Use of soluble fibrin antigen instead of D-dimer as fibrin-related marker may enhance the prognostic power of the ISTH overt DIC score

Carl-Erik Dempfle¹, Michael Wurst¹, Mathias Smolinski¹, Stephan Lorenz¹, Alexandra Osika², Daniela Olenik², Fritz Fiedler², Martin Borggrefe¹

University Hospital of Mannheim, ¹Department of Medicine, and ²Institute for Anaesthesiology and Operative Intensive Care, Mannheim, Germany

Summary
The overt DIC score of the DIC subcommittee of the ISTH includes a fibrin-related marker (FRM) as indicator of intravascular fibrin formation. The type of marker to be used has not been specified, but D-dimer antigen, or fibrin degradation products are used by most investigators. Soluble fibrin complexes have been suggested as more specific indicators of acute intravascular fibrin formation. The aim of the present study was to compare the predictive value of the overt DIC score concerning clinical outcome in a surgical intensive care cohort, using either D-dimer antigen, or soluble fibrin antigen as FRM. The cutoff values for 2 and 3 score points for the FRM were assigned on the basis of the 25% and 75% quartiles of 1870 plasma samples obtained from 359 ICU patients during a period of 6 months. For 331 patients with complete diagnostic workup and day 1 blood samples, the latro SF as FRM component of the overt DIC score displayed the highest prognostic power concerning clinical outcome. The 28-day mortality of patients with overt DIC at day 1, using latro SF as FRM assay was 50.0%, whereas 28-day mortality of patients without overt DIC was 14.0% (p <0.0001). Using MDA D-dimer, and TINAquant D-dimer, 28-day mortality was between 35.5% and 39.3% in patients with overt DIC, and 15.5% to 15.6% in patients without overt DIC. Selection of the FRM as component of the DIC score has a small, but relevant impact on the prognostic performance of the overt DIC score. The present data on the distribution of values may provide a basis for the selection of appropriate cutoff points for assigning 2, and 3 points in the score.

Keywords
Sepsis, disseminated intravascular coagulation (DIC), fibrin monomer, soluble fibrin, D-dimer, score

Introduction
The diagnosis of disseminated intravascular coagulation (DIC) is based on the combination of a clinical condition, which may be associated with DIC, with laboratory parameters for ongoing intravascular coagulation (1).

After release of fibrinopeptides A from the parent fibrinogen molecule by thrombin or other thrombin-like proteases, the resulting fibrin monomers rapidly polymerize, forming fibrin complexes. These complexes serve as cofactors in thrombin-induced factor XIII activation (2), as well as in tPA-induced plasminogen activation (3, 4), making non-crosslinked and non-proteolyzed fibrin complexes a very transient species in vivo (5). Unlike D-dimer antigen assays, which react with a variety of different fibrin compounds containing covalently dimerized D-domains, including high molecular weight fibrin complexes resulting fibrin monomers rapidly polymerize, forming fibrin complexes. These complexes serve as cofactors in thrombin-induced factor XIII activation (2), as well as in tPA-induced plasminogen activation (3, 4), making non-crosslinked and non-proteolyzed fibrin complexes a very transient species in vivo (5). Unlike D-dimer antigen assays, which react with a variety of different fibrin compounds containing covalently dimerized D-domains, including high molecular weight fibrin complexes

Summary
The overt DIC score of the DIC subcommittee of the ISTH includes a fibrin-related marker (FRM) as indicator of intravascular fibrin formation. The type of marker to be used has not been specified, but D-dimer antigen, or fibrin degradation products are used by most investigators. Soluble fibrin complexes have been suggested as more specific indicators of acute intravascular fibrin formation. The aim of the present study was to compare the predictive value of the overt DIC score concerning clinical outcome in a surgical intensive care cohort, using either D-dimer antigen, or soluble fibrin antigen as FRM. The cutoff values for 2 and 3 score points for the FRM were assigned on the basis of the 25% and 75% quartiles of 1870 plasma samples obtained from 359 ICU patients during a period of 6 months. For 331 patients with complete diagnostic workup and day 1 blood samples, the latro SF as FRM component of the overt DIC score displayed the highest prognostic power concerning clinical outcome. The 28-day mortality of patients with overt DIC at day 1, using latro SF as FRM assay was 50.0%, whereas 28-day mortality of patients without overt DIC was 14.0% (p <0.0001). Using MDA D-dimer, and TINAquant D-dimer, 28-day mortality was between 35.5% and 39.3% in patients with overt DIC, and 15.5% to 15.6% in patients without overt DIC. Selection of the FRM as component of the DIC score has a small, but relevant impact on the prognostic performance of the overt DIC score. The present data on the distribution of values may provide a basis for the selection of appropriate cutoff points for assigning 2, and 3 points in the score.

Keywords
Sepsis, disseminated intravascular coagulation (DIC), fibrin monomer, soluble fibrin, D-dimer, score

Introduction
The diagnosis of disseminated intravascular coagulation (DIC) is based on the combination of a clinical condition, which may be associated with DIC, with laboratory parameters for ongoing intravascular coagulation (1).

After release of fibrinopeptides A from the parent fibrinogen molecule by thrombin or other thrombin-like proteases, the resulting fibrin monomers rapidly polymerize, forming fibrin complexes. These complexes serve as cofactors in thrombin-induced factor XIII activation (2), as well as in tPA-induced plasminogen activation (3, 4), making non-crosslinked and non-proteolyzed fibrin complexes a very transient species in vivo (5). Unlike D-dimer antigen assays, which react with a variety of different fibrin compounds containing covalently dimerized D-domains, including high molecular weight fibrin complexes

Summary
The overt DIC score of the DIC subcommittee of the ISTH includes a fibrin-related marker (FRM) as indicator of intravascular fibrin formation. The type of marker to be used has not been specified, but D-dimer antigen, or fibrin degradation products are used by most investigators. Soluble fibrin complexes have been suggested as more specific indicators of acute intravascular fibrin formation. The aim of the present study was to compare the predictive value of the overt DIC score concerning clinical outcome in a surgical intensive care cohort, using either D-dimer antigen, or soluble fibrin antigen as FRM. The cutoff values for 2 and 3 score points for the FRM were assigned on the basis of the 25% and 75% quartiles of 1870 plasma samples obtained from 359 ICU patients during a period of 6 months. For 331 patients with complete diagnostic workup and day 1 blood samples, the latro SF as FRM component of the overt DIC score displayed the highest prognostic power concerning clinical outcome. The 28-day mortality of patients with overt DIC at day 1, using latro SF as FRM assay was 50.0%, whereas 28-day mortality of patients without overt DIC was 14.0% (p <0.0001). Using MDA D-dimer, and TINAquant D-dimer, 28-day mortality was between 35.5% and 39.3% in patients with overt DIC, and 15.5% to 15.6% in patients without overt DIC. Selection of the FRM as component of the DIC score has a small, but relevant impact on the prognostic performance of the overt DIC score. The present data on the distribution of values may provide a basis for the selection of appropriate cutoff points for assigning 2, and 3 points in the score.

Keywords
Sepsis, disseminated intravascular coagulation (DIC), fibrin monomer, soluble fibrin, D-dimer, score

Introduction
The diagnosis of disseminated intravascular coagulation (DIC) is based on the combination of a clinical condition, which may be associated with DIC, with laboratory parameters for ongoing intravascular coagulation (1).

After release of fibrinopeptides A from the parent fibrinogen molecule by thrombin or other thrombin-like proteases, the resulting fibrin monomers rapidly polymerize, forming fibrin complexes. These complexes serve as cofactors in thrombin-induced factor XIII activation (2), as well as in tPA-induced plasminogen activation (3, 4), making non-crosslinked and non-proteolyzed fibrin complexes a very transient species in vivo (5). Unlike D-dimer antigen assays, which react with a variety of different fibrin compounds containing covalently dimerized D-domains, including high molecular weight fibrin complexes
as well as fibrin degradation products (6, 7), assays for fibrin monomer-containing complexes are expected to more accurately reflect acute intravascular fibrin formation. This may be an advantage in the diagnosis and monitoring of patients with sepsis or other conditions associated with DIC.

The current version of the overt DIC score of the DIC subcommittee of the International Society on Thrombosis and Haemostasis (ISTH) contains as laboratory parameters the prothrombin time, platelet count, fibrinogen level, and a fibrin-related marker (FRM) (1). The type of assay to be used as FRM has not been specified. The aim of the present study was to compare the performance of D-dimer antigen assays with a soluble fibrin antigen assay as component of the overt DIC score.

Materials and methods

Plasma samples and laboratory analyses

Analyses were performed prospectively as part of the routine laboratory workup on all plasma samples drawn for coagulation analysis between 07:30 and 10:00 a.m. on Monday through Friday in a 22-bed surgical intensive care unit of a university hospital over a period of six months.

Blood was drawn into syringes containing 1/10 volume of 3.13% (0.105 M) sodium citrate anticoagulant and centrifuged for 10 minutes (2000 x g, 4°C). Functional coagulation assays were performed within three hours after blood sampling. Plasma aliquots for the determination of fibrin derivatives were transferred to polypropylene sample tubes, snap frozen in liquid nitrogen, and stored at -70°C. Before analysis, samples were thawed at 37°C in a water bath and centrifuged at 4000g for 5 minutes.

Reagents for prothrombin time, activated partial thromboplastin time (aPTT), fibrinogen, antithrombin activity, and protein C activity were from bioMérieux Inc., Durham, USA. Analyses were performed on an MDA-180 hemostasis autoanalyzer from the same company.

D-dimer antigen, and soluble fibrin antigen were measured by latex-enhanced photometric immunoassay. MDA D-dimer (D-dimer 1) (bioMérieux Inc.) was measured on the MDA-180 instrument. TINAquant D-dimer (D-dimer 2) (Roche Diagnostics, Mannheim, Germany), and Iatron SF (SFC) (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) (8, 9) were measured on a Hitachi 904 autoanalyzer.

Reagents for coagulation analyses and measurement of fibrin-related markers were provided free of charge by the manufacturers.

Patients and diagnoses

During the study period, 1870 plasma samples from 359 patients (145 female, 214 male patients) were analyzed. The mean age was 59 years, range 17 to 101 years, median 61 years, interquartile range 22 years. Patients were treated according to standard procedures of the intensive care unit. Patients were not treated with antithrombin concentrate. Unfractionated heparin or low molecular weight heparin were used as required, for rinsing of intravascular catheters, extracorporeal circulation procedures (mainly continuous hemofiltration in patients with renal failure), or general prophylaxis of thromboembolism.

Results from 28 patients were excluded from the diagnosis-prognosis-based workup because either laboratory results were not available from the first 24 hours of ICU treatment, or incomplete ICD 10 diagnosis coding, resulting in 331 patients for analysis. In this cohort, 58/331 (17.5%) patients were diagnosed with sepsis according to the guidelines of the American College of Chest Physicians (ACCP) and Society of Critical Care Medicine (SCCM) (10, 11). Septic shock, characterized by systolic blood pressure <90 mm Hg or a reduction of more than 40 mm Hg from baseline in the absence of antihypertensive agents despite adequate fluid resuscitation, or the use of vasopressor agents to maintain blood pressure was present in 42/58 (72.4%) patients with sepsis. Positive blood cultures were found in 17/58 (29.3%) patients. Other diagnoses included acute respiratory insufficiency of various causes, intracerebral hemorrhage, cerebral ischemic stroke, surgery with need for postoperative intensive care treatment, acute severe trauma, obstetrical calamities, and various others.

The category ‘non-survivors’ identifies patients who died during the ICU stay. All other patients were transferred to non-intensive care wards or rehabilitation institutions.

DIC scores

DIC scores were calculated according to the recommendations of the Subcommittee on DIC of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) (1). According to these recommendations, the overt DIC score was only employed if the risk assessment revealed the presence of an underlying disorder known to be associated with overt DIC, including conditions such as sepsis or severe infection, trauma, organ destruction, malignant disease, obstetrical calamities, severe hepatic failure, or severe toxic or immunologic reactions.

Laboratory parameters for calculation of the overt DIC score included platelet count, a fibrin-related marker (FRM), prothrombin time, and fibrinogen. The score points were assigned as follows:

Platelet count >100/µl = 0 points, <100/µl but >50/µl = 1 point, and <50/µl = 2 points. Prolonged prothrombin time <3 seconds = 0 points, >3 seconds, but <6 seconds = 1 point, > 6 seconds = 2 points. Fibrinogen level >1.0 g/l = 0 points, <1.0 g/l = 1 point. Fibrin related marker (FRM) not increased = 0 points, moderately increased = 2 points, and strongly increased = 3 points. A score ≥5 identifies the presence of overt DIC.
**Statistical methods**

Statistical analyses included numerical coefficients of correlation (Pearson), receiver operating characteristic (ROC) analysis, and Fisher’s exact test for a 2 x 2 contingency table. All statistical analyses were performed using JMP Version 5 software from SAS Institute, Heidelberg, Germany.

**Results**

The DIC score uses three levels of the fibrin related marker (FRM): normal range, elevated, and strongly elevated. We first had to specify the cutoff values for these ranges. The distribution of values for D-dimer antigen, and soluble fibrin antigen are shown in Table 1. Median values were 1.7 µg/ml for both D-dimer antigen assays. The 25%-quartile was 1.0 µg/ml for D-dimer 1, and 0.9 µg/ml for D-dimer 2. The 75% quartile was 3.3 µg/ml for D-dimer 1 and 3.1 µg/ml for D-dimer 2. For both assays, the upper limit of normal range declared by the manufacturers is 0.5 µg/ml. For SFC, 25%-quartile, median, and 75%-quartile were 2.6 µg/ml, 3.4 µg/ml, and 5.0 µg/ml, respectively.

The D-dimer antigen assays displayed a high degree of correlation, with numerical correlation coefficient R = 0.69 for all values and R = 0.87 if only day 1 samples were used for calculation. Correlation graphs are shown in Figure 1. There was a low degree of correlation between SFC, and the D-dimer antigen assays 1 and 2, with an R = 0.38 and R = 0.44 for day 1 samples. The correlation coefficients for all plasma samples were R = 0.25 and R = 0.30 for D-dimer 1 and D-dimer 2 respectively.

Presence of high levels of soluble fibrin in the absence of D-dimer antigen may indicate an acute state of intravascular fibrin formation. Of all 331 patients, 19 (5.7%) displayed a SFC concentration ≥75%-quartile in parallel to a D-dimer antigen concentration <25%-quartile. Of these patients, 4 were patients with sepsis.

Overt DIC was diagnosed according to the score system suggested by the DIC subcommittee of the ISTH. As fibrin-related marker (FRM), either D-dimer antigen or SFC were used. Values <25%-quartile of the individual assays were assigned 0 score points, values ≥25%-quartile and <75%-quartile 2 point, and values ≥75%-quartile 3 points. All other components (prothrombin time, platelet count, and fibrinogen level) of the overt DIC score were constant for all variants. Overt DIC was diagnosed on 28-32 of 331 patients on day 1 of ICU treatment (Table 2). Between 50 and 55 patients developed overt DIC during the total ICU stay.

Although the total number of patients with overt DIC seems to be quite similar (Table 2), patients identified by the overt DIC score using different FRM components were not identical. On day 1, 6 patients were identified as ‘overt DIC’ by the overt DIC score using SFC as FRM only, 7 patients were overt DIC positive only in both or one score using D-dimer antigen assay, but not in the overt DIC score using SFC as FRM. Patients with overt DIC only in the score using SFC included patients with sepsis, malignancy, trauma, and hemorrhage. On day 1, 38 patients were classified as ‘overt DIC’ by any version of the overt DIC score. Of these, 23 (60.5%) were identified by all three versions of the score. During the entire ICU stay, 67 patients were classified as ‘overt DIC’ by any version of the overt DIC score. Of these, 40 (59.7%) were identified by all three versions of the score.

Approximately 25% of patients diagnosed with sepsis fulfilled the criteria of overt DIC at day 1 (Table 3). Overt DIC was found in half of patients with sepsis during the total ICU stay.

Overall ICU mortality, and 28-day mortality were used as clinical outcome parameters. Overt DIC is associated with increased mortality. In patients without overt DIC at day 1, ICU mortality was in the range of 16-18%, and 28-day mortality approximately 15% (Table 4). ICU mortality of patients with overt DIC was 35.5%, and 39.3%, using D-dimer 1 and D-dimer 2 as FRM component of the DIC score. Replacement of D-dimer by SFC as component of the DIC score resulted in the identification of a patient group with a mortality of 50.0% (p <0.0001) indicating that replacement of D-dimer by SFC as FRM component of the overt DIC score may lead to improved identification of patients at a high risk of death. Similar observations were made for 28-day mortality, with a mortality of 14.0% in patients without overt DIC and 50.0% in patients with overt DIC, according to the score including the SFC result (p <0.0001). The use of SFC as FRM may thus increase the prognostic power of the DIC score concerning clinical outcome. Similar calculations were made for overt DIC diagnosed during the total ICU stay (Table 5). In this calculation, the diagnostic

<table>
<thead>
<tr>
<th>D-dimer 1 [µg/ml]</th>
<th>D-dimer 2 [µg/ml]</th>
<th>SFC [µg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=1850</td>
<td>n=1869</td>
<td>n=1869</td>
</tr>
<tr>
<td>Maximum</td>
<td>35.9</td>
<td>48.0</td>
</tr>
<tr>
<td>99.5%</td>
<td>22.4</td>
<td>14.6</td>
</tr>
<tr>
<td>97.5%</td>
<td>11.6</td>
<td>9.2</td>
</tr>
<tr>
<td>90.0%</td>
<td>6.2</td>
<td>5.4</td>
</tr>
<tr>
<td>75.0%</td>
<td>3.3</td>
<td>3.1</td>
</tr>
<tr>
<td>50.0% (Median)</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>25.0%</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>10.0%</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>2.5%</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Figure 1: Correlation graphs for D-dimer 1, D-dimer 2, and SFC. Graphs A, C, and E using day 1 samples only, graphs B, D, and F using all values. Graphs and equations are based on a log-log scale.

**Day 1 samples**

**A**
\[ y = 0.96481 \times x^{(0.86466)} \quad R = 0.88955 \]

\[ D\text{-Dimer 2} \text{ [\mu g/ml]} \]

**B**
\[ y = 1.0464 \times x^{(0.78452)} \quad R = 0.69272 \]

\[ D\text{-Dimer 1} \text{ [\mu g/ml]} \]

**C**
\[ y = 4.4515 \times x^{(0.60755)} \quad R = 0.37753 \]

\[ SFC \text{ [\mu g/ml]} \]

**D**
\[ y = 3.2177 \times x^{(0.38718)} \quad R = 0.24649 \]

\[ D\text{-Dimer 1} \text{ [\mu g/ml]} \]

**E**
\[ y = 4.6984 \times x^{(0.68017)} \quad R = 0.43672 \]

\[ SFC \text{ [\mu g/ml]} \]

**F**
\[ y = 3.1916 \times x^{(0.47983)} \quad R = 0.30048 \]

\[ D\text{-Dimer 2} \text{ [\mu g/ml]} \]
benefit of SFC over D-dimer antigen as FRM in the overt DIC score seems to be lost. The total ICU mortality of patients with overt DIC was 52.9% to 57.9%, 28-day mortality 43.1% to 45.6%. This indicates that the diagnostic or prognostic benefit of using SFC as FRM component of the overt DIC score is limited to the initial phase of ICU treatment.

Discussion

The present results indicate that the choice of fibrin assay as fibrin-related marker (FRM) has an impact on the performance of the overt DIC score. Use of a specific assay for SFC, the Iatro SF (8, 9) instead of D-dimer resulted in the identification of more patients with overt DIC, and the selection of a patient group with higher mortality.

Presence of soluble fibrin has been regarded as an indicator of ongoing disseminated intravascular coagulation. In theory, soluble fibrin or fibrin monomer would more closely reflect

### Table 2: Number and percentage of patients with overt DIC at day 1, and overt DIC at any time during ICU stay.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Overt DIC day 1</th>
<th>Overt DIC total</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer 1</td>
<td>31/331 (9.4%)</td>
<td>53/331 (16.0%)</td>
</tr>
<tr>
<td>D-dimer 2</td>
<td>28/331 (8.5%)</td>
<td>50/331 (15.1%)</td>
</tr>
<tr>
<td>SFC</td>
<td>32/331 (9.7%)</td>
<td>55/331 (16.6%)</td>
</tr>
</tbody>
</table>

### Table 3: Number and percentage of patients with sepsis and overt DIC at day 1, and during total ICU stay.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Overt DIC day 1</th>
<th>Overt DIC total</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer 1</td>
<td>14/58 (24.1%)</td>
<td>29/58 (50.0%)</td>
</tr>
<tr>
<td>D-dimer 2</td>
<td>15/58 (25.9%)</td>
<td>28/58 (48.3%)</td>
</tr>
<tr>
<td>SFC</td>
<td>16/58 (27.6%)</td>
<td>30/58 (51.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FRM assay in score</th>
<th>Non-survivors, no overt DIC</th>
<th>Non-survivors, overt DIC</th>
<th>p (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer 1</td>
<td>55/301 (18.3%)</td>
<td>11/31 (35.5%)</td>
<td>0.0319</td>
</tr>
<tr>
<td>D-dimer 2</td>
<td>55/304 (18.1%)</td>
<td>11/28 (39.3%)</td>
<td>0.0122</td>
</tr>
<tr>
<td>SFC</td>
<td>50/300 (16.7%)</td>
<td>16/32 (50.0%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FRM assay in score</th>
<th>Non-survivors, no overt DIC</th>
<th>Non-survivors, overt DIC</th>
<th>p (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer 1</td>
<td>47/301 (15.6%)</td>
<td>11/31 (35.5%)</td>
<td>0.0109</td>
</tr>
<tr>
<td>D-dimer 2</td>
<td>47/304 (15.5%)</td>
<td>11/28 (39.3%)</td>
<td>0.0036</td>
</tr>
<tr>
<td>SFC</td>
<td>42/300 (14.0%)</td>
<td>16/32 (50.0%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FRM assay in score</th>
<th>Non-survivors, no overt DIC</th>
<th>Non-survivors, overt DIC</th>
<th>p (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer 1</td>
<td>36/278 (13.0%)</td>
<td>30/54 (55.6%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-dimer 2</td>
<td>39/281 (13.9%)</td>
<td>27/51 (52.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SFC</td>
<td>33/275 (12.0%)</td>
<td>33/57 (57.9%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FRM assay in score</th>
<th>Non-survivors, no overt DIC</th>
<th>Non-survivors, overt DIC</th>
<th>p (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer 1</td>
<td>34/278 (12.2%)</td>
<td>24/54 (44.4%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-dimer 2</td>
<td>36/281 (12.8%)</td>
<td>22/51 (43.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SFC</td>
<td>32/275 (11.6%)</td>
<td>26/57 (45.6%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4: ICU mortality, and 28-day mortality of patients without and with overt DIC at day 1. Overt DIC scores were calculated using different FRM assays, whereas the other components of the score were identical.

Table 5: ICU mortality, and 28-day mortality according to the presence or absence of overt DIC at any day during ICU stay. Overt DIC scores were calculated using different FRM assays, whereas the other components of the score were identical.
acute fibrin formation than D-dimer assays, which may detect acutely formed crosslinked fibrin as well as proteolytic fragments of intra- and extravascular fibrin clots. Early test systems for soluble fibrin included the ethanol gelation test (12), protamine para-coagulation assays (13), and the erythrocyte agglutination assay (14, 15). Later assays were based on the cofactor role of soluble fibrin in t-PA-induced plasminogen activation (16), and on specific epitopes generated by release of fibrinopeptides A from fibrinogen (17-19), and fibrin polymerization (8, 20-24). To date, no published studies have demonstrated an advantage for the determination of soluble fibrin or fibrin monomer over D-dimer antigen in the diagnosis or prognosis of defined clinical conditions.

In the current version of the overt DIC score, moderately elevated levels of the FRM are assigned 2 points, and strongly elevated levels 3 points. For the identification of appropriate cutoff levels, we used 1870 plasma samples from patients treated in a surgical ICU. The 25%-quartile was chosen as cutoff point for assigning 2 points, the 75%-quartile as cutoff for 3 points in the DIC score. For the two D-dimer antigen assays, these points were quite similar, despite differences in antibody specificity and calibration. In both cases, the 25%-quartile was approximately twice the upper margin of the assigned normal range. Only 10% of ICU plasma samples showed D-dimer antigen values below the cutoff used for exclusion of deep venous thrombosis, indicating that such a cutoff level would not be efficient in the exclusion of venous thrombosis in the ICU patient cohort.

In the majority of patients, increased levels of SFC and D-dimer antigen were observed in parallel. Low D-dimer antigen levels in combination with high SFC levels were present only in 5.7% of patients.

The monoclonal antibody used in the SFC assay, MAb IF-43, was prepared by immunization of mice with a fibrin preparation produced by clotting purified fibrinogen with thrombin, and re-solubilization of the clot in 6 mol/L urea (8). The assay is calibrated with fibrin resolubilized in 0.02 mol/L acetic acid in combination with polymerization inhibitor GPRVVERHQS (8). If plasma is spiked with noncrosslinked desAABB-fibrin resolubilized in 0.02 mol/L acetic acid, the major compound detected by MAb IF-43 is a trimer of one fibrin monomer with two fibrinogen molecules (8). Fibrinogen degradation product D may substitute the fibrinogen. Soe et al. showed that the predominant species detected in clinical plasma samples was a complex of desAA-fibrin monomer with two fibrinogen molecules. In immunoblotting experiments, the monoclonal antibody reacted with fibrin monomer, as well as with thrombin-treated fibrin fragments X, Y, and E (8). The SFC-assay did not correlate with a D-dimer antigen assay (9).

The present results indicate that the replacement of D-dimer antigen assays by Iatro SF (8, 9) may improve the prognostic power of the overt DIC score calculated at admission to the ICU. A possible reason may be that D-dimer antigen may stem from intravascular as well as extravascular sources, whereas SFC appears to be more closely linked to acute intravascular fibrin formation. High molecular weight fibrin complexes formed in the extravascular compartment would not enter the vasculature and appear in venous blood samples. High D-dimer antigen at admission to the ICU may be related to trauma or surgery rather than to intravascular coagulation. Alternatively, the procedure used for assigning cutoff levels according to the quartiles of the distribution of values in ICU patients resulted in more appropriate values for SFC, as compared to D-dimer antigen.

At a later stage of ICU treatment, the diagnostic benefit of SFC over D-dimer antigen as FRM component of the overt DIC score seems to be lost, although the proportion of discrepant results between the versions of the overt DIC score remains constant. This indicates that increased SFC and D-dimer antigen levels are not present in parallel in all patients, but may appear in a sequential manner. The rather low degree of numerical correlation between SFC and D-dimer antigen values supports this assumption.

In any case, an overt DIC score of ≥5 identifies a group of ICU patients with increased mortality, which may profit from additional treatment measures, the selection of which depends upon the underlying disease.

References
exposed in the E domain of fibrin monomer complexed with fibrinogen or its derivatives: its application to the measurement of soluble fibrin in plasma. Blood 1996; 88: 2109-17.