# Platelet activation and clotting cascade activation by dialyzers designed for high volume on-line haemodiafiltration

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Abstract

Introduction

Haemodialysis (HD) patients are pro-thrombotic. Higher volume on-line post-dilutional haemodiafiltration (OL-HDF), with increasing haematocrit increases the risk of clotting in the extracorporeal circuit (ECC). We wished to determine whether OL-HDF increased platelet activation and ECC clotting.

Methods

Coagulation parameters, platelet, white cell and endothelial activation markers were measured at the start and end of dialysis sessions in 10 patients and also pre- and post-dialyzer after 15 minutes using two different dialyzers designed for high volume OL-HDF; cellulose triacetate (TAGP) and polysulphone (PS) and polyvinylpyrrolidone (PVP). Patients were anticoagulated with a heparin bolus.

Findings

At the start of OL-HDF, D dimers, thrombin anti-thrombin complexes (TATs), and soluble adhesions molecules (sICAM-1 and sVCAM-1) were increased. Post treatment soluble P selectin (PS/PVP 26.7±7.1 vs 36.6±9.9; TAGP 28.7±7.2 vs 43.5±8.4 ng/mL, $p<0.001$), and soluble CD40 ligand (PS/PVP 297±228 vs 552±272, TAGP 245±187 vs 390±205 ng/mL, $p<0.05$) increased. Post-dialyzer concentrations increased vs pre-dialyzer for tissue factor (PS/PVP 117±12 vs 136±16, TAGP 100±25 vs 128±40 ng/mL, $p<0.05$), factor VIIIc (PS/PVP 174±54 vs 237±83, TAGP 163±60 vs 247±102 IU/mL, $p<0.01$), sVCAM-1 (PS/PVP 782±64 vs 918±140, TAGP 722±121 vs 889±168 ng/mL, $p<0.01$), and D-Dimers (PS/PVP 292±132 vs 355±167, TAGP 300±129 vs 391±171 ng/mL, $p<0.001$). There was no macroscopic thrombus noted in the ECC, and no increase in microparticles, platelet factor-4, or TATs.

Discussion

Despite being pro-thrombotic, with activation of platelets, and lymphocytes during passage through ECC, no macroscopic clotting, or increased TATs were noted during OL-HDF, and no major differences between cellulosic and polysulphone dialyzers.

## Introduction

Although patients with chronic kidney disease stage 5 who are manged conservatively without dialysis have an increased bleeding time due to a functional platelet defect [1], patients with both acute kidney injury and haemodialysis patients are pro-thrombotic [2,3], with increased risk of clotting in the extra-corporeal circuit [4]. As such, circuit clotting was a major limitation of the early haemodialysis circuits, and it was only overcome with the introduction of heparin [5]. Over time, with the technical advances in circuit and dialyzer design, manufacturing processes to improve internal surface smoothness of dialyzer fibres and blood lines, coupled with the development of bio-incompatible polymers that the risk of extracorporeal circuit clotting has declined to now be a relatively unusual complication of outpatient dialysis sessions. Indeed, the amounts of heparin now administered are much less than those 20 years or so ago [6].

Traditionally haemodialysis treatments have centred on the clearance of urea by diffusion. As such capillary dialyzers, have been designed to have fibres with narrow internal diameters to minimise the distance required for solutes to diffuse. Over the last decade there has been an increased interest in adding convective clearance to standard diffusion, to increase middle sized solute clearances [7]. More recently studies have reported that increasing the amount of convective loss, termed high volume on-line haemodiafiltration is associated with decreased patient mortality [8,9]. However, increasing convective volume exchange in patients with acute kidney injury increases the risk of circuit clotting [10], as the

greater the filtration fraction then the greater the increase in haemoconcentration and protein concentrations as blood flows through the capillary dialyzer [11]. This leads to an increase in pressure within the dialyzer, which will either lead to activation of pressure alarms and so stopping convection, or potentially rupture of the dialyzer casing.

To allow for the delivery of higher convective volume exchanges, a newer generation of dialyzers, designed to have wider diameter capillary fibres have been developed. However, this potentially allows a greater change in hydrostatic pressure to occur as blood passes through the dialyzer and changes in pressure and flow patterns may potentially lead to increased platelet and leukocyte activation and increase the risk of clotting in the extracorporeal circuit. To investigate whether haemodiafiltration is associated with increased risk of clotting we measured markers of platelet activation and activation of the clotting cascade during haemodiafiltration with two dialyzers of different fibre composition.

**Patients and methods**

10 patients attending for thrice weekly outpatient haemodialysis, who had been dialyzing with polyethersulphone and polyvinylpyrrolidone (PVP) dialyzers (ELISIO-21H, Nipro Corp., Osaka, Japan) were enrolled into the study. Patients were then switched to either dialysing with a polysulphone (PS) and PVP dialyzer (Fresenius FX100, Fresenius GMB, Bad Homburg, Germany) or an asymmetric triacetate (ATA) (SOLACEA-21H, Nipro Corp., Osaka, Japan) (table 1). Patients dialysed for a minimum of three dialysis sessions with each dialyzer before they were studied, followed by a washout period returning to dialysing with the ELISIO dialyzer for a minimum of one week, before starting with the other trial dialyzer. All patients were studied during the same weekly dialysis session.

All 10 patients dialysed using arteriovenous fistulae. Dialysis schedules were kept constant in terms of dialysate composition and dialysate flow dialysis machine (BBraun,

Dialogue$^{+}$, BBraun, Melsungen, Germany). (table 2). Constant ultrafiltration rate profiles were used in all cases, and patients dialysed supine in beds. Haemoglobin was measured pre- and post-dialysis (XE-2100 Sysmex Corporation, Kobe, Japan) [12], and serum albumin samples analysed by the bromocresol green method (Roche Integra, Roche diagnostics, Lewes, UK) and total protein by a modified biuret method [13].

All patients were anticoagulated with low molecular weight heparin (tinzaparin, Leo Laboratories, Copenhagen, Denmark), administered into the venous limb of the circuit as a single bolus [14]. No patient was systemically anticoagulated with coumarins, oral anti-Xa or thrombin inhibitors or treated with prophylactic or treatment doses of heparin. Six patients were prescribed 75 mg aspirin and two prescribed 75 mg clopidogrel daily.

Both the dialysers and venous air detector chambers were observed during and at the end of the dialysis session for presence of clot formation, and assessed by a visual analogue scale.

Routine Blood sampling was taken via the arterial blood line following discard of first 10 mL at the start of dialysis and from the venous line at the end of the session. In addition, samples to investigate activation of clotting were also taken pre- and post-dialyzer after 15 minutes, and pre-dialyzer after 60 minutes into 0.109 mol/L$^{-1}$ sodium citrate vacutainers and a 4.5 mL CTAD vacutainer (sodium citrate, citric acid, theophylline, adenosine and dipyridamole and iloprost) (both from Becton Dickinson, Oxford, UK) to prevent platelet activation.

To assess activation of the coagulation pathways we measure tissue factor (TF) and Factor VIIIc, and for thrombus formation and degradation we measured thrombin-antithrombin complexes (TATs), fibrinogen and D-dimers. To determine platelet activation, we measured platelet factor 4 (PF4), plasma microparticles, soluble P selectin, and soluble CD40 ligand, which is expressed and shed by activated T cells activating platelets. To

estimate activation of leukocytes we measured soluble E selectin and vascular cell adhesion molecule 1 (sVCAM-1) as endothelial derived leukocyte adhesion and activation molecules, and soluble intercellular adhesion molecule 1 (sICAM-1) as a marker of monocyte activation.

All plasma samples were prepared by double centrifugation (12 minutes each spin) at 2000 g, separated, aliquoted and frozen at -85$^{o}$C until testing. In addition to standard coagulation screen prothrombin time (PT) and activated partial thromboplastin time (aPTT), fibrinogen was measured using the Clauss method, d-Dimers (ng/mL) using HemosIL D-Dimer kit and factor (F) VIII:C levels were measured by one stage clotting factor assay on an ACLTOP (all Instrumentation Laboratory (IL), Bedford, MA, USA), TAT complexes (Abcam, Cambridge, UK). The CTAD plasma samples were used for the measurement of plasma platelet activation markers PF4 by ELISA (Zymutest, Hyphen Biomed, Neuville-sur-Oise, France) and soluble (s) sPselectin (R&D systems Bio-Techne Ltd, Abingdon, UK). Plasma levels of the following adhesion molecules were determined by ELISA: soluble (s) CD40 ligand (R&D Systems), sICAM-1, sVCAM-1 (R&D Systems). Tissue factor was measured using the Actichrome TF assay (R&D Systems). Plasma microparticles were measured using the Zymuphen MP-Activity assay (Hyphen Biomed) in platelet poor plasma (PPP) [2,15].

Ethical approval was granted by London-Westminster Ethics Committee (15/LO/0102) and the trial registered NCT02546037. All patients provided informed written consent in keeping with the principles of the declaration of Helsinki.

Statistics

Results are expressed as mean ± standard deviation, or median and interquartile range, or percentage. Statistical analysis was by students' paired t test for parametric data and by the

Wilcoxon rank sum pair test for nonparametric data, and by ANOVA or Kuskal Wallis, with appropriate ad hoc correction for multiple testing (Graph Pad Prism version 6.0, Graph Pad, San Diego, CA, USA, and SPSS version 24.0, Univ Chicago, Illinois, USA). Statistical significance was taken at or below the 5% level.

**Results**

We studied 10 patients, 70% male, mean age 74.5±8.8 years, with a median dialysis vintage of 42.9 months (27.1-63.5). The underlying renal diseases included secondary to diabetes in 3, reno-vascular disease in 2, one case each for myeloma (stable paraprotein < 1 g/L), membranous glomerulonephritis, polycystic kidney disease, and 2 cases of undetermined aetiology. There were no differences in dialysis session times, blood flows or convection volumes (table 2). Although the urea clearances obtained with the PS and PVP dialyzer were marginally greater than those for the ATA dialyzer, these differences were not significant. However, post-haemodiafiltration β2 microglobulin was lower with the PS and PVP dialyzer; 5.7±1.2 vs 8.0±2.2 mg/L after correction for the change in haematocrit, with a 81.3±4.5 vs 73.8±6.0 % reduction in serum concentrations, $p<0.05$ (table 3).

Apart from the increase in haematocrit due to net ultrafiltration, there were no significant changes in peripheral white blood cell or platelet counts comparing pre- and post-haemodiafiltration samples. Visual inspection of the dialyzers at the end of the treatment session did not show any significant clotting in the dialyzer headers. Similarly, there was none or < 10% clotting in the venous air-detector chamber. The mean PT remained within normal limits (< 14 seconds) during treatment with both dialyzers. The aPTT was normal at the start of haemodiafiltration, then increased due to the effect of heparin, and returned to

normal at the end of the session. APTT results were not different pre- vs post-dialyzer after 15 minutes, suggesting no significant heparin loss during passage through the dialyzer.

Thrombin-antithrombin complexes (TATs) did not increase during haemodiafiltration treatments (table 4). However, 15 minutes into the dialysis session factor VIII:C, Clauss fibrinogen, tissue factor (TF) and D-dimers were all greater post-dialyzer compared to pre-dialyzer sampling. In addition, both sICAM-1 and sVCAM-1 were also greater post- vs pre-dialyzer after 15 minutes, as was sEselectin. Whereas microparticles and platelet factor 4 (PF4) did not increase with haemodiafiltration, both sPselectin and soluble CD40 ligand (sCD40L) were greater at the end of the haemodiafiltration session compared to the start. Factor VIIIc was also higher at the end of the session with the PS and PVP dialyzer ($p=0.034$, adjusted for multiple testing).

As the coagulation results were similar for the two dialyzers, we combined data and compared the effects of taking anti-platelet agents (aspirin and clopidogrel). We found no demonstrable differences between dialysis sessions in those prescribed and not prescribed anti-platelet agents (supplementary table).

## Discussion

The introduction of cellulosic dialyzers was a major advance in the history of haemodialysis [5]. However, it was soon realised that these dialyzers activated complement, platelets, and leukocytes. This led to the development of modified cellulosic dialyzers, reducing net negative charge, with the introduction of cellulose diacetate and triacetate (CTA) membranes, and the manufacture of synthetic membranes designed to improve haemocompatibility [16]. These newer dialyzer membranes, and changes in dialyzer sterilisation processes, reduced reactions to haemodialysis [17], and as such further developments in dialyzer design were aimed at increasing small solute urea clearances [18].

However clinical trials designed to investigate the effect of greater urea clearance did not show any benefit in terms of patient survival, and despite the technical advances in dialysis technology 5-year patient survival remained lower than that for some of the more common solid organ cancers [19]. Attention then turned to increasing clearance of larger sized azotaemic retention products of metabolism by introducing haemodiafiltration [7]. Once again, the initial studies did not show any survival advantage, but more recently trials of high volume on-line haemodiafiltration [9], in terms of absolute volumes, and also when adjusted for body size [8] showed improved patient survival compared to low flux and high flux haemodialysis. However, to achieve these higher convective clearance targets dialyzers need to be modified to achieve higher hydraulic permeability, without excessive haemoconcentration. Proteins are deposited on the dialyzer membrane during dialysis, including the serine proteases factor XII, factor X1, and fibrinogen. As such high volume haemodialfiltration could potentially increase clotting within the dialyzer capillary fibres, and so reduce volume exchanges.

To investigate the effects of haemodialfiltration on clotting in the extracorporeal circuit we chose to study two dialyzers designed for to increase convective clearances, one with a modified asymmetric triacetate membrane (ATA) and one synthetic membrane (PS and PVP). We did not observe any significant clotting in the extracorporeal circuit, in terms of external examination of the dialyzer header and venous air detector, and standard pressure measurements of the extracorporeal circuit were not different between the dialyzers. There was no difference in small solute, urea clearances when using the two dialyzers, and no difference in convective exchange. Although there were no differences in the absolute pre- and post-haemodiafiltration β2 microglobulin concentrations, the relative reduction was greater with the PS and PVP dialyzers. This small difference may potentially be due to the higher sieving coefficient and larger membrane surface area, although as we only made blood

side measurements cannot exclude any membrane adsorption [20]. All patients were anticoagulated with a single heparin bolus, administered into the venous limb of the circuit at the start of treatment [14]. There was no change in prothrombin times, the aPTT increased, but there was no difference in samples taken pre- and post-dialyzer, suggesting no significant loss during dialyzer passage. To test for activation of the indirect clotting pathway we measured factor VIIIc and tissue factor for direct clotting pathway activation. Previous reports have demonstrated increased factor VIII with older generation cellulosic membranes [21]. However, we found that factor VIIIc concentrations initially fell with both dialyzers, but were increased on passage through the dialyzer. Thereafter they were stable, but factor VIIIc was greater at the end of the session with the PS and PVP dialyzer. Tissue factor concentrations were increased at the start of treatment, and the post-dialyzer concentrations were increased compared to in-let concentrations, but thereafter did not increase with treatment. Other studies have demonstrated that adhesion of cells, including leukocytes to the dialyzer membrane surface, leads to the release of tissue factor [22]. Fibrinogen concentrations similarly increased post-dialyzer compared to in-let, but did not change over time. To study whether these changes in clotting cascade proteins lead to clot formation we measured D-Dimers and TATs. We found that D-Dimers increased in the dialyzer outlet samples with both dialyzers, in keeping with previous reports using PS dialyzers [23], but there was no significant increase in TATs post-dialyzer or increase with treatment duration. These results demonstrate that clot formation was minimal, as earlier studies have reported increased TATs within minutes of starting haemodialysis [24].

More recently the role of activation of the contact coagulation pathway in initiating clotting in the extracorporeal circuit has been questioned [25], with increased attention to platelet activation [26], and the generation of microparticles [27]. Whereas previous studies have reported increased microparticle generation [28], we found that with these dialyzers

designed for high volume haemodiafiltration there were no significant increases in microparticles. Similarly, PF4 was not increased post-treatment. However, we did observe an increase in the post-dialyzer sVCAM-1, sICAM-1, and sEselectin compared to the in-let samples, and post-treatment sCD40L and sPselectin concentrations were increased. Although sEselectin and sVCAM-1 are released from activated endothelial cells, previous reports from dialyzer studies have reported release of endothelial factors during dialysis [21]. sVCAM-1 concentrations at the end of the dialysis sessions were higher with the PS and PVP dialyzer. These soluble adhesion molecules activate lymphocytes and platelets, and both sCD40L which is released from activated T lymphocytes, and sPselectin which is released from activated platelets, increased at the end of dialysis session with both dialyzers. As such our findings are in agreement with previous studies [29].

The majority of our patients were prescribed aspirin or clopidogrel at a standard dose of 75 mg. We did not find any difference in coagulation cascade activation, or activation markers of platelets, leukocytes or monocytes in those prescribed anti-platelet agents. However, patients were prescribed a standard dose without any formal platelet mapping studies, and as such it is unclear as to how many of our patients received an effective therapeutic dose.

Although we have demonstrated increased platelet and leukocyte activation during passage through the dialyzer, with increased production of tissue factor and D dimers with both dialyzers. We performed post-dilution haemodialfiltration, and previous studies have suggested that pre-dilution may cause less clotting in extracorporeal circuits, although potential increased loss of low molecular weight heparins may limit any advantage [30]. Even so, we did not note any macroscopic evidence of clotting in the extra-corporeal clotting. More recently there have been reports suggesting that achieving higher convective exchanges is associated with greater patient survival [31,32], although in part this may relate to more

reliable vascular access and patient factors [33]. The convective volume exchanged achieved in our study was similar to the average reported from the recent trials of haemodiafiltration [32], but was not specifically targeted to what would now be considered to be high volume exchange. As such high volume exchange with greater intra-dialyzer pressures could lead to greater activation of platelets, leukocytes with increased potential for clotting.

Earlier studies reported that dialyzer membranes can activate complement; PS dialyzers by both the alternative and lectin pathways, whereas cellulosic dialyzers activate the classic pathway [34]. As complement activation can increase microparticle production and clotting [27], then, this could potentially have led to differences between the two different dialyzers. However, more recent studies using modern day dialyzers reported minimal complement activation [28], and we did not find any clinically significant differences between the two dialyzers, in terms of small solute clearances or changes in β2-microglobulin concentrations. One previous study has suggested less platelet activation with haemodialfiltration compared to standard haemodialysis [35], and our study would suggest that dialyzer membranes, made from asymmetric triacetate (ATA) and PS and PVP, designed for high volume on-line haemodiafiltration, allow the achievement of high volume exchanges without an increased risk of extracorporeal circuit clotting.

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Table 1. Dialyzer membrane characteristics

| Dialyzer | FX-100 | SOLACEA-21H |
|---|---|---|
| Fibre composition | polysulphone/polyvinylpyrrolidone (PS/PVP) | asymmetric triacetate(ATA) |
| sterilisation | in-line steam | dry gamma irradiation |
| effective surface area | 2.2 $m^2$ | 2.1 $m^2$ |
| Wall thickness | 35 µm | 25 µm |
| Internal diameter | 185 µm | 200 µm |
| Effective length | | 254 mm |
| Priming volume | 116 mL | 118 mL |
| Ultrafiltration coefficient | 73 mL/h.mmHg | 76 mL/h.mmHg |

Table 2. Dialysis session variables when using FX100 and SOLACEA-21H dialyzers. Results expressed as mean ±SD, or median (interquartile range). There were no statistical differences between treatments using the two different dialysers.

| Variable | FX-100 | SOLACEA-21H |
|---|---|---|
| Session time hours | 3.65 ±0.34 | 3.65 ±0.34 |
| Blood flow mL/min | 320 (320-350) | 320 (320-350) |
| Arterial pressure mmHg | -100 (-89.5 to - 123) | -106 (-89.8 to - 130) |
| Venous pressure mmHg | 170 (156 - 182) | 150 (144 - 170) |
| Transmembrane pressure mmHg | 266 (251-289) | 264 (238-316) |
| Needle gauge | 15 (15.0 – 15.3) | 15 (15.0 – 15.3) |
| Heparin dose IU | 2000 (1250-2750) | 2000 (1250-2750) |
| Convection volume L/session | 18.0 ±1.8 | 17.8 ±1.9 |
| Substitution rate mL/kg/min | 1.15 ±0.23 | 1.11 ±0.24 |
| Dialysate flow mL/min | 500 (500-525) | 500 (500-525) |
| On-line $Kt/V_{urea}$ | 1.77 ±0.36 | 1.53 ±0.18 |
| Single pool $Kt/V_{urea}$ | 1.80 ±0.31 | 1.72 ±0.40 |
| Urea reduction ratio % | 78.5 ±5.8 | 76.6 ±7.6 |
| Dialysate temperature °C | 35.0 (35.0 – 35.1) | 35.0 (35.0 – 35.1) |
| Dialysate sodium mmol/L | 137.0 ±1.34 | 137 ±1.34 |
| Dialysate potassium mmol/L | 2.0 (1.0-2.0) | 2.0 (1.0-2.0) |
| Dialysate calcium mmol/L | 1.25 ±0.17 | 1.25 ±0.17 |
| Dialysate magnesium mmol/L | 0.5 | 0.5 |
| Dialysate bicarbonate mmol/L | 32.0 | 32.0 |
| Dialysate acetate mmol/L | 3.0 | 3.0 |
| Dialysate glucose mmol/L | 5.5 | 5.5 |

Table 3. Changes in pre- and post weights, blood pressure (systolic – SBP, diastolic – DBP) and serum biochemistry and full blood counts (total white blood cell (WBC), polymorphonuclear leukocyte (PMN), peripheral blood lymphocytes (PBL using FX100 and SOLACEA-21H dialyzers. Results expressed as mean ±SD, or median (interquartile range). There were no statistical differences pre-dialysis. * $p<0.05$ post-dialysis FX-100 vs SOLACEA-21H.

| | FX-100 | FX-100 | SOLACEA-21H | SOLACEA-21H |
|---|---|---|---|---|
| | Pre-dialysis | Post-dialysis | Pre-dialysis | Post-dialysis |
| Weight kg | 69.4 ±11.4 | 69.3 ±11.5 | 69.7 ±10.9 | 67.7±11.2 |
| Weight loss kg | | 1.58±0.84 | | 1.67±0.69 |
| SBP mmHg | 143 ±18.7 | 132 ±26.6 | 144 ±15.4 | 127 ±17.2 |
| DBP mmHg | 71.2 ±13.7 | 68.4 ±13.1 | 71.4 ±13.5 | 68.5 ±8.7 |
| Sodium mmol/L | 139 ±4.3 | 140 ±1.4 | 139 ±2.9 | 140 ±0.8 |
| Potassium mmol/L | 5.49 ±0.81 | 3.59 ±0.34 | 5.66 ±0.84 | 3.72 ±0.54 |
| Urea mmol/L | 20.9 ±7.5 | 4.5 ±2.0 | 20.9 ±7.2 | 5.1 ±2.9 |
| Creatinine µmol/L | 637 (577-837) | 177 (147-278) | 658 (605-852) | 194 (147-289) |
| Albumin g/L | 39.2 ±2.6 | 41.4 ±3.9 | 39.3 ±1.8 | 41.7 ±2.8 |
| Total protein g/L | 67.3 ±5.0 | 73.2 ±7.0 | 68.0 ±4.9 | 72.6 ±5.4 |
| β2 microglobulin mg/L | 31.7 ±8.3 | 6.0 ±1.4* | 31.2 ±8.3 | 8.3 ±2.3 |
| Haemoglobin g/L | 111 ±9.0 | 122 ±14.4 | 111 ±11.3 | 119 ±13.4 |
| Haematocrit | 0.35 ±0.01 | 0.37 ±0.03 | 0.35 ±0.01 | 0.36 ±0.03 |

| | | | | |
|---|---|---|---|---|
| WBC $10^9$/L | 6.03 ±1.30 | 5.66 ±1.42 | 6.06 ±1.63 | 6.17 ±2.13 |
| Platelets $10^9$/L | 211 (187-240) | 220 (197-272) | 219 (191-238) | 216 (197-251) |
| PMN $10^9$/L | 3.92 ±1.18 | 3.76 ±1.13 | 4.0 ±1.48 | 4.10 ±1.81 |
| PBL $10^9$/L | 1.26 ±0.36 | 1.19 ±0.04 | 1.23 ±0.41 | 1.32 ±0.51 |
| Monocytes $10^9$/L | 0.61 ±0.19 | 0.47 ±0.13 | 0.53 ±0.16 | 0.46 ±0.18 |
| Eosinophils $10^9$/L | 0.23 ±0.09 | 0.22 ±0.09 | 0.28 ±0.13 | 0.28 ±0.13 |
| Basophils $10^9$/L | 0.02 ±0.01 | 0.02 ±0.02 | 0.02 ±0.01 | 0.02 ±0.01 |

Table 4. Changes in clotting studies during haemodiafiltration using FX-100 and SOLACEA-21H dialyzers. PT seconds (s), APTT (s), thrombin-anti-thrombin complexes (TATs), soluble Intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble CD40 ligand (sCD40L), microparticle activity (MP), platelet factor 4 (PF4). Normal reference ranges given in parentheses. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs Start, # $p<0.05$, ## $p<0.01$, ###$p<0.001$ 15 minute pre- vs post- dialyser.

| | Start | Pre-dialyzer 15 min | Post-dialyzer 15 min | 60 min | End |
|---|---|---|---|---|---|
| PT s | (<14 s) | | | | |
| FX-100 | 11.3±0.8 | 12.3±0.8 | 12.1±0.8 | 11.9±0.9 | 11.1±0.8 |
| SOLACEA-21H | 11.2±0.8 | 12.3±0.8 | 11.9±0.8 | 11.6±0.7 | 11.2±0.7 |
| aPTT s | (<45 s) | | | | |
| FX-100 | 31.9±2.5 | 66.9±23*** | 77.4±33.8* | 47.3±16.2 | 33.4±4.3 |
| SOLACEA-21H | 32.1±1.7 | 68.7±23.9** | 82.0±30.5* | 50.0±16.9* | 35.2±11.2 |
| Fibrinogen g/L | (1-3) | | | | |
| FX-100 | 3.92±0.99 | 3.65±0.82$^{###}$ | 4.87±1.39* | 4.15±1.79 | 4.19±1.2 |
| SOLACEA-21H | 3.83±1.18 | 3.63±1.21$^{###}$ | 5.73±1.6* | 3.91±1.35 | 3.95±1.65 |
| Factor VIIIc IU/mL | (< 200) | | | | |
| FX-100 | 202±62 | 174±54***$^{##}$ | 237±83 | 195±61 | 224±77* |
| SOLACEA-21H | 191±72 | 163±60*$^{##}$ | 247±102** | 183±68 | 207±86 |
| Tissue Factor pg/mL | (21-49) | | | | |
| FX-100 | 124±16 | 117±12$^{##}$ | 136±15** | 123±11 | 120±15 |
| SOLACEA- | 103±24 | 100±25$^{#}$ | 128±40* | 103±22 | 103±27 |

| | | | | | |
|---|---|---|---|---|---|
| 21H | | | | | |
| D dimers ng/mL | (<250) | | | | |
| FX-100 | 301±146 | 292±132## | 355±167** | 315±167 | 386±240 |
| SOLACEA-21H | 314±136 | 300±129## | 391±171* | 307±126 | 502±492 |
| TATs ng/mL | (0.5-1.0) | | | | |
| FX-100 | 9.2±3.8 | 10.2±5.9 | 10.7±5.0 | 9.4±4.7 | 8.6±4.7 |
| SOLACEA-21H | 9.1±6.7 | 10.5±6.3 | 10.9±5.2 | 10.3±5.9 | 9.1±5.5 |
| sICAM-1 ng/mL | (71-185) | | | | |
| FX-100 | 199±110 | 194±106## | 225±92* | 208±96 | 213±106 |
| SOLACEA-21H | 192±88 | 197±90# | 250±105* | 205±87 | 220±124 |
| sVCAM-1 ng/mL | (395-594) | | | | |
| FX-100 | 780±69 | 782±64### | 918±140** | 804±103 | 829±100 |
| SOLACEA-21H | 733±119 | 722±121## | 889±168* | 747±134 | 729±80 |
| sE-selectin ng/mL | (13-51) | | | | |
| FX-100 | 37.6±11.8 | 36.4±11.8# | 42.9±14.3 | 36.9±12.1 | 40.1±14.7 |
| SOLACEA-21H | 37.3±11.3 | 37.9±12.7# | 47.9±10.4* | 38.7±11.7 | 38.3±11.4 |
| sCD40L ng/mL | | | | | |
| FX 100 | 297±228 | 503±247 | 352±264 | 600±411 | 552±272* |
| SOLACEA-21H | 245±187 | 336±193 | 381±220** | 372±195 | 390±205* |
| MP nM/mL | | | | | |
| FX 100 | 6.2±1.8 | 8.1±3.6 | 5.1±2.4 | 8.8±4.8 | 10.2±8.1 |
| SOLACEA-21H | 11.0±9.0 | 10.8±10.4 | 7.4±5.0 | 12.1±7.4 | 11.3±6.8 |
| sPselectin | (< 60) | | | | |

| | | | | | |
|---|---|---|---|---|---|
| ng/mL | | | | | |
| FX 100 | 26.7±7.1 | | | | 37.6±9.9*** |
| SOLACEA-21H | 28.7±7.2 | | | | 43.5±8.4*** |
| PF4 ng/mL | (<10) | | | | |
| FX 100 | 58.3±9.9 | | | | 55.1±3.3 |
| SOLACEA-21H | 52.7±6.5 | | | | 57.4±2.2 |