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Temporal changes in ROTEM®-measured coagulability of citrated blood samples from coagulopathic trauma patients

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ABSTRACT

Background: Rotational thromboelastometry (ROTEM®) relies on citrated blood samples, which are regarded as biologically stable for up to 4 h after venepuncture. However, this recommendation is based on data from normal volunteers. The aim of this study was to evaluate possible temporal changes in the coagulability of blood samples from coagulopathic trauma patients.

Patients and methods: This is a prospective series of 10 coagulopathic (maximum clot firmness, MCF < 40 mm) trauma patients. ROTEM® EXTEM (tissue factor activated) and FIBTEM (tissue factor activated, cytochalasin D inhibited) analyses were performed on samples obtained on admission, and after approximately 60 min of storage in an incubator, at 37 °C.

Results: There were statistically significant differences between the median EXTEM MCF (22 mm vs 54 mm, $p < 0.001$) and α angle (30.5 vs 59.5°, $p = 0.004$) of the analyses performed immediately after sampling, and 51 min (median) subsequently, but not coagulation time (CT, $p = 0.133$), clot formation time ($p = 0.0625$) or maximum lysis (ML, $p = 0.154$). There were also no differences in median FIBTEM MCF ($p = 1.00$) or CT ($p = 0.877$) between the immediate and delayed analyses.

Conclusions: Repeated ROTEM® EXTEM analysis of citrated samples from coagulopathic trauma patients shows a spontaneous improvement in coagulability with time. The absence of parallel changes on FIBTEM analysis suggests that this effect may be due to a change in platelet function.

Introduction

The use of rotational thromboelastometry (ROTEM®, TEM® Innovations GmbH, Munich, Germany) to evaluate the coagulation status of trauma patients, and guide blood component therapy, is gaining increasing acceptance.[1–4] The recently updated European guideline on the management of bleeding after major trauma recommends "that thromboelastometry [...] be performed to assist in characterising the coagulopathy and in guiding haemostatic therapy".[2] It is also used widely in the diagnosis of coagulation disorders following cardiac and liver transplant surgery.[5]

ROTEM® relies on citrated blood samples, which are assumed to be stable, and not subject to degradation or other processes which affect the results of the analysis, at least for a period of time. The manufacturer advises that citrated samples yield consistent and reproducible results for up to 4 h following venepuncture.[6] There is also independent evidence, from a study of healthy volunteers, that citrated samples yield stable results for up to 120 min following sampling.[7]

Stability is presumed to be independent of the coagulation status of the patient. However, whilst using ROTEM® to conduct an in vitro study of a novel coagulation-enhancing agent, we made a chance observation that samples from coagulopathic patients may behave differently. We hypothesised that the stability of citrated samples, and the reproducibility of ROTEM results, may depend on the patient's coagulation status at the time of sampling, and conducted an analysis to investigate this theory.

Patients and methods

This is a prospective analysis of blood samples from coagulopathic military trauma patients, collected for the purpose of an in vitro evaluation of a novel coagulation-enhancing agent. For the purpose of this study, "coagulopathic" was defined as a maximum clot firmness (MCF) <40 mm on ROTEM® analysis. The study was conducted at the role 3 (UK) medical treatment facility (field hospital) at Camp Bastion, Helmand Province, Afghanistan, between October and December 2010, and had received approval from the Ministry of Defence Research Ethics Committee

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Table 1
Baseline characteristics.

Patient	Mechanism	ISS	NISS	Admission temperature (°C)	Units of plasma transfused prior to admission	Interval between sampling and admission ROTEM analysis (h:mm)	Interval between admission and repeat ROTEM analysis (h:mm)
1	IED	21	24	35.7	0	0:05	0:21
2	IED	18	48	[a]	2	0:05	0:54
3	IED	16	33	36.8	2	0:05	0:49
4	Blunt	9	18	[a]	2	0:16	2:34
5	IED	20	24	32.0	1	0:06	1:33
6	GSW	29	34	[a]	0	0:05	0:44
7	GSW	4	4	33.5	0	0:10	0:40
8	IED	20	36	34.2	0	0:10	3:41
9	IED	45	45	[a]	0	0:04	0:34
10	GSW	17	26	34.6	4	0:12	2:14
Median		19	30	34.4	0	0:05	0:51

GSW, gunshot wound; IED, improvised explosive device; ISS, injury severity score; NISS, new injury severity score.
[a] Not recorded.

(MODREC). The results of this original study have not been published yet.

The role 3 medical treatment facility at Camp Bastion receives the majority of coalition and Afghan military casualties. It is staffed by British and US military general trauma and orthopaedic surgeons, emergency medicine physicians, anaesthetists and intensivists. Facilities include four operating tables, a twelve-bedded intensive care unit, and two CT scanners. Virtually all patients arrive by helicopter, often staffed by a physician, and – when in extremis – receive red blood cell and plasma transfusions in flight.

Samples were taken on admission, immediately placed in BD Vacutainer® blood collection tubes containing 0.105 M (3.2%) sodium citrate, and kept in a sample warmer at 37.0 °C. This sample was then used to conduct an initial EXTEM (tissue factor activated) and FIBTEM (tissue factor activated, cytochalasin D inhibited) analysis for clinical evaluation, in accordance with the manufacturer's instructions. The supplementary analysis, which forms the basis of this report, involved an additional EXTEM and FIBTEM analysis, following incubation of the original, untreated, sample at 37.0 °C, for approximately 60 min.

The results, and data on time to analysis of samples, injury severity and mechanism, and pre-hospital blood component administration were collated in a Microsoft Excel® spreadsheet. Statistical analysis was conducted using Minitab® V15. Differences in maximum clot firmness (MCF), clotting time (CT), clot formation time (CFT), α angle and maximum lysis (ML) were compared. Investigation of the residuals and normality tests such as Anderson–Darling were used to assess the distribution of the data. A natural log transformation was required to normalise EXTEM CT and ML, whilst EXTEM CFT was resistant to normalisation. Normal values (normalised where necessary) of the immediate and delayed ROTEM® analyses were compared with paired *t*-tests. Untransformed EXTEM CFT values were compared with a Wilcoxon matched-pairs signed-rank test. Where values were not reported by ROTEM due to weak clotting the statistical analysis was performed twice, by substituting minimum detectable values (MCF 2 mm or angle 0°) and by treating the unreported values as missing. Both approaches yielded the same conclusion when a level of $p < 0.05$ was considered statistically significant.

Results

Ten patients were studied. The baseline characteristics are summarised in Table 1. As expected from the setting, the majority had sustained penetrating or blast injuries. The median injury severity score (ISS) and new injury severity score (NISS) were 19 and 30 respectively, indicating high injury severity. All patients were profoundly coagulopathic, with a median admission EXTEM MCF of 22 mm, in spite of some of the casualties having received plasma (range 0–4 units) prior to arrival at the role 3 hospital. None of the patients had received platelets prior to the initial ROTEM® analysis. Admission temperature was only available for six of the patients. The median temperature was 34.4 °C. Admission samples were tested a median of 5 min (range 4–16 min) after venepuncture, and repeat samples a median of 51 min (range 21 min–3 h 41 min) after commencement of the initial analysis.

There were statistically significant increases between the median EXTEM maximum clot firmness ($p < 0.001$) and α angle ($p = 0.004$) of the analyses performed immediately after sampling, and 51 min (median) subsequently, but no significant change in coagulation time ($p = 0.133$), clot formation time ($p = 0.0625$) or maximum lysis ($p = 0.154$) (Tables 2 and 3). There were also no differences in median FIBTEM maximum clot firmness ($p = 1.00$) or coagulation time ($p = 0.877$) between the immediate and delayed analyses (Table 4). The difference in median MCF is clinically significant, as the value of the repeat samples overlaps the normal reference range.[8]

Discussion

Our study shows that citrated blood samples from coagulopathic trauma patients are not stable, and that repeated or delayed ROTEM® analysis yields markedly different EXTEM results. EXTEM maximum clot firmness, α angle, but not coagulation time or clot formation time, showed a statistically significant tendency to

Table 2
EXTEM maximum clot firmness (MCF) and α angle, measured on admission (immediately after sampling) and subsequently.

Patient	MCF (mm)		α angle (°)	
	Admission	Repeat	Admission	Repeat
1	22	42	34	54
2	18	56	30	66
3	30	63	31	69
4	19	45	25	54
5	5	14		9
6	34	60	47	64
7	36	54	58	62
8	37	53	52	63
9	5	55		57
10	2	23		26
Median (IQR)	22 (18–36)	54 (45–56)	30.5 (0–48.3)	59.5 (47–64.5)
p	<0.001[a]		0.004[a]	

Empty cells denote missing values (analyser unable to measure).
[a] Paired *t*-test.