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Thromboelastometry in critically ill patients with disseminated intravascular coagulation

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Coagulopathy has a high incidence in critically ill patients and is often caused by disseminated intravascular coagulation (DIC). Although the clinical picture of DIC ranges from a prothrombotic state to severe consumption coagulopathy with an increased bleeding tendency, there are no clinical tests that reflect of in-vivo hemostatic profile. Rotational thromboelastometry (ROTEM) may be able to indicate whether a patient has a hypocoagulable or hypercoagulable profile and possibly be able to discriminate patients with and without DIC. The aim of this article was to study the diagnostic ability of thromboelastometry to detect DIC. A predefined subgroup analysis of a clinical trial in critically ill patients with a coagulopathy was done. ROTEM and markers of coagulation and levels of natural anticoagulants were measured in patients with and without DIC. Twenty-three patients were included, 13 fulfilled criteria for overt DIC. Patients with DIC had lower platelet count, lower levels of fibrinogen, factors II, VII and VIII compared with those without DIC. Antithrombin, protein C and S were also reduced in DIC patients. Receiver operator characteristic analyses showed that EXTEM CFT, alpha angle and MCF were capable of discriminating patients with and without DIC. Combination of ROTEM values with protein C or antithrombin further improved discriminatory ability. In patients with DIC, thromboelastometry profiles were more hypocoagulable compared with those without DIC. ROTEM correlates well with ISTH DIC score, diagnostic strength improves when ROTEM values are combined with antithrombin or protein C levels. Thereby, ROTEM may be a useful tool in diagnosing DIC in the critically ill. *Blood Coagul Fibrinolysis* 30:181–187

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Background

Coagulopathy, defined as prolonged prothrombin time or increased international normalized ratio (INR), has a high prevalence in critically ill patients [1]. An important cause of abnormal coagulation test results in critically ill patients is the development of disseminated intravascular coagulation (DIC). DIC is defined as 'an acquired syndrome characterized by intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe, can lead to organ dysfunction' [2]. The clinical picture of DIC ranges from a prothrombotic state with (micro)vascular thrombosis to severe consumption coagulopathy, which can present with bleeding. Presence of DIC is also associated with occurrence of multiple organ failure and adverse outcome [3]. It is important to diagnose DIC appropriately because of the prognostic considerations, in addition to specific treatments, such as soluble thrombomodulin, which may be of benefit in these patients [4,5].

The diagnosis of DIC is made by an aggregate score of various laboratory features, including prothrombin time prolongation, platelet count, fibrinogen level and a marker of fibrin degradation products [6]. However, the conventional coagulation tests used for the diagnosis of DIC lack the ability to discriminate between patients with a prothrombotic state and those with consumption coagulopathy and an increased bleeding tendency. Furthermore, the used tests lack sensitivity and specificity [7].

In recent years, there has been a growing interest in rotational thromboelastometry (ROTEM), a point of care test evaluating whole clot formation and degradation. ROTEM may be of use to detect DIC in the critically ill and may be able to indicate whether a patient has a hypocoagulable or hypercoagulable profile [8,9]. Therefore, we aimed to study the diagnostic ability of thromboelastometry (ROTEM) to detect DIC compared with currently used haemostatic tests as well to experimental markers of DIC.

Methods

Setting and patients

The study was performed as a predefined posthoc substudy of a clinical trial on the efficacy of FFP transfusion

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in critically ill patients with a coagulopathy [10,11]. Patients were randomized to either receiving or not receiving a 12 ml/kg of FFP transfusion before an intervention. Patients were eligible if INR was greater than 1.5 and less than 3.0. Patients with clinically overt bleeding or with a thrombocytopenia less than 30×10^9/l were excluded from participation. Patients using platelet aggregation inhibitors, low molecular weight heparin in a therapeutic dose, vitamin K antagonists, activated protein C or prothrombin complex concentrates were also excluded. Patients treated with heparin were eligible if medication was discontinued for at least 1 h. All patients of whom baseline ROTEM values were available, were included.

The study was conducted in a mixed-medical surgical ICU in a university hospital, in Amsterdam, the Netherlands in accordance with the Declaration of Helsinki. The protocol was approved by our Institutional Review Board (MEC 2010_035). Written informed consent was obtained from patients or legal representative before entry in the study.

Data collection

Baseline data included demographics, admission reason, APACHE IV and SOFA score and medical history. Disseminated intravascular coagulation was assessed using the International Society of Thrombosis and Haemostasis (ISTH) DIC score, which defines DIC as a score of at least 5 points [2]. Blood samples were collected at baseline. In the current study, only samples drawn at $T = 0$ (before plasma transfusion) were used.

Coagulation assays

Routine coagulation tests included INR, APTT, fibrinogen, d-dimer levels (Sysmex CA 7000 and all reagents, Siemens Healthcare Diagnostics; Instrumentation Laboratory; DRG Diagnostics, Erlangen, Germany) and platelet count. In addition, levels of coagulation factors II, V and VII were assessed using a one-stage clotting assay according to manufacturers' instruction (ACL TOP 700, Instrumentation Laboratory, Bedford, MA, USA).

Antithrombin was assessed by chromogenic substrate method (Sysmex CA 7000, Siemens Healthcare Diagnostics) with reagents and protocols of the manufacturer.

Thrombin-antithrombin (TATc) and prothrombin fragment 1 +2 (F1+2) levels were measured using specific commercially available ELISAs according to the instruction of the manufacturer (Siemens Healthcare Diagnostics). Plasmin-α2-antiplasmin complex (PAP) levels were also measured by a specific commercially available ELISAs according to the instruction of the manufacturer (DRG Diagnostics; Marburg, Germany). Protein C activity was assessed by a kinetic assay (Coamatic Protein C, Chromogenix, Mölndal, Sweden) and protein S levels were determined by ELISA, as described previously [12].

Rotational thromboelastometry measurements

Using ROTEM (Tem International, Munich, Germany), three separate assays were performed, including EXTEM to assess tissue factor-initiated coagulation, INTEM to assess the intrinsic pathway and FIBTEM to qualitatively assess fibrinogen status. For EXTEM, 20 μl of 0.2 mol/l $CaCl_2$ (star-tem) and 20 μl of recombinant tissue factor (*r* EXTEM) were added to a test vial, subsequently 300 μl of the citrated blood sample was added. For the INTEM test 20 μl of 0.2 mol/l $CaCl_2$ (star-tem), 20 μl of partial thromboplastin made of rabbit brain (in-tem) and 300 μl of blood were added to the test cuvette. FIBTEM test was carried out by adding 20 μl of recombinant human tissue factor (r EXTEM), 20 μl of platelet inactivating cytochalasin D solution 0.2 mol/l $CaCl_2$ and 300 μl of the blood sample to the test vial. The electronic pipette program guided all test steps. For INTEM and EXTEM clotting time, clot formation time (CFT), clot firmness (MCF), alpha angle and maximum lysis were recorded. For the FIBTEM assay clotting time and MCF were recorded. All treating physicians were blinded for ROTEM results.

Endothelial markers

Von Willebrand factor antigen (vWF:Ag) levels were determined with an in-house ELISA using commercially available polyclonal antibodies against von Willebrand factor (vWF) (Dako, Glostrup, Denmark). ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity was determined as described earlier [13]. Syndecan-1 levels were determined using a commercially available Syndecan-1 (CD138) ELISA kit (IBL international GmbH, Hamburg, Germany).

Statistical analysis

All variables are expressed as median (interquartile ranges). To compare groups Mann–Whitney was used for independent variables. Correlations were assessed using Spearman's rho. To assess value of ROTEM variables to diagnose DIC, receiver operating characteristic (ROC) curves were used, for the combined tests, results of a generalized linear model were used as input for the ROC curves. Results are presented as area under the curve (AUC) and 95% confidence interval (CI), optimal cut-off values and likelihood ratios were determined using the Youden index for each parameter. A P value less than 0.05 was considered significant. Statistical analyses were performed with SPSS 20.0 (SPSS Inc, Chicago, Illinois, USA) and *R* statistical computing and the *R* studio intergace (version 3.3.3, Vienna, Austria, http://www.R-project.org). Prism Version 5.0 (Graphpad Software, San Diego, USA) was used to design the graphs.

Table 1 Characteristics of critically ill patients with and without disseminated intravascular coagulation

	DIC ($N=13$)	No DIC ($N=10$)	P value
General characteristics			
Sex, male, % (*n*)	54 (7)	50 (5)	0.86
Age (years)	61 (45–68)	61 (46–67)	0.95
APACHE IV score	116 (100–135)	107 (81–136)	0.72
SOFA score	15 (14–16)	12 (6–14)	0.08
Sepsis, % (*n*)	69 (9)	50 (5)	0.86

Age, APACHE IV and SOFA scores are expressed as median and interquartile ranges. APACHE, Acute Physiology and Chronic Health Evaluation; DIC, disseminated intravascular coagulation; SOFA, Sequential Organ Failure Assessment.

Results

Patients

Of the 81 patients included in the TOPIC trial, ROTEM data were available for 23 patients. Of these, 13 fulfilled criteria for overt DIC, the majority was admitted to the ICU because of sepsis. Accordingly, patients were ill, as reflected by high disease severity scores (Table 1).

Assessment of coagulation and endothelial status

Patients with DIC had lower platelet counts and lower levels of fibrinogen, factors II, VII and VIII compared with those without DIC. Natural occurring anticoagulants, including antithrombin, protein C and S were also reduced in DIC patients. Patients with DIC had higher TATc and F1+2 levels compared with those without DIC, indicating an activated coagulation system; however, this difference was not statistically significant. Reduction of ADAMTS-13 activity was more outspoken in the patients with DIC. Endothelial marker Syndecan-1 did not differ between both groups. Coagulation profiles of patients with and without DIC are depicted in Table 2.

Table 2 Coagulation status in patients with and without disseminated intravascular coagulation

	DIC ($N=13$)	No DIC ($N=10$)	P value
INR	2.2 (1.6–2.7)	1.6 (1.5–1.6)	<0.01
APTT (s)	43 (39–61)	39 (33–48)	0.17
Platelet count $\times 10^9$/l	47 (34–60)	162 (71–314)	<0.01
Fibrinogen (g/l)	2.1 (1.7–2.4)	4.7 (3.2–5.8)	<0.01
D dimer (mg/l)	10.6 (4.2–20.9)	8.1 (2.3–12.5)	0.28
Factor II (%)	28 (17–41)	45 (35–54)	0.02
Factor V (%)	35 (17–48)	44 (29–74)	0.21
Factor VII (%)	19 (10–36)	33 (24–53)	0.03
Factor VIII (%)	232 (152–189)	356 (206–525)	0.02
Antithrombin (%)	41 (22–54)	57 (36–85)	0.04
Protein C (%)	25 (20–34)	42 (30–77)	<0.01
Total protein S (%)	37 (31–48)	62 (41–76)	0.02
TATc (μg/l)	20 (13–31)	8 (4–35)	0.26
F1+2 (pmol/l)	613 (195–1135)	227 (51–517)	0.08
PAP (μg/l)	819 (418–1320)	1070 (295–1218)	0.87
ADAMTS-13 (%)	15 (6–17)	26 (15–42)	<0.05
vWF antigen (%)	535 (277–632)	493 (355–820)	0.35
Syndecan-1 (ng/ml)	944 (717–1754)	600 (90–1032)	0.34

APTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation; INR, international normalized ratio.

Diagnostic accuracy of rotational thromboelastometry to detect disseminated intravascular coagulation

ROC analyses were performed to investigate the value of ROTEM variables for the diagnosis of DIC. ROC curves for EXTEM CFT, CT, alpha and MCF are shown in Figure 1. Comparison of EXTEM CFT, alpha and MCF in patients with and without DIC showed that all three variables were capable of detecting differences between these groups with high accuracy (Table 3). When variables of the ROTEM were combined with or without the addition of protein C or antithrombin, the AUC further improved (Table 3 and Figure 2).

Thromboelastometry in patients with overt disseminated intravascular coagulation

In patients with DIC, thromboelastometry profiles were more hypocoagulable compared with those without DIC. EXTEM CT: 83 [57–102] sec. in DIC vs. 70 [47–93] sec. in non-DIC patients ($P=0.39$), EXTEM CFT: 183 [142–240] sec. in DIC vs 72 [45–177] sec. in non-DIC patients ($P<0.05$), alpha angle: 60 [51–69] degrees in DIC vs. 79 [66–81] degrees in non-DIC patients ($P<0.01$) and MCF: 49 [43–58] mm in DIC vs. 63 [58–73] mm in non-DIC patients ($P<0.05$) (Figure 3). No differences were observed in lysis index, EXTEM ML 0 [0–5]% in DIC patients vs. 1.5 [0–5]% in non-DIC patients. INTEM profiles showed similar results. FIBTEM MCF was reduced in patients with DIC: 13 [7–16] mm vs. 30 [23–59] mm in non-DIC patients ($P<0.05$).

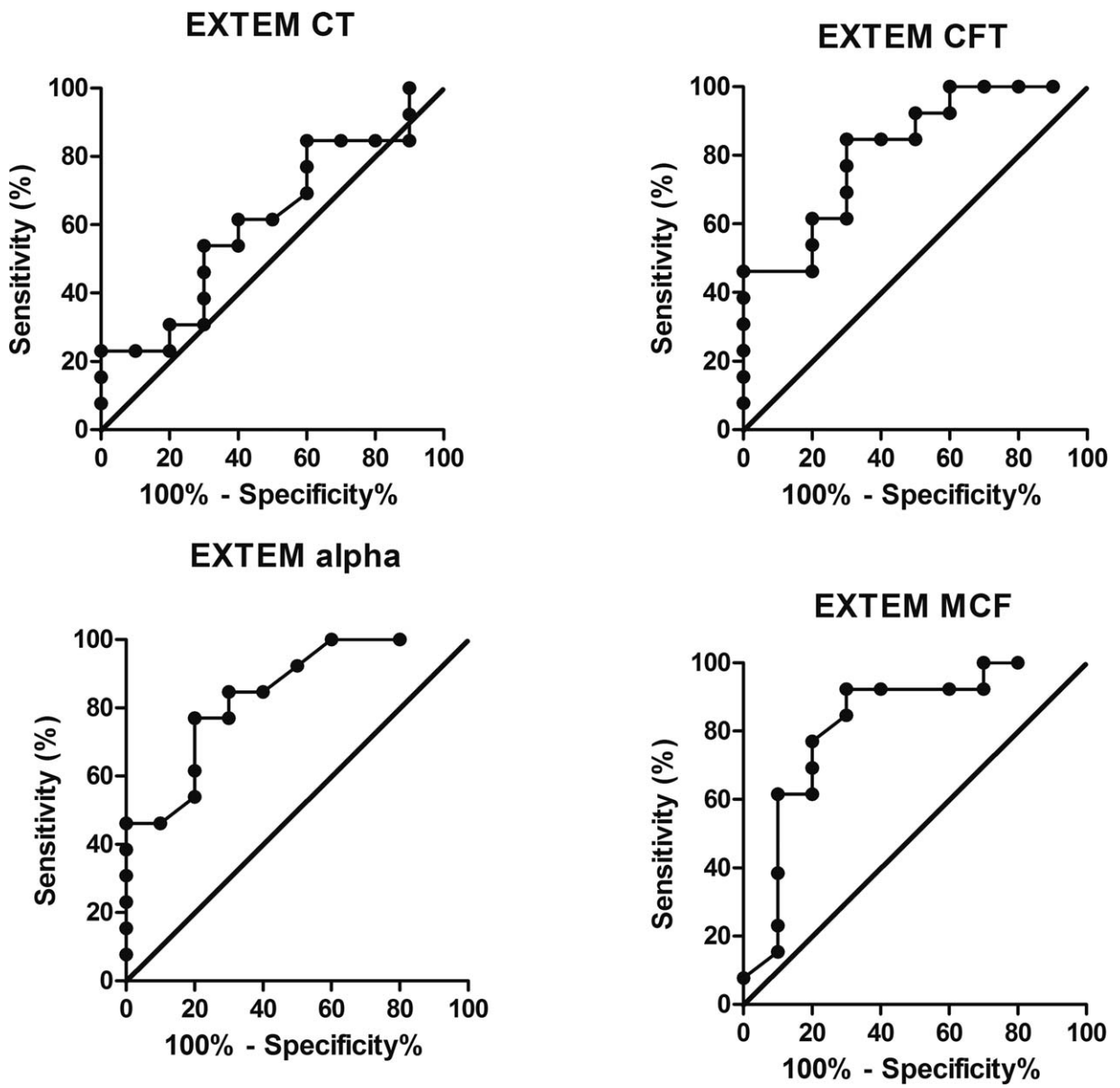
Correlation of thromboelastometry with conventional and experimental markers of disseminated intravascular coagulation

EXTEM variables showed good correlations with platelet count and fibrinogen but not with INR or APTT. EXTEM CFT, alpha angle and MCF correlated modestly with factor II levels but not with other factor levels (Table 4). ROTEM CFT, alpha angle and MCF showed good correlation with protein C levels and CFT and alpha angle correlated moderately with antithrombin levels (Table 4). Lysis indexes did not correlate with markers of fibrinolysis such as D-dimer and plasmin-antiplasmin levels (data not shown). Nor were ROTEM variables correlated with markers of endothelial activation.

Discussion

The current study demonstrates that ROTEM has the ability to discriminate patients with and without DIC, in particular when multiple variables are combined. Overall, ROTEM profiles are more hypocoagulable in patients with DIC compared with those without DIC. ROTEM EXTEM CFT, alpha angle and MCF correlate strongly with DIC score, in addition results correlate well with factor II and the anticoagulant protein C. Correlation with the widely used INR is less clear.

Fig. 1



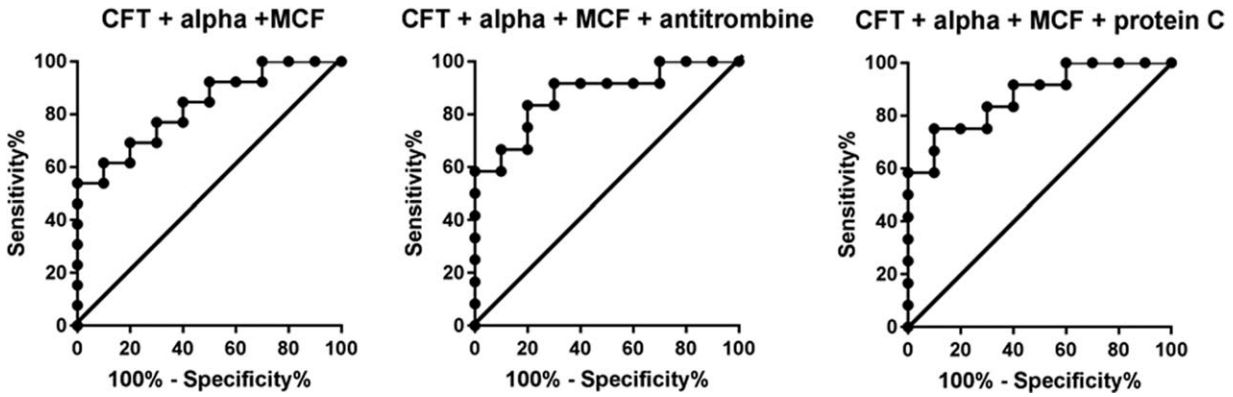
Receiver operating characteristic curves for EXTEM clotting time, clot formation time, alpha and maximum clot firmness for the diagnosis of disseminated intravascular coagulation in critically ill patients with a coagulopathy.

Table 3 Results of receiver operating characteristics curve analysis for EXTEM variables, antithrombin and protein C in patients with and without disseminated intravascular coagulation

	AUC	95% CI	*P* value	Cut off	Sensitivity	Specificity	Likelihood ratio
EXTEM CFT	0.815	0.640–0.990	0.01	>119 s	85%	70%	2.8
EXTEM alpha angle	0.842	0.681–1.000	<0.01	<67°	77%	80%	3.9
EXTEM MCF	0.823	0.633–1.000	<0.01	<58 mm	77%	80%	3.9
Antithrombin	0.733	0.520–0.947	0.07	<50%	75%	70%	2.5
Protein C	0.838	0.665–1.000	<0.05	<27%	75%	90%	7.5
CFT+alpha+MCF	0.831	0.666–0.995	<0.01	NA	54%	100%	∞
CFT+alpha+MCF+antithrombin	0.875	0.728–1.000	<0.01	NA	83%	80%	4.2
CFT+alpha+MCF+protein C	0.875	0.732–1.000	<0.01	NA	75%	90%	3.8

CFT, clot formation time; CI, confidence interval; MCF, maximum clot firmness.

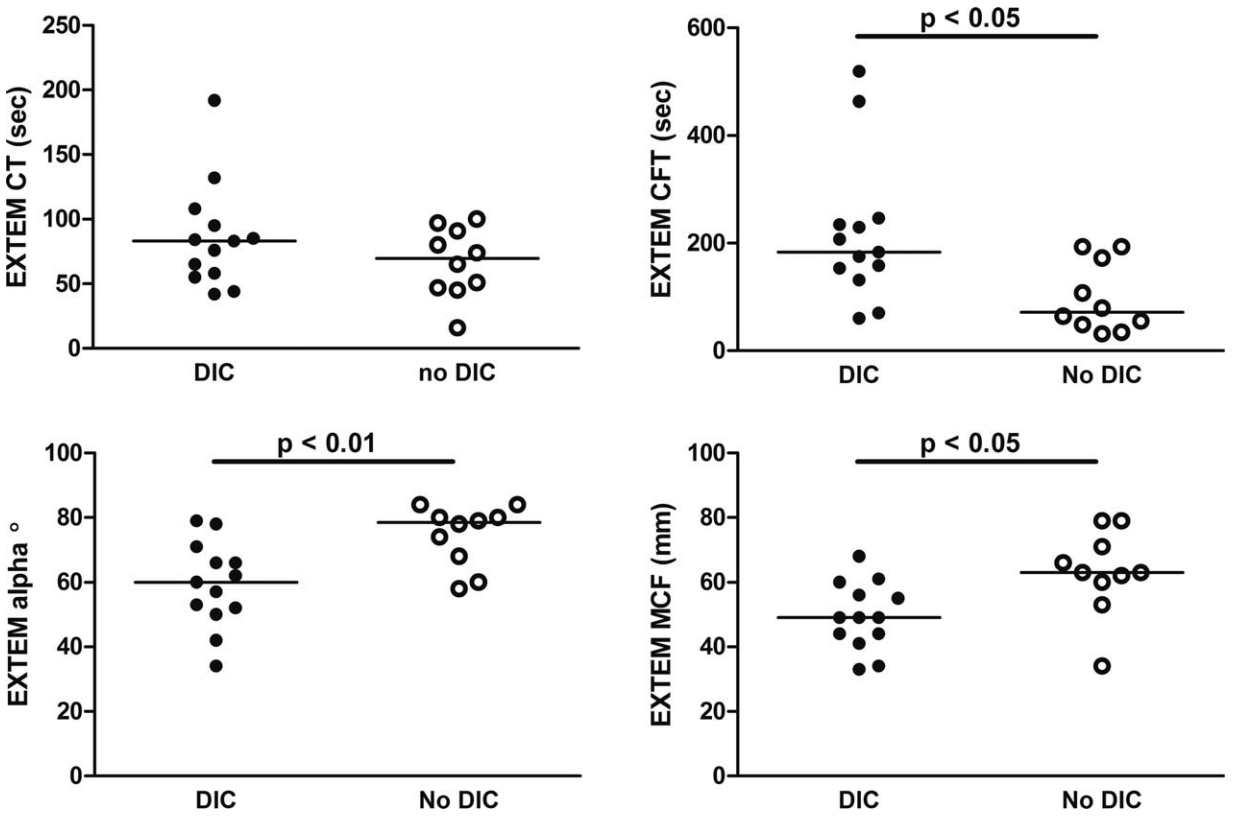
Fig. 2



Receiver operating characteristic curves for EXTEM clot formation time, alpha and maximum clot firmness with and without antithrombin and protein C, for the diagnosis of disseminated intravascular coagulation in critically ill patients with a coagulopathy.

ROTEM EXTEM variables correlated highly with the DIC score. Comparable observations have been reported in sepsis patients [8,14]. Similar to our findings, the combination of CFT, alpha angle and MCF values was able to discriminate between patients with and without DIC [8,15,16]. In addition, we found that the correlation between viscoelastic tests and DIC score is particularly clear once the patient has reduced fibrinogen levels and low platelet counts, both major contributors to viscoelastic test tracings. However, in our cohort, only two patients with DIC had substantially reduced fibrinogen levels (below 1 g/l), hereby fibrinogen hardly contributes to

Fig. 3



EXTEM variables in patients with and without disseminated intravascular coagulation.

Table 4 Correlation of EXTEM rotational thromboelastometry variables with coagulation tests at baseline

	CT	CFT	Alpha angle	MCF
DIC score	0.290	0.731**	−0.807**	−0.705**
INR	0.094	0.159	−0.162	0.280
Platelet count ($\times 10^9$/l)	−0.457*	−0.787**	0.755**	0.558**
Fibrinogen (g/l)	−0.124	−0.642**	0.648**	0.680**
D dimer (mg/l)	−0.114	0.216	−0.241	−0.207
Factor II (%)	−0.316	−0.527*	0.582**	0.497*
Factor V (%)	−0.219	−0.150	0.187	−0.067
Factor VII (%)	0.003	−0.184	0.221	0.412
Factor VIII (%)	−0.239	−0.348	0.396	0.413
Antithrombin (%)	−0.281	−0.505*	0.497*	0.299
Protein C (%)	−0.324	−0.626**	0.631**	0.563**
Total protein S (%)	−0.440*	−0.390	0.468*	0.258
TATc (μg/l)	0.154	0.424*	−0.421	−0.325
F1+2 (pmol/l)	−0.203	0.351	−0.277	−0.405
PAP (μg/l)	−0.312	−0.171	0.133	−0.095
ADAMTS-13 (%)	−0.490	−0.435	0.457	0.296
vWF antigen (%)	−0.073	−0.137	0.149	0.342
Syndecan-1 (ng/ml)	0.447	0.502*	−0.500*	−0.352

APTT, activated partial thromboplastin time; CFT, clot formation time; CT, clotting time; DIC, disseminated intravascular coagulation; INR, international normalized ratio; MCF, maximum clot firmness; ML, maximum lysis; NA, not applicable. * $P < 0.05$. ** $P < 0.01$.

the diagnosis of DIC made by the ISTH DIC score. This observation is in line with previous reports, showing that exclusion of fibrinogen from the ISTH DIC scoring system hardly affects the accuracy [17]. Of note, ROTEM did not correlate with d-dimer a pivotal component of the DIC score, hereby indicating that correlation of ROTEM and DIC score for the most part is determined by platelet count.

Our data demonstrate an enhanced discriminative power by the addition of antithrombin or protein C to ROTEM variables. As mentioned previously, current methods to diagnose DIC consist of scoring systems (JAAM and ISTH) that combine clinical parameters and estimates of thrombin generation. In these scores, the depletion of anticoagulant proteins is not recognized. Our results confirm that integrating reduced levels of the natural anticoagulants antithrombin and protein C, essential contributors to the pathophysiology of DIC, enhances diagnostic accuracy. Combination of ROTEM with these variables could therefore be a useful diagnostic test for DIC.

In patients with DIC, ROTEM CFT was prolonged and alpha angle and MCF were reduced compared with patients without DIC, indicative of a more hypocoagulable profile. EXTEM variables had a clear correlation with fibrinogen, factor II levels and platelet count in our cohort of patients, which is in line with reports in surgical patients [18] and sepsis patients [19]. A modest correlation between INR values and ROTEM was seen in the INTEM assay (data not shown), but not in the EXTEM, as reported previously [18]. Correlation of ROTEM with endothelial markers could not be demonstrated and while ADAMTS13 activity was reduced in patients with DIC, syndecan-1 and VWF did not differ between both groups. However, viscoelastic tests are not capable to detect changes in VWF and endothelial markers, therefore combination of the test with results of endothelial markers could be relevant. Remarkably, most results were within manufacturers reference ranges, which is in line with reports from patients with severe sepsis [9,19]. Thereby, it can be questioned whether reference values as validated in healthy volunteers for a single outcome should be used to diagnose hypocoagulability or hypercoagulability in the critically ill, or rather that new reference values should be formulated for specific profiles by combining several ROTEM values [15].

Our study has several limitations. First, group size is relatively small, although patient characteristics correspond well with those of larger cohort studies on thromboelastometry in the ICU [20] supporting the generalizability of our results on correlation between ROTEM and other haemostatic tests. Another limitation is test precision of ROTEM, which is a subject of debate with reported high coefficients of variance [21]. However, in order to limit variation as much as possible, all tests were carried out on the same device by only two experienced researchers. Finally, our study was substudy of a trial on the administration of FFP (12 ml/kg) to critically ill patients with a coagulopathy, because of the small group size we were not able to analyse the effect of FFP in patients with and without DIC appropriately. However, the limited data indicated that the effect of FFP transfusion on ROTEM variables and occurrence of bleeding did not differ among patients with and without DIC.

In conclusion, ROTEM correlates well with ISTH DIC score and diagnostic strength improves when various ROTEM values are combined with antithrombin or protein C levels. Thereby, ROTEM may be a useful tool in diagnosing DIC in the critically ill. As ROTEM parameters were mostly within reference ranges, further research is warranted enabling development of a thromboelastographic DIC score for critically ill with appropriate reference values. Hereby, ROTEM could provide valuable information on different components of the coagulation system in a bedside manner, abating the need to perform different assays to diagnose DIC.

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Authors contributions: M.M. and N.J. designed the study, acquired the data and analysed and interpreted the results. M.M. drafted the manuscript. J.M. performed the coagulation analyses, interpreted the results and critically reviewed the manuscript. D.v.M. performed statistical analyses and critically reviewed the manuscript. J.T. interpreted the results and critically reviewed the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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