Anticoagulation in severe sepsis and the multiple organ dysfunction syndrome

de Pont, A-C.J.M.

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Chapter 5

The effect of continuous venovenous hemofiltration on the activation of coagulation

Catherine S.C. Bouman, Anne-Cornélie J.M. de Pont, Kamran Bakhtiari, Joost C.M. Meijers, Dorina Roem, Sacha Zeerleder, Gertjan Wolbink, Marcel Levi and Evert de Jonge
Abstract

Background
During continuous venovenous hemofiltration (CVVH), circuit shut down is usually ascribed to thrombogenesis, but its mechanism has not been elucidated. Aim of the present study was to analyze the effect of CVVH on circuit thrombogenesis.

Methods and Results
Ten critically ill patients were treated with predilutional CVVH without anticoagulation. Blood samples were collected at eight different time points for determination of coagulation parameters. Evidence of increased thrombin generation during CVVH was found in 4 out of 10 patients with significantly lower baseline parameters of contact activation. No evidence of tissue factor pathway activation was found. In circuits expiring before the median of 7.4 h, the increase in thrombin generation was inversely correlated with circuit survival time.

Conclusions
Thrombin generation during CVVH was found in a subset of patients with relative impairment of contact activation. In circuits running shorter than the median of 7.4 h, the increase in thrombin generation was inversely correlated with circuit survival time.
Effect of CWH on coagulation

Introduction

Acute renal failure requiring renal replacement therapy, occurs in approximately 4% of patients admitted to the Intensive Care Unit (ICU) [1]. Usually, these patients are treated with some form of continuous renal replacement therapy, such as continuous venovenous hemofiltration (CVVH). Both premature clotting of the hemofilter and bleeding due to the use of systemic anticoagulation are common complications during CVVH in the ICU. Several studies have addressed the pathophysiology of circuit thrombogenesis, but the exact mechanism by which it occurs, has not yet been elucidated. Multiple factors may play a role: the extracorporeal circuit itself, treatment modalities, platelet factors, coagulation factors, natural anticoagulants and fibrinolysis [2,3].

Most earlier studies on the activation of coagulation during CVVH have been performed with concurrent administration of anticoagulants, usually unfractionated heparin (UFH) or low molecular weight heparin (LMWH) [4-6] However, as heparin influences both tissue factor mediated coagulation, contact activated coagulation [7] and fibrinolysis [8], studies on the activation of coagulation during CVVH should ideally be performed without anticoagulation.

Aim of the present study was to elucidate the effect of CVVH on the activation of coagulation and fibrinolysis in critically ill patients without the use of systemic anticoagulation.

Material and methods

Study design

The study was approved by the institutional review board and written informed consent was obtained from all participants or their authorized representatives. A cohort of ten critically ill patients was studied. Patients were eligible for the trial if they had acute renal failure requiring CVVH. Patients were excluded if they had used coumarins or platelet aggregation inhibitors within one week prior to starting CVVH, UFH within 12 hours (h) prior to starting CVVH or LMWH within 48 h prior to starting CVVH. Patients having been treated with extracorporeal techniques within 48 h prior to starting CVVH were equally excluded.

Continuous venovenous hemofiltration

Vascular access was obtained by insertion of a 14 F double lumen catheter (Duo-Flow 400 XL, Medcomp, Harleysville PA, USA) into a large vein (femoral, subclavian, or internal jugular vein). Renal replacement therapy was performed by CVVH using a Diapact hemofiltration machine (Braun AG, Melsungen, Germany). The standard blood flow rate
was 150 ml/min and substitution fluid was added in predilution mode at a flow rate of 2000 ml/h. We used a cellulose triacetate filter with a surface of 1.9 m² and a cut off point of 50 kDa (CT190G, Baxter, Deerfield IL, USA). The hemofiltration run was stopped when clotting in the extracorporeal circuit had occurred. During CVVH, no anticoagulant was used, nor was the extracorporeal circuit primed with any anticoagulant.

Evaluation of patients
Baseline characteristics including demographics, preexisting conditions, organ function, markers of disease severity and routine laboratory tests were assessed within 24 h prior to starting CVVH.

Blood collection
Blood was drawn from the venous limb of the hemofiltration catheter before starting hemofiltration, at 5, 15 and 30 min and at 1, 2, 3 and 6 h after the start of hemofiltration. For the determination of contact activation, 4.8 ml blood was collected in siliconized vacutainer tubes to which 0.2 ml of a mixture of EDTA (0.25 M), benzamidine (0.25 M) and soybean-trypsin inhibitor (0.25%) was added to prevent contact activation in vitro. All other blood samples were collected in citrated vacutainer tubes. Plasma was prepared by centrifugation of blood at 2500 g twice for 20 min at 16°C, followed by storage at -80°C until assays were performed.

Assays
The plasma concentrations of prothrombin fragment F1+2 (F1+2) were measured by ELISA (Dade Behring, Marburg, Germany). Normal values vary from 0.3 to 1.6 nmol/l. Soluble tissue factor (sTF) was determined by ELISA (American Diagnostica, Greenwich CT, USA). The plasma concentrations of factor VIIa were determined on a Behring Coagulation System (BCS, Dade Behring) with the StaClot VIIa-rTF method from Diagnostica Stago (Asnières-sur-Seine, France). Normal levels vary from 16 to 142 mU/ml. Tissue factor pathway inhibitor (TFPI) activity was measured on the BCS as described by Sandset et al [9]. Normal values vary from 39 to 149% of the level measured in normal pool plasma. Kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor complexes were measured as described by Nuijens et al. [10]. Normal levels do not exceed 6 x 10⁻⁴ U/ml and 5 x 10⁻⁴ U/ml respectively. Tissue-type plasminogen activator (t-PA) antigen was assayed by ELISA (Innotest, Hyphen Bio Med, Andrésy, France). Normal levels vary from 1.5 to 15 μg/ml. Plasminogen activator inhibitor type I (PAI-1) antigen was assayed by ELISA (Innotest PAI-1, Hyphen BioMed). Normal levels vary from 10 to 70 ng/ml. D-dimers were determined with an ELISA from Diagnostica Stago. Normal values do not exceed 400 mg/l. Antithrombin activity was determined with Berichrom Antithrombin (Dade Behring) on a BCS. Normal values vary from 80 to 140% of the level measured in normal
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pool plasma. Protein C was determined using the Coamatic protein C activity kit from Chromogenix (Mölndal, Sweden). Normal values vary from 65 to 110% of the level measured in normal pool plasma.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 (SPSS, Chicago IL, USA). Group differences in baseline parameters were tested by student’s t-test. Group differences in time courses of certain parameters were tested by analysis of repeated measures, using mixed linear models. Changes from baseline to a certain time point within the same group were analyzed by a paired student’s t-test. Regression analysis was used to determine the influence of different parameters on circuit survival time. Values are given as mean ± SD or median and range if appropriate. Significance was defined as p<0.05.

Results

Baseline characteristics

The baseline characteristics of the 10 enrolled patients are shown in Table 1.

Thrombin generation

Before starting the CVVH procedure, 9 out of 10 patients showed signs of activation of coagulation, as reflected in increased concentrations of F1+2. In four out of ten patients, F1+2 levels increased further during CVVH (group A). In the remaining six patients, the F1+2 levels remained nearly constant throughout the study period (group B) (Figure 1).

Baseline coagulation parameters

The baseline coagulation parameters of both groups are summarized in Table 2. Baseline levels of antithrombin and protein C were depressed in 7 out of 10 patients. Levels of kallikrein-C1-inhibitor and factor XII-C1-inhibitor complexes were significantly lower at baseline in group A compared with group B (p=0.04) (Table 2).
Table 1. Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total, n</th>
</tr>
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<tbody>
<tr>
<td>Age, years</td>
<td>62 ± 11</td>
</tr>
<tr>
<td>Male sex, n</td>
<td>9</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>SAPS II</td>
<td>47 ± 8</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical settings, n</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular surgery</td>
<td>4</td>
</tr>
<tr>
<td>Sepsis</td>
<td>4</td>
</tr>
<tr>
<td>Intracranial bleeding</td>
<td>1</td>
</tr>
<tr>
<td>Malignancy</td>
<td>1</td>
</tr>
</tbody>
</table>

APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score. Data represent mean ± SD.

Figure 1. Time course of F1+2 before and during CVVH. Left panel: patients demonstrating an increase in thrombin generation during CVVH (group A, n=4); right panel: patients with a constant level of thrombin generation during CVVH (group B, n=6). F1+2, prothrombin fragment F1-2; CVVH, continuous venovenous hemofiltration.
Effect of CVVH on coagulation

Table 2. Baseline coagulation parameters

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+2 (nmol/l)</td>
<td>2.42 ± 1.18</td>
<td>4.24 ± 2.0</td>
<td>0.11</td>
</tr>
<tr>
<td>sTF (pg/ml)</td>
<td>150 ± 93</td>
<td>172 ± 113</td>
<td>0.76</td>
</tr>
<tr>
<td>Factor VIIa (mU/ml)</td>
<td>69 ± 53</td>
<td>112 ± 96</td>
<td>0.39</td>
</tr>
<tr>
<td>TFPI (%)</td>
<td>158 ± 38</td>
<td>127± 69</td>
<td>0.39</td>
</tr>
<tr>
<td>Kallikrein-C1-inhibitor (mU/ml)</td>
<td>7.9 ± 2.0</td>
<td>12.2 ± 3.5</td>
<td>0.04</td>
</tr>
<tr>
<td>Factor Xlla-C1-inhibitor (mU/ml)</td>
<td>1.55 ± 0.25</td>
<td>2.63 ± 0.95</td>
<td>0.04</td>
</tr>
<tr>
<td>t-PA (ng/ml)</td>
<td>20.1 ± 16.7</td>
<td>18.0 ± 16.7</td>
<td>0.85</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>140 ± 123</td>
<td>755 ± 755</td>
<td>0.10</td>
</tr>
<tr>
<td>D-dimer (mg/l)</td>
<td>5.9 ± 3.3</td>
<td>56.2 ± 124.1</td>
<td>0.37</td>
</tr>
<tr>
<td>AT (%)</td>
<td>76 ± 27</td>
<td>46 ± 22</td>
<td>0.12</td>
</tr>
<tr>
<td>PC (%)</td>
<td>80 ± 59</td>
<td>45 ± 28</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Group A: patients demonstrating an increase in thrombin generation during CVVH, Group B: patients with a constant level of thrombin generation during CVVH. Data represent mean ± SD. CVVH, continuous venovenous hemofiltration; F1+2, prothrombin fragment F1+2; sTF, soluble tissue factor; TFPI, tissue factor pathway inhibitor; t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; AT, antithrombin; PC, protein C.

Figure 2. Time courses of sTF, FVIIa and TFPI before and during CVVH. sTF (left panel), FVIIa (middle panel), and TFPI (right panel) expressed as a percentage from baseline, before and during CVVH in patients demonstrating an increase in thrombin generation (group A, ○) and patients with constant thrombin generation (group B, ▲) during CVVH. Data represent mean ± SD. sTF, soluble tissue factor; FVIIa, factor VIIa; TFPI, tissue factor pathway inhibitor; CVVH, continuous venovenous hemofiltration.
Tissue Factor pathway
The time courses of sTF, factor Vila and TFPI as markers of the tissue factor pathway were compared between group A and B. The levels of these three markers did not change significantly over time and no significant difference was found between groups (Figure 2).

Contact activation
To evaluate the role of contact activation, the time courses of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor complexes were studied and compared between group A and B. As shown in Figure 3, levels of kallikrein-C1-inhibitor complexes were significantly lower in group A from baseline until t=1 h (p=0.04). Levels of factor XIIa-C1-inhibitor complexes were significantly lower at baseline in group A as compared to group B and stayed lower throughout the observation period (p=0.04). In both groups, levels of kallikrein-C1 inhibitor and factor XIIa-C1-inhibitor complexes did not change significantly over time during the observation period (Figure 3).

![Graph](image)

**Figure 3.** Time courses of kallikrein-C1 inhibitor and FXIIa-C1-inhibitor complexes before and during CVVH. Kallikrein-C1-inhibitor complex (left panel) and FXIIa-C1-inhibitor complex (right panel) before and during CVVH in patients demonstrating an increase in thrombin generation (group A, ○) and patients with constant thrombin generation (group B, ▲) during CVVH. Data represent mean ± SD. FXIIa, factor XIIa; CVVH, continuous venovenous hemofiltration.
Fibrinolysis
To evaluate the role of fibrinolysis, the time courses of t-PA, D-dimer and PAI-1 were studied and compared between group A and B. Levels of t-PA tended to decrease over time in group A, whereas they remained constant in group B. However, because of the small numbers and the great interpatient variability, the difference did not reach statistical significance. Levels of D-dimer did not change over time and did not differ between groups. At baseline, PAI-1 levels were elevated in 8 out of 10 patients. PAI-1 levels did not change significantly over time in group A and tended to decrease in group B. However, because of the great interpatient variability, the difference did not reach statistical significance (Figure 4).

![Figure 4](image)

**Figure 4.** Time courses of t-PA, D-dimer and PAI-1 before and during CVVH. Time courses of t-PA (left panel), D-dimer (middle panel) and PAI-1 (right panel) before and during CVVH in patients demonstrating an increase in thrombin generation (group A, ○) and patients with constant thrombin generation (group B, ▲) during CVVH. Data represent mean ± SD. t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; CVVH, continuous venovenous hemofiltration.

Circuit survival time
Median circuit survival time was 7.4 h, ranging from 1.1 to 31 h. No significant difference in circuit survival time could be demonstrated between group A and B. However, when CVVH circuits were divided into two groups according to survival time, an inverse relationship between the increase in F1+2 and circuit survival time was found in circuits with a survival time shorter than the median of 7.4 h (Figure 5).
Discussion

In the present clinical study, the effect of continuous venovenous hemofiltration on coagulation markers was studied in ten critically ill patients without the use of anticoagulation. During CVVH, only 4 out of 10 patients showed an increase in thrombin generation. In this subset of patients, contact activation was relatively impaired, as reflected by lower baseline levels of kallikrein-C1-inhibitor and factor Xlla-C1-inhibitor complexes and a persistent lower level of the latter parameter throughout the study period. No evidence for tissue factor pathway activation was found and no statistically significant change in fibrinolysis was observed over time. In circuits with a survival time shorter than the median of 7.4 h, the increase in prothrombin fragment F1+2 was inversely related to circuit survival time.

Baseline levels of F1+2 were elevated in 9 out of 10 patients, indicating activation of coagulation due to the primary disease of the patients prior to CVVH. However, only 4 out of 10 patients showed a further increase in F1+2 levels during CVVH. As we only took measurements during the first 6 hours, we might have missed a later increase in F1+2. However, our findings confirm the results of Cardigan et al., who demonstrated that thrombin-antithrombin (TAT) complexes increased in only 8 out of 12 patients during CVVH [5]. It is conceivable that a specific subset of patients responds to extracorporeal circulation with increased thrombin generation, whereas another subset does not.
In order to elucidate the role of the tissue factor pathway in the mechanism of thrombin generation during CVVH, we studied the time courses of sTF, factor VIIa and TFPI. No changes over time were found in the levels of these three markers. These findings are in contrast with those of Cardigan et al., who found a transient decrease in factor VIIa and a transient increase in TFPI during CVVH. However, UFH was used in this study if required. As TFPI levels increase after administration of heparin [11] and high levels of TFPI may bind factor VIIa [12], the changes in TFPI and factor VIIa found by Cardigan et al. may have reflected the effects of heparin, rather than the activation of the tissue factor pathway. Moreover, no significant relationship was found between the increase in tissue factor, the increase in factor VIIa and the generation of TAT complexes. Based on these findings, Cardigan et al. concluded that thrombin generation during CVVH is not solely mediated by the tissue factor pathway, but that it is probably multifactorial. Recently, a tissue factor independent initiation of thrombin generation by activated platelets has been described [13]. A similar mechanism of thrombin generation during CVVH cannot be excluded.

To evaluate the role of the contact system in the mechanism of thrombin generation during CVVH, we studied the time courses of the levels of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor complexes over time. At baseline, both parameters were significantly lower in the group of patients with enhanced thrombin generation during CVVH. Interestingly, levels of factor XIIa-C1-inhibitor complexes stayed significantly lower in this patient group throughout the observation period. One might conclude that contact activation is relatively impaired in this subset of patients. Several authors have described the role of factor XII and kallikrein in the activation of fibrinolysis [14,15]. Factor XII is able to activate fibrinolysis by three different pathways: 1) it activates prekallikrein, which in turn activates urokinase-type plasminogen activator (u-PA), 2) following the activation of prekallikrein, the kallikrein generated can liberate t-PA and 3) factor XII is able to activate plasminogen directly. As a consequence, fibrinolysis is at least partially contact activation dependent. An association between depressed contact activation dependent fibrinolysis and cardiovascular diseases has been described [16]. In the present study, thrombin generation was already enhanced prior to the start of CVVH. It is conceivable that in the subset of patients with relative impairment of contact activation, relative impairment of fibrinolysis leads to enhanced thrombin generation during CVVH. The fact that t-PA levels tended to decrease in patients with enhanced thrombin generation during CVVH is in agreement with this hypothesis. However, larger studies are needed to confirm this finding.

In circuits with a survival time shorter than the median of 7.4 h, the increase in F1+2 was inversely related to circuit survival time. This confirms the finding of Cardigan et al., who demonstrated that the increase in TAT levels was inversely related to circuit survival time [5]. One could postulate that the rapid increase in thrombin generation
occurring during the first 6 h, increases the risk of circuit flow obstruction, whereas this risk diminishes once thrombin generation has stabilized.

**Conclusion**

In the present study, CVVH without anticoagulation elicited an increase in thrombin generation in a subset of critically ill patients with relative impairment of contact activation. No evidence of tissue factor pathway activation was found during CVVH. Circuit survival time was inversely related to the increase in thrombin generation in circuits with a survival time shorter than the median of 7.4 h.
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References
