Comparison of Different Membranes for Continuous Renal Replacement Therapies: An *In Vitro* Study

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Inflammatory mediators play a major role in the development and progression of acute kidney injury (AKI). Continuous renal replacement therapy (CRRT) removes these mediators from the blood using AN69-M, AN69-ST, and HF1400 filters to target low and middle-molecular weight molecules. We characterized the *in vitro* removal performance of each filter in a 72 hour simulated CRRT procedure. Urea clearance with AN69-M and AN69-ST remained stable (52.4 and 51.2 ml/minute, respectively) but decreased with HF1400 (47.0 ml/minute; $p < 0.001$). Vancomycin clearance remained stable for AN69 filters but decreased for HF1400. Interleukin (IL)-8 was removed primarily *via* adsorption with the AN69 filters (92.2 and 91.2 ml/minute for AN69-M and AN69-ST, respectively), but clearance was significantly lower with HF1400 (8.4 ml/minute). Tumor necrosis factor (TNF)-α clearance was higher with AN69-ST compared with AN69-M or HF1400 (10.3, 1.8, and 2.3 ml/minute, respectively). β_2-microglobulin clearance was higher with both AN69-based filters. The hydrogel water repartition of AN69 filters was different, with a higher percentage of bound water in AN69-ST *versus* AN69-M (30.5% ± 0.2% and 19.3% ± 1.5%, respectively; $p < 0.05$). These results suggest that clearance profiles of CRRT filters differ according to their properties; further investigation is needed to translate this into clinical improvements. *ASAIO Journal* 2025; 71:510–518

http://links.lww.com/ASAIO/B414

Key Words: AN69-ST, dialysis membrane, continuous renal replacement therapy, clearance, cytokines

Acute kidney injury (AKI) is currently recognized as a collection of clinical syndromes, characterized by a sudden decrease in estimated glomerular filtration rate, which can lead to poor outcomes and adverse long-term complications.[1,2] The pathophysiology of AKI is thought to be multifactorial, and systemic inflammation may be a major component.[2] During AKI, inflammatory mediators are released into the injured kidney, leading to inflammation.[2,3] Continuous renal replacement therapy (CRRT) removes inflammatory mediators from the blood in a nonselective manner.[4–6] Current evidence suggests that the plasma levels of some inflammatory mediators are lowered by a combination of diffusion, convection, and adsorption, depending on the nature and composition of the membrane used.[7,8]

Middle-weight molecules (17–52 kDa), including IL-1β, IL-6, and IL-1 receptor agonist (RA), span a broad range of molecular sizes and are often inefficiently removed by current dialysis strategies.[9,10] Polyarylethersulfone (PAES)-polyvinylpyrrolidone (PVP) HF1400, AN69-M (M series), and AN69-ST (ST series) filters are used in extracorporeal blood purification techniques that specifically target middle-weight molecules, as well as fluid and electrolytes for kidney support. However, limited evidence is available regarding the respective clearance profiles for these filters over a wide range of middle-weight molecules.

This *in vitro* study aimed to characterize the performance and efficacy of the AN69-M, AN69-ST, and HF1400 filters over a wide range of molecules. The side-by-side comparisons specifically looked at antibiotics, uremic toxins, chemokines, cytokines, and albumin using a model of continuous solute infusion over a 72 hour period. The AN69 membrane composition was also assessed.

Materials and Methods

Given the nature of the *in vitro* experiment, which involved the use of a simulated CRRT procedure, with no human or

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Submitted for consideration June 2024; accepted for publication in revised from November 2024.

Disclosure: B.M. and D.P. are employees of Baxter, Gambro Industries. M.H., J.K., and R.S. are employees of Baxter, Gambro Dialysatoren. J.E. is a full-time employee of Baxter International, with an ownership interest. L.Y. has no conflicts of interest to report.

Editorial support for the development of this manuscript was provided by Niamh Mortimer and Amy Brown of Nucleus Global, with funding provided by Baxter.

B.M., M.H., J.K., R.S., and D.P. conceived and designed the experimental layout. All authors evaluated and analyzed the data, supported the writing of the manuscript, and studied and discussed the literature and experimental results.

The data sets used or analyzed during the current study are available from the corresponding author on reasonable request.

The results presented in this paper have not been published previously in whole or in part. This publication was subject to review by internal employees from Baxter Healthcare Corporation before submission for protection of confidential information. However, the authors retain full responsibility for the content of this publication.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML and PDF versions of this article on the journal's Web site (www.asaiojournal.com).

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DOI: 10.1097/MAT.0000000000002387

animal participants, there was no requirement for evaluation or approval from the Experimental Research Ethics Committee.

Simulated CRRT Procedure

The investigated devices were the AN69-M (M150 set), the AN69-ST (ST150 set), and the HF1400 sets (PAES-PVP), shown in Table 1 (Gambro Industries, Meyzieu, France).

The test solutions were 3 L of fresh citrated frozen plasma (Octapharma Biopharmaceuticals GmbH, Heidelberg, Germany). To this solution was added a selection of cytokines (500 pg/ml of IL-8, 3,400 pg/ml of IL-1 RA, 100 pg/ml of IL-1β, 200 pg/ml of IL-1α, 4,000 pg/ml of IL-6, 150 pg/ml of interferon [IFN]-γ, 2,500 pg/ml of IL-10, and 200 pg/ml of TNF-α [PeproTech, Hamburg, Germany]), uremic toxins (2 g/L of urea [Merck, Readington, NJ] and 320 μg/L of myoglobin [Lee BioSolutions, Maryland Heights, MO]), and antibiotics (40 mg/L of vancomycin, 40 mg/L of meropenem, and 40 mg/L of ceftriaxone [Lyomark, Oberhaching, Germany]) (Table S1, Supplemental Digital Content, http://links.lww.com/ASAIO/B413).

The *in vitro* experimental setup consisted of a CRRT procedure in the continuous veno-venous hemodiafiltration (CVVHDF) modality on a Prismaflex monitor (Baxter, Deerfield, IL). The filters were connected to a human plasma reservoir (Figure 1).

The investigated filters were primed with a saline solution, following the monitor's instructions. The blood circuit inlet/outlet was connected to the test solution. The priming solution was discarded.

The *in vitro* experimental CRRT procedure was then conducted for 72 hours using the test solution and maintained at 37°C with a plasma flow rate (Q_p) of 150 ml/minute, a dialysate flow rate (Q_D) of 2,000 ml/hour, and an ultrafiltration flow rate (Q_{UF}) of 1,000 ml/hour. To simulate a continuous removal process of the test solutes, an infusion flow rate (Q_{inf}) of 60 ml/hour of a 5 L spike solution of albumin solution at 67 g/L was implemented alongside 2.1 μg/L of IL-8, 14.2 μg/L of IL-1RA, 0.42 μg/L of IL-1β, 0.83 μg/L of IL-1α, 16.7 μg/L of IL-6, 0.63 g/L of IFN-γ, 10.4 μg/L of IL-10, and 0.83 μg/L of TNF-α (PeproTech), uremic toxins (19.2 g/L of urea [Merck], 1.33 mg/L of myoglobin [Lee BioSolutions]), and antibiotics (1.33 mg/L of vancomycin, 167 mg/L of meropenem, and 167 mg/L of ceftriaxone [Lyomark]). In addition, for practical considerations and the absence of a steady state in the test solution, β_2-M was added to the plasma test solution as a bolus of approximately 10 ml β_2-M solution (Lee BioSolutions), 15 minutes before sampling point at 1 hour (Figure 1). The selected number of dose mediators was based on pathological concentrations in septic-AKI.[11–14] The concentrations of antibiotics were chosen to match plasma levels of common prescriptions. The urea concentration was chosen to match the level of uremic concentrations. Purified β_2-M and myoglobin were of limited availability, so chosen concentrations were the minimal concentration that allowed reliable clearance measurements.

To compensate for the continuous infusion and maintain the test solution volume at 3 L, the fluid removal rate was set to 60 ml/hour on the Prismaflex monitor, the CRRT monitor controlling fluid balance via an integrated weighting system. Test solution samples were collected in the plasma reservoir before the CRRT procedure start at the filter blood inlet (C_{Bin}), blood outlet (C_{Bout}), and dialysate outlet (C_{Dout}), and at 1, 6, 12, 24, 48, and 72 hours; additionally, samples were collected in the spike solution at 1, 24, 48, and 72 hours. All samples were stored at less than −15°C until assay and thereby protected from light. Samples of 1 ml were additionally taken from every dialysate outlet waste bag and, at the same time, the exact volume in the effluent bag was noted. All effluent samples of each test were combined according to the ratio of the volume of the effluent bags to get a representative effluent sample.

Test solution volume reduction by sampling was below 1%, and the overall reduction of the test solution volume by sampling was in the range of 3%. The CRRT procedure was repeated with three filters of each type: AN69-M, AN69-ST, and HF1400.

Laboratory Analysis

Analyses of chemokines and cytokines were performed at the contract research lab NMI Laboratories (Reutlingen, Germany) using multiplexed immunoassays based on the Luminex platform. Vancomycin, meropenem, and ceftriaxone analyses were performed at the contract research lab Labor Limbach Laboratory (Heidelberg, Germany) using liquid chromatography with mass-spectrometric detection. Urea, β_2-M, myoglobin, and albumin were analyzed at Baxter Laboratory (Hechingen, Germany) using an enzymatic assay with photometric detection for urea on a Konelab Autoanalyzer, and nephelometric assays for the detection of β_2-M, myoglobin, and albumin on a BN ProSpec Analyzer.

AN69-Based Membrane Characterization

The composition of the AN69-M and AN69-ST membranes was characterized, a schematic representation of which can be found in Figure 2. For each filter, 10 ± 1 g of fibers were collected and heated under reflux for 4 hours with methanol using

Table 1. Characteristics of Investigated Dialysis Devices and Membranes

Device	Membrane Material	Structure	Inner Diameter/ Wall Thickness (μm)	Surface Area (m^2)	Sterilization Mode
AN69-M (M150 set)	Copolymer of AN69	Symmetric hydrogel	240/50	1.5	EtO
AN69-ST (ST150 set)	Copolymer of AN69 coated with PEI	Symmetric hydrogel	240/50	1.5	EtO
HF1400 set	PAES	Asymmetrical finger-like	215/50	1.4	EtO

AN69, acrylonitrile and methallyl sulfonate; EtO, ethylene oxide; PAES, polyarylethersulfone; PEI, polyethyleneimine.

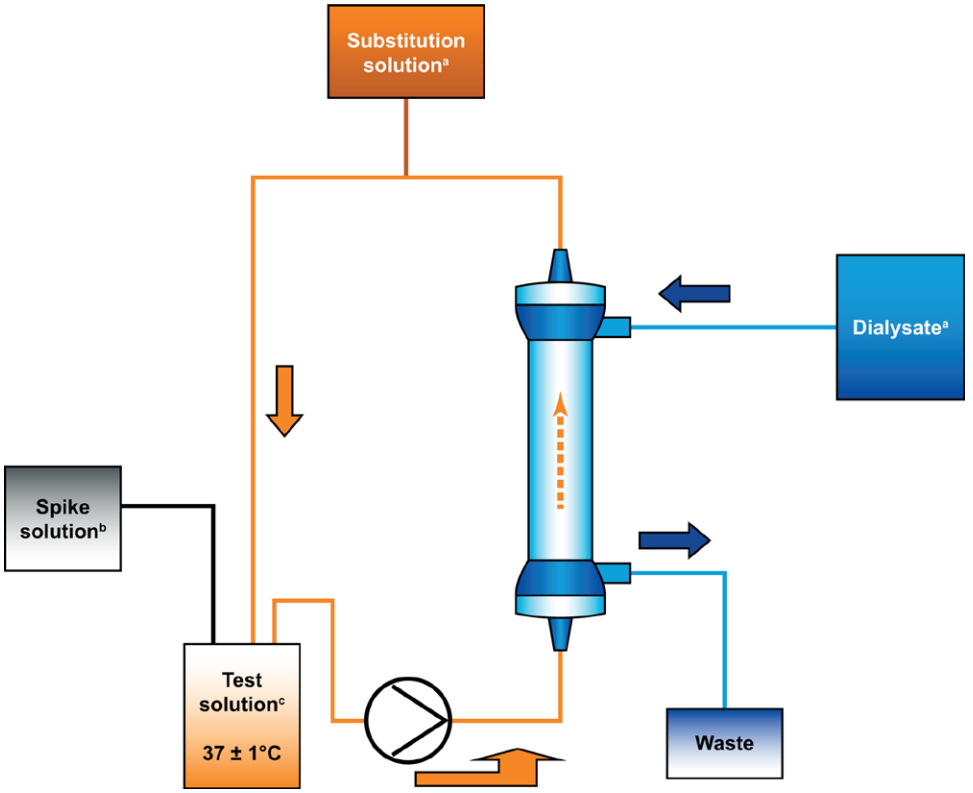

Figure 1. Experimental CRRT setup. [a]Composed of Baxter Prism0cal B22. [b]Consists of albumin solution alongside inflammatory mediators. [c]3 L of fresh citrated frozen human plasma incubated with inflammatory mediators. CRRT, continuous renal replacement therapy.

a Kumagawa extractor (Verre Equipements, Collonges-au-Mont-d'Or, France). The fiber samples were heated at 105°C ± 3°C for 2 hours before being kept in a desiccator for 30 minutes. Glycerol content was quantified in the methanolic extract after the addition of distilled water and methanol evaporation, glycerol oxidation with sodium metaperiodate, and volumetric assay of the reactional formic acid using bromothymol blue titrated with sodium hydroxide. Considering the glycerol amount would be fully replaced with saline solution during the priming procedure, glycerol was assimilated to free water in the device. Finally, the water content in the fiber (bound water) was measured using the Karl Fischer method. The experiment was then repeated with four filters of each type.

Clearance Calculations

Clearances were calculated as instantaneous clearance on the dialysate side ($Clearance_{DS}$) using the following equation:

$$Clearance_{DS} = \frac{(Q_D + Q_{UF}) \times C_{Dout}}{C_{Bin}}$$

wherein C_{Dout} is the dialysate outlet concentration, C_{Bin} is the filter blood inlet concentration, Q_D is the dialysate flow rate, and Q_{UF} is the ultrafiltration flow rate. For filters with adsorptive solute removal (*e.g.*, AN69-based materials), clearances were calculated as instantaneous clearance on blood side ($Clearance_{BS}$) using the following equation:

$$Clearance_{BS} = \frac{Q_p \times C_{Bin} - (Q_p - Q_{UF}) \times C_{Bout}}{C_{Bin}}$$

wherein C_{Bin} is the filter blood inlet concentration, C_{Bout} is the blood outlet concentration, Q_p is the plasma flow rate at the filter inlet, and Q_{UF} is the ultrafiltration flow rate. The nominal Q_{Bin} value was taken for the clearance calculation. Q_{Bin} accuracy was verified after 2 and 70 hours.

For data evaluation and verification, a mean inlet-outlet mass balance (MB_{inout}) was calculated:

$$MB_{inout} = \frac{J_{out} - J_{in}}{J_{in}} = \frac{(Q_p - Q_{UF}) \times C_{Bout} + (Q_D + Q_{UF}) \times C_{Dout}}{Q_p \times C_{Bin}} - 1$$

wherein J_{out} is the mass flux at the filter blood outlet, J_{in} is the mass flux at the filter blood inlet, Q_p is the blood flow rate at the filter inlet, Q_{UF} is the ultrafiltration flow rate, C_{Bout} is the blood outlet concentration, C_{Bin} is the filter blood inlet concentration, Q_D is the dialysate flow rate, Q_{UF} is the ultrafiltration flow rate, and C_{Dout} is the dialysate outlet concentration.

Finally, for the specific case of TNF-α, clearances were derived from the kinetics concentration in the test solution to increase accuracy due to the low concentration differences from the blood side.

The expected kinetics of the test solution concentration assumed TNF-α infusion rate (J_{inf}), the test solution volume ($V0$), and the clearance (K) constant over time.

A nonlinear regression tool is used to derive the above three parameters from the seven concentration records available for each experiment (times 0, 1, 6, 12, 24, 48, and 72 hours) using Minitab software (Minitab Inc., State College, PA), with the following equation:

$$C(t) = (C_0 - C_\infty) \times e^{-K.t/V_0} + C_\infty \text{ with } C_\infty = \frac{J_{inf}}{K}$$

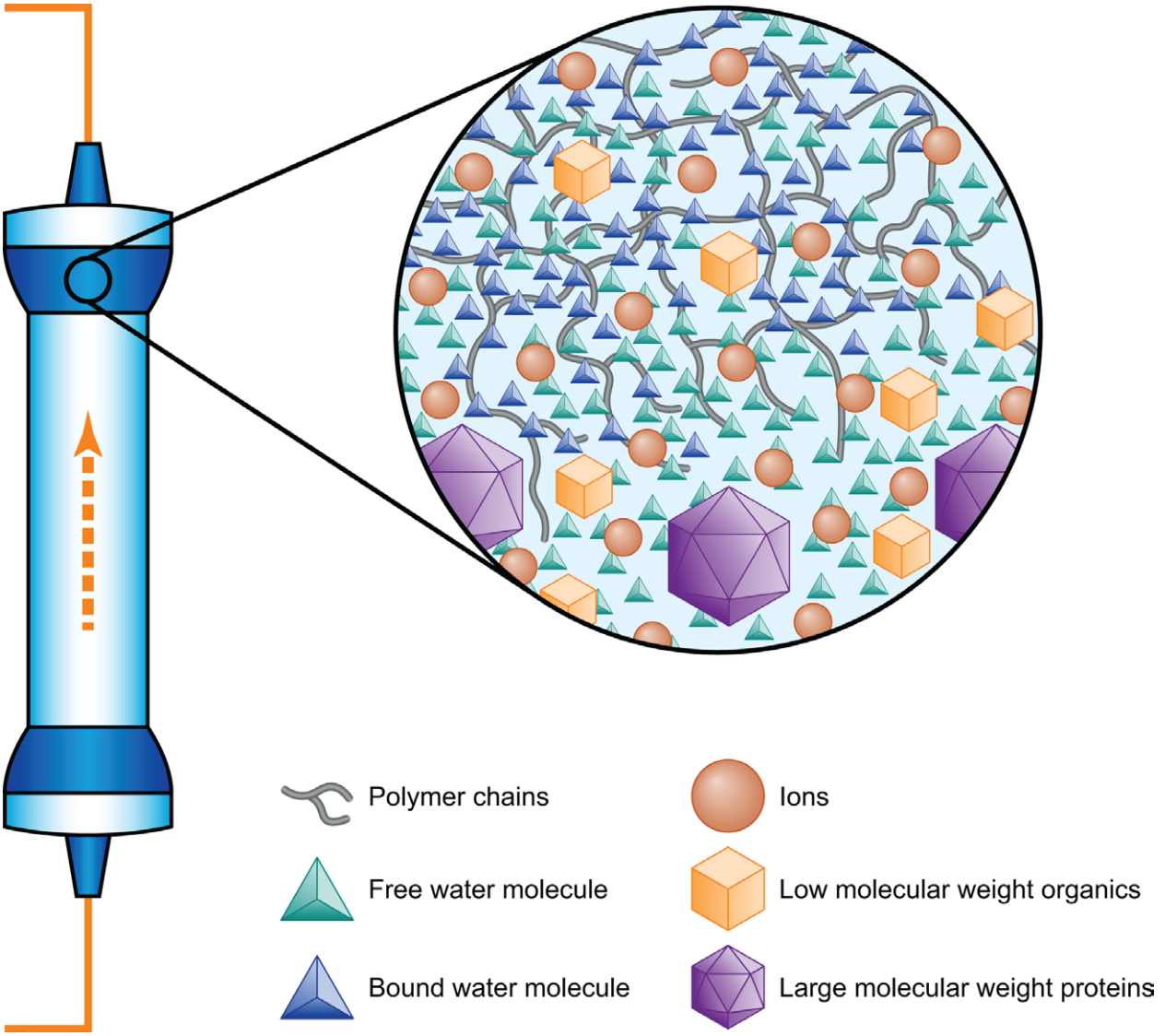

Figure 2. Schematic representation of the AN69 hydrogel-like membrane structure. AN69, acrylonitrile, and methallyl sulfonate.

wherein C_0 is the initial test solution concentration ($t = 0$), C_∞ is the test solution concentration at steady state, J_{inf} is the TNF-α infusion rate, K is the clearance, and V_0 is the test solution volume.

For AN69 membrane composition determination, the following equations were used:

$$\text{Polymer } P\ \%\ (w/w) = 100\ S/E$$

wherein S is the mass of dry fiber after methanolic extraction, heating, and desiccation, and E the mass of starting fiber weight:

$$\text{Free water (glycerol)}\ \%\ (w/w) = (\text{MW} \times V_s\,(V - v) \times N)\,/\,(E \times V_e)$$

wherein MW is the molecular weight of glycerol, V_s is the volume of the methanolic solution, V is the volume of sodium hydroxide used in the reaction, v is the volume of sodium hydroxide used in the blank test, N is the normality of sodium hydroxide, E is the mass of starting fiber weight, and V_e is the volume of the methanolic solution used for the titration.

$$\text{Bound water content } H\ \%\ (w/w) = (d \times V_s \times (C–c))\,/E$$

wherein d is the methanol density, V_s is the volume of the methanolic extraction, C is the water content in the percentage of the methanolic extract, c is the water content in the percentage of the starting methanol solution, and E is the mass of starting fiber weight.

Statistical Analysis

Statistical analysis was performed for mean clearance comparison between the different devices and the AN69-based membrane comparison analysis. Data were expressed as mean values ± standard deviation. Differences between groups were compared for mean clearances by analysis of variance (ANOVA) and for median membrane composition by the Mann–Whitney test using Minitab software (Minitab Inc.). A p value of less than 0.05 was considered to indicate a significant difference.

Regarding mean MB_{inout} evaluation, a criterion (MB_{inout}) of greater than 5.0% was used as a minimum threshold to

Table 2. Mean CVVHDF Clearances Values (ml/minute) Over 72 Hours for Different Devices (Except for Myoglobin and β_2-M)

		HF1400			AN69-M		AN69-ST		
Solute	MW	Mean ± SD	N	p1	Mean ± SD	N	Mean ± SD	N	p2
Urea	60	47.0 ± 4.3	18	<0.001	52.4 ± 0.9	18	51.2 ± 0.9	18	0.01
Vancomycin	1,450	28.1 ± 5.7	18	0.001	33.4 ± 2.3	18	33.0 ± 3.2	16	NS
IL-8	8,900	8.4 ± 2.7	18	<0.001	92.2 ± 7.4	18	91.2 ± 10.1	18	NS
β_2-M	11,731	9.9 ± 5.0	6	<0.001	29.1 ± 8.3	5	29.8 ± 7.3	6	NS
Myoglobin	17,000	3.6 ± 1.0	3	<0.001	13.4 ± 3.1	3	21.3 ± 2.7	3	0.028
IL1-RA	17,126	2.8 ± 1.8	18	<0.001	16.0 ± 10.4	18	23.6 ± 6.3	18	0.021
IL-1β	17,377	2.8 ± 1.4	18	<0.001	10.2 ± 6.0	16	18.3 ± 6.4	18	0.001
IL-6	21,000	0.7 ± 0.4	18	<0.001	4.9 ± 2.8	18	8.6 ± 2.4	18	0.000
TNF-α	52,000	2.3 ± 1.5	3	NS	1.8 ± 1.0	3	10.3 ± 1.2	3	0.000
Albumin	69,000	0.0 ± 0.0	4	N/A	0.0 ± 0.0	3	0.0 ± 0.0	5	N/A

p1, *p* value for identical means between HF1400 and AN69-M; p2, *p* value for identical means between AN69-ST and AN69-M; NS for $p > 0.05$; Plasma flow, 150 ml/minute; Q_D, 2,000 ml/hour; Q_{UF}, 1,060 ml/hour; Q_{Dout}, 3,060 ml/hour (51 ml/minute). Myoglobin was calculated at 1 hour and mean β_2-M was calculated between 1 and 72 hours.

β_2-M, β_2-microglobulin; CVVHDF, continuous veno-venous hemodiafiltration; IL, interleukin; MW, molecular weight; N, number of clearance values; N/A, not applicable; NS, not significant; QD, dialysate flow rate; QD_{out}, total effluent flow rate; QUF, ultrafiltration flow rate; RA, receptor agonist; SD, standard deviation; TNF, tumor necrosis factor.

conclude on the significant contribution of adsorption in the clearance process (Table S2, Supplemental Digital Content, http://links.lww.com/ASAIO/B413). In that case, the calculation of $Clearance_{BS}$ was retained; on the contrary, the calculation of $Clearance_{DS}$ was used as it is more accurate in the context of CRRT.

Results

Clearance Measurements

The mean clearance performances calculated over the study period are presented in Table 2. In addition, the respective clearance kinetics for some representative solutes are presented for each filter in Figures 3 and 4.

Over 72 hours, stable clearances of urea were measured as approximately 51 ml/minute for AN69-based filters (AN69-M and AN69-ST), whereas a decrease over time was noticed for the HF1400 filter. A similar observation was made for vancomycin at a relatively lower clearance rate, at steady state of around 33 ml/minute for AN69-based filters, and 28 ml/minute for the HF1400 filter (Table 2). With respect to chemokines, IL-1β and IL-6 were primarily removed by diffusion-convection with the three filters. An initial peak at 1 hours was observed for AN69-M and AN69-ST filters, followed by a relatively constant clearance profile (Figure 3).

IL-8 was primarily removed by adsorption with the AN69-based filters, with a mean $Clearance_{BS}$ level of approximately 90 ml/minute and limited decrease over time. For the HF1400 filter, the clearance level was significantly lower at approximately 8 ml/minute ($p < 0.05$) (Table 2). Similarly, the relative contribution of adsorption was also observed for IL-1β specifically, with significantly higher clearances for the AN69-ST filter compared with the AN69-M and HF1400 filters ($p < 0.05$); clearance with the AN69-M filter was also found to be significantly higher than for the HF1400 filter ($p < 0.05$) (Figure 4). For the case of TNF-α, relatively low clearances were measured but a significantly higher performance was noted with the AN69-ST filter compared with the AN69-M and HF1400 filters ($p < 0.05$). Additionally, a significant difference was found for myoglobin clearances between each filter type ($p < 0.05$). Significant depletion in the pool only enabled meaningful comparisons at 1 hour. Regarding β_2-M, significantly higher clearances were observed at 1 and 72 hours with the AN69-based filters compared with the HF1400 filter ($p < 0.05$). Considering that a continuous infusion was not performed, and that clearance was measured a few minutes after the plasma reservoir had been spiked with β_2-M, no steady state could be expected at the sampling time, and thus data were expressed at 1 and 72 hours only. Of note, the clearance performances for meropenem, ceftriaxone, IL-1α, IL-10, and IFN-γ are not reported due to the lack of stability in the plasma pool over time (data not shown). Similarly, some clearance values were set as missing data in cases where dialysate side clearance measurement concentration data were below the low detection limit (as in IL-1β and albumin) or when clearance exceeded the effluent flow rate (as in vancomycin).

The clearance profiles of the different filters, as a function of the test solute molecular weight, are represented in Figure 5. The AN69-M and AN69-ST filters had, overall, significantly higher clearances for middle-weight molecules compared with the HF1400 filter, and there were specifically increased clearances in the range of 17–52 kDa with the AN69-ST filter when compared with the AN69-M filter.

Albumin Losses

The total amount of albumin was measured in the effluent pool collected over 72 hours. The respective albumin losses were: 1.0 ± 0.3 g with the AN69-M filter, 0.7 ± 0.3 g with the AN69-ST filter, and 2.5 ± 3.2 g with the HF1400 filter (not significant).

AN69 Membrane Composition

The respective amounts of polymer, free water, and bound water were reported for the AN69-M and AN69-ST filters (Figure 6). For the AN69-M and AN69-ST filters, respectively, the median percentage of polymer was 30.1% and 27.4% ($p < 0.05$), the median percentage of free water was 50.1% and

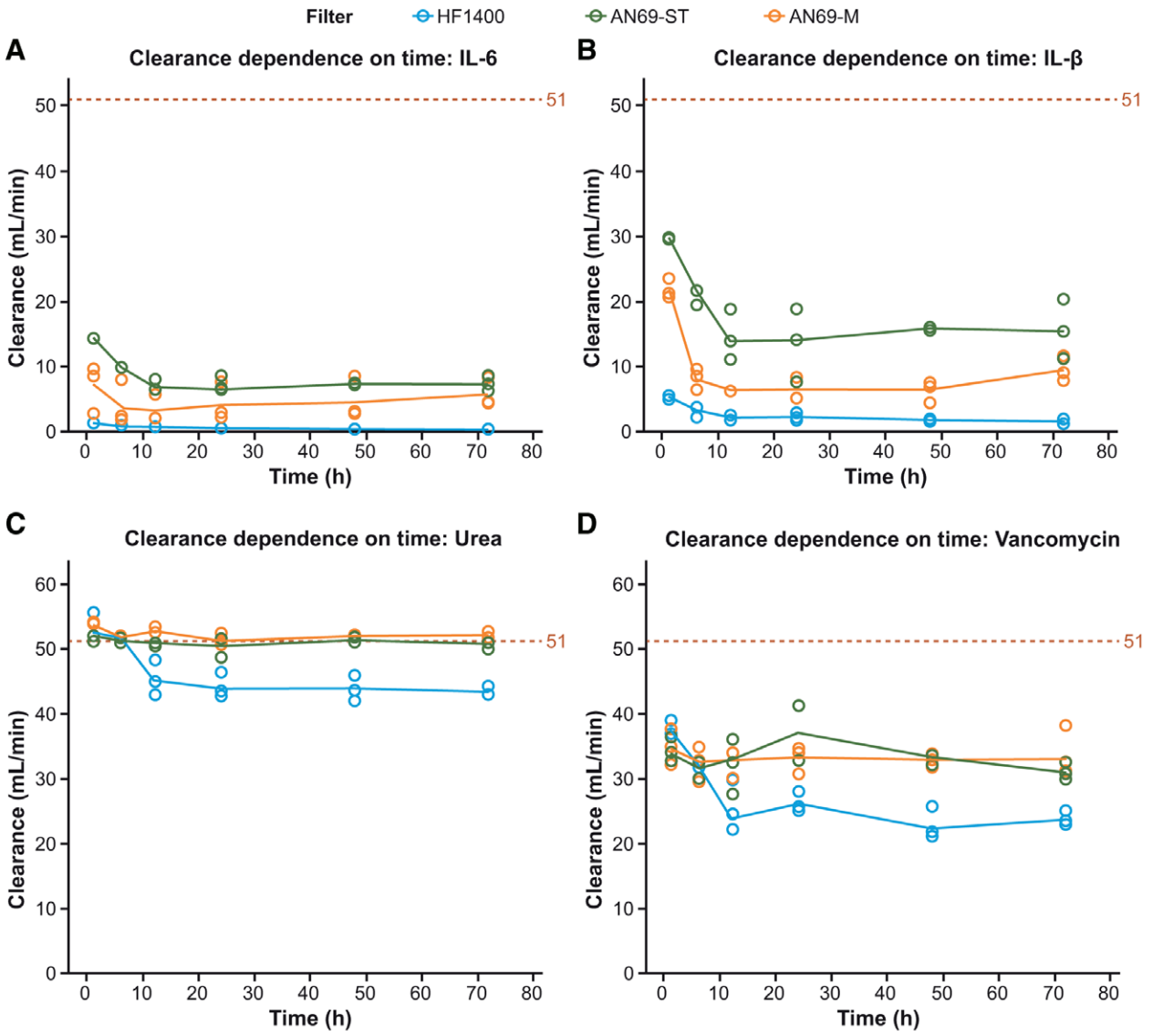

Figure 3. Clearances between the HF1400, AN69-M, and AN69-ST devices for IL-6 (**A**), IL-1β (**B**), urea (**C**), and vancomycin (**D**). h, hours, IL, interleukin. Calculated from the dialysate side. *p* Values for IL-6, IL-β, urea, vancomycin, and the identical means between HF1400, AN69-M, and AN69-ST are presented in Table 2.

41.8% ($p < 0.05$), and the median percentage of bound water was 18.7% and 30.5% ($p < 0.05$).

Discussion

In this study, we have shown that the performances of widely used CRRT filters may vary in terms of the nature of solutes to be removed. AN69-based filters showed an increased clearance profile for middle-weight molecule solutes in the range of 17–52 kDa, compared with the PAES-based HF1400 filter, with a large contribution of adsorption for specific solutes (*ie* IL-8, IL-1β, and B_2-M).

Uremic Toxin and Antibiotic Clearances

With respect to low molecular weight solute removal, the study confirms that the clearance approximates the effluent flow rate.[15,16] Of note, whereas the AN69-based filters showed a highly stable clearance profile over 72 hours, a certain decrease was observed for the PAES-based HF1400 filter. The same phenomenon was confirmed for the evaluation of vancomycin (1,450 Da) clearance and may reflect some evolution of the membrane permeability over time or disturbance from the dialysate compartment circulation. The observed behavior for vancomycin removal is consistent with previous work, in which no adsorption was reported *in vitro* with the AN69- and PAES-based membranes,[17] and for which some dose recommendations were proposed.[18] For middle-weight molecules, the AN69-M and AN69-ST filters showed significantly higher clearances at 1 hour for myoglobin, and at 1 and 72 hours for β_2-M. In accordance with Randoux *et al.*,[19] some adsorption was noticed for β_2-M with AN69 membranes, which was reflected by the difference in β_2-M disappearance from the pool and β_2-M recovered in the filtrate.

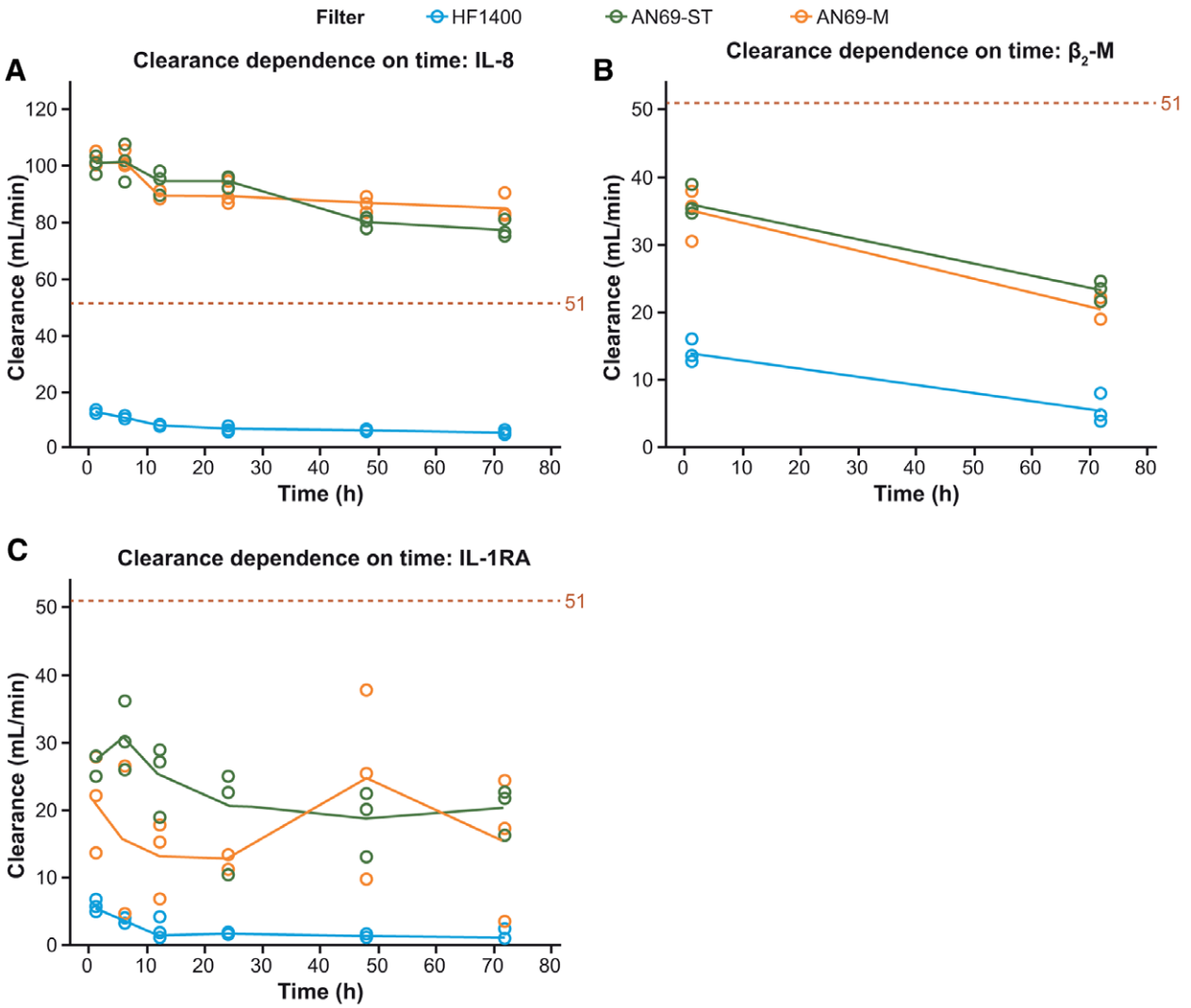

Figure 4. Clearances between the HF1400, AN69-M, and AN69-ST devices over 72 hours for IL-8 (**A**), β_2-M (**B**), and IL-1RA (**C**). β_2-M, β_2-microglobulin, h, hours, IL interleukin, RA, receptor agonist. Clearances calculated from the dialysate side for the HF1400 set and from the blood side for AN69-based devices to account for the adsorption mechanism. *p* Values for IL-8, β2-M, IL-1RA, and the identical means between AN69-M, AN69-ST, and HF1400 are presented in Table 2.

Chemokine and Cytokine Clearances

Among the contributing factors that may lead to AKI, systemic inflammation has been reported as a major characteristic, notably in patients with the sepsis-AKI phenotype.[20–22] Elevated levels of cytokines have been associated with lower renal recovery and higher mortality rates in critically ill patients requiring CRRT.[2] In a multicenter, prospective, observational cohort study of 817 critically ill patients receiving renal replacement therapy (RRT), the risk of RRT dependence and death appeared notably higher for increased IL-8 concentrations.[23] In our study, we have shown specifically high clearance performances for IL-8 with an AN69-based membrane via the adsorption mechanism, with limited signal of membrane saturation. The origin of this phenomenon revolves around the polymer's negatively charged density, which enables ionic interactions with toxins of elevated isoelectric points (above 7) or toxins bearing cationic residues at their periphery.[24] These observations are consistent with previous work, which confirmed the absence of saturation for the AN69-ST membrane's adsorptive capabilities with high mobility group box 1.[25] This also supports the idea that the AN69-ST surface coating has no influence on the accessibility of these solutes to the inner polymer structure compared with the one in the membrane bulk. Compared with IL-8, lower clearances were measured with AN69-based membranes for IL-1RA, IL-1β, IL-6, and TNF-α, all of which are characterized by a lower isoelectric point (below 7.4) and a higher MW. The initial clearance peak at 1 hour may still reflect early adsorption, followed by convection at a constant rate. Finally, clearances measured with HF1400 were lower compared with the AN69 filters, which may also be attributed to a lower permeability. Overall, these observations are consistent with previous work that confirmed better clearances of highly positively charged substances for IL-8 with an AN69-ST membrane.[25,26] Of note, the higher clearance of TNF-α observed with AN69-ST may reflect removal capabilities for higher MW toxins compared

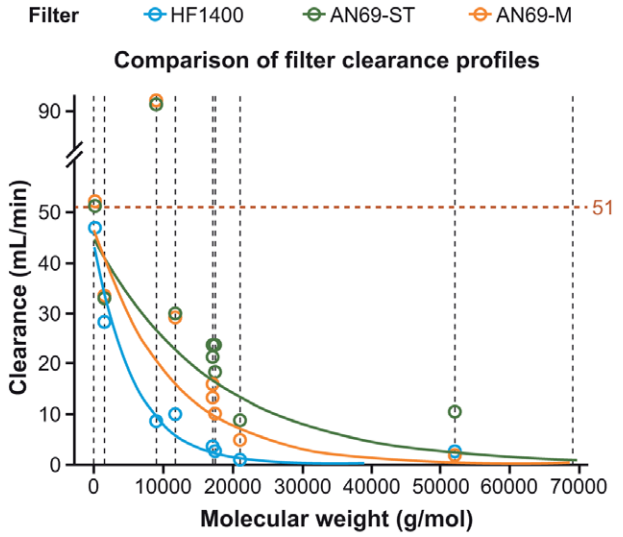

Figure 5. Comparison of clearance profiles between AN69-M, AN69-ST, and HF1400 filters. Theoretical maximum clearance (excluding adsorption contribution) Q_{Dout} 3,060 ml/hour (51 ml/minute).

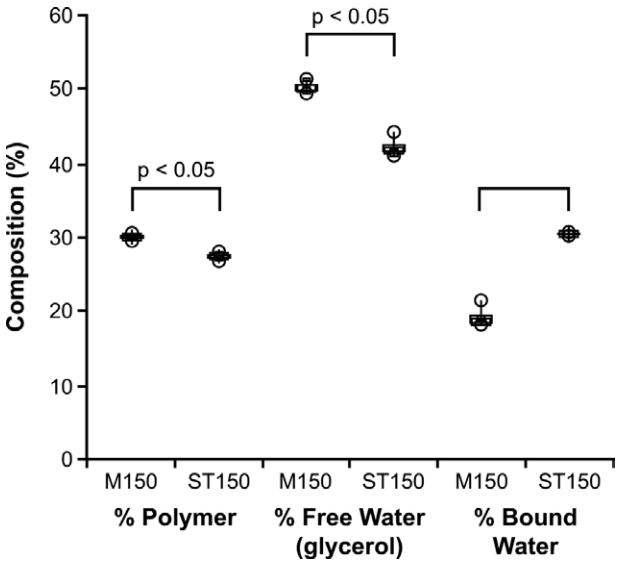

Figure 6. Comparative box plots: relative composition of the AN69-M and AN69-ST filters (n = 4 test samples per filter type). The central horizontal line within each box is the median 50th percentiles. Open circles represent individual outliers of 90th percentiles.

with AN69-M and HF1400, possibly due to increased permeability and/or accessibility to the inner membrane, favoring adsorption.

Albumin Losses

Overall, albumin loss remained low over the 72 hour period, specifically below 1 g with the AN69-based membranes. The net negative charge of albumin, with a low isoelectric point at physiological pH, may lead to some repulsive effects with the AN69 hydrogel-like structure.[27,28]

AN69 Membrane Composition

The dense, symmetrical, and highly hydrophilic structure of the AN69 hydrogel-like membrane offers a unique context for bulk adsorption of low molecular weight proteins.[24] The membrane exhibits the macromolecular structure of a gel (Figure 2), wherein water is present as both freely moving molecules and as molecules trapped and immobilized in the hydrogel network. The higher water content of the hydrogel makes the polymer chains easily accessible[24]; interestingly, in this *in vitro* experiment, a significantly higher proportion of bound water was measured for the AN69-ST filters compared with the AN69-M filters, suggesting a substantial difference in the water repartition process in the two filters. The ST membrane features the same AN69-based polymer and fiber manufacturing process, with the addition of a surface coating made of polyethyleneimine. This coating allows the reduction of surface adsorption for high molecular weight proteins without affecting the bulk adsorption of low- to medium-sized proteins; it also allows the binding of heparin.[24] The observation of increased bound water content with the AN69-ST filter compared with the AN69-M filter supports a mechanism of membrane rehydration during the surface coating. Ultimately, it is hypothesized that this difference may favor the transfer of solutes within the bulk membrane and explain the overall better clearance profile for middle-weight molecules with the AN69-ST filters over the native AN69-M filters.

Limitations

Our study has limitations that should be acknowledged. First, as an *in vitro* study, the outcomes observed here may not be representative of the clinical setting. Second, the experimental setup used human plasma spiked with inflammatory mediators; this system does not allow us to examine the impact of the devices on the inflammatory response itself nor account for the possible influence of blood cells at the filter mass transfer resistance level. Third, the selected anticoagulation strategy using citrate in excess may not accurately reflect the clinical environment, where the method of anticoagulation and other clinical practices can affect the filter's lifespan. Finally, with respect to the contribution of the adsorptive mechanism, the concentrations used were pathological and our data can only be used to comment on the clearances of these mediators, at these concentrations.

Conclusions

The AN69-ST device was shown to have the highest clearance profile for middle-weight molecules in the range of 17–52 kDa compared with HF1400 and the native AN69-M filters. Adsorption was shown to be a major mechanism in the removal performances of AN69-based membranes, specifically for solutes of high isoelectric point, with a major impact of the hydrogel structural water repartition modulating the accessibility to the bulk material. The stability of the clearances over 72 hours varied for the different membranes and solutes, whereas limited saturation was observed in the case of IL-8. Further investigation is warranted as to whether these

observations may translate into any meaningful differences in clinical outcomes.

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