Altered fibrin clot properties in patients on long-term haemodialysis: relation to cardiovascular mortality

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Abstract

Background. Haemodialysis patients are at an increased risk of cardiovascular (CV) morbidity and mortality. Both end-stage renal disease (ESRD) and thromboembolic coronary events have been shown to be associated with the formation of dense fibrin clots resistant to fibrinolysis. The aim of the present study was to investigate the effect of long-term haemodialysis on clot structure/function and analyse an influence of markers of inflammation, oxidative stress and lipoprotein(a). We sought also to investigate if clot features might be related to CV events and mortality in haemodialysis patients.

Subjects and methods. In 33 patients (19 males, 14 females), aged 27 to 89 years, on long-term haemodialysis and 33 age- and sex-matched apparently healthy controls, we investigated fibrin clot properties and susceptibility to lysis using recombinant tissue plasminogen activator by using permeation and turbidity assays.

Results. Haemodialysis patients produced fibrin clots that had less porous structure (P < 0.0001) were less susceptible to fibrinolysis (P < 0.0001), began fibrin protofibril formation more quickly (P < 0.0001) and showed increased overall fibre thickness (P < 0.0001) compared with controls. Clot permeability and lysis time correlated with F2-isoprostanes (P < 0.01), Lp(a) (P < 0.0001) and fibrinogen (P < 0.01). None of the clot variables showed associations with the duration of haemodialysis treatment or the cause of ESRD. During a 36-month follow-up, 10 CV deaths were recorded. Mortality was associated with reduced clot permeability (P < 0.0001), prolonged lysis time (P < 0.0001), faster fibrin protofibril formation (P = 0.0004), thicker fibres (P < 0.0001) and increased fibrin clot mass (P < 0.0001).

Conclusions. Unfavourably altered clot properties can be detected in haemodialysis patients and may be associated with increased CV mortality.

Keywords: fibrin clot; fibrinolysis; haemodialysis; mortality

Introduction

It has been estimated that 40% of end-stage renal disease (ESRD) patients developed premature cardiovascular (CV) disease, with an annual CV mortality of 10% [1]. Patients on maintenance haemodialysis have 10- to 20-fold higher CV mortality than that reported in the age-matched general population. The CV risk factors in haemodialysis patients include older age, male gender, history of coronary artery disease (CAD) and diabetes, while paradoxically, a worse survival has been observed in dialysis subjects with a low rather than with a high blood pressure, body mass index and serum cholesterol [2,3]. Cardiac deaths represent ~40% of all deaths and CAD accounts for >60% of cardiac deaths in haemodialysis patients [4]. Novel CV risk factors identified in such patients include among others endothelial dysfunction, enhanced inflammatory state and increased oxidative stress [5].

Activation of blood coagulation results in fibrinogen conversion to fibrin by thrombin that, along with fibrin cross-linking by factor (F) XIIIa, leads to the formation of a fibrin clot, essential for the integrity of the vascular bed [6]. The structure and function of the fibrin clot are modulated by several environmental and genetic factors [7,8]. Total homocysteine (tHcy) [9], lipoprotein(a) [Lp(a)] [10] and C-reactive protein (CRP) [11], which along with fibrinogen are significantly elevated in haemodialysis patients, have also been reported to reduce clot permeability and susceptibility to lysis.

The clinical relevance of fibrin clot properties is unclear. Fibrin clots composed of dense fibre networks have been found in patients in the acute phase of coronary ischaemia [12], survivors of myocardial infarction (MI) [13], their first-degree relatives [14] and patients with diabetes mellitus [15]. Collet et al. [16] reported that unfavourable clot properties can also be detected in patients with nephrotic syndrome. Whole blood thromboelastography in patients with renal failure showed an increased clot rigidity and accelerated clot formation accompanied by hypofibrinolysis [17]. Very recently, Sjoland et al. [18] described marked alterations in fibrin clot properties evaluated in 22 patients on chronic peritoneal dialysis compared with healthy volunteers. Patients with ESRD had plasma fibrin clots that...
were less permeable and less susceptible to fibrinolysis than clots made from control plasma. We put forward a hypothesis that similar alterations in fibrin properties occur in patients on chronic haemodialysis and are linked to the risk of adverse cardiac events in this high-risk population. Therefore, the aim of this prospective cohort study was to investigate plasma clot characteristics in haemodialysis patients compared to age- and sex-matched healthy subjects. We sought also to investigate if clot features might be influenced by markers of inflammation, oxidative stress and Lp(a). Moreover, we have analysed whether CV mortality during follow-up might be associated with abnormal fibrin clot structure/function.

Subjects and methods

Patients

We enrolled 33 consecutive patients [(19 males (M), 14 females (F)] on maintenance haemodialysis [a median time, 30 (range 6 to 82) months], carried out across Fresenius F6 to F8 polysulfone membranes. Exclusion criteria were age <18 years, active cancer, any acute illness, acute vascular event or coronary intervention within the preceding 30 day and antiagulant therapy. The underlying causes for ESRD included chronic glomerulonephritis (n = 10), diabetic nephropathy (n = 5), autosomal dominant polycystic kidney disease (n = 5), bilateral nephrectomy due to carcinoma (n = 2), hypertensive nephropathy (n = 2) and other renal diseases (n = 9). CV medications taken by the patients studied were as follows: statins (n = 17), β-blockers (n = 14), aspirin (n = 13) and angiotensin-converting enzyme inhibitors (n = 11). Erythropoetin and folic acid 5 mg/day were received by 31 (94%) patients.

Patients were followed for 36 months. MI, stroke and death of any cause were recorded. ST-elevation MI or non-ST-elevation MI was defined according to the ESC/ACC guidelines [19]. Stroke was defined as permanent focal neurological disturbances owing to cerebrovascular infarction verified by computed tomography.

Thirty-three age- and sex-matched apparently healthy individuals who denied taking any medication for at least 4 weeks prior to the enrolment were recruited from the hospital staff and served as controls. Routine blood tests in 4 weeks prior to the enrolment were recruited from the hospital staff and served as controls. Routine blood tests in

Methods

Pre-dialysis blood samples were collected before unfractionated heparin administration. Routine blood tests, including lipid profile, albumin and glucose, were assessed by standard automated laboratory methods. Fibrinogen and high-sensitivity CRP were measured by nephelometry (Dade Behring, Marburg, Germany). The latter variable was estimated by calculating a mean value of two results obtained at a 2-week interval as recommended. Plasma tHcy was determined by reverse-phase liquid chromatography with fluorescence detection, as described previously [20]. Kt/V was calculated according to the Daugirdas model [21]. Venous blood samples for fibrin analysis were taken into 0.13 mM trisodium citrate tubes (Becton Dickinson, Numbrecht, Germany) and centrifuged within 30 min at 2500 g for 20 min. Platelet-poor plasma was frozen in aliquots at −80°C until analysis. Blood samples for tHcy determination were taken into EDTA tubes and placed directly on ice until the plasma separation. Commercially available immunoenzymatic assays were used to determine plasma Lp(a) (Biopool, Umea, Sweden); 8-epi-prostaglandin F2α (8-isopGF2α), a marker of oxidative stress (Cayman Chemicals, Ann Arbor, MI); prothrombin fragments 1 + 2 (F1+2), a marker of thrombin formation (Enzygnost F1+2, Dade Behring); tissue-type plasminogen activator (t-PA) antigen (Biopool), plasminogen activator inhibitor-1 (PAI-1) antigen (Biopool) and D-dimer (American Diagnostica, Greenwich, CT). All the intra-assay and inter-assay coefficients of variation were <7%.

Fibrin permeation analysis

Fibrin clot permeation was determined as previously described [11,12,14]. Briefly, 20-mmol/L calcium chloride and 1-U/mL human thrombin (Sigma) were added to 120-µL citrated plasma samples. After incubation in a wet chamber for 120 min, tubes containing the clots were connected to a reservoir of a buffer (0.05 M Tris–HCl, 0.15 M NaCl, pH 7.5) and its volume flowing through the gels was measured. A permeation coefficient (Kp), which indicates the pore size, was calculated from the equation Kp = Q / L × η / t × A × Δp, where Q is the flow rate in time t, L is the length of a fibrin gel (13 mm), η is the viscosity of liquid (1/100 poise), A is the cross-sectional area (0.049 cm²) and Δp is a differential pressure (in dyne/cm²) [22]. All measurements were performed in duplicates by an investigator blinded to the origin of samples. The intraindividual variability of results was 7.3%.

Turbidity measurements

Plasma samples were diluted 1:1 with 0.05 mol/L Tris–HCl, 0.15 mol/L NaCl, pH 7.4 and addition of 1 U/mL human thrombin (Sigma) and 15 mmol/L calcium chloride to plasma-initiated polymerization [11]. Absorbance was read at 405 nm for 15 min with a Perkin–Elmer Lambda 4B spectrophotometer (Molecular Devices Corp., Menlo Park, CA). The lag phase of the turbidity curve, which reflects the time required for lateral aggregation, and maximum absorbance at plateau reached by all individuals (ΔAbs), which reflects the number of protofibrils per fibre, were recorded [12,15]. Each sample was analysed twice. The inter-assay and intra-assay coefficients of variation were 5.8 to 7.1%, respectively.

Turbidometric clot lysis assay

Plasmin-mediated fibrinolysis was evaluated using a modified method of Williams et al. [23]. Briefly, 100-µL citrated plasma was diluted with 100 µL of a buffer (0.05 M Tris–HCl, 0.15 M NaCl, pH 7.4), containing 20-mmol/L
Table 1. Baseline characteristics of haemodialysis patients and apparently healthy controls

<table>
<thead>
<tr>
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<th>Patients (n = 33)</th>
<th>Controls (n = 33)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>61.0 (51.0–70.0)</td>
<td>59.0 (52.0–64.0)</td>
<td>0.71</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>19/14</td>
<td>20/13</td>
<td>0.80</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>154.4 (150.5–192.9)</td>
<td>178.3 (168.3–192.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>83.7 (67.1–103.1)</td>
<td>102.39 (91.5–120.1)</td>
<td>0.0002</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>46.4 (37.9–60.6)</td>
<td>54.0 (45.2–58.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>132.8 (103.7–201.2)</td>
<td>119.5 (91.2–133.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>98.9 (83.3–134.9)</td>
<td>95.4 (88.2–104.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>8.56 (2.98–14.01)</td>
<td>1.35 (0.98–1.85)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>tHcy (µmol/L)</td>
<td>21.05 (17.80–25.55)</td>
<td>15.10 (11.1–17.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>44.4 (40.0–46.0)</td>
<td>48.0 (45.0–51.0)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.12 (2.83–3.85)</td>
<td>2.72 (2.28–2.96)</td>
<td>0.002</td>
</tr>
<tr>
<td>P AI-1:Ag (ng/mL)</td>
<td>9.8 (8.2–13.4)</td>
<td>9.4 (8.1–11.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>tPA-Ag (ng/mL)</td>
<td>6.9 (5.8–8.0)</td>
<td>6.2 (4.8–7.1)</td>
<td>0.008</td>
</tr>
<tr>
<td>D-dimer (mg/dL)</td>
<td>0.35 (0.32–0.43)</td>
<td>0.15 (0.09–0.24)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>F1+2 (nmol/L)</td>
<td>1.04 (0.9–1.3)</td>
<td>0.9 (0.69–1.11)</td>
<td>0.007</td>
</tr>
<tr>
<td>Lp(a) (mg/L)</td>
<td>15.0 (9.7–22.7)</td>
<td>10.1 (8.7–15.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>8-iso-PGF2α (pg/mL)</td>
<td>240 (227–322)</td>
<td>169 (130–218)</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

Table 2. Fibrin structure and function characteristics in haemodialysis patients and healthy controls

<table>
<thead>
<tr>
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<th>Patients (n = 33)</th>
<th>Controls (n = 33)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ks (10−9 cm2/s)</td>
<td>8.7 (7.9–9.7)</td>
<td>10.7 (10.2–11.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔAbs max. (405 nm)</td>
<td>0.81 (0.77–0.87)</td>
<td>0.72 (0.64–0.75)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lag phase (s)</td>
<td>40 (38–44)</td>
<td>47 (45–50)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>t50% (min)</td>
<td>8.0 (7.1–9.3)</td>
<td>7.0 (6.8–7.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-Dmax (mg/L)</td>
<td>3.66 (3.58–3.84)</td>
<td>3.32 (3.21–3.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-Dmax (mg/L/min)</td>
<td>0.08 (0.07–0.09)</td>
<td>0.07 (0.06–0.07)</td>
<td>&lt;0.0001</td>
</tr>
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</table>

Data are shown as median values (interquartile ranges). Abbreviations: TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; CRP, C-reactive protein; tHcy, total homocysteine; P AI-1:Ag, plasminogen activator inhibitor 1 antigen; tPA-Ag, tissue-type plasminogen activator antigen; F1+2, prothrombin fragment; Lp(a), lipoprotein(a); 8-iso-PGF2α, 8-iso-prostaglandin F2α.

calcium chloride, 1-U/mL human thrombin (Sigma) and 14-µmol/L recombinant tissue plasminogen activator, rtPA (Boerhinger Ingelheim). Assembly kinetics were monitored by spectrophotometry at 405 nm in duplicate aliquots. The time required for a 50% decrease in clot turbidity (t50%) was chosen as a marker of the clot susceptibility to fibrinolysis. The intra-individual variability of results was 8.1%.

Perfusion clot lysis assay

Fibrin clots formed as described above were perfused with the same buffer containing 0.2 µmol/L rtPA (Boerhinger Ingelheim) according to Collet et al. [16]. The lysis rate was determined by measuring the concentration of D-dimers (American Diagnostica), a marker of plasmin-mediated fibrin degradation, every 20 min in the effluent [11]. Maximum rates of increase in D-dimer levels and maximum concentrations detected at 80 or 100 min were analysed in each subject. The experiment was stopped, usually after 80 to 120 min, while the fibrin gel collapsed under the pressure.

Statistical analysis

Data were given as median (interquartile range) unless otherwise stated. Normality of a value distribution was tested using the Kolmogorov–Smirnov test. Intergroup differences for continuous variables were assessed by the t test when normally distributed or the Mann–Whitney U test in the case of non-normal distribution. The χ2 test was used to assess intergroup differences in categorical variables. The Pearson or Spearman rank correlation coefficients were calculated to test the association between two variables with a normal or non-normal distribution, respectively. A P-value <0.05 was considered statistically significant.

Results

Baseline characteristics

Haemodialysis patients did not differ from controls with regard to age and gender (Table 1). Eleven (33%) patients had a history of CAD (MI or hospitalization for unstable angina). Five (15%) had diabetes. Kt/v-values were 1.35 ± 0.16 in the patient group. All controls had normal creatinine levels (64.3 ± 1.9 µmol/L). Glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride levels were lower in patients than in healthy controls (Table 1). Similarly, fibrinogen, tHcy, Lp(a) and CRP were markedly elevated in patients, whereas albumin levels were lower. Thrombin formation, expressed as plasma F1+2 levels, and oxidative stress, reflected by F2-isoprostanes, were higher in the haemodialysis group as well as tPA antigen and D-dimer, but not PAI-1 antigen.

As shown in Table 2, patients on haemodialysis had lower clot permeability and longer clot lysis time compared with healthy individuals. Haemodialysis was also associated with a shorter lag phase and higher maximum
absorbance in turbidimetric measurements, indicating faster protofibril formation and thicker fibrin fibre formation, respectively. Maximum D-dimer levels, indicating the increased fibrin clot mass, and maximum rate of D-dimer release in a pressure-driven clot system were increased in the patients compared with controls.

Patients on haemodialysis diagnosed with CAD prior to enrolment \( n \) were similar to the remaining \( 22 \) patients in terms of age, sex, \( Kt/v \) and medications (data not shown). Comparative analysis of clot fibrin properties in these two groups revealed that the only significant difference was increased maximum absorbance in the former group suggesting the formation of thicker fibres \( 0.87 \) \( (0.81–0.90) \) versus \( 0.79 \) \( (0.75–0.83) \); \( P = 0.04 \).

The permeability coefficient in the haemodialysis group was negatively correlated with F2-isoprostanes \( r = −0.81; P < 0.0001 \), Lp(a) \( r = −0.69; P < 0.0001 \), fibrinogen \( r = −0.70; P < 0.0001 \) and CRP \( r = −0.37; P = 0.04 \). Lysis time was only correlated with Lp(a) \( r = 0.79; P < 0.0001 \), F2-isoprostanes \( r = 0.51; P = 0.002 \) and fibrinogen \( r = 0.46; P = 0.007 \). The lag phase and maximum absorbance in patients were only associated with Lp(a) \( r = −0.48; P = 0.005 \) and \( r = 0.41; P = 0.02 \), respectively. A rate of D-dimer release showed a significant association only with Lp(a) \( r = 0.50; P = 0.003 \), while maximum D-dimer levels in the perfusion lysis assay correlated only with Lp(a) \( r = 0.53; P = 0.001 \) and 8-isoprostanes \( r = −0.35; P = 0.04 \) in haemodialysis patients. No significant associations were observed between any clot variables and age, lipids, albumin, PAI-1, D-dimer, tPA, F1+2, or other variables studied in either group \( r < 0.2; P > 0.1 \). None of the six clot variables showed associations with the duration of haemodialysis treatment or the cause of ESRD \( r < 0.2; P > 0.1 \).

When analysing correlations between medications and fibrin clot characteristics, we found that aspirin treatment was associated with lower clot permeability \( K_c \) \( 8.0 \) \( (7.3–9.0) \) versus \( 9.4 \) \( (8.2–9.9) \) \( 10^{-9} \) \( \text{cm}^2/\text{s} \); \( P = 0.04 \) and increased fibrinogen \( 3.59 \) \( (3.41–3.94) \) versus \( 2.94 \) \( (2.43–3.26) \) \( \text{g}/\text{L} \); \( P = 0.02 \). There was no correlation between aspirin treatment and lysis time. There was no association between administration of other drugs, including statins and clot properties \( P > 0.1 \).

**Characteristics during follow-up**

During follow-up, \( 10 \) \( (30.3\%) \) patients died of CV causes [acute MI \( n = 8 \) and sudden cardiac death \( n = 2 \)] after a median of 14 \( (\text{range}, 4–31) \) months. These patients were similar to the remaining subjects on haemodialysis in terms of age, sex, conventional risk factors, concomitant treatment and cause of dialysis (data not shown). Two patients underwent transplantation, one died of sepsis, and they were excluded from the follow-up analysis. The percentage of patients with CAD among those who died of CV causes was higher than that in the remainder \( 60\% \) versus \( 22\%; P = 0.04 \).

Fibrinogen \( 3.49 \) \( (3.39–4.04) \) versus \( 2.94 \) \( (2.43–3.85) \) \( \text{g}/\text{L} \); \( P = 0.04 \), Lp(a) \( 26.45 \) \( (22.7–32.41) \) versus \( 12.7 \) \( (8.9–18.6) \) \( \text{mg}/\text{L} \); \( P < 0.0001 \) and F2-isoprostanes \( 312 \) \( (281–341) \) versus \( 230 \) \( (202–251) \) \( \text{pg}/\text{mL} \); \( P = 0.002 \) were higher among patients who died of CV causes as compared with the remaining subjects. A corresponding intergroup difference in CRP levels was of borderline significance \( 11.96 \) \( (9.64–15.99) \) versus \( 5.81 \) \( (2.8–10.04) \) \( \text{mg}/\text{L} \); \( P = 0.052 \). Other laboratory variables measured, including glucose, tHcy, F1+2 and albumin, did not differ between two subgroups (data not shown).

Clots made from baseline plasma taken from 10 patients who died of CV causes were significantly less permeable \( P < 0.0001 \) and were lysed less efficiently \( P < 0.0001 \) than those from plasmas of the remaining patients (Table 3). The 10 patients who died of CV causes showed a shorter lag phase \( P = 0.0004 \) and higher maximum absorbance \( P < 0.0001 \), indicating longer time required for fibrin protofibrils to grow enough to allow lateral aggregation to occur and higher average fibrin fibre size, respectively, while maximum D-dimer levels and their rate of increase were increased in the perfusion lysis assay \( P < 0.0001 \) for both comparisons.

**Discussion**

The current study shows that in patients on chronic haemodialysis fibrin clot properties are markedly altered. Fibrin clots from plasma of the patients display significantly reduced permeability, faster protofibril formation, increased fibre size and clot mass, along with decreased susceptibility to fibrinolysis, compared with healthy well-matched individuals. Our findings indicate that clots from haemodialysis patients are much tighter than those from controls. Associations between low permeability and impaired fibrinolysis in haemodialysis patients confirmed that haemodialysis patients are characterized by a reduced lysis rate, likely due to less efficient transport of fibrinolytic agents through a fibrin clot \[24,25\]. In the perfusion lysis assay, higher rate of D-dimer release from clots observed in patients reflects a markedly increased fibrin clot mass combined with reduced volume of the percolate as a result of lower clot permeability.

Fibrinogen has been reported to be an independent risk factor for overall and CV mortality in patients on chronic haemodialysis treatment \[26\]. This study shows that altered clot features may be associated with CV mortality. Particularly unfavourable clot features distinguished haemodialysis patients who died of CV causes during a 36-month
follow-up. This finding could be only in part attributable to other elevated CV death risk factors such as age, Lp(a), CRP, previous CAD event or oxidative stress. Abnormal fibrin clot characteristics, if corroborated in larger groups, may contribute to an increased risk of CV mortality in haemodialysis patients.

Our data corroborate and extend previous findings [18] by showing that unfavourable features of fibrin clot structure and function observed in patients on peritoneal dialysis are also present in individuals on chronic haemodialysis, regardless of the time on dialysis, and these properties are associated with CV mortality. However, comparing our findings with those published by Sjoland et al. [18], there are some discrepancies that warrant consideration. First, the only feature of a fibrin clot correlated with CRP levels in our study is clot permeability ($r = -0.37$; $P = 0.03$). Potential reasons for this include different dialysis methods in the two ESRD populations, inclusion of CAD patients receiving CV medications (no data on this in the paper by Sjoland et al. [18]) and much higher CRP levels in the current study (median, 8.56 versus 4.12 mg/L [18], respectively). Secondly, in the current study there was a significant difference in the lag phase among haemodialysis patients and controls. In contrast to the study on patients on peritoneal dialysis [18], our data suggest that the rate of protofibril formation is higher in ESRD patients, which is in agreement with elevated fibrinogen levels in these subjects because hyperfibrinogenemia, typically results in a shorter lag phase [27]. Differences in clinical patient characteristics, along with much lower fibrinogen and higher CRP levels in our study, could account for this discrepancy.

A novel finding is the significant associations observed between the clot features and the extent of oxidative stress that is considered to be involved in the pathogenesis of atherosclerosis and other complications of ESRD in haemodialysis patients [28]. Elevated plasma F2-isoprostanes that are derived from oxidative nonenzymatic modification of arachidonic acid and serve as a stable in vivo marker of oxidative stress were detected in haemodialysis patients [29], which is in line with our findings. Oxidative stress has been shown to be the independent risk factor for CV mortality in chronic haemodialysis patients [30]. In our study, CV deaths were also associated with enhanced oxidative stress at baseline as well as with predisposition to the occurrence of tight and poorly lysable clots. Mechanisms by which oxidative stress affects fibrin properties in haemodialysis patients are related to protein oxidative modification. In vitro plasma fibrinogen is particularly susceptible to oxidative modification [31]. Oxidized fibrinogen has been reported to promote fibrinogen conversion to fibrin, enhance platelet aggregation and support less efficiently plasminogen activation by t-PA [32].

A highly atherogenic Lp(a) that consists of an LDL-like particle with covalently bound apolipoprotein(a) occurs at high concentrations in haemodialysis patients largely because of reduced clearance but not increased hepatic synthesis [33]. We found that fibrin clot characteristics were correlated with Lp(a) levels in haemodialysis patients, which represented a major independent predictor of clot variables. Our data suggest a novel proatherothrombotic effect of raised Lp(a) levels in haemodialysis patients, similar to that observed in CAD patients with normal serum creatinine levels [10]. A mechanism of the influence of Lp(a) on fibrin clot features is likely related to the fact that the fibrinogen alpha C domains, which are involved in alpha-chain cross-linking and alpha-2-antiplasmin binding to fibrin [6], contain high affinity apolipoprotein(a)-binding sites [34].

Our study has several limitations. First, the study population is limited, which may have introduced type II errors, especially in calculations of the correlation coefficients. However, the number of our patients was similar to those evaluated by other investigators who analysed clot variables in various diseases. Moreover, our observations on mortality also require further studies on larger haemodialysis populations. Second, our analysis was based on a determination of each variable at a single time point, and some changes in clot variables after 3 years cannot be excluded. Third, fibrin structure was not investigated by using scanning electron microscopy. However, using this technique, clots are fixed and further processed; therefore measurements are hard to be extrapolated to the in vivo situation, which was supported by inconclusive data from electron microscopy in patients on peritoneal dialysis [20]. Finally, given variability in fibrinogen concentrations, DAbs might be misleading as a measure of fibre thickness when measured on a single wavelength. However, crucial features, important from a pathophysiologic point of view in patients at risk of CAD, are clot permeability and susceptibility to lysis, which have been convincingly shown to differ between patients and controls.

Concluding, our study indicates that patients on maintenance haemodialysis display unfavourable features of fibrin clot structure/function, which might represent a novel prothrombotic effect involved in the occurrence of fatal CV events. Decreased clot permeability, faster fibrin polymerization and impaired fibrinolysis could contribute to the progression of atherothrombotic vascular disease in haemodialysis patients. Since elevated Lp(a) and F2-isoprostanes were correlated with unfavourable clot features, it might be hypothesized that the correction of the oxidant/antioxidant imbalance and reduction of Lp(a) levels may decrease CV risk in haemodialysis patients at least in part through improvement of clot properties. Larger studies are needed to determine a role of abnormal fibrin network properties in patients with chronic renal failure.

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Conflict of interest statement. None declared.

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