Activation of the tissue factor pathway occurs during continuous venovenous hemofiltration

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Background. Activation of the tissue factor pathway occurs during continuous venovenous hemofiltration (CVVH). Despite adequate exogenous anticoagulation, the occlusion of CVVH circuits can occur within minutes to a few hours of use and is associated with evidence of thrombin generation. Having found no evidence of activation of the contact factor (intrinsic coagulation) pathway during CVVH, we sought to examine the effect of the first episode of CVVH on the tissue factor (extrinsic) pathway of coagulation and thrombin generation.

Methods. Twelve critically ill patients were studied prior to the commencement of hemofiltration and at regular intervals thereafter until the filter clotted.

Results. Prior to hemofiltration, most patients had increased levels of plasma tissue factor, thrombin–antithrombin (TAT) complexes, and tissue factor pathway inhibitor (TFPI); during hemofiltration, further generation of TAT complexes occurred. Initially, levels of activated factor VII (FVIIa) fell and TFPI increased, but during the course of hemofiltration, the levels of TFPI fell and FVIIa increased. Levels of tissue factor increased during CVVH in some patients, but this was not related to the generation of FVIIa.

Conclusions. These data indicate that activation of FVII occurred during CVVH, which was related to levels of TFPI, but not tissue factor, and was coincidental to thrombin generation.

Hemofiltration is a well-established supportive treatment for critically ill patients with acute renal failure. Although hemofilter circuits have an expected lifespan of 48 to 72 hours, they often clot prematurely. This appears to be particularly frequent in acutely ill, unstable patients who may clot their hemofilters within a few hours of use. This has not only cost implications for the intensive care unit in terms of hemofilter circuits and staff time, but also has implications for the patient because the effects on systemic cardiovascular and hematological perturbation are unknown. We have previously shown that significant amounts of unfractionated heparin are not cleared across the membrane of the hemofilter, presumably because of its high negative charge, and that local plasma levels fall within the desired therapeutic range [1]. If adequate exogenous anticoagulation is provided during hemofiltration, alternative mechanisms must be responsible for premature clotting of the hemofilter.

In other extracorporeal circuits such as cardiopulmonary bypass, the binding of plasma factor XII to the surface of the extracorporeal circuit has been implicated in the activation of the contact system [2, 3]. We postulated that contact activation could occur on exposure of blood to the foreign surface of the hemofilter circuit, resulting in activation of the coagulation system with subsequent thrombin and fibrin generation. We tested this hypothesis [4], but, contrary to our expectations, there was no evidence of further activation of the contact system of coagulation over the lifespan of the hemofilter. Nonetheless, premature filter clotting was related to low plasma levels of the naturally occurring anticoagulants antithrombin III (ATIII) and heparin cofactor II (HCII) at the onset of hemofiltration and with increasing thrombin generation as indicated by rising levels of thrombin–antithrombin (TAT) complexes.

These data suggested that although thrombin was being generated in the filters that clotted prematurely, the stimulus for this was not initiated through activation of the contact system, and these patients may actually have a reduced thrombin inhibitory capacity prior to hemofiltration. We postulated that the stimulus for this thrombin generation may come from activation of the tissue factor pathway of coagulation.

The tissue factor (TF) pathway of coagulation is the principal initiating pathway of coagulation in vivo [5].

Key words: continuous venovenous hemofiltration, thrombin, blood flow, dialysis, anticoagulation, tissue factor.

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The pathway is triggered when tissue factor in the subendothelium is exposed following vessel trauma or cell damage, binding plasma factor VII (FVII), or activated FVII (FVIIa). The FVII:TF catalytic complex exerts sufficient protease activity to activate factor X (FX). The activated FX (FXa) then formed can rapidly convert the FVII:TF complex to FVIIa:TF, thus potentiating the system. The FVIIa:TF complex can also cleave factor IX to factor IXa, which can then itself activate FX. The FXa forms a complex with prothrombin and factor V (the prothrombinase complex), cleaving prothrombin to thrombin.

The main effector in controlling tissue factor-mediated coagulation is tissue factor pathway inhibitor (TFPI). TFPI binds reversibly to the active site of FXa, inhibiting the protease; this TFPI:FXa complex then interacts with FVIIa:TF, forming an inactive quaternary complex [6]. TFPI is present in three circulating pools: bound to lipoproteins, contained in platelets, and free in plasma. However, the majority of TFPI is bound to the vascular endothelium.

It is possible that during hemofiltration, tissue factor may become exposed because of endothelial injury. We have previously shown an increase in markers of endothelial injury in some patients during continuous venovenous hemofiltration [7]. Furthermore, although peripheral blood cells do not constitutively express tissue factor, monocytes and endothelial cells contain an inducible form of tissue factor [8]. Monocyte tissue factor is known to be up-regulated in sepsis [9], and because many patients undergoing hemofiltration have sepsis, this is another potential source of tissue factor during hemofiltration.

The aim of this study was to investigate the effect of hemofiltration on activation and control of the tissue factor pathway. Proteins and inhibitors of the tissue factor pathway—namely, plasma tissue factor, FVII, FVIIa, and TFPI (by both immunologic and functional assays)—were studied during the first episode of hemofiltration in critically ill patients. Thrombin formation was assessed by measuring TAT complexes and exogenous anticoagulation by an anti-Xa amidolytic assay for heparin. Baseline levels and changes during hemofiltration in these variables were related to (a) the filter lifespan and (b) thrombin generation.

METHODS

Patients

Patients with acute renal failure requiring continuous venovenous hemofiltration (CVVH) on the intensive care unit of University College London Hospitals were studied. Ethical permission to undertake the study was obtained from the joint UCL/UCLH Committee on the Ethics of Human Research, and informed patient consent (or relative assent) was obtained beforehand. Patients were excluded if they were receiving aprotinin, required hemodiafiltration rather than hemofiltration, or if discontinuation of hemofiltration was necessary for any reason other than clotting of the circuit, for example, transfer for computerized tomography scan. Clinical and routine hematological and biochemical laboratory investigations were recorded, including underlying condition, circulatory status, and vasoactive drug requirement. Twelve patients not previously hemofiltered were studied: six clotted their filters within 24 hours, and six clotted their filters after 24 hours of use. The results of studies of endothelial damage in these patients have been published elsewhere [7].

Hemofiltration

A pumped venovenous hemofiltration system (either BSM 22 or Prisma; Hospal Ltd., Loughborough, UK) using polyacrylonitrile AN69 hollow-fiber hemofilters (Hospal Ltd.) was primed with heparin as recommended by the manufacturer. Blood flow through the circuit was set at 150 to 200 ml/min, and filtrate volumes were removed at a rate of 1000 ml/hr. The replacement fluid used was either Hemofiltrasol 22 (Gambro Ltd., Sidecup, Kent, UK) or Hemosol LG2 (Hospal Ltd.), the amount given depending on the patient’s clinical status and the desired hourly fluid balance. If required, unfractionated heparin (Multipar; CP Pharmaceuticals Ltd., Wrexham, UK) was infused directly into the circuit prefiltor to maintain satisfactory exogenous anticoagulation.

Sample collection

Blood samples were taken from the “arterial” limb of the hemofilter circuit at the following times: prefiltration and following commencement of hemofiltration at 15 minutes, 1 hour, 3 to 4 hours, 8 to 12 hours, 24 hours, and every 24 hours thereafter until the filter blocked. Whole blood was collected into plastic tubes containing 0.106 mol tri-sodium citrate (9 volumes blood:1 volume anticoagulant), EDTA Vacutainers (Becton Dickinson Ltd., Cowley, Oxford, UK) or was allowed to clot in plain glass tubes for serum. Citrated plasma and serum were separated by centrifugation at 2000 × g for 15 minutes at room temperature, removed, aliquoted into plastic tubes, and then frozen at −70°C. The ethylenediaminetetraacetic acid (EDTA) sample was used for a full blood count estimation using a Coulter STAKS counter (Coulter Electronics Ltd., Luton, Bedfordshire, UK).

Measurement of hemostatic factors

Commercially available solid-phase enzyme-linked immunosorbent assays (ELISA) were used to measure tissue factor (American Diagnostica Inc., Greenwich, CT, USA), total TFPI (American Diagnostica Inc.), and
TAT complexes (Behring Diagnostics Ltd., Hounslow, Middlesex, UK).

Amidolytic substrate assays were used to measure heparin, FVII, and TFPI. The TFPI assay was modified from Sandset et al., based on its ability to inhibit FVIIa:TF activation of factor X in the presence of factor Xa as previously described [10, 11]. A two-stage amidolytic assay for factor VII (FVII) was modified from that of Seligsohn et al. and was automated using a Sysmex CA-6000 random access analyzer (Sysmex UK Ltd., Milton Keynes, UK) as previously described [12, 13]. FVII and TFPI assays were standardized using Reference Plasma 100% (Immuno Ltd., Sevenoaks, Kent, UK). Heparin was assayed using an antifactor Xa amidolytic substrate assay (Unistest™ Heparin; Unicorn Diagnostics Ltd., London, UK) standardized using unfractionated heparin (Multiparin; CP Pharmaceuticals Ltd.).

FVIIa was assayed according to the method of Morrissey et al using a recombinant mutant (truncated) form of tissue factor that had selectively lost its ability to promote factor VII activation while retaining its cofactor function for the FVIIa-catalyzed activation of factor X [14]. To standardize for other hemostatic factors that may influence the clotting time, test plasmas were diluted 1/10 in factor VII-deficient plasma (Diagnostic Reagents Ltd., Thame, Oxon, UK). The assay was performed using an ACL-300 coagulometer (Instrumentation Laboratory Ltd., Warrington, Cheshire, UK) and standardized using human FVIIa (Novo Nordisk Ltd., Bagsvaerd, Denmark). We have previously established that unfractionated heparin up to a plasma concentration of 1 IU/ml does not affect this assay [11].

Statistical analysis

Changes in variables over the period of filtration were assessed using the Wilcoxon rank test between prefiltration levels and those prior to clotting. Correlation between variables was assessed using Spearman’s rank coefficient of correlation. A P value of less than 0.05 was considered statistically significant.

RESULTS

The characteristics of the 12 patients studied are shown in Table 1. Patients had not been previously filtered during their intensive care unit admission and received unfractionated heparin as an anticoagulant at a rate of 500 to 1000 IU/hr. The lifespan of the filter was not related to prefiltration levels of any of the hemostatic parameters studied nor to plasma heparin levels. Monocyte, neutrophil, and platelet counts as well as the hematocrit did not significantly change during hemofiltration. Unless otherwise stated, baseline levels of the variables studied were not significantly different between patients with a filter lifespan of less than 24 hours and more than 24 hours. Eight of the 12 patients were septic. Eight had acute respiratory distress syndrome, and eight were thrombocytopenic prior to hemofiltration (Table 2).

Thrombin generation

The distribution of the variables studied was non-Gaussian; therefore, all results are represented by the median (first and third quartiles). TAT complexes were elevated [14.40 (11.73 to 20.90) μg/liter] in all patients prior to hemofiltration (Table 2). In 8 of the 12 patients, there was a further increase in TAT complexes over the filter lifespan [13.71 (8.14 to 17.98) baseline vs. 26.20 (19.07 to 28.74) prior to clotting, P = 0.018]. In the four patients in which there was no further increase in TAT, the filter remained patent for longer than 24 hours. The increase in TAT complexes over the lifespan of the filter inversely correlated with the filter running time (Fig. 1).

Activation and inhibition of the tissue factor pathway

Tissue factor levels were increased in eight patients prior to hemofiltration (Table 2). During hemofiltration there were further increases in plasma tissue factor in six subjects. The percentage change in tissue factor was significantly correlated with the percentage of change in TAT complexes over the lifespan of the filter (r = 0.49, P = 0.02) and inversely correlated with filter lifespan (r = −0.72, P = 0.03).

Baseline FVIIa and FVII levels were below the reference range in three and four subjects, respectively, prior to commencement of hemofiltration (Table 2). Levels of FVIIa were not correlated with either TFPI or FVII prior to hemofiltration. After 15 minutes of filtration, there was a significant decrease in FVIIa followed by an increase over the lifespan of the filter (Fig. 2). The increase in FVIIa during hemofiltration did not correlate with either TAT complex generation, the percentage increase in tissue factor, or filter lifespan. FVII levels did not change significantly during hemofiltration. Pre-filtration total TFPI levels measured by both amidolytic and immunologic assays were above the reference range in 7 out of 12 patients, three of whom (subjects 1, 5, 7) were receiving heparin therapy/prophylaxis prior to institution of hemofiltration (Table 2). However, one patient receiving heparin therapy prior to institution of hemofiltration had normal baseline levels of TFPI (subject 3). There was a good correlation between amidolytic and enzyme-linked immunosorbent assays for total TFPI at baseline (r = 0.80, P = 0.0076).

After 15 minutes of filtration, TFPI levels were significantly increased in most patients (Fig. 3), except in two patients who had received heparin prior to hemofiltration (subjects 5 and 7). The percentage increase in TFPI was not significantly correlated with plasma heparin concentrations. TFPI levels then gradually decreased over the period of filtration such that, prior to the filter clot-
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Precipitating condition</th>
<th>ARDS</th>
<th>Vasopressor/ inotropes</th>
<th>Sepsis</th>
<th>Filter life hours</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>M</td>
<td>Aspiration pneumonia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>38</td>
<td>died</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>F</td>
<td>Rhabdomyolysis 2° to epilepsy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>survived</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>M</td>
<td>Post-CABG, sternal wound infection, pancreatitis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>4</td>
<td>died</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>M</td>
<td>Sigmoid volvulus</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>30</td>
<td>died</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>M</td>
<td>Chest infection post-Whipples procedure</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>15</td>
<td>survived</td>
</tr>
<tr>
<td>6</td>
<td>74</td>
<td>M</td>
<td>Pneumonia/sepsis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>28</td>
<td>died</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>F</td>
<td>Pneumonia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>16</td>
<td>died</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>M</td>
<td>Sepsis post-laparoscopy</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>12</td>
<td>died</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>F</td>
<td>Post-CABG heart failure, arrhythmias</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>18</td>
<td>died</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>M</td>
<td>Lymphoma/sepsis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>68</td>
<td>died</td>
</tr>
<tr>
<td>11</td>
<td>29</td>
<td>M</td>
<td>Pancreatitis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>41</td>
<td>survived</td>
</tr>
<tr>
<td>12</td>
<td>77</td>
<td>M</td>
<td>Rhabdomyolysis 2° to epilepsy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>48</td>
<td>survived</td>
</tr>
</tbody>
</table>

Abbreviations are: CABG, coronary artery bypass graft; ARDS, acute respiratory distress syndrome. Used with permission from reference 7.

Table 2. Prefiltration levels of coagulation variables

<table>
<thead>
<tr>
<th>Subject</th>
<th>TAT</th>
<th>TF</th>
<th>FVII</th>
<th>FVIIa</th>
<th>TFPI am</th>
<th>TFPI ag</th>
<th>PLTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.99</td>
<td>333</td>
<td>1.01</td>
<td>1.62</td>
<td>3.15</td>
<td>102</td>
<td>78</td>
</tr>
<tr>
<td>2°</td>
<td>15.08</td>
<td>208</td>
<td>0.38</td>
<td>0.63</td>
<td>2.79</td>
<td>153</td>
<td>176</td>
</tr>
<tr>
<td>3°</td>
<td>7.29</td>
<td>108</td>
<td>0.69</td>
<td>0.33</td>
<td>1.23</td>
<td>38</td>
<td>640</td>
</tr>
<tr>
<td>4°</td>
<td>13.71</td>
<td>380</td>
<td>1.05</td>
<td>3.96</td>
<td>2.94</td>
<td>148</td>
<td>104</td>
</tr>
<tr>
<td>5</td>
<td>12.75</td>
<td>601</td>
<td>0.70</td>
<td>2.11</td>
<td>1.86</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>10.71</td>
<td>446</td>
<td>1.09</td>
<td>2.23</td>
<td>3.93</td>
<td>123</td>
<td>321</td>
</tr>
<tr>
<td>7°</td>
<td>44.42</td>
<td>598</td>
<td>0.10</td>
<td>0.16</td>
<td>3.17</td>
<td>109</td>
<td>21</td>
</tr>
<tr>
<td>8°</td>
<td>108.44</td>
<td>&lt;15</td>
<td>0.11</td>
<td>0.60</td>
<td>0.86</td>
<td>59</td>
<td>28</td>
</tr>
<tr>
<td>9°</td>
<td>18.95</td>
<td>336</td>
<td>0.48</td>
<td>1.82</td>
<td>1.77</td>
<td>57</td>
<td>174</td>
</tr>
<tr>
<td>10</td>
<td>17.78</td>
<td>19</td>
<td>0.71</td>
<td>0.38</td>
<td>1.08</td>
<td>42</td>
<td>67</td>
</tr>
<tr>
<td>11</td>
<td>22.84</td>
<td>1492</td>
<td>0.81</td>
<td>0.87</td>
<td>2.44</td>
<td>100</td>
<td>73</td>
</tr>
<tr>
<td>NR</td>
<td>1.00-4.10</td>
<td>&lt;220</td>
<td>0.45-1.45</td>
<td>0.60-5.90</td>
<td>0.40-1.90</td>
<td>14-60</td>
<td>150-400</td>
</tr>
</tbody>
</table>

Units: TAT, mg/liter; TF, pg/ml; FVII, FVIIa, ng/ml; TFPI am, µg/ml; TFPI ag, 10°/liter

ABBREVIATIONS: NR, normal range; TAT, thrombin-antithrombin complex; FVII, factor FVII; FVIIa, activated factor VII; TF, tissue factor; TFPI, tissue factor pathway inhibitor; am, by amidolytic assay; ag, by immunological assay; PLTS, platelets.

Filter life of 24 hours

DISCUSSION

Thrombin-antithrombin complex levels were increased in all patients prior to hemofiltration, suggesting an underlying degree of coagulation activation. TAT complexes are known to be increased in patients with sepsis [15, 16] and chronic renal failure [16-18]. Further increases in TAT complexes over the lifespan of the filter were seen in most patients; in those not showing a rise, the filter remained patent for longer than 24 hours.

The rise in TAT complex levels was inversely correlated with filter lifespan. This confirms the results of our previous study [4], namely, that activation of coagulation with subsequent thrombin generation occurs during hemofiltration and is related to filter occlusion. Interestingly, an increase in TAT complexes has been found during hemodialysis in patients with acute renal failure, but not with chronic renal failure [15]. All patients in this study were in acute renal failure.

Plasma tissue factor levels were increased in most patients prior to hemofiltration commencement. Plasma tissue factor levels are increased in disseminated intravascular coagulation (DIC) [19-22], malignancy [21], vasculitis [20], and chronic renal failure [20, 21]; however, the clinical relevance of these observations is not fully understood. Tissue factor is a trans-membrane protein and requires phospholipid for its FVII-dependent coagulant activity. The cellular origin of soluble plasma tissue factor is unclear, and it is not known whether this soluble form can support the activation of coagulation. It is possible that plasma tissue factor is derived from endothelial cell injury [23] or activated monocytes. However, increased plasma tissue factor levels are not observed in sepsis, a syndrome known to increase monocyte tissue factor expression [20, 21].
Fig. 1. Correlation between the increase in thrombin–antithrombin (TAT) complexes during hemofiltration and filter lifespan. $P = 0.0436$ by the Spearman’s correlation $(N = 12; r = -0.61)$.

Fig. 2. Activated factor VII (FVIIa) levels prior to hemofiltration, after 15 minutes of hemofiltration, and prior to the hemofilter clotting. The horizontal line indicates the median, the box the interquartile range, and the bars the 95% confidence interval $(N = 12)$. $^*P = 0.0022$ by the Wilcoxon rank test.

Some patients in this study showed a marked increase in plasma tissue factor during the period of hemofiltration that correlated with an increase in TAT complexes. Others have reported that levels of TAT complex and soluble thrombomodulin (a marker of endothelial dysfunction) are correlated in hemodialysis patients [24]. This implies that thrombin generation may be responsible for the endothelial dysfunction seen in these patients or, alternatively, that plasma tissue factor may enhance the generation of thrombin. Whether plasma tissue factor could be mediating the generation of thrombin remains speculative, but in this study, it is unlikely given that the increase in tissue factor during hemofiltration was not related to the degree of FVII activation.

If increased baseline levels of TAT complexes seen in the subjects studied here were due to increased activation of the tissue factor pathway, one might expect to observe increased levels of FVIIa prior to hemofiltration. However, this was not the case. Indeed, some patients had low levels of FVIIa at baseline. In other clinical situations in which there may be enhanced activation of the tissue factor pathway, such as DIC and sepsis, FVIIa levels are normal or decreased [25, 26]. The latter authors suggest that the low levels of FVIIa observed may be due to increased turnover, binding of FVIIa to cell-associated tissue factor, impaired synthesis, or increased proteolytic degradation. After 15 minutes of hemofiltration, plasma FVIIa levels were significantly decreased compared with baseline. This coincided with an increase in plasma TFPI at this time point. Because TFPI is the main inhibitor of the FVIIa:TF complex, this is not surprising. However, after this initial decrease, FVIIa levels then increased over the lifespan of the hemofilter until the filter occluded, suggesting activation of FVII during this period. The increase in FVIIa during hemofiltration coincided with the generation of TAT complexes, although no significant correlation existed between them. This indicates that activation of FVII over this period does not solely mediate thrombin generation.

Likewise, there was no correlation between the increase in FVIIa and the increase in plasma tissue factor during hemofiltration, suggesting that activation of FVII is not mediated by endothelial dysfunction in these patients. Others have shown that levels of FVIIa are increased in nondialyzed patients with chronic renal failure and that this may be mediated by endothelial damage.
However, further increases in FVIIa seen in patients who are dialyzed were not related to endothelial cell injury, and in both groups, plasma tissue factor did not correlate with FVIIa [24, 27].

Total TFPI levels were increased prior to hemofiltration in most patients. TFPI levels are known to be enhanced in sepsis [28], DIC [22], and acute respiratory distress syndrome [29]. This may reflect mobilization of TFPI caused by endothelial damage. However, several of the patients were receiving heparin therapy prior to hemofiltration, and heparin induces a rapid and substantial release of TFPI from the endothelium [30]. Nonetheless, baseline TFPI levels correlated with soluble tissue factor, suggesting that the observed increased levels of TFPI may be due to endothelial injury. After 15 minutes of hemofiltration, there was an increase in TFPI levels in most patients, presumably because of the release of TFPI by heparin. Of the four patients who received heparin therapy prior to hemofiltration, there were further increases in TFPI during hemofiltration in two, but not in two others. From 15 minutes after starting filtration to the time of filter occlusion, TFPI levels decreased. At this point, they were not significantly different from baseline levels. The percentage increase in FVIIa correlated to the percentage decrease in total TFPI from 15 minutes of filtration to prior to the filter clotting. This indicates that FVII activation can occur during hemofiltration in the face of falling TFPI levels.

Repeated or continuous infusion of heparin is known to deplete intravascular pools of TFPI [31]. In septic patients, it is possible that if the amount of TFPI bound to the endothelium is already reduced, a continuous infusion of heparin will quickly deplete the intravascular pool of TFPI. Without increased TFPI levels, if the stimulus for tissue factor-induced coagulation is enhanced, activation of FVII could occur. Although the maximal rise in TFPI generally mirrored plasma heparin concentrations, this correlation was not significant. However, TFPI release during hemofiltration may not be due entirely to heparin, as some TFPI may be released as a result of endothelial injury [7].

Plasma TFPI activity is increased in uremic patients and further enhanced in uremic patients who receive maintenance hemodialysis [32]. In this study, there were further increases in TFPI during the period of hemodialysis, which correlated with heparin concentration. However, increases in TFPI have been observed during hemodialysis when the patients are not anticoagulated [33], indicating that some TFPI may be released due to endothelial or platelet modulation.

The role of TFPI in controlling coagulation in vivo is illustrated by animal models; immunodepletion of TFPI in rabbits produced DIC following low doses of tissue factor or endotoxin, which were ineffectual in normal animals [34, 35]. Normal levels of TFPI may thus be able to control TF/FVIIa adequately under basal conditions, but when the stimulus is overwhelming (for example, severe trauma or sepsis), it is insufficient. Conversely, infusion of TFPI ameliorates DIC induced by endotoxin in rabbits [36] and decreases mortality in a baboon model of *E. coli* septic shock [37].

In summary, prior to hemofiltration, most of the patients in this study had (a) increased levels of plasma tissue factor, (b) evidence of thrombin generation, and (c) increased levels of TFPI. In most patients, there was a further generation of thrombin during hemofiltration, which inversely correlated with the filter lifespan. Initially, there was a fall in FVIIa levels, presumably because of the heparin-induced release of TFPI at this time point. As TFPI levels decreased over the lifespan of the filter, activation of FVII occurred, but this was not related to endothelial dysfunction. The generation of thrombin was not related to baseline levels of any of the parameters studied. Although thrombin generation was coincidental to activation of FVII during hemofiltration, there was no significant correlation between them.

The mechanism by which FVII becomes activated during hemofiltration is not clear from these results. It does not appear to be related to endothelial cell activation. It is possible that in septic patients, increased expression of monocyte tissue factor could promote FVII activation. The mechanisms by which thrombin is generated during hemofiltration are likely to be multifactorial. Decreased levels of naturally occurring anticoagulants may reduce the inhibitory capacity against thrombin. Increased activation and reduced inhibition of the tissue factor pathway may contribute to thrombin generation, but it is possible that other atypical stimuli may also be important, such as the activation of coagulation factors on cell surfaces. The direct activation of factor X on the surface of monocytes has been shown to occur during cardiopulmonary bypass [38]. A similar phenomenon may occur during hemofiltration, which could contribute to the observed thrombin generation.

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**APPENDIX**

Abbreviations used in this article are: CVVH, continuous venovenous hemofiltration; FVII, factor VII; FVIIa, activated factor VII; FX, factor X; FXa, activated factor X; TAT, thrombin-antithrombin; TF, tissue factor; TFPI, tissue factor pathway inhibitor.
REFERENCES


