Original Article

The AN69 ST haemodialysis membrane under conditions of two different extracorporeal circuit rinse protocols—a comparison of thrombogenicity parameters

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Abstract

Background. Thrombogenicity is an important parameter of haemodialysis (HD) membrane biocompatibility. The surface of the polyacrylonitrile AN69 ST membrane is coated with a polyethylenimine. This modification allows heparin adsorption. The binding of heparin to the membrane surface occurs during priming of the extracorporeal circuit (ECC) by rinsing it with saline and heparin. Our aims were to assess and compare the thrombogenicity of the AN69 ST membrane under conditions of two extracorporeal circuit (ECC) rinse protocols—with and without unfractionated heparin (UFH).

Methods. In a prospective, crossover and randomized study, we examined 10 patients during HD after ECC preparation with either rinse protocols. Prior to HD and at 15, 60 and 240 min, we determined plasma levels of the thrombin–antithrombin complexes (TAT), platelet factor 4 (PF4), heparin concentration (antiXa) and thrombocyte count. Systemic anticoagulation was performed using UFH.

Results. During HD after ECC rinse without UFH, there was a significantly earlier and more marked increase in TAT compared with UFH-containing rinse (P < 0.05). Using Spearman coefficient, we demonstrated a significant correlation between TAT and antiXa at 60 min (r = −0.534) and 240 min (r = −0.538). A comparison of the TAT/antiXa ratios between rinses at 60 min revealed a significantly higher increase in TAT following UFH-free rinse (P < 0.05). There was no difference in PF4 between the rinses. Platelet count did not change significantly during HD using either rinse protocol.

Conclusion. Based on plasma TAT levels, ECC priming with an UFH-containing solution reduces the thrombogenicity of the AN69 ST membrane. There is no significant difference between both types of priming concerning PF4 and thrombocyte count.

Keywords: biocompatibility; haemodialysis; platelet factor 4; polyacrylonitrile; thrombin–antithrombin complexes; thrombogenicity

Introduction

Dialysis membrane thrombogenicity is an important feature of biocompatibility. The AN69 ST membrane is a polyacrylonitrile membrane whose surface is treated with the polycationic biopolymer polyethylenimine (PEI) during manufacture. This modification results in a significant increase in surface charge. The zeta potential of the common AN69 polyacrylonitrile membrane is approximately −70 mV. The PEI coating reduces the electronegativity to values of approximately −15 mV. This surface treatment prevents contact phase system activation and bradykinin generation [1]. Another important feature of the membrane, also related with decreased electronegativity, is its ability to bind heparin. To explain, PEI immediately upon binding to the native polyacrylonitrile membrane neutralizes the anionic sulphonate groups scattered over the surface [2]. Such a surface, in contact with blood, is theoretically able to bind heparin into the layer coating the synthetic membrane, at least through electrical interactions [3–5]. The binding of heparin to the membrane surface occurs during priming of the extracorporeal circuit (ECC) by rinsing it with saline and unfractionated heparin (UFH). Unfractionated heparin and various low molecular weight heparin (LMWHs) seem to show a comparable affinity to the membrane (adsorption capacity of 900–1300 IU/m²). The stability of the bond is proportionate to the molecular weight. This explains why ECC rinse in human citrate plasma results in complete desorption of LMWH, but only 10–20% of UFH.
Based on these experimental studies, it is recommended to rinse the ECC with an UFH-containing solution (not using LMWH) at a concentration of 5000 IU/l. Under these conditions, UFH binds relatively quickly to the membrane, with the adsorption plateau reached within 3–5 min [6]. The information and results from studies available in the literature (relatively infrequent) suggest, the AN69 ST membrane is able to adsorb UFH, which could allow a reduction of systemic anticoagulation without the risk of blood clotting in the ECC. Using an animal model, experimental 6 h HD without systemic heparin was undertaken (only after ECC rinse with a heparinized solution) without visual signs of clotting. A comparison of the AN69 and AN69 ST membranes showed a later increase in the plasma levels of the thrombin–antithrombin complexes (TAT) in the AN69 ST membrane. The values also suggested higher thrombogenicity of the plate dialyser as compared with the hollow-fibre one [7]. The same group of authors (Lavaud et al.) performed successful HD with UFH without systemic anticoagulation, only after priming the ECC with saline and UFH 5000 IU/l, in 28 chronic HD patients at acute risk of bleeding complications and treated at an intensive care unit (ICU) [8]. In the above studies, maximum emphasis was placed on assessing the visual signs of blood clotting in the ECC. In our view and, in particular in connection with chronic HD, it is most important to assess thrombogenicity and, hence, biocompatibility of material, not only based on visual examination but, primarily using sensitive and specific laboratory tests.

The Consensus Conference on Biocompatibility recommended the following criteria to assess material biocompatibility: (i) evaluation of thrombosis formation upon contact with blood with the artificial material (in this particular case, clot formation in the ECC); (ii) signs of coagulation system activation and (iii) signs of thrombocyte activation [9,10]. Generally, contact of blood with an artificial material is followed by adsorption of blood proteins with subsequent adhesion and activation of blood elements and coagulation factors [11]. As regards visual assessment of clot formation in the ECC, this is a simple method yet inaccurate and biased by subjective assessment by staff (despite criteria designed to make assessment at least partly objective). Among laboratory tests, coagulation activation is best characterized by levels of fibrinopeptide A, TAT or prothrombin 1 + 2 fragments. The parameters used most often in practice are TAT. Their advantages include adequate molecular weight (88 000 Da), which prevents them from crossing the HD membrane, and acceptable conditions for handling samples before analysis. In addition, TAT levels correlate closely with fibrinopeptide A levels.

To make thrombogenicity assessment valid, it is critical to have, in addition to the above parameters, information about the intensity of anticoagulation therapy, if there is any. As UFH is used most often, the level of coagulation intensity is characterized by heparin concentration and, possibly, activated partial thromboplastin time (aPTT).

Other parameters characterizing the degree of biocompatibility are signs of thromboocyte activation based on thrombocyte count and/or changes in thrombocyte count during contact of blood with an artificial material. A decrease in thrombocyte count at the start of the procedure reflects their adherence to a material and, hence, suggests a certain degree of bioincompatibility. A much more sensitive method is determination of platelet factor 4 (PF 4) released from platelet granules upon the activation.

Generally, the potential for reducing systemic heparinization when using the AN69 ST membrane for chronic HD patients seems to be promising. At present, UFH is the most common method of anticoagulation used in extracorporeal blood purification procedures. In addition to potentially causing acute bleeding complications, heparin may have also a variety of long-term adverse effects. These include thrombocytopenia and adverse effects on bone and lipid metabolism [12–14]. Given the fact that a dialysis patient is exposed to an approximate 600 000–1 200 000 IU of UFH each year, it is self-evident any possibility to reduce the dose would be beneficial.

The aim of our previous study was a comparison of the thrombogenicity of two high-flux HD membranes, specifically the AN69 ST and the Helixone polysulfone membrane [15]. While no difference in thrombogenicity was seen between the two membranes, ECC priming was performed according to the manufacturer’s recommendations, that is, with saline but without UFH. As first reports were published at that time about the benefits of ECC rinse with UFH, we decided to use a similar design and to test the polyacrylonitrile membrane again using both rinse protocols.

**Aim**

The specific goal of our study was to compare, using sensitive and specific thrombogenicity parameters, AN69 ST biocompatibility during haemodialysis under conditions of two different ECC priming protocols: (i) rinse with a heparinized solution and (ii) rinse with a heparin-free solution.

**Patients and methods**

A total of 10 patients on chronic HD (for at least 3 months) in stable clinical condition were included into a clinical, prospective, randomized and crossover study. Exclusion criteria included laboratory signs of altered liver function, haemostasis disorder, diabetes mellitus, malignancy, treatment with ACE inhibitors, treatment with drugs affecting haemostasis except for antiplatelet drugs (administration of acetylsalicylic acid because of cardiovascular disease (CVD) and atherosclerosis prevention was allowed without dose.
The patients had good vascular access (AV fistula) allowing double-needle dialysis at blood flow rates of at least 200 ml/min. If treated with recombinant human erythropoietin (rHuEPO), they had reached target hematocrit prior to enrollment and were on maintenance doses during the study. There were six men and four women with an age of 72 years (61–78; median, 25–75 percentiles).

Causes of chronic renal failure included polycystic disease (two cases), nephrosclerosis (four), chronic tubulointerstitial nephritis (two cases), chronic glomerulonephritis (one case) and obstructive nephropathy (one case). Other patient characteristics included pre-HD serum creatinine of 661 μmol/l; Kt/V 1.43; leukocyte count of 6.68 × 10^9/l; thrombocyte count of 211 × 10^9/l and aPTT of 32 s.

The study was approved by the local ethics committee. All patients signed their informed consent.

The dialyser used in our study was the hollow fibre Nephral ST 300 dialyzer (Hospal Industrie, Meyzieu, France) with a declared effective area of 1.3 m² and ultrafiltration (UF) coefficient of 40 ml/mmHg/h. The dialyser is sterilized by irradiation and features a high-flux polyacrylonitrile AN69 ST membrane.

Extracorporeal circuit rinse was performed with 1000 ml of saline, either with 5000 IU UFH or without UFH in a random sequence in each patient. In the next study HD, the rinse protocol was just the opposite (with each patient, study HD was the first of the week; with individual rinse protocols used at an interval of 2 weeks). Extracorporeal circuit rinse was performed at a blood pump rate of 100 ml/min for 5 min; upon connecting the dialyser circuit, the blood pump rate was set at 150 ml/min and UF at 2000 ml/h. Once the rinse was completed, UF was decreased to a minimum.

The venous access included two 16G needles in an arteriovenous fistula. At first, the arterial line was connected to the arterial needle and after blood passing through ECC entered the venous chamber, the venous line was connected. Blood flow during the procedure was in the range of 200–300 ml/min, being the same for each patient throughout the dialysis sessions with either priming. The flow rate of a bicarbonate dialysis solution, at a temperature of 36°C, made of water treated by reverse osmosis was 500 ml/min. Thirty minutes before blood collection, UF was set at 10 ml/min and subsequently adjusted to each patient’s needs. Unfractionated heparin made of bovine lungs of the same batch was used as anticoagulant (Heparin, Zentiva, Prague, Czech Republic). The heparin was administered to the arteriovenous fistula as a bolus 5 min prior to connecting the patient to the ECC. The bolus was followed by heparin in continuous infusion delivered to the arterial side of the blood set. The infusion was stopped 30 min prior to dialysis completion. The techniques of anticoagulation and heparin doses were established in patients long before study initiation when they allowed adequate dialysis without resulting in clinically manifest complications. The anticoagulation remained unchanged throughout the study (5000 IU UFH as a bolus and 500 IU/h as a continuous infusion — values are medians). No drugs or blood products were given to patients during study dialysis procedures.

For dialysis, 4008 S monitors (Fresenius Medical Care, Bad Homburg, Germany) were used 4008 S. Identical blood sets (FA204B/FV204B Fresenius Medical Care, Bad Homburg, Germany) were used for all dialysis procedures.

Plasma thrombin-antithrombin complex levels were measured by ELISA (Enzygnost TAT micro, Behring Diagnostics GmbH, Marburg, Germany) as were the plasma levels of platelet factor 4 (PF4, Asserachrom PF4, Boehringer, Mannheim, Germany). Heparin levels—antiXa—were assessed using the commercially available chromogenic test Coamatic® Heparin Art. No. 823393 (Chromogenix, Milan, Italy). Platelet count was determined using an SE 9000 autoanalyzer (Sysmex, Kobe, Japan).

The above substances were determined in the plasma obtained from blood prior to HD initiation and during HD at 15, 60 and 240 min. Before dialysis, blood was collected from the arteriovenous fistula needle still before heparin administration. During HD, blood was collected from blood sets at dialyser outlet. The samples were processed following the instructions of manufactures of the respective kits.

Before statistical analysis, the levels of the investigated parameters obtained during HD were adjusted using hematocrit to eliminate the effect of haemoconcentration and, possibly, haemodilution. Paired Wilcoxon’s test was used to compare variables before and during HD and between both types of priming. Correlation between variables was assessed using Spearman’s rank coefficient of correlation.

Results
The plasma levels of PF4 during HD with either priming tend to rise. A significant increase occurs as early as 15 min, with significantly increased values persisting up to the end of HD (Tables 1 and 2). There is no difference in PF4 between the rinses (Figure 1). Platelet count does not change significantly during HD using either rinse protocol (Tables 1 and 2).

A rising trend during HD with either priming was also seen in plasma TAT levels. The increase reached significance when using UFH-free saline rinse at 60 min, with significantly increased values persisting at HD completion (Table 1). While saline rinse with UFH is also associated with an increase in TAT, it does not become significant until 240 min (Table 2). Consistent with these results, comparison of TAT levels between both rinse protocols at 60 and 240 min also reveals a significant difference (Figure 2).

Heparin levels reflect the technique of systemic anticoagulation. A significant rise occurs as early as 15 min, persist at 60 min. When using saline UFH-free rinse, antiXa levels at 240 min are no longer significantly different from pre-HD values (Table 1). Saline rinse with UFH is associated with significantly increased heparin levels persisting even at HD completion (Table 2). Comparison of values between both rinse protocols shows heparin levels are significantly higher with rinse using saline and UFH at 15, 60 and 240 min (Figure 3).

Spearman’s rank correlation analysis documented a correlation between TAT and antiXa at 60 min ($r = -0.534; \ P < 0.05$) and 240 min ($r = -0.538; \ P < 0.05$) (Figure 4).
Table 1. Plasma levels of parameters investigated during haemodialysis with the AN69 ST membrane and extracorporeal circuit priming without unfractionated heparin

<table>
<thead>
<tr>
<th>Time on dialysis (min)</th>
<th>(0) Before</th>
<th>15</th>
<th>60</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF4 (µg/l)</td>
<td>30.6 (16.1–62.7)</td>
<td>70.75 (68.9–77.2)</td>
<td>70.65 (58.4–73.0)</td>
<td>73.7 (66.3–77.1)</td>
</tr>
<tr>
<td></td>
<td>P = 0.005</td>
<td>P = 0.005</td>
<td>P = 0.005</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>TAT (µg/l)</td>
<td>0 (0–1)</td>
<td>0 (0–0)</td>
<td>2.9 (1.03–8.37)</td>
<td>36.85 (20.5–56.22)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>P = 0.006</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>AntiXa (IU/ml)</td>
<td>0 (0–0)</td>
<td>0.55 (0.49–0.59)</td>
<td>0.4 (0.29–0.40)</td>
<td>0 (0–0.1)</td>
</tr>
<tr>
<td></td>
<td>P = 0.005</td>
<td>P = 0.005</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombo (x10^9/l)</td>
<td>181 (177–228)</td>
<td>176 (165–217)</td>
<td>183 (151–216)</td>
<td>175 (152–221)</td>
</tr>
</tbody>
</table>

TAT, thrombin–antithrombin complexes; PF4, platelet factor 4; antiXa, heparin concentration; thrombo, platelet count. Blood sampling at dialyser outlet. Values during haemodialysis are adjusted using hematocrit to exclude haemoconcentration or haemodilution. Values are medians (25–75 percentiles), P values indicate comparison with baseline, paired Wilcoxon’s test, NS, non-significant.

Table 2. Plasma levels of parameters investigated during haemodialysis with the AN69 ST membrane and extracorporeal circuit priming with unfractionated heparin

<table>
<thead>
<tr>
<th>Time on dialysis (min)</th>
<th>(0) Before</th>
<th>15</th>
<th>60</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF4 (µg/l)</td>
<td>16.1 (14.6–37.6)</td>
<td>72.9 (65.9–76.2)</td>
<td>65.55 (60.4–71.2)</td>
<td>67.8 (60.1–73.2)</td>
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<tr>
<td></td>
<td>P = 0.005</td>
<td>P = 0.013</td>
<td>P = 0.007</td>
<td>P = 0.007</td>
</tr>
<tr>
<td>TAT (µg/l)</td>
<td>0 (0–1)</td>
<td>0 (0–0)</td>
<td>1 (0–3.60)</td>
<td>20.2 (6.75–49.98)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>P = 0.005</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>AntiXa (IU/ml)</td>
<td>0 (0–0.1)</td>
<td>0.65 (0.57–0.81)</td>
<td>0.5 (0.46–0.71)</td>
<td>0.15 (0.1–0.2)</td>
</tr>
<tr>
<td></td>
<td>P = 0.005</td>
<td>P = 0.005</td>
<td>P = 0.017</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombo (x10^9/l)</td>
<td>202 (160–227)</td>
<td>191 (170–231)</td>
<td>190 (156–233)</td>
<td>183 (160–202)</td>
</tr>
</tbody>
</table>

TAT, thrombin–antithrombin complexes; PF4, platelet factor 4; antiXa, heparin concentration; thrombo, platelet count. Blood sampling at dialyser outlet. Values during haemodialysis are adjusted using hematocrit to exclude haemoconcentration or haemodilution. Values are medians (25–75 percentiles). P values indicate comparison with baseline, paired Wilcoxon’s test, NS, non-significant.

Fig. 1. Plasma levels of platelet factor 4 during haemodialysis with the AN69 ST membrane, priming without and with unfractionated heparin. Paired Wilcoxon’s test, values are medians (25–75 percentiles), *P < 0.01, #P < 0.05—indicate comparison with baseline, NS, non-significant.

Fig. 2. Plasma levels of thrombin–antithrombin complexes during haemodialysis with the AN69 ST membrane, priming without and with unfractionated heparin. Paired Wilcoxon’s test, values are medians (25–75 percentiles), *P < 0.01 indicates comparison with baseline, #P < 0.05 indicates comparison between both ECC primings, NS, non-significant.
A comparison of the plasma levels of TAT/antiXa ratio at 60 min between both rinse protocols clearly shows a significant difference; specifically, the ratio is significantly higher with UFH-free rinse compared with UFH-containing rinse ($P < 0.05$) (Figure 5).

Discussion

The bio(in)compatibility of materials used during HD but, also, other methods of renal replacement therapy, is a problem yet to be solved. This is true despite the technological advances made in this area during the last years. The most important role is played by the dialysis membrane, representing more than 90% of the area with which blood is in contact within the ECC. But of course, the influence of other parts (needles, blood lines) and other factors (contact of blood with air in the bubble trap chamber, type of procedure, type of patients) is considerable.

Thrombogenicity is a parameter, which can be used to characterize bio(in)compatibility. It is defined as the property of a material, device, or extracorporeal system inducing or promoting thrombus formation [16]. These include thrombocyte and coagulation system activation. Platelet activation is characterized by their count, or better, plasma concentration of PF4 released from platelet granules upon thrombocyte activation (as a much more sensitive marker). Our study did not show any significant changes in thrombocyte count throughout HD. However, PF4 levels were significantly higher at 15 min (compared with predialysis values) with significant elevation persisting until HD completion. The values did not differ between both rinse protocols. We, therefore, conclude that thrombocyte activation (as indicated by an increase in the plasma levels of PF4) occurs during HD using the AN69 ST membrane and the degree of activation is identical regardless of whether the ECC...
significant difference of TAT/antiXa ratio between antiXa and TAT (it means we wouldn't find below). If there is only the simple indirect correlation minimal dose of UFH from ECC after priming—see from the bond to the membrane or by an additional coagulation (that could be caused by UFH release somewhat higher degree of effective systemic anticoagulation with systemic heparin administration. As the dose of systemic heparin administered during HD to each patient was always identical, our results obtained from comparisons of rinse protocols should not be biased. It is true, a minimal amount of heparinized saline from ECC could enter the patient’s circulation after connection to the HD machine. Because the part of ECC from the venous chamber to the end of the venous line contains only several millilitres, we believe it does not influence significantly our final results. Moreover, the difference of TAT/antiXa ratio excludes the possibility this could be the reason for lower thrombogenicity of ECC after UFH-containing priming. It would seem likely that the elevated antiXa levels seen during HD after UFH-containing rinse are due to UFH release from its bond to the membrane, its entering the circulation and subsequent systemic effect. However, data from literature are not fully straightforward in this respect. There are reports on the course of HD without systemic anticoagulation therapy, with the only intervention being heparin-containing priming of the ECC. In their preclinical study using an animal model, Chanard et al. performed 6 h HD without systemic anticoagulation. Prior to HD, the ECC had been rinsed with heparinized saline at a concentration of UFH 5000 IU/l. The values of aPTT measured during HD did not differ from those obtained before HD. Thus, the results of this study, in contrast with ours, suggested that contact of blood with the membrane is not followed by appreciable UFH release from the bond and its systemic effect [6]. There is no information on the eventual flushing out of the heparinized saline after priming ECC in this study. On the other hand, a clinical study of the same group of authors showed, consistent with our data, clear increases in antiXa and aPTT at 15 min of HD [7]. In this case, the ECC had also been rinsed with heparinized saline with UFH (5000 IU/l). In addition, the solution was subsequently flushed out with 1000 ml of saline. As a result, not even a small amount of UFH-containing solution could have reached the circulation and the increase at 15 min seems to be most likely due to
release of a certain amount of UFH bound to the membrane. The likelihood of partial UFH release is supported also by in vitro testing of the AN69 ST membrane; specifically, the kinetics of UFH and various types of low molecular weight heparin (LMWH) used for rinse [6]. In every case, based on antiXa values and thrombogenicity parameters during our study, the appropriate priming of the AN69 ST membrane is sufficient to ensure a 4 h HD procedure with lower thrombogenicity markers compared with priming without UFH.

**Conclusion**

In this first stage of AN69 ST membrane testing, we decided to monitor thrombogenicity parameters under conditions of systemic heparinization (in stable patients with no bleeding complications). The potential benefit of the membrane for routine practice is the possibility to perform HD without any systemic anticoagulation in patients in whom systemic heparinization would be associated with a risk of bleeding. Extracorporeal circuit priming with a heparinized solution is simple, inexpensive and less time-consuming and would provide the anticoagulation effect throughout the whole HD procedure. From the clinical point of view, no adverse effects or other complications were registered in our patients during the study. In the long run, the possibility of reducing the UFH dose when using the AN69 ST membrane seems most promising in terms of the complications associated with chronic UFH therapy (thrombocytopenia, osteopathy, lipid metabolism disorders). Based on our results, we can assume that priming of the extracorporeal circuit comprising the AN69 ST membrane with a UFH-containing solution leads to reduction of membrane thrombogenicity (there are significantly lower TAT levels with no significant differences to PF4 and thrombocyte count). The next step is to examine and compare these specific and sensitive parameters of thrombogenicity in a sufficient number of patients also during HD sessions without systemic anticoagulation. Should this confirm our assumptions, the properties of the AN69 ST membrane would allow, after adequate ECC priming, to perform safe HD without an additional systemic anticoagulation therapy.

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**Conflict of interest statement.** None declared.

**References**