Plasma hypercoagulability in haemodialysis patients: impact of dialysis and anticoagulation

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Abstract

Background. Thrombotic complications are common in patients with endstage renal disease and contribute substantially to the morbidity and mortality in this population. The aim of the present study was to: i) determine the prevalence and the extent of hypercoagulability in patients undergoing dialysis treatment by measuring parameters that directly reflect thrombin concentrations; ii) assess changes in coagulation status during haemodialysis (HD); iii) quantify the relative impact of heparin, dialysis and their combined effects on coagulation status and iv) detect factors that modify coagulation haemostasis in dialysis patients.

Methods. A total of 39 patients (HD: n = 29, CAPD: n = 10) was analysed for procoagulatory and fibrinolytic activity determined by measurements of partial thromboplastin time, prothrombin fragments F1+2, thrombin-antithrombin complexes and D-dimer concentrations. HD patients were investigated prior to and during dialysis. A subgroup of patients was infused heparin alone without dialysis or was dialysed without heparin administration. Furthermore, subgroup and correlation analyses were performed for the type of dialysis (HD vs CAPD), dialyzer and shunt, Kt/V, underlying disease and treatment with recombinant erythropoietin (rhEPO).

Results. Baseline levels of all parameters — procoagulatory and fibrinolytic — were substantially elevated in all patients, but to a higher degree among those on CAPD. Moreover, haemodialysis treatment increased procoagulatory markers even further, suggesting stimulated coagulation and/or insufficient anticoagulation during dialysis. However, after 3 h of dialysis thrombin concentrations, determined by quantification of prothrombin fragments, were inversely correlated with Kt/V. Selective heparin infusion diminished procoagulatory activity only slightly and incompletely, whereas HD without heparin resulted in excess thrombin accumulation. Finally, subgroup analyses revealed more pronounced thrombin formation among patients treated with polysulfon dialyzers, whereas erythropoietin dosage was positively related with lower procoagulatory activity.

Conclusion. A majority of patients on dialysis are in a hypercoagulable state, which is further aggravated by the haemodialysis procedure itself and may not be sufficiently controlled with current anticoagulation regimes. Intensified heparin treatment and the use of rhEPO are likely to improve coagulation haemostasis, whereas the type of dialyzer should be considered as a relevant procoagulatory factor.

Key words: Blood coagulation; erythropoietin; haemodialysis; heparin; prothrombin fragments; thrombin-antithrombin complex

Introduction

Thrombotic events are frequent among patients with end stage renal disease (ESRD) and contribute substantially to the high cardiovascular morbidity and mortality in this population [1]. They either originate on the ground of hypertensive, diabetic or inflammatory vasculopathies or occur secondarily from coagulopathies due to proteinuria in glomerular disease [2]. Furthermore, a role of uremia by itself promoting a hypercoagulable state has been proposed [3]. Thus, previous studies have described changes in coagulation and fibrinolytic factors in patients with chronic and acute renal failure compatible with increased procoagulatory activity [4–7]. Among these patients, those undergoing chronic haemodialysis treatment represent a high risk group for thromboembolic complications because they are exposed to additional exogenous factors with impact on their coagulation system. Mainly, contact activation by extracorporeal devices has to be counterbalanced by anticoagulants such as heparin. Coagulation control in haemodialysis patients is of further importance with regard to vascular access quality and prevention of access thrombosis, one of the single most important factors for adequate dialysis treatment and therefore longterm uremic control, mor-
bidity and mortality [8]. Consequently, it is crucial to determine coagulation status in patients on renal replacement therapy, especially haemodialysis, and to determine changes occurring during dialysis in order to develop adequate anticoagulation regimens. However, only a limited number of studies examining these questions is available and they have been conducted either in the setting of acute haemodialysis, only prior to dialysis [9] or in a small number of patients [5]. Based on the available data it seems likely, that commonly used heparin regimens do not sufficiently suppress thrombin formation during HD.

In order to test this hypothesis, we assessed coagulation status of HD patients before and during dialysis. Prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT) and D-dimer concentrations were determined, parameters that directly reflect thrombin formation and thrombolysis, respectively. They have previously proven their usefulness in the diagnosis of deep venous thrombosis [10,11], as predictive parameters of postoperative thromboembolic complications from hip surgery [12] and to guide oral anticoagulation for mechanical heart valve prosthesis and after coronary bypass graft surgery [13]. To determine factors that further affect coagulation haemostasis and to identify patients at risk for thromboembolic complications we evaluated the impact of underlying disease process, type of dialysis (HD vs CAPD), dialyzer and vascular access, the Kt/V and therapy with recombinant erythropoietin.

**Subjects and methods**

**Patients**

Thirty-nine patients suffering from ESRD undergoing regular haemodialysis (HD) were recruited randomly out of our HD population. Patient characteristics are given in Table 1. The only exclusion criteria was oral anticoagulation therapy.

All patients received unfractionated heparin (Liquemin® Roche) in order to prevent clotting of dialyzer membranes and artificial surfaces of extracorporeal devices. The ‘adequate’ dose of heparin during dialysis had been determined earlier and individually for each patient aiming for a partial thromboplastin time (PTT) being 2- to 2.5-fold the unheparinized baseline value. Dose adaptations were made as necessary according to the following criteria: coagulation of the dialyzer during HD; retention of blood in the dialyzer at the end of dialysis after rinsing with NaCl; or prolonged bleeding after HD. All patients under investigation had received the same dose of heparin for at least 3 months prior to the study and were negative with respect to the criteria mentioned before. Accordingly, heparin was administered initially as a mean intravenous bolus of 1850 ± 215 IU (range: 1500–2500 IU) that was followed by continuous infusion of 875 ± 61 IU (range: 500–1500 IU) per hour. Infusion was stopped 30 min before completion of HD.

Different protocols were performed with subgroups of patients and are described subsequently. In each protocol all parameters were determined before initiation of HD and heparin treatment and at least after 180 min of treatment (except for CAPD patients).

Protocol A, including ten individuals, was designed to detect changes in coagulation parameters during regular HD with heparinization. Determinations were performed repetitively on three consecutive HD sessions. These patients were uniform with regard to the duration of haemodialysis (3.5 h), blood flow (250 ml/min) and dialyzer membrane (F8, Fresenius). Blood was drawn prior to initiation of HD and the administration of heparin (timepoint 0). Further blood sampling was performed 30 (1), 90 (2) and 120 min (3) into HD, after 180 min (4), when heparin infusion was stopped, and at completion of HD after 210 min (5). Blood was drawn without prior congestion from the arterialized blood proximal to the addition of heparin and the dialyzer membrane. To exclude that results were affected by the site of blood collection, we drew further blood in

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**Table 1. Patient characteristics of all individuals investigated**

<table>
<thead>
<tr>
<th>Group</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>CAPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (n)</td>
<td>10</td>
<td>19</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 ± 3</td>
<td>59 ± 2</td>
<td>58 ± 4</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67 ± 4</td>
<td>59 ± 3</td>
<td>57 ± 6</td>
<td>–</td>
</tr>
<tr>
<td>Heparin bolus (IU)</td>
<td>1500 ± 220</td>
<td>2026 ± 290</td>
<td>1000 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>Heparin infusion (IU/hr)</td>
<td>750 ± 110</td>
<td>947 ± 65</td>
<td>600 ± 100</td>
<td>0</td>
</tr>
<tr>
<td>Dialyzer</td>
<td>Polysulfon</td>
<td>Hemophan</td>
<td>Cellulose</td>
<td>–</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>Chronic GN</td>
<td>Diabetic nephropathy</td>
<td>Interstitial nephropathy</td>
<td>Vascular nephropathy</td>
</tr>
<tr>
<td>Chronic GN</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>0</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Interstitial nephropathy</td>
<td>4</td>
<td>9</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Vascular nephropathy</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>1</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Treatment since (months)</td>
<td>54 ± 16</td>
<td>46 ± 10</td>
<td>23 ± 8</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>Dialysis time (h/week)</td>
<td>10.5 ± 0</td>
<td>10.1 ± 0.5</td>
<td>10.5 ± 0</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.14 ± 0.25</td>
<td>1.35 ± 0.25</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Group (A–D, CAPD) denotes study protocols to which patients were assigned (note: except for CAPD patients may belong to more than one group). (A) Dialysis with heparin (timecourse); (B) dialysis with heparin (determinations before start and 30 min prior to completion of HD); (C) selective heparinization without haemodialysis; (D) haemodialysis without heparin.
Protocol B investigated an additional 19 patients with single blood samplings at time points 0 and 4. Duration of HD, blood flow and type of dialyzer varied among patients in this group.

Protocol A and B were analyzed together for changes in coagulation parameters during haemodialysis (A + B).

Protocol C was designed to analyze the isolated effect of heparin on coagulation in dialyzed patients, i.e. heparinization without haemodialysis, excluding contact activation induced by extracorporeal devices and the dialysis procedure itself. For this purpose, five patients were recruited from the protocol A group, serving as their own control. Studies were performed within 7–14 days after the three consecutive dialyses for protocol A. Heparin was administered via a haemodialysis canula inserted in the HD shunt (AV-fistula or synthetic graft) in equivalent doses and for the same time as during regular HD treatment. Blood was drawn from a separate canula placed in a peripheral vein.

Protocol D was designed to analyze the isolated effect of haemodialysis on blood coagulation, i.e. haemodialysis without heparinization, excluding the anticoagulatory action of heparin. In this setting coagulation parameters exclusively reflect contact activation by the extracorporeal device and the haemodialysis procedure itself. Thus, protocol D is complementary to protocol C, together representing different components of protocol A (i.e. heparinization and contact activation). Three patients were chosen from the protocol A group to undergo HD without heparinization. Again, studies were performed within 7–14 days after the three consecutive dialyses for protocol A. To prevent blood clotting dialyzers and blood lines were rinsed every 30 min with 100 ml of 0.9% sodium chloride (NaCl). Haemodialysis was aborted when regular dialysis time was completed or when dialyzer membranes or blood lines started to clot.

Finally, a control group of ten patients undergoing chronic ambulatory peritoneal dialysis (CAPD), was investigated. They were analyzed to determine the impact of uremia on blood coagulation independent of chronic haemodialysis treatment and heparinization. The rationale to use CAPD patients for control rather than untreated patients with ESRD was to avoid bias from severe uremia due to lack of treatment. Moreover, this approach was used to compare the effect of HD and CAPD on coagulation status. PD patients were selected randomly from our CAPD cohort (patient characteristics see Table 1). They underwent a single blood collection when presenting for routine control in our outpatient clinic.

All analyses of factors with a potential impact on coagulation parameters were based on data obtained from patients of protocols A and B (n = 29) determined before and 180 min after start of haemodialysis.

The majority of the HD study patients (24 out of 29) received treatment with intravenous recombinant human erythropoietin (rhEPO; Eprex®, Cilag, or Recormon®, Boehringer Mannheim) over an extended period of time. The target range determining the amount of rhEPO to be administered was defined as a hematocrit between 30 and 35%. The average weekly dose was 7027 ± 1002 IU, administered three times a week after completion of each haemodialysis session. During study sessions, rhEPO was administered after collection of the last blood sample.

The study protocol was approved by the hospital’s ethical committee. All patients gave their informed consent for participation.

**Materials and methods**

Blood was sampled from the dialysis access site without prior congestion in cuvettes containing 0.025 mol/L calcium chloride solution. Activated partial thromboplastin time (PTT) was measured immediately. Aliquots for determination of prothrombin fragments F1 + F2, TAT complexes and D-dimers were processed by centrifugation within maximal 2 h and stored at −70°C until analyzed.

Reagents for determination of PTT (Pathromtin®) were provided by Behring Diagnostics (Marburg, Germany). The intraassay coefficient of variation (CV) for this test ranges between 0.9 and 3.5%, the interassay CV is 2.8–4.9% [14]. The reference interval for adults ranges from 28–40 seconds.

F1 + 2 are components of the procoagulatory system. In brief, activation of prothrombin represents the key event in the activation of the coagulation cascade. During this reaction factor Xa cleaves the peptide bond Arg273-Thr274 of the prothrombin molecule which results in liberation of thrombin generation process [16]. For determination of TAT-complexes and D-dimers were processed by centrifugation within maximal 2 h and stored at −70°C until analyzed.

Thrombin anti-thrombin complexes (TAT-complexes) are rapidly generated in the final process of blood coagulation by the association of antithrombin III (ATIII) to thrombin, a process that is enhanced by heparin and inactivates thrombin, a healthy subjects a reference range from 0.37–1.11 nmol/l F1 + 2 (2.5–97.5th percentile) was reported. In order to standardize the test for our own laboratory we determined prothrombin fragment concentrations in individuals from a healthy control population (n = 36). The mean ± SD for F1 + 2 concentrations in this group is 0.97 ± 0.12 nmol/l, ranging from 0.71–1.24 nmol/l. Our own reference range (5th–95th percentile) derived from these data is 0.71–1.12 nmol/l.

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In vivo thrombin generation process [16]. For determination of TAT-complexes the enzyme immunonassay Enzygnost TAT thermo® from Behring Diagnostics was purchased [17]. Concentrations were quantified photometrically. The intraassay and interassay CVs for this test reportedly are 4–6 and 6–9%, respectively, the reference range (2.5th–97.5th percentile) 1.0–4.1 µg/l (data according to the manufacturer). In order to determine whether procoagulatory events were counterbalanced by changes in fibrinolysis we measured D-dimer plasma concentrations. D-dimers are generated by the proteolytic action of plasmin on fibrin polymer crosslinked by factor XIII, reflecting thrombolytic activity [18]. Measurements of D-dimers were performed by an ELISA method (D-Dimer ELISA, Boehringer Mannheim, Rotkreuz, Switzerland).

All assays were performed in duplicate and the mean of the two values was used. Kt/V was calculated from the ultrafiltration coefficient of
the patient’s dialyzer (K), the duration of the individual dialysis session (t) and the urea volume of distribution estimated as 58% of dry body weight (V).

Statistics

Data are presented as means ± SEM. Analyses were performed by simple ANOVA or ANOVA for repeated measurements. Correlation of different parameters was tested by simple and multiple regression analysis, or by Spearman’s correlation, as appropriate.

Results

Patient groups were similar in age, body weight, Kt/V and time on haemodialysis or peritoneal dialysis, respectively (Table 1). Comparable amounts of heparin were used for protocols including anticoagulation during HD.

The extent of coagulation determined with all parameters did not vary among different sites of blood collection, i.e. afferent vs efferent shunt vs remote vein (data not shown). However, samples taken from the afferent and efferent blood lines showed less interindividual variation than venous samples. Correlation of predialysis values on two different days was high for any performed assay (PTT: r = 0.74, P < 0.01; F1 + 2: r = 0.8, P < 0.01; TAT: r = 0.83, P < 0.01), reflecting stable pretreatment conditions.

Regular haemodialysis (A and B)

All standard haemodialysis treatments according to protocol A and B were performed without complications in all patients. Neither clotting of dialyzers or blood lines nor bleeding complications were observed.

Baseline PTT values were within normal range before the administration of heparin and/or initiation of haemodialysis (Table 2). After start of regular haemodialysis, PTT doubled within 30 min of treatment (P < 0.01 vs baseline), and remained elevated for 60 min. Values slowly decreased but remained prolonged until the end of dialysis, heparin being withdrawn 30 min earlier (Figure 1).

Baseline concentrations of prothrombin fragments F1 + F2 (F1 + 2) were significantly elevated in patients on regular haemodialysis (Table 2). Only one out of 29 HD patients had concentrations within the normal range. Standard haemodialysis had a minimal effect on prothrombin fragment generation during the first 2 h of dialysis (n = 10), but concentrations were significantly increased after 3 h compared to baseline (P < 0.05; n = 29). However, the amount of prothrombin fragments after 3 h of dialysis was lower among patients with higher values for Kt/V (r = 0.54, P < 0.01).

Concentrations of thrombin-antithrombin complexes (TAT) prior to HD were significantly increased in patients on regular HD (Table 2). Only four out of 29 patients were within the normal range. TAT complexes were increased markedly during standard dialysis (Figure 1) and were significantly elevated after 3 h of treatment vs values at baseline and 30 min of dialysis (P < 0.02).

Among patients undergoing repeated determinations of all parameters on three consecutive days of dialysis (group A, n = 10), prothrombin fragment and TAT complex concentrations were always lower before the start of dialysis than at the end of treatment (Figure 2). This scheme resulted in a biphasic pattern, suggesting that the dialysis procedure consistently resulted in a stimulation of procoagulatory factors.

D-dimer concentrations were significantly elevated beyond normal range, indicating continuous fibrinolytic activity. Also, D-dimer concentrations were inversely correlated with TAT (r = -0.42, P < 0.03) and F1 + 2 levels (r = -0.36, P < 0.05). In view of these findings the observed increases of prothrombin fragments and TAT complexes are indicative of predominant procoagulatory activity rather than reduced fibrinolysis.

Heparinization without haemodialysis (C)

Results are summarized graphically in Figure 3. Unlike regular dialysis, selective heparin infusion did not result in significant changes of any coagulation parameter determined. Prothrombin fragments were lower towards the end of heparin administration without dialysis and therefore relatively (but not significantly) decreased vs standard HD.

Haemodialysis without heparinization (D)

When dialysis was performed without anticoagulation, treatment had to be aborted earlier than scheduled in two out of three patients because dialyzer and blood lines were clotted. Clotting occurred after 120 and

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**Table 2. Reference range of all coagulation parameters examined and their concentrations determined in patients at baseline and after 180 min of haemodialysis (CAPD: only ‘baseline’ concentrations determined)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference range</th>
<th>HD baseline</th>
<th>HD 180 min.</th>
<th>CAPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT (sec)</td>
<td>28 – 40</td>
<td>37.2 ± 1.3</td>
<td>61.7 ± 4.3†</td>
<td>32.5 ± 0.9‡</td>
</tr>
<tr>
<td>F1 + 2 (nmol/l)</td>
<td>0.71 – 1.24</td>
<td>2.79 ± 0.2</td>
<td>2.99 ± 0.2</td>
<td>3.6 ± 0.3‡</td>
</tr>
<tr>
<td>TAT (µg/l)</td>
<td>1.0 – 4.1</td>
<td>12.6 ± 2.8</td>
<td>40.3 ± 8.9†</td>
<td>15.8 ± 3.1</td>
</tr>
<tr>
<td>D-dimer (µg/l)</td>
<td>4 – 25</td>
<td>176 ± 34</td>
<td>188 ± 39</td>
<td>148 ± 46</td>
</tr>
</tbody>
</table>

Results of HD patients are composed of measurements from group/protocol A and B combined. Except for PTT, all coagulation parameters were significantly beyond the normal range. †P < 0.01 vs. HD baseline; ‡P < 0.05 vs. HD baseline.
Plasma hypercoagulability in haemodialysis patients

**Fig. 1.** Results of serial PTT, F1+2 and TAT measurements in patients of group/protocol A (regular haemodialysis; n = 10). Determinations were made before start of dialysis and administration of heparin (0); after 30, 90, 120, and 180 min into HD, and at the end of HD (210 min), respectively. Heparin infusion was started simultaneously with dialysis and stopped 30 min before completion of HD. Measurements were performed during three consecutive haemodialyses and mean values were calculated for each individual patient and timepoint. Shown are means ± SEM of all patients. PTT: † = P < 0.01 vs. all other timepoints; ‡ = P < 0.01 vs. 30, 90 and 120 min. F1+2: † = P < 0.05. TAT: † = P < 0.02 vs. baseline; ‡ = P < 0.02 vs. baseline and 30 min.

135 min, respectively. Dialysis was regularly completed after 180 min in the third patient. However, the dialyzer membrane could not be rinsed completely despite extensive use of NaCl, a considerable amount of blood remaining in the extracorporeal device.

Figure 4 depicts changes in coagulation parameters from treatment according to protocol D. Baseline levels for F1+2 and TAT in this group were higher than those determined in protocol A (Figure 1). This discrepancy is due to the small number of patients in protocol D (n = 3), with two of them having F1+2 and TAT baseline concentrations above average. PTT did virtually not change during dialysis without heparin in these three patients (Figure 4, open dots), whereas regular treatment had resulted in a 2.5-fold increase immediately after initiation of anticoagulation in the same patients (Figure 4, closed dots). Values stayed significantly lower throughout dialysis compared to the standard regimen. In contrast, generation of prothrombin fragments was pronounced in patients undergoing HD without heparin. This effect became more significant over time. Similarly, TAT complex concentrations were demonstrably elevated during dialysis without heparin compared to regular dialysis. Due to the small number of patients, no statistic calculations were performed.

**Procoagulatory activity in patients on PD**

All coagulation parameters in patients on peritoneal dialysis showed a trend towards higher procoagulatory activity compared to baseline measurements in patients on haemodialysis (Table 2). These differences reachedStatistic significance for PTT and F1+2 values. However, when posthaemodialysis measurements were compared with values from PD patients the opposite was found, indicating more pronounced hypercoagulability after haemodialysis.

**Effect of age and underlying disease**

The age of patients did not correlate with coagulation activity, neither before nor during haemodialysis, revealed by any parameter assessed. However, baseline
coagulation parameters could be identified as discriminatory factors of the renal disease process. Patients with vascular nephropathy had significantly higher prothrombin fragment baseline concentrations (4.3 ± 0.1) than patients with diabetic nephropathy (2.95 ± 0.4; \( P = 0.03 \)) or interstitial kidney disease (2.4 ± 0.3; \( P < 0.01 \)). Similarly, D-dimer concentrations were almost twice as high in patients with vascular nephropathy compared to other etiologies (data not shown). In contrast, partial thromboplastin time and TAT complex levels were similar among patients with different renal pathologies.

Impact of vascular access, type of dialyzer and dialysate
No differences in coagulation parameters were detectable between patients with shunts from native vessels and those with prosthetic shunts, nor for acetate vs bicarbonate dialysate. However, differences in prothrombin fragment generation were obvious among different types of dialyzers (Figure 5). In patients treated with polysulfon membranes a 15% increase in \( F_1+2 \) concentrations was observed during standard dialysis, whereas concentrations decreased in patients treated with hemophan and cellulose filters by 11 and 8.4%, respectively. Consequently, levels of \( F_1+2 \) were markedly lower after 3 h of dialysis in patients treated...
Plasma hypercoagulability in haemodialysis patients

Previous studies in patients on HD have suggested that rhEPO is associated with increased coagulatory and decreased fibrinolytic activity [19,20]. Out of 29 patients in our study 24 were on rhEPO treatment. As shown in Table 3 they had slightly, but insignificantly increased levels of prothrombin fragments and TAT complexes but prolonged partial thromboplastin times compared to patients without rhEPO. However, when we analysed the effect of rhEPO dosage on procoagulatory activity in patients receiving erythropoietin therapy a significant (inverse) correlation between erythropoietin dose and prothrombin levels was detected (Figure 6). Higher rhEPO doses were associated with lower prothrombin fragment concentrations ($r=-0.37, P=0.045$). In accordance, patients on higher rhEPO doses had more prolonged partial thromboplastin times ($r=0.48, P=0.008$). These rhEPO dose effects on F1 + 2 and PTT were unrelated to the amount of heparin administered, patient’s hematocrit, age, underlying renal disease, time on dialysis or type of dialyzer used, as revealed by multiple linear

**rhEPO treatment and coagulation status**

Table 3. Coagulation parameters of patients without and with recombinant human erythropoietin (rhEPO) treatment

<table>
<thead>
<tr>
<th></th>
<th>PTT (sec)</th>
<th>F1 + 2 (nmol/l)</th>
<th>TAT (µg/l)</th>
<th>D-dimer (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No rhEPO ($n=5$)</td>
<td>35.4±1.5</td>
<td>2.55±0.36</td>
<td>10.9±1.9</td>
<td>203±95</td>
</tr>
<tr>
<td>rhEPO ($n=24$)</td>
<td>37.6±1.5</td>
<td>2.85±0.27</td>
<td>13.0±3.3</td>
<td>170±37</td>
</tr>
</tbody>
</table>

Results consist of patients from group/protocol A and B (regular HD with heparin).

![Figure 5](image1.png)

**Fig. 5.** Bars indicate concentration of prothrombin fragment before and after 3 h of dialysis in patients treated with different types of dialyzers. † = $P<0.05$ vs. polysulfon group.

![Figure 6](image2.png)

**Fig. 6.** Scatter plots showing relation between erythropoietin dosage (X-axis) and baseline plasma concentrations of F1 + 2 and partial thromboplastin time (PTT), respectively.
Regression. It is noteworthy that the significance of the statistical analysis is unaffected by the outlayer representing one patient receiving particularly high doses of rhEPO. Therefore, these findings imply a specific dose-related effect of recombinant erythropoietin on procoagulatory activity in haemodialysis patients.

**Discussion**

Hypercoagulability is presumed to occur in renal disease and potential mechanisms have been reviewed recently [21]. In our mixed patient population we found procoagulatory activity to be increased by >2- and 3-fold above normal evidenced by increased levels of prothrombin fragments and TAT-complexes, respectively (Table 2). These findings are in accordance with studies from other groups that measured either TAT complexes [6] or TAT complexes in combination with F1 + 2 [9] in patients with ESRD. Similar results have been described also for subgroups of patients with chronic renal failure. For example, diabetic and non-diabetic patients with ESRD have been reported to have increased procoagulatory activity, as evidenced by elevated platelet aggregation, increased concentrations of D-dimers, von Willebrand factor antigen and platelet factor 4 in both groups [7]. Along with our results of a hypercoagulable state of comparable degree in CAPD patients (Table 2), renal failure per se rather than HD treatment seems to be the cause of increased procoagulatory activity. Notably, we also detected strongly elevated D-dimer levels (Table 2), demonstrating that stimulated thrombin formation and not impaired fibrinolytic activity accounts for the hypercoagulable state. However, no conclusion can be drawn from the present study with regard to whether increased prothrombin fragment and TAT complex concentrations are the result of increased production or decreased clearance.

Determination of prothrombin fragments and TAT complexes in this study revealed ongoing or even accelerated coagulation during haemodialysis (Figure 1). Whereas F1 + 2 concentrations remained steadily elevated over the whole period of dialysis despite heparinization, TAT complex concentrations even rose by 460%, detected 30 min before termination of dialysis. The consistent biphasic pattern observed for F1 + 2 and TAT, undulating between start and end of dialysis in patients examined over three consecutive sessions of HD strongly suggests HD-induced thrombin formation (Figure 2). These findings are in accordance with Sagripanti et al., who found more pronounced activation of various coagulation parameters in patients with ESRD on haemodialysis than in patients with conservative treatment with a very-low-protein diet [6]. However, it is of interest to notice, that despite increased coagulation parameters during haemodialysis in our study prothrombin fragments were lower among patients with higher values for $\text{Kt/V}$ ($r = -0.54, P < 0.01$). This suggests that these products are either dialyzed or generated to a lower extent due to better uremic control. Again, our study does not allow us to distinguish between these two possibilities. Different rates of removal from the circulation probably account for the quantitative differences observed in the degree of prothrombin and TAT complex formation during HD: Half-lives (T1/2) for F1 + 2 and TAT are 30 and 20 min, respectively [17]. In accordance with the longer half-life for F1 + 2 their concentrations were only slightly lowered by heparin without dialysis (protocol C) compared to standard HD (Figure 3). An alternative explanation, however, would be that heparin releases TAT-complexes from thrombotic material, thereby steadily increasing their serum levels. In accordance, TAT complex concentrations dropped immediately both after withdrawal of heparin infusion during regular HD (protocol A) and after selective heparinization (protocol C). Finally, it is important to notice, that TAT-complex formation also depends on the amount of ATIII and heparin present in the blood. Indeed, reduced levels of ATIII and ATIII activity have been described in patients with ESRD [22]. Nevertheless, this cannot explain the higher concentrations of TAT in our patients since the changes in ATIII are in the wrong direction. Also, the same study found no significant changes in ATIII concentrations and activity during haemodialysis. However, differences in blood heparin concentrations among patients and changes in heparin levels during HD may contribute to the amount of measured TAT complexes, which therefore reflect thrombin generation with more variability than prothrombin fragments do.

Determination of adequate heparin therapy for haemodialysis is important with regard to dialysis efficiency and reduction of thrombotic risk. Heparin dosage in our patients was based on standard regimens modified empirically. Consequently, no clotting of extracorporeal devices occurred during the study. Nevertheless, our findings suggest that commonly used heparin doses are not sufficient to effectively control hypercoagulability. To further test this hypothesis we developed a study designed to separately assess the effects of anticoagulation and haemodialysis on procoagulatory activity (protocol C and D, respectively). As anticipated, PTT was prolonged by an additional 27% compared to standard HD after 30 min (ns) from selective heparinization. Thereafter, no differences were detectable between the two groups (Figure 3). However, when standard dialysis was compared to HD without anticoagulation, PTT did not change at all in the latter group (Figure 4). In contrast, F1 + 2 and TAT complex concentrations fell from selective heparin treatment, but rose significantly during dialysis without anticoagulation. These results, first, demonstrate, that the dialysis procedure substantially increases procoagulatory factors, shown by the tremendous rise in prothrombin and TAT levels when anticoagulation was withheld (protocol D, Figure 4). Second and most importantly, the findings support our notion, that current heparin dose regimens are insufficient to limit coagulation processes during haemodialysis. Although a relevant reduction in thrombin accumulation is achieved by
heparin administration (as evidenced by the difference in the course of F1 + 2 and TAT concentrations in Figures 1 and 4), heparin by itself diminished thrombin induction even further (Figure 3). The question remains, how excess thrombin formation and the ‘ideal’ heparin dose could be determined. Clearly, the commonly used determination of PTT is not suitable to quantify thrombin formation, as shown by our data. It also has a limited use in monitoring heparin treatment. Due to the short halftime of TAT complexes their blood concentrations reflect ongoing thrombin accumulation the most accurate among the coagulation parameters evaluated in our experiments. The differences in TAT levels during regular HD with heparin and heparinization alone (Figure 3) allow an approximate estimate of excess thrombin resulting from standard dialysis. TAT complex levels during standard dialysis were higher by 30–50% compared to concentrations during selective heparin infusion. Therefore, it is reasonable to assume that standard heparin doses as used in our study should be increased by 50% in order to suppress thrombin accumulation during haemodialysis. This approach is supported by other data suggesting that TAT-complex levels determined at the end of dialysis are a good indicator for dose adaptations [5]. Moreover, Ireland et al. have found TAT levels to correlate well with heparin doses in haemodialysis patients, lower TAT levels reflecting higher heparin concentrations [23]. However, further investigations need to establish the dose/effect relationship for regular heparin treatment.

Finally, we analyzed our data in order to identify treatment modalities that affect thrombin formation in ESRD patients. All coagulation parameters in patients on peritoneal dialysis reflect higher procoagulatory activity compared to baseline (predialysis) values of patients undergoing HD (Table 2). However, it remains to be determined whether haemodialysis delimits hypercoagulation more effectively than PD. Patients with vascular nephropathy had significantly higher prothrombin levels compared to patients with ESRD from other causes. This may reflect contact mediated induction of coagulation by the altered vasculature or decreased uptake and degradation of thrombin metabolites by damaged endothelium. These results become especially important considering recent data suggesting that people at risk for the development of ischemic heart disease have increased F1 + 2 concentrations [24]. Conversely, comparable levels of F1 + 2 and TAT were determined in patients with native or prosthetic shunts. However, significant differences were revealed for dialyzer type and treatment with recombinant human erythropoietin (rhEPO). Polysulfon membranes were associated with higher prothrombin levels compared to dialyzers made of hemophan or cellulose. Baseline levels, and, most notably, concentrations after 3 h of dialysis were significantly increased with polysulfon membranes. These findings imply, that polysulfon membranes induce thrombin formation through contact activation resulting in permanently increased procoagulatory activity. However, more extended studies need to confirm these conclusions. Treatment with rhEPO has previously been shown to affect blood coagulation and fibrinolysis during the first weeks of administration, having a net procoagulatory effect [19,20]. Despite the fact that only a small number of our study patients did not receive rhEPO, our results show the same trend (Table 3). However, an important observation was made when patients receiving rhEPO were analysed separately as a group with regard to the dose effect of erythropoietin on coagulation status. Whereas TAT complex concentrations were unrelated to rhEPO dosage, higher dose regimens were associated with lower amounts of prothrombin fragments. This apparently paradoxical finding may be explained by a dose related effect of rhEPO. Whereas low doses of erythropoietin seem to induce prothrombotic factors, higher doses of the drug actually might decrease procoagulatory activity in haemodialysis patients.

In summary and conclusion, we have shown that patients with endstage renal disease are in a substantial procoagulable state. Coagulatory activity is further stimulated by haemodialysis. Nevertheless, HD appears to be superior to PD in limiting thrombin formation, possibly by better uremic control. On the other hand, current anticoagulation regimens for haemodialysis are likely to be insufficient. These findings could be relevant with regard to the occurrence of thrombotic events and the efficiency of haemodialysis. Coagulation parameters assessing thrombin formation more directly, such as TAT complex and F1 + 2 concentrations, should be employed individually for prescription of anticoagulation therapy, especially in patients with high risk profiles for thromboses. For routine monitoring, however, these measurements are too elaborate and costly to justify their regular implementation. Also, the pathophysiological relevance of these ‘alternative’ coagulation parameters needs to be further corroborated in order to prove importance beyond a simple laboratory phenomenon. Finally, prospective studies are indicated to confirm our findings about dialyzer and rhEPO dose related effects on procoagulatory activity.

References


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