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The effects of pneumatic tube system transport on ROTEM analysis and contact activation assessed by thrombin generation test ☆

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ABSTRACT

Thromboelastometry (ROTEM) is a popular point-of-care test. It generates results quickly and may benefit individualised guided haemostatic therapy. However, processing of specimens by non-technicians might decrease the quality and reproducibility of results. Centralised laboratory equipment receiving specimens through a pneumatic tube system (PTS) could avoid this. This study aimed to evaluate the influence of PTS transport on ROTEM results and its contribution to contact activation assessed by thrombin generation (TG).
Methods: Specimens from 44 patients were drawn immediately after arterial puncture. Two were anti-coagulated by citrate and two by citrate/corn trypsin inhibitor, a Factor XIIa pathway inhibitor. Both types of samples were transported by walking and PTS. Subsequently, analysis was performed: ROTEM on citrated blood, and TG on citrated and corn trypsin inhibitor (CTI) blood using either 0 or 1 pM tissue factor (TF).
Results: In ROTEM analysis the NATEM assay showed significant differences. The EXTEM assay revealed small significant differences for clot formation time: 65 seconds (SD ± 20) versus 67 seconds (SD ± 17), and alpha angle 79° (SD ± 3) versus 77° (SD ± 3). The results remained within reference range. TG was not significantly affected by the type of tube transport, independent of the amount of TF.
Conclusion: PTS for ROTEM analysis is feasible except for NATEM assays. The amount of contact activation via Factor XIIa in terms of TG is independent of transport type. However, due to the different characteristics of pneumatic systems, hospitals should check its impact on the results before introducing this route of transport.

Introduction

Point-of-care (POC) laboratory monitoring is increasingly used in modern anaesthesiology. The main advantage of POC monitoring is rapid data acquisition, which enables treating patients in a more individualised way. Because anaesthesia teams who are tending to bleeding patients are occupied with stabilising vital functions, additional laboratory work (POC tests) might interfere with their regular workflow. In addition, there is concern about the quality and reproducibility of laboratory tests done by non-technicians, especially when the results are critically important in decision-making [1]. An alternative to the use of POC monitoring in the operating theatre might be to send specimens to the laboratory with minimal time delay. Pneumatic tube systems (PTS) reduce the workload and turn-around time of laboratories by accelerating the transport of blood samples, thus reducing manpower. While most haematological indices and standard coagulation indices are not influenced by transport via PTS [2–4], there have been reports of haemolysis in serum samples and in red blood cell concentrates, due to the acceleration and deceleration of PTS and due to vibration of the sample [5–7]. Haemolysis of red blood cells or platelets could lead to the generation of microparticles, which have been shown to trigger thrombin generation in a factor XII-dependent manner [13]. Most authors interpret these changes as minor and without clinical consequences. However, the transport by PTS of specific specimens such as for blood gas analysis, cerebral spinal fluid analysis, or platelet function analysis using the PFA-100® system is not recommended because results are unreliable [8–12].

On the other hand, Braun et al. showed that multiple electrode aggregrometry is possible by PTS transport of citrated samples of whole blood [14]. The influence of PTS transport on thromboelastometry (ROTEM-Tem International GmbH, Munich, Germany) has not been

Abbreviations: POC, Point of care; PTS, pneumatic tube system; CTI, corn trypsin inhibitor; TG, thrombin generation; CAT, calibrated automated thrombogram; TF, tissue factor; LI, lysis index; CFT, clot formation time; VI, velocity index; TEG, thromboelstograph.

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investigated with the type of PTS installed in our hospital and with the blood collection tubes we use (BD Vacutainer® tubes, Plymouth, UK). One of the drawbacks of PTS might be contact activation which can occur during and after collecting whole blood in test tubes [15]. Contact activation (via Factor XII) can be investigated by using citrated sample tubes with or without the addition of a Factor XII pathway inhibitor: corn trypsin inhibitor (CTI).

The aim of this study was to evaluate the influence of PTS transport on ROTEM results and to determine the contribution of contact activation caused by PTS. The latter was investigated using a thrombin generation (TG) assay and CTI as an activation inhibitor.

Methods

After approval by the local ethics board, 44 patients scheduled for elective cardiothoracic surgery at the Maastricht University Medical Centre gave written informed consent and were included in the study. Exclusion criteria were use of anticoagulants other than acetylsalicylic acid, and age younger than 18 years.

Upon arrival in the operating theatre, patients received standard monitoring for cardiac anaesthesia, including five lead electrocardiography, noninvasive blood pressure measurement and pulse oxymetry, a venous line (Vasofix® Safety, 16G or 14G, B. Braun Melsungen, Germany), which was inserted in the forearm, and an arterial line (Radial Artery Catheterization set, 20G, Arrow International, Reading, Pennsylvania, USA), which was positioned in the radial artery under subcutaneous local anaesthesia using 1 ml of lidocaine 1% solution.

Blood was drawn out of the arterial line immediately after insertion. The first 10 mL were discarded before filling one ethylenediaminetetraacetic acid tube (K_2-EDTA 7.2 mg, BD Vacutainer® tubes, Plymouth, UK), two tubes containing citrate (sodium citrate 1.005 M, BD Vacutainer® tubes, Plymouth, UK) and two tubes with citrate and corn trypsin inhibitor (CTI 40 μg/mL, Haematologic Technologies, Inc, Vermont, USA), a Factor XIIa inhibitor. Two sets of labelled tubes – both sets containing one citrate and one CTI tube – were sent to the central laboratory: one by PTS and one by walking transport. Platelet poor plasma (PPP) was prepared according to the standard protocol at our laboratory, consisting of an initial centrifugation step at 2.000 × g for 5 minutes (min) and a second centrifugation step at 10.000 ×*g* for 10 min. All aliquots were snap frozen in liquid nitrogen and stored at -80 °C until analysis.

The PTS (Swisslog-ErgoTrans BV, Apeldoorn, the Netherlands) connects the central surgical complex on the third floor with the haematological laboratory on the fifth floor. This circuit includes two switching stations for change of direction. There are no heat or cold sources along this route. The system generates a maximum speed of 8 m/s. Transport time from the operating theatres to the laboratory is between 83 and 110 seconds.

Sample analysis for the haematological parameters, haemoglobin, haematocrit and platelet count, was performed on a Beckman Coulter® LH-750 analyser (Beckman Coulter, Woerden, the Netherlands). ROTEM analysis was performed using the following standard assays according to the manufacturer's recommendations at 37 °C: NATEM (recalcification, no trigger), INTEM (partial thromboplastin and ellagic acid), EXTEM (tissue factor) and FIBTEM (tissue factor and cytochalasin D) [16]. To investigate contact activation via Factor XIIa pathway as a possible cause of differences induced by PTS the remaining blood was centrifuged and TG was assessed using the Calibrated Automated Thrombogram® (CAT, Thrombinoscope BV, Maastricht, the Netherlands) with phospholipids (4 μM) using either 0 or 1 pM tissue factor (TF). Previously, our group showed that normalization, with normal pooled plasma as a reference, of the time-independent parameters ETP and peak height is necessary to obtain acceptable inter-assay variations [17].

Considering 20% difference from reference values (ROTEM) as clinically relevant 44 samples are necessary to reach a statistical power of 90% with an α-error of 0.05. Statistical analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, California, USA). After testing for distribution the paired Student's *t*-test analysis for normally distributed variables and the Wilcoxon signed-rank test for nonparametric variables were used to compute the differences where applicable. A p-value of < 0.05 was considered statistically significant.

Results

A total of 44 patients were included in this study. Thirty patients (68.2%) were male and 14 (31.8%) were female. The mean age was 69.7 years (SD ± 11.5). Haematological parameters revealed a mean haemoglobin of 7.7 mmol/L (SD ± 0.8), a mean haematocrit of 0.38% (SD ± 0.04), and a mean platelet count of 210×10^9/L (SD ± 60.1×10^9/L).

ROTEM analysis was done on all pairs of 44 citrated samples (transported by walking or with PTS) using the four ROTEM assays per run. The NATEM assay showed statistically significant differences ($p<0.05$) for the transport method. Only the lysis index at 60 minutes (LI60) did not differ significantly. In the EXTEM assay, the clot formation time (CFT) 65 seconds (SD ± 20) versus 67 seconds (SD ± 17), and the alpha angle 79° (SD ± 3) versus 77° (SD ± 3) revealed small significant differences (Table 1). However, all results were still within their specific reference range.

CAT analysis was performed on the blood samples of 31 of the 44 patients (citrate transported by walking or with PTS, and citrate/CTI transported by walking or with PTS). Blood from thirteen patients could not be sampled for CAT analysis because CTI tubes were not available.

Independent of the transport method, there is a significant inhibition of contact activation in all CTI tubes compared to citrate tubes. This difference is more pronounced when CAT analysis is triggered without tissue factor. There is no endogenous thrombin potential (ETP) without tissue factor triggering, and also no velocity index (VI) detectable in the CTI tubes. Whereas nearly an equal amount of contact activation is detectable within the group of citrate tubes (Table 2).

After triggering with 1 pM tissue factor, the contact activation still was suppressed in the CTI tubes independent of transport method. Again the citrate tubes show an equal amount of contact activation in both groups (Walking versus PTS, Table 3).

Discussion

The present study demonstrates that PTS transport does not inflict clinically relevant consequences on ROTEM analysis results. Even though collection of blood samples is associated with a measurable degree of contact activation, in the CAT assay, this is not further enhanced by PTS transport as compared to manual (walking) transport to the laboratory.

Management of acute haemorrhage demands swift reaction and clinical decision-making. Some current literature recommends transfusing haemorrhagic patients as quickly as possible, even without knowledge of haemostatic properties, in order to prevent haemodilution and aggravation of bleeding [18]. Other authors state that ROTEM analysis provides important information on coagulation parameters in acute bleeding patients [19–21]. An actual debate at this moment is whether to perform ROTEM analysis at the bedside (i.e. operating theatre or emergency department) or at a central laboratory. The main argument supporting bedside testing is fast availability of results for tailored haemotherapy. On the other hand, concerns arise on the quality of POC results and some authors recommend performing laboratory analysis by trained technicians in the hospitals central laboratory [22–25].