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Original Article

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Patient characteristics rather than the type of dialyser predict the variability of endothelial derived surface molecules in chronic haemodialysis patients

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Abstract

Background. Cardiovascular disease (CVD) is a frequent complication in chronic haemodialysis (HD) patients. Endothelial dysfunction, as measured by soluble cellular adhesion molecules (sCAM) and von Willebrand factor (vWf) in plasma, is an early manifestation of CVD. Today, it is unknown if, and to what extent, their levels are influenced by the type of dialyser.

Methods. Four dialysers, low-flux cuprammonium (CU); high-flux and low-flux polysulfone and super-flux polyethersulfone, were compared in 15 chronic HD patients in a randomized cross-over fashion. sCAM and vWf were measured at baseline as well as after 4 weeks, and both intra-dialytical and after 24 h (t24 h). Twenty healthy subjects served as controls.

Results. Baseline levels were considerably higher in chronic HD patients than in controls (soluble intercellular adhesion molecule-1: sICAM-1 732 ± 216 *vs* 572 ± 259 ng/ml, $P = 0.06$; soluble vascular cell adhesion molecule-1: sVCAM-1 1917 ± 492 *vs* 1126 ± 338 ng/ml, $P < 0.001$; vWF: $205 \pm 55\%$ *vs* $98 \pm 52\%$, $P < 0.001$). After 4 weeks, no changes were observed. During and after HD, sCAM did not change, except in the case of CU (sICAM-1: 719 ± 259 to 772 ± 261 ng/ml, $P = 0.04$). CU induced a rise in vWF directly after HD (t4 h; from $188 \pm 48\%$ to $255 \pm 92\%$, $P < 0.01$), whereas all modalities induced a significant increase at t24 h (mean $228 \pm 54\%$, $P = 0.02$). The levels of sCAM and vWf appeared to be dependent on the individual patients rather than on the type of dialyser (explained variance by different patients: 66%–91%, $P < 0.001$; by type of dialyser 0.4–1.2%).

Conclusions. Baseline levels of sCAM and vWf were markedly higher in chronic HD patients than in controls and did not change after 4 weeks with any dialyser. All membranes induced a marked rise in vWf at t24 h, whereas sICAM-1 increased only in the case of CU at t4 h. As sCAM showed no marked changes during HD with any other modality, our study suggests activation of blood cells rather than endothelial cells. As pre-dialysis levels of sCAM and vWf varied noticeably between individual patients, endothelial dysfunction seems to be far more dependent on patient-related factors than on the HD treatment itself.

Keywords: cardiovascular disease; endothelial dysfunction; haemodialysis; platelet activation; soluble cellular adhesion molecules; von Willebrand factor

Introduction

Cardiovascular disease (CVD) is a frequent complication and a major cause of mortality in chronic haemodialysis (HD) patients, accounting for more than half of all deaths [1]. In addition, chronic HD patients suffer from atherosclerotic complications at a relatively young age. The increased cardiovascular risk is probably multi-factorial in origin, and already observed in the pre-dialysis phase [2].

The pathogenesis of vascular disease appears to be determined to a critical extent by endothelial dysfunction [3]. Inflammation of the arterial wall results in

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upregulation of endothelial cellular adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM-1), E-selectin, and von Willebrand factor (vWf) [4]. Interestingly, plasma levels of soluble ICAM-1 (sICAM-1), resulting from proteolysis of membrane-bound ICAM-1, correlated positively with the carotis intima media thickness in both renal [5] and non-renal patients [6]. In addition, evidence suggests that sICAM-1, soluble VCAM-1 (sVCAM-1) [7] and vWf independently predict the risk of future CVD in both apparently healthy men [8] and renal patients [9].

Markedly elevated plasma levels of soluble cell adhesion molecules (sCAM) and vWf have been observed in individuals with renal impairment [10,11] and in dialysis patients [12]. Moreover, several authors reported on an increase in the levels of vascular cell surface molecules during HD [12–14]. Therefore, it is conceivable that HD treatment itself promotes vascular injury, thereby contributing to the increased cardiovascular risk in chronic HD patients.

Depending on the type of dialyser and the degree of dialysate contamination, various blood cell types and protein systems are stimulated during HD. As a result, pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are released. Degranulation of granulocytes and platelets leads to the discharge of various granule products, such as myeloperoxidase and platelet factor 4, respectively. In addition, both the coagulation and complement system are activated, the latter especially by plain cellulose membranes. As a consequence, HD treatment induces oxidative stress, prothrombotic changes, and signs of inflammation. Both stimulated cells, mobilized intracellular products and activated proteins leave the dialyser, enter the systemic circulation of the patients, and activate and/or damage the endothelial cells.

As yet, it is unclear whether the plasma levels and variability of the above-mentioned cell surface molecules are mainly determined by patient-related factors, such as co-morbidity, renal dysfunction and prescribed medication, or by the dialysis procedure itself.

Therefore, a prospective randomized cross-over study was designed. Four types of dialysers, differing in material, biocompatibility and flux, were studied during HD and after 24 h, as well as after a period of 4 weeks. The complement activation product C3a was measured as a marker of bio-incompatibility and β2-microglobulin (β2m) as an indicator of flux. Besides the plasma levels of endothelial cell surface molecules, several parameters of inflammation were measured.

Patients and methods

Patients

Fifteen stable patients (8 women, 7 men), undergoing HD treatment for at least 6 months participated in the study after giving written informed consent. Patient characteristics are depicted in Table 1. Causes of renal insufficiency were hypertension ($n=3$), diabetic nephropathy ($n=2$), polycystic kidney disease ($n=2$), analgesic nephropathy ($n=2$), membranoproliferative glomerulonephritis ($n=1$) and renal failure of unknown origin ($n=5$). Exclusion criteria at the entry of the study were co-morbidity (auto-immune disease and/or acute infections) and/or medication that might interfere with parameters of inflammation (immune-suppressive medication and non-steroidal anti-inflammatory drugs). The study was approved by the Ethics Committee of the VU University Medical Center.

Table 1. Characteristics of patients and controls

	Patients ($n=15$)	Controls ($n=20$)
Age (median, range)	72 (35–83) years	35 (25–53) years
Gender (male/female)	7/8	8/12
Time on dialysis (median, range)	4 (1–15) years	NA
Vascular access (goretex/AV fistula)	8/7	NA
Smoking (yes/no)	5/10	14/6
Known cardiovascular disease (yes/no)	8/7	1/19
Body mass index (mean ± SD)	25.3 ± 5.6 kg/m^2	25.1 ± 3.4 kg/m^2
Systolic blood pressure (mean ± SD)	165 ± 25 mmHg	121 ± 13 mmHg
Diastolic blood pressure (mean ± SD)	86 ± 18 mmHg	69 ± 9 mmHg

NA, not applicable.

Controls

Twenty subjects (8 women, 12 men), age 37 ± 9 years, from the outpatient clinics of the departments of nephrology and internal medicine of the VU University Medical Center, with a creatinine clearance (estimated with the Cockroft and Gault formula) of > 90 ml/min, were used as controls. These controls were also part of another study [11]. Characteristics of the controls are depicted in Table 1.

Design of the study

Before starting the study, all patients were dialysed with low-flux PS dialysers. Patients were randomized to HD with low-flux cuprammonium (CU; AM SD 750 U), low-flux polysulfone (PS; F7HPS), high-flux PS (F70S), or super-flux polyethersulfone (PES; Syntra 160 g) dialysers in a cross-over design and dialysed for 4 weeks with each dialyser. In order to exclude carry-over effects, after each study period, patients were dialysed for 2 weeks (wash out) with the low-flux PS dialyser (F7HPS).

Blood samples were collected from the afferent (arterial) line. After each wash out period, i.e. before the first dialysis session with each of the dialysers mentioned (baseline), blood samples were drawn before the start of HD. After 4 weeks of dialysis with each of the dialysers mentioned, blood samples were collected before the start of HD (t0), after 10 min of HD (t10), and after 4 h (t4 h). Furthermore, an extra sampling was performed by venous puncture 24 h (t24 h) after the start

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Table 2. Dialyser characteristics

Type	Membrane material	Surface area	UF factor (ml/mmHg/h)	Membrane thickness	Sterilization method
Syntra 160 g	Polyethersulphone	1.6 m^2	73	30 μm	Gamma radiation
F 70 S	Polysulphone	1.6 m^2	50	40 μm	Heat
F 7 HPS	Polysulphone	1.6 m^2	9.8	40 μm	Heat
AM SD750U	Cuprammonium rayon	1.5 m^2	9	10 μm	Gamma radiation

of the HD session. Dialysate samples were taken from the dialysate lines during HD.

Dialysis procedure and materials

Patients were dialysed 2 or 3 times per week, according to their routine HD scheme. The dialysis sessions lasted 4–5 h, depending on the individual prescription of the patient. Only first-use dialysers were used. Characteristics of the dialysers used in this study (CU: AM SD 750 U, Asahi Medical, Tokyo, Japan; PS: F7HPS and F70S, Fresenius, Bad Homburg, Germany; and PES: Syntra 160 g, Baxter, Osaka, Japan) are depicted in Table 2.

According to the individual needs of the patients, blood flow and ultrafiltration (UF) rates were kept constant between 200–250 ml/min and 300–1000 ml/h, respectively. Isolated UF was not performed. Bicarbonate powder (BiBag, Fresenius, Bad Homburg, Germany) was used for the preparation of the dialysate. Dialysate flow was 500 ml/min. Anticoagulation was achieved by dalteparin with an initial dose of 2500–6000 IU, followed by an extra dose of 2500 IU during the dialysis treatment if necessary. Individual conditions (blood flow, UF-rate, dalteparin dose) were kept stable throughout the study period.

Analytical methods

Endothelial cell surface markers

Soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1. sICAM-1 and sVCAM-1 were measured in serum in duplicate by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Diaclone, Besançon, France). The intra- and inter-assay coefficients of variation were 4.0% and 7.4% resp. for sICAM-1, and 4.4% and 4.6% resp. for sVCAM-1.

Soluble E-selectin. E-selectin was measured in serum in duplicate by the use of a commercially available ELISA kit (Bender MedSystems, Vienna, Austria). The intra- and inter-assay coefficients of variation were 3.1% and 11.9%, respectively.

von Willebrand factor. Plasma vWf antigen was measured in CTAD-plasma by an in-house sandwich enzyme immunoassay, using rabbit anti-vWf antigen as catching antibody and a peroxidase-conjugated rabbit anti-vWf antigen as detecting antibody (Dako, Copenhagen, Denmark). *O*-Phenylenediamine (Sigma Chemical Co., St. Louis, USA) was used as substrate. Levels of vWf are expressed as a percentage of antigen levels in normal pooled plasma, defined as 100%. The intra- and inter-assay variations were 2.0% and 5.7%, respectively.

Markers of inflammation

Interleukin-6. Human interleukin IL-6 was measured by sandwich enzyme immunoassay (Quantikine High Sensitivity, R&D Systems, Oxon, United Kingdom). The intra- and inter-assay coefficients of variation or IL-6 were <4.0% and <9.0%, respectively, as determined in our laboratory. The lower limit of detection was 0.04 pg/ml.

C-reactive protein (CRP). Serum CRP concentrations were determined by a highly sensitive in-house sandwich enzyme immunoassay, using rabbit anti-CRP immunoglobulin and peroxidase-conjugated rabbit anti-human CRP immunoglobulin as a catching and detecting antibody (Dako, Copenhagen, Denmark), with *O*-Phenylenediamine (Sigma Chemical Co., St. Louis, USA) as a substrate with intra- and inter-assay coefficients of variation of 3.9% and 8.7%, respectively.

Tumor necrosis factor-α (TNFα). Plasma levels of TNF-α were measured by commercially available ELISA kits (R&D Systems, Oxon, United Kingdom), with intra- and inter-assay coefficients of variation of 7.3% and 8.5%, respectively, as determined in our laboratory. The lower limit of detection was 0.12 pg/ml.

Complement

C3a. C3a-desarg was determined in EDTA-plasma. Samples were diluted 1/100 and incubated at 37°C. ELISA plates were coated with monoclonal anti-C3a-desarg antibodies, using a peroxidase-conjugated rabbit anti-C3a-desarg as a detecting antibody. The assay was calibrated using standard curves obtained with recombinant C3a.

Beta-2-microglobulin

Beta-2-microglobulin was determined in undiluted serum, using a microparticle enzyme-immunoassay (MEIA, Abbott, Illinois, USA) according to the manufacturers' procedure. The lower limit of detection was 0.005 mg/l. The inter-assay coefficient of variation was 4.5%.

Dialysate endotoxin levels and cultures

Endotoxin levels. Dialysate samples for endotoxin determinations were collected in heparinized, pyrogen-free materials (EndoTube, Chromogenix, Mölndal, Sweden) at t4 h. Samples were stored at −70°C until determination. Endotoxin concentrations were measured using a kinetic chromogenic technique with endotoxin-specific Limulus Amoebocyte Lysates (LALs) (Pyrochrome, ACCI, East Falmouth, Massachusetts, USA). 50 μl samples were placed

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in a water bath of 36–38°C in pyrogen-free glass test tubes. After adding 50 µl LAL solution, the mixture was incubated for 8 min at 36–38°C. 100 µl of buffer-substrate solution was added and incubated at 36–38°C after mixing. After 24 min, this reaction was stopped by adding 250 µl 20% acetic acid. After mixing during 5 s, 200 µl of the solutions were pipetted on a microtitre plate, and absorbance was measured at 405 nm. A six-point calibration curve was prepared from 0 to 0.24 EU/ml. The detection limit of the assay is 0.024 EU/ml. The assay had an acceptable trueness and repeatability. For solutions with nominal 0.082 EU/ml control standard endotoxin, the mean ± SD precision of the assay, as determined from 20 assays, was 0.0765 ± 0.0124 EU/ml.

Dialysate cultures. Dialysate samples were drawn in sterile 1000 ml bottles. Dialysate samples were filtered over 0.45 µm cellulose nitrate filters and the filters were incubated on Reasoner's 2 Agar (both Sartorius AG, Göttingen, Germany) at room temperature. Total plate count was performed on day 7 and expressed as colony-forming units (CFU)/ml.

Statistical analysis

Results are expressed as mean ± SD, or median with range when appropriate. Data analysis was performed with the SPSS/PC+ software system (version 11.0), using paired Student's *t*-tests for data with a normal distribution. An independent Student's *t*-test was performed for the comparison of dialysis patients with normal controls. Data, which were not distributed normally, were analysed by a Mann–Whitney U test. The effects of the dialyser and patient characteristics on vWf and sCAM were tested with MANOVA for repeated measurements. Differences were considered significant at $P < 0.05$.

Results

Four weeks study

Soluble cellular adhesion markers. Baseline levels of all sCAM were comparable at the start of the different study periods, suggesting adequate wash-out periods. Actually, these levels were considerably higher than in controls (sICAM-1 732 ± 216 in HD patients *vs* 572 ± 259 ng/ml in controls, $p = 0.06$; sVCAM-1 1979 ± 492 *vs* 1126 ± 338 ng/ml, $p < 0.001$) [11]. After 4 weeks of HD, no changes were observed, (Figure 1a–c).

von Willebrand factor. Baseline levels of vWf were comparable at the start of different periods, and considerably higher as compared to controls (vWf 205 ± 55 in HD patients *vs* 98 ± 52% in controls, $P < 0.001$). After 4 weeks of HD, no changes were observed, Figure 1d.

Markers of inflammation

Baseline levels of CRP and TNFα were comparable at the start of the different study periods (mean of all modalities CRP 11 ± 10 mg/l; TNFα 5.2 ± 2.0 ng/ml), and remained unchanged after 4 weeks of HD (mean of all modalities CRP: 13 ± 21 mg/l; TNFα 5.3 ± 2.0 ng/ml. Baseline values of CRP were notably higher than in controls (1.7 ± 2.5 mg/l, $P = 0.002$).

Beta-2 microglobulin (β2m)

Baseline levels were comparable at the start of the different study periods, suggesting an adequate wash-out period (mean of all modalities 32.8 ± 9.3 µg/l). After 4 weeks of HD on both high- and super-flux dialysers, plasma β2m decreased significantly (super-flux PES from 32.4 ± 10.3 to 25.8 ± 5.3 µg/l: $P = 0.001$; high-flux PS from 32.0 ± 12.6 to 26.3 ± 6.7 µg/l; $p = 0.042$), but remained stable during HD with the 2 low-flux membranes ($P = 0.46$), Figure 2a.

Intra-dialytical study

Soluble cellular adhesion markers. Before HD, sICAM-1 levels were similar (mean of all modalities 727 ± 216 ng/ml). No intra-dialytic changes occurred, except for a moderate increase in the case of CU (from 719 ± 259 at t0 to 772 ± 261 ng/ml at t4 h, $P = 0.026$), which was significantly different from PS and PES (during PS/PES: from 729 ± 207 to 725 ± 181 ng/ml; versus CU $P = 0.018$). After 24 h, sICAM-1 levels were similar to pre-dialysis values, in all modalities (Figure 3a). sVCAM-1 and sE-selectin remained stable during and after HD, and did not differ between the four modalities.

von Willebrand factor. Before HD, vWf levels were similar (mean of all modalities 206 ± 56%). Only with CU dialysers, was a significant increase observed at t4 h (from 188 ± 48 at t0 to 255 ± 92%, $P = 0.008$; Figure 3b), which was markedly different from PS and PES (during PS/PES: from 211 ± 61 to 209 ± 69%; *vs* CU $P = 0.05$). After 24 h, vWf levels were higher than at t0 (t24 h: mean of all modalities 228 ± 54%, $P = 0.02$), albeit not significant in the case of high-flux PS.

Markers of inflammation

CRP. CRP levels remained stable during and 24 h after HD. No differences were noted between the four modalities (data not shown).

IL-6. Before HD (t0), IL-6 levels were comparable in all modalities (10.1 ± 12.3 pg/ml). During HD, no changes were observed (t4 h: 9.3 ± 10.3 pg/ml).

Complement activation product C3a

During the first 10 min of dialysis with all membranes, C3a levels increased significantly (mean increase of PES/PS from 345.8 ± 127 to 655.5 ± 237 ng/ml, $P < 0.01$; CU from 299.4 ± 139 to 1206.4 ± 908.1 ng/ml, $P < 0.01$; Figure 2b). However, the increase in the case of CU was markedly higher as compared to PES and PS ($P < 0.01$).

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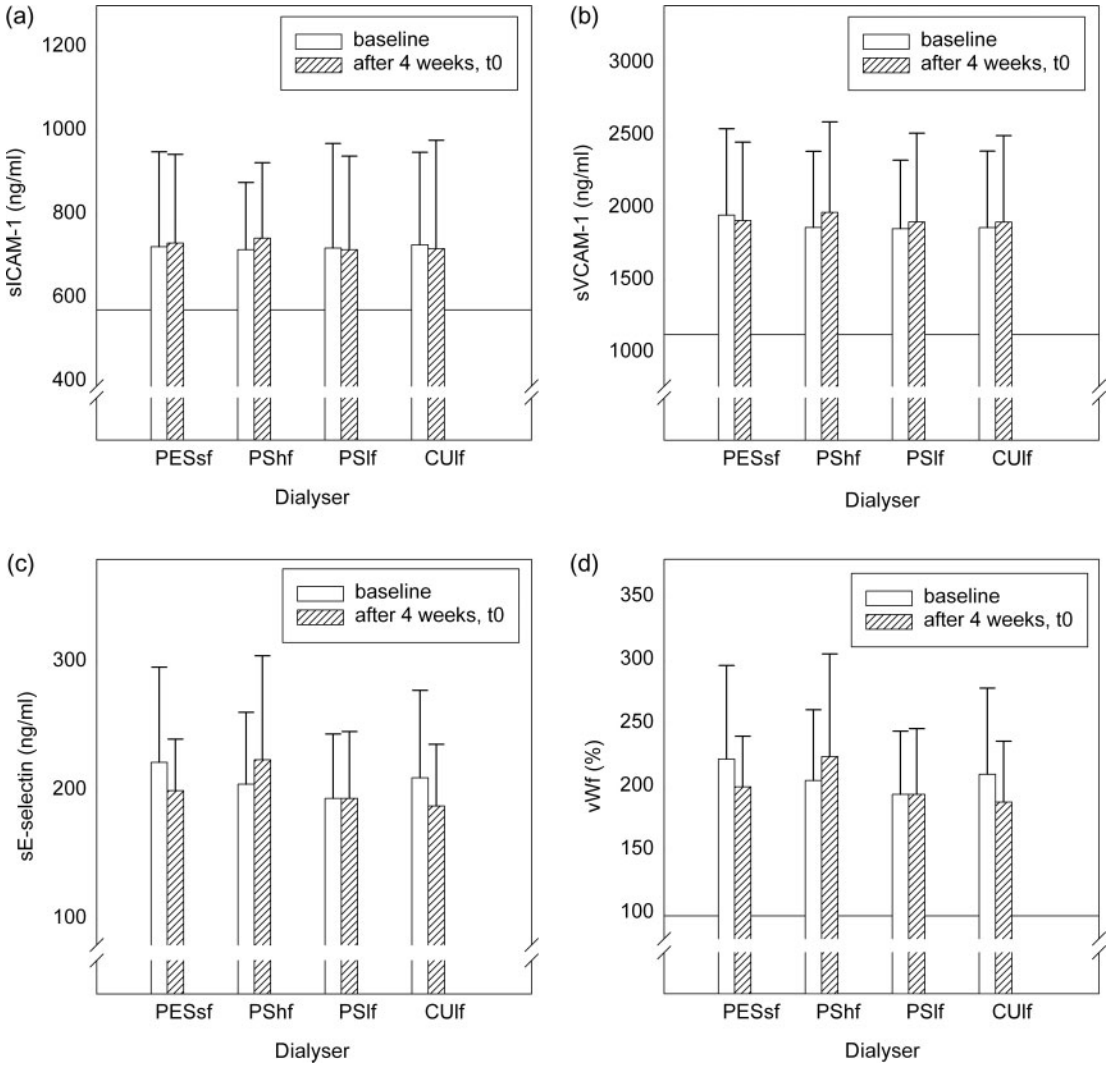

Fig. 1. Baseline levels of sCAM and vWf. Levels of sICAM-1 (a), sVCAM-1 (b), sE-selectin (c) and vWf (d), are depicted at baseline (white bars) and pre-dialysis after 4 weeks (hatched bars) of HD with PESsf, PShf, PSlf and CUlf. The straight line represents the mean value of controls.

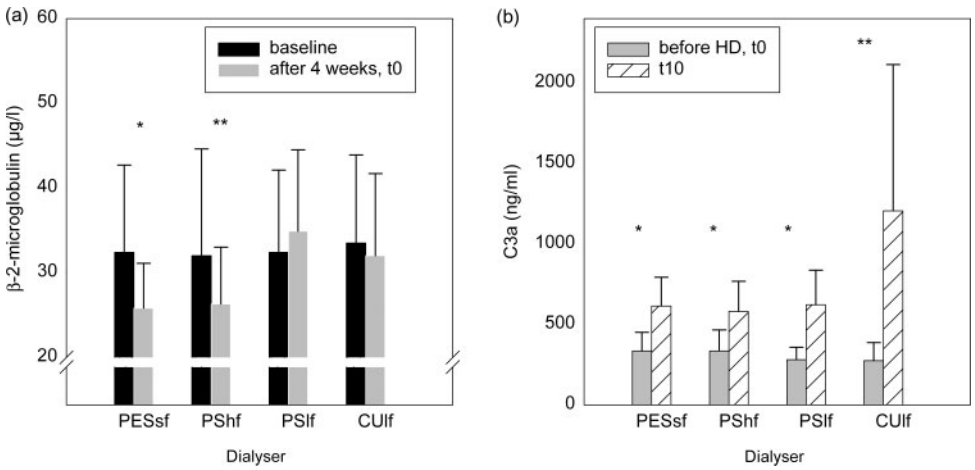

Fig. 2. Pre-dialysis β2m levels at baseline (black bars) and after 4 weeks of HD (grey bars) with PESsf, PShf, PSlf and CUlf (a). The decrease in pre-dialysis β2m was significant during HD with the high permeable dialysers (PESsf and PShf; $*P = 0.001$; $**P = 0.04$). (b) shows the levels of C3a before (grey bars) and after 10 min of HD (hatched bars) with PESsf, PShf, PSlf, and CUlf ($*P \leq 0.01$ t10 *vs* t0; $**P < 0.01$ increase with CU *vs* PES and PS).

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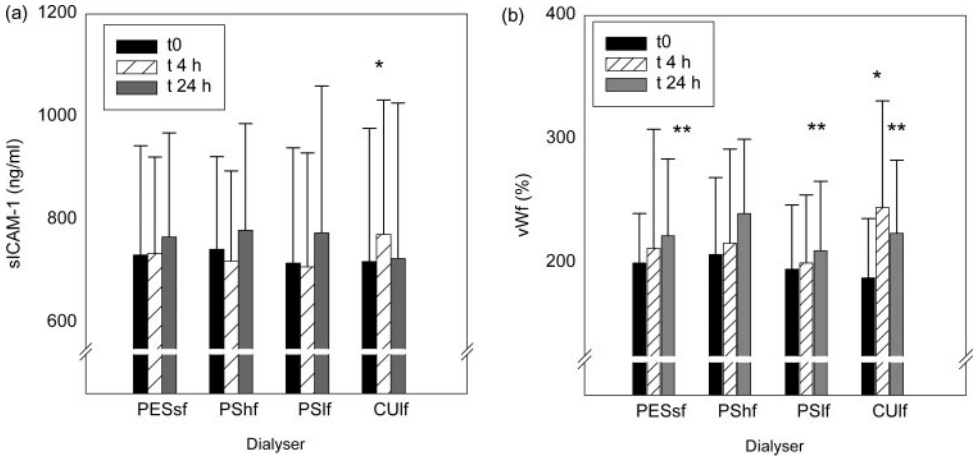

Fig. 3. Levels of sICAM-1 (a) and vWf (b) before (t0; black bars), directly after (t 4 h; hatched bars) and 24 hours after HD (t 24 h; grey bars) with PESsf, PShf, PSlf and CUlf. $*P < 0.01$ *vs* t0; $**P < 0.05$ *vs* t0.

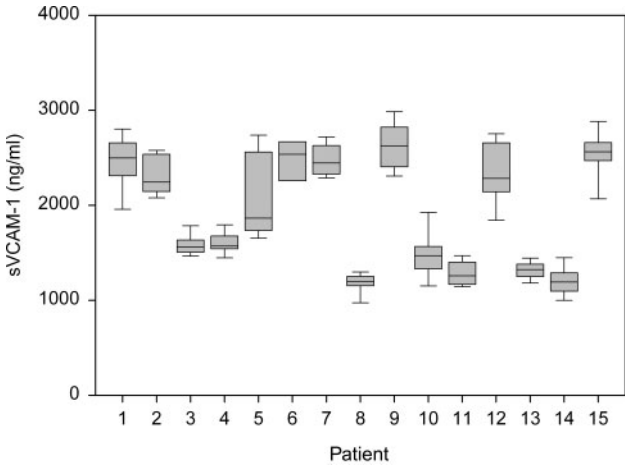

Fig. 4. Levels of sVCAM-1 in individual patients during HD with PESsf, PShf, PSlf, and CUlf, showing a large variation between different patients (mean 89%), whereas the levels in individual patients appear relatively stable throughout the study period (0.4%).

Variability of soluble adhesion molecules and von Willebrand factor

As depicted in Figure 4, the plasma levels of sVCAM-1 differed widely between the patients, but remained relatively stable for each patient during the entire study period (22 weeks). After analysing all data (except for baseline values, which may have been influenced by the low-flux PS dialyser during wash-out periods), the levels of sCAM and vWf were mostly explained by differences between patients (vWf 66%, sICAM-1 77%, sVCAM-1 89%, sE-selectin 91%, all $P < 0.001$), and only marginally by the different dialysers (i.e. vWF 1.0%, $P = 0.67$; sICAM-1 0.6%, $P = 0.8$; sVCAM-1 0.4%, $P = 0.56$; sE-selectin 1.2%, $P = 0.046$). The development over time only explained a significant amount of variance for vWF (7.2%, $P = 0.003$).

Dialysate quality. In water for dialysis (6 random samples assessed monthly, total 30 samples) endotoxin levels were 0.078 EU/ml (median; range 0.001–0.400 EU/ml). In 20 random samples taken during the study, culture results were 0.005 CFU/ml (median, range 0.000–0.420 CFU/ml).

Discussion

In the present study, four dialysers were compared in a randomized cross-over fashion to find out if the various membranes applied are implicated in endothelial cell activation, as measured by the release of sCAM (sICAM-1, sVCAM-1, sE-selectin) and vWf. The dialysers used differed in bio(in)compatibility and flux, as reflected by an intra-dialytical rise in C3a in the case of CU and a decrease in β2m after 4 weeks in the case of high-flux PS and super-flux PES. Regarding the parameters of inflammation measured, both plasma CRP and IL-6 remained unchanged, irrespective of the membrane used.

In line with previous reports, our study clearly shows that pre-dialysis plasma levels of vascular endothelial cell surface markers, such as sCAM and vWf, which are commonly used as markers of endothelial injury, are considerably higher in chronic HD patients than in a group of unmatched controls [11,15,16]. Of note, this difference might be overestimated by an increased prevalence of cardiovascular disease and diabetes in the patient group and underestimated by a higher number of smokers in the control group. Overall, however, the difference seems plausible and consistent with relevant literature on the subject.

Particularly sCAM are thought to be involved in the pathogenesis of atherosclerosis through their actions on leukocyte activation, adhesion, migration, and smooth muscle cell proliferation. As mentioned, both sCAM and vWf have been correlated with CVD and mortality in renal and non-renal patients [8,9]. Neither dialyser induced significant changes in the pre-dialysis levels of these molecules over a 4 week period, suggesting that the type of dialyser does not substantially contribute to plasma levels of sCAM and vWF in stable chronic HD patients. As mentioned, both CRP and

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IL-6 remained unchanged as well during the study, and no correlation could be demonstrated between changes in endothelial activation and inflammation (data not shown).

Only CU induced a moderate but significant rise in plasma levels of vWf , whereas all modalities induced a marked increase 24 h after HD. As vWf is exclusively synthesized in the endothelium and platelets, these data suggest either endothelial damage or release from activated platelets. Interestingly, no correlation was found between the increase in vWf and complement activation, suggesting that other stimuli, such as shear stress, platelet activating factor [17], reactive oxygen species or fibrinogen might be involved. Interestingly, apart from vWf, sICAM-1 increased slightly during CU dialysis. As ICAM-1 is not only expressed on endothelial cells, but also on monocytes, and blood cell activation is a well-known manifestation of bio-incompatibility of CU devices [14,18], the HD-induced increase in vWf and sICAM-1 may originate from activated blood cells. Therefore, it is conceivable that these changes do not represent endothelial injury, but reflect activation of circulating blood cells. Recently, we reported an early increase in plasma levels of platelet factor 4 (PF4) during HD with heparin anticoagulation, which was almost completely abolished during HD with citrate [19]. As PF4 is stored in the α-granula of platelets, which also contain vWf, these findings indicate that the rise in plasma vWf within 24 h after the start of HD may originate, at least partly, from activated platelets.

Only a limited number of non-randomized studies have evaluated the influence of a single HD session on the plasma levels of vascular cell surface molecules. As for sCAM, both a decline [11,13,16] and an increase [13,14] were observed during HD with CU membranes, whereas an increase was reported in the case of modified cellulose membranes and PS [12,13]. As the molecular weight of these molecules is 80–100 kDa and protein adsorption onto cellulose membranes is negligible, both clearance and adsorption seem highly unlikely. Therefore, binding to activated leucocytes has been proposed as an alternative mechanism explaining the decrease of these molecules during HD [13]. At first sight, a rational explanation for the discrepancy between the above-mentioned studies and our investigation seems lacking. However, besides biochemical dissimilarities, such as different assays for the determination of the vascular cell surface molecules, and flaws related to methodology, such as selection bias in cross-sectional and observational follow-up studies, the microbiological quality of the dialysate might be an important issue. Although the dialysate used in our study did not meet the stringent criteria for 'ultra-pure' dialysate, the degree of contamination was extremely low (culture results: median 0.005 CFU/ml). As the microbiological dialysate quality was not mentioned in any of the studies cited, it is conceivable that the transfer of bacterial products from non-sterile dialysate into the blood compartment, which has been implicated in both inflammation and vascular injury [20], has influenced the results.

Interestingly, the levels of sCAM and vWf varied markedly more between individual patients (on average 66–91, 96%) than between the different dialysers within the same patient (on average <1, 0.4–1.2%). Hence, patient-related factors seem to be more predictive of these levels than HD treatment itself, whether or not performed with plain cellulose or synthetic membranes. Although it is likely that cardiovascular disease is one of the most important factors in this respect, the small patient number and cross-over design did not permit the application of a regression analysis to analyse specific patient-related factors which might influence levels of sCAM and vWf.

To summarize, in this randomized prospective cross-over study, comparing four dialysers differing in material, biocompatibility and flux, we showed that baseline levels of sCAM and vWf in chronic HD patients are markedly higher than in healthy controls, and did not change after 4 weeks of HD with either membrane. Only CU induced a modest intra-dialytical rise in sICAM-1 and vWf, whereas all membranes induced an increase in the plasma levels of vWf after 24 h. As no sCAM showed marked changes during HD with any other modality, our study suggests stimulation of blood cells rather than endothelial activation. Whereas none of the dialysers used had any effect on the pre-dialysis levels of sCAM and vWf, these values varied noticeably between individual patients. Hence, in a population of chronic HD patients, the degree of endothelial activation seems to be far more dependent on patient-related factors than on HD treatment itself, regardless of the biocompatibility and flux characteristics of the dialyser applied. The nature of these patient-related factors remains to be investigated.

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