completely mixed with the anticoagulant.

Platelet-fibrin clot strength was measured with the TEG Hemostasis System (Haemoscope Corporation, IL, USA) according to the manufacturer's instructions. Quantitative and qualitative values for the physical properties of clots were obtained with the automated analytical software TEG Hemostasis Analyzer (Haemoscope Corporation, IL, USA) according to the manufacturer's instructions.

Heparinized blood (360 μL) was rapidly added dropwise to a heparinase-coated cup and analyzed with TEG to detect the maximum amplitude of thrombin-induced clot strength ($MA_{Thrombin}$). The mixed blood (340 μL) was added dropwise to a noncoated cup containing activator F and reptilase to generate a blood cross-linked clot without thrombin generation or platelet stimulation (MA_{Fibrin}). The last sample (340 μL) of heparinized blood was added dropwise to a nonheparinase-coated cup containing activator F and adenosine diphosphate-induced platelet-fibrin (ADP) or arachidonic acid (AA) to induce a blood-crosslinked clot with platelet activation (MA_{ADP} or MA_{AA}).

2.3. *Definitions and clinical follow-up*

Platelet aggregation induced by ADP was defined as $\%Aggregation = [(MA_{ADP}-MA_{Fibrin})/(MA_{Thrombin}-MA_{Fibrin})] \times 100\%$, and this value was calculated with software. The cut-off point for high on-treatment platelet reactivity (HPR) was expressed as ≥ 70% ADP-induced platelet aggregation with 2 μmol of ADP as measured with TEG (*6*).

Patients were followed for 24 months during hospitalization depending on the occurrence of adverse events. Patients that complied with antiplatelet medication were contacted by phone or by the clinic and an appointment was made for the postoperative follow-up. Endpoints included stent thrombosis, cardiac death, ischemic stroke, unplanned revascularization, and myocardial infarction. Stent thrombosis was determined using the definition of the Academic Research Consortium (*7*). Cardiac death included a death due to any cardiovascular cause. Myocardial ischemia correlated with the overexpression of troponin I was regarded as myocardial infarction (*8*).

2.4. *Statistical analysis*

Statistical analysis was performed with SPSS version 21.0 (SPSS, Inc., IL, USA). Categorical variables were expressed as *n* (%) and continuous variables were expressed as the mean ± standard deviation (SD). The chi-square test and Student's *t*-test were respectively used to compare categorical variables and continuous variables among groups. Receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic accuracy of MA_{ADP} in relation to ischemic events. Demographic and procedural variables were included in a multivariate Cox proportional hazards model to identify prognostic factors. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. *Patients and demographic characteristics*

Seven hundred and fifty-nine patients who underwent non-emergent PCI were enrolled and administered aspirin and clopidogrel therapy. One hundred and eighty-two of these patients were diagnosed with unstable angina and the remainder were diagnosed with stable angina. The baseline characteristics of patients are shown in Table 1. The average age was (66.0 ± 9.6) years old and 72% of the patients were men. Fifty-eight patients (7.6%) experienced an ischemic event in the 2 years after elective PCI. In short, patients who

Table 1. Baseline characteristics

Demographic characteristics	Total (n = 759)	Ischemic group (n = 58)	Non-ischemic group (n = 701)	P
Age (yrs)	66.0 ± 9.6	66.8 ± 9.2	65.5 ± 9.8	0.653
Gender				
Male, n (%)	547 (72)	33 (57)	514 (73)	0.001
Female, n (%)	212 (28)	25 (43)	184 (27)	
BMI (kg/m^2)	25 ± 4	25 ± 5	25 ± 4	0.986
Risk factors				
Smoking (%)	76 (10)	4 (7)	72 (10)	0.610
Hypertension (%)	539 (71)	48 (82)	491 (70)	0.519
Diabetes (%)	197 (26)	21 (36)	176 (25)	0.485
Hyperlipidemia (%)	30 (4)	4 (7)	26 (4)	0.364
Prior PCI (%)	175 (23)	16 (27)	149 (21)	0.718
Prior MI (%)	99 (13)	16 (27)	83 (12)	0.172
Diagnosis				
SA (%)	577 (76)	51 (88)	526 (75)	0.469
UA (%)	182 (24)	7 (12)	175 (25)	
Laboratory data				
eGFR (mL/min/1.73 m^2)	88.1 ± 39.7	80.9 ± 39.6	88.4 ± 39.7	0.540
Platelets (×10^9/L)	193.5 ± 67.5	214.3 ± 65.3	193.1 ± 67.6	0.590
FBG (mmol/L)	6.9 ± 2.9	8.7 ± 3.4	6.8 ± 2.9	0.040
CRP (mmol/L)	4.2 ± 12.3	18.1 ± 32.3	3.7 ± 11.1	0.326
TC (mmol/L)	4.3 ± 1.1	4.6 ± 2.0	4.3 ± 1.1	0.609
TG (mmol/L)	2.0 ± 1.5	2.9 ± 3.3	2.0 ± 1.3	0.498
LDL (mmol/L)	2.5 ± 0.9	2.6 ± 1.8	2.5 ± 0.9	0.880
HDL (mmol/L)	1.0 ± 0.3	0.9 ± 0.1	1.0 ± 0.3	0.480
CK-mb (ng/mL)	2.7 ± 9.3	1.7 ± 0.7	2.8 ± 9.4	0.795
TnI (ng/mL)	0.4 ± 2.5	0.1 ± 0.1	0.5 ± 2.6	0.641

BMI: Body mass index; PCI: Percutaneous coronary intervention; MI: Myocardial infarction; SA: Stable angina pectoris; UA: Unstable angina pectoris; eGFR: Estimated glomerular filtration rate; FBG: Fasting blood glucose; CRP: C reactive protein; TC: Total cholesterol; TG: Triglycerides; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; CK-MB: Creatine kinase MB; TnI: Troponin I.

Table 2. Parameters for platelet function

Items	Ischemic group	Non-ischemic group	P
MA_{AA}	62.0 ± 9.0	60.4 ± 6.8	0.669
MA_{ADP}	40.8 ± 10.1	26.7 ± 13.7	< 0.001
AA%	88.8 ± 41.9	81.0 ± 23.1	0.298
ADP%	81.1 ± 32.4	71.4 ± 28.5	0.271

MA_{AA}: Maximum amplitude of arachidonic acid-induced platelet-fibrin clot strength; MA_{ADP}: Maximum amplitude of ADP-induced platelet-fibrin clot strength; AA%: Inhibition of platelet aggregation induced by arachidonic acid; ADP%: Inhibition of platelet aggregation induced by adenosine diphosphate.

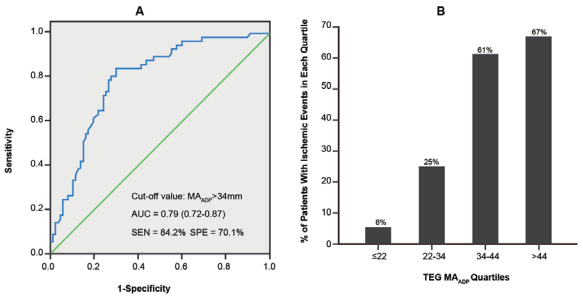

Figure 1. ROC curve and quartile analyses of MAADP values. (A) The receiver operating characteristic curve for MAADP. The cut-off value for MAADP is 34 mm. The diagnostic accuracy of MAADP for ischemic events is 0.79 (0.72-0.87), with a sensitivity of 84.2% and a specificity of 70.1%. **(B)** The observed frequency of patients with ischemic events in each quartile of MAADP values is shown in the figure.

experienced an ischemic evens were more often female (43% *vs*. 27%, $P < 0.05$) and had a higher level of fasting blood glucose (FBG) compared to patients in the non-ischemic group ((8.7 ± 3.4) mmol/L *vs*. (6.8 ± 2.9) mmol/L, $P < 0.05$).

3.2. *Association between platelet aggregation and ischemic events*

Blood samples of all 759 patients were analyzed with the TEG system. The prevalence of HPR was 36% according to TEG (n = 273). As shown in Table 2, post-treatment platelet AA-induced aggregation according to TEG did not differ markedly between the two groups ($P > 0.05$) (88.8 ± 41.9% in the ischemic group *vs*. 81.0 ± 23.1% in the non-ischemic group). Post-treatment platelet ADP-induced aggregation according to TEG was not correlated with ischemic events ($P > 0.05$) (81.1 ± 32.4% in the ischemic group *vs*. 71.4 ± 28.5% in the non-ischemic group). However, patients who experienced ischemic events had significantly greater ADP-induced platelet-fibrin clot strength (MA_{ADP}) than did patients who did not experience ischemic events (40.8 ± 10.1 mm *vs*. 26.7 ± 13.7 mm, $P < 0.05$). ROC analysis, which assessed the association between MA_{ADP} and ischemic events, indicated that MA_{ADP} had good predictive significance, resulting in an area under the curve (AUC) of 0.79 (95% CI: 0.72-0.87, $P < 0.05$, Figure 1A).

Quartile analysis of the MA_{ADP} consistently indicated that the incidence of ischemic events increased with higher quartiles (Figure 1B). Sixty-one percent of patients in the third quartile (an MA_{ADP} of 34-44 mm) experienced ischemic events and 67% of patients in the fourth quartile (an MA_{ADP} > 44mm) experienced ischemic events. Independent predictors of long-term ischemic events were assessed with a multivariate Cox proportional hazards model. MA_{ADP}, being female, and FBG were independently and dramatically related to ischemic events, with a respective risk ratio of 8.9 (95% CI: 4.2-18.3), 5.3 (95% CI: 2.7-11.1), and 2.2 (95% CI: 1.1-3.9) ($P < 0.05$, Table 3).

4. Discussion

Patients undergoing PCI usually suffer from ischemic events, and the standard therapy for these patients has been the combined use of aspirin and clopidogrel (*4*). The PREPARE-POST-STENTING study first indicated a relationship between MA_{ADP} and the occurrence of ischemic events in 2005 (*2*). Light transmittance aggregometry was used to determine platelet reactivity. Several studies have used TEG to predict platelet reactivity and ischemic events after PCI (*2,9*). The current study hypothesized that the MA_{ADP} according to TEG would provide supererogatory information for post-stenting risk assessment. Results indicated that an MA_{ADP} greater than 34 mm according to TEG suggested a higher risk of ischemic events. However, Gurbel *et al*. reported that an MA_{ADP} greater than 47 mm according to TEG significantly predicted long-term ischemic events in contrast to other measurements (*9*). Race is known to be an independent predictor of survival of coronary disease and individual differences in platelet reactivity are known to be heritable; several studies have found a lower frequency of epistaxis in Asians and African Americans than Caucasians (*10,11*).

Multifactorial processes such as clinical, demographic, and hemostatic components influence ischemic events, and especially those after PCI (*12*). The current results suggested that only gender and the level of FBG differed significantly between ischemic and non-ischemic groups. Interestingly, the current results based on a Cox model indicated that female patients had a much higher risk of experiencing ischemic events than did male patients, and a previous study yielded a similar finding. Several studies have indicated that HPR is more prevalent in females than males because of a stronger platelet aggregation reaction to platelet agonist stimulation (*13-15*). This might be caused by a high of clotting at the baseline, the effects of female hormone, or a diminished reaction to clopidogrel in female patients.

MA parameters according to TEG can detect the maximum amplitude of platelet aggregation and fibrin-platelet binding by GP IIb/IIIa (*9*). Circulating platelets are variably inhibited by platelet inhibitors. The MA_{ADP} indicated the level of platelet reactivity induced by ADP. According to the current results, MA_{ADP} may accurately predict the long-term ischemic events after PCI based on platelet inhibition by ADP. However, there has been reluctance to monitor antiplatelet therapy over the long term. There are several reasons for this reluctance, such as the introduction of artifacts due to laboratory methods, incomplete reflection of the actual thrombotic process *in vivo*, and failure to unequivocally establish a causal relationship between test results and the occurrence of thrombotic events. Over the past few years, knowledge of platelet receptor physiology has improved significantly and more patient-friendly assays of platelet function have been introduced. However, a large-scale prospective trial has yet to yield definitive evidence indicating that improved antiplatelet therapy based on a platelet function test actually helps patients. At present, TEG is a preferable technique to identify patients at risk of experiencing ischemic events, but the effectiveness of this technique needs to be assessed further in large-scale trials in the future. Thrombotic events occurred at a relatively low rate in previous prospective trials, and larger samples are needed to assess the potential for TEG to provide personalized treatment for patients with CHD (*16*).

That said, the current study had several limitations. First, this subject was a prospective study that was not randomized, and findings still need to be verified in larger randomized clinical trials. Second, the clopidogrel loading dose used in this study may result in different levels of antiplatelet action in different patients.

In summary, the quantitative measurement of MA_{ADP} with TEG allows more individualized antiplatelet treatment to prevent ischemic events, and this approach may help to improve predictive accuracy and facilitate personalized antiplatelet treatment.

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