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Prompt prediction of fibrinogen concentration during cardiopulmonary bypass: a pilot study

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ABSTRACT

Platelet Mapping can measure both the degree of platelet inhibition and fibrinogen activation, was not originally designed to measure fibrinogen concentration. Traditional laboratory fibrinogen concentration testing requires around 60 minutes; however, fibrinogen activation only takes 10 minutes, and is indicated as maximum amplitude of activator f. If Platelet Mapping can predict fibrinogen concentration during cardiopulmonary bypass, this could facilitate rapid hemostasis management. The aim of this study was to verify whether fibrinogen concentration could be predicted using Platelet Mapping results. Thus, a pilot study was conducted to evaluate this concept during cardiopulmonary bypass. This prospective, observational pilot study investigated 15- to 90-year-old patients who underwent cardiac or aortic surgery from August 2019 to September 2019. Twenty-one patients enrolled in this study, and 43 blood samples were obtained for both fibrinogen activation measurements using Platelet Mapping and traditional laboratory-based tests, respectively. Correlations between results were analyzed using linear regression and the receiver operating characteristic curve. Correlation by Pearson's correlation analysis indicates a significant relationship (correlation coefficient of $r = 0.91$), and a receiver operating characteristic curve indicated that sensitivity, specificity, and receiver operating characteristic area were 100% (95% confidence interval, 75.3–100%), 93.8% (79.2–99.2%), and 0.995 (0.984–1.00), respectively. Our results indicate a strong correlation between fibrinogen activation and serum fibrinogen concentration. The maximum amplitude of activator f can estimate low fibrinogen concentration faster than traditional methods; this method quickly provides important information for anesthesia and hemostatic management in cardiac surgery.

Keywords: thromboelastography, platelet mapping, fibrinogen, cardiopulmonary bypass

Abbreviations:
CPB: cardiopulmonary bypass
TEG6s: thromboelastography
ROTEM: rotational thromboelastometry
PM: Platelet Mapping
ActF-MA: maximum amplitude of activator f
ROC: receiver operating characteristic

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INTRODUCTION

Multiple factors like hypothermia, acidosis, and induction of the inflammatory cascade are responsible for hemostatic disorders during cardiopulmonary bypass (CPB).[1-4] Viscoelastic hemostatic devices such as thromboelastography (TEG6s®, Haemonetics Japan G.K., Japan), rotational thromboelastometry (ROTEM®, TEM International GmbH, Munich, Germany), and sonic estimation of elasticity via resonance sonorheometry (Quantra®, HemoSonics LLC, USA) have been recommended to help guide transfusion during cardiovascular surgery.[5-7] Viscoelastic hemostatic devices have the advantage of being point-of-care tests that can be performed at the bedside and provide fast results compared to conventional laboratory blood tests. Measuring coagulation function with these devices has been shown to improve transfusion outcomes in cardiovascular surgery.[8] A previous report indicated that hemostatic management using a blood transfusion algorithm incorporating these devices was more effective in reducing the use of allogeneic blood products compared to conventional empirical transfusion.[9] In addition, determination of fibrinogen concentration is important for hemostasis management.[10] The intervention lower fibrinogen concentration limit is considered to be 150 mg/dl in cardiac surgery in Japan; it is a general standard to keep the fibrinogen concentration above 150–200 mg/dl. However, unlike with ROTEM, the standard TEG assay (Global Hemostasis assay) during CPB with heparinization does not provide accurate information regarding coagulation function.[11]

Platelet Mapping (PM) is a separate TEG6s assay that was designed to measure the degree of platelet inhibition by aspirin and P_2Y_{12} inhibitors such as clopidogrel. PM also provides maximal amplitude for fibrinogen evaluated by activator f (ActF-MA), which indicates clot strength alone without platelet function. Since the measurement is performed using heparinized blood, PM can provide results during heparinization. If PM can predict fibrinogen concentration during CPB, it would help facilitate rapid hemostasis management tasks such as transfusion. The aim of this study was to verify whether ActF-MA results can predict fibrinogen concentration, and if the lower limit for measurement of ActF-MA can predict the fibrinogen concentration of 150 mg/dl. Therefore, we hypothesized that there is a significant relationship between ActF-MA and fibrinogen concentration during CPB, and a pilot study was conducted to evaluate this concept using results obtained during cardiovascular surgeries with CPB. Platelet Mapping (PM) is a separate TEG6s assay that was designed to measure the degree of platelet inhibition by aspirin and P_2Y_{12} inhibitors such as clopidogrel. PM also provides maximal amplitude for fibrinogen evaluated by activator f (ActF-MA), which indicates clot strength alone without platelet function. Since the measurement is performed using heparinized blood, PM can provide results during heparinization. If PM can predict fibrinogen concentration during CPB, it would help facilitate rapid hemostasis management tasks such as transfusion. The aim of this study was to verify whether ActF-MA results can predict fibrinogen concentration, and if the lower limit for measurement of ActF-MA can predict the fibrinogen concentration of 150 mg/dl. Therefore, we hypothesized that there is a significant relationship between ActF-MA and fibrinogen concentration during CPB, and a pilot study was conducted to evaluate this concept using results obtained during cardiovascular surgeries with CPB.

METHODS

Study population

This study was a prospective, observational pilot study approved by the Nagoya University Hospital Institutional Review Board (IRB #2019–0202). The trial was registered with the University Hospital Medical Information Network (Study ID: UMIN000037138) prior to patient enrollment. After obtaining written informed consent, patients 15–90 years old who were scheduled for cardiac or aortic surgery with CPB from August 2019 to September 2019 were enrolled. The primary endpoint was correlation between fibrin as measured by ActF-MA and traditional laboratory blood tests of fibrinogen concentration (Clauss fibrinogen assay).

Standard monitoring for cardiovascular surgery patients (non-invasive arterial blood pressure measurements, electrocardiography, pulse oximetry, bispectral index monitoring, radial artery cannulation, and pulmonary artery catheterization) were performed in all patients. Fentanyl and midazolam were administered intravenously to induce general anesthesia. Remifentanil and rocuronium were additionally used to facilitate tracheal intubation, and general anesthesia was maintained with air, oxygen, remifentanil, and volatile anesthetics. Porcine heparin (300 U/kg) was administered before starting cannulation for CPB, and additional heparin boluses (50 U/kg) were administered to maintain an activated clotting time of at least 400 seconds. Ventilation was re-started before separating from CPB, and CPB was terminated with inotropic drug support. Protamine (3 mg/kg) was administered to antagonize the heparin effect, and the patient was intubated and admitted to the intensive care unit. Red blood cell concentrates were transfused to maintain a hemoglobin level over 8 mg/dl during CPB. Coagulation function was monitored using standard TEG6s assay, and flesh frozen plasma and platelet concentrate were administered appropriately.

Blood sampling protocol

Blood samples were collected via a radial artery cannula system with a closed circuit for blood sampling (Tru Wave with VAMP system, Edwards Lifesciences Co., Ltd., CA, USA). Each blood sample was dispensed for both ActF-MA measurements for TEG6s and fibrinogen concentration measurements for our hospital's central laboratory to obtain results at the same time points. Each one milliliter blood sample was collected into a tube containing heparin for PM and into 3.2% sodium citrate for fibrinogen concentration measurement. Blood was collected at two time points: 30 minutes after starting CPB, and during the injection of warm cardioplegia solution just before coming off CPB. Usually, two measurements were performed; if hemostasis after removal from CPB was poor, a third blood sample was collected. The TEG6s instrument was used to measure ActF-MA in the operating room immediately after the blood was drawn by the anesthesiologist. Traditional laboratory-based testing was used to measure fibrinogen concentration; specimens were submitted to our hospital's central laboratory and measured by the laboratory technician using Clauss fibrinogen assay.

Statistical analyses

Based on a measurement error of less than 10% in quantitative measurement of fibrinogen concentration, a pilot study was planned in which over 10 samples were measured. First, we evaluated the correlation between fibrinogen concentration via traditional laboratory-based testing and ActF-MA using Pearson's correlation analysis. Second, we performed a receiver operating characteristic (ROC) analysis to identify the optimal cut-off value of ActF-MA for the outcome of fibrinogen concentration < 150 mg/dl. The optimal cut-off value was identified using Youden index. In addition, the lower limit of ActF-MA measurement is 2 mm; therefore, we evaluated

the correlation between fibrinogen concentration and ActF-MA at 2 mm. Third, we removed the results below the lower measurement limit for ActF-MA and developed a conversion equation of fibrinogen concentration based on linear regression. All analyses were performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) and Stata 16 MP (StataCorp, College Station, TX, USA).

RESULTS

Between August and September 2019, 21 patients were enrolled in the study after providing consent. Patient information and surgical characteristics are shown in Table 1. Blood samples were collected twice from 17 patients. Three times blood collection was performed for three patients because of prolonged hemostasis. Therefore, we obtained a total of 43 samples of blood for PM measurement by TEG6s in the operating room and 43 samples for fibrinogen concentration measurement by traditional Clauss method in the laboratory.

All 86 samples were measured normally with no missing values and 43 sets of results were obtained. The ActF-MA and fibrinogen concentration measurements are shown (Figure 1). The lower measurement limit for ActF-MA is 2 mm, so results less than 2 mm are displayed as zero. The correlation between ActF-MA and fibrinogen concentration by Pearson's correlation analysis is also shown in Figure 1. The results (correlation coefficient of $r = 0.91$) indicate a significant correlation between ActF-MA and blood fibrinogen concentration.

The optimal ActF-MA cut-off value was 3.3 mm to predict fibrinogen concentration < 150 mg/dl, where sensitivity was 100% (95% confidence interval (CI), 75.3–100%), specificity was 93.8% (95% CI, 79.2–99.2%), and ROC area was 0.995 (95% CI, 0.984–1.00) (Figure 2). The performance of ActF-MA < 2 mm, which is the lower limit of PM measurement, was sensitivity

Table 1   Patients demographic and surgical characteristics

Demographic (n=21)	Median (IQR)
Demographic Information	
Age (years)	64 (54.0–73.0)
Height (cm)	167.3 (164.9–169.0)
Body weight (kg)	59.3 (55.0–69.2)
Body mass index (kg/m2)	21.1 (20.3–24.1)
Male:female	16:5
Surgical Information	
On-pump CABG	5
Off-pump CABG	1
Single valve	9
Aorta	6

Data are expressed as medians and interquartile range (IQR) range; 25th–75th percentiles (n=21). On-pump CABG, coronary artery bypass graft with cardio-pulmonary bypass. Off-pump CABG, coronary artery bypass graft without cardio-pulmonary bypass. Single valve, aortic valve replacement, mitral valve replacement or mitral valve repair. Aorta, total arch replacement, ascending aorta replacement or descending aorta replacement.

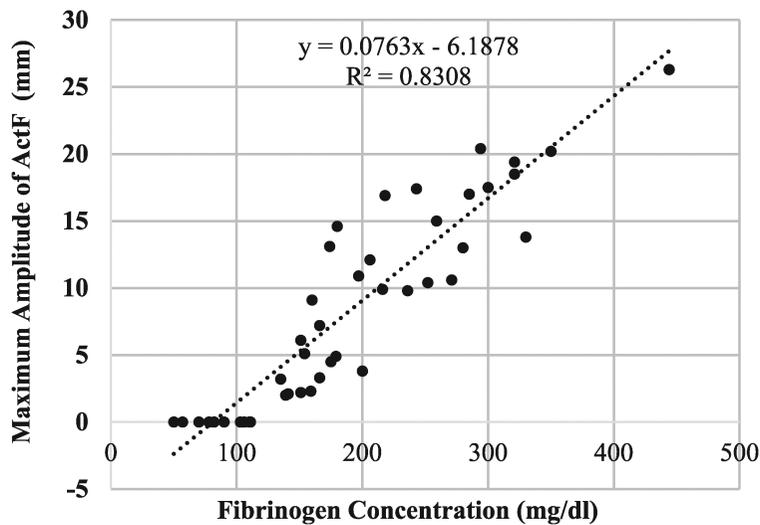Prompt Prediction of Fibrinogen Concentration

Fig. 1 Relationship between fibrinogen concentration and ActF-MA

ActF, activator f.

The correlation between fibrinogen concentration via traditional laboratory-based testing and ActF-MA was evaluated using Pearson's correlation analysis. The result indicates a strong correlation coefficient (r = 0.91) and statistically significant relationship between fibrinogen concentration and ActF-MA.
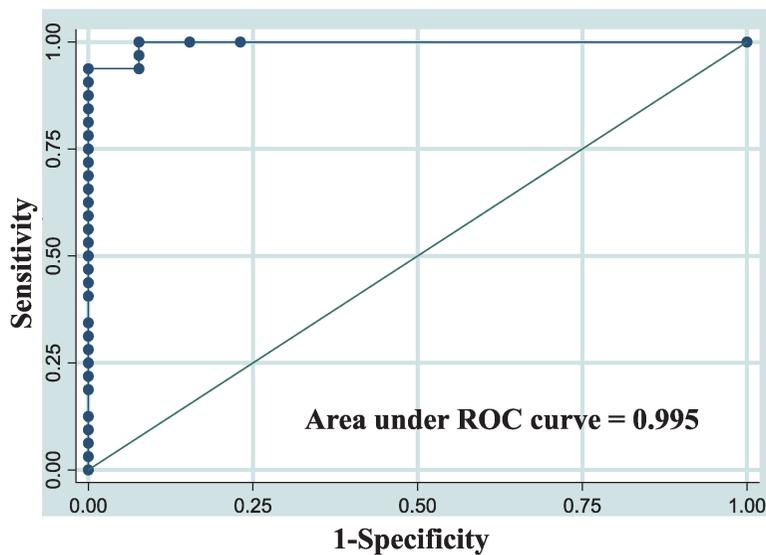
Fig. 2 Receiver operating characteristic analysis

A receiver operating characteristic (ROC) analysis to identify the optimal cut-off value of ActF-MA for the outcome of fibrinogen concentration < 150 mg/dl was evaluated. The optimal cut-off value was identified using Youden index. The optimal cut-off value of ActF-MA was 3.3 mm in order to predict fibrinogen concentration < 150 mg/dl, where the sensitivity was 100% (95% confidence interval (CI), 75.3–100%), specificity was 93.8% (95% CI, 79.2–99.2%), and ROC area was 0.995 (95% CI, 0.984–1.00).
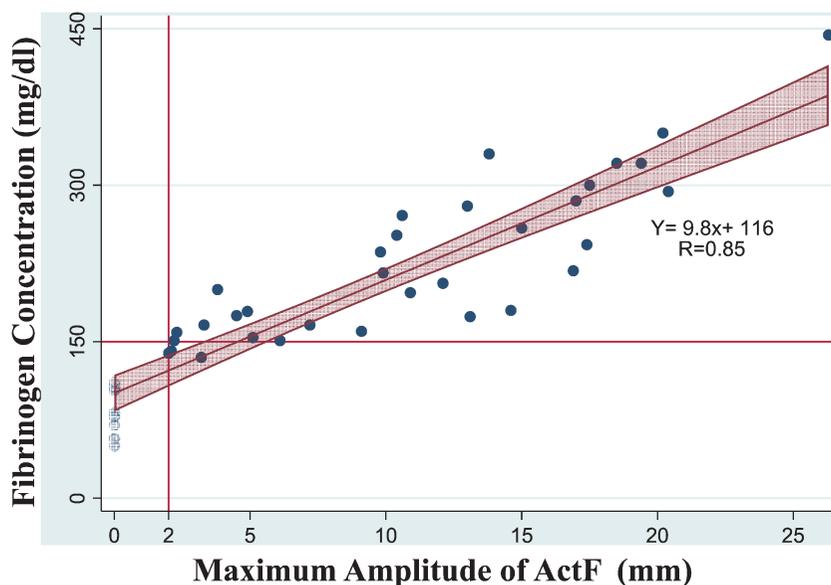
Fig. 3 The conversion equation of fibrinogen concentration based on linear regression

ActF, activator f.

The results below the lower measurement limit for ActF-MA were removed, and the conversion equation of fibrinogen concentration based on linear regression was developed: "predicted fibrinogen concentration"=9.8×"ActF-MA"+116.

of 76.9% (95% CI, 46.2–95%), specificity of 100% (95% CI, 89.1–100%), and ROC area of 0.885 (95% CI, 0.765–1.00). These results strongly predict a fibrinogen concentration of below 150 mg/dl when ActF-MA is approximately 3 mm, and an almost certain fibrinogen concentration of less than 150 mg/dl if ActF-MA is below the measurement limit. In addition, we removed results below the lower measurement limit for ActF-MA and developed the following conversion equation:" predicted fibrinogen concentration"=9.8×"ActF-MA"+116. (Figure 3)

DISCUSSION

This pilot study analyzed the correlation between fibrinogen concentration via traditional laboratory-based testing and ActF-MA evaluated by the TEG PM assay; as far as the authors know, no previous literature has reported these correlations. The results of this study support our hypothesis and revealed one major finding – a significant relationship between ActF-MA and serum fibrinogen concentration, and the ability of ActF-MA to diagnose low fibrinogen concentration.

Since previous data indicate that the use of red blood cell transfusion increases with fibrinogen concentration below 200 mg/dL[10], it is common to keep the fibrinogen concentration above 150–200 mg/dl and at a lower limit of 150 mg/dl in Japan. For hemostatic coagulation management, it is important to know the fibrinogen concentration after taking off CPB. Tamura indicated a previous study showing a correlation between the standard TEG6s assay and fibrinogen blood concentration.[11] However, these values seem to vary in the low fibrinogen concentration range of less than 150 mg/dl, including ROTEM.[11,12] In addition, Tamura previously indicated that standard TEG6s could not measure coagulation function during the heparinized state of CPB.[11] It can take

nearly 40–60 minutes for the traditional fibrinogen concentration measurement assay to report test results. Therefore, if fibrinogen concentration during CPB can be quickly determined using PM, which is another TEG6s assay providing measurement results in about 10 minutes, rapid anesthesia management is possible. From the ROC results in Figure 2, the fibrinogen concentration is predicted to be less than 150 mg/dl, if the ActF-MA is below the measurement limit. The conversion equation in Figure 3 shows the possibility that the fibrinogen concentration will be below 200 mg/dl when the ActF-MA would be below around 8.0 mm. Our results indicate that PM can diagnose low fibrinogen concentration during CPB, which allows for faster transfusion preparation than conventional blood tests.

PM is not a device meant to measure fibrinogen concentration; it is meant to determine the inhibition rate of platelet function induced by antiplatelet drugs as indicated by adenosine diphosphate receptor antagonism and the following four tests: (1) Thrombin-MA; maximally activates all platelets and represents the potential maximal amplitude of platelets and fibrinogen, (2) and (3) adenosine diphosphate -MA and arachidonic acid -MA; demonstrates thrombus strength due to be activated only through these specific receptors, because adenosine diphosphate and arachidonic acid stimulate platelet function through pathways other than thrombin, (4) ActF-MA; abciximab inhibits platelet function. Fibrinogen reaction occurs through reptilase, an enzyme found in the venom of snakes such as the Fer de lance; fibrinogen is converted to fibrin, and stabilization of the fibrin is facilitated by activated factor XIII and composes what has been called activator f. Fibrin formation is shown promptly as ActF-MA. This reflects fibrinogen activation via a pathway that does not involve thrombin and that fibrin intensity does not involve platelets. Thus, ActF-MA can be determined by the above thrombin-independent mechanism of fibrin formation, and we hypothesized that ActF-MA, which is not related to platelet function, correlates highly with blood fibrinogen concentration. Our results strongly support our hypothesis.

This study has four major limitations that should be addressed. First, it was a pilot study, and the number of cases was small. Based on our results, there are strong correlations between ActF-MA and fibrinogen concentration. Therefore, we believe our results are sufficient for a pilot study and reflect results in daily clinical practice. Second, viscoelastic devises (TEG6s, ROTEM, and Quantra) were not originally designed to directly measure fibrinogen concentration. These devices measure comprehensive coagulation functions, including coagulation factors, platelets, and fibrinogen; diagnose blood transfusions requirements such as fresh frozen plasma and platelet concentrate; and/or conduct post-transfusion assessment of coagulation functions.[5-7,13] The Clauss fibrinogen assay may be suitable for diagnosis of reliable fibrinogen concentration; however, we believe that this study demonstrates a method to rapidly evaluate low fibrinogen concentration. Third, as can be determined from measured values, the prediction equation is not absolute, and variations exist. Finally, blood samples were collected at intervals of 60 minutes or more. Therefore, the blood fibrinogen concentration was suspected to be completely different even in the same patient. Repeated measurements of the same patient taken in this study were affected primarily by external factors such as CPB time rather than internal factors. And the assumption of independence is acceptable.

In summary, our results show a significant relationship between ActF-MA and serum fibrinogen concentration. There have been no previous reports measuring low CPB fibrinogen concentration using PM. Low fibrinogen concentration can be estimated more quickly by ActF-MA than by traditional methods, and ActF-MA allows fast prediction of fibrinogen concentration and rapid transfusion preparation without waiting a long time for laboratory results in cardiac surgery. Further research is necessary to explore more useful coagulation management using the TEG6s.

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DISCLOSURE STATEMENT

None of the authors has any conflicts of interest to declare in relation to this work.

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