Abstract

Aim: This study evaluated, using thrombo-elastography (TEG), the efficacy of antiplatelet therapies in retired Chinese officers and explored the factors influencing the efficacy of antiplatelet therapies.

Methods: Nine hundred and fifty-five retired male Chinese officers (≥ 60 years old), who had undergone TEG between June and August 2015 at the Chinese People’s Liberation Army General Hospital (PLAGH), were enrolled in this study. The subjects were divided into four groups according to the antiplatelet drug(s) that they were administered: aspirin, clopidogrel, dual drugs (combination of aspirin and clopidogrel) and no antiplatelet drug. TEG was used to evaluate the efficacy of antiplatelet therapy in the four groups.

Results: The inhibition of platelet aggregation induced by arachidonic acid (AA%) was 48.0 ± 19.3% in the aspirin group, and the inhibition induced by adenosine diphosphate (ADP%) was 63.0 ± 18.2% in the clopidogrel group. The AA% and ADP% in the dual-drug group were 51.0 ± 16.5 and 46.0 ± 15.3%, respectively. The total efficacy of antiplatelet therapy was 45.9% in the aspirin group, 51.2% in the clopidogrel group and 81.4% in the dual-drug group. A multivariate logistic regression analysis of the maximum amplitude of ADP-induced platelet–fibrin clot strength (MA-ADP) indicated that in the population with MA-ADP < 31 mm, an increased white blood cell count (OR = 1.262, p = 0.011) was a protective factor for bleeding. In the population with MA-ADP > 47 mm, increased platelet count (OR = 1.358, p = 0.011) and glycated haemoglobin levels (HbA1c, OR = 1.016, p = 0.013) were risk factors for thrombosis.

Conclusion: This quality-controlled TEG procedure was an efficient method to evaluate the efficacy of antiplatelet therapies in the clinic. White blood cell and platelet counts, and eGFR and HbA1c levels may influence the efficacy of an antiplatelet therapy.

Keywords: thrombo-elastography, aspirin, clopidogrel

Submitted 7/11/17, accepted 18/7/18
Cardiovasc J Afr 2018; 29: online publication www.cvja.co.za
DOI: 10.5830/CVJA-2018-041

Atherosclerotic cardiovascular and cerebrovascular diseases, including coronary heart disease (CHD) and stroke, have become the leading causes of death in China and around the world. Arterial thrombosis caused by the activation, adhesion and aggregation of platelets in atherosclerotic lesions is the pathophysiological basis of acute cardiovascular and cerebrovascular diseases. Antiplatelet therapy is essential for the treatment of these diseases.1-3 Clinical research has shown that even with adequate standardised antiplatelet treatment, such as aspirin at 100 mg/day and/or clopidogrel at 75 mg/day, some patients with CHD still had repeated episodes of acute coronary syndrome, and others may develop ecchymosis or gastrointestinal bleeding. This suggests that there may be differences in the efficacy of antiplatelet therapy in different populations.4,5 Evaluation of the efficacy of antiplatelet therapy is therefore important for the treatment and prognosis of cardiovascular and cerebrovascular diseases.

The thrombo-elastography (TEG) method can be used to monitor the processes of blood coagulation and fibrinolysis. Because TEG is widely available and is easy and convenient, it has been widely used in clinics.6 In this study, we evaluated the efficacy of antiplatelet therapies in retired Chinese officers, using the TEG method, and we also explored the factors influencing the efficacy of antiplatelet therapies.

Methods

This was a cross-sectional study and 955 retired male Chinese officers were enrolled. The subjects voluntarily underwent TEG during their routine examination at the Chinese People’s Liberation Army General Hospital (PLAGH) between June and August 2015.

We excluded subjects who had any of the following conditions from our study: those who had received antiplatelet therapy for less than two weeks, were treated with any kind of anticoagulant, had severe organ dysfunction, an advanced malignant tumour, a mental disorder and/or cognitive dysfunction, and those who exhibited any factors that were deemed unsuitable by researchers to take part in this study.
All subjects signed an informed consent form. The study was approved by the ethics committee of PLAGH.

The 955 subjects were divided into four groups according to the antiplatelet drug(s) administered: aspirin group (368), clopidogrel group (115), dual-drug group (43), and no-drug group (429). Demographic and clinical data were collected using a domestic questionnaire, which included age, regimen of any antiplatelet drugs and combined medications, current smoking habit, and history of hypertension, coronary heart disease, diabetes mellitus and cerebrovascular events. Smoking was defined as having more than one cigarette every day for more than one year, and cerebrovascular events included cerebral haemorrhage, cerebral ischaemia, cerebral infarction and transient ischaemic attack.

Body height, body mass, waist circumference and blood pressure were measured and body mass index (BMI) was calculated. The information was collected by face-to-face consultation, and the investigators were all trained physicians at our hospital.

TEG was used to measure the percentage of platelet inhibition after anti-platelet therapy. The TEG instrument (TEG 5000 Haemoscope) and the related reagents were provided by the Haemonetics Company in the United States. The patients took the antiplatelet drugs as usual on the day of taking blood.

### TEG parameters

Reaction time (R) is the time required from the start of a blood sample test to fibrin formation. The normal range is 5–10 min, and > 10 and < 5 min are considered enzymatic hypercoagulability.

Maximal amplitude (MA), which represents the maximum strength and stability of a clot, can be ascertained by the binding of activated platelets to a fibrin mesh. Using the TEG instrument, 360 µl of heparinised blood was added to 10 ml of activator F (reptilase and factor XIIIa) in channel 1. The contribution of each fibrin meshwork to the clot strength (MA-fibrin) was assessed in channel 1.

In channels 2 and 3, 360 µl of heparinised blood was added to 10 ml of ADP (final concentration 2 µM) and 10 ml of arachidonic acid (AA; final concentration 1 mM), respectively, along with 10 ml of activator F to each. Channels 2 (MA-ADP) and 3 (MA-AA) calculate the contribution of platelets, as activated by ADP or AA, respectively, to the clot strength.

Maximal clot strength with maximally stimulated platelets (MA-thrombin) were assessed in channel 4 by adding 360 µl of kaolin-activated citrated blood to 20 µl of 0.2 M calcium chloride. The normal range of MA-ADP is 31 to 47 mm; when it was < 31 mm, the risk of bleeding was increased, and > 47 mm, the risk of thrombosis was increased. When MA-ADP was between 31 and 47 mm, the subject was considered to have the lowest risk of bleeding and thrombosis.\(^1\)

ADP% is the percentage of platelet inhibition due to clopidogrel, which was defined by the extent of non-response of the platelet ADP receptor to exogenous ADP, as measured by TEG-MA.

\[
ADP\% = \frac{(MA-ADP) - (MA-fibrin)}{(MA-thrombin) - (MA-fibrin)} \times 100\%
\]

ADP% was used as a measure of the therapeutic effect of clopidogrel. The reference values were as follows: < 30% was considered to be ineffective, 30% ≤ ADP% ≤ 75% was considered to be effective, and > 75% was considered to work well.\(^7\)

AA% is the percentage of platelet inhibition due to aspirin, which was defined by the extent of non-response of the platelet TXA\(_2\) receptor to exogenous AA, as measured by TEG-MA.

\[
AA\% = \frac{(MA-thrombin)- (MA-fibrin)}{(MA-thrombin) - (MA-fibrin)} \times 100\%
\]

AA% was used as a measure of the therapeutic effect of aspirin. The reference values were as follows: < 50% was considered to be ineffective, 50% ≤ AA% ≤ 75% was considered to be effective, and > 75% was considered to work well.

### Detection of biochemical parameters

The whole blood count was determined by an automatic haematology analyser (Nihon Kohden MEK-7222K, Japan). The blood lipid and glucose, and serum creatinine (SCr) values were determined by an automatic biochemical analyser (Hitachi 7400, Japan).

Renal function was further assessed by the estimated glomerular filtration rate (eGFR), which was calculated by the following formula:

\[
eGFR (ml/min/1.73 m^2) = 175 \times \text{standard SCr (mg/dl)} - 1.234 \times \text{age} - 0.179 \text{ (or 0.79 for females)}
\]

[The standard SCr (mg/dl) = SCr (mg/dl) (detected by an enzymatic method) × 0.795 + 0.29]\(^9\)

Glycated haemoglobin (HbA\(_1c\)) was measured using high-performance liquid chromatography (Variant II from Bio-Rad, Hercules, California, USA). The amount of D-dimer, the international normalised ratio (INR) and the activated partial thromboplastin time (APTT) were determined by an automatic coagulometer (SYSMAX CA-1500, Sysmex Shanghai Ltd, Japan).

### Statistical analysis

Two data-entry clerks carried out the data input. The data were analysed using the statistical package program SPSS (version 19.0). Categorical variables are expressed as a percentage and continuous variables as mean ± standard deviation (SD). The chi-squared and Student’s \(t\)-tests were used to compare categorical variables and continuous variables, respectively, among groups. A multivariate logistic regression analysis was applied to identify variables independently associated with the efficacy of an antiplatelet therapy. Values of \(p < 0.05\) were regarded as statistically significant.

### Results

The mean age and the prevalence of cerebrovascular events were significantly higher in the clopidogrel group than in the other groups, while the eGFR in the clopidogrel group was lower than in the other groups (\(p < 0.01\)). Compared with the other groups, the aspirin group had a higher prevalence of dyslipidaemia and diabetes mellitus, and higher levels of fasting blood glucose and HbA\(_1c\) (\(p < 0.01\)), while the dual-drug group had a significantly higher prevalence of coronary heart disease (\(p > 0.01\)). The platelet count in the aspirin group was higher than that in the clopidogrel group (\(p = 0.03\)).
Compared with the no-drug group, the levels of cholesterol and low-density lipoprotein cholesterol (LDL-C) were lower in the dual-drug group ($p < 0.01$). There was no statistically significant difference among the four groups in indicators such as systolic and diastolic blood pressure, body mass index, waist circumference, triglycerides, high-density lipoprotein cholesterol (HDL-C), INR and APTT (see Table 1).

As shown in Table 2, according to TEG, the inhibition of platelet aggregation that was induced by AA% was $48.00 \pm 19.30\%$ in the aspirin group, and the inhibition induced by ADP% was $63.00 \pm 18.20\%$ in the clopidogrel group. In the dual-drug group, the inhibition of platelet aggregation was $51.00 \pm 16.50\%$ for AA% and $46.00 \pm 15.30$ for ADP%. The values in the no-drug group were $20.00 \pm 19.20\%$ for AA% and $36.00 \pm 19.50\%$ for ADP%.

The values of ADP-induced platelet-fibrin clot strength of the four groups were all between 31 mm and 47 mm, suggesting a low risk of both bleeding and thrombosis for all subjects. The reaction times of coagulation in the four groups were all in the normal range.

The efficacy rate (including the platelet inhibition classifications of ‘effective’ and ‘works well’) of the dual-drug group was higher than that of the aspirin group through the AA pathway, but there was no statistically significant difference ($51.16$ vs $45.92\%$; $p > 0.05$). The efficacy rate of the dual-drug group was lower than that of the clopidogrel group through the ADP pathway, but there was no statistically significant difference ($46.46$ vs $76.32\%$; $p > 0.05$). The total efficacy rate of the clopidogrel group was significantly higher than that of the aspirin group ($76.32$ vs $45.92\%$; $p < 0.05$).

It is important to note that there were eight subjects in the dual-drug group whose antiplatelet therapy was regarded as ineffective in both pathways, but this group still had the highest total efficacy rate ($81.40\%$) of the antiplatelet therapies, through either the AA or ADP pathway (data not shown in Table 3).
As shown in Table 4, the safety evaluation data showed that, compared with the aspirin group, the bleeding risk of the clopidogrel group was significantly increased ($p < 0.05$). However, there was no significant difference in the risk of bleeding between the dual-drug and no-drug groups. Compared with the aspirin group or the no-drug group, the risk of thrombosis in the clopidogrel group was significantly decreased (both $p < 0.05$). There was also no significant difference in the risk of thrombosis between the other two groups. Overall, there was no significant difference in the total risk of bleeding and thrombosis between the four groups ($p > 0.05$).

The multivariate logistic regression analysis of MA-ADP indicated that in the subjects with MA-ADP < 31 mm, an increased white blood cell count (OR = 1.262, $p < 0.001$) was a risk factor for bleeding, while an increased platelet count (OR = 0.995, $p = 0.013$) was a protective factor for bleeding. In the subjects with MA-ADP > 47 mm, an increased platelet count (OR = 1.006, $p < 0.001$), eGFR (OR = 1.016, $p = 0.013$) and HbA1c level (OR = 1.358, $p = 0.011$) were risk factors for thrombosis. The other factors, such as the age, APTT and LDL-C were not risk factors for bleeding or for thrombosis (Table 5).

### Discussion

According to the Chinese guidelines, aspirin antiplatelet therapy is essential for patients with coronary artery disease, cerebrovascular disease or diabetes. This study showed that these retired male Chinese officers had a high prevalence of cardiovascular and cerebrovascular diseases, diabetes mellitus and dyslipidaemia. The use of antiplatelet drug treatments in this population was approximately 50%.

Currently, aspirin and clopidogrel are the most common antiplatelet drugs used in clinical practice. The options of antiplatelet drugs vary depending on different clinical conditions.

### Table 4. Safety of antiplatelet therapies through different pathways

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aspirin group (n = 368)</th>
<th>Clopidogrel group (n = 115)</th>
<th>Dual-drug group (n = 45)</th>
<th>No-drug group (n = 429)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding risk, n (‰)</td>
<td>92 (25.0)</td>
<td>41 (35.7)†</td>
<td>14 (32.6)</td>
<td>94 (21.9)</td>
</tr>
<tr>
<td>Safe range, n (‰)</td>
<td>143 (38.9)</td>
<td>54 (47.0)</td>
<td>17 (39.5)</td>
<td>179 (41.7)</td>
</tr>
<tr>
<td>Thrombosis risk, n (‰)</td>
<td>133 (36.1)</td>
<td>20 (17.4)‡</td>
<td>12 (27.9)</td>
<td>156 (36.4)</td>
</tr>
</tbody>
</table>

†Bleeding risk refers to MA-ADP < 31 mm;
‡Safe range refers to MA-ADP 31–47 mm;
*Compared with the aspirin group, $p < 0.05$ ($p = 0.026$);
*Compared with the aspirin group, $p < 0.05$ ($p = 0.000$);
*Compared with no-drug group, $p < 0.05$ ($p = 0.000$).

In this study, using TEG, we evaluated the efficacy of different antiplatelet therapies in retired elderly male Chinese officers and explored the risk factors influencing the efficacy of different antiplatelet therapies.

The data showed that the inhibition of platelet aggregation by AA was 48.0 ± 19.3% in the aspirin group, and the inhibition by ADP was 63.0 ± 18.2% in the clopidogrel group. The effective percentage of platelet inhibition in the aspirin and clopidogrel groups was 45.9 and 76.5%, respectively, which suggests that clopidogrel might be a more effective antiplatelet therapy than aspirin. We speculated that this effect might be associated with the older age of patients in the clopidogrel group. Elderly patients have a slower metabolic rate (eGFR: 71.69 ± 14.48 ml/min/1.73 m² in the aspirin group, and 76.87 ± 20.37 ml/min/1.73 m² in the clopidogrel group, $p = 0.0015$), which would lead to an accumulation of antiplatelet drug and therefore enhance the antiplatelet effect.

Both aspirin and clopidogrel treatments have a risk of bleeding, and poor antiplatelet effects increase the risk of thrombosis. The TEG variable MA-ADP is one of the predictors of adverse cardiovascular events, and the best range of MA-ADP in the Chinese population is between 31 and 47 mm, according to the study by Tang et al. In our study, patients treated with either aspirin or clopidogrel monotherapy or a combination of the two drugs showed average MA-ADP values that were all in the best range, suggesting that the lowest risk of bleeding and thrombosis can be achieved with aspirin plus clopidogrel alone or in combination.

Compared with aspirin, clopidogrel reduced the risk of thrombosis and increased the risk of bleeding, which could be associated with the inhibition of neovascularisation by the mechanism of action of clopidogrel. The risk of bleeding and thrombosis in the dual-drug antiplatelet group was not significantly different from the other three groups. This may have been related to the small number of subjects in this group. Overall, there was a low risk of bleeding and thrombosis and a high safety level for antiplatelet therapy with aspirin and clopidogrel in combination.

The logistic regression analysis showed that the increased leukocyte count was a risk factor for bleeding. This is consistent with the results of other studies. A study showed that leukocyte overload could result in significant bleeding in patients with primary thrombocytosis and that a leukocyte count over 1.1 × 10⁹ cells/l was an independent risk factor for acute vascular events. Another study showed that leukocytes may infiltrate the endothelial barrier through the Goαi signalling pathway, be recruited to the inflammatory site, and then cause inflammatory bleeding.

Thirty years ago, the relationship between platelets and TEG was studied, and it was recently revalidated with the modified version of TEG. A study from Holland showed that platelets were significantly and positively associated with MA. When the platelet count was < 100 × 10⁹ cells/l, the clot formation rate decreased, and when the platelet count was < 50 × 10⁹ cells/l, the MA decreased significantly. However, in our study, the platelet count varied according to the patient's condition. It was a protective factor for patients who were prone to bleeding, while it was a risk factor for the patients who were prone to thrombosis.

The research on TEG in other related diseases is limited. There are a variety of reasons for renal failure to cause bleeding, and the most important one may be due to accumulated toxins...
that affect the function of platelets, and the interaction between platelets and the vascular wall. Our study found a slightly positive association between eGFR and thrombosis in the patient population with MA-ADP > 47 mm, but the specific pathogenesis is not clear and further research is needed.

A study by Yao and co-workers showed that in patients with diabetes, the level of MA was significantly increased, and higher MA levels and diabetes were independent predictors for unfavourable one-year functional outcomes of recurrent ischaemic events. Our study also showed that there was a positive correlation between HbA1c level and MA-ADP, but the relevance reflected by this correlation and to the outcomes of elderly male officers merit further observation.

Balancing the risk of bleeding and thrombosis is the first consideration of an antiplatelet therapy in elderly patients with atherosclerotic diseases. To establish an individualised antiplatelet therapy, an accurate, rapid and convenient method for platelet function testing is urgently needed. As a method of detecting platelet function, TEG can guide the decision of antiplatelet drug options in elderly patients.

There are some limitations to this study. First, it is important to note that the number of subjects and the levels of baseline in each group were different. This may have affected our results and further research is needed. Second, the efficacy of antiplatelet therapy in the aspirin group was approximately 45% in our study, to note that the number of subjects and the levels of baseline in antiplatelet drug options in elderly patients. Officers merit further observation.

Conclusion

This quality-controlled TEG procedure was an efficient method to evaluate the efficacy of antiplatelet therapies in the clinic. White blood cell and platelet counts, eGFR and HbA1c levels may influence the efficacy of antiplatelet therapy.

The authors thank the subjects for agreeing to participate in the study. This work was supported by the National Natural Science Foundation of China for Youth (No. 81500316) and the National Key Research Program of China (2017YFC0840100 and 2017YFC0840103).

References