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High haematocrit in cyanotic congenital heart disease affects how fibrinogen activity is determined by rotational thromboelastometry

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ABSTRACT

Introduction: Viscoelastometry enables rapid evaluation of coagulopathy in settings such as cardiac surgery but may be influenced by red cell concentration.
Methods: In order to study the effects of supra-physiological red cell concentrations on viscoelastometry, we compared ROTEM® viscoelastometry and plasma coagulation assay results in high haematocrit (HCT; 0.55-0.76 L/L) blood from patients with cyanotic congenital heart disease (CCHD), and in model high HCT blood (HCT 0.45-0.70 L/L).
Results: High HCT blood from CCHD patients (median HCT 0.66 L/L) displayed prolonged clot initiation in the EXTEM® test compared to controls and reduced maximum clot firmness (MCF) in the EXTEM (median 51 mm vs 64 mm in controls) and FIBTEM® (7 mm vs 14 mm) tests. The plasma fibrinogen (Clauss; CF) was similar in CCHD blood to controls (median 2.94 g/L vs 2.49) but the whole blood fibrinogen concentration (WBFC) was reduced (1.27 g/L vs 1.58). The FIBTEM MCF correlated linearly with the CF ($r^2 = 0.68$; $p < 0.0001$) and WBFC ($r^2 = 0.65$; $p < 0.0001$) in control blood but this relationship was maintained only with WBFC in CCHD blood. Model high HCT blood showed abnormal ROTEM test results that were similar to CCHD blood, including reduced FIBTEM MCF (14 mm with HCT 0.32-0.44 vs 6 mm with HCT 0.63-0.70). The ROTEM results were HCT dependent but independent of plasma clotting times and fibrinogen concentration.
Conclusion: Supra-physiologic HCT causes abnormal ROTEM test results consistent with increased dilution of fibrinogen and coagulation factors in whole blood by red cells. High HCT should be considered during interpretation of ROTEM test results in clinical settings.

Introduction

Rotational thromboelastometry (ROTEM®, Haemonetics, Braintree, MA, USA) and thromboelastography (TEG®, Hemoscope-Hemonetics, Niles, IL, USA) are rapid, whole blood assays that enable clot viscoelastic strength to be determined after coagulation activation by standardised reagents [1]. Both techniques may identify defects in clot initiation [2], platelets and fibrinogen [3,4] and fibrinolysis [5]. Thus, ROTEM and TEG have the potential to be clinically useful tests for diagnosis of coagulopathy in acute trauma [6,7], liver transplantation [8] and in vascular [9] and cardiac surgery [10–12]. In some settings, the use of ROTEM or TEG test results to guide treatments for coagulopathy may reduce the use of allogeneic blood components [10,12] and improve clinical outcomes [13].

In contrast to plasma coagulation tests such as prothrombin time (PT), activated partial thromboplastin time (APTT) and Clauss fibrinogen (CF), the endpoints of the ROTEM and TEG assays are influenced by the cellular components of blood [1]. In both assay systems, this property enables detection of defects in platelet number or function, which may affect the rate of clot formation and clot viscoelastic strength [3,14]. However, the concentration of red-cells in whole blood also influences ROTEM and TEG assay endpoints which may give valuable additional insights into the contribution of red cells to haemostasis *in vivo*. For example, anaemic adults with sub-physiological haematocrit (low HCT: <0.35 L/L) displayed reduced clot formation times (CFT) and increased alpha-angle (α-angle) and maximum clot firmness (MCF) using the ROTEM assay, compared to healthy donor controls [15] that could not be explained by defects in plasma coagulation assays or platelet tests [16,17]. These effects of low HCT include an increase in MCF obtained with the ROTEM FIBTEM reagent [16,17]. In blood with physiological HCT (normal HCT; approximately 0.35-0.55 L/L), the FIBTEM MCF correlates with the plasma fibrinogen concentration determined using tests such as the CF assay [18–20]. However, at low HCT, the FIBTEM

Abbreviations: HCT, Haematocrit; CCHD, Cyanotic congenital heart disease; CT, Clot time; CFT, Clot formation time; MCF, Maximum clot firmness; CF, Clauss fibrinogen concentration; WBFC, Whole blood fibrinogen concentration; PT, Prothrombin time; APTT, Activated partial thromboplastin time; PRC, Packed red cell; VKA, Vitamin K antagonists.

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MCF increases independently of the CF result. Therefore, coagulopathy treatment protocols that utilise the FIBTEM MCF to guide fibrinogen replacement therapy in setting such as cardiac surgery [9,13] and trauma [6] may therefore direct interventions differently than treatment protocols that utilise the CF as a measure of fibrinogen activity [10,12].

At supra-physiological HCT (high HCT; >0.55 L/L), such as in patients with cyanotic congenital heart disease (CCHD), increased relative red cell concentration also affects ROTEM and TEG test results to cause a pattern suggestive of hypocoagulability and low fibrinogen activity [21–23]. This has a high potential impact in patients with high HCT undergoing surgery, particularly patients with CCHD in which there is a strong clinical need for rapid evaluation of coagulopathy because of the high risk of bleeding, and in some cases, thrombosis. In order to improve understanding of the mechanism of how high HCT affects viscoelastometry test results, we have compared ROTEM thromboelastometry and plasma coagulation tests results in CCHD patients with high HCT. We have focussed on the FIBTEM and EXTEM tests reflecting the importance of these tests in guiding fibrinogen and coagulation factor replacement therapy in current cardiac surgery coagulopathy treatment protocols [9,13]. We also provide a unique mechanistic insight into how high HCT affects ROTEM test results using a simple model of high HCT blood.

Materials and Methods

Cyanotic congenital heart disease patient and control blood samples

As part of routine outpatient review for adults with cyanotic congenital heart disease (CCHD) and stable HCT ≥ 0.55 L/L, custom made blood collection tubes were prepared in advance. The volume of 0.105 M tri-sodium citrate per tube was calculated according to the formula- volume 0.105 M tri-sodium citrate = ((1-HCT) x blood draw volume)/(0.595-HCT) as previously described [24]. After informed consent, venous blood samples were obtained by peripheral venepuncture from 5 CCHD patients and from 20 healthy volunteer donors. Blood samples were also obtained during a laboratory evaluation exercise from a further group of 70 healthy volunteer donors (HCT 0.33-0.44 L/L) into standard blood collection tubes (VACUTAINER®; Becton Dickinson, Meylan, France) containing 1 part 0.105 M tri-sodium citrate to 9 parts blood. All patient activities were performed in accordance with the WMA Declaration of Helsinki 2008.

In vitro high haematocrit model

Venous blood samples from 20 healthy volunteer donors were collected by peripheral venepuncture into unmodified tri-sodium citrate blood collection tubes. For each donor, a packed red cell (PRC) suspension was generated by centrifuging 10 mL of the blood sample at 3200 rpm for 10 minutes. Approximately 5 mL of plasma was then aspirated and the remaining pellet resuspended to create the PRC suspension with minimal agitation in order to minimise platelet activation. The PRC suspensions were then gently mixed with un-manipulated venous blood from the same donor in the ratios 1:1 and 3:4 (whole blood: PRC) to create model high HCT blood.

Laboratory evaluation

The haemoglobin concentration, haematocrit, platelet count and white cell count in the model high HCT bloods and from patient and control samples were determined using the Advia 2120 haematology system (Siemens AG, Erlangen, Germany). Plasma for coagulation assays was prepared by centrifugation of anticoagulated blood at 3200 rpm for 10 minutes. The prothrombin time (PT- Innovin; Dade-Behring, Marberg, Germany); activated partial thromboplastin time (APTT- Actin FS; D-B) and Clauss fibrinogen (CF- thrombin reagent (33 u/mL bovine thrombin; D-B)) were determined using a CS-21000i coagulometer (Sysmex AG, Horgen, Switzerland). In order to express the concentration of functional fibrinogen relative to both the plasma and cellular components of blood, we also calculated the whole blood fibrinogen concentration (WBFC) the formula WBFC (g/L) = (1-HCT) x CF (g/L) [17].

Clot formation in whole blood was monitored in duplicate by rotational thromboelastometry (ROTEM®, Haemonetics, Braintree, MA, USA) using the EXTEM® test (20 μL of star-TEM reagent (0.2 mol/L $CaCl_2$ in HEPES buffer pH 7.4 with 0.1% sodium azide) and 20 μL ex-TEM reagent (recombinant tissue factor, phospholipids, heparin inhibitor, preservatives and buffer) and FIBTEM® test (20 μL ex-TEM reagent followed by 20 μL fib-TEM reagent (DMSO/cytochalasin D platelet inhibitor, 0.2 mol/L $CaCl_2$ in HEPES buffer pH 7.4) with 300μL aliquots of whole blood according to the manufacturer's instructions. The following parameters were recorded; i) clot time (CT): time from addition of reagent to formation of clot with amplitude of 2 mm, ii) clot formation time (CFT; EXTEM only): time taken from CT until formation of clot with amplitude of 20 mm, iii) alpha angle (α-angle; EXTEM only): the angle of the tangent of the curve at the point that the CFT is reached and, iv) maximum clot firmness (MCF): the peak amplitude of the clot formed.

Statistical analysis

All statistical comparisons were performed using GraphPad Prism® version 4.03. Plasma and ROTEM laboratory test results are expressed as medians and ranges and were compared between CCHD patients and controls and between groups in the model high HCT experiment using the Mann–Whitney test, with a p value of <0.05 considered significant. Spearman linear regression was used to define the relationship between FIBTEM MCF and CF and between FIBTEM MCF and WBFC in healthy donor controls and in the model high HCT samples. Relationships with a p value of <0.05 were considered significant.

Results

Coagulation and ROTEM parameters in patients with CCHD

The CCHD patient group comprised five subjects (2 male; median age 52 years; range 23–58 years; 2 atrio-ventricular septal defects, 2 ventriculoseptal defects, 1 double inlet left ventricle) including 2 patients receiving long-term vitamin K antagonist treatment (VKA). In both cases, the indication for anticoagulation was deep vein thrombosis and neither patient has experienced any haemorrhagic or further thrombotic complications to date. Two of the remaining patients have experienced infrequent episodes of minor haemoptysis only. The control group comprised 20 healthy donors (10 male; median age 35 years; range 26–44). The CCHD patients displayed higher haemoglobin concentration (median 217 g/L vs 122 g/L; $p < 0.001$) and HCT (median 0.66 L/L vs 0.37 L/L; $p < 0.001$) compared to the controls (Fig. 1A). In the CCHD group, the platelet count was lower (median 79 $x10^9$/L vs 177 $x10^9$/L; $p = 0.001$) than in healthy donor controls. Plasma fibrinogen concentration determined by the Clauss assay (CF) was slightly higher in CCHD patients than in controls (median 2.94 g/L vs 2.49 ($p < 0.01$)). However, the fibrinogen level expressed as WBFC was lower in the CCHD group compared to controls (1.27 g/L vs 1.58 ($p < 0.01$): Fig. 1A and B). The PT and APTT of blood samples CCHD patients not receiving VKA were similar to controls (Fig. 1A).

With the ROTEM EXTEM test, the CCHD group displayed prolonged CT (median 126 s vs 51 s; $p < 0.01$: excluding the patients receiving VKA) and CFT (median 174 s vs 72 s; $p < 0.001$), reduced α-angle (median 55 vs 76°; $p < 0.001$) and reduced MCF (51 mm vs 64 mm; $p < 0.001$) compared to controls (Fig. 1A and B). With the FIBTEM test, the CCHD group also displayed prolonged CT (median 202 s vs 47 s; $p < 0.01$: excluding the patients receiving VKA) and reduced