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Fibrinogen levels measured by the dry hematology method are lower than those measured by the Clauss method under a high concentration of heparin

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ABSTRACT

The activity of fibrinogen has been reported to decrease soon after the onset of major bleeding and to be an important determinant of the final extent of bleeding and postoperative outcome. A device that measures the perioperative fibrinogen level using the dry hematology (DH) method has recently become available. The aim of this study was to compare perioperative fibrinogen levels measured by the DH method with those measured by the conventional Clauss method and to assess the effects of heparin on these measurements. The study included 206 samples from 36 patients undergoing major surgery who received high-dose heparin (HH group, 23 samples), low-dose heparin (LH group, 57 samples), or no heparin (C group, 126 control samples). Each sample was measured using the DH and Clauss methods. After excluding samples outside the effective measurement range, the three study groups (HH group, n=23; LH group, n=49; C group, n=115) were compared. The mean fibrinogen level measured by the DH method in the HH group (87.9 ± 3.1%) was significantly lower than that measured by the Clauss method. There were no significant differences between the fibrinogen measurements obtained by the two methods between the LH and C groups. In patients on high-dose heparin, the mean fibrinogen level measured by the DH method was significantly lower than that measured by the Clauss method. When hemorrhage requires emergency treatment, a method that can measure the fibrinogen level rapidly is important. The DH method may be useful for decision-making with regard to perioperative coagulation factor replacement.

Keywords: fibrinogen, dry hematology method, heparin

INTRODUCTION

The activity of fibrinogen has been reported to become inadequate soon after the onset of hemorrhage.[1-3] An association has also been found between fibrinogen levels in patients undergoing

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cardiac surgery who suffer massive postoperative hemorrhage and the amount of bleeding, blood transfusion needs, and the prognosis.[4-8] Therefore, the fibrinogen level may be an important index of coagulation in patients who have suffered massive bleeding. In view of the rapid reduction in the fibrinogen level during hemorrhage, a rapid measurement method is crucial. However, blood collection, transport, and diagnosis takes more than 30 minutes using the conventional Clauss and prothrombin time (PT)-derived methods. Heparin is often administered perioperatively for anticoagulation. Reports on the use of heparin before and after cardiopulmonary bypass[9] and on use of the recently marketed CG02N whole blood coagulation analyzer for blood gas analysis in heparinized blood[10] indicate that low-dose heparin has no effect on the results of measurements. However, there is no report on the effects of high-dose heparin on fibrinogen levels in patients undergoing cardiopulmonary bypass surgery. In general, the effects of anticoagulants on the fibrinogen level are masked by anticoagulant inhibitors and dilution of samples; the effect of anticoagulant inhibitors and dilution is inadequate, and the fibrinogen measurements obtained in patients who have received heparin may vary depending on the measurement method used.

The aims of this study were to compare the fibrinogen measurements obtained in patients undergoing surgery by the rapid dry hematology (DH) method using the recently marketed CG02N whole blood coagulation analyzer (A&T Corporation, Kanagawa, Japan) with those obtained by the standard Clauss method used in our central laboratory and to assess the effects of perioperatively administered heparin on these measurements.

MATERIALS AND METHODS

The study was approved by the Nagoya University Hospital ethics committee (approval number 2014-0026) and included patients who were scheduled for surgery and had a high risk of hemorrhage. Blood samples were collected from a volunteer on a single occasion to measure the effect of addition of heparin on the fibrinogen measurements obtained by the two methods. Written informed consent was obtained from all patients who participated in the study. The target number of samples was 200.

Methods

Heparin were added to the samples obtained from the volunteer and the fibrinogen levels were measured using the DH method and the Clauss method. The heparin concentrations used were 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 IU/ml.

Samples for measurements obtained using the DH method were collected from patients during routine blood sampling and the results were compared with those obtained by the Clauss method. The DH method is suitable for use at the bedside. The cartridge-type fibrinogen reagent used in the device contains lyophilized bovine plasma thrombin and magnetic particles. When the reagent cartridge is placed in the device and samples are added, the components in the reaction cell dissolve and the magnetic particles start to move. When an intermittent vertical magnetic field is applied to the magnetic particles by turning on and off the horizontal magnetic field of a permanent magnet and an electromagnet, the magnetic particles in the reaction cell start to move immediately after dissolution of the components. The analytic device performs measurements by optically monitoring the reduction of signals from the magnetic particles as a result of progression of the coagulation reactions. Using the Clauss method, the time until fibrinogen in the plasma is converted to fibrin by the thrombin reagent is calculated from the relative ratio to the value at baseline. The time measurement is recorded as the time when the coagulation reaction curve reaches the absorbance at which almost no change in the coagulation reaction is observed. The

fibrinogen measurements using the DH method were made with 5-fold and 10-fold dilution solutions (A&T Corporation). The samples were collected from patients who received high-dose heparin (HH, body surface area × 9000 IU) or low-dose heparin (LH, ≥1000 IU) within four hours of blood collection or no heparin (control, C). For the whole blood samples, the required correction of hematocrit was made using the following formula: quantitative value = measured value × (dilution ratio/15) × (100/[100 – hematocrit × 0.9]). For measurement in the central laboratory, the CS-5100 system and Thrombocheck Fib (L) (Sysmex Corp., Kobe, Japan) were used as the device and reagent, respectively. A 3.2% citric acid blood collection tube (Venoject II: Terumo Corp., Tokyo, Japan) was used for both the DH and Clauss methods.

Statistical analysis

The ratio of the value measured using the DH method to that measured using the Clauss method is expressed as the mean ± standard error of the mean. The paired t-test and Bland-Altman analysis were used to compare the results obtained using the DH method with those obtained using the Clauss method; r-values and p-values were obtained by correlation analysis. All statistical analyses were performed using IBM SPSS version 24 software (SPSS IBM Japan Inc., Tokyo, Japan). A p-value <0.05 was considered statistically significant.

RESULTS

In the sample obtained from the volunteer, heparin concentrations up to 10 IU/ml had no effect on the fibrinogen level measured by the Clauss method, but a heparin concentration of 1 IU/ml tended to give a lower value by the DH method (Figure 1).

Two hundred and six samples were collected from 36 patients (20 men, 16 women; mean age 59.8 years) undergoing cardiac surgery (n=21), cardiovascular surgery (n=11), liver grafting surgery (n=3), or gastrointestinal surgery (n=1); 23, 57, and 126 samples were collected from the HH, LH, and C groups, respectively. One sample that was below the sensitivity threshold (<25 mg/dl), was excluded, leaving 205 samples for inclusion in the analysis.

The linear correlation between the Clauss method and the DH method was lost at a

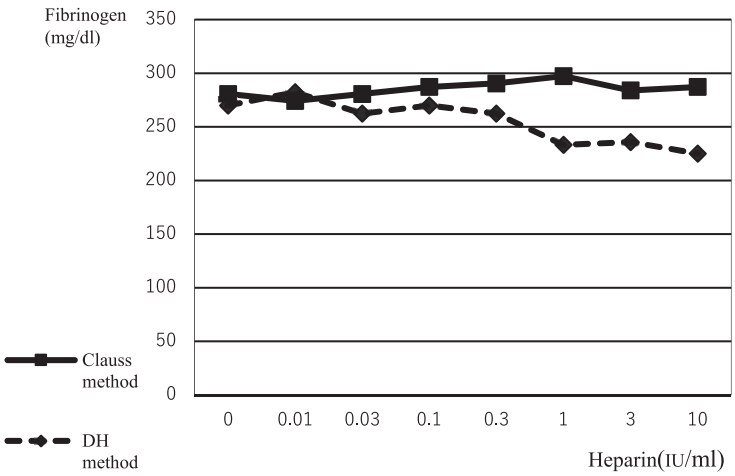

Fig. 1 Effect of addition of heparin in a sample from a volunteer.

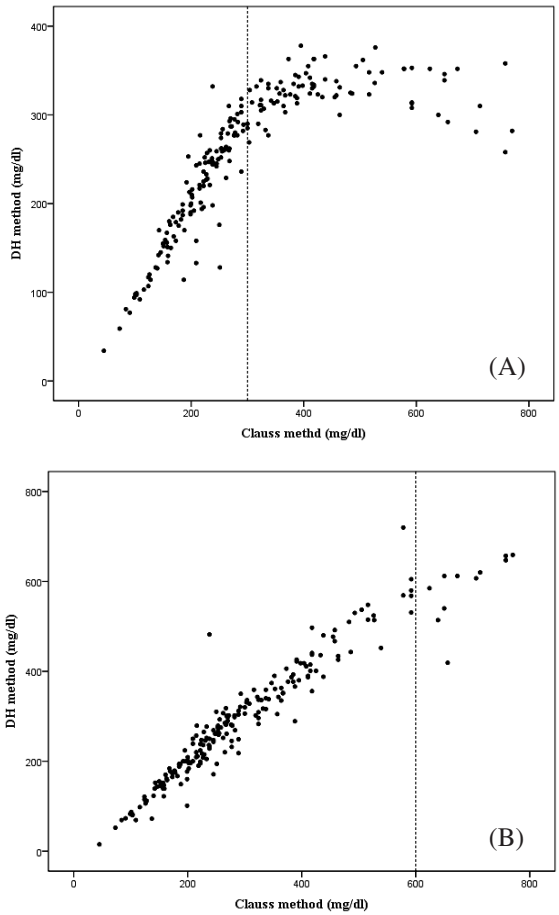

Fig. 2 Correlation of fibrinogen levels (g/l) in solutions diluted by (A) 5-fold and (B) 10-fold when measured by the Clauss method and the dry hematology method.

concentration of ≥300 mg/dl with the 5-fold dilution solution (Figure 2A) and at ≥600 mg/dl with the 10-fold dilution solution in all samples (Figure 2B). The regression equation for the correlation with a 5-fold dilution (<300 mg/dl) was y = 1.0514 – 10.223 (r=0.92152) and with a 10-fold dilution (<600 mg/dl) was y = 0.9139x + 21.407 (r=0.957). Therefore, a subsequent test was performed using the 10-fold dilution solution. For the Clauss method, samples with a concentration of 50 to 500 mg/dl were included in the analysis. After excluding samples outside the effective measurement range, comparisons were made between 23 samples in the HH group, 49 in the LH group, and 115 in the C group.

There was a strong positive correlation between the two methods in group C (r=0.954, p<0.001). The regression equation for the correlation was y = 0.985x + 8.300 (Figure 3A). Furthermore, the mean for the DH method was 100.4 ± 1.1% of that for the Clauss method; the difference was not statistically significant (p=0.65). In the Bland-Altman analysis, the bias was –1.978 mg/dl (95% confidence interval –32.508, 28.552; Figure 4A).

In the patients on continuous renal replacement therapy or extracorporeal membrane oxygenation who received low-dose heparin. A strong positive correlation between the two methods was

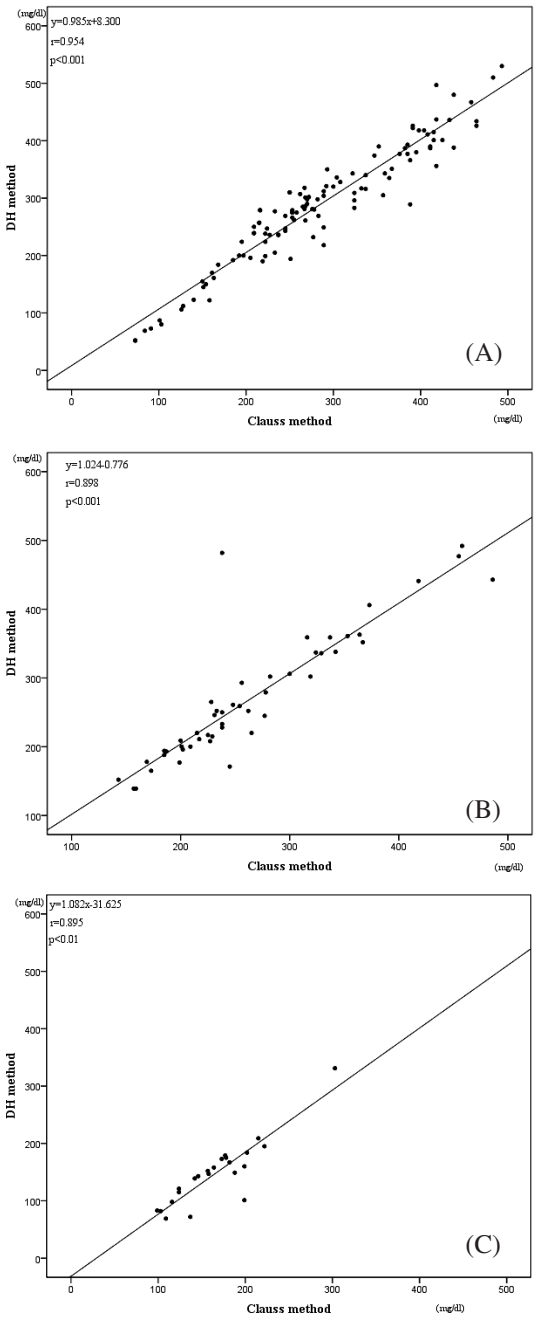

Fig. 3 Correlation between fibrinogen levels (g/l) obtained by the Clauss method and those obtained by the dry hematology method in patients undergoing major surgery.

Fig. 3A: Control group.

Fig. 3B: Low-dose heparin group.

Fig. 3C: High-dose heparin group.

found in the LH group (r=0.898, p<0.001). The regression equation for the correlation was y = 1.024x – 0.776 (Figure 3B). The mean for the DH method was 102.0 ± 2.4% of that for the Clauss method, and the difference was not statistically significant (p=0.42). In the Bland-Altman analysis, the bias was 2.826 mg/dl (95% confidence interval –38.383, 44.036; Figure 4B).

There was also a strong correlation in the HH group (r=0.895, p<0.001). The regression equation for the correlation was y = 1.082x – 31.625 (Figure 3C). In the DH group, the mean fibrinogen level obtained using the DH method (87.9 ± 3.1%) was significantly lower than that for the Clauss method (147.913 ± 11.834 mg/dl vs 165.957 ± 9.794 mg/dl) (p<0.001). In the Bland-Altman analysis, the bias was –9.022 mg/dl (95% confidence interval –34.610, 16.567; Figure 4C).

DISCUSSION

The CG02N system measures fibrinogen levels in whole blood using the DH method and is suitable for use at the bedside. Rapid measurement of the fibrinogen level is crucial in the event of massive bleeding, and the CG02N system has the advantages of not involving transport to a test room or centrifugation.

Major conventional measurement methods, e.g., the Clauss and PT-derived methods, use a reagent solution to measure the fibrinogen levels in plasma samples. Therefore, the measurements usually require centrifugation in the central laboratory. Depending on the institution, conventional measurements require 30–60 minutes for collection of blood, transport to the laboratory, centrifugation, and diagnosis, whereas the DH method allows measurements of fibrinogen in whole blood to be made at the bedside. Measurement of 100-mg/dl and 50-mg/dl samples takes <2 minutes and <5 minutes, respectively. Thus, the DH method may be very useful in surgical, intensive care, and emergency settings that require a rapid measurement result.

In this study, the DH method and the Clauss method were used to measure the perioperative fibrinogen level in 206 samples from patients, including some with acquired hypofibrinogenemia who had received high-dose heparin, low-dose heparin, or artificial colloid solution perioperatively. In the group of patients who received high-dose heparin, there was a significant positive correlation between measurements obtained using the DH method and those obtained using the Clauss method, and the decrease in fibrinogen levels was not marked. However, caution is required when using high-dose heparin.

In Japan, the cost of fibrinogen concentrate is covered by insurance only for patients with congenital hypofibrinogenemia, and the importance of rapid measurement and early administration of fibrinogen, for example, in obstetric patients with disseminated intravascular coagulation and massive hemorrhage, has been widely recognized. Moreover, many institutions allow off-label use of fibrinogen with the approval of an appropriate ethics committee. A rapid measurement system is particularly important for effective treatment of hypofibrinogenemia.

The reliability of low-concentration measurements in our study may be low because of the small number of samples with fibrinogen levels ≤100 mg/dl. However, there have been many studies in surgical and intensive care settings in which a fibrinogen level of 1 to 150 mg/dl was used as the criterion for fibrinogen replacement therapy.[13-17] Therefore, it should be straightforward to decide on fibrinogen replacement in the event of a sample with a fibrinogen level ≤100 mg/dl.[18]

In one study, there was a discrepancy between the measurements obtained by the Clauss method and those obtained by the PT-derived method because of an increase in the concentration of fibrin degradation products (FDP).[19] Although the authors of that study suspected that FDP inhibited polymerization of fibrin, the cause of the discrepancy was unknown. Our present

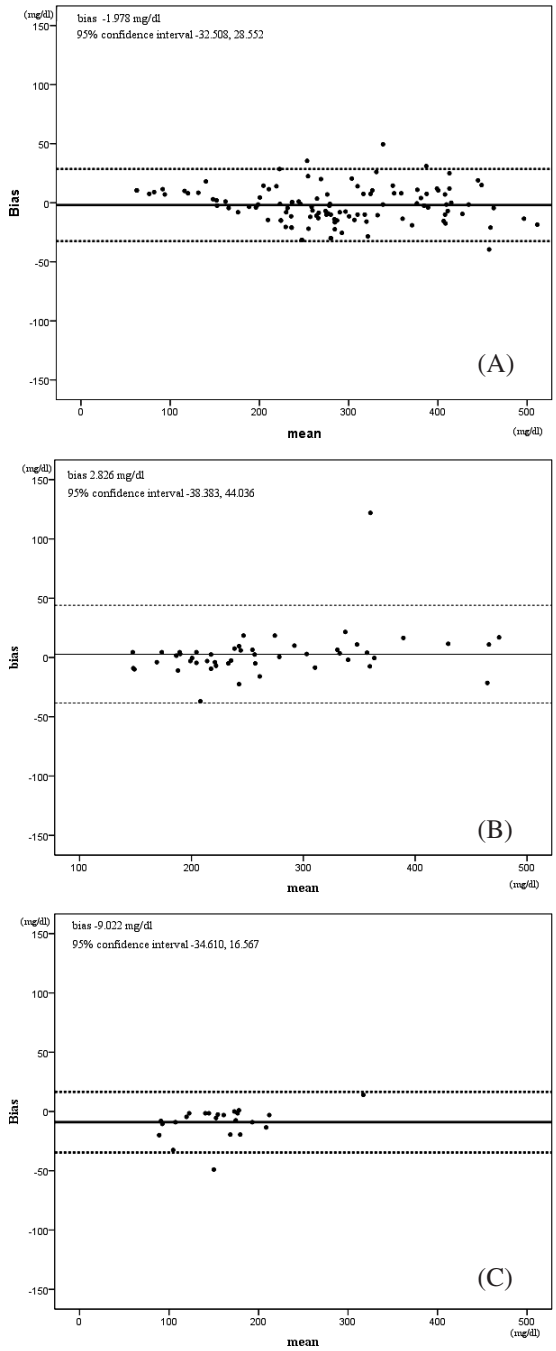

Fig. 4 Results of Bland-Altman analysis of fibrinogen levels (g/l) measured perioperatively by the Clauss method and by the dry hematology method in patients undergoing major surgery. (A) Control group. (B) Low-dose heparin group. (C) High-dose heparin group.

study did not investigate the effects of FDP on the fibrinogen level, so it is unknown whether the significantly lower fibrinogen levels measured using the Clauss method when compared with those measured using the DH method in the samples from patients undergoing cardiopulmonary bypass reflect an increase in FDP.

Artificial colloid solution may affect the coagulation system. This study did not examine the amount of artificial colloid solution administered. However, there has been an in vitro study in which fibrinogen levels measured by the DH method were not affected when citrated whole blood samples were diluted with 6% hydroxyethyl starch to achieve 75% volume replacement.[9]

When using the DH method, the samples are diluted manually, so there is a risk of error. In the present study, all the measurements were made by the same experienced examiner; however, the risk of error could be higher when measurements are made by examiners with less clinical experience.

This study compared fibrinogen measurements obtained by the DH method and the Clauss method. There was a significant positive correlation between the two methods in the samples obtained from patients who had received low-dose heparin and those who had not received heparin; the difference between the groups was not significant. However, although there was a positive correlation between these two groups in samples obtained during cardiopulmonary bypass, the mean fibrinogen level measured using the DH method was significantly lower (87.9 ± 3.1%) than that measured using the Clauss method. Therefore, caution is required when using the DH method because the fibrinogen level may be reduced by heparin.

Rapid measurement of the fibrinogen level is particularly important in the emergency treatment of massive bleeding in women with disseminated intravascular coagulation in the obstetric setting. The time needed to perform the measurement using the DH method is much shorter than that required when using the Clauss method, Furthermore, the DH method seems to be useful when deciding on coagulation factor replacement therapy in patients with perioperative bleeding events.

CONFLICTS OF INTEREST

This clinical study was conducted in collaboration with A&T Corporation.

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