

TEG® Functional Fibrinogen Analysis May Overestimate Fibrinogen Levels

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Fibrinogen is of crucial importance in patients with ongoing bleeding. In this study, we compared fibrinogen concentration measured by thrombelastography (TEG®) with fibrinogen plasma concentration determined by Clauss. Sixty-three surgical patients and 38 healthy controls were included. For the whole group (patients and controls, $n = 101$), TEG® functional fibrinogen was on average 1.0 g/L higher than the plasma fibrinogen concentration (3.5 vs 2.5 g/L, 95% confidence interval for difference 0.8 to 1.2 g/L, $P < 0.0001$). Similar patterns were observed when patients and healthy controls were analysed separately. The fibrinogen level may be overestimated when assessed using TEG® compared with the fibrinogen plasma concentration measured by the conventional method. (Anesth Analg 2014;118:933–5)

Sufficient fibrinogen levels are important for adequate hemostasis in trauma and surgical patients with ongoing bleeding.¹ It has been shown that low preoperative fibrinogen plasma concentration is associated with increased risk of perioperative bleeding and transfusion requirements,^{2,3} and that fibrinogen concentrate treatment in patients undergoing thoracic surgery or cystectomy reduces the use of blood transfusions.^{4–6} Hence, accurate and timely measurement of fibrinogen levels is important. As such, the possibility to rapidly measure functional fibrinogen using thrombelastography (TEG®) is a potentially important new tool. However, because functional fibrinogen measures are different than traditional fibrinogen measures, it seems pertinent to investigate how the 2 measures compare.

Measurement of functional fibrinogen level using TEG® was introduced at the Department of Anesthesiology and Intensive Care at Karolinska University Hospital in 2011. Soon doubts were raised whether high TEG® functional fibrinogen levels, in patients with ongoing bleeding, were accurate. Since we have previously noted poor agreement between conventional coagulation tests and TEG® results,⁷ we compared TEG® functional fibrinogen levels with plasma fibrinogen concentration measures, using standard methods in a series of surgical patients and healthy control subjects.

METHODS

The study was approved by the local scientific ethics committee. All the healthy blood donors signed informed consent, but patient consent was waived because there was no intervention related to the coagulation testing.

Between March 2011 and September 2012, a series of patients with ongoing bleeding were prospectively included in our study at Karolinska University Hospital, Stockholm, Sweden. The inclusion criteria were trauma or surgery with ongoing bleeding. The decision to include the patient was based on the type of surgery or trauma only, and not on the bleeding history or previous laboratory tests. To ensure the external validity of the study, patients were representative of normal surgical patients, with different diagnoses and variation in age and sex. In trauma patients, samples were drawn on admission, when patients were bleeding, and in the elective surgical group of patients, samples were obtained when the patient was bleeding, and replacement of fibrinogen might be considered. None of the patients received colloid fluids. In addition, a number of healthy blood donors with a previous donation >4 weeks earlier served as controls and were included between June 2012 and December 2012. There were no explicit exclusion criteria. No clinical end points were assessed. Only 1 sample was taken in each patient and control.

Laboratory Analyses

Venous samples from patients and controls were collected in citrated tubes for TEG® functional fibrinogen and fibrinogen plasma concentration analyses. Analysis of the fibrinogen plasma concentration (The Clauss method; Dade Thrombin Reagent/Siemens Healthcare Diagnostics®, Marburg, Germany) was performed with the Sysmex CS 2100i® (Sysmex Corporation, Japan), and the normal range was 2.0 to 4.2 g/L. Internal and external controls of the method were done regularly, with a coefficient of variation of 6%.

For TEG® assays (TEG® 5000 Thrombelastograph® Hemostasis system, Analytical Software Version 3, Hemoscope Corp®, IL) recalcified citrated whole blood was processed according to the manufacturer's instruction. Functional fibrinogen, with a normal range of 2.0 to 4.5 g/L, was calculated automatically by the TEG® system based on the maximal amplitude (MA_{ff}), and the result was presented

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on the computer screen. Calibration and method errors for functional fibrinogen in TEG® were excluded through repeated interaction with the manufacturer.

Both TEG® and fibrinogen samples were analysed concurrently, approximately 30 minutes after sampling.

Statistical Analyses

Mean differences between TEG® functional fibrinogen and standard measures of plasma fibrinogen concentration were assessed using paired *t* tests. In vitro models have suggested that a fibrinogen level above 2 to 2.5 g/L is necessary for optimal hemostasis,⁸ so the percentage of the study population with fibrinogen concentration below 2 g/L was also examined using the 2 methods. As hemoglobin (Hb) concentration has been reported to influence results of thrombelastographic systems,^{9,10} we stratified the analyses by whether or not the patients were anemic (Hb <120 g/L). We also tested whether such effect could be seen by fitting the difference between the results of the 2 assays in a linear regression model with Hb concentration as the independent variable. As a graphical comparison of the 2 different measures, scatter plots and Bland-Altman plots were used.¹¹ SAS statistical analysis software, version 9.3 was used (SAS Institute, Cary, NC). *P* values < 0.05 were considered statistically significant.

RESULTS

Sixty-three surgical patients were included, of whom 20 were women (32%). The mean age (SD) was 51 ± 20 years. Cancer surgery was the most common type of operation (*n* = 13), followed by traffic accidents (*n* = 11) and stab wounds (*n* = 10). In addition, we also analysed samples from 38 healthy blood donors, of whom 18 were women (47%). The mean age (SD) of healthy blood donors was 44 ± 15 years. The hematocrit for patients was 0.34 ± 0.07 and controls 0.42 ± 0.03.

For the whole group (patients and controls, *n* = 101), TEG® functional fibrinogen was on average 1.0 g/L higher than the plasma fibrinogen concentration (3.5 vs 2.5 g/L, 95% confidence interval [CI] for difference 0.8–1.2 g/L,

P < 0.0001). Removal of 2 outliers with very high TEG® functional fibrinogen values did not affect the differences between the methods (data not shown). Similar patterns were observed when patients and healthy controls were analysed separately, with consistently higher estimated levels of fibrinogen in functional fibrinogen in TEG® than the fibrinogen plasma concentration. The mean differences were 1.0 g/L (3.3 vs 2.3 g/L, 95% CI for difference 0.7–1.4 g/L) in surgical patients, and 1.0 g/L (3.7 vs 2.7 g/L, 95% for difference CI, 0.8–1.1 g/L) in healthy blood donors. The corresponding MA_{ff} was 18.3 ± 7.5 mm in patients and 20.1 ± 3.2 mm in controls. The correlation between level of functional fibrinogen (g/L) and MA_{ff} (mm) was 100%. The mean differences did not differ significantly when we separated anemic patients from nonanemic patients (mean differences 1.19 and 0.87 g/L, respectively; *P* = 0.20). When tested in a linear regression model, the difference between the 2 Clauss and TEG® methods was not significantly affected by patient hematocrit (*P* = 0.70). Of 101 samples, 28 (28%) had a plasma fibrinogen level below 2 g/L, according to the standard method, but only 4/101 (4%) were detected when functional fibrinogen in TEG® was used.

Figure 1A presents a scatter plot of TEG® functional fibrinogen and standard fibrinogen values, and Figure 1B shows a Bland-Altman plot. While the former shows a near-linear association between the 2 measures, although with clear outliers, the latter confirmed the systematic overestimation of TEG® functional fibrinogen. The discrepancy between measures did not seem to increase at higher values.

DISCUSSION

Overall in this study, the fibrinogen level was on average overestimated by 1.0 g/L using TEG® functional fibrinogen compared with the fibrinogen plasma concentration, with similar findings in patients and healthy blood donors. It is important to note that 86% of the fibrinogen levels below 2 g/L⁸ were missed when analyses were done using functional fibrinogen instead of conventional methods. The

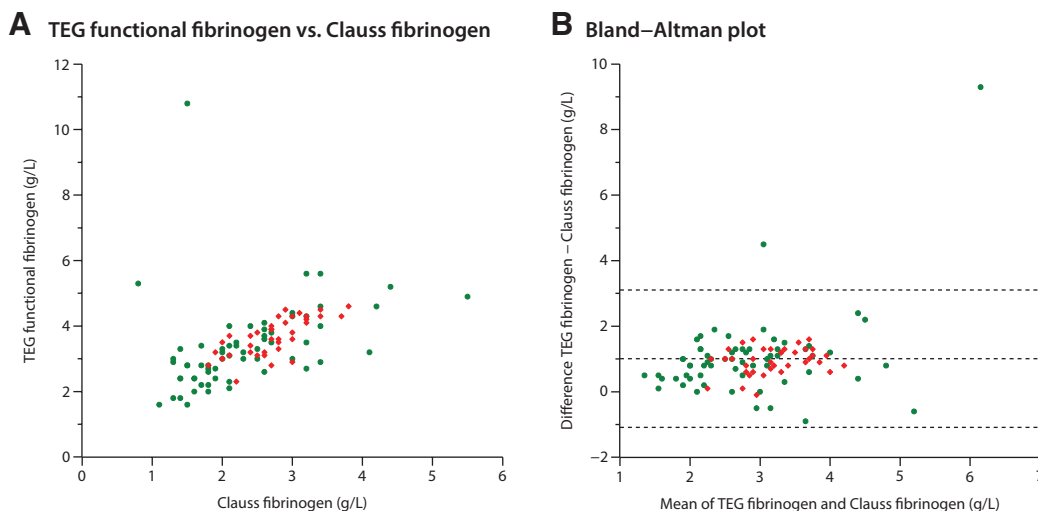


Figure 1. A, Scatterplot of fibrinogen plasma concentration (g/L) vs TEG® functional fibrinogen (g/L) for surgical patients (·) and healthy controls (♦). B, Bland-Altman plots showing the agreement between the 2 methods, visualised as a plot of the difference between measures and their mean for surgical patients (·) and healthy controls (♦). The middle dashed line shows the mean difference (1.0 g/L) with the top and bottom lines showing the mean ± 1.96.

heterogeneous group of patients allowed us to compare the 2 methods across a wide range of fibrinogen levels that ensured the external validity of this investigation.

The results of the study could be compared with those of a study published in *Anesthesia & Analgesia* in 2012 by Solomon et al.,¹² where TEG® produced higher values for fibrin-based clot than ROTEM®. MA and maximum clot firmness (MCF) were compared in TEG® and ROTEM® with the 2 different reagents for fibrin-based clot (fibtan and functional fibrinogen). For instance, MCF (Fibtan) was only 72% of MCF (functional fibrinogen) in nondiluted blood samples, when the analyses were performed on ROTEM®. This corresponds almost exactly to the calculated difference in our study between plasma fibrinogen and functional fibrinogen in TEG®.¹²

There may be various reasons for our results. For example, it has been demonstrated that low hematocrit affects clot strength with similar fibrinogen concentrations,^{9,10} and that changes in FXIII significantly affect clot strength¹³ in thrombelastographic systems. Platelets contain significant FXIII activity, and the TEG® assay only prevents platelet binding to fibrin strands. The assay does not prevent degranulation and release of FXIII. So especially in trauma patients, the hematocrit may be low and FXIII activity released, resulting in greater clot strength than that associated with normal patients. Furthermore, it is possible that platelet inhibition may not be complete with abciximab, which is the platelet inhibitor used in the functional fibrinogen assay.¹⁴ However, we did not see any significant differences in our study, when we separated anemic patients from nonanemic patients.

There is a possibility that raw MA_{ff} values (mm) in TEG® could be used instead of functional fibrinogen (g/L) to observe changes in an individual patient, but it is unlikely that the MA_{ff} values would correspond to actual plasma fibrinogen concentration.

Since it has been shown in previous studies that plasma fibrinogen concentration may predict the risk for perioperative bleeding,^{2,3} the possibility of missing a pathological value, when only TEG® is used, may have negative consequences for patients with increased bleeding risk. ■■

DISCLOSURES

Name: Anna Ågren, MD, PhD.

Contribution: This author helped design the study, collected the data, prepared the manuscript, and attests the integrity of the original study data and the analysis reported in this manuscript.

Attestation: Anna Ågren approved the final manuscript and attests the integrity of the original study data and the analysis reported in this manuscript and is the archival author.

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