Establishing a reference range for thromboelastography maximum amplitude in patients administrating with antiplatelet drugs

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Abstract
Objective: We aimed to establish the reference range of thromboelastograph (TEG) maximum amplitude (MA) in patients taking antiplatelet drugs.

Methods: Between August 2015 and July 2018, a total of 4614 patients administrating with antiplatelet drugs (clopidogrel and aspirin) were retrospectively analyzed in this study. For MA parameter, we used the 10th and 90th percentiles to establish a reference range. The Spearman correlation was used for the correlation analysis among the inhibition rate of adenosine diphosphate (ADP%) and MA_{ADP}, inhibition rate of arachidonic acid (AA%) and MA_{AA}. Then, through receiver operating characteristic (ROC) curve analysis of the best cutoff point, the reference ranges of MA_{ADP} and MA_{AA} could be deduced. Consistency evaluation was performed by statistical analysis of ADP% and MA_{ADP}, AA% and MA_{AA} pairing for 4459 patients.

Results: The reference range of MA_A was 8.1-25.8 mm. The reference range of MA_{ADP} was 19.8-43.2 mm, and the corresponding sensitivity of two endpoints was 0.796, 0.856 and specificity were 0.897, 0.904, respectively. The reference range of MA_{AA} was 18.9-37.7 mm, and the corresponding sensitivity of two endpoints was 0.819, 0.829 and specificity were 0.922, 0.896, respectively. The inconsistency rate of ADP% and MA_{ADP} and AA% and MA_{AA} was 20.1% (898 cases) and 16.6% (738 cases), respectively.

Conclusions: The reference range of MA_{ADP} and MA_{AA} established by us were better in sensitivity and specificity. MA_{ADP} and MA_{AA} were more accurate than conventional inhibition rate analysis in guidance of antiplatelet therapy, especially in patients with excessive low MA or high MA_A.

KEYWORDS
antiplatelet agents, aspirin, clopidogrel, thromboelastography
1 | INTRODUCTION

Thrombus is one of the most common lethal diseases in non-malignant diseases, including myocardial infarction, cerebral infarction, pulmonary embolism, and so on. Once onset, long-term anticoagulant therapy is needed. For arterial thrombus, long-term treatment with antiplatelet agents is usually required, such as aspirin and clopidogrel. It is necessary to monitor the blood coagulation functions of patients administrating with antiplatelet drugs to test the effectiveness of the drug.

Routine coagulation tests (such as activated partial thromboplastin time (APTT), prothrombin time (PT), and platelet count) are the most commonly used method for evaluating coagulation function. These tests are often used as a starting place when investigating the cause of bleeding. However, routine tests possess only limited capacities to reveal patient's risk of bleeding and do not provide information on the risk for thrombus. Besides, they do not provide specific data about clot quality or stability.

In comparison to the conventional tests, the thromboelastograph (TEG) hemostasis analyzer system can objectively reflect the blood clotting, fibrinogen/fibrin/platelet interactions, and the processes of formation, development, and dissolution of thrombus in the body. TEG can reflect the function of platelet. The therapeutic effect of antiplatelet drugs can be evaluated by adding ADP or AA inducer and deducting the coagulation effect of fibrin reticulum. Maximum amplitude (MA) parameters reflect the strength of blood clots. The strength of the blood clot is composed of platelet aggregation, contraction, and fibrin network. Platelets account for about 80%, and fibrin network accounts for about 20%. According to different inducers and detection types, MA can be divided into four types, including Kaolin activity (MA\textsubscript{CK}), fibrin activity (MA\textsubscript{A}), ADP-stimulated platelet activity (MA\textsubscript{ADP}), and AA-stimulated platelet activity (MA\textsubscript{AA}).

It is generally believed that drugs are more effective when platelet function is inhibited by more than 50%. According to clinical experience and manufacturer’s recommendation, our hospital has established a reference range of 40%-90% induction inhibition rate of ADP and 50%-90% induction inhibition rate of AA. However, the platelet inhibition rate needs to consider the MA\textsubscript{CK} value of the common detection and the MA\textsubscript{A} value of the fibrin network. When these two values are too high or too low, the calculated inhibition rate will be affected. Therefore, this study retrospectively analyzed the previous thromboelastograph (TEG) data, aiming to directly establish a reference range of MA\textsubscript{A}, MA\textsubscript{ADP} and MA\textsubscript{AA}, and guide the rational use of drug in clinical practice.

2 | METHODS

2.1 | Patients

We retrospectively reviewed 4614 patients administrating with antiplatelet drugs (clopidogrel and aspirin), who were treated in Renji Hospital Affiliated to Medical College of Shanghai Jiaotong University from August 2015 to July 2018. The inclusion criteria were as follows: (a) patients administrating with both aspirin and clopidogrel; (b) patients undergoing the TEG test; and (c) patients having complete TEG data. Considering the abnormal activation of platelet (MA\textsubscript{A} >35 mm), the original data with MA\textsubscript{A} >35 mm were excluded. Finally, the remaining 4459 cases were included in the subsequent analysis. This study was approved by the ethics committee of Renji Hospital Affiliated to Medical College of Shanghai Jiaotong University. Written informed consent was obtained from each patient.

2.2 | TEG analysis

Platelet-fibrin clot strength measurements were carried out using the TEG Hemostasis System (Haemoscope Corporation). The TEG Hemostasis Analyzer with automated analytical software provides quantitative and qualitative measurements of the physical properties of a clot.

All analyses were performed with TEG disposable cups and pins as devised by the manufacturer and measurements were performed within 4 minutes of sampling. Briefly, a stationary pin is suspended into an oscillating cup that contains the whole blood sample. As the blood clots, it links the pin to the cup. Clot strength is determined by measuring the amplitude of the rotation of the pin, which increases proportionally with clot strength. Maximum amplitude represents maximum clot strength, expressed as the MA parameter.

2.3 | Statistical analysis

All statistical analyses were performed by using SPSS version 17.0 (SPSS Institute). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as means ± standard deviation (SD); non-normal variables were reported as median...
(interquartile range [IQR]). For MAₐ parameter, we used the 10th and 90th percentiles (P₁₀-P₉₀) to establish a reference range. The Spearman correlation was used for the correlation analysis among the ADP% and MAₐADP, AA% and MAₐAA. Then, through receiver operating characteristic (ROC) curve analysis of the best cutoff point, the reference ranges of MAₐADP and MAₐAA could be deduced. Consistency evaluation was performed by statistical analysis of ADP% and MAₐADP, AA% and MAₐAA pairing for 4459 patients. Statistical significance was set at P < .05.

3 | RESULTS

3.1 | Demographic characteristics

We retrospectively reviewed 4614 patients administrating with clopidogrel and aspirin, who were treated in our department from August 2015 to July 2018. Considering the abnormal activation of platelet (MAₐ > 35 mm), the original data with MAₐ > 35 mm were excluded. Finally, the remaining 4459 patients (2512 males, 1947

FIGURE 1  Data distribution of MAₐ, MAₐAA, and MAₐADP. ADP-stimulated platelet activity; MAₐ, fibrin activity; MAₐADP, MAₐAA, AA-stimulated platelet activity
females; mean age: 54.4 years; range from 39 to 85 years) were included in the subsequent analysis. The baseline characteristics of included patients are shown in Table 1.

3.2 | Establishment and evaluation of MA_A reference range

Considering the skewed distribution of MA_A values (Figure 1A), the reference range was 8.1-25.8 mm ($P_{10}-P_{90}$). To further evaluate the feasibility of the MA reference range, TEG results of 155 patients (MA_A ≥ 30 mm) were retrospectively analyzed. Only 11 cases did not show large opening pattern. Especially for patients with MA_A ≥60 mm, the inhibition rate showed a higher false value (Table 2).

3.3 | Establishment of MA_AD and MA_AA reference range

The values of MA_AA and MA_AD were skewed distributed (Figure 1B,C). To establish the reference range of MA_AD and MA_AA, the Spearman correlation was used for the correlation analysis among the parameters firstly. MA_AA was significantly negative correlated with AA% ($r = -0.864, P < .001$) (Figure 2A). Besides, a significant negative correlation was also found between MA_AD and ADP% ($r = -0.892, P < .001$) (Figure 2B).

For MA_AA, ROC curves (Figure 3) of the dichotomous data MA_AA and AA% (with [50, 90] as normal) were analyzed and the best cutoff points were found. The ROC curve analysis results showed that AA% was a predictor of MA_AA (AA%≥50: AUC = 0.896; sensitivity, 0.819; specificity, 0.922. AA% ≤90: AUC = 0.971; sensitivity, 0.829; specificity, 0.896). The best cutoff values of AA% for MA_AA were 18.9 (AA%≥50) and 37.7 (AA%≤90), respectively. The reference range of MA_AA was 18.9-37.7 mm.

For MA_AD, ROC curves (Figure 4) of the dichotomous data MA_AD and ADP% (with [40, 90] as normal) were analyzed and the best cutoff points were found. The ROC curve analysis results showed that ADP% was a predictor of MA_AD (ADP% ≥40: AUC = 0.989; sensitivity, 0.796; specificity, 0.897. ADP% ≤90: AUC = 0.960; sensitivity, 0.856; specificity, 0.904). The best cutoff values of ADP% for MA_AD were 19.8 (ADP% ≥40) and 43.2 (ADP% ≤90), respectively. The reference range of MA_AD was 19.8-43.2 mm.

3.4 | Feasibility analysis of MA_AD and MA_AA reference range

To further evaluate the feasibility of the MA reference range, consistency evaluation was performed by statistical analysis of ADP% and MA_AD, AA% and MA_AA pairing for 4459 patients. The results showed that the inconsistency rate of ADP% and MA_AD was 20.1% (898 cases), AA% and MA_AA was 16.6% (738 cases). The analysis of inconsistent cases, it was found that there was a significant correlation with MA_A and MA_CK values. When the MA_A and MA_CK values were too large or too small, the inconsistency rate increased significantly (Table 3). Such as, for the smallest 100 cases of MA_A, the inconsistency rate of ADP% and MA_AD was 30%, AA% and MA_AA was 37%, which was significantly higher than the median 100 cases of MA_A (the inconsistency rate of ADP% and MA_AD was 3%, AA% and MA_AA was 2%).

4 | DISCUSSION

In recent years, the TEG has attracted much attention in assessing blood coagulation function and guiding blood transfusion in the preoperative period. Compared with routine coagulation function tests, TEG can reflect the first and second stages of hemostasis and fibrinolysis and can also reflect clinical bleeding more sensitively. The TEG can be more comprehensive and effective in evaluating the coagulation status. According to TEG manufacturer’s instructions, the acquisition of ADP% and AA% depended on MA_A and MA_CK values. However, in practice, when the two values were abnormal, the accuracy of inhibition rate would be affected. For example, when MA_A was abnormally increased, the inhibition rate would be falsely increased (Table 1). Clinical workers also

| Case | MA_CK (mm) | MA_A (mm) | MA_AD (mm) | MA_AA (mm) | ADP% Original | Corrected* | AA% Original | Corrected* |
|------|------------|-----------|------------|------------|---------------|-------------|--------------|------------|
| 1    | 75.0       | 66.6      | 69.4       | 70.2       | 66.7          | 9.3         | 57.1         | 8.0        |
| 2    | 72.7       | 60.0      | 69.0       | 64.1       | 29.1          | 6.4         | 67.7         | 14.9       |
| 3    | 77.0       | 65.9      | 61.1       | 32.4       | 100.0         | 25.6        | 100.0        | 71.8       |
| 4    | 70.6       | 60.5      | 60.8       | 61.2       | 97.0          | 17.6        | 93.1         | 16.9       |
| 5    | 72.3       | 60.4      | 51.1       | 61.7       | 100.0         | 36.9        | 89.1         | 18.5       |

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; MA, maximum amplitude; MA_CK, Kaolin activity; MA_A, fibrin activity; MA_AD, ADP-stimulated platelet activity; MA_AA, AA-stimulated platelet activity.

*Corrected data: unified calculation with MA_A = 14.9 mm.
explored the direct evaluation of $\text{MA}_{\text{ADP}}$ or $\text{MA}_{\text{AA}}$ to guide clinical application. For example, the study of Sinai Hospital in Baltimore, USA, tracked 225 patients who received percutaneous coronary intervention (PCI). It was considered that $\text{MA}_{\text{ADP}} > 47 \text{ mm}$ was a predictor of re-infarction risk, while $\text{MA}_{\text{ADP}} < 31 \text{ mm}$ was prone to bleeding risk.\(^\text{22}\) However, the risk value was not equivalent to the reference value of whether the drug was effective. So our laboratory directly evaluated the $\text{MA}_{\text{ADP}}$ or $\text{MA}_{\text{AA}}$ reference range of whether the drug was effective by retrospective analysis of previous results of 4614 patients administrating with antiplatelet drugs (clopidogrel and aspirin). The results showed that the reference range of $\text{MA}_{\text{ADP}}$ and $\text{MA}_{\text{AA}}$ established by us were better in sensitivity and specificity. $\text{MA}_{\text{ADP}}$ and $\text{MA}_{\text{AA}}$ were more accurate than conventional inhibition rate analysis in guidance of antiplatelet therapy, especially in patients with excessive low MA or high MA.\(^\text{21}\) Besides, a significant negative correlation was also found between $\text{MA}_{\text{ADP}}$ and ADP%.

Since the reference range was negatively correlated with the inhibition rate, attention should be paid to clinical use. Exceeding the upper limit of the reference range indicated poor drug efficacy, and the closer the result was to the $\text{MA}_{\text{DX}}$ value, the worse the efficacy was. Below the limit of the reference range, the original inhibition

**FIGURE 2** Correlation analysis. A, Correlation analysis between $\text{MA}_{\text{AA}}$ and AA%. AA, arachidonic acid; $\text{MA}_{\text{AA}}$, AA-stimulated platelet activity. B, Correlation analysis between $\text{MA}_{\text{ADP}}$ and ADP%. ADP, adenosine diphosphate; $\text{MA}_{\text{ADP}}$, ADP-stimulated platelet activity

**FIGURE 3** ROC curve of AA%. A, ROC curve of 3086 patients with AA% ≥ 50%; B, ROC curve of 2284 patients with AA% ≤ 90%. AA, arachidonic acid; ROC, receiver operating characteristic
rate exceeded 90%, indicating that the drug was very effective, but did not indicate whether there was a risk of bleeding. Especially in the clinical use of antiplatelet membrane glycoprotein IIb/IIIa classes of antiplatelet aggregation drugs,23 the fibrin and platelet junction sites were inhibited. The MA_{ADP} values and MA_{A} values were almost identical, and below the limit of the reference range only showed that the drug was effective, but did not indicate whether there was a risk of bleeding.

By analyzing the cases of ADP% and AA% inconsistent with MA_{ADP} and MA_{AA}, we found that when the values of MA_{A} and MA_{CK} were too large or too small, the inconsistency rate increased significantly (Table 2). For the smallest 100 cases of MA_{A}, it was found that the inhibition rate was generally calculated in the appropriate range, MA_{ADP} or MA_{AA} was below the reference range, and the ADP and AA inconsistency rates were 30% and 37%, respectively. However, they all suggested that the drug was effective and therefore did not affect clinical treatment. However, for the largest 100 cases of MA_{A}, it was found that if the MA_{A} was falsely increased, the inhibition rate would incorrectly indicate that the drug was effective.

Since the MA_{A} value was easy to affect the calculation of inhibition rate, it was necessary to establish a reference range. Only
when the MA_A value was in a reasonable range, the calculation of inhibition rate would be more reliable. MA_A reflected the patient’s fibrin formation ability. When the value was too high and the TEG tracing continues to grow wider, it reflected the abnormal activation of platelets. It was recommended to try glycoprotein IIb/IIIa inhibitor.

This study has several limitations. Firstly, this study was a retrospective, single-center and non-control study, which had certain limitations in clinical application. Secondly, this study only studied the MA parameters and did not analyze other TEG parameters such as r-time, k-time, and α-angle. Therefore, multi-centered prospective studies should be conducted in the future to establish a more accurate reference range of TEG in patients taking antiplatelet drugs.

In conclusion, TEG could be used as a relatively reliable method for monitoring antiplatelet drugs. Compared with the traditional inhibition rate analysis, the direct establishment of MA_A, MA_ADP and MAAA reference range could be more rationally in guide the use of drug in clinical practice.

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