Fibrin clot structure in patients with end-stage renal disease


Introduction

Patients with end-stage renal disease (ESRD) have a 20-fold increased risk of cardiovascular disease (CVD) compared with the general population (1). Traditional risk factors alone do not fully explain the excessive risk of CVD in these patients, which suggests that non-traditional risk factors may also be important (2).

Fibrin is the end-product of coagulation, and the formation and degradation of fibrin are essential components in all tissue repair mechanisms, including atherosclerosis and arteriosclerosis (3–5). During clot formation thrombin cleaves off fibrinopeptides A and B from the fibrinogen molecules leaving fibrin monomers, which spontaneously polymerise into a network of multimeric strands held together by non-covalent bonds (6–8). These multimeric strands grow into two-stranded protofibrils from which they then aggregate laterally into fibrin fibers, which then merge into fiber bundles (9). When factor XIII is activated by thrombin and calcium, it covalently cross-links the fibrin strands, which leads to increased fibrinolytic resistance (10).

Patients with previous or incipient CVD and first degree relatives of patients with premature CVD produce fibrin clot structures that are tighter and more rigid than clots from healthy controls (11–14). Such fibrin structures have been reported to display fibrinolytic resistance, which might add to the genesis of CVD (14, 15). A common denominator for most CVD patients is the presence of inflammation which is strongly involved in the development of atherosclerosis (16). The plasma concentration of the acute phase protein fibrinogen, the precursor of fibrin, strongly affects fibrin structure properties (17). In addition, other acute phase proteins such as orosomucoid, C-reactive protein (CRP) and interleukin 6 (IL-6) have been reported to correlate with various fibrin structure characteristics in patients with CVD (14, 18–20), which suggests that the presence of an inflammatory plasma milieu is strongly associated with the production of altered fibrin clot structures.

Summary

Fibrin clots with reduced permeability, increased clot stiffness and reduced fibrinolysis susceptibility may predispose to cardiovascular disease (CVD). Little is known, however, about the structure of fibrin clots in patients with end-stage renal disease (ESRD). These patients suffer from a high risk of CVD in addition to their chronic low-grade inflammation. Using permeability, compaction and turbidity studies in 22 ESRD patients and 24 healthy controls, fibrin clots made from patient plasma were found to be less permeable (p<0.001), less compactable (p<0.001), and less susceptible to fibrinolysis (p<0.001) than clots from controls. The maximum rate of turbidity increase was also higher for the patients than controls (p<0.001), and scanning electron microscopy revealed higher clot density of fibrin fibers in clots from patients than clots from controls (p<0.001). Patients had higher plasma concentrations of fibrinogen, C-reactive protein and interleukin 6 than controls. These plasma markers of inflammation correlated significantly with most of the fibrin structure characteristics observed in the patients. In contrast, plasma markers of azothemia showed no such correlations. The results suggest that in ESRD patients fibrin clots are significantly different from healthy controls, and that the fibrin structure characteristics in the patients are associated primarily with the inflammatory plasma milieu rather than with level of azothemia.

Keywords

Fibrinogen, fibrinolysis, azothemia, inflammation

Patients with ESRD suffer from chronic low-grade inflammation with relatively high plasma levels of fibrinogen, CRP and IL-6 (21, 22). Fibrin networks formed in these patients are thus likely to be different from fibrin networks that are formed in healthy controls. Studies using whole blood thromboelastography in renal disease patients have indicated an increased clot rigidity with an accelerated rate of clot formation and reduced fibrinolysis speed (23, 24). However, little is known about the fibrin clot structure characteristics derived from plasma in these patients. Here we present a comprehensive study on fibrin clot structure characteristics in plasma obtained from ESRD patients versus healthy controls.

Materials and methods

Subjects

Twenty-two ESRD patients were included in the study. All patients were on chronic peritoneal dialysis (PD) with at least one daytime dialysis bag. The median time on PD was 23.4 months (interquartile range: 13.2 – 31.2). Exclusion criteria were age below 18 years, treatment with haemodialysis, oral anticoagulants, a priori known coagulation defects, ongoing infectious disease, and patients suffering from peritonitis within the last two months before sampling. The underlying renal diagnoses included polycystic renal disease (n=4), diabetic nephropathy (n=3), hypertensive nephropathy (n=4), chronic glomerulonephritis (n=6), and other renal diagnoses (n=5). The protocol was approved by the local scientific ethics committee (approval number 2564–03). Twenty-four apparently healthy volunteers among the laboratory staff and their relatives served as a control group. All subjects gave their informed consent.

Biochemical variables

Blood was drawn from an antecubital vein into sterile vacuum plastic tubes containing either 0.129 mM citrate, 0.47 M EDTA or no anti-coagulants, and the plasma or serum isolated after 20 minutes (min) centrifugation at 2,000 g was stored in aliquots at −65°C until analysis. The following variables were measured: high sensitive CRP (Behring Nephelometer Analyzer II, Dade Behring GmbH, Marburg, Germany), IL-6 (Quantikine, R&D Systems Inc., Minneapolis, MN, USA), plasminogen activator inhibitor type 1 (PAI-1) antigen (TintElize, Biopool, Umeå, Sweden), tissue plasminogen activator (tPA) antigen (TintElize, Biopool), fibrinogen (clotting method of Clauss (25) on a STA-R analyzer, Diagnostica Stago, Asnières, France), and coagulation factor XIII activity (Berichrom, Dade Behring). Creatinine, urea, albumin, glucose, sodium, potassium, phosphorus and albumin corrected total serum calcium were analyzed on a Cobas In-tegra 800 (Roche Diagnostics GmbH, Mannheim, Germany).

Permeability

Plasma clots were made by mixing two parts citrated plasma with one part of a reaction mixture based on TBS (50 mM Tris-HCl, 100 mM NaCl, pH 7.4) resulting in 5 KIE/ml aprotinin (Trasylol, Bayer AG, Leverkusen, Germany), 0.5 IU/ml thrombin (Bie & Berntsen A/S, Rødovre, Denmark) and 25 mM CaCl₂ (final concentrations). After overnight incubation the permeability measurements were performed as previously described (26). The resultant permeability coefficient (or Darcy constant, KS) calculated from the experiments expresses the permeability per clot surface area, which is an indirect measure of clot porosity. All clots were made in quadruple.

Compaction

Citrated plasma was mixed (2:1) with 0.5 IU/ml thrombin, 0.1% (v/v) Tween 80 and 25 mM CaCl₂ (final concentrations) in TBS. Two hundred thirty μl of this clotting solution were quickly transferred into polypropylene tubes previously affixed with a thin layer of paraffin oil at the inner surfaces. After 3.5 hours (h) of incubation at 22°C the tubes were centrifuged at 8,000 g for 60 seconds. The supernatant was then carefully evacuated and discarded with a pipette. The expelled volume of the clots was calculated as the reduction in weight of the tubes after liquid evacuation, and compaction was defined as the ratio of the expelled volume to that of the initial plasma clot volume (27). All clots were made in duplicate.

Turbidity

The polymerization of plasma clots was investigated by mixing 110 μl citrated plasma with 65 μl of a TBS-buffered reaction mixture to give final concentrations of 0.11 IU/ml thrombin, 28 mM CaCl₂ and 0.1% (v/v) Tween 80. Turbidity was followed at 405 nm every 30 seconds for 60 min at 22°C on a Sunrise Remote platereader (Tecan Austria, Grödig/Salzburg, Austria). Vₘₐₓ was calculated as the slope of the steepest part of the curve, and lag phase was defined as the time for optical density (OD) to reach 0.01 from baseline (13). The area under the curve (AUC) was calculated using the trapezoid rule. All clots were made in duplicate.

To investigate fibrin fiber properties and subsequent clot fibrinolysis, another set of plasma clots were made containing (in final concentrations) 66% (v/v) citrated plasma, 0.4 IU/ml thrombin, 0.1% (v/v) Tween 80 and 25 mM CaCl₂. After overnight incubation the OD was read at 405, 540, 608 and 690 nm, and the fiber mass-length ratio, fiber diameter and fiber mass density were calculated as described elsewhere (28, 29). As we used a platereader to measure the wavelength dependence turbidity of the clots, the results obtained on fibrin fiber properties were qualitative rather than quantitative (30). All clots were made in duplicate.

Flufenamic acid (N-[α, α, α-trifluoro-m-tolyl] anthranilic acid) is a compound that inhibits most fibrinolysis inhibitors, including α₂-antiplasmin, α₂-macroglobulin, α-proteinase inhibitor, PAI-1, antithrombin and C1-esterase inhibitor (31, 32). Adding a cocktail of flufenamic acid and recombinant tPA onto mature plasma clots allows fibrinolysis of fibrin networks to be studied without major interference from the native fibrinolysis inhibitors (31). To initiate fibrinolysis of the clots 45 μl of a lysis buffer containing 50 μg/ml rtPA (Actilyse, Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany), 15.0 mM flufenamic acid (pH 7.3), 2.4 mM EDTA, and 0.1% (v/v) Tween 80 in TBS were added onto the clots, followed by 45 μl of mineral oil to prevent evaporation. The 405 nm OD was then followed every 5 min for 5 h at 25°C. The rate of fibrinoly-
sis was determined from the slope of the curve when the slope became constant (between 3 and 5 h), and was normalized with respect to the maximum absorbency value before lysis initiation.

**Scanning electron microscopy (SEM)**

A patient plasma pool was made from an equal amount of citrated plasma from each patient. A control plasma pool was made similarly. Three replicate clots from each pool were made in perforated caps from small plastic tubes by mixing plasma (2:1) with 0.5 IU/ml thrombin and 20 mM CaCl$_2$ (final concentrations). After clotting for 2 h in humid atmosphere the clots were prepared for SEM essentially as described elsewhere (33). Briefly, the clots were extensively washed in 67 mM sodium cacodylate (pH 7.4), and then fixed in 2% (v/v) gluteraldehyde for 40 min. After another extensive wash the clots were dehydrated by a serial acetone-gradient followed by CO$_2$ critical point drying. Finally, the clots were mounted and sputter coated with 6 nm gold. Digital micrographs were recorded at six different places within each clot using a LEO 1550 Gemini Column scanning electron microscope. For image analysis every micrograph was cropped into three images at fixed coordinates. Subsequently, these images were light and contrast auto-adjusted using Adobe Photoshop (version 7.0, Adobe Systems Inc.). The diameter of every visual fiber crossing one of three inserted vertical grid-lines was measured using ImageJ software (version 1.33b, Rasband, National Institutes of Health, USA). Fiber clot density (total number of visible fibers per $\mu$m$^2$) in each small image was also measured.

To objectively estimate the overall architectural complexity of the images, the angle measure technique (AMT) was used (34). First, each small image was ‘unfolded’ into 1-dimensional vectors as previously described (35). Basically, each horizontal line of pixels within an SEM image is accumulated end-to-end into one single string. This renders a 1-dimensional line of vectors (pixels with gray-scale intensities) for each particular SEM image. The average complementary angles between the gray-scale intensities of the pixels at increasing pixel distances (scales) can then be measured using Image Analysis Toolbox (36) run under Matlab 6.5 (MathWorks, Inc.). The resultant AMT spectrum (i.e. mean angle vs. scale) is a measure of image complexity, i.e. it reflects the sum of the imaged features of the specimen in question (34, 37). The image complexity of a fibrin clot therefore relates to levels of fiber clot density, size and shape of the pores, fiber lengths and more. The higher the mean angle values of the AMT spectra the higher the degree of image complexity. As a consequence, SEM images of clots with tight network configurations give higher AMT curves than clots with coarser network configurations. To compare image complexities between patient and control plasma pool clots, the area under each AMT spectrum was calculated.

**Polymorphic genotyping**

All patients and controls were genotyped for the factor XIII(val34leu) polymorphism (38) and the fibrinogen $\alpha$(thr312ala), $\beta$(arg448lys) and $\beta$(h-455G/A) polymorphisms (39–41), as these common polymorphisms can affect fibrin structure characteristics (42).

**Statistics**

The Mann-Whitney U test and the Fisher exact test were used to compare study groups. The Spearman rank order correlation test was used to test for correlations. The level of significance was set to p<0.05. Data are presented as median values with the interquartile range in brackets.

**Table 1: Baseline characteristics.** Median values (interquartile range) are shown for continuous data.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 22)</th>
<th>Controls (n = 24)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>59.0 (47.8 – 64.7)</td>
<td>50.2 (45.0 – 59.5)</td>
<td>0.248</td>
</tr>
<tr>
<td><strong>Gender, males / females</strong></td>
<td>12 / 10</td>
<td>10 / 14</td>
<td>0.555</td>
</tr>
<tr>
<td><strong>Smokers / non-smokers</strong></td>
<td>8 / 14</td>
<td>5 / 19</td>
<td>0.330</td>
</tr>
<tr>
<td><strong>Fibrinogen, g/l</strong></td>
<td>5.7 (4.9 – 6.3)</td>
<td>3.4 (2.8 – 4.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>CRP, mg/l</strong></td>
<td>4.12 (1.73 – 10.00)</td>
<td>0.75 (0.42 – 4.09)</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>IL-6, pg/ml</strong></td>
<td>4.98 (3.13 – 6.24)</td>
<td>1.16 (0.99 – 1.93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>PAI-1:Ag, ng/ml</strong></td>
<td>7.6 (5.2 – 11.2)</td>
<td>12.7 (8.2 – 22.3)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>tPA:Ag, ng/ml</strong></td>
<td>7.8 (5.7 – 11.6)</td>
<td>9.5 (6.4 – 12.5)</td>
<td>0.257</td>
</tr>
<tr>
<td><strong>Factor XIII activity, %</strong></td>
<td>136 (129 – 140)</td>
<td>98 (89 – 113)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Creatinine, µM</strong></td>
<td>569 (464 – 832)</td>
<td>74 (49 – 81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Urea, mM</strong></td>
<td>18.3 (16.0 – 21.2)</td>
<td>5.5 (4.0 – 6.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Albumin, µM</strong></td>
<td>575 (530 – 612)</td>
<td>642 (628 – 680)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Glucose, mM</strong></td>
<td>5.6 (5.1 – 6.8)</td>
<td>4.9 (4.3 – 5.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em><em>Calcium</em>, mM</em>*</td>
<td>2.49 (2.35 – 2.59)</td>
<td>2.29 (2.17 – 2.44)</td>
<td>0.017</td>
</tr>
<tr>
<td><strong>Phosphor, mM</strong></td>
<td>1.59 (1.30 – 1.81)</td>
<td>1.28 (1.02 – 1.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Sodium, mM</strong></td>
<td>138 (133 – 139)</td>
<td>139 (138 – 140)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Potassium, mM</strong></td>
<td>4.1 (3.8 – 4.8)</td>
<td>4.2 (4.1 – 4.5)</td>
<td>0.461</td>
</tr>
</tbody>
</table>

*Albumin corrected total serum calcium.
Table 2: Fibrin structure characteristics. Median values (interquartile range) are shown.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 22)</th>
<th>Controls (n = 24)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability (Ks), x10^{-8} cm²</td>
<td>4.1 (3.5 - 5.1)</td>
<td>8.4 (6.4 - 9.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Compaction, %</td>
<td>47.2 (44.5 - 54.5)</td>
<td>61.9 (54.2 - 68.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3: Correlations between fibrin structure characteristics and markers of inflammation and azothemia in the ESRD patients (n=22). Spearman rank order correlation coefficients are shown.

<table>
<thead>
<tr>
<th></th>
<th>Fibrinogen</th>
<th>IL-6</th>
<th>CRP</th>
<th>PAI-1:Ag</th>
<th>Creatinine</th>
<th>Urea</th>
<th>Calcium</th>
<th>Phosphor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability (Ks)</td>
<td>-0.71†</td>
<td>-0.38</td>
<td>-0.48†</td>
<td>-0.24</td>
<td>-0.06</td>
<td>0.11</td>
<td>-0.24</td>
<td>0.33</td>
</tr>
<tr>
<td>Compaction</td>
<td>-0.26</td>
<td>-0.09</td>
<td>-0.06</td>
<td>-0.14</td>
<td>0.19</td>
<td>0.24</td>
<td>0.25</td>
<td>0.45†</td>
</tr>
<tr>
<td>Lag phase</td>
<td>-0.31</td>
<td>-0.26</td>
<td>-0.44†</td>
<td>-0.43†</td>
<td>0.18</td>
<td>0.29</td>
<td>0.33</td>
<td>0.15</td>
</tr>
<tr>
<td>V_max</td>
<td>0.56†</td>
<td>0.40</td>
<td>0.22</td>
<td>-0.11</td>
<td>0.23</td>
<td>0.17</td>
<td>0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>Mass-length ratio</td>
<td>0.81†</td>
<td>0.49†</td>
<td>0.56†</td>
<td>0.08</td>
<td>0.23</td>
<td>0.06</td>
<td>0.07</td>
<td>-0.00</td>
</tr>
<tr>
<td>Fiber diameter</td>
<td>0.87†</td>
<td>0.48†</td>
<td>0.52†</td>
<td>0.13</td>
<td>0.20</td>
<td>0.10</td>
<td>0.03</td>
<td>-0.07</td>
</tr>
<tr>
<td>Fiber mass density</td>
<td>-0.81†</td>
<td>-0.35</td>
<td>-0.33</td>
<td>-0.16</td>
<td>-0.13</td>
<td>-0.27</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>Fibrinolysis rate</td>
<td>-0.87†</td>
<td>-0.52†</td>
<td>-0.64†</td>
<td>-0.20</td>
<td>-0.16</td>
<td>0.06</td>
<td>-0.04</td>
<td>0.12</td>
</tr>
</tbody>
</table>

†Albumin corrected total serum calcium; †p<0.05; †p<0.01; †p<0.001.

Results

Age, gender and smoking status did not differ between patients and controls (Table 1), and no differences in the polymorphic genotype distributions were found (data not shown). As expected, the level of azothemia and inflammation was higher in the patients than in controls (Table 1). In particular, the level of plasma fibrinogen in the patients was nearly double that of the controls (5.7 vs. 3.4 g/l, respectively, p<0.001). PAI-1 antigen levels were lower in the patients compared with controls (7.6 vs. 12.7 ng/ml, p=0.005), whereas no difference was found for tPA antigen levels (7.8 vs. 9.5 ng/ml, p=0.257).

Table 2 shows the overall results of the clot structure analysis. Clots from the patients were significantly less permeable (4.1 vs. 8.4 x10^{-8} cm²; respectively, p=0.001) and showed less compaction (47.2 vs. 61.9 %, p<0.001) than clots from controls. Whilst the level of compaction correlated significantly to Ks in both patients (r=0.59, p=0.004) and controls (r=0.60, p=0.002), compaction did not correlate to factor XIII activity in either patients (r=0.13, p=0.554) nor controls (r=0.26, p=0.210).

The polymerization profile of the patients’ clots was significantly different from controls (AUC: 61.0 vs. 51.3 OD x min, p=0.003, see Fig. 1). No difference in lag phase was observed (103 vs. 96 sec. for patients and controls, respectively, p=0.150), whereas V_max was significantly higher for the patients compared with controls (0.283 vs. 0.179 OD/min, p<0.001).

The fibrin fibers in the patients had higher fiber mass-length ratios (13.1 vs. 11.6 x10^{-2} Da/cm, p<0.001), larger diameters (0.254 vs. 0.224 Da/cm, p<0.001) and lower fiber mass densities (2.46 vs. 2.12 Da/cm, p<0.001), respectively). Neither PAI-1 nor tPA antigen levels correlated with lysis rates in the patients (r<sub>1</sub>=0.20, p=0.378, and r<sub>1</sub>=0.15, p=0.504, respectively) nor in the controls (r<sub>1</sub>=0.01, p=0.950, and r<sub>1</sub>=0.01, p=0.953, respectively).

Correlations between fibrin structure characteristics and markers of inflammation and azothemia in the patients are shown in Table 3. Markers of inflammation correlated negatively to clot permeability and rate of fibrinolysis, but positively to fiber diameters. Interestingly, no significant correlations were found between the fibrin structure characteristics and most markers of azothemia, also when addressing glucose, albumin, potassium and sodium (data not shown). A significant cor-
relation, however, was observed between the level of clot compaction and serum phosphor ($r_s=0.45$, $p=0.035$).

Figure 2 shows representative SEM images of clots made from pooled patient plasma and pooled control plasma, respectively. The fiber clot density was 6.4 (5.1 – 7.4) and 4.3 (3.5 – 5.5) fibers per $\mu m^2$ for the patient and control plasma pool clots, respectively ($p<0.001$). When measuring the diameter of the fibers in the images, no differences could be found between the patient and control plasma pool clots: 0.129 $\mu m$ (0.100 – 0.160) and 0.128 $\mu m$ (0.101 – 0.154), respectively, $p=0.265$. When analyzing for overall image complexity, however, the AMT spectra (Fig. 2, panel C) were significantly higher for the patient pool clots than for the control pool clots (the area under the AMT spectra was 13838 (13167 – 14942) $^o$ scales and 12898 (11783 – 13796) $^o$ scales for the patient and control pool clots, respectively, $p=0.003$).

**Discussion**

In the present study we have investigated fibrin structure characteristics in patients with ESRD using assays for permeability, compaction, turbidity and SEM. The permeability of the patients’ clots was significantly lower compared with controls. This is in good agreement with the increased plasma fibrinogen concentrations in the patients (43). As permeability is directly proportional to the average pore size of fibrin networks (44), low permeability values indicate smaller pore sizes and thus tighter clots. Subjecting clots to centrifugal forces resulted in significantly less compaction in the patients’ clots compared with controls, which suggests that clots from the patients were more rigid than those from controls. The patients had higher factor XIII activity levels than controls (Table 1), but these activity levels did not correlate to compaction. In contrast, compaction correlated significantly to the level of permeability, indicating that the observed rigidity was predominantly governed by the tightness of the clots.

During fibrin polymerization $V_{max}$ was significantly higher for the patients compared with the controls. As the plasma concentration of fibrinogen in the patients was higher than in controls, the increased $V_{max}$ is likely to reflect a higher number of fibers being formed causing a more rapid increase in turbidity at the time of lateral aggregation of the protofibrils (9). No significant differences were found between patients and controls regarding lag phase, which indicates that the rate of protofibril...
formation was not any different. However, this is in conflict with the elevated concentration of fibrinogen found in the patients, as increased fibrinogen concentrations usually lead to shortening of the lag phase (9). It is therefore tempting to suggest that in ESRD patients, plasma constituents other than fibrinogen may influence fibrin monomer assembly rates. On the other hand, caution must be taken when interpreting lag phase results from plasma samples as some degree of inter-assay variation may be reflected in this parameter (45).

Clots from the patients displayed significantly slower fibrinolysis rates than clots from controls despite lower PAI-1 antigen levels. The rate of fibrinolysis in the patients correlated significantly to the level of clot permeability \( r_s=0.66, p<0.001 \) and inversely to the diameter of the fibers \( r=-0.88, p<0.001 \). These results are in agreement with observations made by Collet et al. that tight networks are less susceptible to fibrinolysis than coarser networks, and that fibrinolysis of thick fibers can be slower than that of thin fibers (15, 46). Obviously, the increased fibrin content in clots from the patients may have contributed to the reduced fibrinolysis rates. However, as the fibrinolysis rates were normalized to the initial maximum turbidity value, it is likely that differences in network structures may have contributed to the differences in fibrinolysis rates as well. In any case, the observations are important as they demonstrate that clots made from plasma from ESRD patients have reduced fibrinolysis rates when compared with clots from healthy controls, by this due to increased fibrinogen levels or other mechanisms.

In the SEM images clots made from pooled patient plasma showed significantly higher fiber clot densities than clots made from pooled control plasma (Fig. 2). These results are in good agreement with our permeability and compaction data, and also with the elevated fibrinogen concentration in the patients (9). The higher AMT curves observed for the SEM images of the patient plasma pool clots confirm the results on reduced clot permeability and increased fiber clot density in the patients as high mean angle AMT values indicate reduced pore sizes and increased fiber clot density due to narrowing of the distances between pixels having bright gray-scale intensities within the unfolded images.

In contrast to our turbidity experiments, however, the SEM images showed no differences in fiber diameters between patients and controls. In the turbidity experiments the fiber mass density of the fibrin fibers was lower in the patients compared with controls (Table 2), which indicates a higher degree of fiber hydration and thus differences in fibrin fiber constitutions. It is therefore tempting to hypothesize that during sample dehydration for SEM, the diameter of the patients’ fibers became reduced to a larger extent than the fibers from controls. This may be one of the reasons why we did not observe a significant difference in fiber diameter using SEM.

Patients with manifest CVD have been reported to produce tight and rigid clot structures that are resistant to fibrinolysis (11, 12, 14, 19, 20). Since we have observed similar clot properties in our ESRD patients, it is tempting to suggest that the excessive increased risk of CVD in ESRD patients may be influenced by their pathologic clot structure characteristics.

In Table 3 it was shown that resistance to fibrinolysis as well as level of clot permeability in the patients correlated significantly with plasma concentrations of fibrinogen, CRP and IL-6, but not with markers of azothemia. This suggests that the fibrin structure characteristics observed in the ESRD patients may be influenced to a greater extent by the inflammatory plasma milieu than by azothemia. As a consequence, reducing the azothemic load through increased dialysis would have a smaller impact on fibrin structure characteristics than amelioration of systemic inflammation. Promising results on altered fibrin structure characteristics following reduced systemic inflammation has recently been reported in patients with coronary artery disease (20). We have previously demonstrated that systemic inflammation in ESRD patients on PD can be reduced through a reduction of the intraperitoneal inflammation (47). However, studies investigating the effect of reduced inflammation on fibrin structure characteristics in ESRD patients are warranted as are studies addressing the impact of dialysis on fibrin structures.

The present study is not without drawbacks. The sample size was relatively small, which may have introduced type II errors, especially when addressing correlations between variables. Additionally, the ESRD patients in the study were represented by PD patients only. As heparins have been shown to affect fibrin structure characteristics (48), we chose not to include hemodialysis patients in the study because these patients are routinely treated with small quantities of heparin at the start of each haemodialysis session. Despite these weaknesses the present study is the first study to address fibrin structure characteristics developed from plasma samples in patients with ESRD using assays for turbidity, compaction, permeability and SEM.

In conclusion, the ESRD patients presented were shown to produce fibrin clots that were significantly less permeable, more rigid, and less susceptible to fibrinolysis than clots from healthy controls. The fibrin fibers had larger diameters and were more numerous than in control clots. The fibrin structure characteristics in the patients appeared to be associated primarily with the inflammatory plasma milieu than with level of azothemia. Larger, prospective studies are, however, needed to examine whether these findings correlate with the development of accelerated CVD in these patients.

Acknowledgements

The authors wish to thank laboratory technicians Anette Larsen, Kathrine Overgaard Sorensen, and Gunhild Andreassen from the Department of Clinical Biochemistry, Ribe County Hospital, Esbjerg, Denmark, and Thomas Sorensen, Department of Mechanical Engineering, Aalborg University, Denmark, for excellent technical support.
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