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Intercurrent clinical events are predictive of plasma C-reactive protein levels in hemodialysis patients

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Intercurrent clinical events are predictive of plasma C-reactive protein levels in hemodialysis patients.

Background. In chronic hemodialysis (HD) patients, the repetitive induction of the acute phase response (APR) may induce a chronic micro-inflammatory state, leading to various long-term complications.

Methods. The present prospective study was designed to assess the alterations in the APR in 74 patients who were randomized to HD with a high-flux polysulfone (PS; F 60S), a super-flux PS (F 500S), or a super-flux cellulosic tri-acetate (CTA and CTA with filtered dialysate, CTA_f) dialyzer. Blood samples collected at the start of the study and after twelve weeks were analyzed for interleukin-6 (IL-6) and C-reactive protein (CRP). In addition to the microbiological quality of the dialysate, the appearance of a "clinical event" was assessed.

Results. At baseline, mean IL-6 levels were within the reference range whereas mean CRP levels were slightly elevated. Mean values did not change after 12 weeks of HD with either modality. After subdividing the patients in quartiles with increasing change in plasma CRP, 23.0% of the patients showed a change of more than 8.0 mg/L. In a multiple regression analysis, CRP levels appeared to be independent of the degree of dialysate contamination, the material and the flux characteristics of the devices. In fact, the variable "clinical events" was the only significant predictor of the plasma CRP levels ($P < 0.001$).

Conclusions. Based on these results, both PS and CTA super-flux dialyzers appear safe for clinical use. Whether changes in CRP values, which are associated with intercurrent clinical events, influence the long-term prognosis of chronic HD patients remains to be established.

In chronic hemodialysis (HD) patients, the repetitive induction of the acute phase response (APR) may induce a chronic micro-inflammatory state, leading to a variety of long-term complications, including erythropoietin resistance [1, 2], dialysis related amyloidosis [3], malnutrition [4], and both cardiovascular [5, 6] and overall mortality (abstract; Bergström J, *J Am Soc Nephrol* 54:573, 1995) [7]. According to recent literature, an increased APR has been associated with both the type of dialyzer [8, 9] and the presence of contaminated dialysate [10, 11]. As for the latter, apparently contradictory viewpoints have been described. On the one hand, dialyzers with high permeability characteristics have been associated with an increased risk of back-transport, defined as the transfer of bacterially derived substances from the dialysate to the blood compartment, leading to the induction of pro-inflammatory cytokines [12, 13]. On the other hand, however, in the case of high flux polysulfone (PS) and polyamide membranes, it has been demonstrated that back-transport is largely prevented by the formation of a protein layer at the blood side and/or its ability to adsorb bacterially derived products onto the hydrophobic domains within the membrane. As (modified) high-flux cellulosic dialyzers consist entirely of hydrophilic domains that lack the capacity to adsorb these substances [14], concern has been raised that patients who are treated with these devices are at a high risk of exposure to bacterial contaminants in the dialysate [15–17].

Recently, devices with superior permeability characteristics for potentially harmful middle and large molecular weight substances, such as leptin [18, 19], β_2-microglobulin [20], uremic toxins involved in the extra-renal homocysteine metabolism [21], and advanced glycation end products (AGEs) (abstract; Van Tellingen A, *J Am Soc Nephrol* 10: 308A, 1999), were introduced at our center [21]. In a previous study of these super-flux dialyzers that compared low to moderately contaminated dialysate with ultra-pure dialysate, endotoxin (ETX) concentrations in the plasma, as measured by the limulus amebocyte lysate (LAL) assay, were consistently below the values in the dialysate [22]. Moreover, during HD with CTA dialyzers these values even decreased, sug-

Key words: super-flux dialyzers, interleukin-6, C-reactive protein, dialysate contamination, back-transport, polysulfone, cellulosic tri-acetate.

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gesting that back-transport of LAL-positive material did not occur. However, apart from LAL-positive material, other cytokine-inducing substances (CIS), such as exotoxins and outer membrane proteins (peptidoglycans, muramyl-peptides) [14], may cross the membrane of the dialyzer and elicit an APR in HD patients. Therefore, the present study was designed to detect changes in the APR, as measured by plasma interleukin-6 (IL-6) and C-reactive protein (CRP), in chronic HD patients who were randomized either to HD with highly permeable super-flux dialyzers (PS, CTA and CTA with an ETX filter, CTA_f) or to HD with a standard high-flux dialyzer (PS). As not only alterations in the treatment modality, but also intercurrent clinical events might have a marked influence on changes in plasma CRP levels, the medical conditions of the patients were recorded both at the time of inclusion and regularly during the follow-up period.

METHODS

Study design

Seventy-four patients, (median age 70 years, range 26 to 87), undergoing HD for at least six months (median 37, range 8 to 301) with a high-flux PS dialyzer (F60S), which is the "gold standard" in our center, participated in the study after giving written informed consent. Exclusion criteria at the entry of the study were use of a percutaneous HD catheter, co-morbidity (malignancy, auto-immune disease and/or acute infections), and/or medication (NSAIDs, prednisone) that might interfere with the immune system.

All patients were randomized to HD with either a high-flux PS (F 60S), a super-flux PS (F 500S), a super-flux CTA (Tricea 150G), or a super-flux CTA dialyzer with filtered dialysate (Tricea $150G_f$) for 12 consecutive weeks. Blood samples were collected both at the start of the study and after twelve weeks. Blood samples were drawn from the afferent line before dialysis (t_0) and analyzed for IL-6 and CRP. Dialysate samples were taken at t_{180} from the outlet port of the dialysis machine and were cultured for bacterial growth and analyzed for LAL-reactivity.

Dialysis procedure and materials

The dialysis sessions lasted three to five hours, depending on the individual prescription of the patient. Only first-use dialyzers were used. Characteristics of the three dialyzers used in this study (PS F 60S and F 500S from Fresenius, Bad Homburg, Germany; CTA Tricea 150G; Baxter, Osaka, Japan) are depicted in Table 1. As for the super-flux dialyzers, these devices have been designed to maximize convective transport by increasing the pressure drop along the fibers of the membrane. Furthermore, the pore size and/or the distribution of the pores influence the permeability. These characteristics result in a better convective clearance of middle and high molecular weight substances, as measured by β_2-microglobulin (Vienken J, personal communication). Filtered dialysate was obtained by the interposition of a PS filter (SPS 600; Fresenius) [23]. According to the individual needs of the patients, blood flow and UF rates were kept constant between 200 and 250 mL/min and 300 and 1000 mL/h, respectively. Isolated UF was not performed. Bicarbonate powder (BiBag; Fresenius) 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 to 6000 IU, followed by an extra dose of 500 to 1000 IU during the dialysis treatment if necessary. Individual conditions (blood flow, UF, dalteparin dose) were kept stable throughout the study period.

Table 1. Membrane characteristics

	F 60S	F 500S	CTA
Inner lumen μm	200	155	200
Wall thickness μm	40	35	15
UF coefficient $mL/mm\ Hg/h$	40	300 (H_2O)	29
Surface area m^2	1.3	1.2	1.5
Clearance mL/min			
β_2-microglobulin (MW 11.8 kD)	38	65–80	no data
Sieving coefficient			
β_2-microglobulin	0.65	0.9	0.8

Abbreviations are: F 60S, high-flux polysulfone; F 500S, super-flux polysulfone; CTA, super-flux cellulosic tri-acetate; UF, ultrafiltration; MW, molecular weight.

Clinical events

Clinical conditions that might interfere with the APR, including infections, surgical interventions, and inflammatory or injurious conditions that may elicit tissue damage, were assessed prospectively during the follow-up of the study. In addition, each hospitalization was recorded.

Analytical methods

Microbiological evaluation of the dialysate. Dialysate samples were drawn in sterile tubes. Total plate counts were performed on Reasoners $_2$A-agar (Difco Laboratories, Detroit, MI, USA) after 24 hours of incubation at 37°C and 7 days at 21°C. Isolation of pathogens were performed on McConkey-agar (Becton-Dickinson, Heidelberg, Germany) after 48 hours of incubation at 37°C. Identification of micro-organisms was achieved using Analytical Profile Index identification (Analytical Profile Index system S.A., Montalieu Verceu, France).

Endotoxin assay. Dialysate samples for ETX determinations were collected in pyrogen-free and sterile FALCON 2063 polypropylene tubes (Becton Dickinson). Samples were stored at −20°C until determination. ETX-activity in dialysate was quantified by a kinetic chromogenic method based on the LAL-assay (BioWhittaker, Walkersville, MD, USA). Standard series of purified *Escherichia coli* 055:B5 lipopolysaccharide (LPS) were

Table 2. Patient characteristics

	F 60S	F 500S	CTA	CTA_f	Total
Patients *N*	19	19	19	17	74
Age *years*	65 ± 15	67 ± 11	66 ± 14	68 ± 15	66 ± 13
Gender *female/male*	9/10	6/13	9/10	10/7	34/40
Time on HD *months*	76 ± 78	50 ± 51	58 ± 68	56 ± 44	60 ± 62
Type of vascular access *fistula/graft*	8/11	4/15	3/16	8/9	23/51

Data are mean ± SD.

Table 3. Medical conditions at the time of CRP measurements during the follow-up of the study

Clinical events	*N*
Infections	9
Shunt complications	5
Diabetic/vascular ulcer	3
Heart failure	1
Total hip replacement	1
Subdural haematoma	1

made in the range 0.005 to 50.0 EU/mL. Inhibition and interference testing was performed on each sample by an ETX spike. To overcome inhibition/enhancement, all dialysate samples were diluted 10 times. All determinations were performed in duplicate and recoveries of spikes between 50 and 150% were accepted. Limit of determination was 0.05 EU/mL.

Interleukin 6. IL-6 was determined in K_3-EDTA plasma by a sandwich-type immunoassay, according to the manufacturer's procedures (Central Laboratory, Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) [24]. After centrifugation (10 min, $1500 \times g$), the plasma samples were stored immediately at −70°C until required for testing. The lower limit of detection for IL-6 was 0.3 pg/mL. IL-6 reference value of healthy individuals was below 10 pg/mL.

C-reactive protein. Serum CRP concentrations were determined by nephelometry (BN II; Dade Behring B.V., Leusden, The Netherlands). The lower limit of detection of CRP was 2 mg/L. CRP reference values for healthy individuals were below 5 mg/L.

Statistical analysis

Data are expressed as mean (± SD). Analysis was performed with the Statistical Package for Social Sciences/PC+ software system using paired and unpaired *t* tests to study the differences between groups. Categorical variables were compared using the χ^2-test. Correlation coefficients were calculated with the Pearson method. Multiple linear regression, stepwise method (entry at 5% and removal at 10%), was used to evaluate the influence of the independent predictors on the change of CRP levels after 12 weeks of HD. After subdividing the patients into four groups with increasing change of CRP levels, a logistic regression analysis was applied to each quartile separately. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Patient characteristics

There were no significant differences in age, gender, time on HD, or vascular access type (fistula or graft) between the four patient groups (Table 2). Fifty-four patients had arteriovenous fistulas for vascular access, whereas twenty patients had artificial subcutaneous grafts.

Clinical events

The patients were free of acute events at the start of the study, whereas the clinical events occurred during the observation time. A summary of the patients with medical conditions are depicted in Table 3. Infections were generally mild and included influenza in seven patients and cystitis in two patients. Shunt complications consisted of one contusion, one hematoma and an infection of the shunt in three patients, and repair of the arteriovenous fistula in two patients. Other medical conditions included: diabetic or vascular ulcer in three patients, and congestive heart failure, a total hip replacement and a subdural hematoma in one patient. Of these patients, seven (35.0%) were hospitalized at the time of the blood sample measurements. Marked differences in the number of clinical events were not observed between the four study groups.

Microbiological quality of the dialysate

For the bacterial counts, gram-positive organisms were cultured in 8 of 74 cases, whereas 7 out of 8 also showed gram-negative organisms. When comparing filtered with non-filtered dialysate, a significant difference was observed [filtered dialysate 0 (0–3) CFU/mL; non-filtered dialysate 26 (0–310) CFU/mL, $P < 0.001$]. Pseudomonas species were not isolated.

As for ETX concentrations, marked differences were not found between filtered and non-filtered dialysate (filtered dialysate 0.051 ± 0.005 EU/mL, non-filtered dialysate 0.051 ± 0.005 EU/mL; P = NS; Table 4).

Interleukin-6

At baseline, mean values were within the reference range. IL-6 levels remained stable after 12 weeks of HD with all four modalities (F 60S week 1, 2.3 ± 1.2 pg/mL and week 12, 2.6 ± 1.5 pg/mL, P = NS; F 500S week 1, 2.9 ± 2.0 pg/mL and week 12, 3.3 ± 3.3 pg/mL, P = NS; CTA week 1, 1.9 ± 0.9 pg/mL and week 12, 2.0 ± 1.0 pg/mL, P = NS; CTA_f week 1< 4.2 ± 3.8 pg/mL and week 12, 2.7 ± 2.1 pg/mL, P = NS; Fig. 1).

Table 4. Dialysate cultures and endotoxin concentrations

Characteristic	Non-filtered dialysate ($N = 55$)	Filtered dialysate ($N = 19$)
Dialysate culture *CFU/mL*[a]	26 (0–310)[b]	0 (0–3)[b,c]
Gram stain *number*		
Positive	1	0
Negative	43	1
Positive and negative	7	0
None	4	18
Endotoxin *EU/mL*[d]	0.051 (<0.05–0.086)	0.051 (<0.05–0.071)[e]

[a] Colony-forming unit
[b] Median (range)
[c] $P < 0.001$, filtered versus non-filtered dialysate
[d] Endotoxin unit per milliliter
[e] $P = 0.47$, filtered versus non-filtered dialysate

C-reactive protein

Baseline CRP levels were slightly elevated when compared to the reference value for healthy individuals. Significant differences were not observed between the four modalities at the start of the study (F 60S, 6.9 ± 5.6 mg/L; F 500S, 6.7 ± 6.8 mg/L; Tricea 150G, 6.7 ± 5.9 mg/L; Tricea 150G_f, 16.7 ± 20.2 mg/L; P = NS). Marked fluctuations were not observed after 12 weeks of HD (Fig. 1). After subdividing the patients into four groups (quartiles) with increasing change in CRP after 12 weeks of HD, 29.7% of the patients showed no change, 20.3% showed a change between 0.1 and 3.0 mg/L, 25.7% showed a change between 3.1 and 8.0 mg/L, and 23.0% showed a change of more than 8.0 mg/L (Fig. 2).

Relationship between markers of dialysate quality (bacterial counts and ETX), type of dialyzer, type of vascular access, clinical events, IL-6 and CRP levels

By univariate regression analysis, significant correlations were not found between the bacteriological quality of the dialysate and CRP levels. A strong positive correlation was found between IL-6 and CRP levels ($r = 0.85$, $P < 0.001$; Fig. 3). Moreover, a significant correlation was observed between the appearance of a "clinical event" and CRP levels ($r = 0.44$, $P < 0.001$). Interestingly, the parameter "clinical events" showed an even stronger correlation with the change of CRP levels in the highest quartile (that is, CRP change >8.0 mg/L). Using a stepwise multiple linear regression model with the change of CRP levels after 12 weeks of HD as the dependent variable and the already mentioned parameters as independent factors, the appearance of a "clinical event" during the follow-up was the only significant predictor of the CRP changes, accounting for 22.1% of its variance ($P < 0.001$; Table 5). Moreover, using subsequently the highest quartile (that is, CRP change >8.0 mg/L) as the dependent variable in this model, the appearance of a clinical event again remained the only significant predictor of CRP, accounting for 36.2% of its variance ($P < 0.001$).

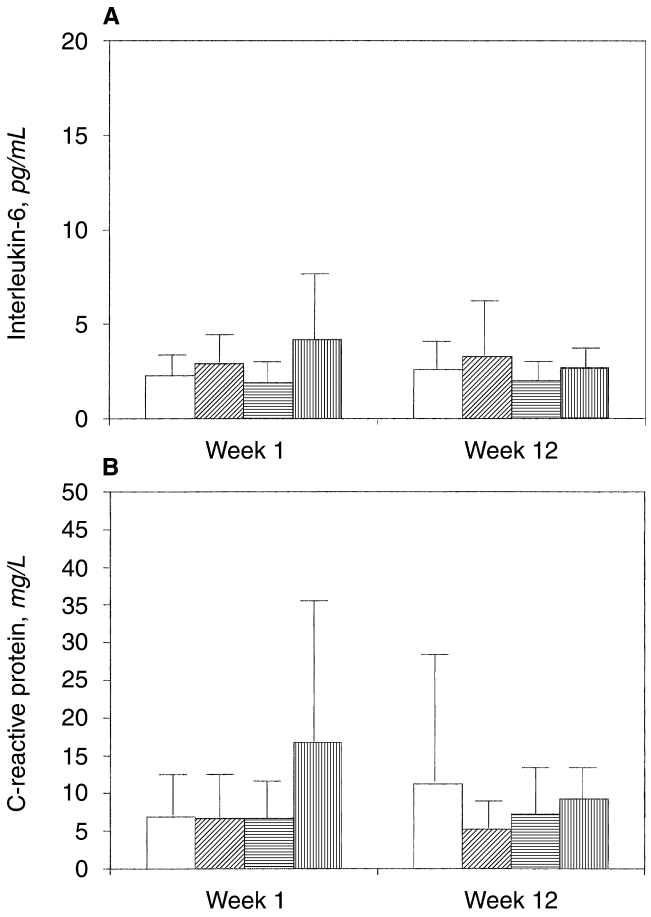

Fig. 1. Plasma interleukin-6 (IL-6; *A*) and C-reactive protein (CRP; *B*) levels at the start of the study and after 12 weeks of HD with four dialyzer modalities. Significant changes in mean values were not observed, nor were there marked differences between the four dialyzer modalities. Symbols are: (□) F 60; (▨) F 500S; (▤) CTA; (▥) CTA_f.

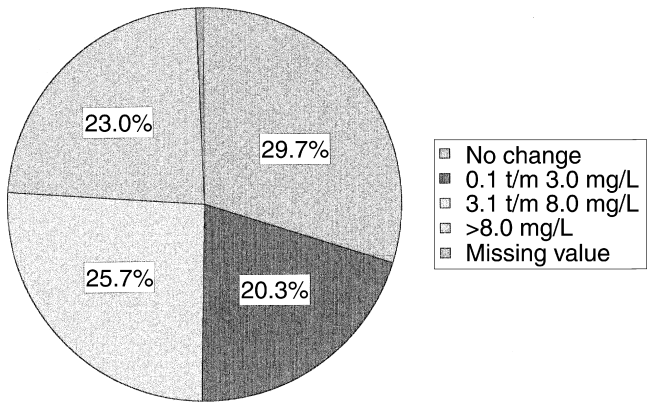

Fig. 2. Subdivision of the patients into four groups with increasing change of CRP levels during the follow-up of the study.

DISCUSSION

The present study was designed to assess potential changes in the APR over a period of twelve weeks in chronic HD patients who were randomized to two types

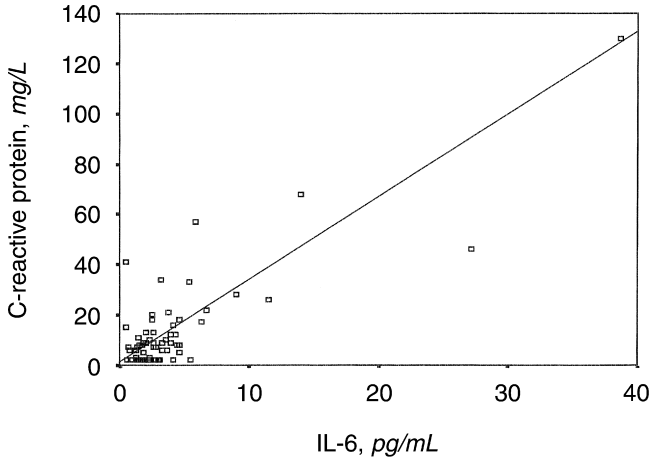

Fig. 3. Correlation between IL-6 and CRP levels. A strong correlation was noted ($r = 0.85$, $P < 0.001$).

Table 5. Multiple linear regression, stepwise method, with the change of CRP levels after 12 weeks of HD as the dependent variable

	R^2	beta	*P* value
(Constant)		4.6	0.16
Clinical event	0.221	27.4	<0.001
Bacterial counts in dialysate culture (*CFU/mL*)[a]			0.59
Endotoxins (dialysate) (*EU/mL*)[b]			0.67
Membrane material			0.77
Type of vascular access dialyzer			0.44
F 60S			0.18
F 500S			0.30
CTA			0.54
CTA_f			0.77

The appearance of a clinical event during the follow-up remained the only significant predictor of CRP accounting for 22.1% of its variance.

[a] Colony-forming unit per milliliter

[b] Endotoxin unit per milliliter

of super-flux dialyzers, the F 500S consisting of synthetic material (PS) and the CTA consisting of modified cellulose. In the case of CTA, both a modality with standard dialysate (26 CFU/mL, range 0 to 310) and ultra-pure dialysate was used (0 CFU/mL, range 0 to 3). Patients randomized to a high-flux PS device (F 60S) served as controls, as this type of dialyzer is the "gold standard" in our center.

At baseline, mean IL-6 levels were within the reference range in all groups, whereas plasma CRP levels were slightly elevated. After 12 weeks of HD, mean values of these measurements did not change, in either modality. In line with previous studies on IL-6 and CRP in chronic HD patients [8, 25], a strong correlation was found between these two markers of inflammation. In a multiple linear regression analysis, CRP levels appeared independent of the degree of dialysate contamination, despite significant differences in the number of CFU/mL between filtered and non-filtered dialysate. In addition, neither the material (CTA and PS), nor the flux characteristics of the devices (high-flux and super-flux) influenced CRP levels. In fact, the appearance of a "clinical event" was the only significant predictor of CRP, accounting for 22.1% of its variance. After subdividing the patients into quartiles with increasing change in CRP levels, 23.0% showed a change of more than 8.0 mg/L. Moreover, after the application of a logistic regression analysis to each quartile separately, the appearance of a "clinical event" during follow-up was the only significant predictor of CRP changes in the highest quartile (that is, CRP change >8.0 mg/L), accounting for 36.2% of its variance. In a separate analysis restricted to the patients showing no acute events during the study, marked changes in CRP levels did not occur (data not shown). In addition, in this patient group CRP levels were not correlated with either the type of dialyzer or the bacterial quality of the dialysate.

Recently, patient-related factors, such as chronic bacterial infections (*Helicobacter Pylori, Chlamydia Pneumoniae*), smoking habit and the presence of cardiovascular disease have been recognized as important contributors to plasma CRP levels in individuals with and without renal diseases [26, 27]. However, as the change in CRP levels over a period of 12 weeks was the principal objective of this study, these relatively stable patient-dependent determinants of plasma CRP were not considered in the present analysis.

Only a limited number of investigations have addressed the influence of HD-related factors on the APR in clinical bicarbonate HD. Recently, we published an intra-dialytic study comparing both cuprammonia (CU), CU with a bacterial filter (CU_f) and PS dialyzers [8]. Plasma IL-6 and CRP were assessed both before, at the end, and 24 hours after HD. From this study it appeared that, whereas PS induced no changes at all, both CU and Cu_f induced a marked rise in IL-6 at t_{180}, which correlated with an increase in plasma CRP after 24 hours. Comparable data were recently published in a crossover analysis of stable HD patients [9]. According to this report the HD-induced inflammatory reaction was markedly affected by the type of material, CU eliciting higher CRP levels than dialyzers with the synthetic membranes polyamide and polycarbonate. Of note, the degree of bacterial contamination of the dialysate in the latter study was comparable with the standard dialysate in the present analysis: 20.5 ± 5.8 and 26 (range 0 to 310) CFU/mL, respectively. Likewise, in a study of chronic HD patients who were treated with PS membranes only, plasma CRP levels were independent to the presence of a bacterial filter, whereas there was a large difference in the degree of contamination between ultra-pure and non-filtered dialysate (0 and 95 CFU/mL, respectively; range 14 to 1000 CFU/mL) [14]. Our findings are in line

with the latter two clinical investigations, suggesting that low to moderate dialysate contamination has no marked influence on CRP levels in chronic HD patients.

As mentioned earlier, back-transport of ETX, as measured by the degree of LAL-positivity in both the dialysate and plasma, was not observed in a previous study on super-flux dialyzers comparing standard with ultrapure dialysate [22]. Moreover, with respect to preliminary data on the cytokines interleukin-1β and tumor necrosis factor α in plasma, differences were not observed between these modalities (abstract; Van Tellingen A, *Neth J Med* 54:A23, 1999). Taking these data together, our findings do not support the concept that CIS, including LAL-positive material, exotoxins and outer membrane proteins (peptidoglycans, muramylpeptides), permeate the membrane of the dialyzer and elicit relevant monocyte activation in clinical practice. Therefore, it seems justified to conclude that both synthetic and modified cellulosic super-flux dialyzers are safe for clinical use, at least during bicarbonate HD with relatively low levels of dialysate contamination.

Thus far, with respect to HD-induced micro-inflammation, clinical studies have suggested that the APR is influenced mainly by the choice of the dialyzer, CU eliciting higher values than both synthetic and modified cellulosic materials. Previously, it had been shown that CRP levels are not only elevated in chronic HD patients, but also in individuals with end-stage renal disease not yet on dialysis and in patients who are treated with peritoneal dialysis [28, 29]. Moreover, our current analysis as well as several other studies have shown that a substantial number of chronic HD patients exhibit normal CRP levels [30]. Therefore, with the exception of HD treatment with CU dialyzers, it is highly doubtful that the contact between blood and the dialyzer or the exposure of blood to the dialysate is the main inducer of the APR in this patient group.

As for other HD-related inducers of the APR, it has been suggested that the presence of indwelling catheters and artificial grafts may play a substantial role [31]. However, to date it has never been clearly demonstrated that intercurrent clinical events have such a profound influence on plasma CRP levels in chronic HD patients. As these patients are prone to develop various direct and indirect HD-related complications regularly, the appearance of intercurrent clinical events may substantially contribute to the chronic micro-inflammatory state in these individuals.

To summarize, in this long-term study neither the type of dialyzer, nor the bacterial quality of the dialysate predicted CRP values in chronic HD patients. In fact, only the variable "clinical events" correlated with alterations in plasma CRP levels in this patient group. Whether changes in CRP values, which are associated with intercurrent clinical events, influence the long-term prognosis of chronic HD patients remains to be established.

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