Received: 7 December 2020 | Revised: 22 January 2021 | Accepted: 18 February 2021

DOI: 10.1111/aor.13944

MAIN TEXT ARTICLE

Artificial Organs WILEY

Clinical hemocompatibility of double-filtration lipoprotein apheresis comparing polyethersulfone and ethylene-vinyl alcohol copolymer membranes

Detlef H. Krieter[1] | **Jarline Jeyaseelan**[1] | **Marieke Rüth**[2] | **Horst-Dieter Lemke**[2] | **Christoph Wanner**[1] | **Christiane Drechsler**[1]

[1]Division of Nephrology, Department of Medicine I, University Hospital Würzburg, Würzburg, Germany

[2]eXcorLab GmbH, Obernburg, Germany

Correspondence
Detlef H. Krieter, Division of Nephrology, Department of Medicine I, University Hospital Würzburg, Oberdürrbacher Street 6, D-97080 Würzburg, Germany.
Email: krieter_d@ukw.de

Funding information
3M Deutschland GmbH

Abstract

Activation of the complement system and leukocytes by blood–membrane interactions may further promote arteriosclerosis typically present in patients on lipoprotein apheresis. As clinical data on the hemocompatibility of lipoprotein apheresis are scarce, a controlled clinical study comparing two different types of plasma separation and fractionation membranes used in double-filtration lipoprotein apheresis was urgently needed, as its outcome may influence clinical decision-making. In a prospective, randomized, crossover controlled trial, eight patients on double-filtration lipoprotein apheresis were subjected to one treatment with recent polyethersulfone (PES) plasma separation and fractionation membranes and one control treatment using a set of ethylene-vinyl alcohol copolymer (EVAL) membranes. White blood cell (WBC) and platelet (PC) counts, complement factor C5a and thrombin–antithrombin III (TAT) concentrations were determined in samples drawn at defined times from different sites of the extracorporeal blood and plasma circuit. With a nadir at 25 minutes, WBCs in EVAL decreased to 33.5 ± 10.7% of baseline compared with 63.8 ± 22.0% at 20 minutes in PES ($P < .001$). The maximum C5a levels in venous blood reentering the patients were measured at 30 minutes, being 30.0 ± 11.2 μg/L with EVAL and 12.3 ± 9.0 μg/L with PES ($P < .05$). The highest C5a concentrations were found in plasma after the plasma filters (EVAL 56.1 ± 22.0 μg/L at 15 minutes vs PES 23.3 ± 15.2 μg/L at 10 minutes; $P < .001$). PC did not significantly decrease over time with both membrane types, whereas TAT levels did not rise until the end of the treatment without differences between membranes. Regarding lipoprotein(a) and low-density lipoprotein (LDL) cholesterol removal, both membrane sets performed equally. Compared with EVAL, PES membranes cause less leukocyte and complement system activation, the classical parameters of hemocompatibility of extracorporeal treatment procedures, at identical treatment efficacy. Better hemocompatibility may avoid inflammation-promoting effects through blood–material interactions in patients requiring double-filtration lipoprotein apheresis.

KEYWORDS

biocompatibility, fractionation membrane, hypercholesterolemia, LDL cholesterol, lipoprotein apheresis, lipoprotein(a)

1 | INTRODUCTION

Transient leukopenia in blood together with pulmonary dysfunction in the early minutes of hemodialysis with cellulosic dialysis membranes was first observed in the 1970s, when it could be attributed to the activation of the complement system.[1] These symptoms of relative hemoincompatibility of cellulose membranes were linked to the presence of free hydroxyl groups on the surface, which trigger intense complement system activation via the alternative pathway with the cleavage of several complement products, resulting in fragments C3a and C5a and the assembly of the membrane attack complex C5b-9.[2] The extent of leukocyte and complement system activation are recognized as classical parameters of hemocompatibility. Synthetic dialysis membranes lacking free hydroxyl groups induce much lower complement and cell activation and, therefore, are considered as hemocompatible.[3]

Hemoincompatibility may have consequences for the outcome of patients repeatedly exposed to artificial surfaces. It is long undisputed that inflammation contributes importantly to the initiation and progression of atherosclerosis in the general population.[4] In patients on maintenance hemodialysis, blood–dialysis membrane interactions, particularly complement activation, may further promote chronic inflammation leading to cardiovascular disease, the major cause for the excess mortality and morbidity observed in this population.[5] Blood–membrane interactions are not limited to hemodialysis. Depending on the materials involved, they occur analogously in any kind of extracorporeal procedure, but only in dialysis have these interactions been extensively examined. For double-filtration lipoprotein apheresis, an extracorporeal procedure to remove proatherosclerotic lipoproteins, which has proven to decrease the incidence of cardiovascular events in patients with elevated lipoprotein (a) (Lp(a)),[6,7] the availability of such data is very limited. An ex vivo model passing human whole blood through different lipoprotein apheresis systems demonstrated strong activation of the alternative way of the complement system by ethylene-vinyl alcohol (EVAL) membranes.[8] This observation was confirmed by a very small clinical study on only three patients showing an increase of complement C3a and Bb after lipoprotein apheresis with EVAL.[9]

Interestingly, from our own clinical experience with double-filtration lipoprotein apheresis using synthetic plasma and fractionation membranes based on EVAL, leukocytosis was frequently observed at the end of the treatment. Similar to hemodialysis with cellulosic membranes, this finding must be considered as a typical consequence of leukocyte rebound from pulmonary sequestrated neutrophils returning into blood and recruitment from the marginated pool or bone marrow stores after complement-induced cell activation.[10] This hypothesis is supported by the fact that EVAL, although classified as a synthetic and, hence, hemocompatible membrane, presents hydroxyl groups on its surface and activates the complement system in vitro.[11,12] Furthermore, in a clinical study on hemodialysis, complement activation with EVAL was higher compared with the synthetic, nonhydroxyl groups-presenting membrane AN69 and similar to that of the synthetically modified cellulosic dialysis membrane Hemophan, whereas it was lower with respect to a regenerated cellulosic membrane.[13] Activation of the complement system and leukocytes may further promote arteriosclerosis typically present in patients on lipoprotein apheresis.[5] Therefore, a controlled clinical study investigating the classical parameters of hemocompatibility in double-filtration lipoprotein apheresis was urgently needed, as its outcome may influence clinical decision-making.

2 | PATIENTS AND METHODS

The study was executed in adherence to the Declaration of Helsinki. Study approval was obtained from the Ethics Committee of the University hospital Würzburg (registration no. 12/18). The study was registered at the German Register for Clinical Trials (DRKS00014075). All patients participating in the study gave written informed consent including the approval for publication of the study data.

2.1 | Study design

The present trial compared the hemocompatibility of the EVAL membrane with a recently introduced polyethersulfone (PES)-based membrane during double-filtration lipoprotein apheresis.

The study was prospective, randomized, and controlled in a crossover manner. Eight patients of at least 18 years of age routinely on double-filtration lipoprotein apheresis at two different sites (University Hospital Würzburg and Dialysis Center Elsenfeld, Germany) were eligible for the trial.

2.2 | Study execution

After prerinsing of the extracorporeal circuit with saline according to the machine's routine procedure, each patient was subjected to one treatment with a polyvinylpyrrolidone-blended

polyethersulfone (FPES) plasma (MicroPES TF10, 0.6 m^2, 3M PlasCure 0.6, 3M Deutschland GmbH, Wuppertal, Germany) and a fractionation (FractioPES 200, 1.9 m^2, 3M SelectiCure H19) membrane, and one control treatment using a set of EVAL membranes (0.5 m^2, Asahi Plasmaflo OP-05W; 2.0 m^2, Asahi Cascadeflo EC-50W, Asahi Kasei Medical Co., Ltd., Tokyo, Japan). All filters were gamma-ray sterilized. Double-filtration lipoprotein aphereses were performed with the OctoNova apheresis platform. Intraindividual treatment conditions were kept identical including blood and plasma filtration flow rates, which were always set at 100 and 30 mL/min, respectively. Anticoagulation with standard heparin and target plasma volumes to be treated were unchanged adopted from the patients' routinely used regimen.

2.3 | Sampling

At baseline, after 5, 10, 15, 20, 25, 30, and 60 minutes as well as at the end of the treatments, samples were drawn at different sites of the extracorporeal blood and plasma circuit (Figure 1). Due to the machine presetting, plasma filtration was initiated 5 minutes after the blood pump had been started to fill the blood circuit. Therefore, except for the samples at 60 minutes and at the end of treatment, sampling from the plasma circuit was accordingly adapted, that is, it was done 5 minutes later.

2.4 | Analytical methods

For each substance, the mean value of a repeat determination was provided. To assess hemocompatibility, white blood cell (WBC) and platelet (PC) counts were quantified with an ABX Pentra 60 cell counter (Agon Lab AG, Reichenbach/Stuttgart, Germany). Complement factor C5a (DRG C5a ELISA, DRG Instruments GmbH, Marburg, Germany) and thrombin–antithrombin III (TAT) (Enzygnost TAT micro, Siemens Healthcare Diagnostics Products GmbH, Erlangen, Germany) were measured by ELISA.

Determination of treatment efficacy was performed by calculating reduction rates of the serum concentrations of low-density lipoprotein (LDL), Lp(a), cholesterol, triglycerides, high-density lipoprotein (HDL), fibrinogen, and albumin based on samples drawn from the patients' arterial blood at baseline (C_0) and at the end (C_f) of the therapy, respectively. To account for extracellular volume changes, C_f was corrected based on the differences in the baseline and post-treatment hematocrits. The reduction rates (RR) were calculated according to the equation[14]:

$$RR = \frac{C_0 - C_f}{C_0} \times 100\,[\%]\,.$$

2.5 | Data analysis

For descriptive analysis of the results, mean values ± standard deviations were calculated. Within-subject between-treatment differences were analyzed by paired t-test if data were normally distributed and by Wilcoxon–Mann–Whitney test if not normally distributed. Within-subject within-treatment changes from baseline were analyzed in series by analysis of variance (ANOVA) and a Tukey post hoc test for normally distributed samples. The Friedman

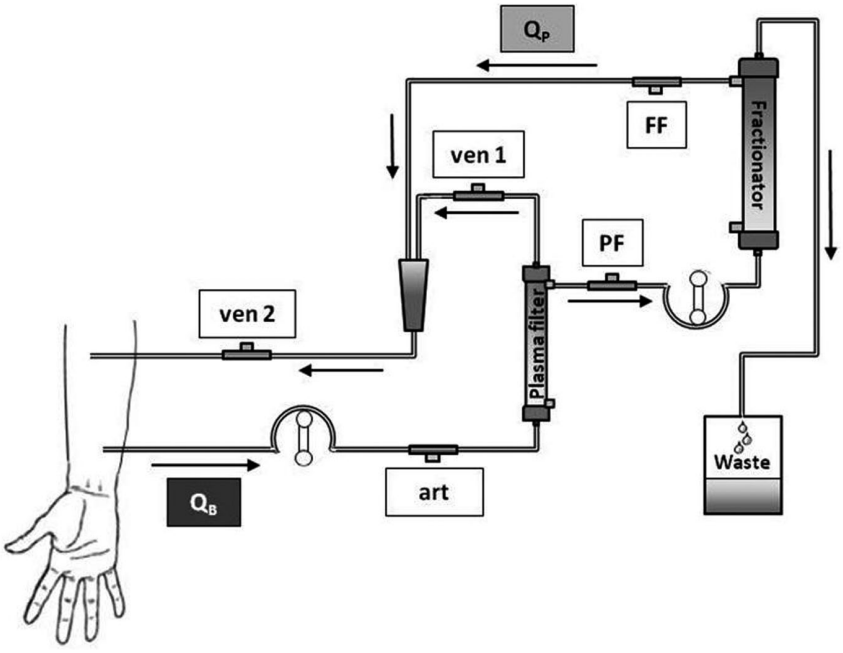

FIGURE 1 Flow diagram of double filtration lipoprotein apheresis indicating the sampling sites used during the trial. Sampling sites were "art" (patient blood entering the plasma filter), "PF" (plasma leaving the plasma filter), "FF" (dead-end filtered plasma leaving the fractionator), "ven1" (hemoconcentrated blood leaving the plasma filter), and "ven2" (blood after adding of the processed plasma before reinfusion into the patient). Q_B, blood flow; Q_P, plasma flow

test was used if normal distribution did not apply. A P value of <.05 was considered statistically significant. The statistical analysis was performed with the "Minitab 17 Statistical Software" package (Minitab, Inc., State College, PA, USA).

3 | RESULTS

3.1 | Patient characteristics

Seven males with isolated Lp(a) hyperlipoproteinemia, all affected by cardiovascular disease, and one female with homozygous hypercholesterolemia type IIa without evidence for vascular lesions were enrolled. Mean age was 51.3 ± 12.0 years (54 years; range 26 to 66 years). The patients were previously for 64.9 ± 65.5 months (median 50 months; range 3 to 195 months) on once weekly or biweekly lipoprotein apheresis. Their body mass index was 25.6 ± 3.3 kg/m^2. Of the seven patients with cardiovascular disease, six suffered from three-vessel and one from two-vessel coronary artery disease. Four patients had a history of myocardial infarction, four had aortocoronary bypass operations, and six patients had received percutaneous coronary interventions. Two patients had an arteriovenous fistula for blood access, whereas in six patients, lipoprotein apheresis was performed via the cubital veins of both arms.

3.2 | Treatment parameters

Blood flow rates during treatment with EVAL and FPES were 101 ± 4 and 100 ± 0 mL/min, respectively. Plasma flow rates averaged at 29 ± 1 and 29 ± 1 mL/min. The treatments lasted 117 ± 18 and 116 ± 22 minutes obtaining mean processed plasma volumes of 3,370 ± 523 and 3,314 ± 598 mL for EVAL and FPES, respectively. No significant differences were noted.

3.3 | Hemocompatibility parameters

The effect of the two membrane types on leukocyte activation was clearly different (Figure 2). Being already lowered at 5 minutes (P < .001), the WBCs in EVAL reached a nadir at 25 minutes with 33.5 ± 10.7% of baseline. A reduction of WBCs with PES was noted first at 10 minutes (P < .001) with the lowest value of 63.8 ± 22.0% at 20 minutes. The nadirs with the membranes were highly different (P < .001), but differences between EVAL and PES were also observed between 15 and 30 minutes at the same time (P < .05). At the end of apheresis, WBC count with PES normalized in the range of the baseline values (102.2 ± 19.7%), being much lower compared with EVAL (130.4 ± 21.0%; P < .001) (Figure 2). Differential count of the leukocytes revealed that the course of WBCs was primarily determined by the activation of neutrophils. The lowest (15.7 ± 12.1% of baseline) and highest

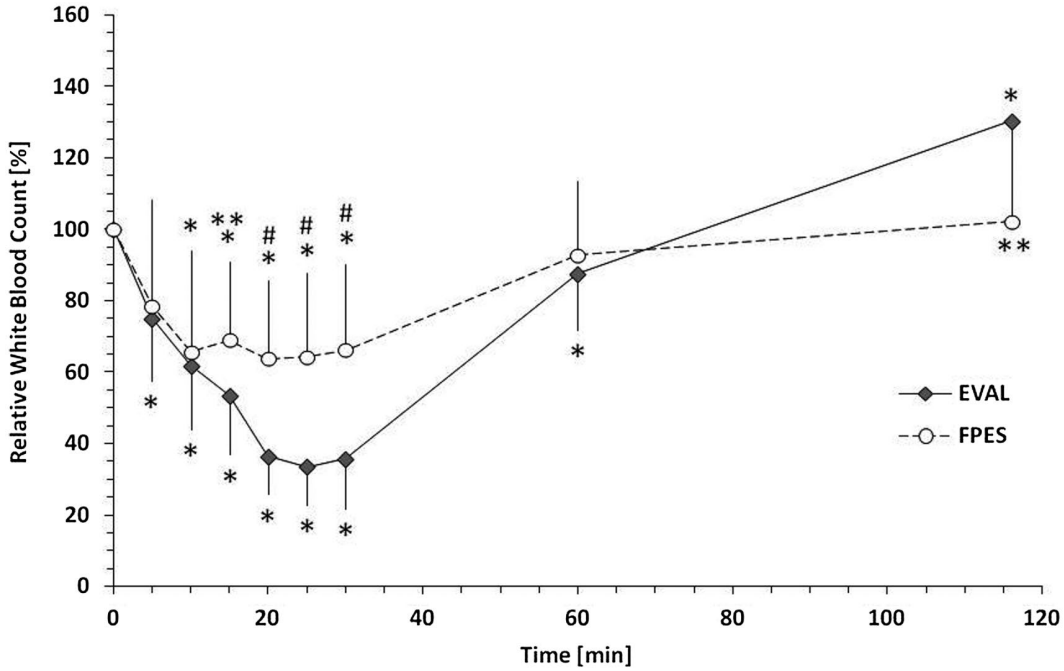

FIGURE 2 Course of the white blood cell count in arterial blood relative to baseline during lipoprotein apheresis with FPES and EVAL. The figure reflects mean values ± standard deviations. *P < .001 versus 0 minute; **P < .05 versus EVAL; #P < .01 versus EVAL. EVAL, ethylene-vinyl alcohol; FPES, polyvinylpyrrolidone-blended polyethersulfone

counts (145.3 ± 35.5%) with EVAL paralleled the WBC count at 25 minutes and at the end of the treatment. This finding was also true with PES, in which neutrophil counts were 53.2 ± 12.1% and 101.1 ± 21.5% of baseline at 20 minutes and at the end, respectively (no further data reported).

Regarding the relative PC, the membranes performed almost identically. After an initial drop at 5 minutes to 93.6 ± 4.3% and 95.9 ± 4.4% of baseline with EVAL and PES, respectively, the PC decreased slowly, but continuously over time with both membrane types to finish at 91.0 ± 6.2 and 91.2 ± 4.6%, respectively.

Compared with the baseline values of 0.06 ± 0.02 μg/L, C5a in blood after the plasma filter and in venous blood reentering the patient started to rise from 5 minutes on with both filter sets ($P < .001$) (Table 1). With EVAL, the maximum in venous blood was reached at 30 minutes with 30.0 ± 11.2 μg/L, whereas, with PES, it was 59% lower and noted at the same time (12.3 ± 9.0 μg/L; $P < .05$). C5a levels in venous blood further declined during the treatment, remaining significantly elevated at the end with both EVAL and PES (12.5 ± 7.6 and 10.8 ± 3.8 μg/L, respectively). The highest C5a concentrations were determined in the plasma circuit immediately after the plasma filters with both sets of membranes. At this location, C5a had a maximum of 56.1 ± 22.0 μg/L with EVAL at 15 minutes and was significantly higher compared with PES (23.3 ± 15.2 μg/L at 10 minutes; $P < .05$). Plasma passage through the fractionation filters did not further increase C5a levels, but the maxima were reached 20 minutes later at 35 minutes with EVAL (50.6 ± 19.4 μg/L; $P < .01$ vs. PES) and at 30 minutes with PES (18.7 ± 14.8 μg/L; $P < .05$ vs. EVAL).

TAT levels did not rise before the end of the treatment without differences between membranes (refer to Figure 3). Compared with the baseline level of 3.6 ± 2.9 μg/L, TAT concentrations in venous blood and in plasma after the plasma filter at the end of the therapy with EVAL were increased to 80.4 ± 210.0 and 89.5 ± 206.7 μg/L ($P < .01$), respectively. With PES, TAT behaved similarly and rose to 25.8 ± 49.2 and 38.9 ± 35.8 μg/L (each $P < .05$ vs. baseline, 4.4 ± 3.8 μg/L). No change in TAT concentrations was observed in plasma after passage of the fractionators.

3.4 | Treatment efficacy

Regarding treatment efficacy, both membrane sets performed equally. Reduction rates of the target lipoproteins Lp(a) (−69.5 ± 6.0 vs. −69.8 ± 5.7%) and LDL (−65.2 ± 6.9 vs. −64.9 ± 8.8%) as well as of fibrinogen (−48.8 ± 4.3 vs. −46.8 ± 5.6%) were almost identical with EVAL and PES. The same was true for the retention of HDL (−18.2 ± 7.3 and −17.4 ± 13.6%, respectively) and albumin (−15.6 ± 6.6 and −13.4 ± 5.5%, respectively) (Figure 4).

4 | DISCUSSION

Although, recently developed second generation antisense oligonucleotides targeting apolipoprotein(a) represent a promising future option,[15-17] lipoprotein apheresis is the only treatment with proven benefits on the incidence of cardiovascular events in patients suffering from Lp(a) hyperlipoproteinemia, progressive cardiovascular disease, and maximized lipid-lowering medication to date.[6,7] A positron emission tomography study on patients with familial hypercholesterolemia attributed the beneficial outcome of lipoprotein apheresis to anti-inflammatory effects on arterial wall inflammation through the reduction of atherogenic apoprotein B-containing lipoproteins.[18] It is obvious that inflammation-promoting effects through blood–material interactions during therapy may cause the opposite. Already decades ago, complement system and leukocyte activation exerted by cellulosic dialysis membranes in patients on maintenance hemodialysis were regarded as a major cause for chronic inflammation and cardiovascular disease.[5,19,20] In double-filtration lipoprotein apheresis, the effects of different plasma separation and fractionation membranes on the classical parameters of biocompatibility have not even been investigated in a controlled clinical study.

Significant complement system activation in lipoprotein apheresis is known from heparin-induced extracorporeal LDL-precipitation, in which it is induced by the plasma separation membrane.[21] Depending on the membrane material used, the generation of activated complement factors during this therapy form differed. Treatments with polyethylene or polymethylmethacrylate filters led to very high concentrations of complement C3a and were accompanied by transient granulocytopenia, whereas polypropylene and polysulfone membranes caused less complement activation without transient leukocytopenia.[22] Strong activation of the alternative way of the complement system by synthetic EVAL plasma separation membranes was demonstrated in an ex vivo model of lipoprotein apheresis using both a dextran sulfate adsorber column and a membrane fractionator for the elimination of lipoproteins from separated plasma.[8] A very small, uncontrolled clinical study on only three patients demonstrated highly increased complement C3a and Bb plasma concentrations after lipoprotein apheresis with a set of EVAL plasma separator and fractionator. Unfortunately, as values were determined exclusively before and after treatment, the study examined neither the kinetics of activated complement nor the impact of the two membranes differing considerably in surface area.[9]

The present trial not only investigated the individual effect of the two serial filters of double-filtration lipoprotein apheresis on the classical parameters of hemocompatibility. In a randomized, crossover controlled approach, a set of EVAL membranes, which were already introduced

TABLE 1 Time course of complement factor C5a plasma levels drawn at different sites of the extracorporeal blood and plasma circuits during lipoprotein apheresis with the two sets of plasma and fractionation filter membranes

		Complement factor C5a (µg/L)									
		0 minutes	**5 minutes**	**10 minutes**	**15 minutes**	**20 minutes**	**25 minutes**	**30 minutes**	**35 minutes**	**60 minutes**	**End**
EVAL	After PF in blood	0.06* ± 0.02	6.3 ± 3.0	12.2 ± 4.2	13.6 ± 3.0	17.5 ± 6.8	14.8 ± 5.7	13.8 ± 5.1	–	8.0 ± 4.6	3.2 ± 2.0
	After PF in plasma	0.06* ± 0.02	–	47.2 ± 19.2	56.1 ± 22.0	55.9 ± 22.9	53.4 ± 22.3	48.3 ± 22.5	46.2 ± 22.6	30.8 ± 17.0	19.3 ± 9.6
	After FF in plasma	0.06* ± 0.02	–	1.5 ± 2.1	14.3 ± 5.7	32.5 ± 11.6	42.7 ± 15.1	48.1 ± 17.9	50.6 ± 19.4	41.3 ± 19.8	26.6 ± 10.7
	Venous blood	0.06* ± 0.02	5.4 ± 3.0	6.5 ± 2.1	11.3 ± 4.1	20.3 ± 7.4	26.8 ± 8.3	30.0 ± 11.2	–	25.4 ± 10.7	12.5 ± 7.6
FPES	After PF in blood	0.06* ± 0.02	7.5 ± 6.3	13.1 ± 7.7	9.9 ± 6.4	9.3** ± 6.4	7.7*** ± 5.9	6.6*** ± 4.7	–	4.9 ± 2.9	4.3 ± 2.4
	After PF in plasma	0.06* ± 0.02	–	23.3** ± 15.2	21.8*** ± 17.0	20.5*** ± 17.5	19.9*** ± 16.9	18.4*** ± 15.5	17.0** ± 14.0	15.3** ± 10.5	13.4** ± 8.5
	After FF in plasma	0.06* ± 0.02	–	1.4 ± 1.5	9.0*** ± 3.9	14.7** ± 9.1	16.9** ± 12.3	18.7*** ± 14.8	18.6** ± 14.5	16.8**** ± 12.4	16:7*** ± 6.7
	Venous blood	0.06* ± 0.02	5.3 ± 4.6	7.5 ± 3.4	8.5 ± 4.9	11.1*** ± 7.8	11.9*** ± 8.0	12.3*** ± 9.0	–	10.6**** ± 6.9	10.8 ± 3.8

Note: C5a baseline values before treatment were 0.06 ± 0.02 µg/L in both EVAL and FPES, respectively. To note, due to the machine presetting, plasma filtration started 5 minutes after the blood pump. Sampling from the plasma circuit was accordingly adapted, that is, it was done 5 minutes later. Mean values ± standard deviations are given. Data significantly different compared with EVAL are highlighted in grey.

Abbreviations: EVAL, ethylene-vinyl alcohol; FF, fractionation filter; FPES, polyvinylpyrrolidone-blended polyethersulfone; PF, plasma filter.

*$P < .001$ versus all other times.

**$P < .01$ versus EVAL; ***$P < .05$ versus EVAL; ****$P < .001$ versus EVAL.

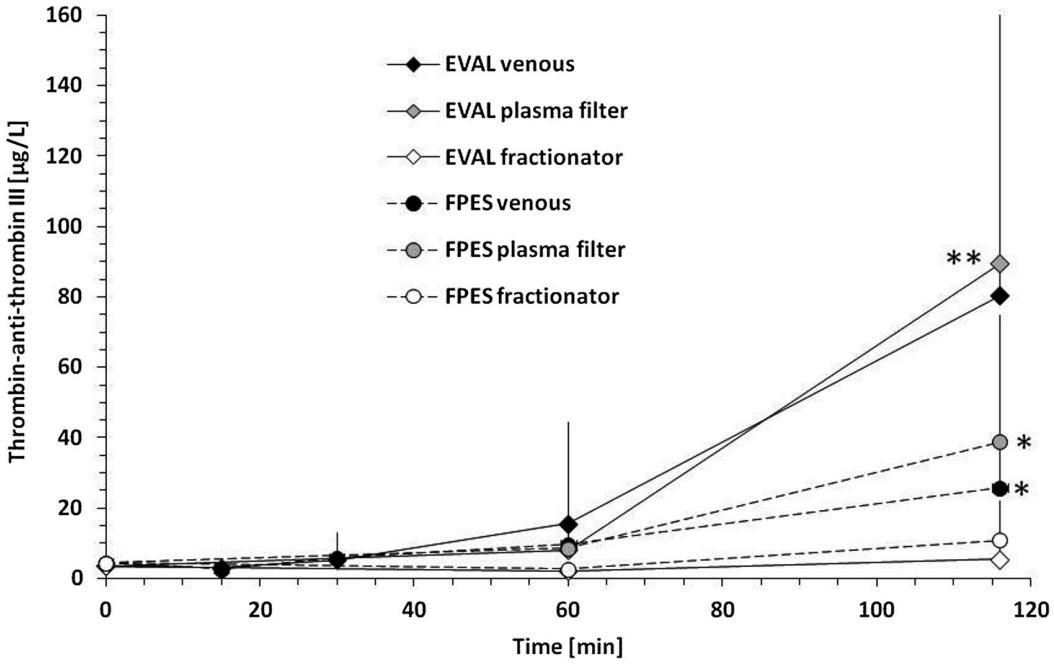

FIGURE 3 Course of the thrombin–antithrombin-III concentration in venous blood and in plasma after passage of the plasma filter as well as the fractionator during lipoprotein apheresis. No significant differences between FPES and EVAL filters were observed. Mean values ± standard deviations are presented. *$P < .05$ versus 0 minute; **$P < .01$ versus 0 minute. EVAL, ethylene-vinyl alcohol; FPES, polyvinylpyrrolidone-blended polyethersulfone

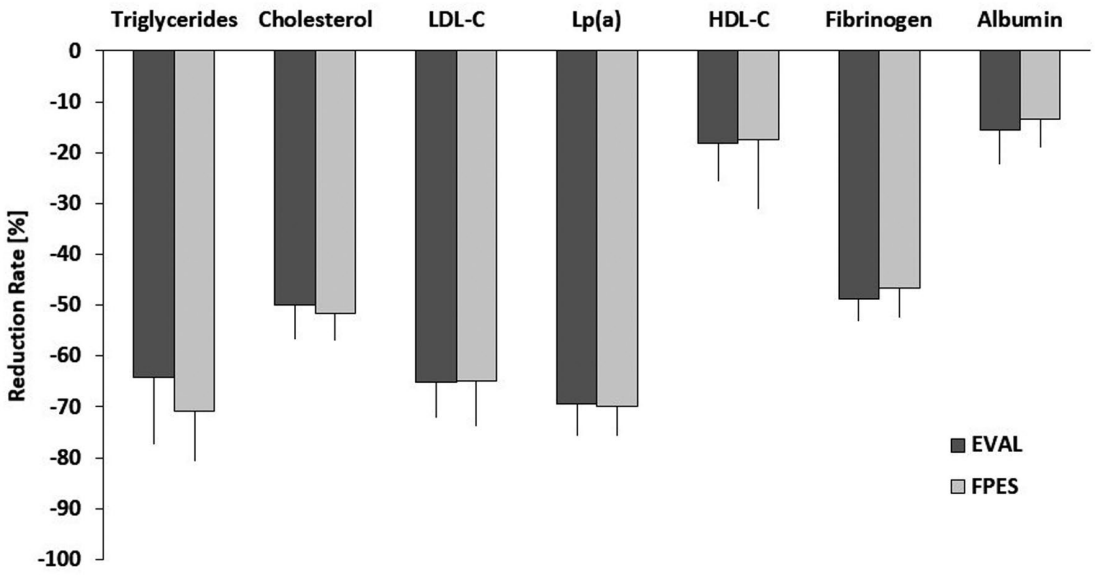

FIGURE 4 Reduction rate of lipoproteins and fibrinogen did not show significant differences between FPES and EVAL. Mean values ± standard deviations are displayed. EVAL, ethylene-vinyl alcohol; FPES, polyvinylpyrrolidone-blended polyethersulfone

in the early 1980s,[23] was compared with a recently introduced set of PES-based plasma separation and fractionation membranes.[24]

Both types of membrane materials caused a significant complement C5a concentration increase beginning with the first sampling after 5 minutes and not returning to baseline values until the end of the treatments. Compared with PES, complement activation with EVAL was throughout much higher in the respective extracorporeal blood and plasma circuit at the same times. The maxima of the C5a concentrations in the blood and plasma containing sections of the circuit occurred at different times because of the serial arrangement of the plasma filters. They were also a consequence of the differences in the blood (100 mL/min) and plasma (30 mL/

min) flow rates as well as of the different volumes required to fill the plasma separation and the fractionation filters. Furthermore, as the plasma pump was only started 5 minutes after the blood pump, it was expected that C5a levels in plasma after the plasma filter would rise accordingly later. Because in the downstream fractionator, the saline also was displaced by plasma first, reaching the maximum C5a increase after this filter would take even longer.

Therefore, it was conclusive that the maximum complement C5a concentration in venous blood, that is, the most relevant location with regard to adverse effects where blood and plasma had merged before reentering the patient after having passed both filter modules, was measured at 30 minutes within lipoprotein apheresis. At that time, the increase of C5a was significant with both types of membranes, being 500-fold with EVAL and, much less prominent, 205-fold with PES in relation to baseline (Table 1). When compared with data generated in hemodialysis with the synthetic membranes PES (only threefold increase at 10 minutes) and polysulfone (sevenfold at 5 minutes), these concentrations were not only much later within the treatment, but were also far higher.[25] With respect to the blood and plasma samples immediately drawn after the plasma filter in lipoprotein apheresis, the maximum with PES was also observed each at 10 minutes, whereas, with EVAL, it was reached at 20 and 15 minutes, respectively. This was similar to observations in hemodialysis with the two membrane types.[13,25] Differences in the patient populations may explain discrepancies in C5a kinetics to some extent. Whereas patients in the present study did not suffer from renal failure, in patients with end-stage chronic kidney disease on maintenance dialysis, the unspecific immune defense is inhibited due the accumulation of uremic toxins.[26] This may be a reason for the considerably higher baseline complement C5a levels in the dialysis patient cohort.[25] However, a significantly greater influence is likely to result from the much larger surface area of the filters in lipoprotein apheresis. The dialysis membrane surface areas were 1.7 and 1.8 m^2.[25] In the present trial investigating double-filtration lipoprotein apheresis, the blood passed not only the plasma filter membranes of 0.5 (EVAL) and 0.6 m^2 (PES), but different to dialysis, filtered plasma subsequently came into contact with the plasma filter wall structures and the outside surface as well as with the surfaces of the fractionation membrane inside (2.0 and 1.9 m^2, respectively), wall layer, and membrane outside. Therefore, the contacting net surface areas were much larger than only adding the nominal membrane surface information specified by the manufacturers for the respective filters. Consequently, the time-delayed C5a concentrations in plasma after passage of the plasma filter, where they already reached their maxima because no further increase was noted after the fractionator, were much higher than in the blood. When reinfused into the extracorporeal blood circuit, complement C5a from plasma was diluted and fueled the concentrations measured in venous blood.

Complement activation plays a causative role in leukocyte activation and correlates with transient leukopenia in hemodialysis.[27] Synthetic membranes, such as EVAL and PES, were attributed as biocompatible because of only limited complement and cell activation.[13,25,27] In accordance with the results on complement C5a generation, the present data evidenced a marked difference in leukocyte activation between the two membrane types tested as well as in comparison with the much more favorable results from past studies in hemodialysis.[13,25] EVAL led to a linear relative reduction of leukocyte counts of 66.5% after 25 minutes with an excess recovery to 130% of baseline until the end of the treatment. Apart from the rather slow decline, which is most probably caused by the longer-lasting intense complement generation in lipoprotein apheresis, these kinetics are in accordance with leukocyte rebound from sequestrated neutrophils returning into blood and recruitment from bone marrow stores as described earlier for less biocompatible dialysis membranes.[11] Compared with EVAL, PES must be regarded as more biocompatible because its relative decrease in WBC was only 36.2% at 20 minutes without excess recovery at the end. For both membrane types, the observed transient leukopenia was essentially the result of granulocyte activation, which is perfectly in line with previous reports.[25] The plasma filter membrane is the largest surface in contact with blood during double-filtration lipoprotein apheresis to exert direct membrane–blood cell interactions. Compared with dialyzers, the plasma filter membrane surface areas are generally small, in the present setting only one third of a standard dialysis membrane. Therefore, to explain the differences between EVAL and PES in inducing transient leukopenia, only the extent of complement activation appears conclusive because it correlates with the leukocyte drop.[27]

In accordance with routine practice patterns for lipoprotein apheresis, anticoagulation with unfractionated heparin was performed in the present study to prevent clotting, which involves the activation of platelets and the hemostatic system through the foreign surfaces of the extracorporeal circuit.[28] No abnormalities were observed for the course of PC and TAT concentrations, reflecting both an adequate level of anticoagulation and no pronounced pro-coagulatory effects of the plasma and fractionation membranes. After a drop at 5 minutes due to dilution from the saline used for rinsing of the extracorporeal circuit, PC with EVAL and PES averaged at 91% of baseline at the end of the treatment. TAT concentrations were assessed to measuring coagulation activation, but again, no differences between the two membrane types were determined. TAT levels had risen in venous blood and in plasma after the plasma filter only at the end of the therapy with EVAL and PES, indicating that the previously effective anticoagulation had subsided as anticipated.[29] In contrast, TAT concentrations were not increased in the plasma after passage of the fractionators, a finding most probably attributed to elimination by retention of the large TAT complexes of 230 kDa in its nonaggregated form.[30]

In terms of treatment efficacy, both membrane sets performed equally. Elimination of the target lipoproteins Lp(a) and LDL was almost identical with both EVAL and PES and far exceeded the 60% minimum reduction rate target for a single-lipoprotein apheresis treatment.[31] Furthermore, the undesired retention of the protective HDL and of albumin was far below 20%.

CONCLUSION

In conclusion, compared with EVAL, PES membranes cause less leukocyte and complement system activation at identical treatment efficacy. Inflammation promotes atherosclerosis, and repeated complement activation through blood–dialysis membrane interactions is regarded to cause chronic inflammation leading to cardiovascular disease in patients on maintenance hemodialysis.[4,5] Therefore, implementing more hemocompatible plasma separation and fractionation PES membranes instead of EVAL membranes would be consequent as it may reduce inflammation-promoting effects from blood–material interactions in the patients on double-filtration lipoprotein apheresis already suffering from progressive cardiovascular disease. Whether such a change actually affects patient outcome is currently completely unclear. However, suppliers of EVAL membranes for lipoprotein apheresis need to be confronted with these unfavorable data. A comparable constellation in hemodialysis during the 1990s led to the disappearance of complement-activating cellulosic dialysis membranes from the market.[32] Therefore, an increased demand for PES-based or chemically similar filters can be expected.

ACKNOWLEDGMENTS

The study was supported by a grant from 3M Deutschland GmbH. Parts of the manuscript were presented at the XLVI Annual Congress of the European Society for Artificial Organs, 2019, Hannover, Germany.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

ORCID

Detlef H. Krieter https://orcid.org/0000-0001-9173-4859

REFERENCES

1. Craddock PR, Fehr J, Brigham KL, Kronenberg RS, Jacob HS. Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. N Engl J Med. 1977;296:769–74.
2. Deppisch R, Schmitt V, Bommer J, Hänsch GM, Ritz E, Rauterberg EW. Fluid phase generation of terminal complement complex as a novel index of bioincompatibility. Kidney Int. 1990;37:696–706.
3. Clark WR, Hamburger RJ, Lysaght MJ. Effect of membrane composition and structure on solute removal and biocompatibility in hemodialysis. Kidney Int. 1999;56:2005–15. https://doi.org/10.1046/j.1523-1755.1999.00784.x.
4. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993;362:802–9.
5. DeAngelis RA, Reis ES, Ricklin D, Lambris JD. Targeted complement inhibition as a promising strategy for preventing inflammatory complications in hemodialysis. Immunobiology. 2012;217:1097–105. https://doi.org/10.1016/j.imbio.2012.07.012.
6. Jaeger BR, Richter Y, Nagel D, Heigl F, Vogt A, Roeseler E, et al. Longitudinal cohort study on the effectiveness of lipid apheresis treatment to reduce high lipoprotein(a) levels and prevent major adverse coronary events. Nat Clin Pract Cardiovasc Med. 2009;6:229–39.
7. Leebmann J, Roeseler E, Julius U, Heigl F, Spitthoever R, Heutling D, et al. Lipoprotein apheresis in patients with maximally tolerated lipid-lowering therapy, lipoprotein(a)-hyperlipoproteinemia, and progressive cardiovascular disease: prospective observational multicenter study. Circulation. 2013;128:2567–76.
8. Hovland A, Hardersen R, Nielsen EW, Enebakk T, Christiansen D, Ludviksen JK, et al. Complement profile and activation mechanisms by different LDL apheresis systems. Acta Biomater. 2012;8:2288–96. https://doi.org/10.1016/j.actbio.2012.02.017.
9. Lappegård KT, Enebakk T, Thunhaug H, Ludviksen JK, Mollnes TE, Hovland A. LDL apheresis activates the complement system and the cytokine network, whereas PCSK9 inhibition with evolocumab induces no inflammatory response. J Clin Lipidol. 2016;10:1481–7. https://doi.org/10.1016/j.jacl.2016.09.001.
10. Hörl WH. Hemodialysis membranes: interleukins, biocompatibility, and middle molecules. J Am Soc Nephrol. 2002;13:S62–71.
11. Sawada K, Malchesky PS, Ohshima T, Goldcamp JB, Guidubaldi JM, Omokawa S. Evaluation of materials for use in extracorporeal circulation. In vitro serum-material interaction. ASAIO Trans. 1991;37:M144–6.
12. Sawada K, Shimoyama T, Malchesky PS, Goldcamp JB, Omokawa S. Evaluation of a relationship between polymer bulk hydroxyl and surface oxygen content and in vitro serum-material interaction. J Biomed Mater Res. 1993;27:547–55.
13. Ward RA, Schaefer RM, Falkenhagen D, Joshua MS, Heidland A, Klinkmann H, et al. Biocompatibility of a new high-permeability modified cellulose membrane for haemodialysis. Nephrol Dial Transplant. 1993;8:47–53. https://doi.org/10.1093/oxfordjournals.ndt.a092271.
14. Krieter DH, Hackl A, Rodriguez A, Chenine L, Moragues HL, Lemke H-D, et al. Protein-bound uraemic toxin removal in haemodialysis and post-dilution haemodiafiltration. Nephrol Dial Transplant. 2010;25:212–8.
15. Tsimikas S, Viney NJ, Hughes SG, Singleton W, Graham MJ, Baker BF, et al. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. Lancet. 2015;386:1472–83. https://doi.org/10.1016/S0140-6736(15)61252-1.
16. Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, Yu RZ, et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. Lancet. 2016;388:2239–53. https://doi.org/10.1016/S0140-6736(16)31009-1.
17. Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, Tardif JC, Baum SJ, Steinhagen-Thiessen E, et al. Lipoprotein(a) reduction in persons with cardiovascular disease. N Engl J Med. 2020;382:244–55. https://doi.org/10.1056/NEJMoa1905239.
18. van Wijk DF, Sjouke B, Figueroa A, Emami H, van der Valk FM, MacNabb MH, et al. Nonpharmacological lipoprotein apheresis

reduces arterial inflammation in familial hypercholesterolemia. J Am Coll Cardiol. 2014;64:1418–26. https://doi.org/10.1016/j.jacc.2014.01.088. PMID: 25277610.

19. Hakim RM. Influence of the dialysis membrane on outcome of ESRD patients. Am J Kidney Dis. 1998;32:S71–5. https://doi.org/10.1016/s0272-6386(98)70165-9.
20. Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD. The role of complement in biomaterial-induced inflammation. Mol Immunol. 2007;44:82–94. https://doi.org/10.1016/j.molimm.2006.06.020.
21. Würzner R, Schuff-Werner P, Franzke A, Nitze R, Oppermann M, Armstrong VW, et al. Complement activation and depletion during LDL-apheresis by heparin-induced extracorporeal LDL-precipitation (HELP). Eur J Clin Invest. 1991;21:288–94. https://doi.org/10.1111/j.1365-2362.1991.tb01372.x.
22. Böhler J, Donauer K, Köster W, Schollmeyer PJ, Wieland H, Hörl WH. Biocompatibility of four plasmapheresis membranes in patients treated for hypercholesterolemia. Am J Nephrol. 1991;11:479–85. https://doi.org/10.1159/000168363.
23. Agishi T, Kaneko I, Hasuo Y, Hayasaka Y, Sanaka T, Ota K, et al. Double filtration plasmapheresis. Trans Am Soc Artif Intern Organs. 1980;26:406–11.
24. Krieter DH, Lange F, Lemke HD, Bonn F, Wanner C. Characterization of fractionation membranes in an animal model of double filtration lipoprotein apheresis. Ther Apher Dial. 2018;22:189–95. https://doi.org/10.1111/1744-9987.12649.
25. Krieter DH, Morgenroth A, Barasinski A, Lemke HD, Schuster O, von Harten B, et al. Effects of a polyelectrolyte additive on the selective dialysis membrane permeability for low-molecular-weight proteins. Nephrol Dial Transplant. 2007;22:491–9. https://doi.org/10.1093/ndt/gfl610.
26. Cohen G, Haag-Weber M, Hörl WH. Immune dysfunction in uremia. Kidney Int Suppl. 1997;62:S79–82.
27. Cheung AK. Biocompatibility of hemodialysis membranes. J Am Soc Nephrol. 1990;1:150–61.
28. Lee G, Arepally GM. Anticoagulation techniques in apheresis: from heparin to citrate and beyond. J Clin Apher. 2012;27:117–25. https://doi.org/10.1002/jca.21222.
29. Klingel R, Schaefer M, Schwarting A, Himmelsbach F, Altes U, Uhlenbusch-Körwer I, et al. Comparative analysis of procoagulatory activity of haemodialysis, haemofiltration and haemodiafiltration with a polysulfone membrane (APS) and with different modes of enoxaparin anticoagulation. Nephrol Dial Transplant. 2004;19:164–70. https://doi.org/10.1093/ndt/gfg459.
30. Pletcher CH, Cunningham MT, Nelsestuen GL. Molecular weight analysis of antithrombin III-heparin and antithrombin III-thrombin-heparin complexes. J Biol Chem. 1986;261:4143–7.
31. Thompson G, Parhofer KG. Current role of lipoprotein apheresis. Curr Atheroscler Rep. 2019;21:26. https://doi.org/10.1007/s11883-019-0787-5.
32. Krieter DH, Wanner C. Membranes for dialysis and hemofiltration. In Jörres A, Ronco C, Kellum JA, editors. Management of acute kidney problems: Temporary replacement of kidney function. London: Springer Heidelberg Dordrecht; 2010. p. 491 ff.

How to cite this article: Krieter DH, Jeyaseelan J, Rüth M, Lemke H-D, Wanner C, Drechsler C. Clinical hemocompatibility of double-filtration lipoprotein apheresis comparing polyethersulfone and ethylene-vinyl alcohol copolymer membranes. *Artif Organs*. 2021;45:1104–1113. https://doi.org/10.1111/aor.13944