Validity of Thromboelastometry for Rapid Assessment of Fibrinogen Levels in Heparinized Samples During Cardiac Surgery: A Retrospective, Single-center, Observational Study

Hamish Mace, MBChB, FANZCA, DRANZCOG,* Nicholas Lightfoot, MBChB, FANZCA,*
Stuart McCluskey, MD, FRCPC, PhD,* Rita Selby, MBBS, FRCPC, MSc,† Debashis Roy, MD,‡
Tarik Timoumi, MD,§ and Keyvan Karkouti, MD, FRCPC, MSc*

Objective: **To assess the validity of fibrinogen assay of rotational thromboelastometry (FIBTEM)-derived estimates of fibrinogen in samples collected during cardiopulmonary bypass in cardiac surgical patients by comparison to Clauss method fibrinogen concentration.**

Design: **Retrospective observational study.**

Setting: **Single university hospital center.**

Participants: **Human participants.**

Interventions: **Retrospectively obtained laboratory assays including rotational thromboelastometry (ROTEM) and Clauss fibrinogen assay.**

Measurements and Main Results: **A retrospective review was performed of anesthesia records at a single university teaching hospital during a 1-year period. From paired samples taken near the end of cardiopulmonary bypass, fibrinogen concentrations (Clauss method) were compared with FIBTEM-derived measures of maximal clot firmness (MCF) and clot amplitude at 10 minutes (A10) using Spearman's rank correlation, linear regression, and receiver operating characteristic curve analysis. The study included 1,077 patients. Clauss fibrinogen was correlated strongly with FIBTEM amplitudes ($r = 0.78$ for MCF and A10; $p < 0.01$). The correlation was related inversely to hemoglobin concentration ($p < 0.01$). The area under the receiver operating characteristic curve was 0.95; the optimal FIBTEM A10 cutoff for diagnosis of a fibrinogen concentration of <1.5 g/L was ≤ 8 mm.**

Conclusions: **The FIBTEM was a valid point-of-care method for estimating the fibrinogen concentration during cardiopulmonary bypass and may be used for prediction of hypofibrinogenemia before separation from the extracorporeal circuit.**

KEY WORDS: fibrinogen, FIBTEM, ROTEM, cardiopulmonary bypass, cardiac anesthesia, Clauss method

COAGULOPATHY IS COMMON during surgical procedures involving cardiopulmonary bypass (CPB). Fibrinogen is an important component of clot formation and stability; thus, perioperative hypofibrinogenemia is associated with an increased risk for excessive hemorrhage and blood transfusion.[1–5]

There is increasing evidence that timely correction of hypofibrinogenemia after separation from CPB reduces hemorrhage and the need for transfusion.[6–8] To appropriately guide therapy after CPB, accurate fibrinogen assays that have short turnaround times and can be conducted during CPB (ie, are not influenced by heparin) are required.

Fibrinogen is measured conventionally in the laboratory on citrated plasma samples using the Clauss method, in which a high concentration of thrombin is added to diluted plasma with the resultant clotting time proportional to the fibrinogen concentration (determined from a calibration curve). However, as this is a plasma-based assay that cannot be performed at the bedside, it typically has a long latency.[9,10] Rotational thromboelastometry (ROTEM; Tem International GmbH, Munich, Germany) is a point-of-care (POC) assay that examines the viscoelastic properties of whole blood by dynamically measuring clot firmness during its formation and subsequent fibrinolysis (if present). Because it is a POC test conducted on whole blood, results are available within 15 minutes of sample collection. The fibrinogen assay of ROTEM (FIBTEM) specifically measures the contribution of fibrinogen to clot strength by largely eliminating the influence of platelets through the addition of cytochalasin D (a potent inhibitor of platelet function).[11] FIBTEM-derived maximal clot firmness (MCF) and clot firmness at 10 minutes (A10) have been shown to correlate well with the Clauss method in nonheparinized samples, and have been used successfully to guide therapy with fibrinogen concentrate in major bleeding, including cardiac surgery.[10,12–22] The utility of FIBTEM for estimating fibrinogen concentration in samples taken during CPB to allow for timely correction of hypofibrinogenemia after CPB, however, needs further investigation.

The objective of this retrospective study was to assess the validity of FIBTEM-derived estimates of fibrinogen concentration in samples collected during CPB in cardiac surgical patients by comparison to the gold standard Clauss method.

METHODS

This retrospective observational study was approved by the University Health Network Research Ethics Committee. The tests were conducted as a part of routine care, thus the requirement for informed consent was waived.

*From the *Department of Anesthesia and Pain Management, Toronto General Hospital, University Health Network, Toronto, Ont, †Department of Medicine, Laboratory Medicine Program, University Health Network, Toronto, Ont, ‡University of Toronto, Toronto, Ont; and §Department of Anesthesia, Université Paris-Diderot, Paris, France.*

This study was funded by an Innovation Grant from the Peter Munk Cardiac Centre at the Toronto General Hospital, University Health Network. Keyvan Karkouti is funded in part by a Merit Award from the Department of Anesthesia at the University of Toronto. K. Karkouti received research support from TEM, the makers of ROTEM, subsequent to completion of this project.

Address reprint requests to Hamish S. Mace, MD, Fiona Stanley Hospital, 102-118 Murdoch Drive, Murdoch WA 6150, Australia. E-mail: hamish.mace@health.wa.gov.au

1053-0770/2601-0001$36.00/0
http://dx.doi.org/10.1053/j.jvca.2015.04.030

Patient Selection

Patients who underwent cardiac surgery with CPB at the Toronto General Hospital during 2013 were included if simultaneous Clauss and ROTEM fibrinogen assays were performed during CPB. Repeat samples (eg, in the bleeding patient) were excluded from analysis, as were patients in whom POC testing was not performed (eg, out-of-hours procedures). These assays were conducted routinely as part of a POC-based blood management algorithm that was instituted at the hospital in January 2013. Toronto General Hospital is a university teaching hospital where a range of cardiac surgical procedures is performed on adult patients.

Patient Management

CPB practice during the study period was as follows. Heparin (400 IU/kg initial bolus with additional dosing as needed) was administered to achieve an activated clotting time of $\geq$480 seconds before initiation of CPB. If heparin resistance was suspected, the patient was treated with antithrombin or fresh frozen plasma. After weaning from CPB, protamine was administered at a ratio of 1 mg to 100 IU of the initial heparin dose and titrated thereafter to obtain an activated clotting time within 10% of baseline. The CPB circuits were phosphorylcholine coated (Sorin, Vancouver, Canada) and were primed with 10 g of mannitol and 1.8 L of lactated Ringer's with or without 25% albumin. CPB management included cooling to 34°C, targeted mean arterial pressure of 50 to 70 mmHg, and pump flow rates of 2.0-to-2.4 L/min/m^2. For myocardial protection, intermittent anterograde cold blood cardioplegia was used, with retrograde cardioplegia as required. In certain patients, hypothermic circulatory arrest was achieved with cooling to 25°C to 28°C with or without retrograde cerebral perfusion. During CPB, salvaged blood from pericardial suction was reinfused into the heparinized CPB circuit. Tranexamic acid (Pharmacia & UpJohn Inc, Mississauga, Canada) was administrated routinely (50-100 mg/kg) providing there were no absolute contraindications.[23]

Blood Collection

Blood was collected via a 10-mL syringe from the CPB circuit during the rewarming phase (temp $\geq$36°C) and transferred into two 2.7-mL sodium citrate tubes containing 0.3 mL of 3.2% sodium citrate (BD, Franklin Lakes, NJ, USA). One sample was delivered to the core laboratory for estimation of fibrinogen concentration by the Clauss method (STAR Evolution [Stago, Paris, France]),[24] whereas the second was used for the ROTEM (TEM innovations, GmbH, Munich, Germany) assay in a validated POC laboratory situated within the surgical complex. This was performed by one of two POC technicians under the supervision of staff anesthesiologists.

The extrinsic thromboelastometry (EXTEM) and FIBTEM assays of ROTEM were conducted at 37°C. For the FIBTEM analysis, 20 μL of cytochalasin D and calcium chloride (fib-tem reagent); with recombinant tissue factor, heparinase, and phospholipids (r ex-tem reagent) were transferred to the cup followed by 300 μL of citrated blood using an automated sequence. For the EXTEM assay, the same reagents were used, but without cytochalasin D. The blood and reagents were mixed via automated signaling in the pipette and the cup was set into the pin to start the tests. Parameters obtained via the ROTEM assays included A10 (EXTEM and FIBTEM), MCF (EXTEM and FIBTEM), and clotting time (CT).

Data Collection and Statistical Analyses

Data were collected retrospectively from patients' records and existing clinical databases. Statistical analysis was conducted with SAS version 9.3 (SAS Institute, Cary, NC, USA). Values are presented as median (interquartile range) unless otherwise specified. The Shapiro Wilk test was performed for normality. Thereafter, Spearman's rank correlation was used to measure interactions of interest. As hemoglobin concentration has been shown to influence the results of the FIBTEM assay,[25,26] the correlations were recalculated after categorizing patients into quartiles based on their hemoglobin concentration at the time of fibrinogen measurement. A Fisher r-z calculation compared correlation coefficients between groups.

The relationship between the fibrinogen measurements was further assessed by 2×2 contingency tables of various FIBTEM A10 cutoffs against a Clauss-derived fibrinogen concentration threshold of $<$1.5 g/L. This value was chosen to reflect a common transfusion trigger for fibrinogen replacement in this institution, and is supported by international guidelines.[27,28] A receiver operating characteristic (ROC) curve was derived using logistic regression modeling, which included FIBTEM A10 as a continuous independent variable and the Clauss-derived fibrinogen concentration as a categorical dependent variable ($<$1.5 g/L).

RESULTS

The study included 1,077 consecutive patients who underwent cardiac surgery with CPB during the study period and had both fibrinogen assays conducted during CPB. The total number of procedures with CBD during the study period was 1,265, with 188 patients excluded (all emergency cases performed out of hours when POC analysis was unavailable). Patient characteristics and laboratory values are shown in Table 1. Mean (SD) tranexamic acid dose was 4.6 g ($\pm$1.9 g).

The correlation coefficient (r) between FIBTEM A10 and FIBTEM MCF was 0.98 ($p < 0.01$). FIBTEM measures of clot firmness were correlated strongly with Clauss method fibrinogen ($r = 0.78$; Table 2). The correlation between FIBTEM A10 and the Clauss method in the entire sample is represented graphically in Figure 1. After categorization into quartiles, the correlation between FIBTEM A10 and the Clauss method was inversely related to the hemoglobin concentration ($p <0.01$; Table 3).

The area under the ROC curve for FIBTEM A10 prediction of a fibrinogen of $<$1.5 g/L was 0.95 (95% confidence interval, 0.92-0.97; Fig. 2). A FIBTEM A10 cutoff of $\leq$8 mm had the best balance of sensitivity and specificity for predicting fibrinogen of $<$1.5 g/L (Table 4).

DISCUSSION

In this retrospective study, the authors found that FIBTEM measurements of clot stability strongly were correlated ($r = 0.78$) with fibrinogen concentration as measured by the Clauss method on samples taken during the rewarming phase of CPB. Based on the analysis of the ROC curve, a FIBTEM A10