Comparative analysis of procoagulatory activity of haemodialysis, haemofiltration and haemodiafiltration with a polysulfone membrane (APS) and with different modes of enoxaparin anticoagulation

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Abstract

Background. Treatment modalities of renal replacement therapy differ in their diffusive and convective mass transfer characteristics. It was the goal of this study to clarify whether an increase in convective mass transfer as performed with haemofiltration (HF) and haemodiafiltration (HDF) in comparison with high-flux haemodialysis (HD) is associated with an alteration in procoagulatory activity or with complement activation.

Methods. Ten stable chronic HD patients were monitored during 120 treatments in a randomized cross over design. A high-flux polysulfone dialyser (APS 900) was used for high-flux HD, pre-dilution HF and pre-dilution HDF. Constant flow of on-line substitution fluid for HF and HDF was 200 ml/min. The low molecular weight heparin (LMWH) enoxaparin was used for anticoagulation (i) as single bolus (50 IU/kg body weight, median 3700 IU) and (ii) as bolus of 1200 IU followed by a median continuous dose of 400 IU/h. Blood samples were collected before the LMWH bolus, after 10 min, 60 min, 120 min and at the end of treatment in venous and arterial blood lines to determine antiXa activity, thrombin–antithrombin-III complex (TAT), D-dimer and C5a generation.

Results. Net ultrafiltration did not significantly differ between HD, HF and HDF but total ultrafiltration in HF and HDF far exceeded total ultrafiltration in HD. With conditions of single bolus, or bolus and continuous anticoagulation with enoxaparin, after comparable treatment times (median duration 4.25 h), TAT and D-dimer generation at identical anti-Xa levels revealed significantly higher coagulation activity during HF and HDF, compared with high-flux HD as assessed by comparative area under the curve (AUC) analysis. Plasma concentration of C5α in venous bloodlines did not significantly differ during HD, HF and HDF.

Conclusion. A higher convective mass transfer during HF and HDF, in comparison with high-flux HD caused by a greater total ultrafiltration volume was associated with increased procoagulatory activity in the extracorporeal circuit. Molecular markers assessing the activation of coagulation are appropriate to adjust the anticoagulation regime to high UF volumes in order to minimize bleeding risk and optimize patency of the extracorporeal circuit.

Keywords: haemodiafiltration; haemodialysis; haemofiltration; low molecular weight heparin; polysulfone; procoagulatory activity

Introduction

In order to reproduce the removal characteristics of the natural kidney renal replacement therapy modes have been developed which either use convection exclusively, as in haemofiltration (HF), or a combination of convective and diffusive processes as in high-flux haemodialysis (HD) and haemodiafiltration (HDF) for solute removal. Convection favours the elimination of higher molecular weight (MW) substances; low MW solutes are mainly transferred from the blood to the dialysis fluid by diffusion. In order to optimize convective clearances the fluid volume driven through the membrane must be increased far beyond the volume needed to attain the patient’s dry weight. Survival of critically ill patients was significantly improved when
using continuous veno-venous HF at an increased rate of ultrafiltration [1]. The total ultrafiltration capacity in HDF is 12–15 l/session, and in HF volumes corresponding up to 1–1.2 times the bodyweight of the patient. Therefore, a substitution fluid is necessary in HF and HDF, to be infused into the patient either before (pre-dilutional) or after the dialyser (post-dilutional). It is possible that these huge amounts of fluid filtered from the blood increase the fluid shear stress (i.e. the force by unit area generated by flow of a viscous liquid, e.g. blood) that the cellular components of blood are exposed to. Platelets at the periphery of the membrane fibre may be especially affected, probably activating the haemostatic system [2].

The activation of the coagulation system during HD is usually prevented by heparin. With respect to lipid and bone metabolism, polymorphonuclear cell stimulation, induction of antibody mediated thrombocytopenia and aldosterone suppression in chronic HD, low molecular weight heparin (LMWH) is supposed to be advantageous compared with unfractionated heparin for chronic HD. It was additionally shown in the case of enoxaparin that endothelial cells are protected from activation and that the expression of cell adhesion molecules is inhibited [3]. Because of the longer biological half-life, LMWHs offer the possibility of single bolus administration, which can be safely and effectively used for chronic high-flux and low-flux HD [4]. Significant coagulation within the extracorporeal circuit would result in decline of the maximal ultrafiltration rate over the duration of dialysis thus decreasing efficiency, especially in HDF.

The thrombin–antithrombin-III complex (TAT), a marker of intravascular thrombin formation, directly reflects activation of the haemostatic system and is not filtered through dialysis membranes (MW ~90 000) [5]. D-dimer, a degradation product of cross-linked fibrin split by plasmin, represents a reflection of prior coagulation activity or thrombin generation, respectively [6]. We investigated both parameters together with antiXa activity to compare the state of anticoagulation in all experiments. Two different regimes of anticoagulation were used differing in their time course of coagulation activation: LMWH was applied either as a bolus alone or as bolus with additional continuous infusion. We further investigated the influence of flux on complement activation by determination of C5a concentrations during all three treatment modalities [7].

More convective therapies need filters with high hydraulic permeabilities including membranes with pore sizes allowing the elimination of higher MW uraemic toxins without significant albumin loss. We used in our experiments the APS 900 dialyser with a polysulfone membrane of enhanced permeability. Dialysers of this series exhibited significantly improved sieving coefficients, clearances and reduction ratios for proteins with a MW up to 32 000 in comparison with standard polysulfone dialysers without a higher loss of albumin [8]. This polysulfone dialyser was recently characterized to be highly efficient in preventing the passage of cytokine-inducing bacterial products from contaminated dialysis fluid into the patient’s blood [9].

Subjects and methods

Patient characteristics, dialysis protocol and anticoagulation

Ten chronic HD patients with end-stage renal disease, three female, seven male, with a median age of 56 years (range 33–80 years) who gave their informed consent were treated randomly four times with each modality (HD, HF and HDF). Of these four times, twice was with a regime of special anticoagulation (single bolus or bolus and continuous infusion, respectively). In total, 120 treatments were analysed. All treatments were performed with two-needle vascular access and new gamma sterilized APS 900 polysulfone dialysers with a surface area of 1.8 m², and an UFcoeff. of 75 ml/h-mmHg (Asahi Medical, Tokyo, Japan; Diamed, Cologne, Germany). Median duration on dialysis was 45 months (range 1–91 months) median duration of a dialysis session was 4.25 h (range 3–5 h), median blood flow was 300 ml/min (range 190–365 ml/min) and dialysate flow was 500 ml/min with HD, HF and HDF were performed with 200 ml/min pre-dilution; dialysate flow with online-HDF was 800 ml/min. Median net-UFR was 600 ml/h (range 100–1000 ml/h), total ultrafiltration volumes are summarized in Table 1. LMWH enoxaparin (Clexane®, Aventis, Frankfurt, Germany) served as anticoagulant and was, respectively, applied either as a single bolus (50 IU/kg body weight) or bolus (1200 IU) with additional continuous infusion (related to the dose of heparin during chronic dialysis patients were assigned to receive either 400, 600 or 800 IU/h) during the entire treatment. The individual dose was not changed throughout the entire investigation.

Blood samples and laboratory measurements

Blood samples were taken from the arterial and venous bloodline before anticoagulation and treatment, after 10 min, 1 h, 2 h and at the end of the session. AntiXa activity was determined with the COATEST® LMW Heparin/Heparin (Chromogenix, Monza, Italy), the TAT complex with the Enzygnost® TAT micro (Behringwerke AG, Marburg, Germany) and C5a with the ELISA C5a EIA-3327 (DRG Instruments, Marburg, Germany) following the manufacturer’s instructions and as otherwise described [10]. D-dimer was determined with the enzyme immuno assay Tina-quant® [a] D-Dimer (Roche Diagnostics, Mannheim, Germany)] [6].

Statistical analysis

The area under the curve (AUC) vs time of dialysis represents the appropriate parameter for the assessment of haemocompatibility parameters, as were the generation of procoagulatory molecular markers during time of dialysis and were calculated as described previously [10]. Results of the coagulation tests are reported as the median and interquartile range (25th to 75th percentiles). The statistical significance was defined as P < 0.05. Comparative data were analysed by paired t-test. The Wilcoxon’s Matched Pairs Signed
Ranks test and the Shaffer procedure for multiple tests were used for statistical analysis with \( P \)-level < 0.05. Significance was tested by comparison of AUC.

Results

One hundred and twenty treatments were performed. No complete coagulation of the extracorporeal circuit occurred. Randomized crossover of patients from HD to HE and HDF using a high-flux polysulfone dialyser was well tolerated.

Table 1. Net and total ultrafiltration volumes during HD, HF and HDF with single and combined bolus + continuous anticoagulation (median values with interquartile range) (each \( n = 20 \))

<table>
<thead>
<tr>
<th>Anticoagulation</th>
<th>HD</th>
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<th>HF</th>
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<th>HDF</th>
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<tr>
<td>Net UF (ml)</td>
<td>2802 (1200–3129)</td>
<td>2618 (1678–3125)</td>
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<td>2895 (2550–3525)</td>
<td>2700 (1783–3125)</td>
<td></td>
<td>2560 (1650–3525)</td>
<td>2804 (1725–3277)</td>
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<td>Median (interquartile range)</td>
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<tr>
<td>Total UF (ml)</td>
<td>2801 (1200–3128)</td>
<td>2619 (1678–3125)</td>
<td></td>
<td>54244 (50 550–63 125)</td>
<td>53 950 (53 475–62 692)</td>
<td></td>
<td>53 102 (50 150–63 525)</td>
<td>53 969 (49 700–63 277)</td>
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<td>Median (interquartile range)</td>
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Table 2. Plasma antiXa-activity in venous blood sides during HD, HF and HDF with single bolus anticoagulation and with combined bolus + continuous anticoagulation at 10 min and end of treatments, and anti Xa activity on the venous blood side calculated as AUC of the complete treatment time (median values with interquartile range) (each \( n = 20 \))

<table>
<thead>
<tr>
<th>Anticoagulation</th>
<th>HD</th>
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<th>HF</th>
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<th></th>
<th>HDF</th>
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<tbody>
<tr>
<td>Anti Xa (IU/ml) median (interquartile range)</td>
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<tr>
<td>10 min</td>
<td>1.36 (0.98–1.53)</td>
<td>0.48 (0.39–0.58)</td>
<td></td>
<td>1.17 (0.93–1.41)</td>
<td>0.49 (0.44–0.58)</td>
<td></td>
<td>1.17 (0.90–1.44)</td>
<td>0.56 (0.47–0.66)</td>
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<tr>
<td>End of treatment (median 4.25 h)</td>
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<tr>
<td>0.62 (0.56–0.68)</td>
<td>0.71 (0.55–1.25)</td>
<td></td>
<td>0.61 (0.53–0.80)</td>
<td>0.76 (0.61–1.49)</td>
<td></td>
<td>0.59 (0.46–0.72)</td>
<td>0.79 (0.61–1.27)</td>
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<tr>
<td>AUC (IU/ml-min) median (interquartile range)</td>
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<td>227.28 (165.13–263.03)</td>
<td>196.46 (117.73–322.88)</td>
<td></td>
<td>233.01 (150.95–294.25)</td>
<td>195.86 (139.53–349.98)</td>
<td></td>
<td>208.60 (158.53–265.38)</td>
<td>202.96 (114.50–292.38)</td>
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or combined bolus and continuous anticoagulation with enoxaparin after comparable treatment times (median duration: 4.25 h) revealed significantly higher coagulation activity during HF and HDF. In contrast, the differences in TAT and D-dimer generation were not statistically significant.

TAT and D-dimer generation was determined during treatment time in the arterial and venous blood sides for all three treatment modalities.

Table 3 shows TAT measurements of venous samples. Figure 2A–F illustrates the pattern of TAT generation during HD, HF and HDF treatments in arterial and venous blood sides for both anticoagulation regimes.

Data of the AUC of TAT and D-dimer during HD, HF and HDF were also measured for AUC of arterial (HD vs. HF; \( P = 0.006 \)) and venous TAT (HD vs. HF; \( P = 0.027 \)).

### Table 3. Plasma concentration of TAT during HD, HF and HDF with single bolus anticoagulation and with combined bolus + continuous anticoagulation depending on treatment time [h] (median values with interquartile range) (each \( n = 20 \)). TAT and D-dimer generation (AUC) during treatment in arterial and venous blood sides with single bolus and combined bolus + continuous anticoagulation (median values with interquartile range) (each \( n = 20 \)).

<table>
<thead>
<tr>
<th>Anticoagulation</th>
<th>HD</th>
<th>HF</th>
<th>HDF</th>
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<tbody>
<tr>
<td>TAT (µg/l) median (interquartile range) venous blood side</td>
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<tr>
<td>10 min</td>
<td>0.35 (0.24–0.5)</td>
<td>0.34 (0.22–0.53)</td>
<td>0.34 (0.26–0.56)</td>
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<tr>
<td>End of treatment (median 4.25h)</td>
<td>0.36 (0.26–0.49)</td>
<td>0.41 (0.25–1.19)</td>
<td>0.40 (0.26–0.56)</td>
</tr>
<tr>
<td>AUC (µg/l-min) median (interquartile range) arterial</td>
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<tr>
<td>TAT arterial</td>
<td>3402 (2448–5008)</td>
<td>6459 (3409–8477)</td>
<td>9199 (8061–12042)</td>
</tr>
<tr>
<td>D-dimer arterial</td>
<td>77.74 (59.65–137.23)</td>
<td>117.26 (78.25–155.70)</td>
<td>18 913 (123.35–263.63)</td>
</tr>
<tr>
<td>D-dimer venous</td>
<td>74.51 (59.38–144.35)</td>
<td>120.05 (61.73–299.28)</td>
<td>132.90 (99.35–165.28)</td>
</tr>
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Fig. 1. Plasma concentration of TAT during HDF with single bolus anticoagulation (A) and combined bolus + continuous anticoagulation (B) depending on time of dialysis [h] (median values with interquartile range) (each \( n = 20 \)).
HD vs HDF: $P = 0.002$) with bolus and continuous anticoagulation. Significant differences were detected for AUC of venous D-dimer with single bolus anticoagulation for HD compared with HF ($P = 0.047$) and HD vs HDF ($P = 0.014$). Also the AUC of arterial D-dimer with bolus + continuous anticoagulation showed significant differences (HD vs HDF: $P = 0.004$; HF vs HDF: $P = 0.037$).

**C5a generation**

Measurements of C5a concentration in arterial and venous blood sides were low per se with the APS dialyser, and did not reveal significant differences between high-flux HD, HF and HDF for both anticoagulation regimes as assessed by comparative AUC analysis (data not shown).

Fig. 2. Plasma concentration of TAT during HD (A), HF (B), HDF (C) with single bolus anticoagulation and HD (D), HF (E) and HDF (F) with combined bolus + continuous anticoagulation depending on time of dialysis [h] (median values with interquartile range) (each $n = 20$).
Discussion

The results of this study demonstrate that convective mass transfer is associated with increased procoagulatory activity. The greater total ultrafiltration volume applied in HF and HDF, in comparison with high-flux HD, was linked to higher TAT (as a measure for thrombin generation) and D-dimer (as a measure for subsequent fibrinolysis) generation in the blood at an equal grade of anticoagulation (as determined by antiXa activity) in all three methods. These results were identical for both anticoagulation regimes used, single bolus or bolus plus continuous anticoagulation.

The pathogenesis of surface-induced thrombosis in extracorporeal therapies involves both plasma coagulation and platelet function. It is influenced by uremic alterations of the thrombotic system, by characteristics of the artificial surface (e.g. surface roughness, electrical charge, quality and quantity of adsorbed proteins), and by blood flow [2]. Although blood flow was kept constant in all three treatment modes the higher total ultrafiltration in HF and HDF may have altered local flow conditions possibly increasing shear forces at distinct regions of the fibres. Platelets are especially susceptible for the mechanical shear forces generated by flowing blood. Shear-induced platelet activation and aggregation is not an artifact of platelet lysis, but rather depends on the presence of the van Willebrand factor (vWF) and functional platelet receptor complexes GpIb/IX/V and GpIIb–IIIa [2]. When high shear stress is applied to platelets the vWF binds to GpIIb–IIIa as well as the GpIb/IX/V complexes leading to platelet activity and aggregation [2]. Activated platelets further induce activation in the clotting cascade. Flow conditions affect further coagulation by regulating the formation and dissociation of the cofactor/enzyme complexes: tissue factor/factor VIIa, factor VIIa/ factor IXa and prothrombinase (factor Va/factor Xa).

The development of these complexes increased with the flow and concentration of coagulation factors [11]. But in summary there is not yet definite conclusion about the mechanisms explaining the observed effects.

However, although a higher procoagulatory activity has been observed with HF and HDF in comparison with high-flux HD they could not be related to clinical effects as increased dialyser redness or clotted dialysers. This could be clinically relevant where there is an increased bleeding risk with low dose anticoagulation strategies, or with continuous renal replacement therapy in the intensive care unit [12]. The well described therapeutic benefits of higher convective treatments in comparison to high-flux HD, e.g. maintaining stable blood pressure, increased removal of higher MW uraemic toxins like β2-microglobulin, leptin and advanced glycation end products (AGE) and their influence on dialysis-related long-term effects in cardiovascular disease, amyloidosis, malnutrition and atherosclerosis should not be curtailed here [13].

Survival of critically ill patients was significantly improved when using continuous veno-venous hemofiltration (CVVH) at an increased rate of ultrafiltration [1]. Total UF was recommended to exceed 35 ml/h/kg, resulting in total UF volume > 58 l/24 h in a 70 kg patient [1]. Patients with acute renal failure often present with co-morbid conditions of increased bleeding risk and require minimal anticoagulation regimes. The prognosis of end-stage renal disease patients is significantly determined by inflammatory processes related to multiple pathogenic mechanisms. Activation of coagulation is one important phenomenon of blood membrane interactions in extracorporeal circuits. Therefore, in chronic as well as acute renal replacement therapy high ultrafiltration volumes could potentially act as an additional pro-inflammatory stimulus. The results presented here imply to monitor high volume CVVH not only by parameters of anticoagulatory activity like ACT for heparin or antiXa for LMWH, but also by markers assessing activation of coagulation like TAT. The anticoagulation regime could specifically be tailored to minimize bleeding risk and optimize blood–membrane interactions thus improving the patency of the extracorporeal circuit.

The effect of higher convective treatment on complement activity could not be observed with the parameter C5a used in this study. Complement generation (factor C3a) with the Asahi polysulfone membrane was shown recently to be very low [8]. Furthermore, C5a with its MW of approximately 11 000 is probably effectively filtered due to the sieving characteristics of this polysulfone high-flux membrane [7]. Measured C5a concentrations reflect the dynamic balance between generation and elimination of this molecule. The same can be expected for C3a with a MW of approximately 9000 [14]. However, as C3a and C5a are regarded as anaphylatoxins their elimination might improve overall biocompatibility. Only portions of generated C3a and C5a remaining in the fluid phase would reach the bloodstream of the patient in order to act as a potential inflammatory mediator [15]. C5a can induce IL-1, causing different acute responses observed in patients on HD [16]. Because of the sieving properties of high-flux membranes differences in the generation of complement factors between treatment modalities can hardly be detected. Determination of the terminal complement complex may be a better parameter in order to detect small differences [17,18].

This study confirmed further earlier findings (i) that LMWH applied as a bolus is as effective as bolus and continuous infusion in preventing clotting [4,19,20] and (ii) that LMWH activity is obviously not removed with any treatment mode. Because of its low MW of 3000–8000 enoxaparin could theoretically be filtered with the highly permeable polysulfone membranes used in our study. However, antiXa activity decreased slowly during bolus anticoagulation to the same extent in all treatment modes. This decline has obviously to be linked to the consumption of the enoxaparin rather than to a loss in the ultrafilter. Ljyngberg et al. came to the same results in their investigation [19]. The low permeability of LMWH could be explained by their negative electrical charge. The antithrombin complex...
and factor Xa that is not eliminated is preferentially responsible for the anticoagulatory effect of LMWH.

In conclusion, increased convective mass transfer associated with higher total ultrafiltration volume during HF and HDF, in comparison with high-flux HD, was associated with increased procoagulatory activity in the extracorporeal circuit of both convective methods. This result was consistent with two different regimes of anticoagulation. Molecular marker assessing the activation of coagulation like TAT are appropriate to adjust the anticoagulation regime to high UF volumes. Increasing levels of procoagulatory markers were not paralleled by differences in the concentration of activated complement factor C5a, probably due to the elimination characteristics of the used polysulfone dialysis membrane.

Conflict of interest statement. None declared.

References


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