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Comparison of citrated and fresh whole blood for viscoelastic coagulation testing during elective neurosurgery

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ABSTRACT

Background: Previous viscoelastic haemostatic tests studies have often indicated a hypercoagulative test signal with citrated blood, which could influence clinical decision makings.
Purpose: The aim of this study was to compare fresh and citrated whole blood using two non-automated viscoelastic ROTEM and Sonoclot tests. Our hypothesis was that citrated blood would demonstrate a hypercoagulative response in this setting, not tested before.
Methods: Perioperative viscoelastic coagulation changes were evaluated with a ROTEM and Sonoclot in 38 patients undergoing elective brain tumor surgery. The citrated samples were recalcified with $CaCl_2$. Wilcoxon non-parametric-paired tests and Bland-Altman plots were performed to compare the fresh and citrated blood analyses.
Results: The citrated blood showed a hypercoagulative response in ROTEM NATEM-clot formation time and α-angle, Sonoclot-clot rate and platelet function, as compared to fresh blood ($p < 0.0001$).
Conclusions: Fresh whole blood may theoretically reflect in vivo haemostasis more closely than citrated analyses, which indicated a hypercoagulative response as compared to the fresh whole blood analyses Bland-Altman plots also indicated that ROTEM reference ranges in patients undergoing brain surgery should be redefined.
Future studies must establish the correlation between viscoelastic test results using fresh or citrate anticoagulated blood and clinical outcomes, such as bleeding, transfusion or reoperation for postoperative haematoma.

1. Introduction

The thromboelastography (TEG) method was first described by Hartert in 1948 and improved in the 1960s. Both TEG and the thromboelastometry (ROTEM) method introduced later, as well as their interpretations, have been further developed [1]. However, it was first after Kang et al. in Pittsburgh, US, showed that TEG reliably and rapidly detected coagulation defects during liver transplantation [2] and reduced blood transfusion that it spread further to cardiac surgery, other types of surgery and obstetrics [3]. Currently, both TEG and ROTEM viscoelastic haemostasis testing (VHT) methods are used to optimize blood component therapy and coagulation factor concentrate treatment in trauma care. It is especially used in patients with low plasma fibrinogen levels [4] and in critical care [5]. VHT also detects coagulopathies secondary to haemodilution with synthetic colloids and human albumin [6,7]. Other whole-blood VHTs include free oscillation rheometry (FOR) and Sonoclot [3,5], but new commercial VHT devices, such as the sonorheometry [8], have been introduced into clinical research.

TEG initially used fresh blood added to non-disposable cuvettes. Testing had to being within 4 min after sampling to avoid artifacts secondary to pretest clotting. Introduction of plastic disposable cuvettes, activated tests with different reagents and the use of citrated vacutainer test tubes shortened the analysis time and improved test quality [3]. However, preanalytic errors, due to inadequate sampling techniques, are also a challenge with citrated blood coagulation analyses [9]. Testing the citrated reference plasma in different laboratories indicated user, device and test reagent variability issues with the VHTs [10–12].

In some centers, blood for VHT analyses is sent with pneumatic transport tube systems. The velocity of the tube system can enhance contact activation and affect platelet functioning [13,14].

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Citrated whole blood has been shown to differ from fresh whole blood with regards to coagulant properties. Gilman et al. noted a slightly hypercoagulable TEG appearance with citrated whole blood not seen in healthy volunteers, as compared to fresh whole blood after cardiopulmonary bypass cardiac surgery [15]. In patients on extracorporeal membrane oxygenation, citrated samples indicated partial or complete heparin reversal on the TEG, which could lead to heparin overdosing [15]. Citrated whole blood samples are also sensitive to inadequate recalcification before analysis, especially in patients with high haemoglobin (with a small plasma fraction) and, therefore, excessive citrate concentration [16].

We have previously studied the effect of different fluid regimes on ROTEM in vitro [6] and in vivo during neurosurgery using citrated blood [7]. However, no real-time comparison between fresh and citrated blood VHT analyses has been performed to study the effects of the sample anticoagulant on perioperative haemostasis during neurosurgery. We have previously used the Sonoclot in our research [12,17–19] and found it to be very easy to use both with citrated and fresh blood with complete test results often within 15 min as compared to up to 1 h and even more for TEG/ROTEM analyses [20]. We therefore wanted to test it together with ROTEM. Sonoclot is a less documented VHT and a recent published guideline recommends it only for research [21].

The aim of the present study was to compare fresh and citrated whole blood using ROTEM and Sonoclot analyses on patients undergoing elective neurosurgery. Our main hypothesis was that citrated blood would indicate a hypercoagulative response with ROTEM and Sonoclot, as compared to fresh whole blood analyses.

2. Materials and methods

This study was performed as a prospective unblinded observational cohort study on patients undergoing elective neurosurgery at the Skåne University Hospital in Lund, Sweden. The Regional Ethical Review Board approved the study (Lund, Protocol DNR 406/2015). The work was carried out in accordance with the Code of Ethics of the World Medical Association (Helsinki Declaration) of 1975, as revised in 1983.

The study participants included a total of 38 patients undergoing elective brain tumor resection/biopsy with craniotomy from September to December 2015. Written and informed consent was obtained from all patients before inclusion. Only patients over 18 years of age were included.

A previous study [7] from our department showed that a group size of 14–15 patients with our fluid regimes (saline versus saline plus albumin) would give a statistical power of 0.80 at a significance level of $p < 0.05$ to identify a decrease in clot strength (decreased ROTEM-MCF of >5 mm) from preoperative as compared to postoperative analyses.

None of the patients had any known coagulation defects with a risk for increased perioperative bleeding. Furthermore, none were taking prescription anticoagulants, antiplatelet drugs (also aspirin/NSAID) or had any abnormal preoperative coagulation analyses (prothrombin time [PT], activated partial thromboplastin time [aPTT] and platelet count [PLC]).

2.1. Protocols

Total intravenous anaesthesia (TIVA) was induced and maintained according to the standard Lund neuroanaesthesia departmental protocol. Remifentanil (Ultiva®, GlaxoSmithKline, Sweden), Propofol (Diprivan®; AstraZeneca, Sweden) and Rocuronium (Esmeron®; MSD, USA) (0.5–0.8 mg/kg) were given to facilitate the trachea intubation and maintain relaxation during the neurosurgery. Ventilation was conducted using positive pressure ventilation in an attempt to maintain normocapnia ($PaCO_2$: 4.5–5.5 kilo Pascal (kPa)) and normal PaO_2 values (>12.0 kPa).

After the anaesthesia was administered, a radial arterial catheter was inserted for continuous blood pressure monitoring and arterial blood gas sampling. A bladder catheter was inserted in all but two patients. Mechanical calf compression (Kendall SCDTM Express Sleeves; Covidien, USA) was applied to all patients for thrombosis prophylaxis. Finally, a Bair Hugger™ was used (3 M, St. Paul, USA) to avoid hypothermia during surgery.

The anaesthetist in charge exclusively decided upon transfusions and infusions based on a standard departmental fluid protocol for neurosurgery [7].

2.1.1. Blood sampling

Blood was sampled for the ROTEM and Sonoclot analyses from the radial arterial catheter with a continuous flush system, with no heparin and through a sampling membrane by an experienced anaesthesiologist or anaesthetic nurse immediately after the arterial line had been established and prior to any surgical incision had been performed(annotated: *preoperative sample*). A new sample was taken 20 min prior to the end of surgery (annotated: *postoperative sample*): 0.5 ml of blood was drawn and disposed of before sampling. Citrated blood was then sampled with a vacutainer system, using 2.7 ml plastic tubes (3.2 % citrate; BD, Plymouth, UK), followed by fresh (non-citrated) blood that was collected in a 1.0 ml plastic syringe. Heparinized blood was sampled for routine arterial blood gas analysis after these research samples were collected.

The blood was transported to the point of care (POC) laboratory at a very fast walking pace. All fresh samples were started within 4 min after sampling (see Results), as recommended for ROTEM/Sonoclot. The citrated tube was immediately placed in a test tube heater, set at 37 °C and citrated ROTEM/Sonoclot analyses began within 20 min.

2.1.2. Sonoclot

A volume of 0.36 ml fresh blood was pipetted into a standard glass bead test cuvette (Sienco® gbACT™ Kit) and analysed. A volume of 500 μl citrate blood was added to an Eppendorf Tube with a manual pipette, and 20 μl of 0.25 Molar (M) $CaCl_2$ was added. The tube was tilted for mixing and then 0.36 ml was withdrawn and placed into another standard glass bead test cuvette.

The Sonoclot measures the viscoelastic drag impedance that fibrin and platelets in a whole blood sample impose upon the Sono-probe [3]. The time-based graph that reflects the different steps in the clotting of the whole blood sample, which is called the Sonoclot signature. The Sonoclot parameters are as follows: activated clotting time (ACT) is the time required for the first fibrin to form. ACT corresponds to aPTT, e.g. clot initiation [1] and normal range is 100–155 s. The clot rate (CR) corresponds to clot propagation and the initial formation of a platelet-fibrin clot and normal range is 9–35 units/min. Platelet function (PF) is the point at which the squeezing out of trapped serum in the contracting clot - clot retraction (sign of functioning platelets - GPIIb/IIIa-dependent) exceeds the accumulation of clot bulk on the probe and its normal range is >1.5 units. The Sonoclot variation coefficients for the different parameters are 6–10% [3]. They are highest for activated clotting time (ACT) and lowest for the clot structure parameter platelet function (PF).

2.1.3. ROTEM

ROTEM (TEM Innovations GmbH, Munich, Germany) is a viscoelastic test that measures coagulation in whole blood [3]. Whole blood (300 μL) is installed in a fixed disposable cuvette with a rotating pin. When the blood starts to clot, a fibrin bridge is created between the pin and the cup, and the resistance to rotation is displayed as a graph that outlines the time for the blood clot to form and the strength of the clot. The ROTEM device has several settings and different reagents can be used. For this study, the NATEM protocol, which is simply recalcification of the citrated blood with the addition of 20 μl of $CaCl_2$ 0.2 M (star-tem), was chosen to compare with the *fresh whole blood* analyses (non-