ARTICLE

Residual platelet reactivity is preferred over platelet inhibition rate in monitoring antiplatelet efficacy: insights using thrombelastography

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INTRODUCTION
Platelet activation plays a key role in the development of atherosclerosis and the occurrence of arterial thrombosis [1, 2]. Dual antiplatelet therapy (DAPT), including aspirin and clopidogrel, provides a substantial reduction in ischemic events in the setting of acute coronary syndrome (ACS). Arachidonic acid (AA)- or adenosine diphosphate (ADP)-induced platelet-fibrin clot strength (MAAA or MAADP) was indicative of the net residual platelet reactivity after the treatment with aspirin or clopidogrel, respectively. AA% or ADP% was indices of the relative platelet inhibition rate on AA or ADP pathway. We found that each parameter alone was predictive of the risk of 6-month ischemic event, even after adjusting for confounding factors. However, the association between AA% and clinical outcome disappeared when further adjusted for MAAA. Likewise, inclusion of MAADP changed the significant relation between ADP% and clinical outcome. MAADP > 47.0 mm and MAAA > 15.1 mm were identified as the optimal cutoffs by receiver operating characteristic analysis. High MAAA (HR = 3.963; 95% CI: 1.152–13.832; P = 0.029) and high MAADP (HR = 5.185; 95% CI: 2.228–12.062; P < 0.001) were independent predictors when both were included in multivariable Cox regression hazards model. Interestingly, an even higher risk was found for the coexisting high MAAA and high MAADP (HR = 7.870; 95% CI: 3.462–17.899; P < 0.001). We conclude that when performing TEG to predict clinical efficacy, residual platelet reactivity has superiority over platelet inhibition rate as a measure of thrombotic risk in patients treated with aspirin and clopidogrel after ACS.

Keywords: thrombelastography; clopidogrel; aspirin; platelet function; ADP; arachidonic acid

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METHODS
Study population
A total of 447 ACS patients who underwent PCI with DES were included in this prospective observational study. The details have been described previously [11–13]. All participants received DAPT with the combination of aspirin (300 mg loading dose, 100 mg once daily) and clopidogrel (300 mg loading dose, 75 mg once daily). Patients who received other antiplatelet agents or oral anticoagulants were excluded.

However, much confusion remains. First, the predictive role of various TEG parameters has never been investigated in a large cohort of patients with ACS. Second, there is a lack of consensus on the optimal TEG parameter to identify high ischemic risk patients treated with aspirin and clopidogrel. Third, data regarding threshold definition are limited.

To address the abovementioned issues, we conducted this post hoc analysis of a prospective, single-center cohort to quantify the relationship between TEG parameters and the clinical efficacy of DAPT in Chinese patients who received drug-eluting stents (DES) implantation after ACS.

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The study protocol was approved by the hospital’s medical ethics committee, and informed consent was obtained from each patient. The trial was registered (URL: www.chictr.org, number: ChiCTR-OCH-11001767).

Platelet function

The antiplatelet effect of aspirin and clopidogrel was assessed by using a Thrombelastograph Hemostasis Analyzer (Haemoscope Corp, Niles, Illinois, USA) with platelet mapping. A blood sample for TEG analysis was obtained 24 h after DAPT loading. TEG is a point-of-care test for the evaluation of platelet and fibrin contributions to clot strength. A particular advantage of the TEG platelet mapping system is that it can evaluate the treatment effects of aspirin and clopidogrel simultaneously. Moreover, the reporting of results includes the relative inhibition rate (responsiveness) of platelet aggregation and net residual (post-treatment) platelet activity.

The direct parameter measured by this system is the maximum amplitude (MA), which is indicative of the strength of the final clot. Parameters of platelet-fibrin clot strength were measured in four channels. For the kaolin channel, 1 mL of whole blood was mixed with 1% kaolin solution (Haemoscope Corp). Then, 360 µL of neutralized blood was immediately added to a heparinase-coated cup and assayed in the TEG analyzer to measure the thrombin-induced clot strength (MA\textsubscript{thrombin}), which is indicative of the baseline maximal platelet reactivity. AA 100 µL (1 mmol/L) was placed in the AA channel cup to measure the thrombin-induced clot strength (MA\textsubscript{AA}), which is indicative of the absolute residual platelet reactivity in the AA pathway. Similarly, 100 µL of ADP (2 µmol/L) was placed in the ADP channel cup to measure the thrombin-induced clot strength (MA\textsubscript{ADP}), which is indicative of the absolute residual platelet reactivity in the ADP pathway. The contribution of fibrin to clot formation can be measured by the addition of the agonist F (MA\textsubscript{fibrom}).

The platelet inhibition rate on the AA or ADP pathway (AA% or ADP%) was calculated with computerized software according to the formula AA% or ADP% = (MA\textsubscript{thrombin} – MA\textsubscript{AA or ADP})/ (MA\textsubscript{thrombin} – MA\textsubscript{fibrom}) \times 100.

Main outcomes

The main outcome of the study was ischemic events during 6 months of follow-up. Ischemic events were the composite of cardiovascular death, nonfatal myocardial infarction (MI), or stroke. Cardiovascular death was regarded as any death with a demonstrable cardiovascular cause or any death not clearly attributable to a noncardiovascular cause. The diagnosis of MI was based on a new rise in troponin T ≥ 0.03 ng/mL associated with typical symptoms and/or typical electrocardiogram changes. The diagnosis of ischemic stroke required confirmation by computed tomography or magnetic resonance imaging of the head.

Statistical analyses

Continuous variables were expressed as the mean ± SD. Categorical variables were expressed as frequencies and percentages. For analysis of the relations between categorical variables, we used the chi-square test or Fisher’s exact test when appropriate. The Kolmogorov-Smirnov test was used to check for a normal distribution of continuous data. The T-test or the Wilcoxon rank sum test for unpaired samples was used to compare any continuous variable with a normal or nonnormal distribution, respectively. A receiver operator curve (ROC) analysis was used to determine the ability of the TEG parameter to predict ischemic events. The predictive utility of TEG parameters was investigated by a multivariable Cox regression hazards model. All tests were two-sided with a significance level of $P < 0.05$.

The statistical analyses were performed with SPSS software package, version 18 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Study population and clinical outcome

The baseline clinical characteristics and procedural variables of patients with and without ischemic events are depicted in Table 1. During the 6-month follow-up, ischemic events occurred in 28 (6.3%) patients. A total of three patients died (0.7%), 22 patients presented with nonfatal acute myocardial infarction (4.9%), and three subjects experienced nonfatal ischemic stroke (0.7%). Compared with patients without ischemic events, those with events were significantly older.

Antplatelet effect of aspirin measured by TEG and clinical outcome

For all subjects, the mean MA\textsubscript{AA} was 21.1 ± 12.0 mm, and the mean AA% was 79.9% ± 19.6%. Compared with individuals without ischemic events, those experiencing an ischemic event had a reduced response to aspirin (AA%: 80.5% ± 19.3% vs. 70.8% ± 20.9%, $P = 0.01$, Table 1) in addition to greater post-treatment platelet reactivity (MA\textsubscript{AA}: 20.6 ± 11.8 vs. 29.1 ± 13.6 mm, $P < 0.001$, Table 1).

The independence of the association between TEG parameters and clinical outcome was assessed by a multivariable Cox regression hazards model. Baseline and procedural characteristics with a $P < 0.10$ in Table 1 and previous reported predictors of ischemic events [14], including age, LVEF ≤ 45%, sex, BMI, diabetes, smoking status, use of proton pump inhibitor, and stent length, were included in the multivariate analysis. As demonstrated in Table 2, after adjustment for confounding factors, MA\textsubscript{AA} (HR = 1.047, 95% CI: 1.019–1.076, $P = 0.001$) or AA% (HR = 0.978, 95% CI: 0.962–0.994, $P = 0.009$) was significantly associated with adverse ischemic events. However, when these two parameters were both included in the multivariate analysis, MA\textsubscript{AA} was independently and significantly associated with adverse ischemic events, with a hazard ratio of 1.067 (95% CI: 1.009–1.129, $P = 0.024$), whereas there was no significant relationship between AA% (HR = 1.013, 95% CI: 0.979–1.049, $P = 0.451$) and clinical outcomes.

Antplatelet effect of clopidogrel measured by TEG and clinical outcome

For all subjects, the mean MA\textsubscript{ADP} was 35.0 ± 15.0 mm, and the mean ADP% was 54.2% ± 24.8%. Subjects with events had lower mean ADP% (HR = 1.064, 95% CI: 1.032–1.096, $P < 0.001$) and higher residual platelet reactivity (MA\textsubscript{ADP}: 48.4 ± 14.2 vs. 34.2 ± 14.7 mm, $P < 0.001$).

As demonstrated in Table 2, after adjustment for confounding factors, MA\textsubscript{ADP} (HR = 1.046, 95% CI: 1.004–1.093, $P < 0.001$) or ADP% (HR = 0.967, 95% CI: 0.950–0.984, $P < 0.001$) was significantly associated with adverse ischemic events. Likewise, when these two parameters were both included in the multivariate analysis, MA\textsubscript{ADP} (HR = 1.049, 95% CI: 1.001–1.110, $P = 0.046$) was independently and significantly associated with adverse ischemic events, whereas there was no significant relationship between ADP% (HR = 0.990, 95% CI: 0.963–1.017, $P = 0.451$) and clinical outcomes.

The predictive value of MA\textsubscript{AA} and MA\textsubscript{ADP}

The inclusion of residual platelet reactivity as a covariate in the multivariate regression model changed the significant relationship between the relative platelet inhibition rate and clinical outcomes, suggesting that the latter parameters obtained by calculation could not provide greater prognostic value.

Next, we investigated the predictive value of MA\textsubscript{AA} and MA\textsubscript{ADP} by ROC analysis. The ROC analysis demonstrated that MA\textsubscript{AA} and MA\textsubscript{ADP} were able to distinguish between patients with and without ischemic events at the 6-month follow-up, as shown in Fig. 1. The areas under the ROC curves of MA\textsubscript{AA} and MA\textsubscript{ADP} were 0.692 (95% CI: 0.647–0.735, $P < 0.001$) and
MAAA > 15.1 mm was identified as the optimal cutoff, with a sensitivity of 89.3% (71.8%–97.7%), a specificity of 42.7% (37.9%–47.6%), a negative predictive value of 98.4% and a positive predictive value of 9.4%. MAADP > 47.0 mm was identified as the optimal cutoff, with a sensitivity of 67.9% (47.6%–84.1%), a specificity of 80.0% (75.8%–83.7%), a negative predictive value of 97.4% and a positive predictive value of 18.4%.

Patients above the optimal cutoff level determined by ROC analysis were considered to exhibit high MA. High MAAA (HR = 3.963; 95% CI: 1.152–13.632; P = 0.029) and high MAADP (HR = 5.185; 95% CI: 2.228–12.062; P < 0.001) were independent predictors when both were included in the multivariable Cox regression hazards model. The event-free survival curves according to the presence of high MA by different agonists are shown in Fig. 2.

Since the positive predictive value of each MA parameter alone was low, a combination of both high MA was used for further investigation. Interestingly, an even higher risk was found for the coexistence of high MA due to AA and ADP (HR = 7.870; 95% CI: 3.462–17.899; P < 0.001).

Then, patients were categorized into four groups according to the presence or absence of high MAAA or high MAADP. As demonstrated in Fig. 3, among those without high MAAA or high MAADP (n = 158), high MAAA alone (n = 186), high MAADP alone (n = 20) and both high MAAA and high MAADP (n = 83), the incidence of ischemic events was 1.3%, 3.8%, 5.0%, and 21.7%, respectively (P < 0.001).

**DISCUSSION**

This study confirms the prognostic utility of the TEG platelet mapping system to individually assess the antiplatelet effect of aspirin and clopidogrel in ACS patients receiving DES implantation. To the best of our knowledge, the present study is the first to
determine which parameter, platelet inhibition rate or residual platelet reactivity, best predicts the clinical efficacy of the drug. Important findings of the present study are as follows: (1) residual platelet reactivity is preferred over the relative platelet inhibition rate as a risk assessment tool; (2) prothrombotic phenotype other than high-residual platelet reactivity to ADP may be responsible for post-PCI ischemic events; and (3) the concurrence of high MA_AA and high MA_ADP resulted in the highest risk for 6-month ischemic events.

Accumulated data from large studies have demonstrated a clear association between platelet function testing and ischemic event occurrence. However, platelet function monitoring to define a high-risk population is not recommended by recent guidelines. The reasons for this are that (1) no standard platelet function testing has been unanimously adopted by clinicians to assess the antiplatelet effect and (2) there is a lack of consensus on the optimal parameter for quantifying the high-platelet reactivity phenotype. Some studies have suggested that TEG MA parameters have potential superiority over ADP-induced light transmittance aggregation as a risk assessment tool [9, 10]. A particular advantage of the TEG platelet mapping system is that it measures not only the rate of platelet responsiveness but also the extent of platelet aggregation. Another plausible explanation is that platelet-fibrin clot strength denotes the function of the maximum dynamic properties of fibrin-platelet binding via GPIIb/IIIa (secondary aggregation), whereas the light transmission aggregometry method is based on platelet aggregation mediated by fibrinogen (primary aggregation) and ignores the contribution of platelet-fibrin interactions to thrombosis [10].

Fig. 1 Receiver operating characteristic (ROC) curve for platelet-fibrin clot strength induced by arachidonic acid (AA) and adenosine diphosphate (ADP)

Fig. 2 Cumulative Kaplan–Meier estimates of the rates of the first ischemic event (cardiovascular death, nonfatal myocardial infarction, or ischemic stroke) according to the platelet reactivity phenotype to arachidonic acid (a) or adenosine diphosphate (b), as measured by thrombelastography

Fig. 3 The rate of ischemic clinical endpoints in patients stratified according to the presence or absence of high MA_AA and high MA_ADP
inhibition rate and residual platelet reactivity have been used to assess treatment effects. The former parameter, acting as a reliable pharmacodynamic indicator, is the most commonly used estimate of platelet responsiveness to drugs. However, residual platelet reactivity might be more appropriate for estimating the clinical thrombotic risk, as suggested by Gurbel and colleagues [4, 15, 16]. Of note, there is limited clinical evidence to support this idea.

Our results suggest that residual platelet reactivity is more suitable than the platelet inhibition rate for predicting the clinical efficacy of DAPT. Although the platelet inhibition rate induced by AA or ADP was able to discriminate between participants with and without ischemic events at the 6-month follow-up, it lost its predictive ability when further adjusted for residual platelet reactivity, whereas residual platelet reactivity remained predictive. This result might be explained by the interindividual variability in the baseline level of platelet activity. The measurement of platelet inhibition rate may underestimate ischemic risk in responders with high-baseline platelet reactivity and may overestimate risk in nonresponders with low-baseline platelet reactivity [4]. The advantage of the residual platelet reactivity phenotype is that it represents the sum of baseline platelet activity and responsiveness to antiplatelet drugs. Hence, based on its superiority and lower cost, it is recommended to use residual platelet reactivity when performing TEG to predict the clinical efficacy of DAPT.

In line with previous studies [10, 17, 18], our study showed that MAAPD was significantly associated with post-PCI clinical endpoints and could be used as an important indicator of ischemic events occurrence. Our optimal diagnostic cutoff value of 47 mm for MAAPD, obtained in patients with ACS and with a 6-month follow-up, is consistent with that reported in a previous report [10]. However, it should be noted that even though the observed cut-off value for MAAPD had a very high-negative predictive value, the positive predictive value was fairly low. In the PLATO study and the TRITON-TIMI38 study, residual ischemic risk remained substantial even when the platelet P2Y12 receptor was potently and consistently blocked [19, 20]. Consequently, a prothrombotic phenotype other than high platelet reactivity to ADP may be responsible for post-PCI ischemic events. Few studies have been conducted to investigate the predictive role of MAAAP in patients receiving DES implantation and DAPT. Our ROC analysis suggested that MAAAP > 15.1 mm was significantly associated with ischemic events and was an independent predictor after adjustment for confounding factors. The mean AA% was relatively high (79.9% ± 19.6%) in our study population. Given that aspirin provides potent platelet inhibition, the high MAAAP phenotype may be due to the high intrinsic activity of platelets and fibrin. Our results suggested that the simultaneous measurement of residual platelet reactivity to AA and ADP was more comprehensive when performing TEG to assess individual risk in patients treated with DAPT after ACS. These findings will be important for clinical implementation and interpretation.

Additionally, the low-positive predictive value of the platelet phenotype may also be explained by its variation over time. Recent studies by us [21] and Campo et al. [22] have demonstrated that platelet reactivity showed a significant reduction from index hospitalization to the follow-up phase. Patients still exhibiting high-platelet reactivity at 1 month after the index procedure had a better predictive value [22]. Accordingly, multiple dynamic measurements may provide more prognostic information.

LIMITATIONS

The present study has limitations that merit mention. First, this study was a post hoc analysis, which may limit definite conclusions. Second, in this observational study, we cannot completely exclude possible bias by various risk factors and patient characteristics, although the multivariable adjustment models confirmed the primary analyses. Third, there may be limitations to generalizing these results to western patients. Given that intrinsic thrombogenicity differs, the relationship between platelet function parameter and ischemic events might differ according to race. Finally, the conclusions may not be applicable to other platelet function tests.

CONCLUSION

Among TEG parameters, residual platelet reactivity was superior over platelet inhibition rate as a risk assessment approach in patients treated with aspirin and clopidogrel after ACS. Our results would help to avoid unnecessary tests. Moreover, patients exhibiting a high-platelet phenotype to AA and ADP are associated with the highest risk for 6-month ischemic events.

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AUTHOR CONTRIBUTIONS

Study design: HYW, JBG. Data collection: CZ, XZ. Providing patients: JYQ, QBW. Data analysis: HYW, XZ. Writing: HYW, CZ.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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