Diagnostic Performance and Therapeutic Consequence of Thromboelastometry Activated by Kaolin versus a Panel of Specific Reagents

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ABSTRACT

Background: Thromboelastography/metry (TEG®; Haemoscope, Niles, IL/ROTEM®; Tem International GmbH, Munich, Germany) is increasingly used to guide transfusion therapy. This study investigated the diagnostic performance and therapeutic consequence of using kaolin-activated whole blood compared with a panel of specific TEM®-reagents to distinguish: dilutional coagulopathy, thrombocytopenia, hyperfibrinolysis, and heparinization.

Methods: Blood was drawn from 11 healthy volunteers. Dilutional coagulopathy was generated by 50% dilution with hydroxyethyl starch 130/0.4 whereas thrombocytopenia (mean platelet count 20 × 10^9/l) was induced using a validated model. Hyperfibrinolysis and heparin contamination were generated by tissue plasminogen activator 2 nM and unfractionated heparin 0.1 U/ml, respectively. Coagulation tests were run on ROTEM® delta.

Results: Kaolin-activated whole blood showed no differences between dilutional coagulopathy and thrombocytopenia (mean clotting time 450 s vs. 516 s, alpha angle 47.1° vs. 41.5°, maximum clot firmness 35.0 mm vs. 34.2 mm, all P values <0.001). Hyperfibrinolysis specifically disclosed an increased maximum lysis (median: 100%, all P values less than 0.001), and heparin induced a distinctly prolonged clotting time (2283 s, all P values less than 0.02). The coagulopathies were readily distinguishable using a panel of TEM-reagents. In particular, dilutional coagulopathy was separated from thrombocytopenia using FIBTEM (maximum clot firmness 1.9 mm vs. 11.2 mm, P < 0.001). The run time of analysis to achieve diagnostic data was shorter applying a panel of TEM-reagents. A transfusion algorithm based on kaolin suggested platelets in case of dilutional coagulopathy, whereas an algorithm applying TEM-reagents suggested fibrinogen.

Conclusion: Monoanalysis with kaolin was unable to distinguish coagulopathies caused by dilution from that of thrombocytopenia. Algorithms based on the use of kaolin may lead to unnecessary transfusion with platelets, whereas the application of TEM-reagents may result in goal-directed fibrinogen substitution.

What We Already Know about This Topic

• Goal-directed transfusion and hemostatic therapy using point-of-care diagnostic tests such as thromboelastography (TEG) or thromboelastometry (ROTEM) may improve outcome.

What This Article Tells Us That Is New

• Simultaneous assessment of extrinsic and intrinsic coagulation pathways, investigating fibrin polymerisation and the influence of heparin or fibrinolysis (TEM) provides more detailed and accurate diagnosis than using kaolin (TEG) alone, shortens the time for diagnosis, and helps to avoid administration of platelet concentrates when fibrinogen substitution would be more appropriate.

SEVERAL published studies indicate that goal-directed transfusion and hemostatic intervention may improve clinical outcome in continuously bleeding patients and reduce unnecessary use of allogeneic blood products such as...
erythrocytes, fresh frozen plasma (FFP), concentrated platelet pools, or cryoprecipitate.1–6

So far, thromboelastography (TEG®; Haemoscope, Niles, IL) and thromboelastometry (ROTEM®; Tem International GmbH, Munich, Germany) are among the most commonly used point-of-care laboratory technologies.7,8 Both technologies provide viscoelastic continuous profiles of whole blood (WB) clot formation adopting various assay compositions. They differ slightly in the way they record the viscoelastic changes occurring during the coagulation process; in the TEG® the plastic cup is slowly oscillating and changes in viscoelasticity are detected by a pin attached to a suspended torsion wire, whereas in ROTEM® the pin is oscillating while the cup remains fixed.9

Various TEG® or ROTEM®-guided “theranostic” (therapy goal-directed by diagnosis) algorithms have been suggested and evaluated.1–4,10–14 However, current practices demonstrate considerable variations in the applied assays and activators. Published algorithms using TEG® are predominantly based on monoaalysis using kaolin activation,12,13 sometimes celite,1,2 tissue factor,10 or combined kaolin and tissue factor,14 with supplemental analysis on heparinase-neutralized WB being performed in some protocols.1,13 In contrast, centers using ROTEM® generally rely on a panel of supplementary assays, simultaneously triggering both extrinsic and intrinsic coagulation pathways, and specifically investigating fibrin polymerization as well as the influence of heparin or excessive fibrinolysis (collectively named TEM-reagents).4,11,15 This approach has resulted in algorithms based on use of a panel of reagents.3,6

Importantly, initial reports outline considerable differences in transfusion outcomes whether the “theranostic” algorithm is based on TEG® kaolin-activated thromboelastography or a panel of TEM-reagents. Johansson and Stensballe12 used a transfusion protocol partly guided by kaolin-activated TEG® and showed a reduced mortality, but found no changes in the consumption of erythrocytes or FFP, together with a 294% increase in the use of concentrated platelet pools. In contrast, Theusinger et al.13 used a panel of TEM-reagents and demonstrated a reduction in the use of erythrocytes, FFP, and platelets, but an increase in use of fibrinogen supplementation. The pronounced divergence in transfusion practices is most likely rooted in overall different transfusion approaches, but it may be speculated that differences in the diagnostic performance of the applied thromboelastographic assays may contribute to the disparity observed.

The time from diagnosis to hemostatic intervention in management of massive bleeding is an important determinant of patient outcomes.16 Delay of hemostatic intervention may potentially be influenced by the treatment protocol applied,17 the nature of the therapy,4 and the time until laboratory diagnostic data are available. A cardinal argument for using point-of-care devices is the expected quicker availability of diagnostic data in comparison with traditional laboratory screening. Faster-developing thromboelastographic profiles have been suggested to be beneficial for earlier recognition of a coagulopathy,18 but so far, systematic reporting on the total time required to establish confirmatory diagnostic data has been sparse. Assays based on TEM-reagents provide faster developing profiles in comparison with kaolin activation,19,20 and it may be speculated that this will also result in an earlier availability of diagnostic data suitable for goal-directed hemostatic intervention.

This study aimed to investigate the diagnostic performance of kaolin-activated WB against a panel of extrinsic activated WB, intrinsic activated WB, a fibrinogen assay, aprotinin-treated WB, and heparinase-neutralized WB in disclosing isolated coagulopathies of dilutional coagulopathy, thrombocytopenia, hyperfibrinolysis, and heparin. Furthermore, we attempted to evaluate the time spent from start of analysis until diagnostic data were available; eventually, diagnostic conclusion and derived treatment strategies were compared based on two published algorithms.

Materials and Methods

Blood Sampling

The study was approved by the Regional Research Ethics Committee of Midtjylland, Viborg, Denmark (reference No. 20090090). After written informed consent was received, blood samples were drawn from 11 healthy adults (5 males and 6 females) with a mean age of 37 yr (range, 26–60). None of the study subjects had received drugs known to affect platelet function or coagulation, and all displayed laboratory coagulation parameters within the normal reference range: activated partial thromboplastin time (32.2 ± 2.0 s, mean ± 1 SD), relative prothrombin time (0.90 ± 0.07), and fibrinogen levels (2.98 ± 0.38 g/L). Using minimum stasis and a 21-gauge butterfly needle, the blood was drawn into citrated plastic tubes (Venosafe®; Terumo Europe, Leuven, Belgium; trisodium citrate 0.109 M: 3.2 w/v%), at a volume ratio of 1:10.

In Vitro Development of Investigated Coagulopathies

Dilutional Coagulopathy. Dilutional coagulopathy in WB was produced by a 50% in vitro dilution with hydroxyethyl starch 130/0.4 (Voluven®; Fresenius Kabi AG, Bad Homburg, Germany) as previously reported,21 resulting in an assumed mean fibrinogen level of approximately 1.50 g/l and a mean platelet count above 100 × 10^9/l.

Thrombocytopenia. Thrombocytopenia in WB was induced using a validated laboratory model.22 Platelet-rich plasma and platelet-poor plasma were processed immediately after blood collection. The blood was centrifuged at 114 g for 15 min at room temperature or at 3,300 g for 25 min for the preparation of platelet-rich plasma or platelet-poor plasma, respectively. The platelet-rich supernatant was substituted with equal amounts of autologous platelet-poor plasma and

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subsequently reconstituted. This procedure was repeated up to four consecutive times for induction of severe thrombocytopenia in healthy WB (mean platelet count 20 × 10⁹/l (range 7–28)). The platelet count was assessed on a Sysmex SP-1000i instrument (Sysmex Corporation, Kobe, Japan).

Hyperfibrinolysis. Hyperfibrinolysis was generated adopting a setup previously used by our group in evaluation of clot stability. Recombinant human single-chain tissue-type plasminogen activator (American Diagnostica Inc., Stamford, CT, final concentration 2 nM) was added to the WB sample immediately before processing the coagulation analysis.

Heparin-induced Coagulopathy. Heparin-induced coagulopathy was produced by adding unfractionated heparin (SAD, Copenhagen, Denmark) to the WB, reaching a final concentration of 0.1 U/ml.

**WB Coagulation Analyses**

Continuous WB clot formation profiles were recorded using ROTEM® delta analyzers (Tem International GmbH). A maximum runtime of 60 min was activated. Coagulation was activated with kaolin (Haemoscope) and a panel of assays consisting of INTEM, EXTEM, FIBTEM, APTEM, and HEPTEM (Tem International GmbH).

Kaolin is a standardized reagent that activates clotting through the contact activation (intrinsic) pathway. According to manufacturer recommendations, 1 ml citrated WB was transferred to a kaolin vial and mixed by five times of inversion. From the kaolin vial 340 µl was added to a ROTEM cup preloaded with 20 µl 0.2 M CaCl₂ whereby the analysis was started immediately. The INTEM assay was performed by addition of 20 µl in-TEM® reagent (containing ellagic acid as intrinsic activator), 20 µl 0.2 M CaCl₂ in HEPES buffer, and 300 µl WB to the cup. The HEPTEM assay also uses 20 µl in-TEM® reagent for activation, but in addition holds 20 µl hep-TEM® reagent containing 0.2 M CaCl₂ and heparinase to eliminate the influence of heparin in the sample. The EXTEM test is performed by addition of 20 µl ex-TEM® (containing rabbit brain tissue factor as extrinsic activator) and 20 µl 0.2 M CaCl₂, whereas the FIBTEM assay applies 20 µl fib-TEM® (0.2 M CaCl₂ with the addition of cytochalasin D (a potent platelet antagonist inhibiting the actin/myosin system) together with the extrinsic activation. The APTEM assay applies ex-TEM® activation together with 20 µl ap-TEM® reagent consisting of fibrinolysis inhibitor aprotinin and 0.2 M CaCl₂. The following standard thromboelastometry parameters were recorded: clotting time (CT, s), clot formation time (CFT, s), amplitude at 10 min after CT (A10, mm), α-angle (α, degree), maximum clot firmness (MCF, mm), time to MCF (MCF-t, s), lysis index 30 min after the CT (LI30, %), and maximum lysis (ML, %). Please refer to figure 1 for additional details.

**Comparison of Published Treatment Algorithms**

Two recently published transfusion protocols based on kaolin or TEM-reagents for activation were selected for comparison of diagnostic strategies and consequent first-line choices of hemostatic intervention (table 1). Comparison of data obtained from TEG® and ROTEM® equipment in the medical literature may potentially be limited by the difference in sample volume applied (TEG® 360 µl vs. ROTEM® 340 µl), differences in type and concentration of activator, and the plastic composite of the proprietary reaction cups. However, it has been demonstrated that the TEG® and ROTEM® provide similar data when activated with the same exogenous activator. Therefore, it was considered feasible to evaluate the therapeutic outcome of a kaolin-activated treatment algorithm, based on the data obtained from a ROTEM® coagulation analyzer. However, the lysis parameters of the TEG® and ROTEM® differ. The parameter LY30 on the TEG® is defined as the decrease in amplitude 30 min after the achievement of the MCF (maximal amplitude), whereas the LI30 of the ROTEM® is defined as the decrease in amplitude 30 min after reaching the CT. Hence, the algorithms were not evaluated for differences in use of antifibrinolytic agents.

**Statistical Analysis**

Statistical analyses were performed using Stata 11.0 (StataCorp, College Station, TX). Descriptive statistics were performed. Continuous data following a Gaussian distribution were expressed as means and SDs and evaluated using a parametric two-sample Student t test or a paired Student t test as appropriate. Nonparametric data were expressed as medians and interquartile ranges (25–75%) and analyzed using Wilcoxon signed-rank test.
coxon rank sum test or Wilcoxon signed-rank test. The observed differences between thrombocytopenia and dilutional coagulopathy when using kaolin versus FIBTEM, INTEM, and EXTEM were used as primary endpoints and evaluated applying multivariate ANOVA. The parameters CT and MCF were used as factors being the parameters available for all assays and applied in both investigated algorithms (see table 1). The differences were well described by a Gaussian distribution and a model allowing heterogeneity in SDs and correlations was applied. A two-tailed \( P \) value less than 0.05 was considered significant.

### Results

**Diagnosing Using Kaolin versus a Panel of TEM-Reagents**

As illustrated in figure 2 and table 2, profiles based on kaolin activation distinguished the investigated coagulopathies

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**Table 1. Simplified Summary of Two Recently Published TEG/ROTEM-based Transfusion Algorithms**

<table>
<thead>
<tr>
<th></th>
<th>Kaolin (Johansson and Stensballe(^ {12} ))</th>
<th>Panel of TEM-reagents (Theusinger et al.(^ {3} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (CT)</td>
<td>11–14 min</td>
<td>Fibrinogen 2–6 g</td>
</tr>
<tr>
<td>R (CT)</td>
<td>( &gt;14 ) min</td>
<td>Protamine sulfate</td>
</tr>
<tr>
<td>MA (MCF)</td>
<td>46–50 mm</td>
<td>Tranexamic acid</td>
</tr>
<tr>
<td>MA (MCF)</td>
<td>( &lt;46 ) mm</td>
<td>Platelet concentrate*</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>( &lt;52^\circ )</td>
<td></td>
</tr>
<tr>
<td>Ly30</td>
<td>( &gt;8% )</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIBTEM MCF ( \leq 7 ) mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INTEM CT and CFT prolonged, HEPTEM normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXTEM and INTEM decline after MCF, APTEM normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXTEM and INTEM MCF ( &lt;40 ) mm, Platelets ( &lt;50 \times 10^9/)l</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Platelet concentrate is only suggested for ongoing bleeding after administration of initial treatment suggestions, and the finding of a reduced MCF combined with a platelet count less than \( 50 \times 10^9/\)l.

\( \alpha = \alpha \)-angle; APTEM = tissue factor and aprotinin; CFT = clot formation time; CT or R = time to initiation of clot formation; EXTEM = tissue factor; FFP = fresh frozen plasma; FIBTEM = tissue factor and cytochalasin D; HEPTEM = ellagic acid and heparinase; INTEM = ellagic acid; Ly30 = lysis 30 min after the maximum amplitude; MCF or MA = maximum amplitude of clot formation.

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![Fig. 2.](https://example.com/fig2.png)

Fig. 2. Representative profiles of normal control samples (A) and modeled coagulopathies: dilutional coagulopathy (B), thrombocytopenia (C), hyperfibrinolysis (D) and heparin contamination (E), after activation with kaolin (row 1) or TEM*-reagents: INTEM (row 2), EXTEM (row 3), FIBTEM (A4–C4), APTEM (D4) and HEPTEM (E4). APTEM = tissue factor and aprotinin; EXTEM = tissue factor; FIBTEM = tissue factor and cytochalasin D; HEPTEM = ellagic acid and heparinase; INTEM = ellagic acid.
Table 2. Thromboelastometric Data of Normal Whole Blood and Modeled Coagulopathies (n = 11) after Activation with Kaolin or TEM-reagents

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Dilutional Coagulopathy</th>
<th>Thrombocytopenia</th>
<th>Hyperfibrinolysis</th>
<th>Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (s)</td>
<td>490 ± 52</td>
<td>450 ± 83</td>
<td>516 ± 168</td>
<td>470 ± 98</td>
<td>2,283 ± 1,241</td>
</tr>
<tr>
<td>A10 (mm)</td>
<td>43.8 ± 5.8</td>
<td>28.1 ± 3.1</td>
<td>25.3 ± 6.1</td>
<td>45 ± 5.7</td>
<td>N/A</td>
</tr>
<tr>
<td>α (°)</td>
<td>57.4 ± 6.9</td>
<td>47.1 ± 5.1</td>
<td>41.5 ± 10.5</td>
<td>61.9 ± 6.2</td>
<td>N/A</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>189 ± 56</td>
<td>316 ± 70</td>
<td>395 ± 206</td>
<td>148 ± 38</td>
<td>N/A</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>55.8 ± 3.3</td>
<td>35.0 ± 3.3</td>
<td>34.2 ± 7.4</td>
<td>48.7 ± 7.0</td>
<td>21.7 ± 21.5</td>
</tr>
<tr>
<td>LI30 (%)</td>
<td>100 (100–100)</td>
<td>99 (98–100)</td>
<td>100 (100–100)</td>
<td>54 (20–87)</td>
<td>N/A</td>
</tr>
<tr>
<td>ML (%)</td>
<td>1.5 (1–3)</td>
<td>11 (9–14)</td>
<td>6 (3–9)</td>
<td>100 (100–100)</td>
<td>6 (3–11)</td>
</tr>
<tr>
<td>INTEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (s)</td>
<td>160 ± 21</td>
<td>251 ± 26</td>
<td>157 ± 15</td>
<td>176 ± 16</td>
<td>351 ± 153</td>
</tr>
<tr>
<td>A10 (mm)</td>
<td>56.3 ± 3.9</td>
<td>27.2 ± 2.9</td>
<td>28.7 ± 7.4</td>
<td>56.4 ± 3.8</td>
<td>51.9 ± 6.1</td>
</tr>
<tr>
<td>α (°)</td>
<td>77.5 ± 1.8</td>
<td>44.9 ± 3.3</td>
<td>64.7 ± 12.5</td>
<td>77.1 ± 1.7</td>
<td>68.9 ± 9.0</td>
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<tr>
<td>CFT (s)</td>
<td>63 ± 10</td>
<td>343 ± 68</td>
<td>309 ± 214</td>
<td>66 ± 11</td>
<td>110 ± 51</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>61.2 ± 3.9</td>
<td>33.3 ± 3.5</td>
<td>36.2 ± 7.9</td>
<td>59.6 ± 3.3</td>
<td>59 ± 3.8</td>
</tr>
<tr>
<td>LI30 (%)</td>
<td>99 (98–99)</td>
<td>98 (97–100)</td>
<td>100 (99–100)</td>
<td>51 (6–80)</td>
<td>100 (99–100)</td>
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<tr>
<td>ML (%)</td>
<td>4 (3–6)</td>
<td>13 (9–15)</td>
<td>7 (4–10)</td>
<td>100 (100–100)</td>
<td>6 (3–11)</td>
</tr>
<tr>
<td>EXTEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (s)</td>
<td>63 ± 19</td>
<td>147 ± 39</td>
<td>55 ± 11</td>
<td>59 ± 9</td>
<td>69 ± 25</td>
</tr>
<tr>
<td>A10 (mm)</td>
<td>54.1 ± 4.2</td>
<td>31.1 ± 2.8</td>
<td>28.1 ± 6.9</td>
<td>54.3 ± 3.3</td>
<td>53.8 ± 5.0</td>
</tr>
<tr>
<td>α (°)</td>
<td>72.6 ± 2.8</td>
<td>49.1 ± 2.0</td>
<td>61.6 ± 14.4</td>
<td>73.1 ± 3.0</td>
<td>73.4 ± 2.6</td>
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<tr>
<td>CFT (s)</td>
<td>87 ± 16</td>
<td>276 ± 31</td>
<td>329 ± 181</td>
<td>85 ± 14</td>
<td>87 ± 11</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>60.4 ± 4.7</td>
<td>39.7 ± 3.9</td>
<td>38.9 ± 7.7</td>
<td>58.7 ± 3.6</td>
<td>58.8 ± 5.6</td>
</tr>
<tr>
<td>LI30 (%)</td>
<td>100 (99–100)</td>
<td>100 (100–100)</td>
<td>100 (100–100)</td>
<td>35 (74–174)</td>
<td>99 (94–100)</td>
</tr>
<tr>
<td>ML (%)</td>
<td>3 (1–4)</td>
<td>9 (7–10)</td>
<td>7 (6–10)</td>
<td>100 (99–100)</td>
<td>12 (8–17)</td>
</tr>
<tr>
<td>FIBTEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (s)</td>
<td>66 ± 25</td>
<td>2,278 ± 1,514</td>
<td>51 ± 15</td>
<td>60 ± 6</td>
<td>162 ± 19</td>
</tr>
<tr>
<td>A10 (mm)</td>
<td>11.6 ± 3.1</td>
<td>2.3 ± 0.5</td>
<td>10.3 ± 2.8</td>
<td>54.4 ± 3.9</td>
<td>54 ± 4.5</td>
</tr>
<tr>
<td>α (°)</td>
<td>67.8 ± 7.4</td>
<td>N/A</td>
<td>65.1 ± 9.6</td>
<td>72.2 ± 4.0</td>
<td>76.4 ± 2.2</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>89 ± 19</td>
<td>69 ± 14</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>12.4 ± 3.3</td>
<td>1.9 ± 1.9</td>
<td>11.2 ± 2.8</td>
<td>60.8 ± 3.4</td>
<td>59 ± 3.9</td>
</tr>
<tr>
<td>LI30 (%)</td>
<td>100 (98–100)</td>
<td>100 (100–100)</td>
<td>N/A</td>
<td>99 (98–100)</td>
<td>97 (97–100)</td>
</tr>
<tr>
<td>ML (%)</td>
<td>0 (0–1)</td>
<td>N/A</td>
<td>0 (0–1)</td>
<td>12 (8–13)</td>
<td>13 (8–14)</td>
</tr>
</tbody>
</table>

Data are shown as mean values ± SDs or median values and interquartile ranges (25–75%).

α = α-angle; APTEM = tissue factor and aprotinin; A10 = amplitude 10 min after CT; CFT = clot formation time; CT = clotting time; EXTEM = tissue factor; FIBTEM = tissue factor and cytochalasin D; HEPTEM = ellagic acid and heparinase; INTEM = ellagic acid; LI30 = lysis index 30 min after CT; MCF = maximum clot firmness; ML = maximum lysis; N/A = not available.

Comparing the coagulopathies based on kaolin profiles, hyperfibrinolysis was specifically disclosed evaluating the LI30 and ML (all P values less than 0.001), and heparin-induced coagulopathy was clearly distinguished from other coagulopathies by a fourfold increase in the mean CT (thrombocytopenia P = 0.001; hyperfibrinolysis P = 0.008). In contrast, no significant differences between thrombocytopenia and dilutional coagulopathy were observed evaluating kaolin profiles (CT P = 0.26, α P = 0.14, A10 P = 0.19, CFT P = 0.27, MCF P = 0.74; table 2 and fig. 3).

Using a panel of TEM-reagents data also distinguished all the investigated coagulopathies from healthy control samples; dilutional coagulopathy showed significant differences in the INTEM and EXTEM (CT, α, A10, CFT, MCF; all P values less than 0.001), as well as FIBTEM profiles (CT, A10, MCF; all P values less than 0.001). Thrombocytopenia disclosed significant changes in the INTEM (α P = 0.007; CFT P = 0.005; A10 and MCF P < 0.001) and EXTEM (α P = 0.03; CFT P = 0.002; A10 and MCF P < 0.001), whereas no significant changes were disclosed in the FIBTEM compared with healthy control samples (CT P = 0.14, α P = 0.60, A10 P = 0.31, MCF P = 0.37).

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Hyperfibrinolysis could be distinguished from healthy control samples and the other coagulopathies by combined interpretation of the EXTEM and APTEM (MCF $P = 0.004$; LI30 $P = 0.003$; and ML $P = 0.003$), but could also be distinguished by isolated analysis of the EXTEM or INTEM (LI30 and ML, all $P$ values $=0.003$). Finally, heparin contamination could be disclosed applying combined interpretation of the HEPTEM and INTEM (CT, 162 s vs. 351 s, respectively, $P = 0.02$). Heparin contamination prolonged the INTEM CT in all cases, but three values were within a published reference range (137 s-246 s).

Multivariate ANOVA of the difference observed between thrombocytopenia and dilutional coagulopathy using the parameters CT and MCF showed clear evidence against the hypothesis of equal means comparing kaolin, FIBTEM, INTEM, and EXTEM ($P < 0.001$). Likewise, when comparing kaolin (CT: 66 s $\pm 145$; MCF: 0.8 mm $\pm 5.3$) and FIBTEM (CT: 2,227 s $\pm 1,516$; MCF: 9.3 mm $\pm 2.8$) a highly significant difference was also observed ($P < 0.001$), whereas no significance was observed when comparing the differences using kaolin with INTEM (CT: 94 s $\pm 22$; MCF: 2.9 mm $\pm 5.4$; $P = 0.65$) or EXTEM (CT: 93 s $\pm 38$; MCF: 2.8 mm $\pm 5.7$; $P = 0.68$).

**Time Duration until Establishing a Confirmatory Diagnosis**

In kaolin-activated WB no definite conclusion of dilutional coagulopathy or thrombocytopenia could be made (fig. 3, table 2). In contrast, dilutional coagulopathy could be clearly distinguished applying combined interpretation of EXTEM and FIBTEM after a mean analysis time of 12.5 min (mean CT $+10$ min; SD = 0.7 min) by the finding of a reduced A10 in the EXTEM (fig. 2, A3 and B3) and the observation of a reduced and almost absent clot development in the FIBTEM (fig. 2, A4 and B4) at the corresponding time point. Thrombocytopenia could be diagnosed after a mean analysis time of $10.9 \pm 0.2$ min by a reduced A10 in the EXTEM (fig. 2, panel A3 and C3) and normal clot development in the FIBTEM (fig. 2, A4 and C4).

Hyperfibrinolysis may be suspected by a small reduction in MCF in the EXTEM compared with the APTEM (fig. 2, D3 and D4), but was only clearly distinguishable when reaching the LI30. These observations suggest that definitive diagnosis could be made during the period from the EXTEM-MCF at a mean analysis time of 14.4 min (CT + MCF-t, SD = 3.0 min) until the EXTEM-LI30 at a mean analysis time of 31.0 min (CT + 30 min, SD = 0.1 min) was obtained. Likewise, in kaolin-activated WB an assured diagnosis should be possible during the period from the MCF (mean analysis time to MCF 24.0 $\pm$ 3.8 min) to a time point corresponding to the LI30 (mean analysis time to LI30 37.8 $\pm$ 1.6 min).

In kaolin-activated WB all heparin contaminated samples revealed abnormally prolonged CT, and heparin contamination may be suspected when the analyses had run longer than the upper limit of the reference range (8 min). Applying TEM-reagents, heparin contamination could be disclosed by the observation of a prolonged CT in the INTEM compared with the HEPTEM. Confirmative diagnosis was possible by the time when the INTEM-CT was reached (mean analysis time 5.8 $\pm$ 2.6 min).

**Choice of Hemostatic Intervention Based on Diagnostic Approach of Published Algorithms**

Table 1 summarizes two recently published transfusion algorithms used for patients with ongoing bleeding. The algorithms are based on different diagnostic principles using activation with kaolin or TEM-reagents. In the experimental condition of thrombocytopenia the algorithm using kaolin-activated WB would initially suggest intervention with FFP for correction of the CT in 2 of 11 cases (fig. 4). The reduced MCF would suggest intervention with platelet concentrate in all cases of thrombocytopenia (all MCF $\leq 43$ mm) (fig. 4). In dilutional coagulopathy none of the 11 cases would be eligible for FFP based on the CT (all CT values less than 10.1 min). However, the algorithm based on kaolin would suggest platelet transfusion in all cases of dilutional coagulopathy (all MCF $\leq 40$ mm) (fig. 4). In contrast, the algorithm by Theusinger et al. based on a panel of TEM-assays (table 1) would conclude that fibrinogen substitution be used in all cases of dilutional coagulopathy (all FIBTEM-MCF $\leq 4$ mm) and in 1 case of severe thrombocytopenia (FIBTEM-MCF = 7 mm at platelet count $8 \times 10^9/l$), and platelet concentrate would only be suggested in 6 of 11 cases of thrombocytopenia (fig. 4).
Fig. 4. Suggested first-line interventions of a published treatment algorithm based on kaolin12 and a treatment algorithm based on a panel of TEM-assays.3 FFP = fresh frozen plasma (red bars), platelet concentrate (blue bars) and fibrinogen concentrate (green bars) are depicted. In modeled dilutional coagulopathy the algorithm based on kaolin would suggest platelet concentrates in all cases, whereas the algorithm based on TEM-assays would recommend fibrinogen. In thrombocytopenia, the algorithm based on TEM-assays was also less likely to suggest intervention with platelets.

Discussion

In the current study, we found that using a panel of TEM-reagents provides more detailed and accurate diagnoses than using kaolin alone; the time until diagnostic data were available was shorter using a panel of TEM-reagents; and a kaolin-based algorithm would suggest the use of platelet concentrates in more cases whereas an algorithm based on TEM-reagents would lead to fibrinogen substitution in more individuals.

The findings outline advantages and drawbacks of point-of-care monitoring and challenges in design of theranostic algorithms. Kaolin-activated WB coagulation failed to distinguish thrombocytopenia from a coagulopathy caused by dilution, whereas the difference between the two induced coagulopathies was easily identified applying the FIBTEM functional fibrinogen assay. Several studies have documented that dilutional coagulopathy by the administration of crystalloids, and particularly by colloids, is characterized by abnormal fibrinogen polymerization that constitutes an important component of massive bleeding.26–28 Furthermore, randomized clinical studies have suggested that substitution therapy with fibrinogen can control hemorrhage and reduce the need for allogeneic blood products.29,30 Other studies propose an important clinical effect of platelet transfusion.31 Thromboelastometry has been suggested useful to predict the need for massive transfusion in trauma patients.32 Ideally, point-of-care laboratory monitoring should assist in minute-to-minute management of coagulopathy.18 Profiles of TEM-assays became available more quickly than following kaolin activation. In addition, diagnostic data feasible for goal-directed hemostatic intervention were achieved more rapidly with TEM-reagents than with kaolin, which may be cases where fibrinogen substitution may be a more rational and effective treatment.

A key dissimilarity between using monoanalysis with kaolin compared with the panel of TEM assays is the FIBTEM assay, specifically analyzing functional fibrin polymerization. This assay has been used in clinical studies to detect dilutional coagulopathy33 and to calculate relevant dosages of fibrinogen.34 Although significant difference where found between the levels of dilutional coagulopathy and thrombocytopenia applied in this study using the INTEM and EXTEM, distinction based on monoactivation without FIBTEM does not seem feasible in a clinical setting with complex coagulopathies of widespread severity. A functional fibrinogen assay is also available from Haemoscope,35 but has so far not found widespread application in published treatment algorithms and was not evaluated in this study.

Coagulopathies encountered in various clinical settings are often highly complex.36 Acidosis causes irreversible loss of platelets and fibrinogen.37 Perioperative and traumatic coagulopathy is associated with tissue damage and hyperfusion leading to excessive activation of protein C, inhibition of thrombin generation, and imbalanced overstimulation of plasmogen activation causing hyperfibrinolysis.38,39

In the current study, a condition of facilitated fibrinolysis was investigated by in vitro addition of 2 nM tissue plasminogen activator. Hyperfibrinolytic profiles were detectable by isolated analysis of kaolin, INTEM, or EXTEM assays by evaluation of lysis parameters LI30 and ML, whereas there was no significant change in other parameters including the MCF. Apparently, current basic assays are capable of detecting excessive fibrinolysis. Recent studies have indicated that the severity of fibrinolysis is an important determinant of survival or death during traumatic coagulopathy,40 and it may be hypothesized that assays with increased sensitivity to low-grade fibrinolysis would be advantageous. The APTTEM assay has been suggested useful for rapid diagnosis of excessive fibrinolysis41; however, in this experimental setting the assay was of limited value.

Kaolin was more sensitive to low levels of heparin than the INTEM assay. However, in this study heparin-induced coagulopathy could be specifically disclosed in all cases when applying the supplementary HEPTEM assay, and the combined interpretation of INTEM and HEPTEM reduced the time to diagnosis.

Attention has been focused on the total time duration from initiating point-of-care or central laboratory analyses until commencing rational transfusion or hemostatic intervention. Results show that kaolin requires an activation phase of several minutes before coagulation starts, and this delay has been suggested to be critical in patients requiring minute-to-minute management of coagulopathy.18 Profiles of TEM-assays became available more quickly than following kaolin activation. In addition, diagnostic data feasible for goal-directed hemostatic intervention were achieved more rapidly with TEM-reagents than with kaolin, which may be
beneficial in a clinical setting. In this study, the diagnosis of thrombocytopenia and dilutional coagulopathy was possible using the A10, which is supported by recent findings that more than 98% of the final MCF was reached after 10 min runtime in patients scheduled for nonemergent surgery.42 Furthermore, this also corresponds to clinical observations in noncardiac surgery where A10 was found suitable for fast interpretation of thromboelastometric analysis.#

Several study limitations merit attention. The current study was performed on modeled coagulopathies in WB. Samples from patients with various clinical coagulopathies would have been preferable. However, the main focus was directed at assay performance. Thus, modeled coagulopathies enabled us to perform a systematic assessment with high internal validity by eliminating the vast variability that would be encountered applying pathologic patient blood samples. Differences in the overall profile development of the coagulopathies may have been disclosed applying an ANOVA model. However, we chose to evaluate the individual parameters independently to reflect the clinical practice, and the observed effects on thrombocytopenia versus dilutional coagulopathy following kaolin activation (relative difference of means: 2–20%) would not be useful distinguishing the coagulopathies in the clinic.

Patients suffering from severe bleeding often have a coagulopathy of multifactorial etiology in which the combined use of ROTEM®/TEG® and standard hematology coagulation tests may be beneficial.3,32 All assays were processed on the ROTEM® equipment. We assumed there would be limited differences between applying ROTEM® or TEG® analyzers, as has been forwarded in recent studies,43 but minor dissimilarities cannot be excluded. In the current study design, it was not possible to address clinical outcome parameters or aspects of health economics.

The “ideal” transfusion strategy remains elusive and the optimal theranostic approach is further complicated as more and more assays and treatment modalities become available. The best theranostic algorithm for patients ultimately requires evaluation of the current practical performance of rapid point-of-care diagnostic tests, the current study suggests that the application of a panel of supplementary assays and in particular disclosure of abnormal fibrinogen polymerization improves the quality and speed of the diagnosis.

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References


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