

The effect of sepsis and septic shock on the viscoelastic properties of clot quality and mass using rotational thromboelastometry: a prospective observational study

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Conflicts of interest

PAE and PRW have signed the International Committee of Medical Journal Editors (ICMJE) form for declaration of interest and have declared all conflict of interests. All other authors declare no competing conflicts of interest.

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Abstract

Purpose: The study purpose was to define changes in coagulation across the sepsis spectrum using rotational thromboelastometry (ROTEM).

Methods: Sepsis patients were recruited on admission to the Emergency Department and Intensive Care Units of a large teaching hospital in Wales. ROTEM markers of clot development and fibrinolysis were determined, as well as standard coagulation markers. A healthy control group matched for age and gender was also recruited (n = 44).

Results: 100 patients were recruited (50 sepsis, 20 severe sepsis and 30 septic shock). Maximum clot firmness was significantly higher in the sepsis ($p < 0.001$) and severe sepsis ($p = 0.012$) groups than the healthy control (71.6 ± 4.5 and 70.4 ± 4.1 vs 64.4 respectively). In septic shock there was prolonged clot development; however, maximum clot firmness remained normal. Fibrinolytic function was significantly impaired in septic shock, which was also significantly associated with 28-day mortality ($p < 0.001$).

Conclusions: ROTEM indicated significantly enhanced clot structural development in sepsis and severe sepsis, which could be indicative of a hypercoagulable phase. In septic shock, despite there being a prolongation of clotting pathways and impaired fibrinolysis, clot mass was comparably normal, suggestive of the development of a clot with healthy characteristics.

Key Words

Sepsis

Biomarkers

Coagulation

ROTEM

Introduction

Sepsis is known to exert a complex effect on the coagulation system, globally affecting all aspects of clot formation, namely kinetic activity, clot development and fibrinolysis [1, 2]. It is critically important for the clinician to know how the underlying pathophysiological progression of sepsis affects the coagulation system in order to deliver and target appropriate therapy. Clinically, it is well recognised that the earlier phases of sepsis induce a hypercoagulable state, but in septic shock, there still remains uncertainty on how the underlying pathophysiology affects the coagulation profile, and eventual clot formation, particularly the effects on clot structural development.

The hypercoagulable effect of sepsis leads to increased thromboembolic risk [3], but this is not detected by standard markers of coagulation [4]. Due to the limitations of conventional tests such as PT and aPTT, and the need for a dynamic, sensitive marker of coagulation, point of care tests of coagulation are increasingly utilised in the clinical setting [4]. Rotational Thromboelastometry (ROTEM) is a viscoelastic point of care test and provides bedside information on both clot structural development and fibrinolytic function. ROTEM can be performed using blood which has been activated through the extrinsic pathway (EXTEM test) or the intrinsic pathway (INTEM test). Whereas standard markers focus on the kinetics of coagulation, or concentration of individual components, ROTEM provides a global picture of the coagulation status of patients and their overall effect on clot outcome at point of care. ROTEM has the advantage that it is a point of care test, and not only gives the global effects of kinetic coagulation activity and fibrinolysis but also has the added advantage of being able to determine clot quality and mass. Therefore more recently studies have utilised either ROTEM or thromboelastography (TEG) as an improved measurement to quantify the effect of sepsis progression on coagulation in the various stages of severity [5–13].

These studies have utilised several parameters of the global test as a main outcome in treatment as well as assessing disease severity and outcome. One of the main parameters utilised to assess thrombogenicity has been maximum clot firmness (MCF) which is known to be a marker of

clot development and mass in sepsis and other disease states. Increased clot mass is indicative of a hypercoagulable state, whereas reduced clot mass and firmness is indicative of a hypocoagulable state [14] and is also associated with poor outcomes [7]. Although previous studies have looked at the different sepsis stages in isolation or comparatively, no previous study has assessed how progressive pathophysiological change across the sepsis spectrum affects the coagulation profile using ROTEM. In this prospective observational study ROTEM was used to determine global coagulation change across all stages of sepsis and compared to conventional markers of coagulation in patients presenting to the emergency department. All of these were compared against a healthy matched control independently.

Some of the data related to the research in this manuscript was presented and published as an abstract in the International Symposium of Intensive Care and Emergency Medicine 2016 prior to the completion of this study [15, 16].

Material and Methods

Ethical Approval

Full ethical approval was given by the South West Wales Research Ethics Committee. Informed 2-stage written consent was given by patients with capacity to do so. Assent was obtained from personal or legal representation in cases where capacity to give informed consent was lacking.

Recruitment of Subjects with Sepsis

Subjects that met sepsis criteria as outlined by the American College of Chest Physicians/Society of Critical Care Medicine in 1991 [17] were recruited to this study within 24 hours of admission. Patients on any medication likely to affect coagulation such as any anticoagulant, antiplatelet or blood component replacement therapy were excluded. Patients with any history of disease that affects the coagulation profile (malignancy, renal failure, liver disease, thromboembolic disease)

were excluded. Patients that were deemed insensitive to include i.e. impending death were also excluded from the study.

At the time of recruitment, patients were categorised as sepsis, severe sepsis or septic shock. Categorisation was blinded, and carried out by an intensive care specialist independent of the study. Definitions were followed as outlined by the American College of Chest Physicians / Society of Critical Care Medicine in 1991.[17] Sepsis was defined as two or more of the SIRS criteria, with evidence of infection, severe sepsis was defined as sepsis with acute organ dysfunction of at least one organ and septic shock was defined as sepsis with perfusion abnormality (lactate > 2 mmol/L) and refractory hypotension in the absence of cardiogenic shock or bleeding. All patients were defined at point of entry.

A group of healthy volunteers matched for age, gender and from a demographically similar population group was recruited as a healthy control.

Blood Sampling

Blood was sampled at the time of inclusion to the study, and the first 5mls of blood was always discarded. Blood was drawn into 3.2% sodium citrate vacutainers (Greiner Bio-One GmbH, Austria, REF: 454327) to assess standard coagulation markers (aPTT, PT, Fibrinogen) and ROTEM thromboelastometry. A further blood sample was drawn into EDTA vacutainers (Becton, Dickinson and Company, UK, REF: 367839) to assess platelet count, white blood count and haemoglobin.

Laboratory Markers

Standard coagulation markers (aPTT (intrinsic), PT (extrinsic) and fibrinogen) were determined using a Sysmex CA1500 automated analyser and platelet count, white blood count and haemoglobin was determined using a Sysmex XE 2100 automated haematology analyser. All samples were analysed within 2 hours of collection.

Rotational Thromboelastometry

Thromboelastometry was performed using a ROTEM delta whole blood haemostasis system. Blood coagulation was assessed with ROTEM by both intrinsic (INTEM) and extrinsic (EXTEM) activation. Tests were carried out in accordance with the manufacturer's recommendations using disposable cups and pins (Cup and pin pro, Tem Innovations GmbH, Martin-Kollar-Str, Germany, REF: 200011). The automated pipetting system was used to recalcify and activate the blood with the appropriate reagents for each test. 20µl CaCl₂ 0.2M, STARTEM reagent (Tem innovations GmbH, Munich, Germany, REF: 503-10) and 20µl thromboplastin-phospholipid INTEM reagent (Tem innovations GmbH, Munich, Germany, REF: 503-02) were added to 300µL of blood for the INTEM test. 20µL CaCl₂ 0.2M STARTEM reagent and 20µL tissue factor EXTEM reagent (Tem innovations GmbH, Munich, Germany, REF: 503-05) were added to 300µL blood for the EXTEM test. The Clotting Time (CT), Maximum Clot Firmness (MCF) and Lysis Index at 60 minutes (LI60) were recorded to assess the kinetic, structural and fibrinolytic aspects of clot development.

Statistical Analysis

All statistical analysis was carried out on IBM Statistical Package for Social Sciences (SPSS) for Windows, version 22.0 (Armonk, NY: IBM Corp.). Values are reported as mean and standard deviation or median and interquartile range where appropriate. Pearson's chi-squared test was used to assess differences in the frequency distribution of nominal parameters across groups. Student's T-test was used to assess differences between two normally distributed groups whereas Kruskal-Wallis was used to determine differences between two non-normally distributed groups. One-way ANOVA was used to assess differences in normally distributed data between more than two groups, and Kruskal-Wallis was used to assess differences between more than two non-normally distributed groups. Data normality was assessed using Shapiro-Wilk test with an α value of 0.05. Bonferroni corrected posthoc analysis was used to assess multiple comparisons across groups.

Results

Patient Characteristics

100 sepsis patients in total were included in the study. This included 50 patients with sepsis, 20 with severe sepsis and 30 with septic shock. The screening 44 healthy volunteers matched for gender and age were also recruited as a healthy control group. Patient groups were matched for age, gender and source of infection. SOFA score, hospital length of mortality and 28 day mortality were all significantly increased with increasing severity of group ($p < 0.05$). Information on patient characteristic and demographics can be found in Table 1.

Changes in Standard Markers of Coagulation

The results of the standard markers of coagulation are shown in Table 2. Performing multiple comparison tests using Bonferroni corrected post hoc analysis we found that standard kinetic markers of coagulation PT and aPTT were significantly prolonged in septic shock ($p < 0.001$) when compared to all other groups. PT was also significantly prolonged in severe sepsis compared to the healthy group ($p < 0.005$). An increased fibrinogen concentration was observed in sepsis and severe sepsis ($p < 0.001$) consistent with the underlying inflammatory response of the disease process. In the septic shock group, although fibrinogen concentration was significantly lower than in sepsis ($p < 0.01$), it remained significantly higher than in the healthy group ($p < 0.05$). A significantly reduced platelet count was observed in patients with septic shock compared to all other groups ($p < 0.05$).

Haemoglobin was significantly lower in the septic shock group when compared to all other groups ($p < 0.05$). The healthy value of haemoglobin was significantly increased when compared to the sepsis and severe sepsis groups ($p < 0.05$). A significantly different White blood count was observed for the healthy patients when compared to all stages of sepsis ($p < 0.001$), however, no significant differences were observed between the different sepsis groups. We found that the healthy group had a significantly higher value of haemoglobin than the sepsis groups ($p < 0.001$) and the sepsis and severe sepsis groups had significantly higher values than the septic shock group ($p < 0.01$). We found no significant difference between the C-reactive protein results between the sepsis groups.

Changes in Thromboelastometry Measurements (ROTEM) across the Sepsis Spectrum

Baseline ROTEM measurements CT, MCF and LI60 are shown in Figure 1. A significant trend towards prolongation of EXTEM clotting time (CT) was observed with increasing severity of stage ($p < 0.001$). Overall a hypercoagulable effect was observed in sepsis and severe sepsis, with a significant increase in the clot firmness and rate of formation. Furthermore, EXTEM clot formation time (CFT) was significantly shortened in the sepsis group compared to the healthy control (62.5 ± 15.3 vs 81.8 ± 18.3 ($p = 0.004$)). In septic shock, ROTEM measurements indicated normal but delayed clot formation and significantly impaired fibrinolysis ($p < 0.001$).

Comparison between Survivors and non-Survivors at 28 Days

All patients were followed up for all cause 28-day mortality. Characteristics of survivors and non-survivors at 28 days are shown in Table 3. A significant trend towards hypocoagulability was observed in non-survivors compared to survivors. Poor outcome was also associated with prolonged EXTEM CT and impaired fibrinolysis, as indicated by significantly increased LI60. Structural ROTEM parameters (MCF, α) were not significantly different in patients that did not survive 28 days.

Discussion

In this study for the first time several ROTEM parameters were determined against severity of stage of sepsis across the whole sepsis spectrum as a diagnostic test in the emergency setting at time of presentation. In this single study we demonstrated that in the earlier stages of sepsis progression (sepsis and severe sepsis), ROTEM was more sensitive in detecting a hypercoagulable phase when compared to standard markers of coagulation, indicating enhanced clot development in sepsis and severe sepsis when compared to the standard healthy control group and normal ROTEM ranges. These findings provide more evidence that a clear hypercoagulable state is associated with sepsis, with increased clot mass and viscoelasticity (MCF), as has been shown in previous studies [1, 18, 19]. Although there was no change in the initial time to clot formation (CT), the clot formation time (CFT) remained shortened in the earlier stages of sepsis indicating enhanced clot mass formation, suggesting altered mechanical clot properties. These findings were also reflected by a rapid increase in clot mass development, as measured by the α angle, and higher resultant MCF possibly indicative of the hypercoagulable phase and potential thromboembolic risk in the earlier stages of sepsis and severe sepsis as noted in previous studies [3]. In the later stage of septic shock both the CT and CFT were prolonged, indicative of a hypocoagulable phase. However, despite a prolongation of the time based ROTEM markers, both the alpha angle and MCF remained within the normal range in septic shock, indicating normal clot development and mass as indicated by all structural markers of ROTEM thromboelastometry. This is indicative of a normally formed clot in septic shock, despite there being significant alterations in clotting time and fibrinolysis and is in contrast to recent studies which have specifically measured new markers of clot elasticity and strength [19]. These studies have shown that clot strength was significantly weakened when going from severe sepsis to the septic shock phase which was thought to be due to the effect on the mechanical strength by alteration of the cross-linked fabric leading to a looser, weaker clot. Furthermore, an increase in kinetic time in septic

shock was mirrored by increases in the aPTT and PT, both of which were prolonged. Although ROTEM parameters in the earlier phases of sepsis indicated a hypercoagulable effect with increased clot mass, surprisingly there was no change in clot formation or clot mass in the septic shock group, despite a profound effect of a decrease in the kinetic activity of clot formation. This may be partly explained by there being an elevated fibrinogen concentration and a normal platelet count.

Markers of fibrinolysis remained unchanged in the earlier phases of sepsis, but there was pronounced fibrinolytic activity and impaired fibrinolytic function in the septic shock group as measured by D-dimers and LI60 respectively. Although fibrinolytic function was impaired, D-dimer concentration indicated an increase in the fibrinolytic activity in septic shock. This suggests the ongoing intravascular fibrin formation (consumption), which could contribute to the impairment of the fibrinolytic and procoagulant systems. Previous studies have suggested that fibrinolysis is regulated in part by clot structure [20, 21], with denser, more elastic clots being more difficult to lyse. However, the results herein do not support this, as ROTEM indicated impaired fibrinolysis in weaker clots associated with septic shock. This again suggests the overall effects on coagulation could possibly be due to the overall consumption of procoagulant and fibrinolytic proteins that inevitably increases with increasing severity of sepsis, leading to an overall impairment of both clot development and breakdown. It has been shown previously that fibrinolytic shutdown is also significantly associated with increased mortality in trauma patients, highlighting the complexity of coagulation in critical illness.[22]

Previous studies have attempted to investigate this impairment of fibrinolysis in septic shock by measuring individual concentrations of fibrinolytic proteins in sepsis, and found that a reduced plasminogen concentration was present in patients with severe sepsis/septic shock [2]. Furthermore, studies have shown there is a reduced tPA and PAI-1 concentration [23, 24, 25], which would suggest the fibrinolytic system is progressively overloaded as activation of the coagulation system is enhanced, which could help to explain the underlying development of DIC in sepsis. These

mechanisms could help to explain the progressively increased risk of both bleeding and thrombosis that are observed with increasing severity of sepsis.

In this study ROTEM gave some understanding and new information in profiling in the earlier stages of sepsis, indicating a hypercoagulable phase, but there was no consistent marker to indicate the progression from the early stages from sepsis to septic shock. Although ROTEM gave us some information regarding the kinetic information (time-based markers) and clot rigidity, ROTEM does not provide a measurement of elasticity, and even Newtonian fluids have been shown to provide substantial readings using thromboelastographic techniques [26].

Although ROTEM has been utilised in the diagnosis of haemorrhage blood loss and component replacement in major haemorrhage associated with surgery [27–29] or trauma [30, 31], no studies have yet determined its usage as a marker of hyper and hypocoagulable states in patients attending the emergency department who were subsequently admitted to the ward or ICU. Although ROTEM determined a hypercoagulable phase indicative of an increased clot mass in sepsis, it did not differentiate between or show major change in clot mass in those patients that presented with septic shock. What remains uncertain is why ROTEM defines patients in septic shock still produce a normal profile of a clot. It may be that the measurement of clot development and strength is in mm, which is an arbitrary description, and not an exact scientific marker of elasticity or true clot strength.

Further larger studies are required to ascertain the potential clinical utility of ROTEM within the emergency setting. Although ROTEM shows a normal MCF trace in septic shock, this may not reflect accurately the viscoelastic change, as determined previously. Further work is needed to determine the role that sepsis plays in altering the viscoelastic profile and its relationship to disease severity and its effect on clot quality. There may very well be a discrepancy between clot mass, as measured by ROTEM and final clot strength in terms of its elastic mechanical properties, which warrants further studies to determine this relationship.

Study Limitations

As with other similar studies involving critically ill patients, this study has a number of limitations. It is difficult to account for comorbidities between the groups, and there could also be differences in concomitant medications. In an attempt to address this, strict inclusion/exclusion criteria were adopted. Furthermore, although several results were significant, this study was not powered for outcome.

Conclusions

ROTEM indicated enhanced clot mass and development in sepsis and severe sepsis, consistent with a hypercoagulable phase and increased thrombotic risk. However, in septic shock, despite prolongation of laboratory markers and clotting time, ROTEM indicated a normal clot mass and development consistent with a normocoagulable state. Despite the development of a normal clot mass in septic shock, ROTEM indicated an impairment of fibrinolysis, which could potentially lead to increased thrombotic risk, despite the lack of a perceived hypercoagulable state.

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Tables

Table 1: Baseline characteristics of healthy volunteers and sepsis patients

	Healthy	Sepsis	Severe Sepsis	Septic Shock	Significance Value
N	44	50	20	30	
Age (years)	60.8 ± 18.6	59.6 ± 19.8	66.3 ± 17.4	66.6 ± 14.9 0.268	
Male Gender (n[%])	22 [50]	24 [48]	9 [45]	18 [60]	0.693
Source of Infection					0.398
<i>Respiratory</i>	-	29 [58]	12 [60]	11 [37]	
<i>Urinary Tract</i>	-	8 [16]	4 [20]	7 [23]	
<i>Abdominal</i>	-	9 [18]	1 [5]	9 [30]	
<i>Soft Tissue</i>	-	2 [4]	1 [5]	0 [0]	
<i>Other</i>	-	2 [4]	2 [10]	3 [10]	
SOFA Score	-	3 (1, 3.5)	5 (4, 6)	9.5 (6.75, 12)	<0.001
Hospital Length of Stay (days)	-	6 (2, 9)	9 (5, 18)	15 (4, 38)	0.006
28 Day Mortality (n [%])	-	1 [2]	3 [15]	13 [43]	<0.001

Table 2: Changes in standard markers of coagulation and ROTEM parameters (CFT and Alpha Angle) across the sepsis spectrum. Standard laboratory markers of coagulation and ROTEM parameters are shown for the different sepsis groups. Data presented as mean \pm SD. Significance values indicated (One-way ANOVA).

	Healthy	Sepsis	Severe Sepsis	Septic Shock	Significance Value
PT (sec)	10.5 \pm 0.6	11.0 \pm 1.0	11.5 \pm 1.2	13.3 \pm 2.3	<0.001
aPTT (sec)	25.4 \pm 2.0	26.6 \pm 3.1	29.1 \pm 6.7	35.6 \pm 8.9	<0.001
Fibrinogen (g/L)	3.1 \pm 0.5	4.6 \pm 0.6	4.5 \pm 1.0	3.7 \pm 1.6	<0.001
Platelets ($\times 10^9/L$)*	231 (205, 299)	245 (221, 334)	234 (185, 337)	155 (102, 249)	<0.001
White Blood Count ($\times 10^9/L$)*	6.7 (5.1, 7.9)	18.0 (14.4, 21.4)	17.5 (10.6, 19.8)	11.4 (7.3, 22.1)	<0.001
CRP (mg/dL)	-	79 (39.5, 221.5)	193 (124, 422)	127.5 (21.0, 127.5)	0.078
Haemoglobin (g/dL)*	14.1 (13.4, 15.0)	12.7 (11.9, 14.6)	12.3 (12.3, 13.6)	10.6 (9.1, 11.6)	<0.001
ROTEM EXTEM					
CFT (sec)	80 (69, 96)	61 (51, 75)	66 (56, 73)	85 (67, 103)	<0.001
Alpha (°)	77 (71, 79)	78 (76, 80)	77 (76, 79)	75 (70, 76)	<0.001
ROTEM INTEM					
CFT (sec)	64 (57, 77)	58 (48, 63)	58 (56, 67)	73 (59, 89)	<0.001
Alpha (°)	77 (74, 78)	78 (77, 81)	78 (77, 78)	75 (72, 79)	<0.001

*not normally distributed represented as a median and interquartile range)

Table 3: Comparison of ROTEM parameters in survivors and non-survivors. ROTEM parameters in sepsis patients that survived and did not survive 28 days are shown. Data presented as mean \pm SD, median (IQR). Comparisons made using students t-test or Kruskal-Wallis test, with respective significant values indicated.

	Survived (n=83)	Died (n=17)	Significance Value
ROTEM EXTEM			
CT (sec)*	62.0 (53.2, 70.5)	75.5 (58.3, 101.0)	0.032
MCF (mm)	69.6 \pm 7.0	63.9 \pm 13.5	0.215
Alpha (°)*	55.00 (31.0, 64.00)	66.5 (38, 78.5)	0.228
LI60 (%)*	94 (90.25, 96)	95.5 (92.5, 98)	<0.001
ROTEM INTEM			
CT (sec)*	156.0 (137.0, 189.5)	178.0 (135.3, 210.3)	0.623
MCF (mm)	69.1 \pm 7.2	64.8 \pm 14.3	0.405
Alpha (°)*	60.0 (50, 67.5)	66.0 (55.0, 116.3)	0.305
LI60 (%)*	94 (90, 96)	96 (94.75, 98)	<0.001

*not normally distributed represented as a median and interquartile range)

Figure Legend.

Figure 1: Changes in the different ROTEM parameters across the sepsis spectrum. ROTEM markers of coagulation across the sepsis and in the healthy control group are shown. Data presented as box plots (median and IQR) with whiskers representing the range. Healthy reference ranges as recommended by ROTEM are also shown on each graph respectively. Significant differences between groups assessed using Kruskal-Wallis test with multiple comparisons (Bonferroni correction).

