Effect of haematocrit on fibrin-based clot firmness in the FIBTEM test

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Background. Point-of-care thromboelastometry (ROTEM®) can be used to assess coagulation in whole blood. In the ROTEM® FIBTEM test, cytochalasin D eliminates the contribution of platelets to the whole blood clot; hence, only the remaining elements, including fibrinogen/fibrin, red blood cells and factor XIII, contribute to clot strength. We investigated the relationships between FIBTEM maximum clot firmness (MCF), whole blood fibrinogen concentration and plasma fibrinogen concentration to determine the impact of haematocrit on these parameters during cardiac surgery.

Materials and methods. The relationships between FIBTEM MCF and both whole blood fibrinogen concentration and plasma fibrinogen concentration (Clauss assay) were evaluated pre-operatively and after cardiopulmonary bypass/protamine administration in haematocrit-based subgroups.

Results. The study included 157 patients. The correlation coefficient rho between FIBTEM MCF and plasma fibrinogen concentration was 0.68 at baseline and 0.70 after protamine, while that between FIBTEM MCF and whole blood fibrinogen concentration was 0.74 at baseline and 0.72 after protamine (all $P < 0.001$). In subgroup analyses based on haematocrit levels, pre-operative FIBTEM MCF and whole blood fibrinogen concentration were both significantly higher ($P < 0.05$) for the lowest haematocrit subgroup, but plasma fibrinogen concentration was similar in all groups. After protamine, no significant differences were observed between the lowest haematocrit group and the other groups for any of the three parameters.

Conclusions. The effect of haematocrit on blood clotting is not reflected by plasma fibrinogen concentration, in contrast to FIBTEM MCF which incorporates the contribution of haematocrit to whole blood clot firmness. This effect does, however, appear to be negligible in haemodiluted patients.

Keywords: cardiac surgery, fibrinogen, FIBTEM, haematocrit, thromboelastometry.

Introduction

Thromboelastometry (ROTEM®, Tem International GmbH, Munich, Germany) has proven useful for the rapid assessment of haemostasis in cardiac surgery patients with coagulopathic bleeding, e.g., after cardiopulmonary bypass (CPB)[1-5]. The method can be used to measure the quality of the fibrin-based clot via the FIBTEM test, which uses cytochalasin D to eliminate the contribution of platelets to clot strength[6]. Since this type of clot results mainly from the conversion of fibrinogen to fibrin, a degree of correlation between FIBTEM clot firmness and plasma fibrinogen concentration exists and has been described in different clinical settings, including cardiac surgery[1,7-9].

Importantly, cytochalasin D eliminates only the contribution of platelets to the formation of the clot; the remaining elements, including fibrinogen converted into fibrin, factor XIII (FXIII), red blood cells (RBC) and other cellular and plasma factors, all contribute to the strength of the whole blood clot. However, a recent study has shown that FXIII makes only a minimal contribution to clot formation[10]. Fibrinogen plays several key roles in haemostasis and has been previously identified as a primary haemostatic target. It is also the first factor to fall to critically low levels following major surgery. Thus, when FIBTEM is performed on whole blood, clot firmness is evaluated in relation to fibrinogen converted to fibrin plus the volume of RBC[11]. However, in samples with similar plasma fibrinogen concentrations and platelet counts, clot firmness was shown to be greater in anaemic whole blood samples than in control samples with a normal haematocrit, in both experimental and clinical

anaemia[11,12]. Therefore, for measurements of fibrinogen concentration in plasma, it follows that an adjustment should be made to the theoretical total volume in order to account for the absence of red cell mass (as indicated by the haematocrit value of the whole blood sample).

If RBC are a part of the whole blood coagulation measurement, monitoring fibrinogen replacement by whole blood tests such as FIBTEM would include the impact of haematocrit in settings such as acute bleeding, while the measurement of plasma fibrinogen concentration would be more adequate for use with congenital fibrinogen deficiencies, with stable haematocrit and non-acute bleeding. Furthermore, the plasma fibrinogen concentration threshold for fibrinogen replacement in the peri-operative setting may need to be re-considered if haematocrit affects whole blood clot strength and whole blood fibrinogen concentration but not plasma fibrinogen concentration. In this study we investigated the relationships between FIBTEM clot firmness, whole blood fibrinogen concentration, and plasma fibrinogen concentration pre-operatively and at the end of CPB (after protamine administration) in order to determine the impact of haematocrit on these parameters.

Materials and methods

Following Research Ethics Committee approval and informed consent from participants in the study, prospective data were collected from patients undergoing surgery with CPB at the Hannover Medical School, Hannover, Germany. The surgical procedures included coronary artery bypass grafting (CABG), reoperation for CABG, aorta and/or aortic valve surgery, and other cardiac interventions.

Prior to surgery, general anaesthesia was induced with etomidate, fentanyl and cisatracurium. During induction of anaesthesia, all patients received 500 mL of lactated Ringer's solution and 500 mL of gelatin polysuccinate. For maintenance of anaesthesia, sevoflurane was titrated to an end-tidal concentration of 1-2% until aortic cross-clamping on CPB. Propofol and fentanyl were administered continuously during CPB as an infusion and as a bolus, respectively. One million kallikrein-inhibiting units (KIU) of aprotinin were administered before CPB and the same dose was added to the CPB prime solution. Before weaning from CPB, reperfusion was performed for a period of time equal to around 40% of the aortic cross-clamping time. Heparin was neutralised with protamine sulphate (1 mg protamine/100 U of total heparin dose) immediately after weaning from extracorporeal circulation. Arterial blood gases were monitored according to α–stat and the haematocrit was maintained above 25% by RBC transfusion. At the end of the operation, patients were re-warmed to a bladder temperature of 36-37 °C when necessary and transported to the intensive care unit (ICU). Blood was collected into 3.8% citrate and ethylenediaminetetraacetic acid (both Sarstedt collection vials, Sarstedt, Germany) at baseline (before induction of anaesthesia) and at the end of CPB (after protamine administration). At each time point plasma fibrinogen concentration was measured using the Clauss method and whole blood fibrinogen concentration was calculated as plasma fibrinogen concentration × (100 − haematocrit)/100. Haematocrit was measured at baseline and after administration of protamine. Subgroups based on haematocrit levels were defined separately for samples at both time points to account for the decrease in haematocrit that typically occurs during cardiac surgery. Haematocrit subgroups were defined empirically, based upon haematocrit distribution of the total study population. This investigation had focused on the haematocrit values observed at the end of CPB, as they are relevant for RBC transfusion, together with haemostatic therapy in the course of elective cardiac surgery. Post-CPB, the groups were defined according to the following rationale: a haematocrit of 25% is used in a number of centres, including ours, as a maximum acceptable target on CPB (we aim to maintain a haematocrit of 23-25% on CPB); the limit of 28% is used as a target after CPB, including in the ICU; if the haematocrit is below this limit, RBC are administered; a haematocrit >30% is a potential indicator of stabilisation of the post-operative status in the ICU[4,13]. Pre-CPB groups were empirically defined to ensure that the groups were comparable in terms of numbers of patients and the range of haematocrit values covered.

Fibrinogen concentration was measured by a STA-R Evolution analyser (STAGO Diagnostica & Roche, Düsseldorf, Germany) using the Clauss method, and platelet count, haemoglobin and haematocrit were measured by the Sysmex XE-2100 (Roche Diagnostics, Mannheim, Germany).

Changes in the viscoelastic properties of whole blood clotting were evaluated using a ROTEM® device according to the manufacturer's instructions. In the FIBTEM test the contribution of platelets to whole blood coagulation was inhibited by cytochalasin D. All measurements were performed at 37 °C using 300 μL whole blood, which was re-calcified in the reaction cup with 20 μL star-TEM® reagent and activated with 20 μL ex-TEM® reagent containing recombinant tissue factor (both from Tem International GmbH, Munich, Germany). Maximum clot firmness (MCF; mm) was recorded at each time point.

Data distribution was evaluated graphically and, in the absence of a normal distribution, data were expressed as medians (with interquartile ranges [IQR]). Given the non-parametric distribution, Spearman's rho correlation

coefficient was determined between laboratory test results and ROTEM® variables. Subgroups were analysed using one-way analysis of variance with Bonferroni's correction. A P value <0.05 was considered statistically significant. Statistical analyses were performed using MedCalc Version 11.6.1.0 (MedCalc Software, Mariakerke, Belgium) and GraphPad Prism Version 5.04 (GraphPad Software Inc, La Jolla, United States of America).

Results

One-hundred and fifty-seven patients who underwent cardiac surgery were enrolled in the study. Ninety-three patients (59%) were male; the median age of all the patients was 66 years (IQR: 57, 73) and their median body mass index was 27 (IQR: 24, 30). Patients were on CPB for a median of 93 min (IQR: 72, 131).

In the analysis of baseline samples, a positive correlation was observed between FIBTEM MCF and plasma fibrinogen concentration (rho =0.68; P <0.001), and between FIBTEM MCF and whole blood fibrinogen concentration (rho =0.74; P <0.001) (Figure 1). In the subgroups created based on haematocrit level, FIBTEM MCF and whole blood fibrinogen concentration were significantly higher (P <0.05) in the subgroup with haematocrit <36% than in the subgroups with haematocrit 36-38.9%, 39-41.9% and >42% (Table I). The plasma fibrinogen concentrations were comparable across all four haematocrit subgroups at baseline, as were the platelet counts (Table I).

At the end of CPB, after protamine administration, the rho correlation coefficient for the relationship between FIBTEM MCF and plasma fibrinogen
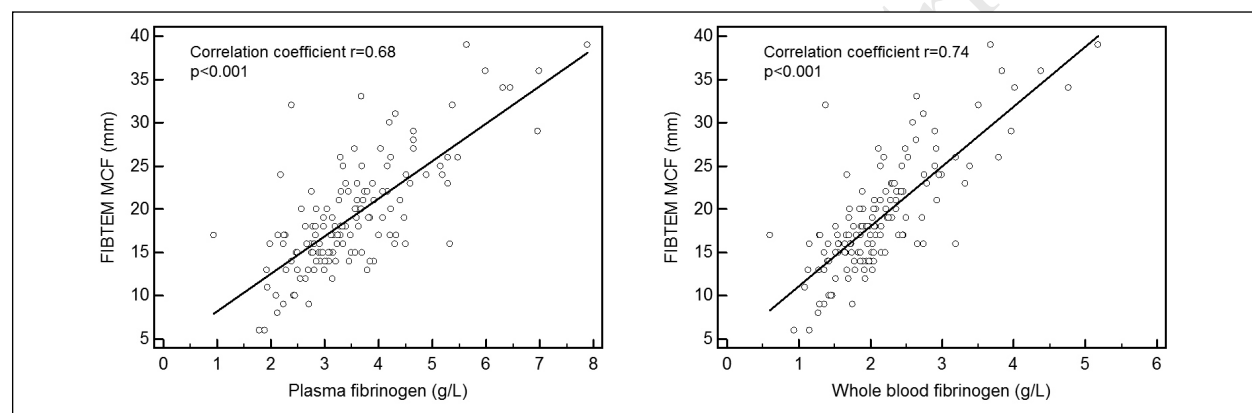
Figure 1 - Correlation between FIBTEM maximum clot firmness (MCF) and plasma or whole blood fibrinogen concentration at baseline (pre-operative).
Whole blood fibrinogen concentration was calculated as plasma fibrinogen level × (100 − haematocrit)/100.

Table I - Baseline coagulation data in cardiac surgery patients: comparison between haematocrit subgroups.

Parameters	All samples (n=157)	Samples with haematocrit <36% (n=34)	Samples with haematocrit 36-38.9% (n=41)	Samples with haematocrit 39-41.9% (n=36)	Samples with haematocrit >42% (n=46)
FIBTEM MCF (mm) (normal range, 9-25 mm)	18 (15, 22)	22 (19, 25)	17 (15, 21)*	16 (15, 20)*	17 (14, 21)*
Plasma fibrinogen concentration (g/L) (normal range, 2.0-4.5 g/L)	3.3 (2.8, 3.9)	3.4 (2.8, 4.4)	3.2 (2.9, 3.7)	3.3 (2.5, 3.9)	3.3 (2.8, 3.9)
Whole blood fibrinogen concentration (g/L)	2.0 (1.7, 2.4)	2.4 (1.9, 2.9)	2.0 (1.8, 2.3)*	2.0 (1.6, 2.3)*	1.8 (1.5, 2.1)*
Platelet count *1,000/μL (normal range, 150-450*1,000/μL)	218 (180, 261)	224 (170, 284)	231 (170, 287)	217 (182, 270)	202 (182, 242)
Haemoglobin (g/dL) (normal range, 13.5-17.5 g/dL)	13.6 (12.3, 14.6)	11.3 (10.4, 11.5)	12.9 (12.4, 13.3)	14 (13.7, 14.3)	15.2 (14.6, 15.7)
Haematocrit (%) (normal range, 42-50%)	39.2 (36.2, 42.7)	33.2 (30.4, 34.7)	37.6 (36.6, 38.3)	40.2 (39.7, 41.2)	44.2 (43.1, 45.3)

Legend Data presented as median (interquartile range).
*: P <0.05 compared to samples with haematocrit <36%; comparisons performed between all groups for FIBTEM MCF, plasma fibrinogen concentration, whole blood fibrinogen concentration, and platelet count.
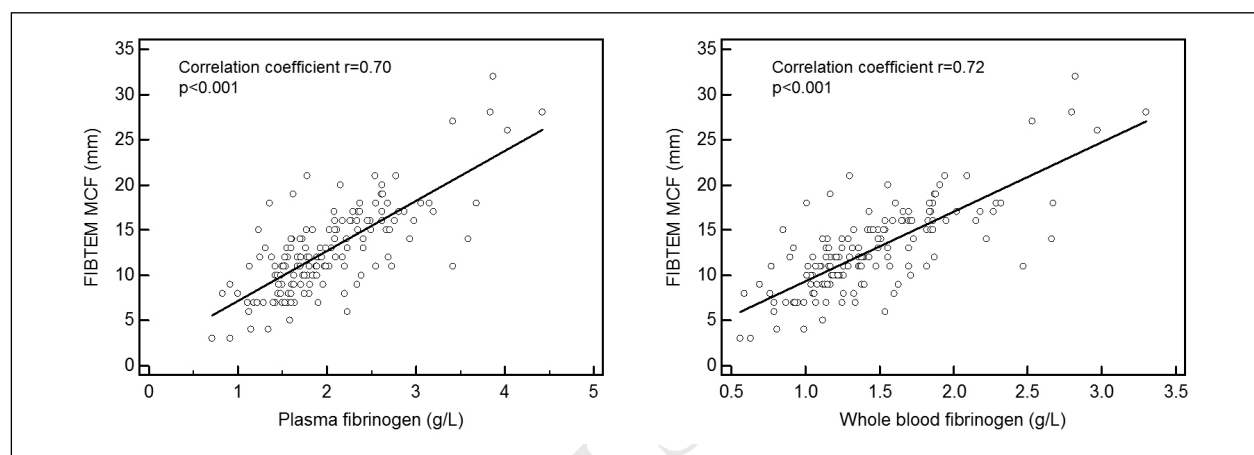FIBTEM, whole blood thromboelastometry fibrin-based test; MCF, maximum clot firmness.
Whole blood fibrinogen concentration calculated as plasma fibrinogen level × (100 − haematocrit)/100.

concentration was 0.70 (P <0.001), and it was 0.72 (P <0.001) for the relationship between FIBTEM MCF and whole blood fibrinogen concentration (Figure 2); in both cases the values at the end of CPB were very similar to the preoperative values. After administration of protamine, new subgroups based on haematocrit (<25%, 25-27.9%, 28-29.9% and >30%) were assigned to evaluate the effect of red cell mass on MCF. Fibrinogen concentrations in both whole blood and plasma were comparable for all four haematocrit subgroups after protamine administration, as were platelet counts (Table II). The FIBTEM MCF was slightly higher for the subgroup with haematocrit 25-27.9% compared to

the subgroups with haematocrit 28-29.9% and >30% (P <0.05). However, no significant differences were observed between the lowest haematocrit group (<25%) and the other groups for FIBTEM MCF or fibrinogen concentrations in whole blood and plasma.

Discussion

In pre-operative whole blood samples from cardiac surgery patients, FIBTEM MCF and whole blood fibrinogen concentration were both increased in samples with low haematocrit, while plasma fibrinogen concentration was unchanged with different haematocrit levels. At the end of CPB, after protamine administration,

Figure 2 - Correlation between FIBTEM maximum clot firmness (MCF) and plasma or whole blood fibrinogen concentration at the end of cardiopulmonary bypass (CPB), after protamine administration.
Whole blood fibrinogen concentration was calculated as plasma fibrinogen level × (100 − haematocrit)/100.

Table II - End of CPB (after protamine administration) coagulation data in cardiac surgery patients: comparison between haematocrit subgroups.

Parameters	All samples (n=157)	Samples with haematocrit <25% (n=33)	Samples with haematocrit 25-27.9% (n=44)	Samples with haematocrit 28-29.9% (n=40)	Samples with haematocrit >30% (n=40)
FIBTEM MCF (mm) (normal range, 9-25 mm)	12 (9, 16)	13 (9, 16)	13 (11, 17)	12 (8, 14)*	11 (10, 14)*
Plasma fibrinogen concentration (g/L) (normal range, 2.0-4.5 g/L)	1.9 (1.6, 2.4)	1.8 (1.5, 2.4)	1.9 (1.6, 2.4)	1.8 (1.5, 2.4)	1.9 (1.7, 2.2)
Whole blood fibrinogen concentration (g/L)	1.3 (1.1, 1.7)	1.4 (1.1, 1.9)	1.4 (1.2, 1.7)	1.3 (1.1, 1.7)	1.3 (1.2, 1.5)
Platelet count *1,000/μL (normal range, 150-450 *1,000/μL)	100 (67, 125)	98 (75, 127)	103 (77, 126)	111 (78, 125)	84 (58, 113)
Haemoglobin (g/dL) (normal range, 13.5-17.5 g/dL)	9.6 (9.2, 10.3)	8.5 (8.1, 8.8)	9.4 (9.2, 9.6)	10 (9.7, 10.2)	10.9 (10.5, 11.2)
Haematocrit (%) (normal range, 42-50%)	28 (26.5, 30)	24.1 (23.8, 25.1)	27.2 (26.6, 27.5)	28.9 (28.5, 29.3)	31.3 (30.5, 32.3)

Legend Data presented as median (interquartile range).
*: P <0.05 compared to samples with haematocrit 25-27.9%; comparisons performed between all groups for FIBTEM MCF, plasma fibrinogen concentration, whole blood fibrinogen concentration, and platelet count
CPB, cardiopulmonary bypass; FIBTEM, whole blood thromboelastometry fibrin-based test; MCF, maximum clot firmness
Whole blood fibrinogen concentration calculated as plasma fibrinogen level × (100 − haematocrit)/100.

whole blood fibrinogen concentration and plasma fibrinogen concentration were unchanged at different degrees of anaemia, while FIBTEM MCF varied slightly. Taken together, these results suggest that FIBTEM MCF incorporates the contribution of haematocrit to whole blood clot firmness in different peri-operative situations. While this contribution is evident pre-operatively when haematocrit levels are in the low-to-normal range, the effect is less pronounced at the end of CPB, after protamine administration, when haematocrit levels are severely decreased. Clinicians should, therefore, be aware that the impact of haematocrit on blood clotting is not accounted for by plasma fibrinogen concentration, in contrast to FIBTEM MCF; however, at low haematocrit values, when haemostatic therapy for bleeding may also be necessary, the impact of haematocrit is negligible.

The finding of increased FIBTEM MCF and whole blood fibrinogen concentration with haematocrit in the low-to-normal range and constant plasma fibrinogen concentration is in agreement with previous reports in the literature. Spiezia *et al*.[12] reported that FIBTEM MCF values were significantly higher in samples from patients with sideropenic anaemia than in those from healthy matched subjects. Importantly, these patients were not in a haemodiluted state, as would be seen following surgical intervention. Plasma fibrinogen level was normal in both groups and, after adjusting for the haematocrit level, whole blood fibrinogen concentration in the anaemic group is likely to have been higher than in the control group, in agreement with the FIBTEM MCF values and with our findings.

The effect of RBC on clot formation has also been addressed by experiments *in vitro* and in animal models. Using blood samples from healthy volunteers, Iselin *et al*.[11] separated packed RBC from plasma and reconstituted them with 0.9% saline to produce samples with haematocrit values from 10% to 40%. A decreased haematocrit did not compromise clot strength; moreover, with decreasing haematocrit, all thrombelastography variables indicated improved clot strength. Also, in a transgenic mouse model with a haematocrit reaching 85%, clot formation was prolonged and thrombus formation inhibited[14]. These results may be explained by the findings of Gersh *et al*.[15], who demonstrated that incorporation of RBC into a fibrin clot significantly affects clot structure and mechanical properties in a concentration-dependent manner. Below a haematocrit of 10%, RBC assembled into holes in the fibrin network without disrupting it; however, above this concentration, RBC integrated fully into the network, thereby decreasing fibre density and resulting in a weaker clot. As assays using spun plasma do not take into account the effect of RBC on clotting, they cannot fully characterise clot formation[15].

Bleeding is common following cardiac surgery and clot formation is typically diminished as a result of haemodilution and the loss of coagulation factors, RBC and platelets. Tests for monitoring haemostatic parameters, including FIBTEM and/or plasma fibrinogen and haematocrit, are essential for the identification and successful treatment of coagulopathy. An increasing number of studies have demonstrated successful use of fibrinogen concentrate as haemostatic therapy in cardiovascular surgery[3,4] and trauma[16,17], in which fibrinogen supplementation therapy is often guided by FIBTEM MCF. It is important to understand that whole blood clot strength does not behave in the same way as plasma fibrinogen concentration in situations in which whole blood components (e.g., haematocrit or FXIII) are altered, as in cardiac surgery. FIBTEM MCF, a measure of the viscoelastic quality of the blood clot, appears to be more meaningful than plasma or whole blood fibrinogen concentration for evaluating the impact on the coagulation system of haemostatic therapy such as fibrinogen concentrate.

As the FIBTEM test eliminates only the contribution of platelets to clot formation, it is possible that other coagulation factors, apart from fibrinogen, contribute to the measured clot firmness and stability. For example, a study by Theusinger *et al*.[18] showed that *in vitro* supplementation of FXIII significantly improved FIBTEM MCF. Additionally, the sensitivity of clot formation to FXIII supplementation has been shown to be more pronounced in haemodiluted blood[10], suggesting that the contribution of FXIII to clot firmness may be of more importance in the post-operative setting, when haemodilution is likely. A recent study has also shown that factors II and X have a role in clot formation[19]. However, as these studies were performed *in vitro*, the results cannot be directly extrapolated to a clinical setting. Further *in vivo* studies are needed to determine the influence that other coagulation factors exert upon clot firmness, and the importance of this influence relative to that of fibrinogen.

There are numerous reports on the correlation between plasma fibrinogen concentration and FIBTEM MCF in cardiac surgery: the correlation ranges from r =0.6–0.85[1,7-9] but has been reported to be as low as r =0.33 following haemostatic therapy[20]. When investigating the relationship between the two tests, it is imperative to consider that they measure different physical properties. The FIBTEM test measures the shear modulus during clot formation in whole blood, and the SI unit (Système International d'Unités) of shear modulus is dyne/cm^2 (gigapascal). The SI units of fibrinogen concentration measured in platelet-poor plasma are g/L or mol/L, and are entirely different[20]. If platelet-poor plasma is used in viscoelastic assays, their

correlation with fibrinogen concentration measurements becomes almost as strong as between other different fibrinogen concentration measurement methods, such as prothrombin time-derived fibrinogen *vs.* an enzyme-linked immunosorbent assay (ELISA). This is because fewer confounding factors, such as FXIII or hydroxyethyl starch, which influence the two measurement methods, are present. Furthermore, because concentration measurements are indirect measurements along a calibration curve, tissue factor or thrombin-activated viscoelastic assays performed in platelet-poor plasma may serve as measures of fibrinogen concentration when similarly calibrated. For example, Kalina *et al.*[21] showed that MCF (EXTEM in platelet-free plasma) correlated excellently with a Clauss assay (r^2=0.93) and a fibrinogen ELISA (r^2=0.95). When the sample was spiked with fibrinogen, the increase in MCF was directly dependent upon fibrinogen concentration. If plasma is used instead of whole blood, the difference between the measurement methods is mainly dependent on the kind of activation (tissue factor versus thrombin) and the type of read-out method (e.g., time to turbidity/ clot strength, delta to maximum turbidity or maximum clot strength). However, if all the cellular components of the coagulation system (platelets, RBC) are removed, the viscoelastic measurements become as artificial as Clauss measurements and no longer reflect the impact of a haemostatic intervention on the entire coagulation system. Clot strength in whole blood may, therefore, be a more reliable surrogate marker than fibrinogen concentration for guiding haemostatic therapy in the peri-operative setting. Accordingly, recent years have seen the publication of a number of treatment algorithms and fibrinogen concentrate dosing formulae based on clot strength measurements instead of concentration measurements[3-5,22,23]. In the setting of acute bleeding, the main advantage of point-of-care viscoelastic testing is its short turnaround time; in contrast, the long turnaround times typically associated with laboratory tests lead to an unacceptable delay and often to unguided, "blind" therapy[24-29].

It is important to consider that although the haematocrit showed a certain impact on clot firmness, the correction of haemoglobin/haematocrit level is generally a precondition for performing haemostatic therapy. This aspect is addressed in numerous transfusion algorithms and guidelines[5,22,23,30]. Therefore, variations in the dosing of haemostatic agents because of severely low haemoglobin/haematocrit levels are excluded.

One limitation of our study is that patients received 500 mL gelatin polysuccinate (Gelafundin 0.026; Serumwerk, Bernburg, Germany) before the initiation of CPB. Although colloids are known to affect clot firmness when given in large volumes in experimental or clinical settings[31-33], the single dose of colloids we used was diluted upon CPB and is, therefore, unlikely to have had a strong impact on our measurements. Another limitation is that the haematocrit subgroups were defined empirically in order to reflect haematocrit intervals common in our clinical setting. Because of the observational nature of the study, there were no pre-defined haematocrit intervals or numbers of patients per subgroup.

In summary, this study showed that the effect of haematocrit on blood clotting is not reflected by plasma fibrinogen concentration, in contrast to FIBTEM MCF which incorporates the contribution of haematocrit to whole blood clot firmness. This effect does, however, appear to be negligible in haemodiluted patients.

Conflicts of interest disclosure

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