Hemocompatibility of Polysulfone Hemodialyzers – Exploratory Studies on Impact of Treatment Modality and Dialyzer Characteristics

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Abstract
Background The hemocompatibility of dialyzers for extracorporeal kidney replacement therapy (KRT) is of importance to minimize harmful reactions between blood constituents and the membrane. We investigated in these exploratory studies the hemocompatibility profile of several types of polysulfone dialyzers.

Methods Hemocompatibility of various high-flux polysulfone dialyzers were compared in two consecutive, prospective, randomized, crossover studies, each including 24 adult patients being at least 3 months on hemodialysis (HD) or on-line hemodiafiltration (HDF). These dialyzers, differing in membrane type, fiber geometry, sterilization method, and production technology, were each applied for 1 week in HD or HDF. Hemocompatibility was assessed through markers of complement activation, cell activation, coagulation, contact activation, and immunologic reactions.

Results The patients in the two studies were on average 67 ± 11 and 68 ± 11 years old, 75% and 67% were male, and were on KRT for 5.4 ± 5.0 and 4.4 ± 3.6 years. The complement factors C3a and C5a increased early and transiently during treatment, less so with HDF than with HD, and with dialyzers combining wider inner fiber diameter (210 versus 185 μm) and advanced membrane type (Helixone plus versus Helixone). sC5b-9 increased in all study phases, reaching its highest level after 60 minutes, with lower values over the entire treatment (area under the curve) for HDF than HD, and for wider inner fiber diameter and advanced membrane type. Leukocytes decreased in the first 10 minutes, without significant differences between dialyzers. PMN elastase increased in the first hour, more so with HD than HDF. Thrombocytes decreased slightly in the first 30 minutes, with differences only between HDF and HD mode. IL-8 decreased from pre- to postdialysis, particularly on HDF. No differences were observed for kallikrein, IgE, and hsCRP.

Conclusions In these exploratory studies we found indications to a comparable hemocompatibility profile of the investigated dialyzers. We observed distinctions in compounds between HDF and HD and for some dialyzer and membrane characteristics.

Introduction
The efficiency and tolerability of dialysis affects outcomes and is dependent on the treatment modality, dialyzer, and membrane type. High-flux hemodialysis (HD) and hemodiafiltration (HDF) modalities are associated with greater elimination of uremic retention solutes and reduced mortality risk compared with low-flux HD (1–3). Dialysis modalities that provide more convective clearance require high-flux dialyzers to effectively eliminate uremic solutes of middle molecular mass (molecules of 500–60,000 D, such as β2-microglobulin and cytokines) (4). Specifically, the better removal of middle molecules in high-flux HD is suggested to reduce the risk of mortality versus low-flux HD (5). HDF provides higher middle molecule clearance compared with low-flux and high-flux HD and is associated with a reduced mortality risk, when a minimum of convection volume is achieved (2,6,7).

The dialyzer membrane is in continuous contact with the blood during HD/HDF treatments, making the hemocompatibility of high importance. Synthetic dialyzer membranes (e.g., polysulfone, polyethersulfone, polymethylmethacrylate) are the most widely used in clinical practice. In vitro and in vivo studies have shown synthetic dialyzer membranes exhibit a higher hemocompatibility compared with cellulose-based membranes. These membranes are associated with more pronounced transient complement activation and drop in leukocyte count than polysulfone and other synthetic membranes (8–11).

The polysulfone dialyzer series FX CorDiax (Fresenius Medical Care, Bad Homburg, Germany) contains

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the Helixone plus membrane, which has, through advances in material and production technologies, a more porous membrane wall for higher clearance of middle molecules, and reduced diffusion resistance compared with the Helixone membrane in the FX dialyzer series. This facilitates convective filtration and clearance of a broad range of uremic toxins (12). FX CorDiax dialyzers have been shown in vitro and in vivo to provide higher removal of middle molecules, including β2-microglobulin, myoglobin, and prolactin, compared with those of the FX series containing the Helixone membrane (12). Hemocompatibility has been assessed in vitro for this new dialyzer series. We conducted two consecutive, randomized, crossover trials to compare the in vivo hemocompatibility and immunologic reactions by modality, and the association with the FX CorDiax and two further types of commonly used polysulfone dialyzers.

Materials and Methods

Study Design and Patients

Two clinical investigations were performed to investigate the hemocompatibility profile of several types of polysulfone dialyzers, and the potential modulatory effect of specific dialyzer and membrane characteristics, production technology of the dialyzers by different manufacturers, and treatment modality.

Both studies had a prospective, randomized, crossover design, and were monocentric. The characteristics of the investigated dialyzers (FX 100, FX CorDiax 100, FX CorDiax 1000; all Fresenius Medical Care, Bad Homburg, Germany, and Xevonta hi23; B. Braun, Melsungen, Germany) are given in Table 1.

In study A, FX 100 and FX CorDiax 100 were compared, with FX 100 having been applied in HD, and FX CorDiax 100 both in HD and on-line HDF. In study B, FX CorDiax 100, FX CorDiax 1000, and Xevonta hi23 were each compared with FX 100, and all were applied in on-line HDF.

Each study phase consisted of the application of a dialyzer/modality for 1 week in randomized order. Total study duration per patient was 3 weeks in study A and 4 weeks in study B.

Adult patients prevalent on high-flux HD or on-line HDF for at least 3 months, with fistula or graft as vascular access allowing blood flow of at least 300 ml/min, without known allergies to trial products or related products or other allergies, were eligible for the study (Supplemental Table 1).

All treatments were performed with the 5008 HD system (Fresenius Medical Care, Bad Homburg, Germany), with a blood flow rate of at least 300 ml/min and a treatment time of at least 240 minutes. Anticoagulation was provided according to center practice without changes to prestudy and between study phases. Dialysis treatment conditions should be kept constant for all study phases, except the dialyzer and treatment modality, which were applied according to randomization. Blood sampling was performed once per study week, at the same day of the week in each phase. Blood samples were drawn from the arterial needle predialysis and during treatment, as well as before treatment end from the arterial line.

The studies were approved by the Ethics Committee of the Hessen State Medical Association. All patients gave written informed consent before starting any study related activities. The execution of the study adhered to the Declaration of Helsinki in its current version.

The studies are registered with the “Deutsches Register Klinischer Studien” (registration identifiers DRKS00008200, and DRKS00009996).

Outcome Variables and Laboratory Methods

Complement factors, blood cell count, and markers of cell and immune activation, coagulation, and contact activation have been measured as outcome parameters in both studies. All laboratory parameters were analyzed in blood samples taken pre- and post-dialysis, blood cell count in addition in samples taken after 10, 30, 60, and 120 minutes, and complement factors, PMN granulocyte elastase, and kallikrein in those taken after 10, 30, and 60 minutes.

Complement factors (C3a, C5a, and sC5b-9) were analyzed by ELISA (MicroVue C3a Plus resp. MicroVue sC5b-9; Quidel, Hannover, Germany, and resp. EIA-3327; DRG, Marburg, Germany), PMN elastase by Human PMN Elastase Platinum ELISA (Affimetrix, Vienna, Austria), and thrombin–antithrombin III (TAT) by Enzygnost TAT microassay (Siemens, Marburg, Germany). Plasma kallikrein-like activity was measured with the chromogenic substrate S-2302 (Haemochrom, Essen, Germany), IgE was measured with an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), high-sensitivity C-reactive protein (hsCRP) was measured with the immunoturbidimetric test CRP Dynamic HIT (Invicon Diagnostic Concepts).

| Table 1. Characteristics of study dialyzers and use in study A and study B |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic                  | FX 100          | FX CorDiax 100  | FX CorDiax 1000 | Xevonta hi23    |
| Membrane                        | Helixone        | Helixone plus   | Helixone plus   | Amembris        |
| Membrane surface area, m²       | 2.2 (polysulfone)| 2.2 (polysulfone)| 2.3 (polysulfone)| 2.3 (polysulfone)|
| Inner fiber diameter, μm        | 185             | 185             | 210             | 195             |
| Sterilization method            | INLINE steam    | INLINE steam    | INLINE steam    | Oxygen-free y-sterilization |
| Use in study and modality       | Study A         | Study B         |                 |                 |
| Study A                         | HD              | HD              |                 |                 |
| Study B                         | HDF             | HDF             | HDF             | HDF             |

HD, hemodialysis; HDF, hemodiafiltration.
München, Germany), and IL-8 was measured with the Quantikine Human CXCL8/ IL-8 HS ELISA (R&D Systems, Wiesbaden-Nordenstadt, Germany). Molecular masses of the measured parameters are given in Supplemental Table 2. Differential blood count measurement was performed with the XS 800i device (Sysmex, Norderstedt, Germany). These analytical methods were equally applied in both studies, but with different batches of the assay, so that certain systematic deviations of the results between study A and study B could occur.

### Statistical Methods

The order of dialyzer/modalities was assigned by randomization with randomly permuted blocks of fixed size, which have been used for allocation of patients to treatment sequences. Randomization lists using a code generated in SAS version 9.2 were prepared by the study statistician.

Linear mixed models were used for inferential statistical analysis, including fixed effects for treatment, period, interaction between treatment and period, and a random subject effect. The corresponding covariance structure was defined as “compound symmetry,” and degrees of freedom were calculated according to Kenward-Rogers (13).

For primary parameters assessed multiple times over the treatment period (C3a, C5a, sC5b-9, kallikrein, and differential blood count), a first model used \( t=10 \) minutes as target parameter, as these parameters showed a positive or negative transient peak at this time point, because of immediate biologic activation processes. For parameters following a log-normal distribution, the respective value was log-transformed (PMN elastase, C3a, C5a, and kallikrein). The second model used the natural logarithm of the area under the curve (AUC) over the whole treatment period as target parameter. For both models, differences in least square means and associated 95% confidence intervals and derived \( P \) values were calculated. For the first model, an additional factor baseline (e.g. parameter value at \( t=0 \) for each period) was added to the model.

Point estimates and confidence intervals of log-transformed variables were transformed via the exponential function, resulting in estimates for the geometric means.

For parameters assessed before and after treatment (TAT, IgE, hsCRP, and IL-8), the target parameter for the mixed model was the relative change over the treatment. Again, differences in least square means, associated 95% confidence intervals, and derived \( P \) values for relative change were calculated.

Because of the explorative character of this study, no formal sample size estimation was performed, and no formal significance level defined, i.e. all calculated \( P \) values serve as descriptive measures.

### Results

#### Patients and Treatments

Twenty four patients were enrolled in each study in the Center for Kidney and BP Diseases, Giessen, Germany, from May to July 2015 (study A) and from February 2016 to January 2017 (study B). These comprised the full analysis sets used for statistical analysis of the respective study. Patient characteristics are given in Table 2.

Major treatment parameters are given in Table 3. On-line HDF was performed in postdilution mode and achieved substitution volumes on average \( >22 \) L; considering the achieved ultrafiltration volume, this resulted in mean convective volumes \( >24 \) L.

#### Factors of Complement Activation

Complement factors C3a and C5a increased to a peak at 10 minutes into the treatment and decreased afterwards, reaching pretreatment concentrations at the end of the session with all settings of dialyzer/modalities (Figures 1 and 2).

Estimated mean values of C3a and C5a at 10 minutes, taking baseline values into account, were different overall between study phases in study A, but only for C3a in study B (Tables 4 and 5).

The peak concentration of C5a at 10 minutes was lower with FX CorDiax 100 than with FX 100 (both applied in HD mode), and for C3a with FX CorDiax 100 in HDF compared with HD mode (Table 4).

The mean estimates for AUC again showed overall differences between dialyzer/modalities for both C3a and C5a in study A, but only for C5a in study B (Tables 6 and 7).

The AUC analysis revealed lower values for C3a in HDF than in HD (study A: both FX CorDiax 100; \( P < 0.001 \)), similarly utilizing dialyzers with a wider inner fiber diameter combined with advanced membrane type Helixoneplus (study B: FX CorDiax 1000 versus FX 100; \( P = 0.02 \)) (Tables 6

### Table 2. Patient characteristics

| Baseline Characteristics | Study A, \( N=24 \) | Study B, \( N=24 \) |
|--------------------------|------------------|------------------|
| Age, yr                  | 66.8±11.4        | 67.8±11.4        |
| Sex, % male              | 75               | 67               |
| Body weight, kg          | 87.0±27.6        | 79.0±15.4        |
| Diabetics, n; %          | 10; 42%          | 6; 25%           |
| Any cardiovascular disease, n; % | 24; 100% | 22; 92%          |
| Time on KRT, yr          | 5.4±5.0          | 4.4±3.6          |
| Treatment modality before inclusion | | |
| On-line HDF, n; %        | 22; 92%          | 24; 100%         |
| HD, n; %                 | 2; 8%            | 0; 0%            |
| Time on current modality, yr | 3.7±4.0 | 3.3±3.1          |

Data are given as number and percentage or mean ± standard deviation, as appropriate. HDF, hemodiafiltration; HD, hemodialysis.
and 7). In study A, the estimate of AUC for C5a was lower with HDF than with HD (FX CorDiax 100; $P<0.001$). In study B, AUC for C5a was lower with dialyzers with an advanced polysulfone (Helixoneplus) membrane (FX CorDiax 100: $P=0.009$), Helixoneplus membrane and wider inner fiber diameter (FX CorDiax 1000: $P<0.001$), and different sterilization/production method (Xevonta hi23: $P=0.001$), all compared with FX 100 (Tables 6 and 7).

| Table 3. Treatment parameters |
|--------------------------------|
| Treatment Parameter | Study A | Study B |
|----------------------|---------|---------|
| Dialyzer/ Modality   | FX 100 HD | FX CorDiax 100 HD | FX CorDiax 100 HDF | FXCorDiax 100 HDF | FXCorDiax 1000 HDF | Xevonta hi23 HDF |
| Blood flow, ml/min   | 376±34 | 379±44 | 379±43 | 381±43 | 373±40 | 381±40 | 382±44 |
| Substitution flow rate, ml/min | n.a. | n.a. | 112±35 | 89.3±10.4 | 87.1±9.0 | 89.5±9.4 | 89.2±9.2 |
| Treatment time, min  | 258±22 | 257±26 | 254±23 | 256±25 | 258±28 | 259±28 | 265±36 |
| Ultrafiltration volume, L | 2.50±1.3 | 2.50±1.2 | 2.55±1.2 | 2.01±1.2 | 2.09±1.3 | 2.11±1.2 | 2.16±1.4 |
| $K_t/V_{OCM}$        | 1.52±0.37 | 1.53±0.36 | 1.55±0.45 | 1.80±0.35 | 1.79±0.37 | 1.84±0.40 | 1.89±0.40 |

Data are given as mean ± standard deviation. HD, hemodialysis; HDF, hemodiafiltration; n.a., not applicable; OCM, on-line clearance measurement.

Figure 1. | Course of concentration of complement factor C3a during treatment by study phase. (A) Study A and (B) study B. HD, hemodialysis; HDF, hemodiafiltration.
The concentration of the sC5b-9 complex increased during the course of the treatment, reaching a maximum at 60 minutes, then slightly decreasing without returning to pretreatment levels (Figure 3).

An overall difference of the estimate of AUC for sC5b-9 between study arms both in study A \( (P<0.001) \) and study B was observed \( (P=0.003; \) Tables 6 and 7).

In study A, the increase of sC5b-9 was less pronounced in the treatment phase of HDF compared with the HD mode (FX CorDiax 100 HDF versus HD \( P=0.001 \)). In study B, for sC5b-9, again the wider inner fiber diameter combined with advanced membrane type in FX CorDiax 1000 was associated with a lower AUC than with FX 100 \( (P=0.02) \) (Tables 6 and 7).

**Markers of Cell Activation and Coagulation**

The estimated mean of leukocyte count decreased in the first 10 minutes of the treatment, but recovered in the following 50 minutes, and remained stable for the remainder of the treatment time (Figure 4).

In both studies, no statistical differences between dialyzers and modality were observed, either at 10 minutes (except for the comparison between FX 100 and Xevonta hi23, Tables 4 and 5), or for the entire treatment (AUC, Tables 6 and 7).

PMN elastase, released from leukocytes upon activation, increased in all dialyzer/modality settings during the first hour and then remained stable on an elevated level for the rest of the treatment. In study A, higher AUC values were found with FX CorDiax 100 in HD and with FX CorDiax 100 in HDF than with FX 100 in HD \( (P=0.002 \) and \( P<0.001 \), respectively; Tables 6 and 7). In study B, when applying all dialyzers in HDF mode, no differences between study phases were observed.

TAT as an indicator for coagulation activation increased with all dialyzer/modality settings from start to end of treatment. An overall difference between the relative changes from pre to postdialysis was observed in both studies (study A: \( P=0.002 \); study B: \( P=0.03 \); Tables 8 and 9).

In study A, the relative change was higher with FX CorDiax
100 in HDF than in HD ($P = 0.03$). In study B, we found lower values of relative changes with FX CorDiax 1000 and with Xevonta hi23 compared with FX 100 ($P = 0.02$ and $P = 0.03$, respectively). Yet, a high interindividual variability of that parameter, resulting from outliers in the measured changes (particularly at the end of the treatment), needs to be taken into account.

The mean values of thrombocytes decreased in the first 30 minutes of the treatment, and then remained constant. The decrease of thrombocytes, as reflected by the AUC values, was more pronounced with FX CorDiax 100 in HDF than in HD in study A ($P \leq 0.001$). In study B, no significant differences were observed overall and between the studied dialyzers (Tables 6 and 7).

### Inflammatory and Other Markers

Kallikrein was analyzed as a measure of contact activation. Mean values showed a small peak at 10 minutes; however, the statistical models did not reveal overall statistical differences, either at 10 minutes or for the entire treatment, except for a lower AUC value with Xevonta hi23 versus FX 100 ($P = 0.03$) (Tables 4–7).

IgE has been measured to preclude hypersensitivity reactions to the membrane material. Mean relative changes of IgE concentrations from pre to postdialysis were comparable with all dialyzer/treatment settings. Likewise, the statistical model confirmed that relative changes of hsCRP from pre- to postdialysis were not differing between the study phases.

IL-8 decreased in all dialyzer/modality settings, with overall differences in both study A and study B. The decrease was more pronounced with FX CorDiax 100 used in HDF compared with HD in study A ($P = 0.01$), and in HDF (study B) comparing membrane type (FX CorDiax 100 versus FX 100; $P = 0.002$) and sterilization/production method (Xevonta hi23 versus FX 100; $P = 0.01$) (Tables 8 and 9).

### Safety Data

All adverse events observed during the study, including two serious adverse events (wound infection, vascular...
access stenosis in study A; and shunt occlusion, tachyarhythmia, and suspected neurologic deficits, all occurring in one patient in study B) were assessed by the investigator as not being related to study material or interventions. Non-serious adverse events observed in one patient (cold sweat, feeling abnormal, CRP increased) were judged by the investigator as definitely or likely related to the Xevonta hi23 dialyzer.

**Discussion**

The presented clinical studies evaluated the hemocompatibility profile of several polysulfone high-flux hemodialyzers, applied in HD and on-line HDF, on the basis of laboratory parameters reflecting complement activation, cell activation, coagulation and contact activation, and inflammatory and immunologic markers.

A transient increase of complement factors (C3a and C5a) was observed with all dialyzers, in a direct comparison of HD and HDF to a lesser extent with HDF, likely because of the fact that these small complement proteins (9–11 kD) are effectively eliminated throughout the treatment by convection (12). Whether a lower level of activation also contributes to the lower increase can only indirectly be answered by the complement factor sC5b-9, the molecular mass of which (1030 kD) is far beyond the cut-off of dialysis membranes. It

### Table 6. Study A: estimated mean values from mixed models of area under the curve of laboratory parameters and P values of specified comparisons, including period, treatment, and interaction term between period and treatment

| Dialyzer/ Modality | FX 100 HD | FX CorDiax 100 HD | FX CorDiax 100 HDF | C3a, mg/min/L | sC5b-9, mg/min/L | Leukocytes, 10^3/μL | Elastase, mg/min/L | Thrombocytes, 10^3/μL | Kallikrein, IU/min/ml | P Value |
|---------------------|-----------|--------------------|--------------------|----------------|-------------------|--------------------|-------------------|-------------------|----------------------|---------|
| Overall             |           | Membrane Type (in HD) | Treatment Modality |                |                   |                    |                   |                   |                      |         |
|                     | FX CorDiax 100 versus FX 100 | FX CorDiax 100 (HDF) versus FX CorDiax 100 (HD) |                |                   |                    |                   |                   |                   |                      |         |
| C3a, mg/min/L       | 24,864    | 24,785             | 18,234             | <0.001          | 0.96              | <0.001             | 0.06              | <0.001            | 0.01                 | 0.01    |
| sC5b-9, mg/min/L    | 80,704    | 84,579             | 65,631             | <0.001          | 0.44              | <0.001             | 0.45              | <0.001            | 0.87                 |         |
| Leukocytes, 10^3/μL | 1338      | 1371               | 1364               | 0.73            | 0.45              | 0.87               |                   |                   | 0.73                 | 0.87    |
| Elastase, mg/min/L  | 9141      | 10,448             | 7999               | <0.001          | 0.02              | <0.001             | 0.25              | <0.001            | 0.31                 |         |
| Thrombocytes, 10^3/μL | 41,271 | 42,453             | 38,631             | 0.01            | 0.25              | <0.001             |                   |                   | 0.31                 |         |
| Kallikrein, IU/min/ml | 4106    | 3783               | 3563               | 0.07            | 0.17              | 0.31               |                   |                   | 0.07                 | 0.31    |

HD, hemodialysis; HDF, hemodiafiltration.

*The overall P value displays the P value for analyzing differences between all three groups.

### Table 7. Study B: estimated mean values from mixed models of area under the curve of laboratory parameters and P values of specified comparisons, including period, treatment, and interaction term between period and treatment

| Dialyzer/ Modality | FX 100 HDF | FX CorDiax 100 HDF | FX CorDiax 100 HDF | C3a, mg/min/L | sC5b-9, mg/min/L | Leukocytes, 10^3/μL | Elastase, mg/min/L | Thrombocytes, 10^3/μL | Kallikrein, IU/min/ml | P Value |
|---------------------|-----------|--------------------|--------------------|----------------|-------------------|--------------------|-------------------|-------------------|----------------------|---------|
| Overall             |           | Membrane Type (in HDF) | Treatment Modality |                |                   |                    |                   |                   |                      |         |
|                     | FX CorDiax 100 versus FX 100 | FX CorDiax 100 (HDF) versus FX CorDiax 100 (HD) |                |                   |                    |                   |                   |                   |                      |         |
| C3a, mg/min/L       | 25,392    | 23,279             | 22,503             | 23,846         | 0.11              | 0.08              | 0.02              | 0.20              | 0.20                 |         |
| sC5b-9, mg/min/L    | 34.60     | 29.57              | 27.11              | 28.58          | <0.001            | 0.009             | <0.001            | 0.01              | 0.01                 |         |
| Leukocytes, 10^3/μL | 77,057    | 76,919             | 71,197             | 80,753         | 0.003             | 0.96              | 0.02              | 0.14              | 0.14                 |         |
| Elastase, mg/min/L  | 1285      | 1267               | 1257              | 1252           | 0.80              | 0.61              | 0.42              | 0.37              | 0.37                 |         |
| Thrombocytes, 10^3/μL | 9816    | 9539               | 9458              | 9880           | 0.92              | 0.70              | 0.62              | 0.93              | 0.93                 |         |
| Kallikrein, IU/min/ml | 43,626 | 43,630             | 42,413             | 44,035         | 0.70              | 1.00              | 0.41              | 0.79              | 0.79                 |         |
|                     | 4678      | 4195               | 4270              | 3961           | 0.17              | 0.15              | 0.23              | 0.03              | 0.03                 |         |

For Elastase, C3a, C5a, and Kallikrein, geometric means are estimated. HDF, hemodiafiltration.

*The overall P value displays the P value for analyzing differences between all four groups.
increased less with HDF than HD, which might be an indicator of lower complement activation. Similarly, a wider inner fiber diameter combined with the new Helixoneplus membrane was associated with a lower increase in complement factors. It remains unanswered whether higher shear stress or a stronger pressure drop in fibers with smaller diameter plays a role. Such observations have only been described in relation to platelet activation (14), which we could not confirm with our data. Overall, the observed increases are lower by far than those observed earlier with cellulose-based membranes (10).

Leukocytes showed a typical transient decrease at the first measurement within the treatment, which recovered quickly almost to pretreatment levels. Leukocyte drop as a consequence of C5a-mediated granulocyte activation and transient adherence to the endothelium of the pulmonary vasculature has been described previously in studies assessing hemocompatibility (15,16). Leukocyte drop was less pronounced with synthetic membranes than with cellulose-based membranes (10,11,16).

Increase of complement and decrease in leukocyte count was both transient, as observed in other studies (10,11). The decrease of complement after an initial peak might be due to elimination across the dialysis membrane for proteins of middle molecular mass, binding to receptors of circulating blood cells, downregulation within the patient (16), deposition on the inner surface of the membrane (17), or reduced activation potential after formation of a protein layer on the membrane (18). As suggested by Martin-Malo et al. (10), this transient nature of complement increase and also of leukopenia might explain the absence of clinical symptoms associated with such activation in the vast majority of patients on dialysis.

An association of HD treatment with decrease of thrombocyte count has been described earlier (19). In contrast, we found only a moderate decrease of thrombocyte count. TAT as an indicator for coagulation activation increased at the end of dialysis, the extent of which was associated with modality, fiber diameter, and type of membrane. This is in line with observations of higher TAT levels at treatment end.
with hemofiltration and HDF as compared with HD treatments using another brand of polysulfone dialyzers. The authors suggested that the higher ultrafiltration volume might alter local flow conditions, affecting shear forces in the fiber, which in turn may affect platelet function (20). Because of observed high inter- and intrapatient variability of this parameter, also resulting from individual outliers in the measured changes, conclusions should be drawn with caution.

PMN elastase, released upon degranulation of leukocytes after cell activation and by complement factors, increased in the first hour of the treatment in all three treatment phases. This course was similar to findings of an earlier study comparing different types of polysulfone/polyethersulfone membranes (21). The highest value we observed with the HD mode coincides with the higher increase of C3a, C5a, and sC5b-9 in this treatment modality. With a molecular mass of close to 30 kD, the stimulation of elastase during treatment might be compensated to a certain extent by transmembrane elimination (12,22), which could explain the difference observed with HDF.

Kallikrein was measured as an indicator of contact activation. This marker, being a precursor of bradykinin, has been chosen because it is analytically more stable than bradykinin. The latter is generated upon contact activation, i.e., blood contact with negative charges of surfaces (23). No evidence of contact activation was observed from the course of kallikrein in this study.

IL-8, a proinflammatory marker, was lowered at treatment end with all dialyzer/modality settings, as similarly observed in other studies (24,25). With a molecular mass of 8 kD it might be eliminated across the membrane both with HD and HDF and/or adsorb to the membranes, masking a potential stimulation of this cytokine during treatment.

The change of hsCRP as inflammatory marker from pre- to post-treatment did not differ either by dialyzer characteristics or treatment modality, similar to findings in an earlier study comparing hemocompatibility of four different

Figure 4. | Course of leukocytes during treatment by study phase. (A) Study A and (B) study B.
high-flux membranes (26). It is very likely that the duration of a dialysis treatment is too short to capture an increase in C-reactive protein, which peaks at 48 hours (27).

IgE levels were slightly higher at treatment end, possibly owing to hemoconcentration. No differences between dialyzer type and treatment modality for the relative changes from pre- to post-treatment could be observed, confirming no acute allergic reaction caused by the membrane material or treatment modality.

We acknowledge the limitations of this study, including its exploratory nature, the substantial variability of some markers, and its limited sample size, and so confirmative conclusions are precluded. Moreover, the studies were performed in a single center, so a center effect cannot be ruled out.

In conclusion, in vivo hemocompatibility of the investigated dialyzers of the FX, FX CorDiax, and the Xevonta series were evaluated by parameters that are established as a standard panel to measure complement, cell and contact activation, stimulation of immune cells, and activation of coagulation. Although some differences related to the treatment modality and some membrane characteristics were observed, these explorative studies indicate a comparable hemocompatibility profile of the investigated dialyzers. This warrants further studies examining hemocompatibility together with established dialyzer performance, to determine their potential to improve patient outcome by applying highly efficient, extracorporeal KRTs.

**Author Contributions**
S. Wagner, S. Zschätzsch, A. Erlenkoetter, M. Stauss-Grabo, and A. Gauly conceptualized the study and were responsible for methodology; S. Wagner, S. Zschätzsch, L. Rauber, and A. Gauly were responsible for data curation; A. Gauly was responsible for formal analysis; S. Wagner, S. Zschätzsch, L. Rauber, M. Stauss-Grabo, and A. Gauly were responsible for project administration; M. Stauss-Grabo was responsible for resources; S. Wagner and M. Stauss-Grabo provided supervision; A. Gauly was responsible for validation and visualization, and wrote the original manuscript; and all authors were responsible for investigation, and reviewed and edited the manuscript.

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Table 8. Study A: estimated mean values from mixed models of relative changes [%] of laboratory parameters from pre to postdialysis and P-values of specified comparisons by study phase, including period and treatment and interaction term between period and treatment

| Dialyzer/Modality | FX 100 HD | FX CorDiax 100 HD | FX CorDiax 100 HDF | FX CorDiax 100 HDF |
|-------------------|-----------|------------------|-------------------|-------------------|
| TAT               | 383.37    | 672.76           | 1068.33           | 0.002             |
| IgE               | 6.86      | 5.68             | 6.47              | 0.75              |
| hsCRP             | 4.42      | 4.61             | 2.92              | 0.76              |
| IL-8              | −20.82    | −24.56           | −34.97            | 0.004             |

HD, hemodialysis; HDF, hemodiafiltration; TAT, thrombin-antithrombin; hsCRP, high-sensitivity C-reactive protein.

*The overall P value displays the P value for analyzing differences between all three or four groups, respectively.

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Table 9. Study B: estimated mean values from mixed models of relative changes [%] of laboratory parameters from pre to postdialysis and P-values of specified comparisons by study phase, including period and treatment and interaction term between period and treatment

| Dialyzer/Modality | FX 100 HDF | FX CorDiax 100 HDF | FX CorDiax 1000 HDF | Xevonta hi23 HDF |
|-------------------|------------|--------------------|---------------------|-----------------|
| TAT               | 279.28     | 262.37             | 140.81              | 145.06          |
| IgE               | 6.07       | 5.68               | 5.90                | 6.02            |
| hsCRP             | 3.01       | 3.30               | −0.28               | 6.49            |
| IL-8              | −29.53     | −41.86             | −34.94              | −39.41          |

HD, hemodialysis; HDF, hemodiafiltration; TAT, thrombin-antithrombin; hsCRP, high-sensitivity C-reactive protein.

*The overall P value displays the P value for analyzing differences between all three or four groups, respectively.
Disclosures
A. Erlenkötter, A. Gauly, L. Rauber, and M. Stauss-Grabov are full-time employees of Fresenius Medical Care. S. Wagner and Z. Scharzhöchz have nothing to disclose.

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