Activation of platelets and coagulation during haemodialysis

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Deviations in coagulation activation due to treatment with different haemodialysis membranes

P. C. M. Barrels, M. Schoorl, M. Schoorl & M. J. Nubè

ABSTRACT

Despite systemic heparinization, extracorporeal circulation will induce activation of blood coagulation. Thrombogenicity is associated with biocompatibility of dialysis membranes. Investigation of procoagulatory and fibrinolytic activity is performed prior to and during treatment with haemodialysis. In this study fluctuations of plasma coagulation factor XII, thrombin antithrombin complexes, prothrombin fragment 1+2 and thrombus precursor protein were monitored in 10 subjects during treatment with haemodialysis. Subjects were treated with both polysulphone high-flux dialyser membranes (F-60) and low-flux polymethylmethacrylate (PMMA) membranes.

Immediately after start of treatment, blood in contact with artificial membrane surfaces resulted in a marked decrease in factor XII activity amounting to a mean reduction of 80% in the case of PMMA and a reduction of 40% in the case of F-60. In due course, a steady, on-going generation of thrombin antithrombin complexes was observed in several subjects, especially after treatment with F-60 membranes, amounting to increases exceeding 100% of initial values. Establishment of fibrinogen, prothrombin fragment 1+2 and thrombus precursor protein plasma concentrations yielded enhanced results for PMMA compared with the results for treatment with F-60 dialysis membranes.

In order to prevent activation of clotting during several stages of haemodialysis, supplementation of anticoagulant can be established on the basis of analytical results of coagulation parameters.
INTRODUCTION

As a consequence of extracorporeal blood circulation the haemostatic balance is disturbed in subjects treated with haemodialysis [1]. Repetitive induction of acute-phase reactant response may induce a chronic micro-inflammatory state. According to recent studies, an increased acute-phase response is associated with type of dialyser membrane [2, 3]. In particular, acute phase response and concomitantly increased degree of hypercoagulability is strongly influenced by surface characteristics of the dialyser membrane and flow conditions within the circuit [4–6].

Thrombus generation in the extracorporeal circuit is demonstrated to be a multifactorial process [4]. If not effectively prevented, thrombogenicity formation will result in reduced haemodialysis efficiency because membrane area is lost [5].

Evaluation of conditions concerning biocompatibility of dialysis membranes, flow rates and appropriate application of anticoagulants is essential in order to minimize damage caused by white blood cell activation [7]. Activation of intracellular constituents in response to inflammatory stimuli is an essential step in release processes of proteolytic enzymes that are initiated by granule products such as myeloperoxidase, elastase and lactoferrin [8, 9]. Haemodialysis induced degranulation of polymorphonuclear leucocytes is considered to be a reactive process, induced by contact between blood cells and artificial dialyser membrane. A Ca^{2+}-free environment within the dialyser lumen is shown to attenuate degranulation of polymorphonuclear leucocytes [10].

Contact of blood constituents with negatively charged dialysis membrane surfaces such as polyacrylonitrile membrane AN-69 is demonstrated to result in a marked activation of coagulation [1].

Because activation of plasma coagulation factor XII is induced by mediation of a prekallikrein kininogen complex, platelet activation will stimulate this conversion by creating a procoagulant surface [11].

Heparinization not only affects haemostasis and thrombogenicity, but also other physiological systems that may become activated during haemodialysis as a result of biocompatibility [12].

Results from clinical studies with respect to thrombogenicity are widely scattered owing to varying subject-to-subject response to treatment with anticoagulants during haemodialysis.

Conversion of prothrombin into active thrombin is a major event in the final stage of the coagulation activation cascade. In the case of suspected activation, plasma concentrations of thrombin antithrombin III complexes (TAT) and prothrombin fragment 1 + 2 (F1 + 2) yield information with regard to thrombin generation.

The aim of the present longitudinal investigation was to evaluate and quantify the effects of application of alternative membranes for treatment with haemodialysis on coagulation activation, particularly the poly-methyl-methacrylate (PMMA) membrane and the polysulphone membrane F-60, both of which have been used subsequently in a selected subject group. For the purpose of appropriate comparison, additional investigations were performed in an apparently healthy subject group and in a group of predialytic uraemic subjects.
MATERIALS AND METHODS

Patients
Ten subjects with ages varying between 20 and 80 years participated in the study. Treatment with haemodialysis (HD) was indicated owing to renal failure caused by pyelonephritis, nephrolithiasis, glomerulosclerosis and membrane proliferative glomerulonephritis. In the remaining cases, the nephropathology was of unknown origin. The subjects were given treatment three times a week in periods ranging from 2 to 12 years.
The subjects were included in the study after they had given their informed consent. Criteria for exclusion included application of salicylates, Warfarin, dipyridamol or any other therapy that might have affected platelet function (Persantin®).
After venipuncture, blood samples were anticoagulated with sodium citrate (0.129 mol, 1/10). Platelet-poor plasma was prepared by centrifugation of blood samples at 2500 g for 10 min at 4°C. Small aliquots of plasma were stored in plastic tubes at -70°C until analysis. Blood samples were collected from the arterial line before dialysis (t=0) and subsequently after 5 (t=5), 30 (t=30), 60 (t=60) and 150 (t=150) min.

Reference group
Apparently healthy subjects.
A reference group of 20 apparently healthy subjects (aged 25 – 53) was used to yield insight on the reference range.

Uraemic subjects.
A group of 17 predialytic subjects with serum urea concentrations in the range of 20 – 35 mMol/L was included in the study in order to establish the effect of uraemia on coagulation activation.

Dialysis protocol
The PMMA membrane (Toray Industries Inc., Tokyo, Japan), the polysulphone membrane F-60 (Fresenius, Bad Homburg, Germany) and the biBAG dialysis system (Fresenius, Bad Homburg, Germany) were used in the same subject group. Blood-flow rates ranged between 200 and 250 ml/min and ultrafiltration flow rates between 300 and 1000 ml/min according to the individual needs of the patient. Dialysate contains sodium at a concentration of 138 mMol/L, potassium at 2.0 mMol/L, calcium at 1.75 mMol/L, magnesium at 1.5 mMol/L, chloride at 107 mMol/L, glucose at 5.5 mMol/L and acetate at 38 mMol/L. The dialysis sessions lasted 3 – 4 h, depending on individual needs and efficacy of treatment.
Before starting HD, the extracorporeal system was rinsed with approximately 500 ml of saline. At the start of HD treatment either with the F-60 membrane or PMMA membrane, a priming dosage depending on individual body weight and amounting to 2000 to 5500 units low molecular weight heparin (LMWH) (Fragmin, Kabi, Stockholm, Sweden) was administered. After 150 min on HD treatment the patients received an additional dose of LMWH (Fragmin 500 – 1000 U). For familiarization, patients were dialysed twice already on either the PMMA or the F-60 membrane before the day of venipuncture.
Factor XII
Factor XII activity is determined by performing a modified activated partial thromboplastin time (APTT) with silica activation (Instrumentation Laboratory, Milan, Italy). Samples were diluted and added to plasma deficient in factor XII. Correction of the prolonged clotting time of the deficient plasma is proportional to the activity percentage of factor XII in the patient’s plasma. Calibration is performed with application of CALplasma as supplied by the manufacturer (Instrumentation Laboratory, Milan, Italy).

TAT
The concentration of TAT complexes was determined by application of a sandwich-type enzyme-linked immunosorbent assay (Enzygnost® TAT micro, Dade Behring, Marburg, Germany)

F1+2
The concentration of prothrombin F1+2 was determined by means of a sandwich-type enzyme-linked immunosorbent assay (Enzygnost® F1+2 micro, Dade Behring, Marburg, Germany).

Thrombus precursor protein
The concentration of thrombus precursor protein polymers (TpP) was determined by application of an enzyme-linked immunoassay (ABS TpP™ Assay, American Biogenetic Sciences Inc., Columbia, USA).

Fibrinogen
Fibrinogen concentration was determined in accordance with the Clauss method by adding excess of thrombin to diluted plasma in order to convert fibrinogen to fibrin (Instrumentation Laboratory, Milan, Italy).

Statistical evaluation of results
Statistical evaluation of data was performed by applying multivariance analysis (ANOVA) and Student’s t-test for paired results (SPSS software 10.0 for Windows).

RESULTS
Investigations within a selected subjects’ group were performed in order to register kinetics along with intra-individual variations due to activation of coagulation during treatment with HD while subsequently applying F-60 and PMMA membranes, respectively. Membrane dependent results (mean value±SD) for coagulation parameters are presented in Table I. For comparison purposes, coagulation parameters which were established in a reference group of apparently healthy subjects (n=20) and a group of predialytic uraemic subjects (n=17) are listed in Table II.
TABLE I. Results for coagulation parameters (mean value, standard deviation) at several stages of haemodialysis: before (t=0) and at t=5, t=30, t=60 and t=150 min after starting HD.

<table>
<thead>
<tr>
<th>Time after starting HD (min)</th>
<th>F60 Mean</th>
<th>SD</th>
<th>PMMA Mean</th>
<th>SD</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XII activity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>58</td>
<td>21</td>
<td>62</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
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<td>21</td>
<td>12</td>
<td>13</td>
<td>**</td>
</tr>
<tr>
<td>30</td>
<td>36</td>
<td>20</td>
<td>17</td>
<td>13</td>
<td>**</td>
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<tr>
<td>60</td>
<td>40</td>
<td>21</td>
<td>24</td>
<td>14</td>
<td>*</td>
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<tr>
<td>150</td>
<td>45</td>
<td>18</td>
<td>36</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>3.5</td>
<td>0.8</td>
<td>4.0</td>
<td>1.1</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>3.3</td>
<td>0.7</td>
<td>4.1</td>
<td>1.2</td>
<td>*</td>
</tr>
<tr>
<td>30</td>
<td>3.3</td>
<td>0.7</td>
<td>4.2</td>
<td>1.2</td>
<td>**</td>
</tr>
<tr>
<td>60</td>
<td>3.5</td>
<td>0.7</td>
<td>4.4</td>
<td>1.2</td>
<td>**</td>
</tr>
<tr>
<td>150</td>
<td>3.5</td>
<td>0.7</td>
<td>4.4</td>
<td>1.1</td>
<td>**</td>
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<tr>
<td>TAT (μg/L)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>0</td>
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<td>0.9</td>
<td>3.5</td>
<td>1.0</td>
<td>**</td>
</tr>
<tr>
<td>5</td>
<td>2.6</td>
<td>0.8</td>
<td>3.3</td>
<td>0.8</td>
<td>NS</td>
</tr>
<tr>
<td>30</td>
<td>3.4</td>
<td>1.8</td>
<td>3.2</td>
<td>0.9</td>
<td>NS</td>
</tr>
<tr>
<td>60</td>
<td>4.2</td>
<td>2.6</td>
<td>3.6</td>
<td>1.4</td>
<td>NS</td>
</tr>
<tr>
<td>150</td>
<td>6.4</td>
<td>5.1</td>
<td>3.8</td>
<td>0.9</td>
<td>NS</td>
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<tr>
<td>TpP (mg/L)</td>
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<tr>
<td>0</td>
<td>1.3</td>
<td>0.8</td>
<td>4.1</td>
<td>1.5</td>
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<tr>
<td>5</td>
<td>1.5</td>
<td>0.8</td>
<td>3.8</td>
<td>1.6</td>
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</tr>
<tr>
<td>30</td>
<td>2.0</td>
<td>1.0</td>
<td>3.8</td>
<td>1.7</td>
<td>**</td>
</tr>
<tr>
<td>60</td>
<td>1.6</td>
<td>0.7</td>
<td>4.0</td>
<td>1.7</td>
<td>**</td>
</tr>
<tr>
<td>150</td>
<td>1.6</td>
<td>1.0</td>
<td>4.8</td>
<td>1.6</td>
<td>**</td>
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<tr>
<td>F1+2 (nMol/L)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.8</td>
<td>0.4</td>
<td>3.6</td>
<td>1.5</td>
<td>***</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
<td>0.4</td>
<td>1.7</td>
<td>0.8</td>
<td>***</td>
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<tr>
<td>30</td>
<td>0.7</td>
<td>0.4</td>
<td>1.7</td>
<td>0.8</td>
<td>***</td>
</tr>
<tr>
<td>60</td>
<td>0.7</td>
<td>0.4</td>
<td>1.9</td>
<td>0.9</td>
<td>***</td>
</tr>
<tr>
<td>150</td>
<td>0.6</td>
<td>0.3</td>
<td>1.9</td>
<td>0.9</td>
<td>***</td>
</tr>
</tbody>
</table>

Degree of statistical significance between F-60 and PMMA:
*p < 0.05; **p < 0.01; ***p < 0.001.

NS = difference is not statistically significant; PMMA = poly-methyl-methacrylate; F-60 = polysulphone F-60; HD = haemodialysis; TAT = thrombin antithrombin III complexes; TpP = thrombus precursor protein; F1+2 = prothrombin fragment 1+2.
### TABLE II. Results for coagulation parameters (mean value, standard deviation) in a reference group of apparently healthy subjects (n=20) and a group of uraemic subjects (n=17).

<table>
<thead>
<tr>
<th></th>
<th>Apparently healthy subjects</th>
<th>Uraemic subjects</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Factor XII activity (%)</td>
<td>92</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.5</td>
<td>0.8</td>
<td>3.2</td>
</tr>
<tr>
<td>TAT (µg/L)</td>
<td>1.3</td>
<td>1.8</td>
<td>4.7</td>
</tr>
<tr>
<td>TpP (mg/L)</td>
<td>1.1</td>
<td>0.8</td>
<td>2.8</td>
</tr>
<tr>
<td>F1+2 (nMol/L)</td>
<td>0.8</td>
<td>0.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Degree of statistical significance between the two reference groups: ***p < 0.001.
NS = Difference is statistically not significant; TAT = thrombin antithrombin III complexes; TpP = thrombus precursor protein; F1+2 = prothrombin fragment 1+2

**Figure 1.** Results for factor XII activity (%) established in 10 subjects at several stages before starting haemodialysis (HD) (1) and at t=5 (2), t=30 (3), t=60 (4) and t=150 min (5) after starting HD. The horizontal, dashed line indicates the upper level of the reference range for apparently healthy subjects.

**Factor XII**
As demonstrated in Figure 1, obvious decreases exceeding the lower level of the reference range were observed in 7 subjects within 5 min of starting HD treatment when using F-60 membranes and in 9 subjects when using PMMA membranes. The rate of decrease was more pronounced when PMMA membranes were used. Afterwards, at T=60 and T=150 min, steady ongoing increases were detected.

**TAT and F1+2**
Thrombin generation was estimated by monitoring TAT (Figure 2a) and F1+2 concentrations (Figure 2b). Determination of plasma TAT concentrations yielded markedly increased results during treatment with F-60 dialysis membranes in four subjects. Conversely, application of PMMA membranes did not result in increased generation of TAT during
treatment. However, in the latter case prothrombin F1+2 concentrations had already increased in the initial stage of HD after flushing the lines with saline. In normal circumstances patients would be dialyzed with a F-60 membrane. During treatment with PMMA, prothrombin F1+2 concentrations remained markedly increased beyond the upper limit of the reference range in three subjects.

However, these patients were not the same subjects as those with increased levels of TAT at t=150 min after treatment with F-60. When compared with the reference group of apparently healthy subjects in the ureamic subjects group, statistically significant increased results were established (Table II).

**Fibrinogen and TpP**

As a result of application of PMMA membranes, fibrinogen concentrations demonstrate a tendency towards higher values if compared with application of F-60 membranes. The results of statistical evaluation of results at any time interval are listed in Table I. In the final stage of treatment with HD, fibrinogen concentrations were higher than those in the initial stage when using PMMA membranes. When using F-60 membranes, alterations in fibrinogen concentrations were not observed during HD (Figure 3a).

TpP concentrations within the reference range were demonstrated in all subjects at the initial stage and during HD (Figure 3b). However, as a result of treatment with PMMA membranes, statistically significantly increased results were obtained in comparison with F-60 membranes (Table I).

![Figure 2](image.png)

**Figure 2.** Results indicating activation of coagulation established in 10 subjects at several stages before starting haemodialysis (HD) (1) and at t=5 (2), t=30 (3), t=60 (4) and t=150 min (5) after starting HD. (a): Thrombin antithrombin III complexes (TAT) (µg/L). (b): Prothrombin fragment 1+2 (F1+2) (nMol/L). The horizontal, dashed line indicates the upper level of the reference range for apparently healthy subjects.
DISCUSSION

Thrombotic events are common among subjects with end-stage renal disease and contribute substantially to the high cardiovascular morbidity and mortality in this population [13]. In agreement with a study elsewhere [6] we demonstrated that concentrations of TAT and F1+2 were significantly increased prior to HD. In four subjects TAT complexes were markedly further increased during treatment with F-60 membranes. Activation of coagulation may be due to mechanical stress as a result of high pressures needed for ultrafiltration. Shear stresses result from friction between blood flow components and the capillary wall of artificial membranes. Within a capillary vessel, blood flow velocity is maximal at the centre, whereas at the bloodwall interface flow velocity is minimal and shear stress is maximal.

Haemorrhagic abnormalities associated with HD treatment implicate activation of platelets, activation of the coagulation system and modifications of the fibrinolytic system. Therefore, routine practice of HD requires systematic treatment for anticoagulation. However, use of heparin in patients with increased risk of bleeding may induce serious complications [14]. Conversely, insufficient anticoagulation treatment will give rise to increased fibrin-fibrinogen deposition in the dialyser membrane, with a subsequent reduction in dialyser efficacy.

During HD treatment activation of the plasma coagulation system occurs, owing to interrelated reactions at the artificial interface between blood cells and membrane. Thrombogenic stimuli may arise from dialyser membranes and other components of the extra-
corporeal circuit such as blood flow rate turbulence, bubble traps, shear stress induced activation of platelets due to access needles and blood roller pumps. It is important to define specific parameters that are of essential interest for evaluation of haemocompatibility [4]. Owing to lack of standardization, the findings of several clinical studies comparing the relative thrombogenicity of HD membranes are inconsistent. As a result of our study, within 5 min of starting HD treatment a sudden decrease in Factor XII activity was demonstrated in both F-60 and PMMA membrane application. After the initial reduction a gradual increase was shown for F-60 membranes but not for PMMA membranes. Activation of the coagulation pathway induced by contact with foreign surfaces can be monitored by the establishment of Factor XII. With application of PMMA membranes, Factor XII concentrations are more markedly decreased than those with application of F-60 membranes. In a previous study, application of AN-69 membranes resulted in only a 9 – 45% decrease [1]. Reduced plasma Factor XII concentrations are associated with contact system activation [15]. Thrombogenicity is monitored by determination of circulating levels of prothrombin fragment 1+2 and TAT complexes. In four subjects, increased TAT generation during HD treatment only occurred when F-60 membranes were used. Thrombin generation and platelet activation may occur because of biomembranes or leucocyte-derived proteases [9]. Evaluation of thrombotic tendency in most cases is reliant on detection of plasma coagulation markers rather than on platelet factors. With application of PMMA membranes, prothrombin F1+2 concentrations are already increased in the initial stage of treatment. Although patients were already dialyzed twice in order to become familiar with the alternative PMMA membrane before venipuncture took place, switching the type of dialysis membrane could still be the causal factor in the increased levels of F1+2 that are detected at t=0. With regard to initially increased F1+2 concentrations, the same phenomenon was demonstrated in a previous study using AN-69 membranes [1]. It is important to gather information concerning activation of coagulation at an early stage. In low shear stress in the extracorporeal circulation, activation of coagulation may occur without being washed away by the force of blood flow. In contrast, higher shear stress in the arterial circulation associated with increased flow tends to dilute procoagulant substances, thus preventing the formation of prothrombin, thrombin and insoluble fibrin. As a result of coagulation activation, the inner luminal capillary diameter is decreased. Ultimately, fibrin generation is increased because of reduced blood flow and blood-flow velocity [16]. Compared with F-60 membrane treatment, slightly increased fibrin concentrations were detected in the subject group that was treated with PMMA membranes. Increased fibrinolysis is hypothesized to result from the dialysis process itself, whereas no difference was observed corresponding to the type of dialysis membrane used [17]. We conclude from our results that dialysis procedures with application of both membranes consistently resulted in stimulation of procoagulatory factors. Variations of fibrin-fibrinogen degradation products between subjects indicate variable degrees of clotting activation during treatment with HD.
REFERENCES


