

Haemodialysis in patients treated with oral anticoagulant: should we heparinize?

Thierry Krummel¹, Elise Scheidt², Claire Borni-Duval¹, Dorothée Bazin¹, François Lefebvre³,
Philippe Nguyen⁴ and Thierry Hannedouche¹

¹Department of Nephrology, University Hospital and School of Medicine, Strasbourg, France, ²Department of Nephrology, General Hospital, Haguenau, France, ³Department of Biostatistics, University Hospital and School of Medicine, Strasbourg, France and ⁴Laboratory of Hematology, University Hospital, Reims, France

Correspondence and offprint requests to: T. Krummel; E-mail: thierry.krummel@chru-strasbourg.fr

ABSTRACT

Background. Anticoagulation for the haemodialysis circuit in patients treated with oral anticoagulation poses additional haemorrhagic risk. The few available data suggest that tapering or even stopping heparinization is feasible and the HeprAN membrane with grafted heparin was developed to decrease heparin dose. The objective of our study was to evaluate the need for additional anticoagulation in patients on long-term oral anticoagulation, according to the type of membrane used.

Methods. This is a prospective, randomized, crossover bifactorial trial in haemodialysed patients on oral anticoagulation. Each patient had four haemodialysis sessions with two different membranes [HeprAN or polysulphone (PS)] and with or without enoxaparin. Clinical coagulation was evaluated by the need for premature ending and by a visual score (Janssen scale). Coagulation activation markers were also measured: D-dimers, prothrombin fragments 1 + 2, thrombin–antithrombin complexes, tissue factor pathway inhibitor and platelet factor-4.

Results. Ten patients were included (M/F = 4/6, mean age 63 ± 15 years). None of the 40 sessions ended prematurely. The clotting scores were similar with or without enoxaparin (dialyser: 1.49 ± 0.19 versus 1.53 ± 0.17, $P = 0.97$; bubble trap: 0.75 ± 0.19 versus 0.78 ± 0.22, $P = 0.62$) and with the polysulphone or the HeprAN membrane (dialyser: 1.54 ± 0.20 versus 1.47 ± 0.16, $P = 0.65$; bubble trap: 0.74 ± 0.22 versus 0.79 ± 0.19, $P = 0.58$). There was no significant difference in coagulation activation markers between dialysis modalities; however, dialysis efficacy was significantly greater with the PS membrane (1.58 ± 0.07 versus 1.43 ± 0.06, $P = 0.02$).

Conclusions. These results suggest that haemodialysis without additional anticoagulation is possible in patients with oral anticoagulation. The HeprAN membrane did not provide any additional benefit compared with a PS membrane.

Keywords: chronic haemodialysis, coagulation, haemodialysis membrane, low molecular weight heparin, oral anticoagulation

INTRODUCTION

Extracorporeal blood treatment is known to activate the blood coagulation cascade and blood platelets, resulting in thrombus formation and premature ending of the haemodialysis session with insufficient dialysis dose and blood loss [1, 2]. Guidelines for clotting prevention recommend either unfractionated (UH) or low-molecular-weight heparin (LMWH), which can potentially increase haemorrhagic risk in patients with end-stage renal disease who frequently exhibit a uraemia-associated platelet dysfunction [3–5].

Up to 25% of haemodialysis patients are treated with oral anticoagulation, usually for atrial fibrillation, but also for other purposes, with a benefit/risk ratio difficult to estimate [6–9]. In these patients, additional anticoagulation for haemodialysis may even further increase haemorrhagic risk.

In the literature, there is no firm data demonstrating that oral anticoagulation with antithrombin K is sufficient to prevent clotting during the haemodialysis procedure or whether additional administration of LMWH or UH is required. Despite this lack of evidence, European Best Practice Guidelines for Haemodialysis suggest tapering the dose of LMWH or UH on an individual basis, e.g. to the dose that results in minimal clotting in the bubble trap [3].

Two small prospective crossover trials have studied activation of coagulation in haemodialysis patients treated with oral anticoagulant in order to examine whether oral anticoagulation was sufficient for haemodialysis. In the study of Ziai *et al.*, the dose of heparin could be reduced by 50% and in a subsequent study of the same group, using AN69ST

membrane, heparin-free haemodialysis was feasible in the absence of prothrombotic disorder [10, 11].

Recently, the HeprAN membrane, derived from the AN69ST membrane and grafted with UH, was developed in order to reduce the need for systemic heparinization. To date, only few studies have been performed with the Evodial® dialyser and more specifically, there are no available data for patients treated with oral anticoagulation [12].

In this context, we conducted a study of the clinical and biological activation of coagulation in haemodialysis sessions with the HeprAN or polysulphone (PS) membrane, with or without additional LMWH, in a small cohort of patients receiving long-term oral anticoagulation.

MATERIALS AND METHODS

Study design

The CHAMO study was a prospective, randomized, bifactorial crossover study conducted in the Department of Nephrology of the University Hospital in Strasbourg from 1 December 2010 to 16 February 2011.

The study protocol was approved by the local Ethics Committee, and all patients gave informed consent before the start of the study.

Patients

The inclusion criteria were age ≥ 18 years, maintenance haemodialysis for at least 3 months, three dialysis sessions per week, chronic oral anticoagulation and written informed consent. Exclusion criteria were participation in another study, known heparin-induced thrombocytopenia, pregnancy or feeding and chronic use of antiplatelet agent within 8 days before inclusion. During the session preceding the study, we also ensured that patients had an international normalized ratio (INR) within the target range (2.0–3.0) and minimal inflammation with a C-reactive protein level >20 mg/L.

Ten patients (4 men, 6 women) on maintenance haemodialysis since 67.3 ± 51.1 months (mean $\pm$ SD) and receiving long-term oral anticoagulation by vitamin K antagonists (VKA) were enrolled into the study (coumadine or phenindione). The aetiology of chronic kidney disease, the indications for oral anticoagulation and the remaining patient characteristics are presented in Table 1.

Haemodialysis procedures

Patients were allocated to a random sequence of four haemodialysis procedures with either no additional anticoagulation or low-dose enoxaparin (2000 UI) and to treatment with either the Evodial 2.2 dialyser (HeprAN membrane, surface area 2.15 m^2 , Hospal SAS, Mezieu, France) or the FX100 dialyser (Helixone PS membrane, surface area 2.2 m^2 ; Fresenius Medical Care, Bad Homburg, Germany). Thus, each patient was studied during four sessions: two with enoxaparin 2000 UI with each of the two membranes (HeprAN and Helixone) and two without additional enoxaparin with the two aforementioned membranes. The study was performed on the second and third sessions of the week in order to achieve a similar ultrafiltration rate. The duration of the dialysis sessions was 4 h in eight patients and 4.5 and 5.5 h in the two remaining patients. Eight patients had a native arteriovenous fistula (AVF), one patient had a pre-thoracic polytetrafluoroethylene (PTFE) loop and the last patient had a two-lumen-tunnelled central venous catheter. All of the sessions consisted of standard haemodialysis (i.e. no haemodiafiltration) using standard dialysis solutions. Standard bicarbonate concentrations were adjusted if necessary (30–35 mmol/L). Two needles were used in all patients with an arteriovenous access. Blood flow was between 250 and 450 mL/min in all patients and maintained stable throughout the four study sessions. Dialysate flow was between 500 ($n=8$) and 800 ($n=2$) and did not change during the study period. The restitution volume was between 400 and 500 mL. In sessions with enoxaparin, a single bolus of 20 mg was administered at the beginning of the haemodialysis

Table 1. Patient clinical characteristics

Patient no.	1	2	3	4	5	6	7	8	9	10
Sex	F	F	M	M	F	M	M	F	F	F
Age (years)	68	49	48	75	87	62	55	79	36	72
Indication for VKA	AF	AT rAPC	AF	AF	PE	AF	AF	AF	APS	AF
Renal disease	HN	TIN	Diab	Diab	HPB	MN	HN	Diab	Lupus	TIN
Duration of ESRD (months)	71	3	142	11	23	140	96	26	96	65
Vascular access	AVF	CVC	Loop	AVF	AVF	AVF	AVF	AVF	AVF	AVF
Malignancy	Womb 1980	No	No	Prostate	Kidney	No	No	Skin	None	MF
Infection last 2 months	No	Pyelo	No	Prostate	Septic	No	No	No	Bronchitis	No
History of haemorrhage	No	No	No	No	No	No	No	No	No	No
History of thrombo-embolism	Yes	No	Yes	No	Yes	No	No	No	Yes	No

AF, atrial fibrillation;

rAPC, repeated vascular access thrombosis and resistance to activated C protein;

PE, repeated pulmonary embolism;

APS, antiphospholipid syndrome; HN, hypertensive nephritis;

TIN, tubulo-interstitial nephritis; Diab, diabetic nephropathy; MN, membranous nephropathy;

ESRD, end-stage renal disease; AVF, arteriovenous fistula;

CVC, central venous catheter;

MF, myelofibrosis;

Pyelo, pyelonephritis;

Septic, septicemia.

session. The oral anticoagulation dose was adjusted at each session for the next days, according to the INR obtained.

Blood sampling

At each dialysis session, blood samples were drawn from the arterial line predialysis (H0) and after 3 h from the arterial (H3A) and venous line (H3V) for D-dimer, TAT, F1 + 2, PF4 and TFPI measurements.

D-dimer concentrations were assayed with an immunoturbidimetric assay (STA® Liatest® D-Di, Stago). Blood samples for determination of TAT complexes, F1 + 2 and PF4 were centrifuged for 45 min at 4500 g in order to separate plasma from the cellular fraction. TAT complexes, F1 + 2 and PF4 concentrations were assayed by ELISA (Enzygnost® TAT micro, Enzygnost® F1 + 2, Siemens Healthcare Diagnostic, Inc., Marburg, Germany; Asserachrom PF4®, Diagnostica Stago, Asnières, France).

TFPI measurements were performed with an ELISA test (DTFPI10, Human TFPI Quantikine ELISA kit, R&D Systems).

Haemostatic markers included INR (H0), aPTT (H0), anti-FXa activity (H0 and H3A), antithrombin (H0) and fibrinogen (H0). In addition, white cell count (H0), platelet count (H0), haemoglobin (H0), haematocrit (H0 and H3A), urea (H0 and end-session) and total proteins (H0) were measured. For all the above markers, measurements were performed in the same laboratory using routinely applied assays.

Dialysis efficiency

Haemodialysis efficiency was assessed by measuring Kt/V by ionic dialysance and by calculating equilibrated Kt/V (eKt/V) according to standard formulae [13, 14].

Statistical analysis

The main end point was the coagulation of the extracorporeal circuit evaluated by the need of premature restitution and with a visual scale of clotting in the bubble trap and in the dialyser. The scale was adapted from Janssen *et al.* [15] and ranged from 0 to 4: Grade 0 was equal to no detectable clotting, Grade 1 to minimal clot formation, Grade 2 to moderate clot formation, Grade 3 to major clots but dialysis still possible and Grade 4 to complete occlusion of air traps or dialyser. The evaluation was performed visually and recorded on digital images and final scores represented the average of the scores

attributed independently by the medical ($n = 2$) and nursing team ($n = 1$).

Secondary end points were changes in plasma concentrations of D-dimer, TAT, F1 + 2, TFPI, PF4 plasma level between H0 and H3A and between H3A and H3V.

Statistical analysis was performed using Stata® software (StataCorp LP, Texas, USA). As data were assumed to be normally distributed, all values are expressed as mean values $\pm$ standard deviation (SD). To explore the effects of the membrane and of LMWH on the measured parameters, ANOVA models for repeated measures were fitted to the data, with membrane as between factor and LMWH as within factor. Differences were considered statistically significant if the corresponding P values were < 0.05 (adjusted according to Greenhouse and Geisser).

RESULTS

Individual characteristics of the 10 patients included in the study are listed in Tables 1 and 2. Each individual patient underwent the four sessions of haemodialysis and none of the 40 sessions ended prematurely due to coagulation.

The mean INR during the study was 2.49 ± 0.11 and for 10 sessions the INR was under 2. The percentage of sessions with an INR under the therapeutic goal was not statistically different between the different dialysis modalities. Conversely, the INR was > 3 for eight sessions.

A statistical interaction between the membrane and the coagulation regimen was found only for basal anti-Factor Xa (anti-FXa) activity of which the mean did not differ between the groups and remained under the threshold of detection.

LMWH effect: haemodialysis with and without low dose LMWH

Baseline haemostatic and haemodialysis parameters did not differ between the two groups (Tables 3 and 4).

The mean dose of enoxaparin was 0.24 ± 0.05 mg/kg. At the third hour, the anti-FXa activity was higher in the LMWH group comparatively with the group without additional anticoagulation (0.17 ± 0.02 UI/mL versus 0.08 ± 0.01 , $P < 0.001$).

There was no statistically significant difference for clotting scores (Figure 1) or dialysis dose (Kt/V) (Figure 2) between the two anticoagulation modalities.

Table 2. Biological characteristics and dialysis parameters

	Patient no.									
	1	2	3	4	5	6	7	8	9	10
Previous anticoagulation (mg enoxaparin)	0	60	60	20	20	40	60	20	0	40
Blood flow (mL/min)	340 $\pm$ 25	290 $\pm$ 50	410 $\pm$ 25	410 $\pm$ 25	390 $\pm$ 25	440 $\pm$ 25	440 $\pm$ 25	400 $\pm$ 0	400 $\pm$ 40	425 $\pm$ 30
End-session body weight (kg)	64.8 $\pm$ 0	68.4 $\pm$ 1	120 $\pm$ 0	86 $\pm$ 0.8	71.3 $\pm$ 0.1	78 $\pm$ 0	151 $\pm$ 2	97.6 $\pm$ 0.4	73.8 $\pm$ 0.6	73.4 $\pm$ 0.3
UF (L)	1.7 $\pm$ 0.3	2 $\pm$ 0.3	3.6 $\pm$ 0.5	2.7 $\pm$ 0.9	1.8 $\pm$ 0.5	2.6 $\pm$ 0.7	4 $\pm$ 0.9	2 $\pm$ 0.3	2.1 $\pm$ 0.4	2.2 $\pm$ 1.8
EPO ($\times 1000$ UI/week)	1.3 $\pm$ 0	1.85 $\pm$ 5	0	5.7 $\pm$ 0.7	1 $\pm$ 1.3	0	2.18 $\pm$ 4.5	1.3 $\pm$ 0	11.1 $\pm$ 0	8.9 $\pm$ 0
Haemoglobin (g/dL)	11.6 $\pm$ 0.7	11.7 $\pm$ 0.4	10.8 $\pm$ 0.3	11.2 $\pm$ 0.3	9.1 $\pm$ 0.3	14 $\pm$ 0.3	12.9 $\pm$ 1	12.2 $\pm$ 0.2	11.3 $\pm$ 0.4	11 $\pm$ 0.4
Platelets (G/L)	139 $\pm$ 20	238 $\pm$ 13	145 $\pm$ 9	237 $\pm$ 13	203 $\pm$ 40	241 $\pm$ 45	182 $\pm$ 6	288 $\pm$ 33	217 $\pm$ 48	148 $\pm$ 18
INR ^a	2.2 $\pm$ 0.3	1.9 $\pm$ 0.3	2.4 $\pm$ 0.5	3.2 $\pm$ 1.1	2.6 $\pm$ 0.5	2.5 $\pm$ 0.6	2.0 $\pm$ 0.4	2.2 $\pm$ 0.8	3.4 $\pm$ 0.6	2.5 $\pm$ 0.4

^aInternational normalized ratio.

Table 3. Predialysis biological parameters according to membrane type and anticoagulation regimen

	Normal values	FX100	EVODIAL	P value	Enoxaparin	No anticoag	P value
D-dimer ($\mu\text{g/L}$)	<400	596 $\pm$ 99	496 $\pm$ 62	0.38	526 $\pm$ 72	565 $\pm$ 93	0.72
Fibrinogen (g/L)	2–4	4.2 $\pm$ 1.7	4.3 $\pm$ 0.2	0.22	4.3 $\pm$ 0.2	4.2 $\pm$ 0.2	0.85
PF4 (UI/mL)	10–27	37.9 $\pm$ 12.3	27.9 $\pm$ 5.1	0.32	33.6 $\pm$ 8.3	32.5 $\pm$ 6.0	0.97
TAT ($\mu\text{g/L}$)	1.0–4.1	4.1 $\pm$ 0.6	3.3 $\pm$ 0.2	0.25	3.5 $\pm$ 0.3	3.9 $\pm$ 0.6	0.57
F1 + 2 (pmol/L)	69–229	124.7 $\pm$ 13.3	118.1 $\pm$ 9.4	0.72	122.2 $\pm$ 11.9	120.1 $\pm$ 11.2	0.93
TFPI (ng/mL)	13–44.9	75.4 $\pm$ 6.0	76.0 $\pm$ 6.4	0.92	71.0 $\pm$ 5.3	80.4 $\pm$ 6.8	0.12
INR		2.65 $\pm$ 0.17	2.32 $\pm$ 0.13	0.16	2.48 $\pm$ 0.17	2.49 $\pm$ 0.14	0.99
aPTT	0.7–1.2	1.46 $\pm$ 0.07	1.36 $\pm$ 0.05	0.16	1.43 $\pm$ 0.07	1.39 $\pm$ 0.05	0.56
Antithrombin	>80	81 $\pm$ 4	83 $\pm$ 3	0.14	80 $\pm$ 3	83 $\pm$ 4	0.13
Anti-FXa (UI/mL)		0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.56 ^a	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.88 ^a
Platelets (G/L)	150–400	204 $\pm$ 13	204 $\pm$ 11	0.97	200 $\pm$ 11	207 $\pm$ 13	0.52
CRP (mg/L)	<4	11.2 $\pm$ 2.9	8.4 $\pm$ 1.3	0.33	10.0 $\pm$ 2.9	9.6 $\pm$ 1.5	0.88
Protein (g/L)	35–50	70 $\pm$ 2	71 $\pm$ 1	0.81	70 $\pm$ 2	71 $\pm$ 2	0.81
Haemoglobin (g/dL)	13–18	11.5 $\pm$ 0.3	11.6 $\pm$ 0.3	0.83	11.6 $\pm$ 0.3	11.6 $\pm$ 0.3	0.93
Haematocrit (%)	40–54	36.0 $\pm$ 1.1	35.9 $\pm$ 0.9	0.86	35.8 $\pm$ 1.0	36.1 $\pm$ 1.0	0.61
Haematocrit H3A–H0 ^b (%)		1.39 $\pm$ 0.43	2.01 $\pm$ 0.39	0.03	1.72 $\pm$ 0.28	1.68 $\pm$ 0.52	0.89

^aSignificant interaction between the anticoagulation regimen and the membrane for the anti-FXa activity ($P = 0.03$).

^bH3A–H0: difference between the third hour in the arterial line and the beginning of the session.

Table 4. Dialysis parameters according to the four treatment groups

	FX100 + Enoxaparin	FX100 without anticoagulation	P value	EVODIAL + enoxaparin	EVODIAL without anticoagulation	P value
End-session body weight (kg)	90.5 $\pm$ 8.7	90.9 $\pm$ 9.1	0.77	90.9 $\pm$ 9.0	90.8 $\pm$ 8.8	0.68
Ultrafiltration (L)	2.43 $\pm$ 0.26	2.50 $\pm$ 0.35	0.90	2.52 $\pm$ 0.30	2.44 $\pm$ 0.25	0.84
Duration (hours)	4.18 $\pm$ 0.09	4.10 $\pm$ 0.67	0.70	4.13 $\pm$ 0.10	4.15 $\pm$ 0.11	1.00
Blood flow (mL/min)	400 $\pm$ 20	385 $\pm$ 15	0.86	383 $\pm$ 22	400 $\pm$ 15	0.86
EPO ($\times 1000$ UI/week)	7.03 $\pm$ 2.51	5.81 $\pm$ 1.7	0.19	6.03 $\pm$ 1.76	5.75 $\pm$ 1.72	0.33

All of the biological haemostatic parameters varied without significant statistical difference between the two anticoagulation groups, whether between the beginning and the third hour, or between the arterial and the venous line at the third hour (Table 5).

Dialyser effect: FX100 versus EVODIAL

Baseline haemostatic and haemodialysis parameters did not differ between the two groups except for a slightly higher haemoconcentration (haematocrit variation between H0 and H3A) in sessions with the HeprAN membrane (Tables 3 and 4).

At the third hour, the anti-FXa activity was similar in the two groups (0.12 UI/mL $\pm$ 0.01 with EVODIAL versus 0.13 $\pm$ 0.01 with FX100, $P = 0.69$).

Clotting scores in the dialyser and in the air trap were not statistically different between sessions performed with HeprAN or Helixone (Figure 1).

In contrast, the obtained Kt/V indexes, both measured and calculated, were significantly lower in sessions with the HeprAN membrane than with the PS membrane (respectively, 1.31 $\pm$ 0.07 and 1.43 $\pm$ 0.06 versus 1.47 $\pm$ 0.06 and 1.58 $\pm$ 0.07, $P = 0.03$ and 0.02, respectively) (Figure 2).

In sessions conducted with the HeprAN membrane, the concentration of platelet factor 4 (PF4) rose significantly more than that in the session with the PS membrane between H0 and H3A; moreover, the concentration of TAT complexes in the circuit rose significantly more (H3V–H3A) when using the

HeprAN membrane (Table 5). No significant difference was observed with regard to the other biological parameters.

DISCUSSION

The need for heparinization during the haemodialysis procedure in patients receiving long-term oral anticoagulation is still poorly defined. This prospective study demonstrates that in patients under adequate oral anticoagulation (mean INR 2.49 $\pm$ 0.14 [2.20–2.77]) and with minimal inflammation, heparin-free haemodialysis can be performed without increasing the risk of clotting in the extracorporeal circuit. Indeed, none of the 40 sessions ended prematurely and clotting scores were similar with or without enoxaparin. Moreover, delivered dialysis dose was not reduced without heparin anticoagulation and all markers of haemostatic activation varied equally with or without additional enoxaparin.

Our results differ from those of Ziai *et al.* [10] which did not favour the use of haemodialysis without additional anticoagulation. In 10 haemodialysis patients on oral anticoagulation, and among the 30 haemodialysis sessions without additional anticoagulation, these authors found a significantly higher coagulation activation (D-dimer variation and clotting) than that observed during the 30 sessions performed with LMWH. Moreover, a complete circuit coagulation occurred during a session performed without LMWH. However, weekly Kt/V indexes were similar in both groups.

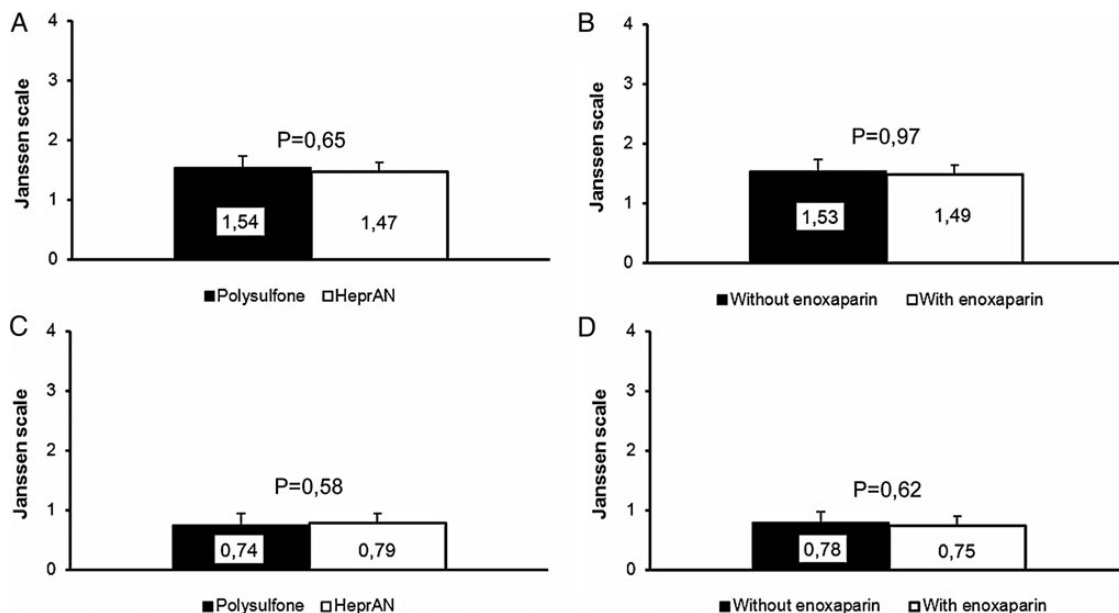


FIGURE 1: Clotting in the extracorporeal circuit (Janssen scale) according to the anticoagulation regimen and membrane used. (A and B) Clotting in the dialyser. (C and D) Clotting in the bubble trap. Results are expressed as mean $\pm$ SD.

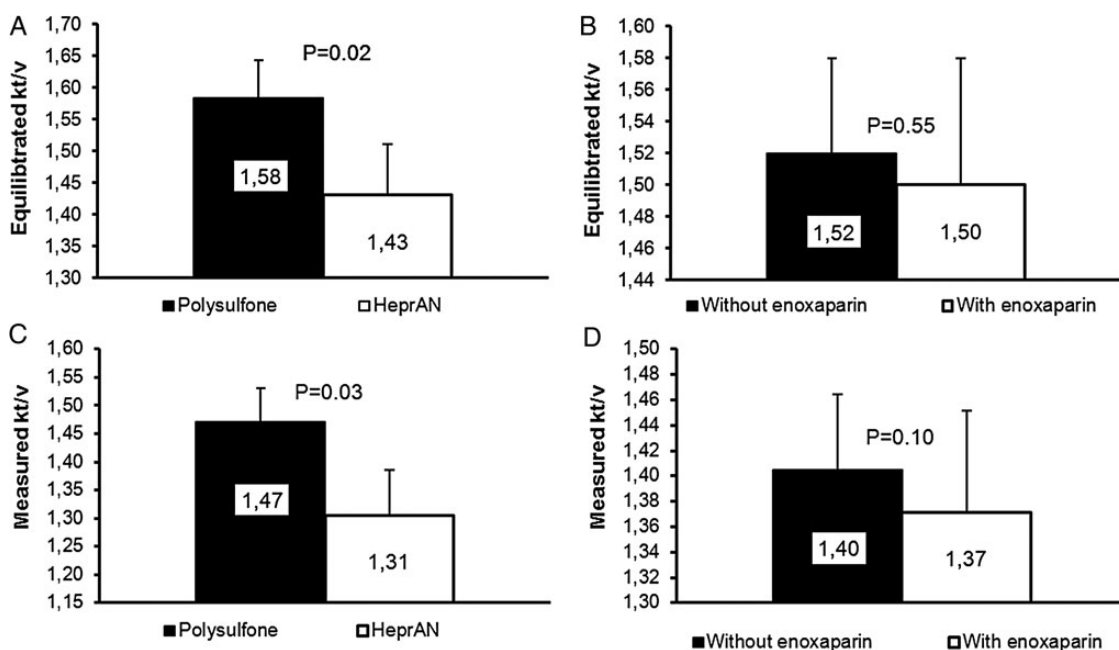


FIGURE 2: Dialysis dose delivered. (A) and (B) are equilibrated Kt/V; (C) and (D) are measured Kt/V; (B) and (D) are results according to the anticoagulation regimen and (A and C) according to the membrane used. Results are expressed as mean $\pm$ SD.

Several reasons could explain these discordant results between our study and those of Ziai *et al.*: first, the better results with LMWH could have been linked to a higher LMWH dosage, dalteparin 40 UI/kg, while in the present study, the dose was fixed at 2000 UI enoxaparin (i.e. 17–31 UI/kg). This different anticoagulation intensity resulted in a higher anti-FXa activity in the Ziai study during the sessions with LMWH [0.33 (0.27–0.38) at the second hour and 0.16 (0.03–0.23) at the fourth hour versus 0.17 (0.15–0.20) at the third hour]. Secondly, the number of

sessions was higher in the Ziai study which may have increased the statistical power and differences between groups. Thirdly, the variation in D-dimers in the Ziai study was measured at the fourth hour of dialysis versus the third hour in this study. Fourthly, in the present study, mean blood flows were higher at 250–450 versus 250–300 mL/min. Lastly, the Ziai study used low permeability membranes (PS, cuprophane, polyarylsulphone) that are potentially more prone to activate the coagulation cascade. Low blood flow and low permeability membranes are

Table 5. Evolution of the biological markers of coagulation activation, according to membrane type and anticoagulation regimen

	FX100	EVODIAL	P value	Enoxaparin	No anticoagulation	P value
H3A-H0^a						
ΔD-dimer	118 ± 158	89 ± 29	0.84	23 ± 27	183 ± 156	0.27
ΔPF4	−6 ± 13	20 ± 17	0.02	3.2 ± 13.8	9.9 ± 17	0.67
ΔTAT	1.9 ± 0.7	4.5 ± 1.5	0.06	3.1 ± 1.2	3.3 ± 1.1	0.74
ΔF1 + 2	53 ± 26	140 ± 60	0.06	74 ± 42	121 ± 51	0.30
ΔTFPI	1.0 ± 7.4	0.04 ± 5.3	0.92	1.6 ± 5.0	−0.5 ± 7.7	0.83
H3V-H3A^b						
ΔD-dimer	−13 ± 76.5	23 ± 20.0	0.85	18 ± 24	−8 ± 75	0.76
ΔPF4	10.2 ± 6.5	6.1 ± 17.6	0.67	10.7 ± 5.1	5.1 ± 19.3	0.70
ΔTAT	1.4 ± 1.0	6.2 ± 1.9	0.01	2.8 ± 1.6	4.8 ± 1.6	0.37
ΔF1 + 2	17.1 ± 14.8	57 ± 24	0.10	23.9 ± 17.3	44.3 ± 14.4	0.55
ΔTFPI	21.6 ± 4.3	14.0 ± 3.8	0.25	21.3 ± 3.3	14.4 ± 4.7	0.29

^aH3A-H0: difference between the third hour in the arterial line and predialysis values.^bH3V-H3A: difference between the third hour value in the venous line and in the arterial line.

two potent risk factors for clotting during dialysis and could explain the better results observed in the present study during the sessions performed without heparin [1, 16]. Kodras *et al.* have already suggested that heparin-free dialysis is feasible in patients with oral anticoagulation under certain circumstances (INR in the target range, no inflammation process, vascular access with adequate blood flow and native AVF) [11, 17]. Nevertheless, in their study comparing FX100 and AN69ST dialysers with priming of the dialyser and no systemic heparinization, 4 of 60 sessions ended prematurely because of complete clotting. This occurred in patients with low INR, high basal D-dimers and/or with antiphospholipid syndrome. Our study enrolled similarly high-risk patients, but yet we did not observe any complete coagulation. Again, a fundamental difference between the present study and the Kodras study was blood flow, which was as low as in the Ziai *et al.* study. These results reinforce the importance of higher blood flow to prevent blood stasis and coagulation activation.

Measuring plasma components of the coagulation cascade may represent a more sensitive approach to quantify coagulation activation in the extracorporeal circuit, and thus we measured the generation of D-dimers, TAT, prothrombin fragments 1 and 2 (F1 + 2), PF4 and tissue factor pathway inhibitor (TFPI) in all of the sessions. With regard to the baseline values of these parameters, we found elevated values for D-dimers, PF4 and TFPI. In the literature, TFPI, F1 + 2, TAT and D-dimers have been shown to be elevated in end-stage renal disease patients, regardless of the type of treatment, i.e. haemodialysis or peritoneal dialysis [2, 18, 19]. However, plasma PF4 values are not modified during chronic kidney insufficiency, even at the end-stage phase [19]. Vitamin K antagonists (VKA) also have an effect on these parameters, especially on D-dimers, TAT and F1 + 2, which are reduced, whereas PF4 is not modified [20–22]. In haemodialysed patients, VKA reduce predialysis F1 + 2 but not TAT [23]. To our knowledge, the effects of VKA on TFPI plasma values have not been reported so far.

D-dimers are products of fibrin hydrolysis and are a general marker for thrombus formation. Their variation during haemodialysis could be a more sensitive marker of coagulation activation and were also measured in both the Ziai and Kodras

studies [10, 11]. In the present study, a slight increase was observed during the sessions but without any difference between sessions with or without additional anticoagulation.

Conversion of prothrombin into active thrombin is a major event in the final stage of the coagulation activation cascade and thrombin generation can be evaluated by the plasma concentration of TAT and F1 + 2. These markers have been used in a few previous studies to evaluate coagulation activation in haemodialysis, with TAT complexes being acknowledged as one of the most reliable markers because of their short half-life (<20 min), thus are more specific of a recent activation of coagulation [1, 24, 25]. However, the concentrations of TAT and F1 + 2 are decreased in dialysis patients treated with oral anticoagulant, although they remain correlated with clotting [23]. In the present study, the absence of significant difference between the two haemodialysis modalities is suggestive of a similar pattern in thrombin generation with or without low-dose LMWH.

Platelet interaction in the extracorporeal circuit results in adhesion and retention of platelets on the artificial membrane and subsequently the release of platelet-derived growth factors. Platelet degranulation is indicated by the release of intracellular products, such as PF4 from α-granules [26]. However, PF4 can also be released *in vivo* after heparin administration by release of its bound form from the endothelium [27]. In spite of the presence of LMWH in one of the groups, the absence of significant difference can be explained by the relatively low-dose LMWH used in our study, but also by the late timing of measurement when compared with the early release of PF4 by heparin. Indeed, in the studies of Schoorl *et al.* [28] and Gritters *et al.* [26], the concentrations of PF4 practically returned to normal after 150 min of dialysis, whereas the doses of LMWH were even more important than those in the present study [26, 28]. Thus, the lack of significant difference in the PF4 concentration between the groups with and without LMWH suggests a lack of platelet overactivation.

TFPI is released from platelets and is a marker of coagulation activation which raises particular interest as a major inhibitor of the dissemination of coagulation. However, TFPI is released from the endothelium in the presence of heparin,

hence its concentration is difficult to interpret, especially in haemodialysis. Consequently, its use in clinical studies is still confidential and there are no data reported to date in haemodialysed patients treated with oral anticoagulant [29–31]. The absence of significant difference between the two groups herein is likely explained by the relatively low-dose LMWH used in our study, but also by the late timing of measurement when compared with the early release of TFPI induced by heparin. Indeed, in the studies by Zemanova *et al.*, the concentrations of TFPI almost returned to normal 30 min after heparin cessation [26, 28]. Thus, the lack of significant difference in the TFPI concentration between the groups with and without LMWH suggests the absence of both coagulation activation and platelet overactivation.

When comparing the two membranes, HeprAN and PS, we noted no difference in clotting scores despite the presence of grafted heparin on the HeprAN membrane. In contrast, the delivered dialysis dose was significantly lower in sessions with the HeprAN membrane. These results were unexpected and in contradiction with those observed in a study comparing HeprAN with a PS membrane which found a similar Kt/V index between two membranes during sessions performed with LMWH in patients without oral anticoagulant [12]. Our results are also in contradiction with those of Kodras *et al.* [11] which found similar weekly Kt/V between AN69ST and Helixone in patients with oral anticoagulation. The blood flows and the duration of the sessions were exactly the same and could not explain this difference in dialysis efficacy; however, the surface of the HeprAN membrane used was very slightly smaller (2.15 versus 2.2 m²).

The only significant differences in haemostasis markers between these two membranes in the present study were a greater rise in TAT and PF4 in sessions with the HeprAN membrane, likely explained by the presence of the heparin coat on its surface. Thus, this marker may not be the most pertinent parameter when evaluating a heparin-coated membrane, a fact which underscores the importance of a multiparameter approach in the assessment of coagulation. On the other hand, the increase in PF4 observed during the sessions with HeprAN could not be merely explained by its release from the endothelium since heparin is coated on the membrane, thus suggesting greater platelet activation as a specific effect of this membrane. Of note, the HeprAN membrane was associated with a slightly but significantly higher haemoconcentration which may have partly affected some of the results regarding haemostatic markers. This higher haemoconcentration was not explained by the ultrafiltration volume which was not significantly higher (2.48 versus 2.46 L, *P* = 0.84). Similar results regarding D-dimers and clotting scores were also found in the study of Kodras *et al.* [11] which compared the Helixone membrane and the AN69ST membrane (from which the HeprAN membrane is derived) in patients with oral anticoagulant. Another study comparing these two membranes in patients without oral anticoagulant found a similar thrombogenicity (TAT, PF4, TFPI) during haemodialysis sessions with UH [29].

The main limitation of the present study is the relatively small number of included patients, a fact compensated by the

double crossover design and the careful and objective definition of the end points. Moreover, both clinical and biological end points were concordant in our results. However, as the conclusions were inferred from a small sample of dialysis patients, any extrapolation to the entire population of haemodialysis patients receiving oral anticoagulation should be made with caution.

In conclusion, haemodialysis in patients treated with oral anticoagulant is feasible and effective without additional heparin in some if not all patients provided that certain conditions are met: INR within the target range, minimal inflammation, native vascular access with adequate blood flow rate and use of synthetic high flux membranes.

When compared with a synthetic high permeability membrane, the novel HeprAN membrane with grafted heparin did not yield significant advantages in this indication both in terms of coagulation activation and efficiency of dialysis.

These results are relevant to everyday practice since up to 25% of dialysis patients are treated by oral anticoagulant and therefore exposed to the excess risk of bleeding with undue additional heparin.

ACKNOWLEDGEMENTS

We are thankful to the nursing staff for their invaluable help in conducting this study.

CONFLICT OF INTEREST STATEMENT

None of the authors declared any personal conflict of interest in this study. Costs for TFPI measurements were supported by Gambro Inc.

REFERENCES

1. Lindhout T. Biocompatibility of extracorporeal blood treatment. Selection of haemostatic parameters. *Nephrol Dial Transplant* 1994; 9: 83–89
2. Ambuhl PM, Wuthrich RP, Korte W *et al.* Plasma hypercoagulability in haemodialysis patients: impact of dialysis and anticoagulation. *Nephrol Dial Transplant* 1997; 12: 2355–2364
3. European Best Practice Guidelines Expert Group on Hemodialysis ERA. Section V. Chronic intermittent haemodialysis and prevention of clotting in the extracorporeal system. *Nephrol Dial Transplant* 2002; 17: 63–71
4. Noris M, Remuzzi G. Uremic bleeding: closing the circle after 30 years of controversies? *Blood* 1999; 94: 2569–2574
5. Sagedal S, Hartmann A, Sundstrom K *et al.* A single dose of dalteparin effectively prevents clotting during haemodialysis. *Nephrol Dial Transplant* 1999; 14: 1943–1947
6. Fiaccadori E, Maggiore U, Regolisti G. Balancing thromboembolic risk against vitamin K antagonist-related bleeding and accelerated calcification: is Fondaparinux the holy grail for end-stage renal disease patients with atrial fibrillation? *Nephrol Dial Transplant* 2013; 28: 2923–2928
7. Ishani A, Herzog CA, Collins AJ *et al.* Cardiac medications and their association with cardiovascular events in incident dialysis patients: cause or effect? *Kidney Int* 2004; 65: 1017–1025

8. Kruger T, Brandenburg V, Schlieper G *et al.* Sailing between Scylla and Charybdis: oral long-term anticoagulation in dialysis patients. *Nephrol Dial Transplant* 2013; 28: 534–541
9. Sood MM, Komenda P, Sood AR *et al.* The intersection of risk and benefit: is warfarin anticoagulation suitable for atrial fibrillation in patients on hemodialysis? *Chest* 2009; 136: 1128–1133
10. Ziai F, Benesch T, Kodras K *et al.* The effect of oral anticoagulation on clotting during hemodialysis. *Kidney Int* 2005; 68: 862–866
11. Kodras K, Benesch T, Neumann I *et al.* Comparison of two dialysers (AN69ST vs. FX100) for heparin-free dialysis in patients with oral anticoagulation. *Blood Purif* 2008; 26: 226–230
12. Morena M, Jaussent I, Chalabi L *et al.* Biocompatibility of heparin-grafted hemodialysis membranes: impact on monocyte chemoattractant protein-1 circulating level and oxidative status. *Hemodial Int* 2010; 14: 403–410
13. Daugirdas JT. Simplified equations for monitoring Kt/V, PCRn, eKt/V, and ePCRn. *Adv Ren Replace Ther* 1995; 2: 295–304
14. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *J Am Soc Nephrol* 1993; 4: 1205–1213
15. Janssen MJ, Deegens JK, Kapinga TH *et al.* Citrate compared to low molecular weight heparin anticoagulation in chronic hemodialysis patients. *Kidney Int* 1996; 49: 806–813
16. Ward RA. Effects of haemodialysis on coagulation and platelets: are we measuring membrane biocompatibility? *Nephrol Dial Transplant* 1995; 10 (Suppl 10): 12–17
17. European Best Practice Guidelines Expert Group on Hemodialysis ERA. Section II: Haemodialysis adequacy. *Nephrol Dial Transplant* 2002; 17: 17–31
18. Adams MJ, Irish AB, Watts GF *et al.* Hypercoagulability in chronic kidney disease is associated with coagulation activation but not endothelial function. *Thromb Res* 2008; 123: 374–380
19. Sagripanti A, Cupisti A, Baicchi U *et al.* Plasma parameters of the pro-thrombotic state in chronic uremia. *Nephron* 1993; 63: 273–278
20. Eritsland J, Seljeflot I, Arnesen H *et al.* Effects of long-term treatment with warfarin on fibrinogen, FPA, TAT, and D-dimer in patients with coronary artery disease. *Thromb Res* 1992; 66: 55–60
21. Jafri SM, Mammen EF, Masura J *et al.* Effects of warfarin on markers of hypercoagulability in patients with heart failure. *Am Heart J* 1997; 134: 27–36
22. Nozawa T, Inoue H, Iwasa A *et al.* Effects of anticoagulation intensity on hemostatic markers in patients with non-valvular atrial fibrillation. *Circ J* 2004; 68: 29–34
23. Sagedal S, Hartmann A, Sundstrom K *et al.* Anticoagulation intensity sufficient for haemodialysis does not prevent activation of coagulation and platelets. *Nephrol Dial Transplant* 2001; 16: 987–993
24. Opatrny K, Jr, Bouda M, Kohoutkova L *et al.* A clinical study to assess the effect of heparin in dialyzer rinsing solutions. *Int J Artif Organs* 1997; 20: 112–118
25. Lane DA, Bowry SK. The scientific basis for selection of measures of thrombogenicity. *Nephrol Dial Transplant* 1994; 9(Suppl 2): 18–28
26. Gritters M, Borgdorff P, Grooteman MP *et al.* Platelet activation in clinical haemodialysis: LMWH as a major contributor to bio-incompatibility? *Nephrol Dial Transplant* 2008; 23: 2911–2917
27. Dawes J, Pumphrey CW, McLaren KM *et al.* The in vivo release of human platelet factor 4 by heparin. *Thromb Res* 1982; 27: 65–76
28. Schoorl M, Nube MJ, Bartels PC. Platelet depletion, platelet activation and coagulation during treatment with hemodialysis. *Scand J Clin Lab Invest* 2011; 71: 240–247
29. Zemanova P, Opatrny K, Vit L *et al.* Tissue factor, its inhibitor, and the thrombogenicity of two new synthetic membranes. *Artif Organs* 2005; 29: 651–657
30. Cella G, Vertolli U, Naso A *et al.* Tissue factor pathway inhibitor (TFPI) activity in uremic patients during hemodialysis. *Thromb Res* 1996; 81: 671–677
31. Naumnik B, Rydzewska-Rosolowska A, Mysliwiec M. Different effects of enoxaparin, nadroparin, and dalteparin on plasma TFPI during hemodialysis: a prospective crossover randomized study. *Clin Appl Thromb Hemost* 2010; 17: 480–486

Received for publication: 10.10.2013; Accepted in revised form: 10.12.2013