

# Biocompatibility of heparin-grafted hemodialysis membranes: Impact on monocyte chemoattractant protein-1 circulating level and oxidative status

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## Abstract

This prospective observational study aimed at evaluating efficacy and biocompatibility performances of the new heparin-coated Evodial dialyzers with/without systemic heparin reduction. After a 4-week wash-out period with reference polysulfone F70S dialyzers, 6 hemodialysis patients were sequentially dialyzed with Evodial, F70S, and Evodial dialyzers using 30% heparin reduction, each period of treatment was 4 weeks. Removal rates (RR) (urea, creatinine, and  $\beta$ 2-microglobulin), dialysis dose, and instantaneous clearances (urea and creatinine) were measured as well as inflammatory (C-reactive protein, fibrinogen, interleukin 6, tumor necrosis factor  $\alpha$ , and monocyte chemoattractant protein-1) and oxidative stress (OS) (superoxide anion, homocysteine, and isoprostanes) parameters at the end of each study period. Patients treated with Evodial or F70S dialyzers for 4 weeks presented comparable dialysis efficacy parameters including urea and creatinine RR, dialysis dose and instantaneous clearances. By contrast, a significantly lower but reasonably good  $\beta$ 2-microglobulin RR was achieved with Evodial dialyzers. Regarding biocompatibility, no significant difference was observed with inflammation and OS except for **postdialysis monocyte chemoattractant protein-1 which significantly decreased with Evodial dialyzers. Thirty percent heparinization reduction with Evodial dialyzers did not induce any change in inflammation but led to an improvement in OS as demonstrated by a decrease in postdialysis superoxide production and predialysis homocysteine and isoprostane.** This bioactive dialyzer together with heparin dose reduction represents a good trade-off between efficacy and biocompatibility performance (improvement in OS with a weak decrease in efficacy) and its use is encouraging for hemodialysis patients not only in reducing OS but also in improving patient comorbid conditions due to lesser heparin side effects.

**Key words:** Hemodialysis, Evodial, heparin-coated membrane, oxidative stress

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## INTRODUCTION

Hemodialysis (HD) patients have long been known to have an increased bleeding tendency,<sup>1</sup> the origin of which appears to be multifactorial: Uremia-associated bleeding

diathesis, comorbid conditions predisposing the patients to bleeding, and widespread use of medications that affect hemostasis.<sup>2</sup> The common use of heparin for anticoagulation of the extracorporeal circuit during dialysis not only increases bleeding risk but also adds metabolic side effects including platelet activation,<sup>3,4</sup> lipoprotein lipase release with contribution to hypertriglyceridemia<sup>5</sup> and aldosterone suppression with subsequent hyperkalemia<sup>6</sup> in high-risk patients.

For more than 30 years, attempts have been made to synthesize heparin-grafted dialysis membranes as a means to minimize systemic administration of heparin.<sup>7</sup> The earlier attempts were associated with significant heparin leaking and concerns regarding the release of toxic detergents from these membranes. The most recent technologies developed to minimize systematic administration of heparin were based on surface treatment of the AN69 membrane (AN69ST and HeprAN membranes).

AN69ST is a modified AN69 membrane whose surface electronegativity has been neutralized by layering polyethyleneimine. This decrease in electronegativity of the membrane surface in AN69ST improves the binding of the membrane to heparin and use of this dialyzer requires priming with heparin solution at the dialysis unit before use. HeprAN (the membrane of Evodial dialyzer) uses improved AN69ST technology where heparin is grafted to the membrane during the manufacturing process thereby eliminating the need for heparin priming at the dialysis center. This results in better chemical and biological stability of the heparin on the membrane. In addition, the amount of heparin fixed per square meter of membrane is approximately twice that achieved when coating the AN69ST membrane with heparin during priming.

Several studies have reported that reduced doses of systemic heparin were required with heparin-coated AN69ST membranes both in animal models and clinical studies.<sup>8-10</sup> By contrast, to date, only few data are available for the Evodial dialyzer.<sup>11</sup>

The objectives of this study were therefore to evaluate in a prospective observational study (1) the efficacy and

biocompatibility (inflammation and oxidative stress) performances of this new bioactive dialyzer in comparison with a polysulfone reference dialyzer and (2) the potential beneficial effects of Evodial dialyzers in lowering the systemic heparin dose required.

## PATIENTS AND METHODS

### Patients

Six end-stage renal disease patients undergoing maintenance HD in 1 Montpellier dialysis facility were enrolled in this study. Only patients older than 18 years, on treatment with 3 times a week HD for more than 3 months, with a stable anticoagulation scheme, a hemoglobin level > 10.5 g/dL, and vascular access allowing a stable blood flow of 300 mL/min during treatment, were recruited after giving written informed consent. Patients were excluded if they had malignancy, symptoms or signs of acute/chronic inflammatory or infectious diseases, oral anticoagulant drugs, antibiotics or immunosuppressive treatments, or allergy to heparin. The study was conducted according to the principles of the Declaration of Helsinki and in compliance with International Conference on Harmonization/Good Clinical Practice regulations. According to the French Law, the study has been approved by our institutional ethical committee with the following number 2007-A00613-50.

### Study design, membranes, and dialysis conditions

This is a pilot observational and sequential “N-of-one” study, each patient being their own control.

Design of the study is described in Figure 1. Briefly, after a 4-week wash-out period with reference dialyzer F70S (polysulfone membrane, effective membrane surface area: 1.6 m<sup>2</sup>; Fresenius Medical care, Bad Homburg, Germany), all patients were sequentially dialyzed for 4 weeks with Evodial 1.6 dialyzers (HeprAN membrane,



**Figure 1** Design of the study. After a 4-wk wash-out period with reference dialyzer F70S (polysulfone membrane, effective membrane surface area: 1.6 m<sup>2</sup>; Fresenius Medical Care), all the patients were sequentially dialyzed with the Evodial 1.6 dialyzer (HeprAN membrane, effective membrane surface area: 1.65 m<sup>2</sup>, Hospal SAS), the F70S dialyzer and finally with the Evodial 1.6 dialyzer with a 30% reduction in heparin dose. Each period of treatment was 4 wk.

effective membrane surface area: 1.65 m<sup>2</sup>, Hospal SAS, Meyzieu, France), for 4 weeks with F70S dialyzers, and finally for 4 weeks with Evodial 1.6 dialyzers with a 30% reduction in heparin dose.

Dialysis conditions remained unchanged for each patient: 3 sessions/wk, 3 to 4 h/session, with a blood flow (QB) of 350 to 400 mL/min, ultrapure bicarbonate buffered dialysate, dialysate flow (QD) of 500 mL/min. The routine anticoagulation protocol consisted of a single bolus intravenous administration of low molecular weight heparin 2 to 3 minutes before launching the dialysis session.

## Sampling

Blood samples were drawn before the start of the midweek HD session from the arterial line, after 60 minutes of HD from the arterial and venous lines, and finally after HD.

First sampling was performed at baseline (week 0, corresponding to the end of the wash-out period). Thereafter, samples were drawn at the end of each study period (after 4, 8, and 12 weeks of treatment from baseline) (see Figure 1).

Removal rates as percentage reduction rates (urea, creatinine, and  $\beta$ 2-microglobulin [ $\beta$ 2M]), dialysis dose (Kt/V), and instantaneous clearances of urea and creatinine were measured as well as inflammatory parameters (including high-sensitive C-reactive protein [CRP], fibrinogen, interleukin 6 [IL-6], tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ], and monocyte chemoattractant protein-1 [MCP-1]), and oxidative stress (superoxide anion production, homocysteine, and isoprostanes).

## Laboratory parameters

Serum creatinine and urea were measured by the enzymatic method (Olympus apparatus, Rungis, France) using reagents from Randox (Randox, Mauguio, France).<sup>12</sup>  $\beta$ 2M and high-sensitive CRP were determined by immunoturbidimetry method (Olympus apparatus, Rungis, France). Fibrinogen was measured by immunonephelometry using Von Clauss method (STA Fibrinogen, Diagnostica Stago, Asnières, France). Cytokines including IL-6, TNF- $\alpha$ , and MCP-1, were determined by Protein Biochip Array Technology with chemiluminescence detection (Randox Investigator, Mauguio, France).<sup>13</sup> Homocysteine was measured using a commercial Recipe HPLC analytical kit (Recipe Chemicals & Instruments, Munich, Germany). Plasma isoprostanes were measured using gas chromatography-mass spectrometry.<sup>14</sup> Basal and PMA-stimulated superoxide (O<sub>2</sub><sup>-o</sup>) anion production in whole blood were determined by the chemiluminescence method as reported previously.<sup>15</sup>

## Expression of results and statistical analyses

Data were expressed as mean  $\pm$  standard deviation for normally distributed variables and as median [minimum–maximum] for non-normally distributed variables.

The comparisons of variables between periods of treatment and between predialysis and postdialysis were performed using Wilcoxon signed rank test.

Values were considered statistically significant at  $P < 0.05$ . All analyses were carried out with SAS software, version 9.1 (SAS Institute, Cary, NC, USA).

## RESULTS

### Characteristics of the patient population at inclusion

General characteristics for the 6 HD patients are summarized in Table 1.

Sex ratio of the patients was 4/2 (male/female), median age was 73.2 with a range of 51.8 to 79.7 years.

Causes of CKD were glomerulonephritis (n=2), cystic renal disease (n=1), diabetic nephropathy (n=1), and angiosclerosis and hypertensive nephropathy (n=1), infectious/obstructive interstitial nephropathy (n=1).

### Systemic heparin dose administered during the study

Low molecular weight heparin dose administered during the 4 periods of study was as follows: 44.3 [27.5–65.0] (F70S), 44.4 [26.8–64.2] (Evodial), 44.3 [27.1–63.8] (F70S), and 31.0 [22.2–47.8] (Evodial with 30% heparin reduction) IU/kg/session.

### Comparisons between Evodial dialyzers (100% heparin dose) and F70S dialyzers after 4 weeks of treatment

*Parameters of dialysis efficacy were not significantly different between dialyzers except for  $\beta$ 2M reduction rate*

As shown in Table 2, patients treated with Evodial (100% heparin dose) or F70S dialyzers for 4 weeks did not present significant differences in terms of dialysis efficacy parameters including urea and creatinine reduction rates, urea Kt/V, and instantaneous urea and creatinine clearances. By contrast, a significantly lower but reasonably good  $\beta$ 2M reduction rate was achieved with Evodial dialyzers (55.7 [37.3–73.9] vs. 71.0 [58.8–75.6] % in Evodial vs. F70S dialyzers, respectively;  $P = 0.03$ ).

**Table 1** General characteristics of the hemodialysis patients at inclusion

| Variables   | N | %    | Median [minimum–maximum] |
|---|---|------|--------------------------|
| No. of patients   | 6 |      |                          |
| Gender (male)   | 4 | 66.6 |                          |
| Age (years)   |   |      | 73.2 [51.8–79.7]         |
| Origin of chronic kidney disease                          |   |      |                          |
| Glomerulonephritis  | 2 | 33.3 |                          |
| Cystic renal disease                                      | 1 | 16.7 |                          |
| Diabetic nephropathy                                      | 1 | 16.7 |                          |
| Angiosclerosis and hypertensive nephropathy               | 1 | 16.7 |                          |
| Infectious/obstructive interstitial nephropathy           | 1 | 16.7 |                          |
| Dialysis vintage years                                    |   |      | 3.9 [1.4–8.8]            |
| Patients with diabetes                                    | 1 | 16.7 |                          |
| Patients with current hypertension                        | 1 | 16.7 |                          |
| Patients with baseline coronary heart disease             | 2 | 33.3 |                          |
| Patients with baseline peripheral atherosclerotic disease | 1 | 16.7 |                          |
| Dry body weight (kg)                                      |   |      | 58.5 [41.9–81.0]         |
| Hemoglobin (g/dL)   |   |      | 12.1 [11.0–12.8]         |

Values were described by using proportions for categorical variables and median [minimum–maximum] for quantitative variables.

No significant difference was observed with parameters of inflammation and oxidative stress

Table 3 depicts the evolution of inflammatory (A) and oxidative stress (B) parameters when using reference F70S or Evodial dialyzers for 4 weeks. No significant difference was observed between dialyzers regarding levels of IL-6, TNF- $\alpha$ , CRP, fibrinogen (Table 3A), O<sub>2</sub>-<sup>o</sup> production, isoprostanes, and homocysteine (Table 3B) both before and after the session. A tendency to intradialytic isoprostane increase was observed with polysulfone dialyzers at week 0 (intradialytic increase: 63.1 [–7.1 to 420.5] %; P=0.075), such an increase being further significantly confirmed at week 8 (intradialytic increase: 29.4 [–2.5 to 91.1] %; P=0.046) (pool of week 0 and week 8: Intradialytic increase: 46.7 [–7.1 to 420.5] %; P=0.0096).

By contrast, use of Evodial during 4 weeks was associated with a decrease in postdialysis MCP-1 level

Indeed, treatment for 4 weeks with Evodial dialyzers was associated with a significant decrease in postdialysis MCP-1 (237.9 [124.7–405.0] vs. 281.6 [181.3–561.1] pg/mL in Evodial vs. F70S dialyzers; P=0.03), whereas no significant difference was observed in predialysis levels (see Figure 2).

### Comparisons between Evodial (100% heparin dose) and Evodial (30% heparin dose reduction) after 4 weeks of treatment

No change in inflammatory parameters (Table 4) was observed after 4 weeks of treatment with Evodial dialyzers

**Table 2** Comparisons of dialysis efficacy parameters between Evodial and F70S dialyzers after 4 wk of treatment

| Variables                                   | Reference F70S (week 0) | Evodial 1.6 (week 4) | P           |
|---|-------------------------|----------------------|-------------|
| Urea reduction rate (%)                     | 73.5 [64.6–84.7]        | 72.0 [64.7–80.6]     | NS          |
| Creatinine reduction rate (%)               | 72.7 [61.0–89.4]        | 71.4 [64.0–79.0]     | NS          |
| Predialysis $\beta$ 2M (mg/L)               | 26.7 [19.2–30.7]        | 29.6 [22.3–33.0]     | NS          |
| Postdialysis $\beta$ 2M (mg/L)              | 9.4 [5.7–12.8]          | 14.5 [8.9–25.4]      | <b>0.03</b> |
| $\beta$ 2M reduction rate (%)               | 71.0 [58.8–75.7]        | 55.7 [37.3–73.9]     | <b>0.03</b> |
| Urea Kt/V Garred                            | 1.7 [1.3–2.9]           | 1.7 [1.4–2.1]        | NS          |
| Instantaneous urea clearance (mL/min)       | 270.9 [195.8–292.0]     | 249.7 [229.9–256.1]  | NS          |
| Instantaneous creatinine clearance (mL/min) | 200.3 [143.1–236.7]     | 197.4 [180.6–216.8]  | NS          |

Bold values indicate statistical significance of P value.  $\beta$ 2M=beta2-microglobulin; NS=not significant.

**Table 3** Comparisons of inflammatory (A) and oxidative stress (B) parameters between Evodial and F70S dialyzers after 4 wk of treatment

| Variables  | Pre/post HD | Reference F70S (week 0) | Evodial 1.6 (week 4) | P  |
|--|-------------|-------------------------|----------------------|----|
| <b>(A)</b>                                       |             |                         |                      |    |
| IL-6 (pg/mL)                                     | Pre         | 2.3 [1.3–5.7]           | 3.9 [0.5–7.9]        | NS |
|  | Post        | 4.0 [1.4–9.2]           | 5.2 [1.1–6.1]        | NS |
| TNF- $\alpha$ (pg/mL)                            | Pre         | 4.5 [3.6–7.2]           | 4.7 [3.8–6.8]        | NS |
|  | Post        | 2.9 [1.4–4.5]           | 3.7 [2.2–4.7]        | NS |
| CRP (mg/L)                                       | Pre         | 1.8 [0.4–8.6]           | 3.0 [0.3–45.3]       | NS |
|  | Post        | 2.0 [0.7–9.9]           | 3.2 [0.3–40.9]       | NS |
| Fibrinogen (g/L)                                 | Pre         | 3.4 [2.8–4.8]           | 3.6 [3.1–5.1]        | NS |
|  | Post        | 3.7 [2.9–5.2]           | 4.2 [3.3–5.6]        | NS |
| <b>(B)</b>                                       |             |                         |                      |    |
| O <sub>2</sub> <sup>-•</sup> (counts/leukocytes) |             |                         |                      |    |
| Basal production                                 | Pre         | 0.3 [0.2–0.5]           | 0.3 [0.2–0.4]        | NS |
|  | Post        | 0.3 [0.2–0.4]           | 0.3 [0.2–0.4]        | NS |
| PMA-stimulated production                        | Pre         | 0.5 [0.3–0.7]           | 0.4 [0.3–0.6]        | NS |
|  | Post        | 0.5 [0.4–0.7]           | 0.5 [0.3–0.7]        | NS |
| % PMA activation                                 | Pre         | 54.6 [41.7–121.4]       | 58.9 [30.4–136.7]    | NS |
|  | Post        | 54.8 [27.9–104.3]       | 50.7 [12.5–136.7]    | NS |
| Homocysteine ( $\mu$ mol/L)                      | Pre         | 38.2 [28.0–44.0]        | 37.2 [27.7–45.0]     | NS |
|  | Post        | 20.0 [12.4–24.0]        | 20.3 [14.5–26.9]     | NS |
| Isoprostanes (pg/mL)                             | Pre         | 151 [20.8–686.7]        | 146.9 [100.0–737.3]  | NS |
|  | Post        | 213.8 [108.4–638.1]     | 161.3 [93.6–698.5]   | NS |

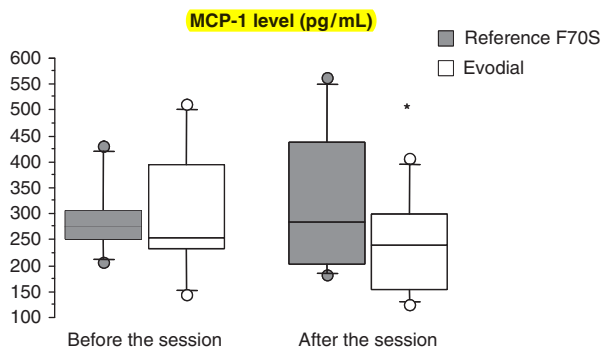
CRP=C-reactive protein; IL-6=interleukin 6; NS=Non significant (P>0.05); PMA=phorbol myristate acetate; TNF- $\alpha$ =tumor necrosis factor  $\alpha$ .

using a 30% heparin dose reduction compared with 100% heparin dose.

Interestingly, the use of Evodial dialyzers with heparin dose reduction led to an improvement in oxidative status as

demonstrated by a decrease in postdialysis PMA-stimulated O<sub>2</sub><sup>-•</sup> production and subsequent intradialytic variation (see Figure 3A and B). Similarly, such a reduction in heparin dose was also accompanied by a significant decrease in predialysis homocysteine (see Figure 3C) and isoprostane (see Figure 3D) levels after 4 weeks of treatment.

Finally, no change in dialysis efficacy parameters including creatinine and  $\beta$ 2M reduction rates and urea and creatinine instantaneous clearances, was observed with Evodial dialyzers using 100% heparin dose vs. 30% heparin dose reduction. By contrast, a significantly lower urea reduction rate and Kt/V were achieved with 30% heparin dose reduction (urea reduction rate=68.6 [58.7–76.8] vs. 72.0 [64.7–80.6] % using 30% heparin reduction vs. 100%; P=0.043) (urea Kt/V=1.5 [1.2–1.9] vs. 1.7 [1.4–2.1] using 30% heparin reduction vs. 100%; P=0.027).



**Figure 2** MCP-1 level after 4 wk of treatment with Evodial dialyzers compared with F70S dialyzers. Patients were dialyzed sequentially (4-wk period each) with reference dialyzer (F70S) and Evodial dialyzer. Level of MCP-1 cytokine was evaluated before and after the session at each end of treatment period. No significant difference was observed in MCP-1 before the sessions with F70S and Evodial dialyzers. By contrast, treatment during 4 wk with Evodial dialyzers was associated with a decrease in postdialysis MCP-1 (\*P<0.05 vs. reference F70S).

## DISCUSSION

This prospective observational study aimed at evaluating (1) the efficacy and biocompatibility performances of the new heparin-coated Evodial dialyzer using the F70S dialyzer with a polysulfone membrane with equal effective membrane surface area as the reference dialyzer and

**Table 4** Comparisons of inflammatory parameters using Evodial dialyzers with 100% heparin dose vs. 30% heparin dose reduction after 4 wk of treatment

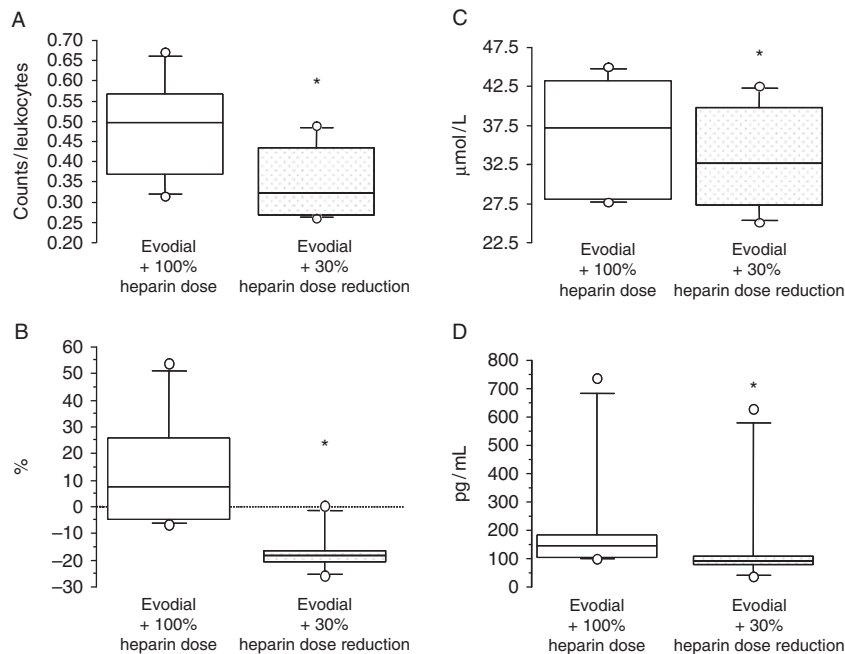
| Variable              | Pre/post HD | Evodial 1.6+100% heparin dose (week 4) | Evodial 1.6+ 30% heparin dose reduction (week 12) | P  |
|-----------------------|-------------|--|---|----|
| IL-6 (pg/mL)          | Pre         | 3.9 [0.5–7.9]                          | 1.9 [1.1–21.0]                                    | NS |
|                       | Post        | 5.2 [1.1–6.1]                          | 3.1 [1.4–16.7]                                    | NS |
| TNF- $\alpha$ (pg/mL) | Pre         | 4.7 [3.8–6.8]                          | 5.0 [3.7–6.6]                                     | NS |
|                       | Post        | 3.7 [2.2–4.7]                          | 3.4 [3.0–4.8]                                     | NS |
| CRP (mg/L)            | Pre         | 3.0 [0.3–45.3]                         | 3.0 [0.3–58.3]                                    | NS |
|                       | Post        | 3.2 [0.3–40.9]                         | 3.4 [0.3–64.3]                                    | NS |
| Fibrinogen (g/L)      | Pre         | 3.6 [3.1–5.1]                          | 3.7 [2.8–5.6]                                     | NS |
|                       | Post        | 4.2 [3.3–5.6]                          | 3.9 [3.0–6.2]                                     | NS |

CRP=C-reactive protein; IL-6=interleukin 6; NS=Non significant ( $P > 0.05$ ); TNF- $\alpha$ =tumor necrosis factor  $\alpha$ .

(2) the potential beneficial effects of Evodial dialyzers in lowering systemic heparin dose.

Our results showed that the dialysis efficacy performances of Evodial dialyzers were comparable with those with the polysulfone dialyzers. Indeed, we did not observe any difference between Evodial and F70S dialyzers regarding dialysis dose, urea, and creatinine reduction

rates and finally instantaneous clearances. A lower (but reasonably good for HD)  $\beta$ 2M reduction rate was achieved with the bioactive dialyzer. However, predialysis  $\beta$ 2M levels were not significantly different between dialyzers and were close to the cut-off value from which  $\beta$ 2M predicts the all-cause mortality described previously in the HEMO study.<sup>16</sup>



**Figure 3**  $O_2^{\circ}$  production after 4 wk of treatment with Evodial dialyzers using 100% heparin dose vs. 30% heparin dose reduction. Patients were dialyzed sequentially (4-wk period each) with Evodial dialyzers using 100% heparin dose or 30% heparin dose reduction. Whole blood  $O_2^{\circ}$  production and plasma homocysteine and isoprostanes levels were evaluated at each end of the treatment periods. A decrease in (A) postdialysis and (B) subsequent intradialytic variation of PMA-stimulated  $O_2^{\circ}$  production, (C) homocysteine, and (D) isoprostanes levels was observed with a 30% reduction in heparin dose (\*  $P < 0.05$  vs. Evodial 100% heparin dose).

In addition, no cellular reactivity with inflammatory and oxidative stress parameters was observed using Evodial compared with F70S dialyzers, attesting to the comparable biocompatibility of these 2 membranes. It is noteworthy that most of the tested parameters were not modified during the sessions performed with either dialyzers, which clearly shows the excellent biocompatibility profile achieved with today's dialysis procedure. Exploration of bioincompatibility phenomena now requires the use of more sensitive biomarkers. Our data showing an increase in intradialytic isoprostanes with the reference polysulfone membrane suggest that molecule should be evaluated as a new microbioincompatibility marker in exploring oxidative stress. Our results could also demonstrate a beneficial effect of Evodial dialyzers on clearance of MCP-1, whereas a tendency to intradialytic increase was observed with F70S dialyzers. The latter result corroborates data in the literature and in particular the study from Papayianni et al.<sup>17</sup> who reported increased intradialytic MCP-1 levels with polysulfone dialyzers, probably resulting from either inadequate clearance or enhanced synthesis and release. Its molecular weight of 16.3 kDa and isoelectric point of 9.81 strongly suggest an adsorption onto Evodial dialyzers explaining decreased levels after the session. This chemotactic cytokine produced by endothelial cells after exposure to proinflammatory cytokines or oxidized lipoproteins plays an important role in the migration and activation of monocytes and T cells. It also regulates the proliferation of vascular smooth muscle cells suggesting a role of this protein in the pathogenesis of atherosclerotic cardiovascular disease.<sup>18</sup> Decreasing its level during dialysis sessions using Evodial dialyzers may be beneficial in reducing the inflammatory process participating in development of atherosclerotic disease.

Despite diminished efficacy performances, use of Evodial dialyzers with 30% lowering of the dose of systemic heparin was associated with a prevention of oxidative status as demonstrated by a decrease in free radical production and predialysis homocysteine and isoprostane levels. We recently reported that in a population of 478 elderly subjects homocysteine constituted a main determinant of superoxide anion production.<sup>15</sup> In the present study, simultaneous reduction in superoxide and homocysteine levels is therefore not surprising; the decrease in isoprostane level being a consequence of this free radical reduction. By contrast, more surprising is the prevention of oxidative status with heparinization lowering. Indeed, several authors observed antioxidant rather than pro-oxidant properties of heparin. Garcia-de-la-Asuncion et al.<sup>19</sup> recently showed that bemiparin sodium, a new second-generation low molecular weight heparin, exerts

an early and brief beneficial antioxidant effect against oxidative stress in plasma from healthy volunteers: Increases total antioxidant capacity, which coincides with an increase in antioxidant enzyme activity (superoxide dismutase and glutathione peroxidase) and decreases levels of oxidative stress markers such as GSSG and MDA. The authors hypothesize that such a favorable influence exerted by bemiparin might be due, at least in part, to the modulation of antioxidant enzyme activity or also to a direct antioxidant effect. Several authors have reported that the type of heparin was determinant of its antioxidant properties. Indeed, Poyrazoglu et al. revealed that only low molecular weight heparin was able to decrease oxidative stress and inflammation in HD patients, whereas standard heparin exerted pro-oxidant activity.<sup>20</sup> Leitienné et al.<sup>21</sup> could also demonstrate beneficial effects of low molecular weight heparin vs. unfractionated heparin upon granulocytopenic effect in this population. Here, we demonstrated heparin as a pro-oxidant agent increasing superoxide anion production and subsequently oxidative stress markers. Of note, the effect of heparin in activating leukocytes to release superoxide anion observed here corroborates the pro-oxidant effect of this molecule on myeloperoxidase release from activated leukocytes documented in the literature.<sup>22</sup> Our results are also in total agreement with the study from Leitienné et al. regarding a better biocompatible treatment with regard to leukocyte stimulation using lower doses of anticoagulant.<sup>21</sup> This suggests a dual effect of heparin as a prooxidant in increasing free radical production and as an antioxidant in improving enzymatic defenses, the real effect of heparin on subsequent oxidative stress markers varying dependent on the relative effect of pro-oxidant production and antioxidant increase.

In conclusion, we have demonstrated that the new bioactive Evodial dialyzer presents comparable biocompatibility performances with reference polysulfone dialyzers when using 100% heparin dose. Its performances are even higher in term of MCP-1 removal. More interesting is the use of this bioactive dialyzer with heparin dose reduction representing a good trade-off between efficacy and biocompatibility performance (improvement in oxidative stress with a weak decrease in efficacy). Beneficial effects of this dialyzer obtained with heparin lowering are all the more encouraging for HD patients by not only reducing oxidative stress but also by improving patient comorbid conditions due to lesser heparin side effects. In this context, reducing heparin use with such new bioactive membrane would improve biocompatibility and subsequent vascular and metabolic complications, offering novel hope to dialysis patients. Further

large-scale studies are now required to fully investigate the beneficial effects of this bioactive dialyzer, especially in diabetics and other high-risk patients.

The major limitation of this study is the small number of patients. Owing to the opposite effects on oxidative stress observed depending on the nature of heparin administered, the use of unfractionated heparin for systemic anticoagulation should also have been tested.

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