Coagulopathy and plasma transfusion in critically ill patients

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Chapter 7

Correlation of thromboelastometry with conventional hemostatic tests in critically ill patients with a coagulopathy treated with fresh frozen plasma

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Abstract

Introduction: Increased INR values have a high prevalence among critically ill patients and are often a trigger to administer Fresh Frozen Plasma (FFP). However, ability of INR to accurately reflect hemostasis is limited. In contrast to INR, rotational thromboelastometry (ROTEM) assesses whole clot formation and degradation.

Aim: Assessing correlation of ROTEM with conventional hemostatic tests in critically ill patients with a coagulopathy and evaluating the response of thromboelastometry variables to a prophylactic transfusion of a fixed dose of FFP in these patients.

Methods: In a sub-study of a prospective trial evaluating the efficacy of FFP to prevent bleeding, ROTEM assays were performed in 16 critically ill patients with a prolonged INR (1.5-3.0) before and after transfusion of a fixed dose of FFP (12 ml/kg).

Results: At baseline, only INTTEM CFT was prolonged to 133 (51-162), while all other variables were within reference ranges. INR showed weak correlation with INTTEM CFT, and was not correlated with EXTEM CFT. In patients with disseminated intravascular coagulation (DIC), EXTEM CFT was increased (218 sec (154-409) vs. 60 sec (38-165), p=0.007), whereas alpha (55° (44-65) vs. 79° (70-83), p=0.003) and MCF (47 mm (36-49) vs. 65 mm (55-77), p=0.01) were decreased compared to those without DIC, indicative of a hypocoagulable state. Also, CFT, MCF and alpha correlated well with platelet count, fibrinogen and factor II levels, as well as with DIC score. EXTEM CFT, alpha and MCF had high accuracy in discriminating patients with and without DIC. Transfusion of FFP decreased EXTEM CT (70 (46-94) before vs. (58 (46-82) after FFP, p=0.04) and MCF (51 mm (43-65) before FFP vs. 55 (47-75) after FFP, p=0.04) and decreased FIBTEM CT (66 sec (50-96) before vs. 49 (40-66) after FFP, p=0.01).

Conclusion: ROTEM parameters were mostly within reference ranges and correlated only modestly with INR, but did correlate with platelet count, fibrinogen and factor II levels. Patients with DIC had more hypocoagulable profiles and ROTEM could be useful to diagnose DIC. Transfusion of FFP improved EXTEM CT and MCF.
Background

Coagulopathy, defined as prolonged prothrombin time (PT) or increased International Normalized Ratio (INR), has a high prevalence in critically ill patients [1]. Audits of transfusion practice show that clinicians consider increased INR and PT values as indicators of increased bleeding risk and thereby they are an important trigger to transfuse Fresh Frozen Plasma (FFP), in particular in patients undergoing invasive procedures [2,3]. However, despite the widespread use of INR and PT to detect coagulopathy, these tests do not accurately reflect \textit{in vivo} hemostatic potential [4], which may be due to the fact that only a part of the coagulation cascade is assessed. Essential contributors to hemostasis, such as platelet count and levels of endogenous anticoagulants, both of which are often decreased in the critically ill, are not accounted for [5,6].

An important cause of abnormal coagulation test results in critically ill patients is the presence of disseminated intravascular coagulation (DIC). The clinical picture of DIC ranges from severe consumption coagulopathy with increased bleeding tendency to a prothrombotic state with (micro)vascular thrombosis. Conventional coagulation tests lack the ability to discriminate between these profiles.

Ideally, a test to assess bleeding risk in the critically ill should take into account all factors contributing to hemostasis and be readily available for clinicians caring for these patients. In recent years there has been a growing interest in rotational thromboelastometry (ROTEM), a point of care test evaluating whole clot formation and degradation. Use of ROTEM was shown to reduce the amount of transfusions in cardiac surgery [7] and ROTEM may also be of use to detect DIC in the critically ill [8,9]. Therefore we aimed to study the correlation of thromboelastometry with other hemostatic tests and ISTH DIC score in critically ill patients with a coagulopathy. Also, the effect of transfusion of prophylactic FFP on ROTEM parameters in non-bleeding patients has not been evaluated before. As a substudy of a trial evaluating efficacy of prophylactic FFP in coagulopathic patients prior to undergoing an intervention, the response of thromboelastometry variables to a fixed dose of FFP was investigated.
Chapter 7

Methods

Setting and patients
The study was performed as a predefined post-hoc substudy of a randomized clinical trial on the efficacy of prophylactic FFP in critically ill patients with a coagulopathy [10]. Patients were either randomized to receive or not to receive 12 ml/kg of FFP transfusion before an intervention. Patients were eligible if INR was ≥1.5 and ≤3.0 and an intervention was scheduled. Defined invasive procedures were insertion of a central venous catheter, thoracocentesis, percutaneous tracheotomy or drainage of abscess or fluid collection. Patients randomized to FFP transfusion were included in the current study. Patients with clinically overt bleeding or with a thrombocytopenia <30 x 10⁹/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 an appropriate period.

The study was conducted in a mixed-medical surgical ICU in a university hospital, the Academic Medical Center in Amsterdam, the Netherlands in accordance with the Declaration of Helsinki. The protocol was approved by our Institutional Review Board. 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 previous medical history. DIC was assessed using the ISTH DIC score, which defines DIC as a score of ≥5 points [11]. Blood samples were drawn just prior to FFP transfusion and immediately after FFP transfusion.

Coagulation assays
Routine coagulation tests included INR, aPTT, fibrinogen, d-dimer levels (Sysmex CA 7000 and all reagents, Siemens Healthcare Diagnostics, 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, USA).

Antithrombin was assessed by chromogenic substrate method (Sysmex CA 7000, Siemens Healthcare Diagnostics, Germany) with reagents and protocols of the manufacturer. Plasmin-α2-antiplasmin complex (PAP) levels were measured using specific commercially available ELISAs according to the instruction of the manufacturer (Siemens Healthcare Diagnostics, 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]. All tests were carried out before and directly after FFP transfusion.

ROTEm measurements

Using ROTEM (Tem International, Munich, Germany), three separate assays were carried out, 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 CaCl₂ (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 CaCl₂ (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 CaCl₂ 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 (CT), clot formation time (CFT), clot firmness (MCF), alpha angle and maximum lysis (ML) were recorded. For the FIBTEM assay CT and MCF were recorded. All treating physicians were blinded for ROTEM results.

Statistical analysis

All variables are expressed as median (interquartile ranges). To compare groups Mann Whitney was used for independent variables and Wilcoxon signed rank test was used for paired data. Fisher’s exact test was used for comparisons in crosstabs. Correlations were assessed using Spearman’s rho. To assess value of ROTEM variables to diagnose DIC, receiver operating characteristic (ROC) curves were used.
Results are given as area under the curve (AUC) and 95% confidence interval (CI). In addition optimal cut-off values ad odds ratio’s were calculated for each parameter. A p value less than 0.05 was considered significant. Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, USA) and Prism Version 5.0 (Graphpad Software, San Diego, USA).

Results

Patients

Of the 16 included patients (table 1), the majority was admitted to the ICU due to sepsis. Accordingly, patients were ill, as reflected by high disease severity scores. Eight patients fulfilled criteria for overt DIC, with a median platelet count of 69 x 10^9/L. Median levels of natural anticoagulants antithrombin, protein C and S were reduced. Most patients underwent central venous catheterization and minor bleeding occurred after six interventions, including hematoma requiring prolonged pressure, suture or application of spongostan. No major bleedings were observed.

Thromboelastometry and assessment of coagulation status

Baseline thromboelastometry results are depicted in figure 1. With the exception of INTEM CFT, which was prolonged, all median variables were within manufacturers reference values. However, EXTEM CFT was at the upper reference limit. In addition, both median INTEM and EXTEM MCF were on the lower reference range, indicating a tendency to hypocoagulability.

Baseline CT for both INTEM and EXTEM did not correlate with INR, nor did EXTEM CFT, while INTEM CFT showed moderate positive correlation with INR. Both INTEM and EXTEM CFT correlated positively with aPTT (tables 2a and 2b). CFT, alpha and MCF (INTEM and EXTEM) correlated well with platelet count, fibrinogen and factor II levels. Other factor levels did not correlate with ROTEM variables (tables 2a and 2b). Lysis indexes did not correlate with markers of fibrinolysis such as D-dimer and plasmin-antiplasmin levels (data not shown). ROTEM parameters did not differ between patients with and without bleeding (data not shown).
Table 1: Characteristics of critically ill patients with a coagulopathy prior to receiving prophylactic FFP for an intervention.

<table>
<thead>
<tr>
<th>General characteristics</th>
<th>n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male, % (n)</td>
<td>63 (10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 (46-69)</td>
</tr>
<tr>
<td>APACHE IV score</td>
<td>115 (81-136)</td>
</tr>
<tr>
<td>SOFA score</td>
<td>14 (8-16)</td>
</tr>
<tr>
<td><strong>Medical condition</strong></td>
<td></td>
</tr>
<tr>
<td>Liver disease, % (n)</td>
<td>25 (4)</td>
</tr>
<tr>
<td>Sepsis, % (n)</td>
<td>56 (9)</td>
</tr>
<tr>
<td>DIC, % (n)</td>
<td>50 (8)</td>
</tr>
<tr>
<td><strong>Coagulation</strong></td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>1.60 (1.54-2.12)</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>40 (35-47)</td>
</tr>
<tr>
<td>Platelet count x10⁹/L</td>
<td>69 (49-177)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.60 (1.95-4.80)</td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>54 (38-68)</td>
</tr>
<tr>
<td>Protein C (%)</td>
<td>34 (22-53)</td>
</tr>
<tr>
<td>Total protein S (%)</td>
<td>47 (39-72)</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
<td></td>
</tr>
<tr>
<td>Central venous catheter, % (n)</td>
<td>69 (11)</td>
</tr>
<tr>
<td>Chest tube, % (n)</td>
<td>13 (2)</td>
</tr>
<tr>
<td>Tracheotomy, % (n)</td>
<td>13 (2)</td>
</tr>
<tr>
<td>Abdominal drain, % (n)</td>
<td>6 (1)</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td></td>
</tr>
<tr>
<td>Occurrence of bleeding, % (n)*</td>
<td>38 (6)</td>
</tr>
</tbody>
</table>

Data expressed as median and interquartile ranges.

*Assessed using a tool validated in critically ill (HEME)[24]

**Acute Physiology and Chronic Health Evaluation**

**Sequential Organ Failure Assessment**

**Disseminated Intravascular Coagulation**

**Activated Partial Thromboplastin Time**

**International Normalized Ratio**

**Thromboelastometry in patients with disseminated intravascular coagulation**

In patients with DIC, thromboelastometry profiles were more hypocoagulable compared to those without DIC (figure 2). Also, prolonged CFT and reduced alpha and MCF were significantly associated with DIC score (tables 2a and 2b). Although the proportion of patients with DIC that had EXTEM test results outside manufacturers...
Figure 1: ROTEM EXTEM variables before and after Fresh Frozen Plasma transfusion (12 ml/kg). Dotted lines indicate reference ranges.

reference range did not differ from those without DIC (p=0.12), a ROC curve analysis was performed to investigate the value of these variables for the diagnosis of DIC. ROC curves for EXTEM CFT, alpha and MCF are shown in figure 3. Comparison of EXTEM CFT, alpha and MCF in patients with and without DIC showed all three variables were capable of detecting differences between these groups with high accuracy (table 3).

Response of thromboelastometry variables to transfusion with FFP

Transfusion with 12 ml/kg FFP reduced median EXTEM CT and improved median EXTEM MCF, indicating enhanced coagulation. However, in most patients, variables
Thromboelastometry and FFP transfusion in critically ill patients with a coagulopathy

Figure 2: ROTEM EXTEM variables before and after Fresh Frozen Plasma transfusion (12 ml/kg) in patients with and without disseminated intravascular coagulation.

Figure 2: ROTEM EXTEM variables before and after Fresh Frozen Plasma transfusion (12 ml/kg) in patients with and without disseminated intravascular coagulation.

only changed marginally after FFP transfusion (figure 1). FIBTEM MCF was unaffected by FFP transfusion and the same applied for the INTEM variables (CT: 183 (175-218) before vs. 175 (159-197) after FFP (p=0.40), CFT: 133 (51-162) before vs. 115 (60-140) after FFP (p=0.33), alpha: 74 (63-80) before vs. 72 (68-78) after FFP (p=0.63) and MCF: 52 (43-70) before vs. 55 (49-69) after FFP (p=0.19)). The effect of FFP transfusion did not differ among patients with or without DIC (figure 2).
**Table 2a:** Correlation of INTEM ROTEM variables with coagulation tests at baseline.

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>CFT</th>
<th>Alpha</th>
<th>MCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>0.174</td>
<td>0.538*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>0.430</td>
<td>0.646*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
<td>-0.270</td>
<td>-0.908**</td>
<td>0.725**</td>
<td>0.811**</td>
</tr>
<tr>
<td>Factor II (%)</td>
<td>-0.609*</td>
<td>-0.785**</td>
<td>0.839**</td>
<td>0.794**</td>
</tr>
<tr>
<td>Factor V (%)</td>
<td>-0.319</td>
<td>-0.187</td>
<td>0.187</td>
<td>0.279</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>-0.187</td>
<td>-0.451</td>
<td>0.487</td>
<td>0.389</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>-0.498</td>
<td>-0.769**</td>
<td>0.865**</td>
<td>0.665**</td>
</tr>
<tr>
<td>DIC score</td>
<td>0.452</td>
<td>0.802**</td>
<td>-0.918**</td>
<td>-0.636*</td>
</tr>
</tbody>
</table>

* *p<0.05
** *p<0.01

INR = International normalized ratio
aPTT = activated partial thromboplastin time
DIC = disseminated intravascular coagulation
CT = clotting time
CFT = clot formation time
MCF = maximum clot firmness
NA = not applicable

**Table 2b:** Correlation of EXTEM ROTEM variables with coagulation tests at baseline.

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>CFT</th>
<th>Alpha</th>
<th>MCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>-0.022</td>
<td>0.369</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>0.318</td>
<td>0.548*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
<td>-0.487</td>
<td>-0.833**</td>
<td>0.818**</td>
<td>0.613*</td>
</tr>
<tr>
<td>Factor II (%)</td>
<td>-0.412</td>
<td>-0.689**</td>
<td>0.732**</td>
<td>0.513*</td>
</tr>
<tr>
<td>Factor V (%)</td>
<td>-0.146</td>
<td>-0.190</td>
<td>0.259</td>
<td>0.018</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>-0.062</td>
<td>-0.306</td>
<td>0.373</td>
<td>0.484</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>-0.233</td>
<td>-0.743**</td>
<td>0.765**</td>
<td>0.724**</td>
</tr>
<tr>
<td>DIC score</td>
<td>0.271</td>
<td>0.796**</td>
<td>-0.860**</td>
<td>-0.790**</td>
</tr>
</tbody>
</table>

* *p<0.05
** *p<0.01

INR = International normalized ratio
aPTT = activated partial thromboplastin time
DIC = disseminated intravascular coagulation
CT = clotting time
CFT = clot formation time
MCF = maximum clot firmness
NA = not applicable

**Table 3:** Results of Receiver Operating Characteristics curve analysis for EXTEM variables in patients with and without disseminated intravascular coagulation.

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95% CI</th>
<th>p value</th>
<th>Cut off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFT</td>
<td>0.906</td>
<td>0.757-1.056</td>
<td>&lt;0.01</td>
<td>&gt;105 sec</td>
<td>100%</td>
<td>75%</td>
<td>3.0</td>
</tr>
<tr>
<td>alpha</td>
<td>0.945</td>
<td>0.841-1.050</td>
<td>&lt;0.01</td>
<td>&lt;67°</td>
<td>88%</td>
<td>88%</td>
<td>7.0</td>
</tr>
<tr>
<td>MCF</td>
<td>0.883</td>
<td>0.688-1.078</td>
<td>0.01</td>
<td>&lt;51 mm</td>
<td>88%</td>
<td>88%</td>
<td>7.0</td>
</tr>
</tbody>
</table>

CFT = clot formation time
MCF = maximum clot firmness
Discussion

The current study demonstrates that most ROTEM variables are within reference values in critically ill patients with an increased INR. Correlation with the widely used INR is moderate, while ROTEM correlates well with factor II, platelet count and fibrinogen. ROTEM profiles are more hypocoagulable in patients with DIC compared to those without DIC and CFT, alpha and MCF correlate strongly with DIC score. Prophylactic transfusion of FFP slightly improved ROTEM EXTEM results and FIBTEM CFT.

In the present study, ROTEM measurements were done in severely ill patients with increased INR values. Thromboelastometry results were heterogeneous ranging from hyper- to hypocoagulable profiles, as shown before in ICU patients [13]. However for the whole cohort, most results were within reference ranges, which is in line with reports from patients with severe sepsis [6,14]. Thereby, it can be questioned whether reference values as validated in healthy volunteers for a single outcome should be used to diagnose hypo- or hypercoagulability in the critically ill, or rather

**Figure 3:** Receiver operating characteristics curves for EXTEM clot formation time, alpha and maximum clot firmness for the diagnosis of disseminated intravascular coagulation in critically ill patients with coagulopathy.

CFT = clot formation time
MCF = maximum clot firmness
that new reference values should be formulated for specific profiles by combining several ROTEM values.

We assessed the correlation of ROTEM with conventional coagulation tests and individual factor levels. INR values increase when levels of factor II, VII, IX and X are reduced and a reduction of these factors would result in concomitant prolonged coagulation times in the ROTEM assay, as was observed in patients with sepsis [15]. Indeed, INTEM CFT was prolonged and EXTEM CFT was at the upper reference limit in our patients. However, correlation between INR values and ROTEM was only seen in the INTEM assay and not in the EXTEM, as reported previously [16]. A possible explanation for this observation could be that compared to activation of the intrinsic pathway, EXTEM is less standardized due to the use of tissue factor [17].

This has led to the recommendation to use INTEM for patients with known bleeding diathesis [18]. Of note, correlation with INR was only modest. A stronger correlation was found between CFT, alpha and MCF with fibrinogen, factor II levels and platelet count in our patients, which is in line with recent reports in surgical patients [16] and sepsis patients [14]. Hereby, ROTEM could provide valuable information on different components of the coagulation system in a bedside manner, abating the need to perform different assays.

Half of the patients in the current study had overt DIC. Compared to patients without DIC, ROTEM CFT was prolonged and alpha and MCF were reduced, indicative of a more hypocoagulable profile. Also, ROTEM correlated highly with the DIC score. Comparable observations have been reported in sepsis patients [6,8]. Similar to our findings, the combination of CFT, alpha and MCF values was able to discriminate between patients with and without DIC [8]. This suggests that ROTEM could be a useful diagnostic test for DIC [19]. Further research is warranted enabling development of a thromboelastographic DIC score for critically ill with appropriate reference values.

Transfusion with a fixed dose of FFP reduced CT in EXTEM and FIBTEM and increased MCF in EXTEM, whereas INTEM variables remained unaffected. Although FFP is widely used to correct coagulopathy in critically ill patients [1], effect of this practice on coagulation parameters is limited. An increase in individual factor levels and decrease of INR following FFP transfusion has been demonstrated in different patient groups [20-22], however to our best knowledge this is the first report of the
effect of FFP on thromboelastometric variables. Of note, observed increments in this study were only small and the clinical significance of the observed improvement is doubtful.

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 [13], supporting the generalizability of our results on correlation between ROTEM and other hemostatic tests. However, although we found no differences in ROTEM parameters between patients with and without bleeding following an intervention, assessment of the ability of ROTEM to predict bleeding complications requires a larger sample size. Another limitation is test precision of ROTEM, which is a subject of debate with reported high coefficients of variance [23]. However, in order to limit variation as much as possible, all tests were carried out on the same device by only two experienced researchers.

**Conclusion**

In critically ill patients with a coagulopathy, ROTEM parameters were mostly within manufacturers reference ranges. Correlation with INR was moderate, but CFT, alpha and MCF correlated well with platelet count and levels of factor II and fibrinogen, as well as with DIC score. Also, EXTEM variables showed high accuracy in discriminating patients with and without DIC. Thereby, ROTEM may be a useful tool in diagnosing DIC in the critically ill. Transfusion of FFP resulted in improved EXTEM CT and MCF and reduced CT in FIBTEM.
References


