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Impact of changes in haematocrit level and platelet count on thromboelastometry parameters ☆

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ABSTRACT

Introduction: To what extent haematocrit levels (Hct) and platelet counts (PLT) influence the measurement of parameters of thromboelastometry when assessed with the ROTEM® device is unclear. We investigated to what extent thromboelastometry measurements depend on Hct and PLT.
Materials and Methods: Whole blood samples were taken for in-vitro preparations of mixtures with three different levels of PLT and a varying Hct. Maximum clot firmness (MCF), clotting time (CT), clot formation time (CFT) and alpha angle (α) for INTEM, EXTEM, FIBTEM and APTEM was recorded.
Results: Measurements depended substantially on Hct and PLT. MCF readings were systematically lower with increasing Hct (0.2 vs. 0.4: −7.8 (−8.3 to −7.2); $p<0.001$, 0.2 vs. 0.55: −14.5 (−17.3 to −14.3); $p<0.001$) but higher with increasing PLT (50 vs. 125×10^9/l: 8.2 (4.2 to 12.3); $p=0.005$, 50 vs. 250×10^9/l: 12.0 (7.2 to 16.8); $p=0.002$). CT readings were systematically higher with increasing Hct (0.2 vs. 0.4: 9.2 (6.2 to 12.1); $p=0.001$, 0.2 vs. 0.55: 38.2 (21.5 to 54.9); $p=0.003$) while increasing PLT had no influence. CFT readings were also systematically higher with increasing Hct (0.2 vs. 0.4: 83.8 (40.2 to 127.6); $p=0.006$, 0.2 vs. 0.55: 226.2 (110.7 to 341.7); $p=0.006$) but systematically lower with increasing PLT (50 vs. 125×10^9/l: −144.0 (−272.3 to −15.6); $p=0.036$, 50 vs. 250×10^9/l: −189.2 (−330.4 to −48.0); $p=0.02$); readings of the alpha angle showed a similar pattern.
Conclusions: Our results suggest that readings of thromboelastometry parameters need to be adjusted by Hct and PLT to avoid potential confounding and miss-interpretations in clinical practice.

Introduction

Thromboelastometry analysis (ROTEM®) is a point-of-care viscoelastic test comparable to thromboelastography (TEG®), which is widely used in acute clinical care settings. Operators are attracted by the ease of use and the fast production of graphs that are easy to grasp, displaying the results [1,2]. Due to its claim to provide global information on all parts of haemostasis including platelet function, coagulation cascade, cross-linking of fibrin and fibrinolysis, it is seen as an innovation in the setting of acute bleeding [3,4] and several other clinical situations [5–10]. However, while thromboelastometry has been thoroughly evaluated in perioperative and trauma settings for the detection of coagulopathies, to guide substitution therapy and to reduce unnecessary transfusions, key factors influencing the measurement of thromboelastometry parameters are still incompletely understood [3,11–14].

For example, the impact of important factors of influence, such as haematocrit level (Hct) and platelet count (PLT), on thromboelastometry parameters as assessed with the ROTEM® device have not been studied in detail. Investigations in different clinical and experimental settings found associations between Hct and PLT with some thromboelastometry parameters [11,15–22]. Other investigations indicate that changing Hct affects thromboelastometry parameters when used as trigger for fibrinogen supplementation [15]. However, due to design and the clinical context of the studies, confounding variables such as dilution phenomena have possibly affected the results. Nevertheless, the type and the extent of changes on particular thromboelastometry parameters due to isolated, specific changes of Hct and PLT (alone and in combination) are uncertain. The problem of Hct and PLT as possible confounders is particularly important in the context of massive bleeding, where rapid changes of Hct and PLT are common [23,24]. Therefore, changes of Hct and PLT may have

Abbreviations: Hct, haematocrit level; PLT, platelet count; PRP, platelet rich plasma; PPP, platelet poor plasma; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; α, alpha angle.

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contributed to the uncertainty of the clinical value, which has been questioned by some authors and two systematic reviews [23,25–28]. Sound evidence on factors of influence of a laboratory test are essential pre-requisite for clinical studies and may facilitate large studies, which are valid and are able to answer questions on clinical value [29]. Therefore, the aim of the present in-vitro evaluation study was to investigate the impact of changes in Hct and PLT on a broad spectrum of thromboelastometry parameters systematically in a strictly experimental design excluding additional confounding variables such as dilution phenomena.

Materials and methods

Study design

Citrated whole blood samples were taken from five healthy volunteers for in-vitro preparations (45 measurements). Individuals did not have any known disease, were taking no medication with an effect on haemostatic parameters and had no history of bleeding or thromboembolic events. As an inclusion criterion, baseline measurements of Hct, PLT, white blood count, routine haemostatic parameters including fibrinogen level and thromboelastometry parameters were within reference range. The study was approved by the local ethical review board (Kantonale Ethikkommission Luzern) and all participants provided written informed consent.

Sampling

Blood withdrawal was done from cubital vein under standardised conditions. A 21-gauge butterfly cannula and plastic syringes (Monovette®, Sarstedt, Nümbrecht, Germany) containing 1 ml trisodium citrate (0.106 mol/l) for 9 ml of blood were used. Citrated sample for thromboelastometry was collected after EDTA sample (for determining platelet count) to avoid tissue factor contamination. Tourniquet application was limited to a maximum of one minute and removed before blood withdrawal to avoid platelet activation. Samples were processed immediately by hand to preparation. Samples for baseline measurements were collected in advance.

In-vitro preparations

For each blood withdrawal, platelet rich plasma (PRP), platelet poor plasma (PPP) and mixtures with three different levels of PLT (50, 125 or 250×10^9/l respectively) and a varying haematocrit (0.2, 0.4 and 0.55) were prepared. Preparations were done in analogy to current standards of light transmission aggregometry [30]. PRP was made by centrifugation at 150×*g* for 15 minutes, PPP by centrifugation at 1500×*g* for 15 minutes. Blood samples with an intended PLT of 50×10^9/l were centrifuged at 150×*g* for 15 minutes, plasma fraction removed and PPP added to the anticipated Hct. Samples with an intended PLT of 125 or 250×10^9/l were centrifuged at 1500×*g* for 15 minutes, plasma fraction removed and PPP and/or PRP added to the anticipated haematocrit and PLT level. Proportions of PPP and/or PRP necessary were determined in a pilot study. Samples were resuspended by gentle agitation. If Hct and/or PLT deviated substantially from intended levels, a limited number of correction measures (ad maximum 3 times) by means of adding PPP or PRP respectively were allowed. Fibrinogen level (as measured by Clauss method) was held constant, as no dilution of the plasma has been done. Each sample was handled identically to limit potential influences such as platelet activation. Preparation was done in the original syringe (only PRP and PPP was transferred into separate tubes), temperature was held stable at 20-24 °C, no brake were used during centrifugation and manipulations were limited to a minimal amount. Samples have been continually agitated and processed immediately to centrifugation, preparation and analysis.

Analysis

PLT, Hct and white blood count were determined using XE 5000 (Sysmex AG, Horgen, Switzerland). Thromboelastometry was conducted using three ROTEM® analysers (ROTEM® delta; Pentapharm GmbH, Munich, Germany). Preparation of blood samples and thromboelastometry procedures were performed according to the manufacturer's instructions by following an automated electronic pipette program. We analysed the following parameters in INTEM, EXTEM and APTEM test: clotting time (CT, s), clot formation time (CFT, s), maximum clot firmness (MCF, mm) and the alpha angle (α). In FIBTEM test we analysed maximum clot firmness (MCF, mm). Details of the ROTEM® analysis, parameters and activating agents have been described in detail elsewhere [11,31]. Time between in-vitro preparations and analysis of the samples was strictly held at 10–15 minutes.

Statistical analysis

For each of the four parameters CT, CFT, MCF and alpha angle we fitted a mixed linear model entering PLT and Hct using two indicator variates. For the CT, CFT and alpha angle model we entered two indicator variates for the INTEM, EXTEM, and APTEM test. For the MCF assessment we also entered the FIBTEM test using a third indicator variate. For all analyses, the subject variable was entered as a random factor to take into account that the same blood sample was used for various measurements. All analyses were performed using the Stata 11.1 statistics software package (StataCorp LP, College Station, TX, USA).

Results

Hct and PLT realised with the different samples are displayed in Table 1. Thromboelastometry values depended substantially on Hct and PLT; the distribution of the measurements with regard to the individual tests (INTEM, EXTEM, FIBTEM, APTEM) are displayed in Tables 2 to 5.

MCF readings were systematically lower with increasing Hct (0.2 vs. 0.4: −7.8 (−8.3 to −7.2); $p<0.001$, 0.2 vs. 0.55: −14.5 (−17.3 to −14.3); $p<0.001$) but systematically higher with increasing PLT (50 vs. 125×10^9/l: 8.2 (4.2 to 12.3); $p=0.005$, 50 vs. 250×10^9/l: 12.0 (7.2 to 16.8); $p=0.002$).

CT readings were systematically higher with increasing Hct (0.2 vs. 0.4: 9.2 (6.2 to 12.1); $p=0.001$, 0.2 vs. 0.55: 38.2 (21.5 to 54.9); $p=0.003$) while increasing PLT had no influence (50 vs. 125×10^9/l: −4.7 (−18.4 to 8.9); $p=0.392$, 50 vs. 250×10^9/l: −4.2 (−18.3 to 9.9); $p<0.001$).

CFT readings were also systematically higher with increasing Hct (0.2 vs. 0.4: 83.8 (40.2 to 127.6); $p=0.006$, 0.2 vs. 0.55: 226.2 (110.7 to 341.7); $p=0.006$) but systematically lower with increasing PLT (50 vs. 125×10^9/l: −144.0 (−272.3 to −15.6); $p=0.036$, 50 vs. 250×10^9/l: −189.2 (−330.4 to −48.0); $p=0.02$).

Table 1
Haematocrit and platelet count realised in the sample series with regard to the intended levels.

Parameter	Intended level	Level realised *median (range)*
Haematocrit	0.2	0.20 (0.17–0.22)*
	0.4	0.39 (0.36–0.41)*
	0.55	0.53 (0.50–0.57)*
Platelet count (x10^9/l)	50	58 (34–81)*
	125	150 (95–186)*
	250	242 (201–282)*

* n = 15.