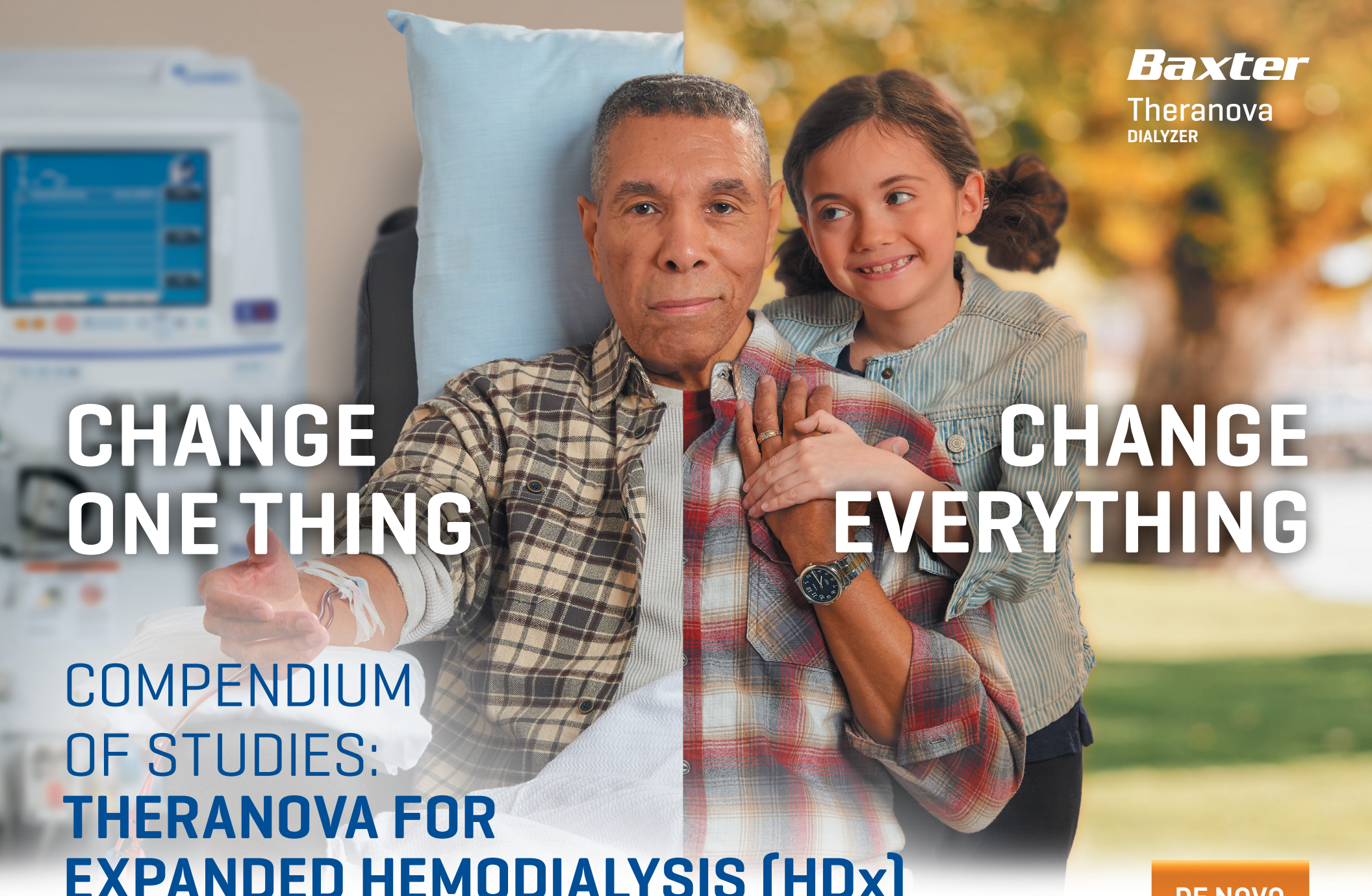

COMPENDIUM OF STUDIES: THERANOVA FOR EXPANDED HEMODIALYSIS (HDx)

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INTRODUCTION

Theranova, the next evolution in hemodialysis, brings us a step closer to the natural kidney by superior removal of conventional/large middle molecular (500-45,000 Da) uremic toxins, compared to traditional hemodialysis.[1-4] These molecules have been linked to the development of inflammation, cardiovascular disease and other dialysis related comorbidities.[3,5,6] The **Theranova** dialyzer's unique membrane design allows for a filtration profile that's closer to the natural kidney. It combines high permeability for uremic toxins (up to 45,000 Da)[2,3,6] while selectively retaining essential proteins and maintaining stable albumin levels.[1,4,7,8]

This compendium includes summaries of 20 key papers, abstracts and posters with evidence supporting use of the **Theranova** membrane and expanded hemodialysis therapy (HDx). In a number of studies, the **Theranova** membrane design is identified as a mid cut-off membrane (MCO) or high retention onset membrane; they are synonymous. This membrane has a pore size between that of a standard high flux and a high cut-off membrane with narrowly distributed pores to enhance membrane permeability and selectivity.[6,7,9]

The compendium is categorized by five topics:

- Safety evidence of the MCO/**Theranova** membrane and HDx therapy
- Performance of the MCO/**Theranova** membrane and HDx therapy
- Clinical Effectiveness of the MCO/**Theranova** membrane
- Quality of Life Improvement by switching to HDx therapy
- Economic Impact of HDx Therapy

While the studies cited in this compendium do not represent a comprehensive review of the literature, they are representative of conclusions drawn in the literature regarding the clinical use and science pertaining to **Theranova** and expanded hemodialysis therapy.

References: 1. Kirsch AH, et al. Performance of hemodialysis with novel medium cut-off dialyzers. Nephrol Dial Transpl 2017; 32(1):165-72. 2. Boschetti-de-Fierro, A., Voigt, M., Storr, M. et al. MCO Membranes: Enhanced Selectivity in High-Flux Class. Sci Rep 5, 18448 (2015). https://doi.org/10.1038/ srep18448. 3. Zweigart C, Boschetti-de-Fierro A, Hulko M, et al. Medium cut-off membranes - closer to the natural kidney removal function. Int J Artif Organs. 2017; 40(7):328–334. doi:10.5301/ijao.500060. 4. Lim JH, Park Y, Yook JM, et al Randomized controlled trial of medium cut-off versus high-flux dialyzers on quality of life outcomes in maintenance hemodialysis patients. 2020; 10:7780. 5. Wolley M, Jardine M, Hutchison CA. Exploring the Clinical Relevance of Providing Increased Removal of Large Middle Molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814. 6. Hutchison CA, Wolley M. The Rationale for Expanded Hemodialysis Therapy (HDx). Contrib Nephrol. 2017;191:142–152. Doi:10.1159/000479262. 7. Krishnasamy R et al. Trial evaluating mid cut-off value membrane clearance of albumin and light chains in hemodialysis patients (REMOVAL-HD): a safety and efficacy study. ASN 2018 Kidney Week Abstract TH-PO353. 8. Bunch A, et al. Long Term Effects of Expanded Hemodialysis (HDx) on Clinical and Laboratory Parameters in a Large Cohort of Dialysis Patients. ASN 2018 Kidney Week Abstract FR-PO766. 9. Krishnasamy et al. A tRial Evaluating Mid Cut-Off Value Membrane Clearance of Albumin and Light Chains in HemoDialysis Patients (REMOVAL-HD): A Safety Device Study. *Blood Purif*. 2020;1-11.

A tRial Evaluating Mid Cut-Off Value Membrane Clearance of Albumin and Light Chains in HemoDialysis Patients (REMOVAL-HD): A Safety Device Study

Krishnasamy R et al. A trial evaluating mid out-off value membrane clearance of albumin and light chains in hemodialysis patients (REMOVAL-HD): a safety device study. *Blood Purif*. 2020; 1-11.

BACKGROUND

Patients with end-stage kidney disease (ESKD) are often burdened with a myriad of complications including cardiovascular disease, infection, and malnutrition resulting in high rates of hospitalization, reduced quality of life and increased risk of death. Retention of uremic toxins, especially middle molecules that are not well cleared by current dialysis therapies, may contribute to the disease burden in the ESKD cohort.

The clearance of middle molecules has continued to improve with the evolution of dialysis technology over the last 20 years. With the advent of high-flux dialyzers and hemodiafiltration (HDF), the efficiency of middle molecule clearance by chronic hemodialysis (HD) has continually increased; however, the clearance of almost a third of the larger middle molecules (>25kDa) is yet to be optimized. Pore sizes of dialysis membranes are crucial in determining the clearance of larger middle molecules. However, membranes with larger pore sizes such as the high cut-off dialyzer (HCO) were associated with substantial albumin loss (molecular weight (MW) (65kDa[1]), requiring albumin supplementation following dialysis treatment. This resulted in the view that HCO membranes were unsafe and impractical for maintenance HD.

Advancements have led to the development of a new class of dialysis membranes called the mid cut-off (MCO) dialyzer. This membrane has a pore size between that of a standard high flux and an HCO membrane with narrowly distributed pores to enhance membrane permeability and selectivity. However, the safety and efficiency data for the MCO dialyzer in a clinical setting are limited.

OBJECTIVE

The primary aim of a tRial Evaluating Mid cut-Off Value membrane clearance of Albumin and Light chains in Hemodialysis patients (REMOVAL-HD) study the was to determine the safety of HD using a MCO dialyzer (**Theranova**; Baxter Healthcare, Sydney, Australia) with regard to its effect on change in serum albumin over 6 months in a prevalent HD cohort.

METHODOLOGY

The study was an investigator initiated, open label, non-randomized, cross over, longitudinal device study conducted across 9 centers in Australia and New Zealand (n=89.). Recruitment commenced in January 2017 and the last participant follow-up occurred in April 2018. The criteria for inclusion included > 18 years of age, had been on chronic in-center HD for at least 12 weeks.

Study Procedures

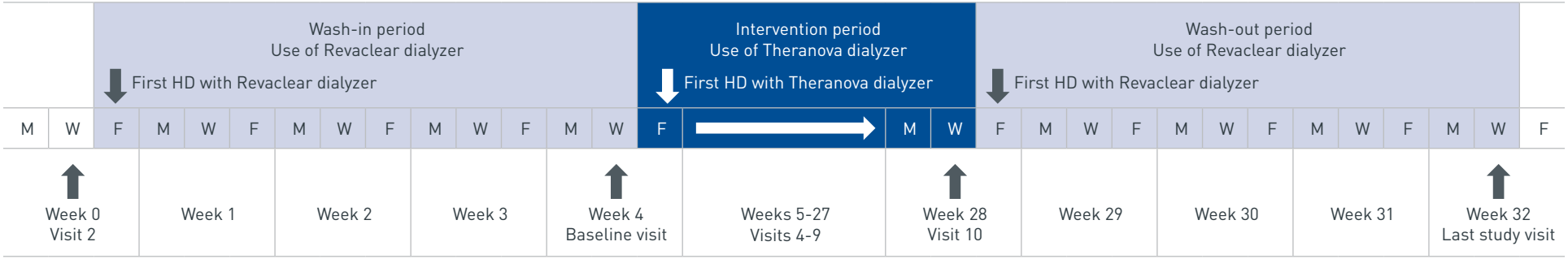

Figure 1. REMOVAL-HD study schema. Adapted from Krishnasamy et al. Abbreviations: HD, hemodialysis.

All visits occurred during participants' mid-week HD session. See Figure 1. Study schema was as follows:

- ***Wash-in period (week 0-4)***
  Participants used a 4-week HD wash-in period using a high flux dialyzer (**Revaclear** R400; Baxter Healthcare, Sydney, Australia).
- ***Intervention period (week 4-28)***
  Participants then received 24 weeks of treatment with the investigational device, the MCO dialyzer (**Theranova** 400; Baxter Healthcare, Sydney, Australia).
- ***Wash-out period (week 28-32)***
  Participants then received a 4-week wash-out period using the **Revaclear** high flux dialyzer again.

Centers were advised to maintain the duration (3.0-5.5 hours/session) and frequency of dialysis (3x/week), target blood flow (>300 mL/minute) and dialysate flow rate (DFR) (500 mL/minute) throughout the study period.

Outcome Measures

Primary Outcomes

The primary outcome was change in centrally measured pre-dialysis serum albumin between baseline (week 4) and 6 months (week 28) during the treatment phase of HD with the MCO dialyzer. Other safety outcomes included change in serum albumin across all study visits during the treatment phase monitoring of any large (>25%) reductions in serum albumin level at every visit. In addition, serious adverse events (SAE) were recorded regardless of whether they were related to the study intervention using standard criteria for clinical trials.

Secondary Outcomes

Secondary outcomes included change in pre-dialysis serum level of middle molecules (lambda-free light chains [FLC] (45 kDa), kappa-FLC (22.5 kDa) and ß2-microglobulin (11.8kDa)).

RESULTS

Eighty-nine (89) participants started the MCO HD intervention and provided analyzable data and 87 were sufficiently compliant with the intervention (at least 80% use of MCO dialyzer) to be included in the main analysis of the primary outcome.

Serum Albumin

Serum albumin 65 kDa[1] levels were stable over 6 months and the overall albumin concentration decline was minimal at 0.7 g/L. See Table 1. In addition, an immediate decline in serum albumin following commencement of the MCO dialyzer was not seen. See Figure 2. A sustained, unexplained reduction in serum albumin of >25% for 2 consecutive visits was not observed in any participant.

Timing	Measurement of Serum Albumin (n=87)	*p* value
Baseline (Week 4)	35.8 ± 3.9 g/L	
6 Months (Week 28)	35.1 ± 4.0 g/L	
Reduction (6 Months-Baseline)	–0.7 g/L (95% CI –1.5 to 0.1)	0.1

Table 1. Measurement of serum albumin and reduction. Adapted from Krishnasamy et al. Abbreviations: CI, confidence interval.

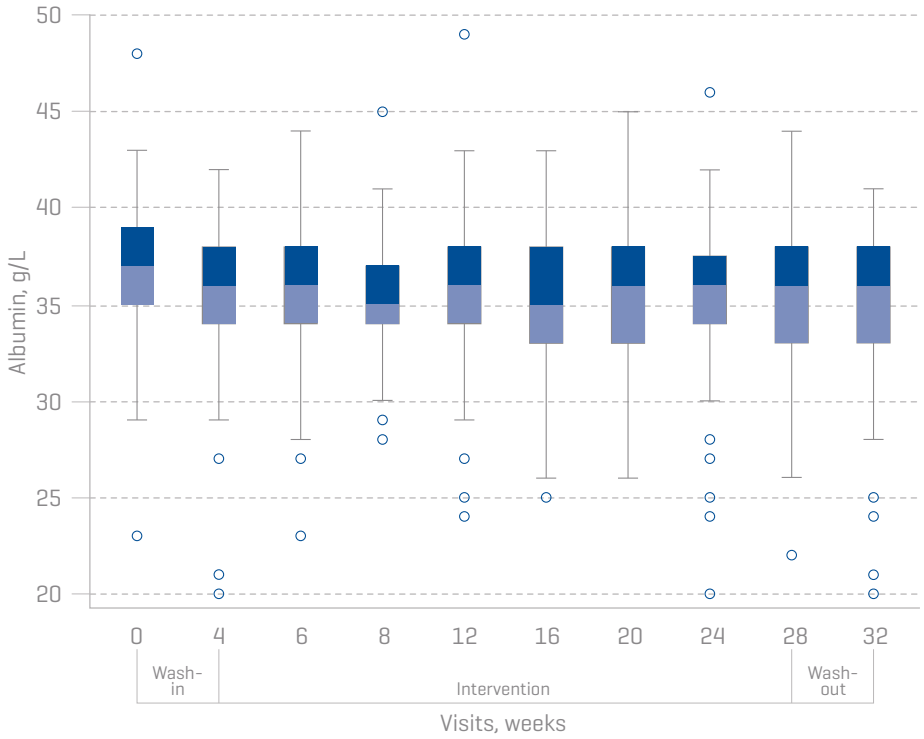

Figure 2. Trend in centrally measured serum albumin during the entire study period. Adapted from Krishnasamy et al.

Middle Molecules

A reduction in FLC was observed 2 weeks into MCO HD (see Table 2), which plateaued and remained unchanged throughout the intervention period. However, levels of both FLC significantly increased following cessation of HD with the MCO dialyzer during the wash-out of high-flux HD phase. (see Table 2). The rebound supports the hypothesis that this dialyzer can result in sustained reduction in middle molecules. The ability to provide sustained removal of large middle molecules such as lambda FLC (45 kDa) suggests that MCO HD is a promising therapy to enhance removal of other large middle molecules such as soluble tumor necrosis factor receptor-1 (27-30 kDa[1]), fibroblast growth factor-23 (32 kDa[1]), advanced glycosylated end products (<1-70 kDa[1]) that are not cleared by current conventional HD therapies, but are strongly implicated in chronic inflammation and accelerated cardiovascular disease in patients with ESKD.

	Average change from week 4-6 (95% CI)	*p* value	Average change from week 28-32 (95% CI)	*p* value
Lambda-FLC, mg/L	-9.1 (-14.4 to -3.7)	0.001	7.9 (0.8 to 14.9)	0.03
Kappa-FLC, mg/L	-5.7 (-9.8 to -1.6)	0.007	8.2 (1.3 to 15.1)	0.02

Table 2. Change in lamda- and kappa-FLC following the initial exposure (week 4-6) and cessation of MCO dialyzer (week 28-32) respectively. Adapted from Krishnasamy et. al. Abbreviations: CI, confidence interval; FLC, free light chains

No significant change in ß-2 microglobulin was observed for the duration of treatment with MCO dialyzer. As standard high-flux HD and HDF provide excellent clearance of ß-2 microglobulin, it is not surprising that this study did not find a change in the levels of ß-2 microglobulin following conversion to the MCO HD from standard dialysis treatments.

Adverse Events
There were no reported SAE's related to the MCO dialyzer for the entire duration of the study.

Study Limitations
The major limitation of the study was the single-arm design. In addition, post-dialysis serum and dialysate concentrations of albumin and middle molecules were not performed and may have provided more in-depth evidence especially regarding the efficacy of this membrane. Participant-level information on the type of dialysate including citrate-based dialysis buffer that may have an impact on middle molecule removal was not collected during the study period. By design, this study excluded major factors that may impact serum albumin measurements and confound the primary outcome in order to assess the independent effect of MCO dialyzer on serum albumin.

CONCLUSIONS

REMOVAL-HD demonstrated that regular use of MCO dialyzer for 6 months in chronic HD patients was safe and did not result in a significant fall in serum albumin. This study's results support the hypothesis that albumin loss will not be a limitation of the future application of the MCO dialyzer in chronic HD. In addition, the significant rebound of FLC levels following cessation of HD with the MCO dialyzer supports the hypothesis that this dialyzer can result in sustained reduction in middle molecules (up to 45 kDa) and represents a promising therapy to enhance removal of other large middle molecules.

REMOVAL-HD study demonstrated MCO (Theranova) dialyzer's effective removal of middle molecules up to 45 kDa, such as lambda-FLC, while maintaining stable serum albumin levels, with only 0.7g/L decline in overall serum concentrations.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

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Medium Cut-Off Dialyzers in a Large Population of Hemodialysis Patients in Colombia: COREXH Registry

Bunch A et al. Medium cut-off dialyzers in a large population of hemodialysis patients in Colombia: COREXH Registry. *Ther Apher Dial*. 2020; 1-11. doi: 10.1111/1744-9987.13506.

BACKGROUND

Recent advances in technology have introduced expanded hemodialysis (HDx) utilizing medium cut off (MCO) membranes with high retention onset membranes. MCO membranes easily clear conventional and large middle molecules with acceptable levels of albumin removal (2-4 g/session), which maintains serum albumin levels within the normal range.

Middle molecules, which accumulate during hemodialysis (HD) are considered to be inflammatory mediators. Inflammation also contributes to decreased serum albumin levels. Importantly, a lower serum albumin level is associated with increased mortality. The higher mortality rate associated with low serum albumin levels has been reported to be dependent on inflammation as assessed by high sensitivity C-reactive protein (hsCRP) levels.

Renal Therapy Services (RTS) is a nationwide provider in Colombia partially owned by Baxter that serves over 9000 patients who are undergoing HD or peritoneal dialysis. RTS dialysis units provide HD to over 5500 patients, accounting for approximately 29% of patients receiving HD in Colombia.

There is a paucity of longitudinal data regarding the clinical outcomes and safety of MCO membranes, especially in the current practice setting.

OBJECTIVE

To describe the outcomes and trends in serum albumin levels among a large cohort of patients switched from conventional high-flux HD to HDx utilizing an MCO membrane and document the long-term safety.

METHODOLOGY

Expanded Hemodialysis Registry Protocol in Colombia (COREXH) is a prospective, observational, multicenter cohort study of patients undergoing HDx in Colombia. Between September 4, 2017 to November 30, 2017 prevalent HD patients (receiving HD therapy for at least 90 days at an RTS renal clinic) were invited to participate in the registry. Patients were required to be at least 18 years of age and receiving HDx for a minimum of 4 hours, 3 times per week using an MCO membrane (**Theranova**, Baxter, Deerfield, IL). Patients with life expectancy less than 6 months or those with an active infection diagnosed within the previous 4 weeks were not invited to participate. Baseline data were obtained of the last seven days before switching to HDx and represent the initial state of the patient's health, serum albumin levels, and other laboratory parameters. Patients were prospectively followed for one year from enrollment into the registry.

RESULTS

Patients

One thousand (1000) patients at 12 clinics across Colombia were invited to participate. A total of 992 patients met the participation criteria and were included in the intention to treat (ITT) group. The majority (62%) of the patients were men, and at enrollment the mean age was 60 years. Over 90% of the patients had a history of hypertension and nearly 50% had a history of diabetes. Two-thirds (67%) of patients had chronic kidney disease (CKD) attributed to hypertension (28%) or diabetes (39%). A total of 638 patients were eligible for the 1-year follow up assessment.

Albumin Levels

The cumulative change in serum albumin levels in the ITT population during the follow-up was -1.8%. See Table 1. A total of 468 patients in the per protocol (PP) population had all six serum albumin measurements taken during HDx. The changes in serum albumin levels was less pronounced, with an accumulated change of -1.2%. See Table 2.

Follow-up	n	Marginal mean[a] (g/dL)	Change from baseline (%)	Change from previous[b] (%)	Cumulative change(%)
Baseline	992	4.05 (4.04-4.07)	–	–	–
15 days	938	3.98 (3.97-4.00)	-1.7	-1.7	-1.7
1 month	951	4.00 (3.98-4.01)	-1.2	0.3	-1.4
3 months	883	3.91 (3.90-3.93)	-3.5	-2.0	-3.5
6 months	728	3.94 (3.92-3.96)	-2.7	0.7	-2.8
9 months	735	3.94 (3.92-3.96)	-2.7	0	-2.8
12 months	587	3.98 (3.96-4.00)	-1.7	1.0	-1.8

Table 1. Change in serum albumin levels over time (ITT population)

Abbreviation: ITT, intention to treat.
[a]Marginal mean is the means estimation based on the fitted model in repeated measures and are presented as 95% confidence interval.
[b]The percentual change from the last measurement value.

Table adapted from Bunch et al.

Follow-up	n	Marginal mean[b] (g/dL)	Change from baseline (%)	Change from previous[c] (%)	Cumulative change(%)
Baseline	468	4.03 (4.01-4.05)	–	–	–
15 days	468	4.00 (3.98-4.02)	-0.9	-0.9	-0.9
1 month	468	3.98 (3.96-4.00)	-1.3	-0.4	-1.3
3 months	468	3.93 (3.91-3.95)	-2.7	-1.4	-2.7
6 months	468	3.95 (3.93-3.97)	-2.0	0.7	-2.0
9 months	468	3.96 (3.94-3.98)	-1.9	0.0	-2.0
12 months	468	3.99 (3.97-4.01)	-1.2	0.8	-1.2

Table 2. Change of serum albumin levels over time (PP[a])

Abbreviation: PP, per=protocol defined as patients who received all treatments with the MCO membrane during the follow-up period or until hospitalization that involveed >12 dialysis sessions without MCO or death.
[a]Only patients in the PP population who had baseline and all six scheduled serum albumin measurements during HDx were included in the analysis.
[b]Marginal mean is the means estimation based on the fitted model in repeated measures and are presented as 95% confidence interval.
[c]The percentual change from the last measurement value.

Table adapted from Bunch et al.

While a slight decrease in albumin over 12 months of observation was statistically significant in the large cohort study, this should be considered clinically insignificant. At all times, the observed variability of serum levels was within 5% from baseline and the mean serum albumin concentration remained within the normal ranges (3.5-5.5 g/dL).

Patient Outcomes

Seventy-four (8%) patients died during 866 patient-years (PY) of follow-up; the mortality rate was 8.54 deaths/100 PY (95% confidence interval (CI), 6.8-10.7). There were 673 hospitalization events with a rate of 0.79 events/PY (95% CI, 0.73-0.85) with 6.91 hospital days/PY (95% CI, 6.74-7.09). The observed mortality rate, hospitalization rate, and number of hospital days were lower than previous experiences with the **RTS network** in Colombia.

Dialysis Parameters
HDx adequacy, as measured by single-pool Kt/V and serum phosphorus. Single pool Kt/V was 1.68, which is considered a very good level of adequacy for small-molecule reduction and is well above the minimum 1.2 Kt/V per HD session for patients treated 3x weekly, as is recommended by the (US) National Kidney Foundation's Kidney Disease Outcomes Quality Initiative. Serum phosphorus levels remained relatively constant throughout the 12 months, with a mean of 4.55 mg/dL at month 12, which is below the recommended level of 5.5 mg/dL.

Safety
During the follow-up period, there were 1019 adverse events during 866 person-years of follow-up for a rate of 1.18 adverse events (AE) per PY (95% CI, 1.10-1.25). For comparison, 130,601 sessions were performed with MCO membranes. A total of 667 (66.4%) AEs were serious, and of these, 91 (8.9%) resulted in withdrawal from the study. No AEs during HDx were deemed related to MCO membrane use, according to investigator and techno-surveillance evaluation. AEs numbering 146 were deemed related to the dialytic procedure, which represents 0.17 events per PY (95% CI, 0.14-0.20), equivalent to 1.12 events per 1000 HD sessions (95% CI, 0.90-1.30).

Strengths and Limitations
Strengths of the present study include the prospective collection of current practice data, an analysis of nearly 1000 patients undergoing HDx, over 130,000 sessions performed, with baseline information and a follow-up for 12 months. Limitations of the study include the absence of a comparison group, which diminishes the strength of adjudging causality to the observed effects. A positive selection bias cannot be excluded, although given the large number of patients and renal clinics involved, it does not appear that the population in this analysis differs much from the general prevalent HD population in the **RTS network** in Colombia.

CONCLUSIONS

No adverse events were related to the MCO membrane. HDx using an MCO membrane maintains serum albumin levels within the normal range among patients undergoing expanded hemodialysis with nonoccurrence of dialyzer related adverse effects.

The MCO (Theranova) membrane is safe and preserves serum albumin levels within the normal range among patients undergoing expanded hemodialysis (HDx).

Assessment of the Association Between Increasing Membrane Pore Size and Endotoxin Permeability Using a Novel Experimental Dialysis Set-Up

Schepers E et al. Assessment of the association between increasing membrane pore size and endotoxin permeability using a novel experimental dialysis set-up. *BMC Nephrol*. 2018; 19:1. doi: 10.1186/s12882-017-0808-y.

BACKGROUND

The current trend is to further increase pore size and permeability of dialysis membranes to enhance removal of uremic toxins in the larger molecular weight range even when used in dialysis mode. High-cut off (HCO) dialyzers allow elimination of molecules up to 45 kDa and remove specific middle molecules more effectively than standard high flux membranes. The use of these membranes decreases inflammation and in vitro calcification, but also results in albumin loss.

More recently membranes with a steeper cut-off at a lower molecular weight than HCO membranes, medium cut off (MCO) membranes have been introduced. These membranes can even remove large toxins such as kappa (22.5 kDa)[1] and lambda (45 kDa)[1] free light chains, two compounds associated with inflammatory markers and mortality in chronic kidney disease (CKD).

The question arises whether these membranes with increasing pore size also have higher permeability for endotoxins and other bacterial degradation products potentially present in dialysis fluids. This permeability issue is relevant, since chronic exposure of hemodialysis (HD) patients to low levels of cytokine-inducing microbial components can potentially contribute to the micro-inflammatory status of these patients, thus neutralizing the potential positive effect induced by their capacity of enhanced removal of pro-inflammatory uremic toxins. Therefore, the request for ultrapure dialysate might become a more important concern as membrane pore size becomes larger, even when applied in hemodialysis mode. Standard methods to determine biological contamination are bacterial culture and the Limulus Amebocyte Lysate (LAL) assay. To test true biologic response with more clinical relevance, bio-assays such as the one using the THP-1 cell line can be applied.

In the present study, a realistic dialysis set-up using full-sized dialyzers was developed that simulates the clinical situation in terms of flow rates and viscosity of the medium perfused in the blood compartment.

OBJECTIVE

To assess commercial dialyzers of comparable composition but with different pore size for their permeability for bacterial degradation products by means of a biological assay sensitive to several bacterial components (THP-1) as read-out in addition to the LAL assay.

METHODOLOGY

Dialysis membranes

The dialysis membranes evaluated for their endotoxin permeability provided by the manufacturer were composed of comparable polymers but with a different pore size: **Polyflux** 17 L (low flux); **Revaclear** 400 (high flux); **Theranova** 400 (MCO); and **Theralite** 2100 (HCO); Baxter, Hechingen, Germany. See Table 1.

Dialyzer	Type	Membrane Polymer	Pore radius[a] (nm)
Polyflux 17 L	Low flux	PAES/PVP/PA	3.1 ± 0.2
Revaclear 400	High flux	PAES/PVP	3.9 ± 0.1
Theranova 400	Medium cut off	PAES/PVP	5.0 ± 0.1
Theralite 2100	High cut off	PAES/PVP	10 ± 2

Table 1. Characteristics of dialyzers. [a] effective Stokes-Einstein radius; calculated from molecular weight cut-off measured with polydisperse Dextran. Abbreviations: PAES: polyarylethersulfone; PVP, polyvinylpyrrolidone; PA, polyamide, UF, ultrafiltration. Adapted from Schepers et al.

Dialysate and blood substitution fluids

Ultrapure dialysis fluid was prepared on-line with an AK200 dialysis machine (Gambro, Lund, Sweden) using a smartbag (Fresenius Medical Care, Willebroek, Belgium) acid concentrate and a BiCart cartridge (Gambro) resulting in a dialysate containing 3 mM K^+, 140 mM Na^+, 1.25 mM Ca^{2+}, 0.50 mM Mg^{2+} and 34 mM bicarbonate. A 1.25% polyvinylpyrrolidone (PVP) (Luvitec® K85 powder, BASF, New Jersey, USA) solution was prepared in sterile phosphate buffered saline (PBS) 10×, pH 7.2 (Gibco, Life technologies, Paisley, UK) and diluted 1:10 in sterile water (Braun, Melsungen, Germany) to achieve a solution with a kinematic viscosity of 4 mm^2/s, to mimic the viscosity of whole blood. Compatibility of PVP dissolved in PBS (PVP_{PBS}) with both the LAL and THP-1 assay was evaluated per se and in combination with lipopolysaccharide (LPS) in comparison to PBS. No interference of the PVP dissolved in PBS could be observed in both assays.

Challenge solution

The ISO11663:2014 standard for LPS allows less than 0.5 endotoxin units (EU)/mL in dialysis fluid. In the *in vitro* experimental set up, the duration of a dialysis session was set to 1 hour. Corresponding to the total exposure during a regular dialysis session of 4 hours, a minimum load of 2 EU/mL should be aimed for. However, to create a worst-case scenario, this load was increased, aiming at a dialysis fluid containing at least 4 EU/mL. To obtain this, a concentrated solution (200 EU/mL) of two clinically relevant water borne bacterial species *Pseudomonas aeruginosa* and *Pelomonas saccharophila* was prepared.

Dialysis Machine Set Up

The AK200 dialysis machine was set in double needle treatment and the tubings for hemodialysis (Gambro) and the dialyzers were connected. The four different membranes were tested in random order; for each membrane type, 6 different dialyzers were tested.

During the actual experiment 3 liters of the PVP solution at 37 °C was recirculated during 60 min at a blood flow rate (Q_B) of 400 mL/min while the PVP pool was continuously mixed. The dialysate flow (Q_D) was set at 500 mL/min and the challenge solution was continuously infused from the sterile bag into the dialysate line before inlet of the dialyzer at a rate of 10 mL/min with a droplet pump aiming at a contamination level of the dialysate above 4 EU/mL. A sampling port was placed between the contamination inlet port and the inlet of the dialyzer to assess the level of contamination. A schematic figure of the experimental set-up is shown in Fig.1

Samples of the dialysate were taken after 5 and 55 minutes. The PVP pool was sampled in duplicate before the start (PVP_{start}) and at the end (PVP_{end}) of the experiment. For the PVP_{post} solutions the LAL activity of the duplicates was reported separately, but the sample was considered positive for endotoxin if at least one contained a measurable endotoxin level.

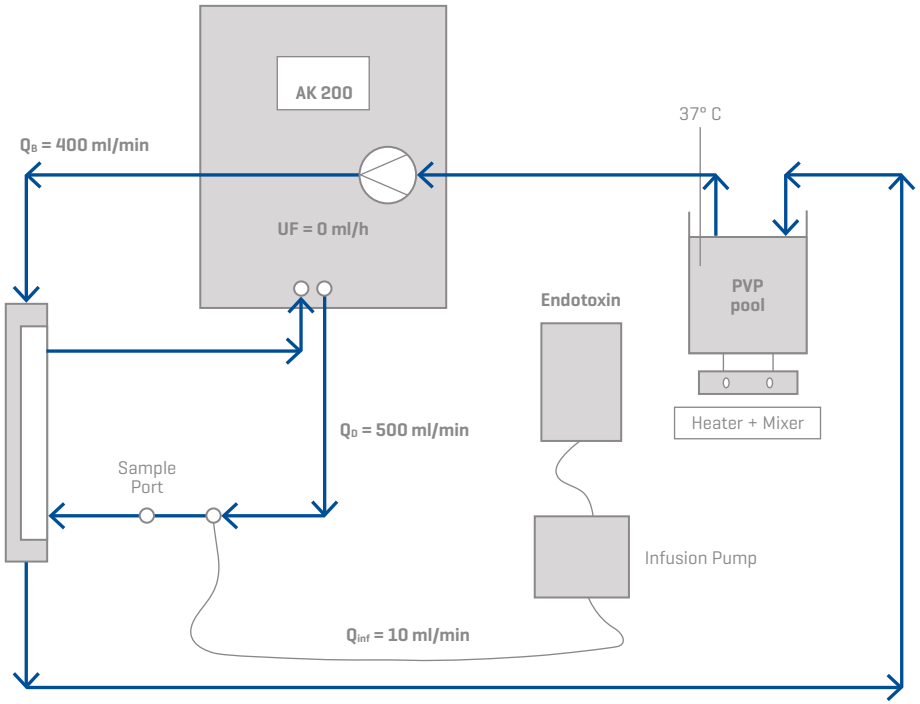


Figure 1. Schematic overview of the dialysis set-up. After priming both blood and dialysate circuit and after coating the test membrane with plasma, 3 L of PVP solution, continuously mixed, was recirculated at 37 °C at the blood side at a blood flow rate (Q_B) of 400 mL/min. Samples of the PVP were taken from the pool before and after the experiment. The dialysate was prepared by the AK200 and circulated at a dialysis flow rate (Q_D) of 500 mL/min. Before entering the membrane, contaminant was infused at a flow Q_{inf} of 10 mL/min by means of a pump. Samples from the dialysate were taken just before the membrane at 5 and 55 minutes. Ultrafiltration QF was set at 0 mL/min. Figure adapted from Schepers et al.

RESULTS

Permeability of dialysis

For the tested membranes, there was a nonsignificant difference in number of the PVP solutions which contained a detectable amount of endotoxin after repetitive circulation through the dialyzer, be it close to the detection limit in the majority of cases.

Table 2 shows the individual and the mean LAL assay responses for the dialysate and PVP solutions per individual experiment, categorized per membrane. Although dialysate-endotoxin concentration varied between 3.2 and 33.7 EU/mL in the individual experiments, mainly due to the difficulty of filtrate preparation and complexity of the experimental set-up, the mean exposure to endotoxins through the contaminated dialysate was above the intended minimum 4 EU/mL for each of the different experiments and did not differ between the different membranes. There was no dose-response correlation between the level of contamination within the tested range of dialysate endotoxin concentration and the detectable concentration of endotoxin in the PVP_{post} solutions.

LAL activity in the PVP solution at the blood side of the dialyzer was below the limit of detection (limit of detection (LOD) =0.005 EU/mL) both before (PVP_{pre}) and after the experiment (PVP_{post}) for 12 out of 24 tested dialyzers. Endotoxin levels were below LOD in all but three (High-flux 2, high cut-off 1) PVP_{pre} solutions. This potentially indicates contamination occurred already before the start of the experiment in these three experiments. Positive PVP_{post} reading higher than the corresponding PVP_{pre} reading, indicating possible contamination from endotoxin in the dialysate, was found in 9 out of 24 experiments (low-flux: 1/6; high-flux: 1/6; MCO 3/6; HCO: 4/6). While more open membranes (MCO and HCO) had a detectable amount of endotoxin, the permeability of endotoxin was not significantly different.

Membrane	Dialysate (EU/mL)	PVP_{pre} (EU/mL)	PVP_{post} (EU/mL)		Statistics PVP_{pre} vs PVP_{post}
			Duplicate 1	Duplicate 2	
Low-flux	3.5	< LOD	< LOD	< LOD	
	3.9	< LOD	< LOD	< LOD	
	5.5	< LOD	< LOD	< LOD	
	9.4	< LOD	< LOD	< LOD	
	10.3	< LOD	***0.011***	< LOD	
	19.1	< LOD	< LOD	< LOD	n.s.
Mean ± SD	**8.6 ± 5.8**				
High-flux	3.6	< LOD	< LOD	< LOD	
	4.1	*0.008*	*0.007*	*0.008*	
	5.9	< LOD	***0.005***	< LOD	
	6.1	< LOD	< LOD	< LOD	
	20.0	*0.007*	< LOD	< LOD	
	33.7	< LOD	< LOD	< LOD	n.s.
Mean ± SD	**12.2 ± 12.2**				
Medium cut-off	6.0	< LOD	***0.023***	***0.005***	
	6.3	< LOD	< LOD	< LOD	
	6.8	< LOD	< LOD	< LOD	
	8.0	< LOD	***0.006***	***0.005***	
	10.6	< LOD	***0.005***	***0.005***	
	11.8	< LOD	< LOD	< LOD	n.s.
Mean ± SD	**8.3 ± 2.4**				
HIgh cut-off	3.2	< LOD	***0.019***	***0.013***	
	4.1	< LOD	***0.005***	***0.005***	
	5.4	< LOD	***0.005***	***0.005***	
	5.8	< LOD	***0.005***	< LOD	
	12.1	< LOD	< LOD	< LOD	
	22.5	*0.006*	*0.005*	*0.005*	n.s.
Mean ± SD	**8.9 ± 7.4**				

Table 2. Endotoxin levels in the dialysate and the PVP solution per membrane measured by the LAL-assay (n=6 for each membrane). LOD was 0.005 EU/mL. Data with measurable endotoxin levels were written in italic and when they were higher than the PVP_{pre} value they were additionally put in bold and underlined. Abbreviations: PVP, polyvinylpyrrolidone; LAL, limulus amebocyte lysate; LOD; limit of detection; EU, endotoxin units; PVP_{pre}, PVP before the experiment; PVP_{post}, PVP after the experiment; SD, standard deviation. Adapted from Schepers et al.

Cytokine induction assay

In none of the experiments, biological activation of the inflammatory system was observed as measured by a IL-1ß (17.5 kDa)[1] production by the THP-1 assay, sensitive for several bacterial components such as intact LPS, LPS fragments, peptidoglycan and short bacterial DNA fragments. As shown in Table 3, 25 EU/mL LPS and the contaminated dialysate significantly induced IL-1ß expression, whereas none of the PVP solutions used in the different experiments induced IL-1ß expression neither before or at the end of the experiments. Moreover, no significant difference in induction of IL-1ß expression was found between the PVP solutions treated with the different membranes.

Despite the fact that in some of the PVP samples endotoxin was detectable, none of the PVP solutions induced a biologic response as assessed by activity of human THP-1 monocytes higher than that of the background culture medium.

The highest measured level of endotoxin in a duplicate sample from the 'patient side' was 0.023 EU/mL. This corresponds to a total amount of about 70 EU transferred during the 1 hour dialysis session, and thus a transfer rate of about 1 EU/kg/h (mean patient of 70 kg), which is still well below the pyrogenicity limit of 5 EU/h/kg body weight (the minimum dose that induces fever) for injectable medications and devices.

	Medium	LPS 25EU/mL	Dialysate	PVP_{pre}	PVP_{post}	Statistics°
Low-flux	11.44 ± 4.05	53.78 ± 21.11*	51.69 ± 57.07	10.87 ± 4.02	10.53 ± 4.22	n.s.
HIgh-flux	12.86 ± 3.43	62.03 ± 22.91*	88.4 ± 122.13	11.87 ± 3.09	11.09 ± 2.82	n.s.
Medium cut-off	11.93 ± 3.54	54.71 ± 20.85*	40.99 ± 60.49	12.11 ± 3.55	11.13 ± 2.87	n.s.
HIgh cut-off	12.25 ± 3.69	59.97 ± 17.22*	22.78 ± 21.88	11.55 ± 3.69	11.30 ± 3.18	n.s.

Table 3. Overview of IL-1ß expression in pg/mL in the THP-1 cytokine induction assay by the dialysate and PVP solutions. *p < 0.05 vs Medium; °PVP_{pre} vs PVP_{post}. Abbreviations: PVP, polyvinylpyrrolidone; LPS, lipopolysaccharide; EU, endotoxin units; PVP_{pre}, PVP before the experiment; PVP_{post}, PVP after the experiment. Adapted from Schepers et al.

Strengths of Study

The experimental set-up was a worst-case scenario in which endotoxin exposure was 4x greater than permitted in standard dialysate, spread over a 4-hour dialysis session. In contrast with the clinical setting, most modern dialysis monitors have an additional ultrafilter between permeate and dialysate, providing an extra safeguard for contamination that was bypassed in the set-up infusing the contaminant at the dialyzer inlet.

The four different membranes were tested in random order; for each membrane type, six different dialyzers were tested. The PVP pool was sampled in duplicate before the start (PVP_{start}) and the end of the (PVP_{post}) of the experiment. Individual data from all experiments was provided as the most exact way to present the data and provide full visibility. The PVP solutions in many cases had a measured LAL activity below the LOD and the individual numbers give a better impression of the limited degree of contamination in case values were above LOD in the blood compartment.

Special emphasis was made in the present study to develop an experimental model mimicking the clinical reality as close as possible.

- The dialysate and blood flows were comparable to the ones applied in the clinic, and in the same context, viscosity of the fluid circulating in the blood compartment was comparable to that of blood, mimicking clinical pressure distributions in the dialyzer and with it, realistic filtration profiles.
- Full size dialyzers were used rather than down-sized models to reflect the migration of endotoxin from the dialysate to the blood side by diffusion and backfiltration. To augment the worst-case scenario, ultrafiltration was set at 0 mL/mn, resulting in maximal backfiltration.
- A protein coating was applied to the tested membranes by circulating a human plasma solution before the beginning of the experiments to mimic the properties of the synthetic membrane during dialysis.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Limitations of Study

Dialyzers of comparable membrane composition from a single manufacturer were chosen to focus the investigation in pore size. Subsequently, the results can not likely be generalized to membranes of different compositions or structure.

Whole blood was not used in the experiments based on cost and difficulty of use. However, use of whole blood in this type of experiment might abrogate activity of endotoxins as several components of blood have the capacity to neutralize endotoxins.

Two water borne bacterial species *Pseudomonas aeruginosa* and *Pelomonas saccharophila* were used as the source of endotoxins. Preparations of bacteria can contain a wide range of contaminants and endotoxins with different molecular weights and transmembrane transport properties. While not all these bacterial products may test positive in the LAL test, they all have a cytokine-inducing capacity as assessed by the production of IL-1ß by the THP-1 cell line in the bio-assay.

CONCLUSIONS

A realistic and feasible model to assess dialysis membrane translocation of bacterial degradation products present in the dialysate was developed and applied to test the retention capacity of 4 different membranes with similar chemical composition but different pore sizes. Although more blood side PVP solutions had a detectable amount of endotoxin using a highly sensitive LAL assay in the more open vs traditional membranes the permeability for endotoxins of the 4 tested dialysis membranes was not significantly different. Moreover, none of these PVP_{post} solutions induced IL-1ß expression in the THP-1 based bio-assay that is sensitive also to other bacterial byproducts.

The present experiments demonstrate that, when using a 4-fold overload of endotoxin, the use of larger pore membranes, MCO and HCO, is likely safe from that regard. Indeed, in none of the experiments, biological activation of the inflammatory system was observed.

The MCO (Theranova) membrane maintains the same endotoxin retention as other high-flux membranes.

Hemodialysis Membranes

Ronco C and Clark WR. Hemodialysis membranes. www.nature.com/nrneph. *Nat Rev Nephrol.* 2018; 14:394-410.

BACKGROUND

Hemodialysis is an extracorporeal process in which the blood is cleansed via removal of uremic retention products by a semipermeable membrane. Traditionally, dialysis membranes have been broadly classified based on their composition (cellulosic or non-cellulosic) and water permeability (low flux or high flux). Incorporation of innovative manufacturing processes have led to consideration of other parameters for classification including new permeability indices, hydrophilic vs hydrophobic balance, adsorption capacity, and electrical potential.

AIM

To provide clinicians with an updated analysis of dialysis membranes and dialyzers, highlighting online hemodiafiltration and new therapies such as expanded hemodialysis, and considerations governing the clinical acceptable balance between large-solute clearance and albumin loss for extracorporeal therapies.

OVERVIEW

History of Dialyzers

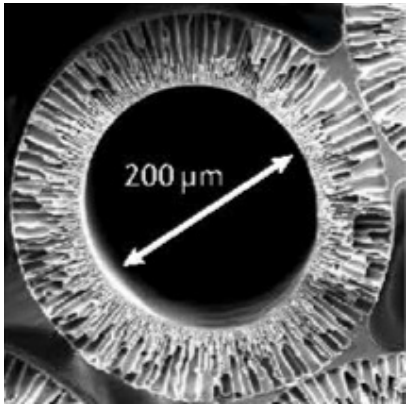
The availability of devices like the rotating drum kidney, coil dialyzer, and Kiil dialyzer allowed the use of dialysis to grow but it had many limitations. Of note were the high blood compartment volumes required and the inefficient mass transfer characteristics. In the late 1960s the hollow-fiber artificial kidney revolutionized dialysis by providing improved geometry in terms of blood rheology and solute mass transfer. Specific advantages included an improved surface-area:volume ratio in the blood compartment and decreased boundary layer effects with acceptable end-to-end pressure drops. These made the hollow-fiber configuration the main choice in dialysis. At present (2018) approximately 300 million hollow-fiber hemodialyzers are utilized worldwide.

History of Hollow-fiber Membranes

Categorization of dialysis membranes have traditionally been based on their material composition, either cellulosic or synthetic membrane groups. The use of unmodified cellulosic membranes has dropped precipitously over the past decades, to the point of effective absence from the market (i.e. no longer manufactured). Dialyzers with synthetic high-flux membranes now dominate clinical practice.

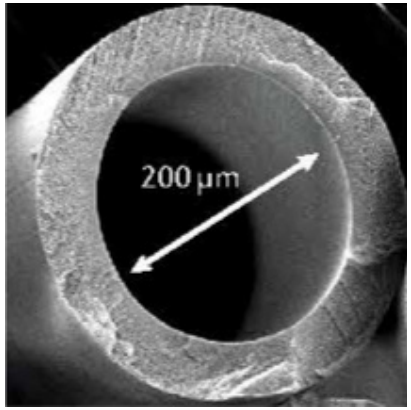
Synthetic membranes were originally developed for a dialysis application more than 40 years ago to address the relative bioincompatibility and limited permeability of unmodified cellulosic membranes. The first highly permeable membrane, sulfonated polyacrylonitrile (AN 69), was introduced in the late 1960s. Subsequent development included polysulfone membranes, which had very thick walls (75-100 μm), and despite higher permeability, were not conducive to diffusion-based therapies. Their use was originally limited to convection-based hemofiltration. Modern synthetic membranes (e.g. polyethersulfone (PES)) have thinner walls (20-50 μm) and higher permeability, permitting diffusion and convection to be employed simultaneously. Please see Figure 1.

Polysulfone Wall thickness 75-100 µm



- Synthetic polymer-asymmetric
- Hydrophobic structure
- High hydraulic permeability
- HIgh sieving properties
- Exclusively used for convective therapy (hemofiltration)

Polyethersulfone Wall thickness 30 µm



- Synthetic polymer-microporous
- Hydrophobic-hydrophilic
- High hydraulic permeability
- HIgh sieving properties
- Combination diffusive-convective therapy (high-flux hemodialysis and hemodiafiltration)

Figure 1. Physical Characteristics of Synthetic Membranes. Adapted from Ronco and Clark.

The majority of synthetic membranes (e.g. PES) used for contemporary dialysis have an asymmetric structure. In contrast, synthetic membranes similar to cellulosic membranes (e.g. AN69 and poly (methyl methacrylate) (PMMA)) are structurally symmetric.

New Membrane Classification

Focus on Solute Permeability Properties

Conventional membranes that are used in hemodialysis generally provide high clearance for small solutes such as urea (60.055 Da)[1] and creatinine (113.12 Da)[2]. However, membranes in current use provide limited clearance of compounds > 10 kDa. Although these membranes have relatively large mean pore sizes (compared to unmodified cellulosic membranes), they still offer mass transfer resistance to the diffusive removal of large solutes. Furthermore, (membrane) fouling has a considerable effect on convective solute clearance, especially for molecules > 10 kDa.

In typical hemodialysis operating conditions, the water permeability characteristics for a standard high-flux dialyzer result in a fairly large drop in the blood compartment axial pressure during treatment. The pressure drop is sufficiently large that the blood compartment pressure is less than the dialysate compartment pressure in normal operating conditions. There is a point at which the ultrafiltrate begins to be driven from the *dialysate to the blood* as opposed to the 'standard' (blood-dialysate) direction. This combination of filtration and backfiltration, termed internal filtration, is considered to be the predominant mechanism by which larger compounds are removed during standard high-flux dialysis. Maximizing the extent of internal filtration during high-flux dialysis through a combination of increased membrane permeability (increased pore size) and higher axial blood compartment resistance (decreased hollow fiber inner diameter) can provide clinically meaningful increases in large solute clearance.

Classification schemes that focus even more on solute permeability properties have been proposed. These new classification systems acknowledge the importance of larger molecules and the need to incorporate additional membrane classes that have extended removal spectra. High-flux and 'protein leaking' membranes have been defined based on a combination of water permeability, beta-2 microglobulin ($ß_2$m) (12 kDa)[3] removal factors (sieving coefficient (SC) or clearance) and albumin (65 kDa)[3] parameters (SC or amount cleared). In this system, the high-flux class is defined by a water permeability of 20–40 m/h/mmHg/m^2, a $ß_2$m SC of 0.7–0.8 and albumin loss of < 0.5 g (on the basis of a 4 h hemodialysis treatment), whereas the same parameters defining a protein-leaking membrane are >40 m/h/mmHg/m^2, 0.9–1.0, and 2–6 g, respectively. Although not explicitly stated, these values correspond to 'virgin' membrane performance and do not reflect potential diminutions during treatment as a result of secondary membrane effects.

Medium Cut-Off (MCO) and High Cut-Off (HCO) Membranes

Two new membrane classes, medium cut-off (MCO) and high cut-off (HCO), have been proposed, extending the earlier classification scheme.

The HCO class is characterized by a substantial increase in water permeability (relative to both high-flux and the protein-leaking classes) and a virgin β_2m SC of 1.0. However, the high albumin loss rates associated with this membrane class generally preclude their long-term use for patients with end stage renal disease (ESRD). In addition, this membrane has been used for fairly limited time periods in clinical conditions in which the potential risks due to albumin loss are considered reasonable to the potential benefits (e.g. for patients with myeloma-associated acute kidney injury to target augmented removal of free antibody light chains (kappa interleukin light chain (22.5kDa)[3]; lambda interleukin light chain (45 kDa)[3]). The role of HCO membranes in clinical practice remains unclear.

In comparison to HCO membranes, the MCO class is intended to preserve the β_2m sieving characteristics and to improve the clearance of other large-molecular-weight solutes (for example, free antibody light chains) while demonstrating a marked reduction in albumin permeability. MCO membranes represent the basis for a new diffusion-based therapy called 'expanded hemodialysis'.

Molecular Weight Retention Onset (MWRO)

A new solute removal parameter for the characterization of modern highly permeable membranes has been proposed. This new parameter, the 'molecular weight retention onset' (MWRO) index, is generated from a standard solute sieving coefficient versus molecular weight profile, as with the classic molecular weight cut off (MWCO). The MWRO is defined as the molecular weight at which the *SC* value first reaches 0.9 (whereas the MWCO corresponds to a *SC* of 0.1). This approach was rationalized by suggesting that the MWRO index, which provides insights about pore size distribution, supplements information provided by the MWCO, which is primarily correlated with mean pore size. The steepness of the sieving coefficient versus molecular weight profile is determined mostly by the proximity of these parameters. A classification scheme has been proposed in which the MWCO and MWRO are utilized in combination to define different dialyzer classes.

Insights

Although extending the removal spectrum of modern dialysis membranes beyond the capabilities of standard of high-flux devices is highly desirable, the design challenge is to maximize the removal of large uremic toxins while also maintaining albumin losses for long-term treatment of patients with ESRD. The updated classification system includes the previously mentioned MCO membranes that incorporate high-retention onset (HRO) properties. This membrane class may hold promise in achieving acceptable albumin losses.

Pore size distribution curves (Fig.2 a-c) and the corresponding sieving coefficient profiles (Figure 2d) and the corresponding sieving coefficient for three classes of membranes (high flux, MCO, and HCO) reveal that as the separation between MWRO and MWCO decreases, the profile of the curve becomes steeper, resulting in increased removal of large uremic toxins and decreased loss of albumin. As shown in Figure 2d, the MCO curve is the steepest of the four curves, stopping before the molecular weight (MW) of albumin, while HCO extends beyond albumin's MW.

By virtue of larger pore sizes, increased membrane diffusivity is one mechanism by which the removal of large solutes is augmented with the MCO class of dialyzers relative to standard high-flux membranes. Although hemodialysis using this type of dialyzer is technically diffusion-based, most large-solute removal still occurs by convection through the mechanism of internal filtration. For MCO and other dialyzers, the effect of this mechanism is intentionally augmented through increases in the mean pore size and reductions in the inner diameter of hollow fibers. Preliminary data suggest that this class of dialyzers has depuration capabilities that approach those of online post-dilution hemodiafiltration without the need for (exogenous) substitution fluid administration.

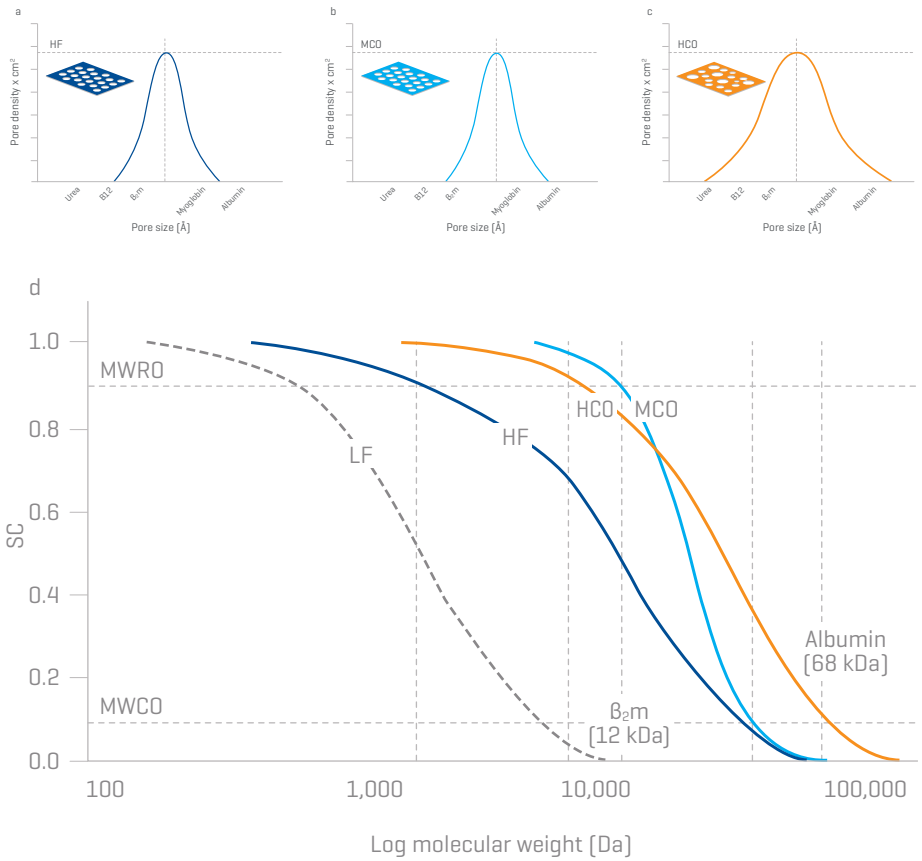


Fig. 2. Performance characteristics of hemodialysis membranes derived from a suggested new classification system. Pore size distribution curves are schematically depicted for three classes of membranes, high flux (HF; part **a**), medium cut-off (MCO; part **b**) and high cut-off (HCO; part **c**), as well as their sieving coefficient (SC) profiles (part **d**). As the interval between molecular weight retention onset (MWRO) and molecular weight cut-off (MWCO) decreases, the profile of the curve becomes steeper, increasing the removal of large uremic toxins (such as ß2-microglobulin (ß2m)) while decreasing the loss of albumin (part d). A low-flux (LF) membrane is shown for comparison. Figures adapted from Ronco and Clark.

CONCLUSIONS

The bidirectional process of mass separation between the blood and the dialysate involves several mechanisms of interaction between the fluid phases and the membrane barrier. The nature of the fluid phases, the characteristics of the solutes and the structure of the membrane represent a combination of elements that are involved in the final process of mass separation and dialysis.

The development of a unifying classification system for dialysis membranes is extremely complex and must be multidimensional. Proposed in the classification system is inclusion of membranes that have substantial differences of potential clinical importance, like MCO membranes. The MCO class is intended to improve the clearance of large molecular-weight solutes, while demonstrating a marked reduction in albumin permeability, influenced primarily by the decreased interval between MWRO and MWCO and resulting steep sieving curve. This membrane class may hold promise in achieving acceptable albumin losses, representing the basis for a new diffusion-based therapy called 'expanded hemodialysis'.

The MCO membrane features an increased pore density with tight pore-size distribution, enhancing ultra-filtration and permeability via a steep sieving-curve — resulting in clearance of larger uremic toxins, while retaining essential proteins.

References: 1.https://biocyc.org/compound?orgid=META&id=UREA Accessed 6/15/20.

2. https://biocyc.org/compound?orgid=META&id=CREATININE Accessed 6/15/20.

3. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

The Rationale for Expanded Hemodialysis Therapy (HDx)

Hutchison CA and Wolley M. The rationale for expanded hemodialysis therapy (HDx). *Contrib Nephrol*. 2017; 191:142-152.

BACKGROUND

To date, the class of uremic toxins known as large middle-molecules has been classified as "difficult to remove" in dialysis membrane technologies. Expanded hemodialysis utilizes a new generation of high-retention-onset hemodialysis membranes; these membranes provide the ability to remove large middle-molecules effectively for the first time, without significant albumin loss. These large middle-molecules appear to be linked to several unsolved clinical complications of end-stage kidney disease (ESKD); their increased removal may potentially lead to improved patient outcomes.

OBJECTIVE

The purpose of this review was to evaluate the removal of large middle-molecules by the new high-retention-onset membranes, clinical relevance of these molecules, and how expanded hemodialysis can be prescribed.

DISCUSSION

High-Retention-Onset Membranes

High-flux dialysis membranes have been designed principally to remove middle-molecules up to the size of ß-2-microglobulin (16 kDa). Due to the non-uniformity of membranes' pores, molecules much larger than ß-2-microglobulin can be removed, but the absolute clearance rates are limited.

In addition, high flux membranes have a low molecular-weight-retention-onset (MWRO) value of 2-5 kDa. The term "retention onset" refers to the molecular weight at which the sieving value for a membrane reaches 0.9; there is no longer "free clearance" of molecules greater than this size. The term "molecular weight cut off" (MWCO) refers to the other end of the sieving curve of the membrane. This is the point when the sieving coefficient has reached 0.1, which means almost no clearance of a given molecule.

The closer the values of MWRO and MWCO, the steeper is the slope of the sieving coefficient. The ability to provide a steep sieving coefficient curve for a dialysis membrane allows the curve to be moved to the right, closer to basement (glomerular) membrane, enabling the removal of larger molecules without the loss of very clinically important large molecules, such as the protein albumin (65 kDa)1.

Technical advances have now allowed the distribution of pore sizes to be narrowed, which in turn has tightened the relationship between the MWRO and MWCO of a membrane. This new generation of dialyzers has been referred to as "mid-cut off membranes" or "high-retention-onset membranes".

Large Middle-Molecules

In comparison with high-flux membranes (polysulfone-PVP blend), high-retention-onset membranes (polyarylethersulfone-PVP blend) have significantly higher clearance rates of middle-molecules with molecular weights greater than 15 kDa. See Figure 1.

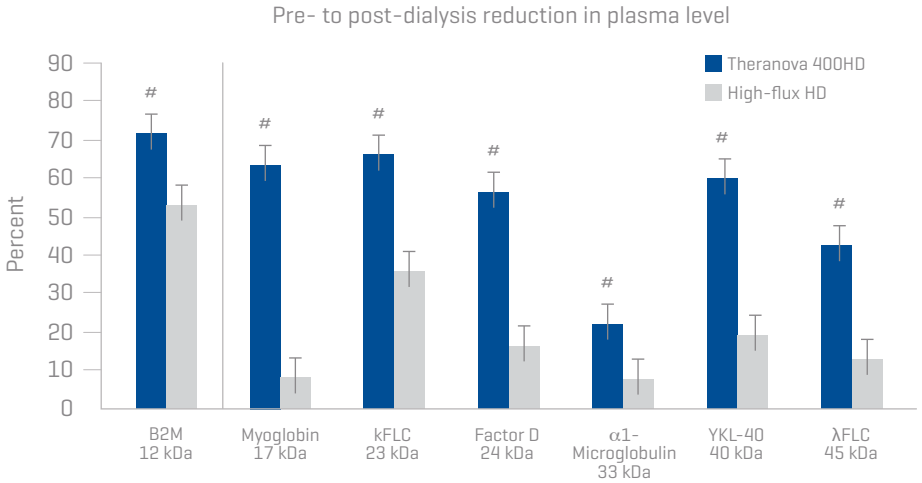


Figure 1. Reduction rates of large middle-molecules on high-flux and Theranova dialyzers. Nineteen patients, blood flow 301 ± 22 mL/min, treatment time 4 hours. High-flux hemodialysis undertaken on FX CorDiax 80 dialyzer (polysulfone-PVP blend membrane). Dialysis with Theranova dialyzer (polyarylethersulfone PVP blend membrane. Bars indicate mean and standard deviation (SD). Post-dialysis data corrected for hemoconcentration. #$p<0.001$ vs high-flux dialysis. Adapted from Hutchison and Wolley.

Currently there are 27 middle molecules with molecular weights greater than 15 kDa described in medical literature (see Table 1).

Serum concentrations of middle-molecules in dialysis are principally influenced by renal rates and production rates. As a result of these two variables, serum concentrations of large middle-molecules are highly variable in dialysis patients compared to healthy controls.

The 27 large middle-molecules can be classified into 5 broad groups: cytokines (n-5); adipokines (n-4); growth factors and other hormones (n-4); immune-mediated molecules (n-8), and other molecules (n-6).

The 5 cytokines had molecular weights between 17 and 28 kDa. The interleukins (IL)-1ß (17.5 kDa), IL-6 (21-28 kDa), IL-18 (18 kDa), and tumor necrosis factor (TNF)-α (17 kDa) are all widely accepted as pro-inflammatory and are likely to be contributing factors to the chronic inflammation frequently seen in dialysis patients. However, IL-10 (18 kDa) is more clearly described as an anti-inflammatory cytokine.

Adipokines are cytokines but they are produced principally by adipose tissue. For the 4 adipokines described as large middle-molecules, their molecular weights range from 16 to 52 kDa with serum concentrations that are typically 2- to 6-fold of those seen in healthy controls. These adipokines have wide ranging biological functions in health and disease.

Three growth factors are found in this group of uremic toxins: vascular endothelial growth factor (34 kDa), fibroblast growth factor 2 (18 kDa), and fibroblast growth factor 23 (32 kDa). These molecules span from 18 to 34 kDa and have been described to be hundreds of fold higher in dialysis patients compared to healthy controls.

Several immune-mediated proteins are found in this group of large middle-molecules including the free light chains (FLC) κ (22.5 kDa) and FLC λ (45 kDa) and complement factor-D (24 kDa). With molecular weights spanning 17–45 kDa, they represent nearly the entire breadth of larger molecules, which can be removed with expanded hemodialysis.

An additional 6 large middle-molecules can be removed by this new therapy. Of these, potentially the most clinically relevant are the advanced glycosylation end products (<1-70 kDa) which are up to 20-fold higher in dialysis patients. These large middle-molecules are implicated in multiple pathways of progressive cardiovascular disease.

See Table 1.

	Class	Molecule	Molecular weight, kDa	Relative increase in dialysis	Clinical relevance
1	Cytokines	Interleukin-18	18	~2-fold higher	• Interleukins, 1β, 6, 18 and tumor necrosis factor-α (TNF-α) can provide pathways to chronic inflammation • Interleukins, 1β, 6, 18 and TNF-α provide pathways for atherosclerosis in combination with raised concentrations of advanced glycosylation end products, adipokines and prolactin • Retention of cytokines associated with flu-like symptoms
2		Interleukin-6	21-28	2- to 5-fold higher	
3		Interleukin-1β	17.5	~2-fold higher	
4		Interleukin-10	18	~1.5-fold higher	
5		Tumor necrosis factor-α	17	4- to 5-fold higher	
6	Adipokines	Adiponectin	30	2- to 3-fold higher	• Adipokines adiponectin and leptin, Interleukins 1β, 6, 18 and TNF-α, advanced glycosylation end products, and prolactin provide pathways for atherosclerosis
7		Visfatin	52	3- to 6-fold higher	
8		Leptin	16	3- to 4-fold higher	
9		Retinol-binding protein 4	21.2	3- to 4-fold higher	
10	Growth factors and other hormones	Vascular endothelial growth Factor	34	~2-fold higher	• Fibroblast growth factors 2 and 23 have been linked to left ventricular hypertrophy, and associated pathologies of atrial fibrillation and heart failure • Hormone prolactin, adipokines adiponectin and leptin, Interleukins 1β, 6, 18 and TNF-α, and advance glycosylation end products provide pathways for atherosclerosis • Fibroblast growth factor 23, α1-acid glycoprotein, polyclonal free light chains (k FLC, λ FLC) have been described to impair normal function of neutrophils (Secondary Immunodeficiency)
11		Fibroblast growth factor 2	18		
12		Fibroblast growth factor 23	32	>200 fold higher	
13		Prolactin	23	2- to 4-fold higher	
14	Immune-mediated proteins	Complement factor D	24	4- to 17-fold higher	• Polyclonal free light chains (k FLC, λ FLC), α1-acid glycoprotein, and fibroblast factor 23 have been described to impair normal function of neutrophils (Secondary Immunodeficiency) • TNF receptors 1 and 2 appear to prolong the circulating half-life of TNF-α in uremia
15		κ-Ig light chains	22.5	2-16	
16		λ-Ig light chains	45	2-18	
17		α1-acid glycoprotein	35-44	<1.5 fold higher	
18		Soluble TNF receptor 1	27-30	3- to 10-fold higher	
19		Soluble TNF receptor 2	17	3- to 10-fold higher	
20		Pentraxin-3	40	2- to 7-fold higher	
21		YKL-40	40	2- to 5-fold higher	
22	Other molecules	β-Trace protein	26	>35-fold higher	• Advanced glycosylation end products, prolactin, adiponectin and leptin, Interleukins 1β, 6, 18 and TNF-α provide pathways for athersclerosis • Retentioin of α-1 microglobulin is associated with restless leg syndrome
23		Myoglobin	17	2- to 7-fold higher	
24		Hyaluronic acid	Variable	3-fold	
25		Advanced glycosylation endproducts	<1-70	5- to 16-fold higher	
26		Clara cell protein	15.8	~30-fold higher	
27		α1-Microglobulin	33	~9-fold higher	

Table 1. Large middle-molecules with molecular weights greater than 15 kDa. Classification, molecular weights, serum concentrations in dialysis patients, and clinical relevance. Table adapted from Hutchison and Wolley.

Clinical Relevance of Large Middle-Molecules

To be classified as a uremic toxin, in addition to having raised concentrations in end stage kidney disease (ESKD), a molecule must also have adverse biologic effects. These large middle-molecules appear to be linked to unsolved complications of ESKD including chronic inflammation, cardiovascular disease, secondary immunodeficiency, erythropoietin resistance, and symptom burden. In symptom burden, the molecules can directly cause symptoms; for example, the retention of α-1 microglobulin (33 kDa) is associated with restless leg syndrome and the retention of cytokines is associated with flu-like symptoms. See Table 1.

Prescription of Expanded Hemodialysis Therapy

The high-retention-onset membranes provide clinicians the opportunity to increase the clearance of large middle-molecules beyond that provided with conventional hemodialysis strategies. Patients with residual renal function may benefit, as well as patients with conditions linked to retention of large middle-molecules, as detailed in the Clinical Relevance section.

As high-retention onset membranes are used to increase the clearance of middle molecules, the factor of time should be considered. In dialysis settings where time is flexible such as home hemodialysis, the high-retention-onset membranes could be utilized for longer or more frequent dialysis treatments to further increase middle-molecule removal.

CONCLUSION

Expanded hemodialysis utilizes a new generation of hemodialysis membranes, which allows for the first time, the effective clearance of large middle-molecules without significant albumin loss. These middle molecules have circulated in chronic kidney disease patients since the use of the first hemodialysis machine by Dr. Kolff in 1943. Biological pathways have been described for the involvement of these molecules in cardiovascular disease, secondary immunodeficiency, chronic inflammation, and symptom burden. Potentially, increased removal of large middle-molecules (molecular weight > 15k Da) can lead to improved patient outcomes. More studies are needed to further understand these biological pathways; therefore, this hypothesis should now be tested in robust clinical studies.

HDx utilizes a new generation of high retention onset/mid cut-off membranes that efficiently remove large middle-molecular uremic toxins that have been linked to the development of inflammation, cardiovascular disease and other dialysis related comorbidities.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Exploring the Clinical Relevance of Providing Increased Removal of Large Middle Molecules

Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

BACKGROUND

End stage kidney disease (ESKD) is accompanied by the retention of uremic toxins, molecules that accumulate in kidney impairment and have an adverse biologic effect. Uremic toxins can be broadly classified into three groups: small water-soluble molecules, middle molecules, and protein-bound solutes. Of these three groups, established dialysis technologies most efficiently remove small water- soluble molecules. However, current dialysis strategies lack the ability to provide effective clearance of middle molecules (500-60,000 Da), having been designed to remove ß2-microglobulin (11.8 kDa).

Recent advances in membrane technology have enabled the development of a new generation of membranes, medium-cut off membranes (MCO), which allow for the removal of middle molecules up to 50kD, surpassing even the range provided by hemodiafiltration (HDF), the established method for clearing middle molecules.

OBJECTIVES

This purpose of the review is three-fold. First, it describes the development of dialysis membranes that allow for removal of large middle molecules without albumin loss. Second, it identifies large middle molecules that can be removed using the MCO membranes, namely those with molecular weight > 15 kDa and assesses their clinical relevance. Third, it evaluates clinical experience to date with these membranes and recommended steps to make these membranes widely acceptable as a new therapeutic option for ESKD.

REVIEW

Adapting Hemodialysis Membranes to Remove Large Middle Molecules

Hollow fiber hemodialysis membranes have pores which have a bell-shaped distribution of size from small to large. See Figure 1. The pore size distribution of standard high-flux membranes shows low clearance of middle molecules with molecular mass > 15 kDa, with the largest of its pores being smaller than albumin (65 kDa) to prevent albumin loss. To increase the size of molecules removed by a membrane, the sizes of the pores needs to be increased by moving the distribution of the pores to the right, which occurred with the high-cut off (HCO) membranes. Although the HCO membranes were able to remove larger molecules, such as the free light chains k (22.5 kDa) and λ (45 kDa) in myeloma kidney, this resulted in the loss of albumin due to the nonuniformity of the pores.

To enable the clearance of larger middle molecules with molecular mass between 15 and 60 kDa without the loss of albumin, the distribution of the pores within the dialysis membranes had to fundamentally change to a tighter distribution. The MCO dialyzers use this new distribution of pores, which should provide in clinical practice the effective clearance of large molecules without excessive albumin loss.

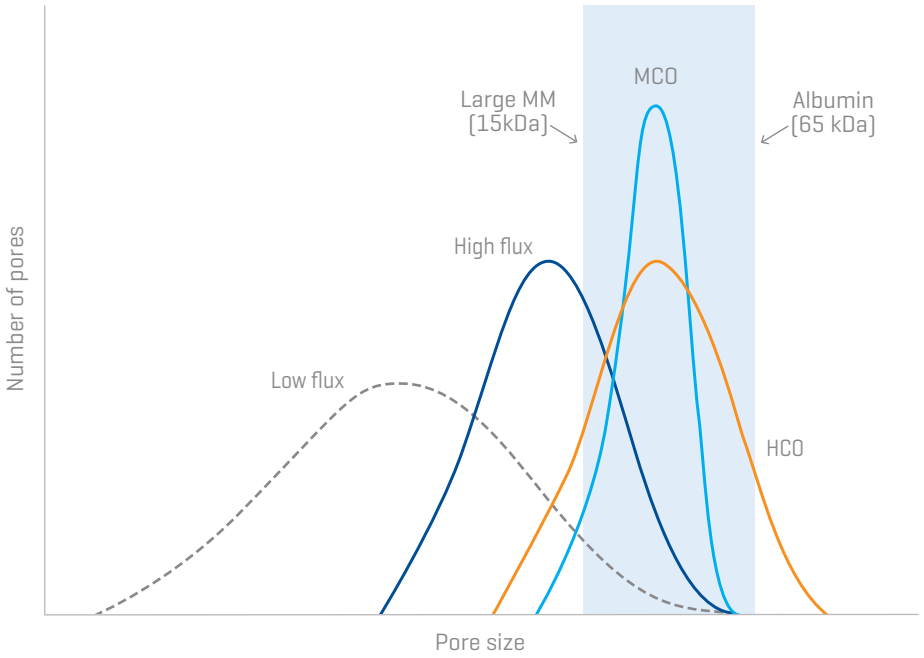


Figure 1. | Medium cut-off membranes provide clearance of large middle molecules without albumin loss. Schematic of pore size distribution in dialysis membranes. As membranes have been developed to allow the removal of large middle molecules (MMs) without albumin loss, the distribution of the pore sizes has had to be "tightened." The blue bar represents the distribution of large MMs before albumin is lost. The dotted line indicates Low flux, the dark blue line indicates High flux, the orange line indicates high cut-off (HCO) and the light blue line indicates medium cut-off (MCO). Adapted from Wolley M, et al.

Identification of Large Middle Molecules

A list of uremic toxins that can be classified as middle molecules was generated from EuTox database, associated publications, and Medline search. The review was limited to middle molecules with molecular mass > 15 kDa, above which clearance by high-flux membranes is reduced and clearance is increased by MCO membranes. Protein-bound solutes are clinically relevant but were not assessed because they cannot be removed by MCO membranes.

Fifty-nine (59) middle molecules are summarized in Table 1. Of the 59 middle molecules, 31 middle molecules had molecular mass under 15 kDa, and therefore can be removed by high-flux dialysis. Fourteen middle molecules had molecular mass between 15 and 25 kDa, and therefore can be removed by HDF. Fourteen middle molecules have molecular mass greater than 25 kDa, not currently removed by high-flux or HDF.

Classification and Biologic Summary of Larger Middle Molecules

The literature review identified 27 middle molecules with molecular mass > 15 kDa, the largest of which was 52 kDa. The serum levels of these middle molecules can range from < 1.5- to > 200-fold higher in patients receiving dialysis or with advanced chronic kidney disease (CKD) than in those with normal kidney function. These molecules were categorized into four broad functional groups: cytokines (n=5), adipokines (n=4), immune-mediated proteins (n=8), growth factors and hormones (n=4), and other molecules (n=6). In Table 2, the molecular mass, usual biologic role, possible adverse effects in uremia, and relative increase in dialysis or advanced CKD are described for each molecule.

Clinical Relevance of Large Middle Molecules as Uremic Toxins

Accelerated Atherosclerotic Cardiovascular Disease

Patients with CKD and especially those reliant on maintenance dialysis are subject to substantially elevated risk of cardiovascular disease and cardiovascular mortality compared to the general population. Many of the large middle molecules are involved in atherosclerosis. Correlations between serum concentrations of these large middle molecules and the rates of cardiovascular disease and overall survival have also been found.

Elevated levels of proinflammatory cytokines interleukin (IL)-18 (18 kDa), IL-6 (21-28 kDa), IL-1ß (17.5 kDa) and tumor necrosis factor (TNF)-α (17 kDa) are all involved with cardiovascular disease. Serum concentrations of IL-18 are associated with plaque burden and instability. IL-6 and IL-1ß have been described to be pathologically involved in the progression of atherosclerosis. TNF-α alters endothelial and vascular smooth muscle cell function.

Removed by High Flux (<15 kD)	Molecular Mass, kD	Removed by HDF (15–24.9 kD)	Molecular Mass, kD	Not Currently Removed (>25 kD)	Molecular Mass, kD
Methionine-enkephalin	0.5	Clara cell protein	15.8	Hyaluronic acid	25
Glutathione	0.6	Leptin	16	ß-Trace protein	26
Angiotensin A	0.8	Myoglobin	17	Soluble TNF receptor-1	27
d–Sleep-inducing peptide	0.8	TNF-a	17	Adiponectin	30
Dinucleoside polyphosphates	1	Soluble TNF receptor-2	17	FGF-23	32
Substance P	1.3	IL-1ß	17.5	a1-Microglobulin	-2.8
Motilin	2.7	FGF-2	18	VEGF	34.2
Orexin B	2.9	IL-10	18	YKL-40	40
Atrial natriuretic peptide	3	Retinol binding protein	21.2	Pentraxin-3	40.2
Desacylgherlin	3.2	Prolactin	22	a1-Acid glycoprotein	43
Vasoactive interstinal peptide	3.3	κ-Ig light chain	22.5	AGEs	45
Calcitonin	3.4	Complement factor D	23.75	λ-Ig light chain	45
Gherlin	3.4	IL-18	24	Visfatin	55
b-Endorphin	3.4	IL-6	24.5	AOPPs	>60
Orexin A	3.5				
Calcitonin gene–related peptide	3.7				
Cholecystokinin	3.8				
Endothelin	4.2				
Neuropeptide Y	4.2				
SIAM-1	4.2				
Adrenomedullin	5.7				
Osteocalcin	5.8				
IGF-1	7.6				
IL-8	8				
Parathyroid hormone	9.5				
Guanylin	10.3				
b2-Microglobulin	11.8				
Uroguanylin	12				
Resistin	12.5				
Cystatin C	13.3				
Degranulation inhibiting protein[a]	14.1				

Table 1. Summary of middle molecules (n=59).
[a]Degranulation inhabiting protein corresponds to angiogenin. Abbreviations: HDF: hemodiafiltration; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; AGE, advanced glycosylation end product; AOPP, advanced oxidative protein products. Adapted from Wolley et al.

Other middle molecules have also been associated with cardiovascular disease. Immune-mediated protein pentraxin (PTX)-3 (40 kDa) has been linked to unstable plaque in coronary and carotid arteries. ß-trace protein (BTP) (26 kDa) is correlated with the severity of coronary disease. The hormone prolactin (23 kDa) levels have been associated with increased risk of cardiovascular events and all-cause mortality in CKD/dialysis populations. Tissue accumulation of advanced glycosylation end products (AGEs) (<1-70 kDa) can contribute to cardiovascular disease by cross-linking with other molecules, causing structural changes and inducing inflammation in heart and blood vessels.

The adipokine visfatin (52 kDa) is strongly upregulated in atherosclerotic plaque, and serum levels are higher in those with unstable versus stable vascular disease. Additionally, the adipokines adiponectin (30 kDa) and leptin (16 kDa) have been implemented in progressive atherosclerosis by contributing to the recruitment of macrophages and the formation of cells.

Contribution to Structural Cardiac Disease

Several growth factors have been linked to cardiac hypertrophy. Experimental animal studies implicated fibroblast growth factor 2 (FGF-2) (18 kDa) and FGF-23 (32 kDa) as having a direct role in this process. Observational studies in humans also revealed a link between FGF-23 and left ventricular hypertrophy.

Influence on Secondary Immune Deficiency

ESKD is associated with significant immune dysfunction. Patients on dialysis experience high levels of infection-related morbidity and mortality. Immunoglobulin (Ig) free light chains (k-Ig (22.5 kDa), λ-Ig (45 kDa) were shown to reduce glucose uptake by polymorphonuclear leukocytes *in vitro* and reduce chemotaxis. In addition, serum free light chains were an independent risk factor for death by infectious cause in a CKD population. Adipokine retinol binding protein (RBP) 4 (21.2 kDa) also inhibits the chemotactic movement of polymorphonuclear leukocytes in a concentration-dependent fashion. FGF-23 (32 kDa) has been shown to exert inhibitory effects on leukocytes in a mouse CKD model, which was reversible with a neutralizing antibody toward FGF-23. α-1 acid glycoprotein

Molecule (Alternative Names)	Group	Size, kD	Usual Biologic Function	Possible Adverse Effects in Excess or Uremia	Relative Increase in Dialysis or Advanced CKD
IL-18	Cytokine	18	Proinflammatory; induction of TH1 response	Proatherogenic; increased amyloid-β production	Approximately 2x higher
IL-6	Cytokine	21–28	Diverse proinflammatory	Proatherogenic; sarcopenia and wasting; anorexia	2-5x higher
IL-1β	Cytokine	17.5	Proinflammatory; upregulation of IL-6	Proatherogenic; contributes to systemic inflammation	Approximately 2x higher
IL-10	Cytokine	18	Anti-inflammatory; downregulation of macrophage	Diminished anti-infectious immune function	~1.5x higher
TNF-α	Cytokine	17	Upregulation of immune response, induction of fever	Enhanced protein catabolism, anorexia, and muscle protein breakdown	4-5x higher
Adiponectin	Adipokine	30	Modulates glucose	Unknown	2-3x higher
Visfatin (Nicotinamide phosphoribosy ltransferase)	Adipokine	52	Intracellularly involved in NAD biosynthesis; extracellularly stimulates angiogenesis and endothelial cell proliferation	Proinflammatory cytokine effects; angiogenic effects, promotion of vascular smooth muscle cell proliferation	3-6x higher
Leptin	Adipokine	16	Regulates appetite and body energy stores	Anorexia and protein- energy wasting	3-4x higher
Retinol binding protein 4	Adipokine	21.2	Delivers retinol from liver to peripheral tissues	Inhibition of leukocyte chemotaxis and function	3-10x higher
Soluble TNF receptor 2	Immune-mediated protein	17	Binds to and limits TNF-α activity	May increase circulating TNF-α $t_{1/2}$	3-4x higher
κ-Ig light chains	Immune-mediated protein	22.5	Unknown	Inhibit leukocyte chemotaxis, apoptosis, and function	2-16x higher
Complement factor D (C3 proactivator convertase)	Immune-mediated protein	24	Component of alternative complement pathway; humoral defense	Overactivity of complement system	4-17x higher
Soluble TNF receptor 1	Immune-mediated protein	27–30	Binds to and limits TNF-α activity	May increase circulating TNF-α $t_{1/2}$ to prolong cytotoxic effects	3-10x higher
a1-Acid glycoprotein (Orosomucoid)	Immune-mediated protein	35–44	Anti-inflammatory acute-phase protein; suppresses local leukocyte activity and promotes immunosuppressive macrophage differentiation	Inhibition of leukocyte migration, contribution to secondary immunodeficiency	<1.5x higher
Pentraxin-3	Immune-mediated protein	40	Opsonization and complement activation; macrophage activity	Prothrombotic actions in endothelial cells; impaired NO production	2-7x higher
YKL-40 (Chitinase-3–like protein 1)	Immune-mediated protein	40	Regulates local inflammatory markers; other functions unclear	Contribution to upregulation of local tissue inflammation and fibrosis	2-5x higher
λ-Ig light chains	Immune-mediated protein	45	Unknown	Inhibit leukocyte chemotaxis, apoptosis, and function	2-18x higher
Vascular endothelial growth factor (Vascular permeability factor)	Growth factor	34	Promotes endothelial cell proliferation, migration, and differentiation; involved in cardiac adaptation to hypoxia and stretch	Involved in cardiomyopathy and left ventricular dysfunction	Approximately 2x higher
Fibroblast growth factor 2	Growth factor	18	Neovascularization; upregulates inflammatory cytokines and chemokines	Cardiac hypertrophy; contribution to local inflammation	
Fibroblast growth factor 23	Growth factor	32	Regulates phosphate homeostasis and kidney hydroxylation of vitamin D	Cardiac hypertrophy	>200x higher
Prolactin	Hormone	23	Primary role in mammary cell proliferation and reproductive function	Amplification of inflammatory cytokine response (IL-12 and TNF-α); increased CVS events	2-4x higher
Clara cell protein (CC16)	Protein	15.8	Phospholipase-A2-inhibitor; immunosuppressive role in respiratory tract	Unknown	Approximately 30x higher
α1-Microglobulin	Protein	33	Inhibitor of heme and neutrophil-induced oxidative damage	Inhibition of leukocyte migration, chemotaxis, and IL-2 secretion	Approximately 9x higher
β-Trace protein (L-prostaglandin D2 synthase)	Protein	26	Catalyzes isomerization of precursor prostanoids to active forms	Observationally associated with atherosclerotic plaque	>35x higher
Myoglobin	Protein	17	Oxygen carrier in muscle tissue	Increased oxidative stress	3x higher
Hyaluronic acid (Hyaluronan)	Glycosa-minoglycan	Variable	Formation of endothelial glycocalyx; structural role in extracellular matrix	Proinflammatory; promotes endothelial dysfunction and damage	5-16x higher
Advanced glycosylation end products	Other	<1–70	Unknown	Adverse structural effects	2-20x higher

Table 2. Classification, levels in ESKD, and methods of measurement of large middle molecules (n=27).Abbreviations: CVS, cardiovascular system; NO, nitroic oxide. Adapted from Wolley et al.

(35-44 kDa) inhibits the migration of neutrophils to infectious foci and is associated with the susceptibility to infections in individuals with diabetes.

Protein-Energy Wasting in CKD

There is evidence linking cytokines IL-6 (21-28 kDa), TNF-α (17 kDa), IL-1ß (17.5 kDa) to anorexia and protein-energy wasting in cancer, AIDS, and geriatric cachexia. Evidence specific to patients with CKD and patients on dialysis is getting stronger. Elevated levels of the adipokine leptin (16 kDa) can contribute to protein-energy wasting by inhibiting food intake and increasing energy expenditure.

In patients on dialysis, IL-6 (21-28 kDa) has a clear inverse relationship with albumin levels in patients on dialysis and have been found to negatively correlate with muscle mass. TNF-α (17 kDa) levels were higher in patients on dialysis with poor appetite, evidence of anorexia, nausea, or vomiting compared with those without. Higher IL-1ß (17.5 kDa) levels were associated with lower physical activity scores and faster declines in bioimpedence-derived measure of body cell mass.

Contributions to Chronic Inflammation

Retention of inflammatory cytokines, proteins, and other proinflammatory factors can cause chronic inflammation in patients on dialysis. For example, cytokine IL-6 (21-28 kDa) release from both leukocytes and peripheral tissues has been found to be upregulated in uremia, along with the increase of cytokine IL-1ß (17.5 kDa). Similarly, cytokine TNF-α (17 kDa) and immune-mediated proteins TNF receptors 1 (27-30 kDa) and 2 (17 kDa) are also increased in CKD, which further contribute to the chronic inflammation of ESKD.

Clinical Evaluation of MCO Dialyzers

With increasingly porous membranes, such as those in MCO dialyzers, there are two principal safety concerns: back filtration of endotoxins and albumin loss.

An in vitro assessment of the back filtration of endotoxins identified no increased back filtration with MCO dialyzers compared with high-flux dialyzers. Hemodialysis with the MCO membrane is associated with albumin loss of approximately 3 g per session. In a study in which patients were converted from online HDF to MCO dialysis, there was no significant change in serum albumin concentrations, suggesting that this level of albumin loss is tolerable. Additionally, in another study evaluating the efficacy of MCO dialysis for the removal of large middle molecules, MCO dialysis increased clearance of complement factor D (24 kDa), YKL-40 (40 kDa) and α1-microglobulin (33 kDa) vs high-flux dialysis.

CONCLUSIONS

The review identified 27 large middle molecules, many of which have biologic pathways through which they can contribute to cardiovascular disease, secondary immune deficiency, protein energy wasting and chronic inflammation. After reporting of these assessments of safety and efficacy of MCO dialysis, robust clinical trials are now required to determine if increasing their removal by dialysis can improve clinical outcomes.

MCO membranes improve removal of middle-molecular uremic toxins that have been linked to the development of chronic inflammation, CVD, and other dialysis related comorbidities; this may result in improved patient outcomes.

MCO Membranes: Enhanced Selectivity in High-Flux Class

Boschetti-de-Fierro A et al. MCO membranes: enhanced selectivity in high-flux class. *Nature/Sci Rep*. 2015; 5:18448. doi:10.1038/srep18448.

BACKGROUND

One of the unmet needs in hemodialysis is the adequate removal of uremic toxins over a broad molecular weight range. As synthetic membranes are less selective than the glomerular membrane, current hemodialysis membranes do not remove higher molecular weight toxins appropriately. Consequently, patients on hemodialysis have higher levels of middle and large molecular solutes in plasma.

Membrane innovation is currently directed towards enhanced removal of uremic toxins and increased membrane permeability. During the last decade, some experience has been gathered with high permeability membranes such as high cut-off (HCO) membranes. Pilot trials with HCO membranes indicated that expanded toxin removal might benefit the patient by decreasing the general inflammatory state.

Medium cut-off (MCO) membranes were designed to deliver expanded toxin removal as observed with HCO membranes while retaining albumin (65 kDa)[1] so that they are appropriate for regular use in conventional treatment schedules and treatment mode (i.e. 4-hour treatments, three times weekly in Europe).

OBJECTIVE

The aim of this study was to present the characterization of four prototypes of novel MCO membranes by dextran filtration[¥]. In addition, the sieving properties of the membranes before and after blood contact were reported, and the pore size during operation (i.e. hemodialysis treatment) was compared to the size of uremic toxins and vital proteins.

[¥]Fractional clearance studies to measure the size selectivity associated with glomerular filtration have universally employed dextran as a test transport probe. It is neither secreted nor reabsorbed by the renal tubules, so its clearance is easily measured.[2]

METHODOLOGY

Four different types of prototype devices denoted as MCO 1 to MCO 4, which differ in permeability, were investigated. As reference, a HCO device (Theralite) and a conventional high flux membrane (**Revaclear**) were also tested. All devices were manufactured by Gambro Dialysatoren GmbH, Hechingen, Germany. The membrane material was a polyarylethersulfone/polyvinylpyrrolidone blend.

Membranes were characterized in minimodules with the same surface area, nominal length, inner diameter and wall thickness. The minimodules were immersed in water before the filtration experiments. Minimodules intended for characterization after contact with blood for simulating in vivo operation conditions were initially perfused with blood (bovine) for 40 minutes and rinsed afterwards with water.

Dextran solutions were prepared, and filtration experiments were carried out. For experiments run on MCO 4, the dextran solution included one additional fraction of 150 kDa, to allow for sieving coefficient (SC) calculation with similar precision as the other MCO membranes.

RESULTS

Characterization of the MCO high-flux membranes by dextran sieving profiles in aqueous solution (pristine; before blood exposure)

As shown in Figure 1, the sieving curves for the MCO membranes are located at the molecular weights between that of the conventional high-flux membrane and HCO membrane in aqueous solution (pristine; before blood exposure). The MCO sieving curves are similar to the ficoll sieving curve for the glomerular membrane; blood purification membranes should mimic the filtration spectrum of the natural (glomerular) membrane.

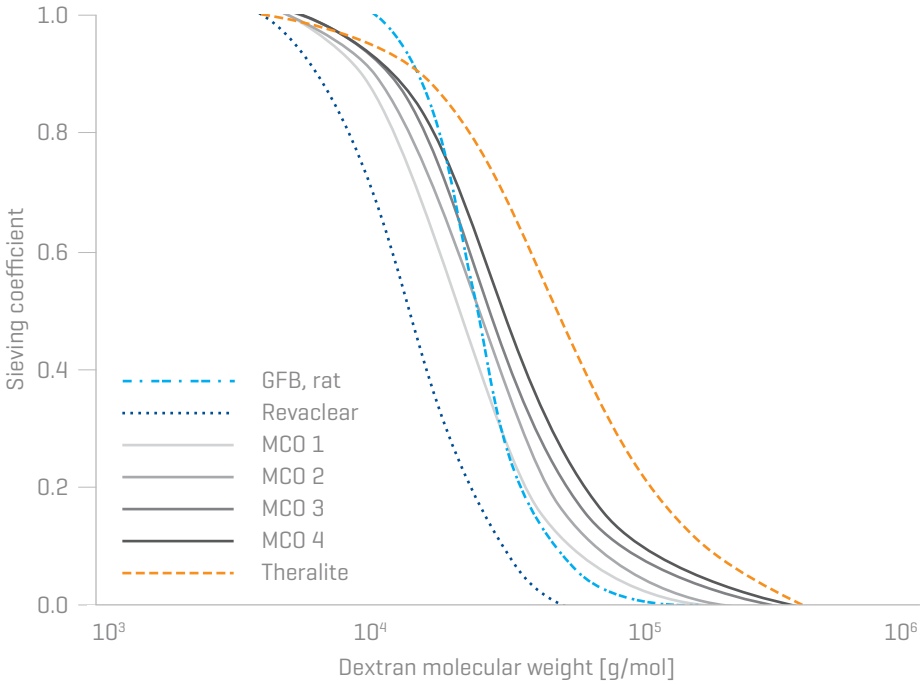


Figure 1. Characteristic *in vitro* dextran sieving curves measured in aqueous solution (pristine/before blood exposure) for different types of blood purification membranes: high-flux (Revaclear, MCO 1-4), HCO (Theralite). Data for glomerural membrane added for comparison (rat specimen, ficoll filtration, measured in vivo). Abbreviations: GFB: glomerular filtration barrier; MCO: medium cut-off. Adapted from Boschetti-de-Fierro et al.

The values for Medium Weight Retention Onset (MWRO), Medium Weight Cut Off (MWCO) and pore radius (i.e. effective Stokes-Einstein radius calculated from MWCO) are depicted for the four membranes, as well as the conventional high flux membrane and HCO membrane in Table 1. The differences between the MCO membranes are evident from 1 to 4, showing increased MWRO and MWCO values which indicate increased permeability.

	Before blood exposure			After blood exposure		
Membrane	MWRO [kDa]	MWCO [kDa]	Pore radius [nm]	MWRO [kDa]	MWCO [kDa]	Pore radius [nm]
Revaclear	5.7 ± 0.5	32 ± 3	3.9 ± 0.1	4.4 ± 0.3	14.2 ± 0.2	2.68 ± 0.02
MCO 1	9.4 ± 0.2	56 ± 3	5.0 ± 0.1	5.0 ± 0.4	18.1 ± 0.8	3.0 ± 0.1
MCO 2	10.0 ± 0.6	64 ± 3	5.4 ± 0.1	5.84 ± 0.09	18.2 ± 0.3	3.0 ± 0.1
MCO 3	11.3 ± 0.4	81 ± 9	6.0 ± 0.3	6.32 ± 0.06	22.7 ± 0.8	3.3 ± 0.1
MCO 4[a]	12.1 ± 0.7	99 ± 7	6.5 ± 0.2	6.77 ± 0.06	25 ± 5	3.5 ± 0.3
Theralite	15 ± 1	300 ± 100	10 ± 2	8.1 ± 0.8	40 ± 8	4.3 ± 0.4

Table 1. Characterization of MCO hemodialysis membranes, conventional high-flux and HCO membranes, based on dextran sieving experiments before and after blood exposure. Values are + standard deviation for n=3.*Experiments with MCO 4 included one dextran fraction of 150 kDa. Abbreviations: MWRO: medium weight retention onset: MWCO; medium weight cut off; MCO, medium cut-off. Adapted from Boschetti-de-Fierro et al.

The parameters describing the pore size distribution calculated from the sieving profiles for the same membranes are detailed in Table 2. The presented values assess the mean and broadness of the pore size distribution. The values of the mean of the distribution increase with membrane permeability. The variance of the respective distribution is larger as the pore sizes increase. The pore size distribution for all membranes is narrower after blood contact, indicating that the selectivity of synthetic membranes improves during the operation.

	Before blood exposure		After blood exposure	
Membrane	**Mean [nm]**	**Variance [nm]**	**Mean [nm]**	**Variance [nm]**
Revaclear	3.0 ± 0.3	2 ± 1	2.42 ± 0.08	0.587 ± 0.003
MCO 1	4.1 ± 0.2	4.7 ± 0.8	2.5 ± 0.1	0.6 ± 0.2
MCO 2	4.0 ± 0.2	4.6 ± 0.4	2.55 ± 0.07	0.7 ± 0.1
MCO 3	4.40 ± 0.03	6.4 ± 0.1	2.9 ± 0.1	1.3 ± 0.4
MCO 4	4.8 ± 0.2	8.8 ± 0.4	3.3 ± 0.6	3 ± 3
Theralite	5.1 ± 0.3	11 ± 2	3.4 ± 0.2	2 ± 1

Table 2. Mean (pore radius) and variance of the log-normal pore size distribution for the 4 MCO prototype membranes, conventional high-flux and HCO membranes before and after blood exposure. Values are average ± standard deviation for n=3. Adapted from Boschetti-de-Fierro et al.

The challenge in developing novel high-flux membranes with toxin removal capabilities similar to HCO membranes while retaining albumin adequately resides in the membrane manufacturing process. Increasing pore sizes usually leads to an increase in the broadness of the pore size distribution, causing undesirable albumin permeation. Controlled membrane manufacture allows some improvement in this direction. As can be seen, the MCO 4 membrane shows similar mean pore size to Theralite (HCO), while having a 20% smaller variance. The less permeable versions, MCO 1 and MCO 2, show mean pore size around 4 nm with less than half the variance of Theralite. This indicates that the MCO membranes offer enhanced selectivity compared to HCO membranes.

Membrane classification after blood contact

The sieving curves before and after exposing the MCO membranes to blood are shown in Figure 2. After blood exposure, the sieving curves were shifted toward lower molecular weights, and the sieving profiles indicate that the MCO membranes are less permeable than the glomerular membrane.

The natural formation of the protein layer on top of the synthetic membrane during hemodialysis gradually affects the solute removal during the first 40 minutes of treatment. This phenomenon is illustrated by the comparison of the sieving characteristics before and after blood contact. While the pristine MCO membrane allows the passage of molecules above 70 kDa to some extent, the sieving profile of the MCO membranes (as that of every artificial membrane) shifts towards lower molecular weights during operation. This circumstance is a compromise to deliver a tailored removal after the inevitable membrane fouling. As the hemodialysis treatment time is around 4 hours in Europe, this means that during more than 80% of the treatment blood purification is accomplished by a membrane the performance of which is governed by protein fouling. The MCO membranes show sieving profiles close to that of the natural kidney after the formation of the protein layer, thereby maintaining the required performance along the treatment.

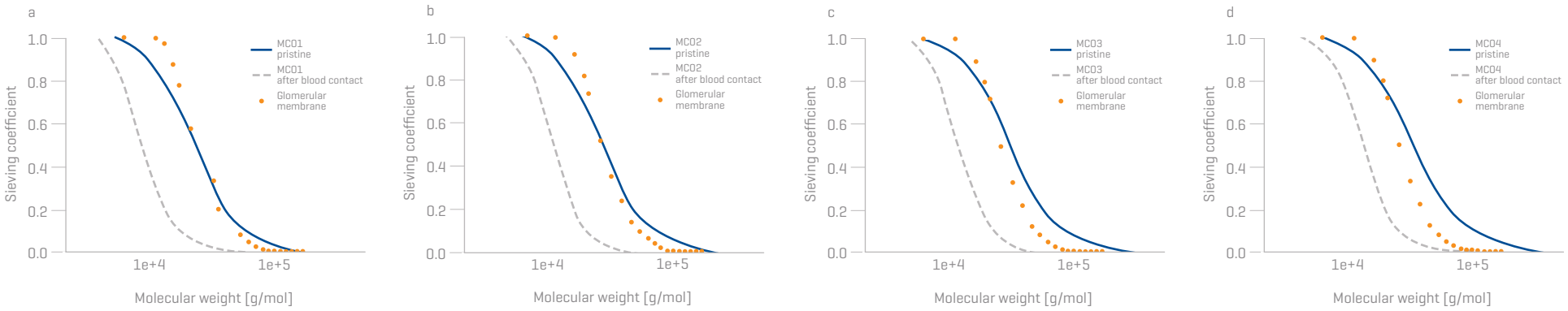


Figure 2. Characteristics of sieving curves for MCO high flux membranes before (solid line) and after blood contact (dashed line), for (a) MCO 1, (b) MCO 2, (c) MCO 3, (d) MCO 4. The data for the glomerular membrane has been added for comparison (rat specimen, ficoll filtration, measured *in vivo*). Abbreviation: MCO; medium cut-off. Adapted from Boschetti-de-Fierro et al.

The effective pore size is an indication for the biggest molecules that will pass through the membranes. The effective pore size of the MCO membranes is between 3.0 and 3.5 nm after blood contact/formation of the protein layer (see Table 1), indicating that the membranes retain albumin (hydrodynamic radius of 3.51 nm) (65 kDa)[1] during treatment. Additionally, the least permeable MCO membrane has an effective pore radius of 3.0 nm during treatment (Table 1), which should allow adequate removal of large uremic toxins, up to lambda free light chains (λ-FLCs) (45 kDa)[1] with a hydrodynamic radius of 2.8 nm. See Table 3 for hydrodynamic radius (R_h) for albumin and representative middle and large uremic toxins.

Molecule	R_h [nm]	Comments	Ref.
ß 2 microglobulin	1.7	calculated from the diffusion coefficient in free solution	16
Tumor necrosis factor (TNFα)	1.9-2.3	depending on its aggregation state, influenced by concentration and pH	17
Free light chains (FLC) monomeric state (mostly κ -FLC)	2.3	Stokes' radius determined by chromatography	18
Free light chains (FLC) dimeric form (mostly λ -FLC)	2.8	Stokes' radius determined by chromatography	18
Albumin	3.51	calculated from the intrinsic viscosity (agrees with Stokes' radii from diffusion and sedimentation coefficient)	19

Table 3. Hydrodynamic radius (R_h) for albumin and some representative middle and large toxins.
Adapted from Boschetti-de-Fierro et al.

Based on the data presented, it can be presumed that some albumin permeation takes place even after the formation of the protein layer for the most permeable membrane MCO 4. This effect, if properly controlled, is not necessarily detrimental to the patients. Albumin loss is tolerated to some extent, as demonstrated in peritoneal dialysis patients where weekly albumin losses of 21–42 g/1.73 m^2 are accepted and not linked to outcome detriment.

CONCLUSION

The novel MCO membranes provide for large pore sizes with appropriate pore size distribution and permeability close to that of the natural kidney. Their MWCO values suggest that, when used in hemodialysis treatments, they allow for removal of an expanded range of uremic toxins compared to conventional high-flux membranes. A formation of a protein layer on top of the synthetic membrane during hemodialysis restricts the removal of molecules above 3.5 nm in radius, ensuring the retention of albumin during a treatment, while still optimizing removal of large uremic toxins.

Tailored pore sizes of MCO membranes promote removal of an expanded range of uremic toxins, while ensuring retention of albumin. MCO's unique membrane design allows for a filtration profile that's closer to the natural kidney.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

2.Editorial Review, Change Selectivity in Kidney Ultrafiltration, *Kidney International*. 1995; 47:1242-1251.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Fluid Dynamics Analysis by CT Imaging Technique of Hollow Fiber Dialyzer with Medium Cut-Off Membrane

Lorenzin A et al. Fluid dynamics analysis by CT imaging technique of hollow fiber dialyzer with medium cut-off membrane. ASN 2019. **Abstract SA-P0054**.

BACKGROUND

Inadequate removal of molecules between 5 and 50K Da,due to their restriction in diffusibility, may cause long-term complication in chronic hemodialysis (HD) patients. Medium Cut-off (MCO) is a new class of membranes with enhanced sieving properties and negligible albumin loss, thanks to its high molecular weight (MW) retention onset and MW cut-off value lower than albumin MW. MCO membrane used in HD allows to perform expanded hemodialysis (HDx), a technique based on high internal filtration (IF). Our previous study quantified the IF of **Theranova** dialyzer leveraging a nuclear imaging technique.

In order to characterize the local distribution of the IF, an *in vitro* study assessing the fluid dynamics inside **Theranova** dialyzer was conducted through CT imaging technique.

METHODS

Dialyzer **Theranova** 400 (Baxter, Deerfield, USA) was placed in vertical position in the CT gantry. Blood and dialysate compartments were analyzed separately. Dye solution was circulated through blood compartment at 300mL/min and through dialysate one at 500mL/min. Longitudinal sections, 0.5cm thick, were recorded for 60 seconds.

RESULTS

In blood compartment, dye solution immediately after its entrance in the dialyzer demonstrates homogeneous progression, while different velocity profiles were observed among the fibers proceeding to the outlet port (Fig b). In dialysate compartment, dye solution is distributed in the periphery first (Fig d), then seeps in the fibers bundle and reaches the complete compartment filling.

CONCLUSION

The homogeneous dye profile immediately after its entrance in blood compartment demonstrated a good design of the inlet port; the optimal dye distribution reached in both blood and dialysate compartments ensure that IF phenomenon is equally achieved in both central and peripheral regions of the dialyzer.

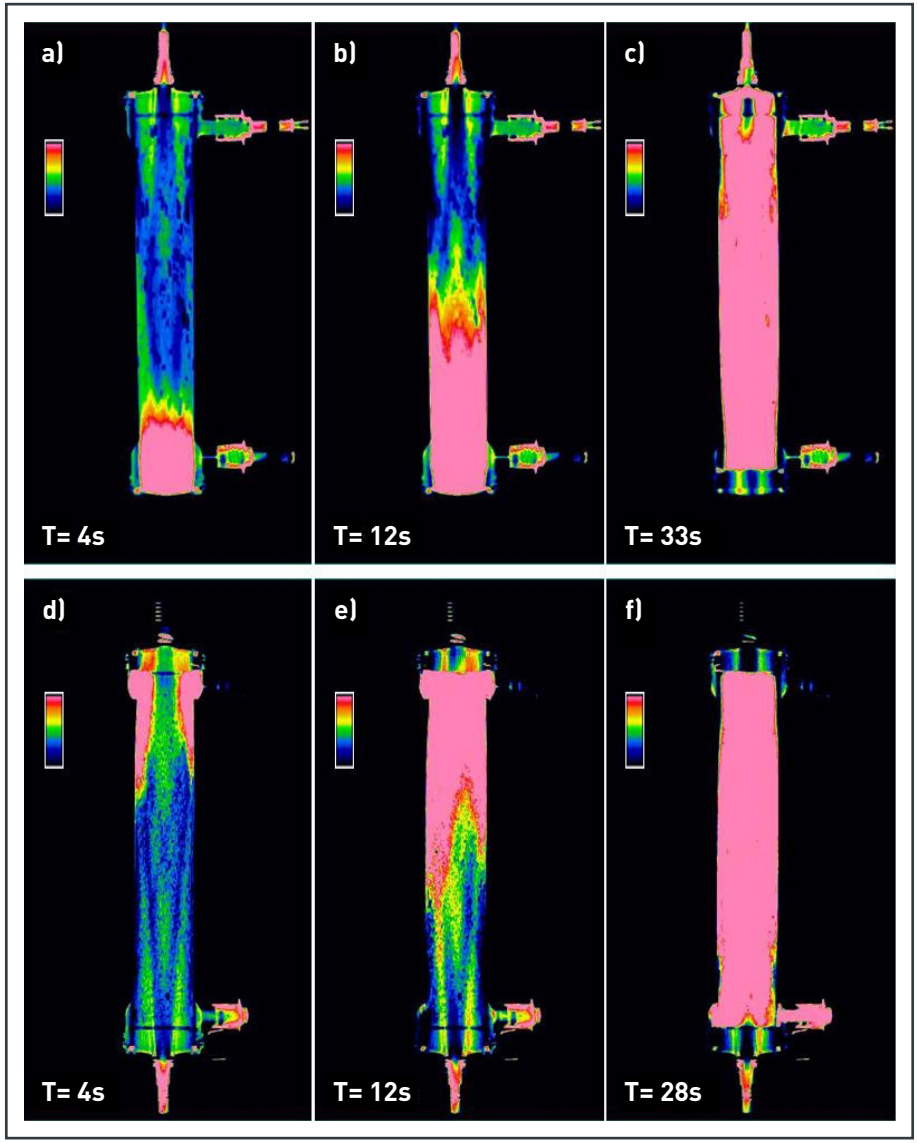


Figure 1. Dye progression in blood (a, b, c) and dialysate (d, e, f) compartments after 4s, 12s, and at total filling. Central longitudinal sections show different profiles in the two compartments: in blood, initial progression in homogeneous, at 12s different velocity profiles emerge along the fiber bundle; in dialysate, dye solution proceeds faster along the dialyzer wall than within the fiber bundle, at the end the whole compartment is reached by the dye solution. Abbreviations. T: time; s: seconds Figure adapted from Lorenzin et al.

Performance of Hemodialysis with Novel Medium Cut-Off Dialyzers

Kirsch AH et al. Performance of hemodialysis with novel medium cut-off dialyzers. *Nephrol Dial Transplant.* 2017; 32:165-172. doi:10.1093/ndt/gfw310.

BACKGROUND

End-stage renal disease (ESRD) results in the retention of uremic toxins, which is associated with high mortality. Uremic toxins are classified into small (<500 Da) and middle molecular (500 Da–60 kDa) water-soluble solutes and protein-bound substances. While conventional hemodialysis (HD) modalities remove small solutes and smaller-sized middle molecules, clearance of larger middle molecules and protein-bound substances is poor.

Studies have associated middle molecules to pathological features of uremia, such as immune dysfunction and inflammation, as well as adverse outcomes in dialysis patients. Free immunoglobulin light chains (FLCs) have a molecular weight (MW) of ~22.5 kDa for kappa FLC (κFLC) and 45 kDa for lambda FLC (λFLC). Importantly, FLC levels have been associated with mortality in chronic kidney disease (CKD) cohorts.

Efforts have focused on improving the clearance of larger middle molecules in dialysis. The introduction of more water-permeable high-flux membranes allowed the clearance of middle molecules such as ß2-microglobulin (12 kDa) and increasing convection with hemodiafiltration (HDF) considerably enhanced middle molecule clearance. However, high flux dialyzers have cut-off values of ~20 kDa and are thus limited in their ability to remove larger middle molecules κFLC and λFLC. Maintenance HD patients who are at high mortality risk seem to benefit from high-flux HD, but large outcome trials comparing HDF to HD have yielded equivocal results.

Medium cut-off (MCO) dialyzers utilize a novel class of membranes designed to increase the removal of larger middle molecules in HD, and in contrast to more permeable high cut-off (HCO) membranes, are intended for routine use in maintenance HD patients.

OBJECTIVES

To compare the performance of three prototypes of MCO dialyzers with HD and high-volume hemodiafiltration (HDF) using contemporary high-flux dialyzers. Specifically, these studies compared the clearance of larger middle molecules, including κFLC (23 kDa) and λFLC (45 kDa), considered to be uremic toxins.

METHODOLOGY

Study Design

The two studies were prospective, open-label, 4-arm, randomized, active-control, crossover pilot studies comparing **Theranova** 400 dialyzer (MCO AA; Gambro Dialysatoren GmbH, Hechingen, Germany, a subsidiary of Baxter International Inc.) and two MCO dialyzer prototypes (MCO BB and CC) with high-flux dialyzers FX CorDiax 80; Fresenius Medical Care Deutschland, Bad Homburg, Germany in studies 1 and 2 and high-volume HDF (FX CorDiax 800; Fresenius Medical Care Deutschland, Bad Homburg, Germany; study 2.

The membranes of the MCO dialyzers had increased permeability (AA < BB < CC). Membranes MCO AA, BB and CC were polyarylethersulfone-PVP blend membrane polymer; Fx CorDiax 80 and Fx CorDiax 800 were polysufone-PVP blend membrane polymer.

Study 1 compared MCO AA (**Theranova** 400), BB, and CC with high-flux dialyzers (FX CorDiax 80), while study 2 compared MCO AA (**Theranova** 400) and BB with high-flux dialyzers (FX CorDiax 80) and high-volume HDF (FX CorDiax 800).

Study 1 was conducted in the dialysis units of a medical university and a hospital in Austria. Study 2 was performed in a dialysis center in Germany. The dialysis sessions in study 1 were 4 hours with a dialysate flow (Q_D) of 500 mL/min and a blood flow (Q_B) of 300 ± 20 mL/min, while the dialysis sessions in study 2 were 4-5 hours with a QB of 400 + 50 mL/min.

Patients

Patients aged 18 years or above, on either HD or HDF treatment for at least 3 months before enrollment, had a κFLC/ λFLC ratio of > 0.37 and < 3.1, and no history of monoclonal gammopathy were enrolled and randomized to each study dialyzer treatment. A total of 39 patients were enrolled: 19 in study 1 and 20 in study 2.

Outcomes

The primary outcome was the overall clearance (K_{ovr}) of λFLC using MCO dialyzer prototypes in HD mode vs. K_{ovr} using high-flux dialyzers used in HD and HDF. Secondary outcomes were the clearance of other middle molecules and small molecules, as well as safety (albumin removal and treatment tolerance) of MCO HD.

RESULTS

Study 1: Free Light Chain Removal During HD Using MCO Dialyzers Compared to a High-Flux Dialyzer

Overall Clearance

In study 1, the λFLC K_{ovr} and the κFLC K_{ovr} with MCO AA, BB, and CC were significantly higher than with high-flux HD. See Figure 1A and Table 1A.

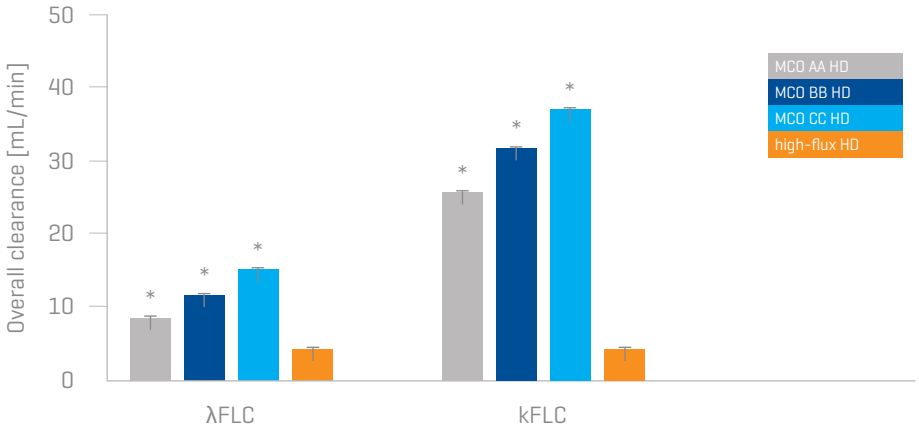


Figure 1A. Overall Clearance. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and high-flux dialyzer in study 1. Data are least square mean + standard error. *p<0.001 compared to high-flux dialyzer. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HD, hemodialysis. Adapted from Kirsch et al.

Test Dialyzer	λFLC K_{ovr} Overall Clearance		κFLC K_{ovr} Overall Clearance	
	Least square mean mL/mn (standard error)	*P* value vs high-flux HD	Least square meanmL/mn (standard error)	*P* value vs high-flux HD
MCO AA HD	8.5 (0.54)	P<0.001	26.2 (1.24)	P<0.001
MCO BB HD	11.3 (0.51)	P<0.001	31.8 (1.17)	P<0.001
MCO CC HD	15.0 (0.53)	P<0.001	37.3 (1.24)	P<0.001
High-flux HD	3.6 (0.51)		3.3 (1.17)	

Table 1A. Overall Clearance. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and high-flux dialyzers in study 1. Data are least square mean + standard error. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HD, hemodialysis. Adapted from Kirsch et al.

Reduction Ratios

The λFLC reduction ratios (RRs) and κFLC RRs of MCO AA, BB, and CC were significantly higher than that of high-flux HD. See Figure 1B and Table 1B.

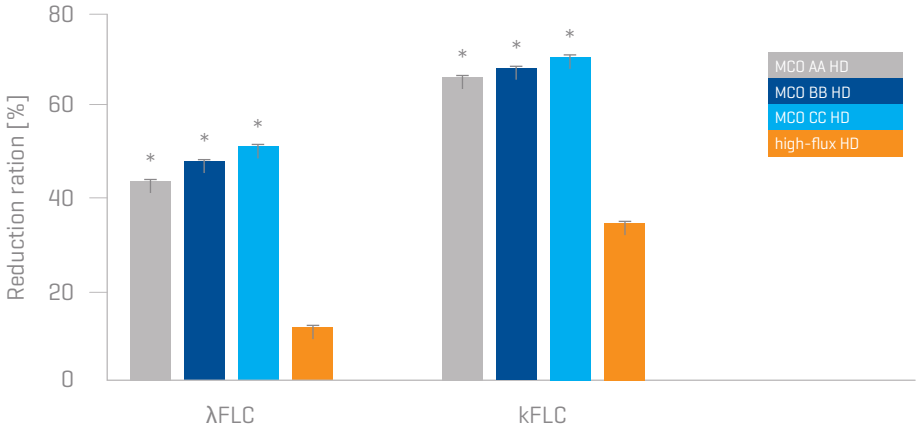


Figure 1B. Reduction Ratio. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and high-flux dialyzer in study 1. Data are least square mean + standard error. P< 0.001 compared to high-flux dialyzer. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HD, hemodialysis. Adapted from Kirsch et al.

Test Dialyzer	λFLC K_{ovr} Reduction Ratio		κFLC K_{ovr} Reduction Ratio	
	% (standard error %)	*P* value vs high-flux HD	% (standard error %)	*P* value vs high-flux HD
MCO AA HD	42.5 (2.06)	P<0.001	66.3 (1.85)	P<0.001
MCO BB HD	47.6 (2.06)	P<0.001	68.4 (1.85)	P<0.001
MCO CC HD	51.5 (2.10)	P<0.001	70.4 (1.88)	P<0.001
High-flux HD	12.9 (2.10)		36.4 (1.88)	

Figure Table 1B. Reduction Ratio. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and high-flux dialyzer in study 1.Data are least square mean + standard error. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HD, hemodialysis. Adapted from Kirsch et al.

Study 2: Free Light Chain Removal During HD Using MCO Dialyzers Compared to High-Flux HD and HDF

Overall Clearance

Like study 1, in study 2, the λFLC K_{ovr} and κFLC K_{ovr} during HD were again significantly greater (p<0.001) when using MCO AA or BB compared to high flux HD (see Figure 2A). The λFLC K_{ovr} and κFLC K_{ovr} with MCO AA and BB were also significantly higher than that of HDF (p<0.001). See Figure 2A and Table 2A.

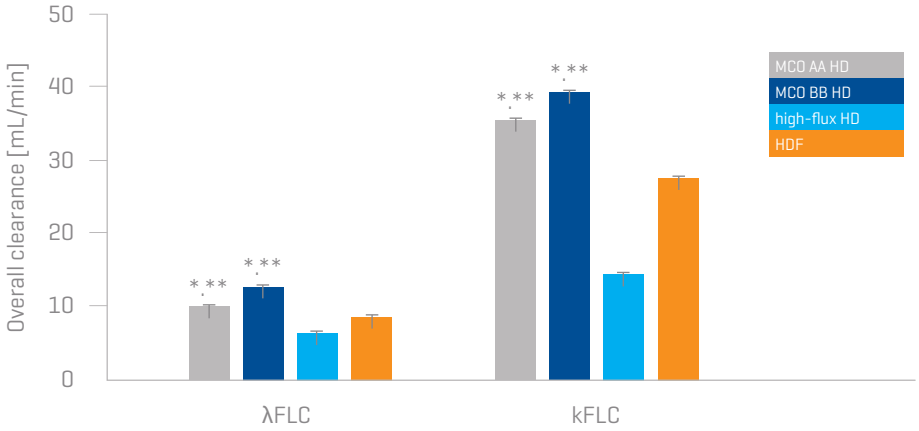


Figure 2A. Overall Clearance. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and high-flux dialyzer and hemodiafiltration in study 2. Data are least square mean + standard error. *p<0.001 compared to high-flux HD; **p<0.001 compared to HDF. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HD, hemodialysis, HDF, hemodiafiltration. Adapted from Kirsch et al.

Test Dialyzer	λFLC K_{ovr} Overall Clearance		κFLC$_{ovr}$ Overall Clearance	
	Least square mean mL/mn (standard error)	*P* value vs HDF	Least square meanmL/mn (standard error)	*P* value vs HDF
MCO AA HD	10.0 (0.58)	P<0.001	35.0 (1.43)	P<0.001
MCO BB HD	12.5 (0.57)	P<0.001	39.4 (1.39)	P<0.001
HDF	6.2 (0.58)		25.4 (1.43)	P<0.001

Table 2A. Overall Clearance. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and hemodiafiltration in study 2. Data are least square mean + standard error. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HDF, hemodiafiltration. Adapted from Kirsch et al.

Reduction Ratios

The λFLC reduction ratios (RR) with MCO AA and BB were superior to that with HDF (p<0.001). There was no difference between κFLC RR achieved with MCO AA vs HDF (p=0.3); whereas MCO BB resulted in a statistically significantly higher κFLC RR than HDF (p = 0.01). See Figure 2B and Table 2B.

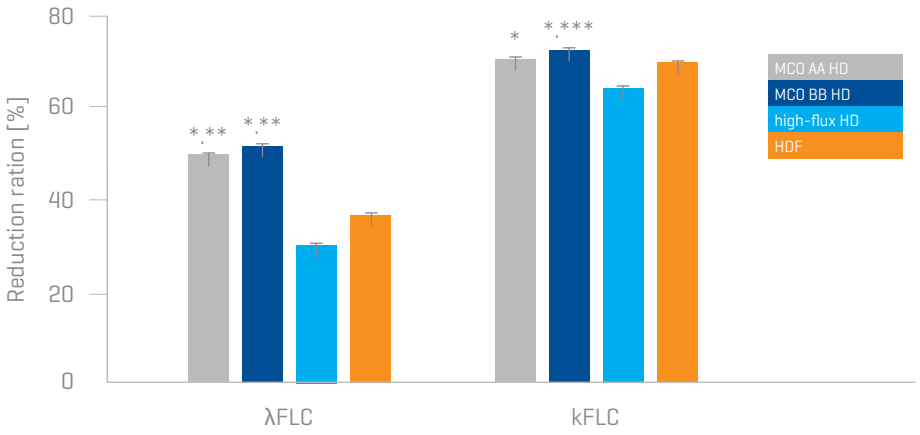


Figure 2B. Reduction Ratio. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and high-flux dialyzer and hemodiafiltration in study 2. Data are least square mean + standard error*p<0.001 compared to high-flux HD; **p<0.001 compared to HDF; *** p p=0.01, compared to HDF. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HD, hemodialysis; HDF, hemodiafiltration. Adapted from Kirsch et al.

Test Dialyzer	λFLC K_{ovr} Reduction Ratio		$KFLC_{ovr}$ Reduction Ratio	
	% (standard error %)	*P* value vs HDF	% (standard error %)	*P* value vs HDF
MCO AA HD	48.1 (1.72)	P<0.001	72.9 (1.35)	P=0.3
MCO BB HD	52.7 (1.72)	P<0.001	74.8 (1.35)	P=0.01
HDF	37.9 (1.76)		71.6 (1.37)	

Table 2B. Reduction Ratio. Free immunoglobulin light chain removal during hemodialysis with medium cut-off dialyzers and high-flux dialyzers and hemodiafiltration in study 2. Data are least square mean + standard error. Abbreviations: FLC, free light chain; MCO, medium cut-off dialyzer; HD, hemodialysis; HDF, hemodiafiltration. Adapted from Kirsch et al.

Removal of other middle molecules during HD and HDF

In addition to κFLC and λFLC, removal of other larger-sized solutes was greater with MCO HD (MCO AA/**Theranova** 400) compared to high-flux HD (FX CorDiax 80) and high-volume HDF (FX CorDiax 800). The overall clearance for α1-microglobulin (33 kDa), complement factor D (CFD) (24 kDa), myoglobin (17 kDa) and β2-microglobulin (12 kDa) as well as λFLC (45 kDa) and κFLC (23 kDa) were significantly greater for MCO AA HD than HD and HDF (p<0.001). The exception was the difference for β-2 microglobulin vs HDF in which the significance was p<0.01. See Figure 3A.

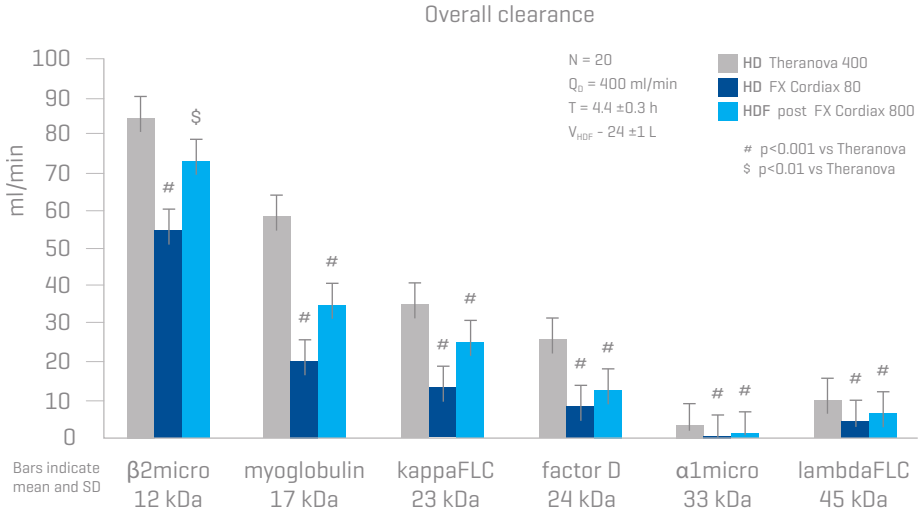


Figure 3A. Overall clearances of medium-sized and small molecules. Comparisons are based on a mixed model with fixed effects of period and study dialyzer type, and the random effect of subject. Abbreviations: HD, hemodialysis; HDF, hemodiafiltration, SD: standard deviation.

The reduction ratios were significantly greater for MCO AA HD (**Theranova** 400) than HD and HDF (p<0.001) for λFLC (45 kDa), YKL-40 (40 kDa), Complement Factor D (24 kDa) and myoglobin (17 kDa). MCO AA HD also showed a significant difference (p<0.001) vs HD in reduction ratios for κFLC (23 kDa) and β2-microglobulin (12 kDa). A slightly higher reduction ratio for β-microglobulin was achieved with HDF, underlining that MCO HD more efficiently removes larger middle molecules. See Figure 3B.

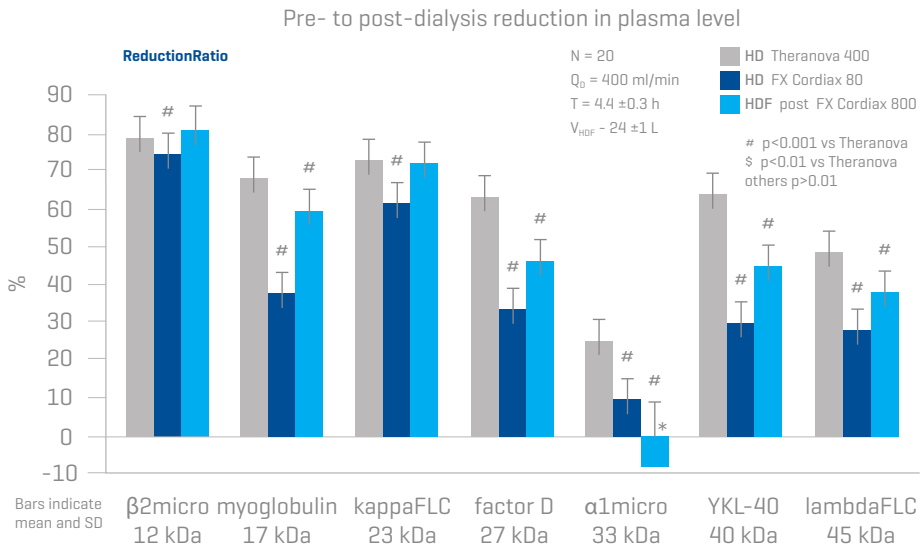


Figure 3B. Reduction ratios of medium-sized and small molecules. Comparisons are based on a mixed model with fixed effects of period and study dialyzer type, and the random effect of subject. Abbreviations: HD, hemodialysis; HDF, hemodiafiltration, SD: standard deviation.

Albumin Removal During MCO HD

Increasing pore sizes, while permitting clearance of larger uremic toxins, bears the trade-off of the leaking of larger molecules such as albumin (65 kDa)[1]. In both studies, MCO dialyzers resulted in larger albumin removal than high-flux HD and HDF. However, for MCO AA, the tightest of the three membranes, the per treatment albumin loss was about 3 g on average, and the controlled loss was between 1 and 4 g. The albumin loss obtained with the MCO AA dialyzer was within range observed in HDF treatment with high flux dialyzers, below transperitoneal albumin losses seen in peritoneal analysis, and less than a third of what has been reported for HD with High Cut-off (HCO) membranes. See Table 3.

Test Dialyzer	Study 1 – Albumin Removal Median g (range)	Study 2 – Albumin Removal Median g (range)
MCO AA	**2.9 g (1.5-3.9)**	**3.2 g (1.9-3.9)**
MCO BB	4.8 g (2.2-6.7)	4.9 g (1.1-7.2)
MCO CC	7.3 g (1.9-9.7)	nv
High-flux HD	0.2g (0.2-0.2)	0.2 g (0.2-0.3)
HDF	nv	0.4 g (0.3-0.8)

Table 3. Albumin removal in studies 1 and 2. Albumin values (median g (range)). Abbreviations: MCO, medium cut-off dialyzer; HD, hemodialysis; HDF, hemodiafiltration; nv, no value. Table adapted from Kirsch et al.

Safety

In study 1, the adverse events (AEs) between the study dialyzers were comparable (total = 23) and 11 patients (58%) experienced at least one AE. No serious AEs were recorded and none of the AEs was considered possible or likely to be related to the investigational product.

Similar AEs distribution was observed in study 2 (total = 31) with 11 patients (55%) experienced at least one AE. Two serious AEs occurred, both requiring intradialytic hospitalization but not related to the study treatments. Six AEs were considered possibly or likely to be related to the investigational product: one patient in MCO BB dialyzer and one in FX CorDiax 80 dialyzer (each had three events).

Limitations

K_{ovr} only includes transmembrane removal and does not take into account any potential adsorption to the membrane. The study design was confined to a single treatment with each dialyzer for each patient and the study did not examine the long-term effects of such membranes on serum levels of middle molecules and albumin.

CONCLUSION

These studies were the first studies that presented a unique characterization of HD membrane clearance for an extensive size range of middle molecules (11.6 – 45 kDa). MCO HD removes a wide range of middle molecules more effectively than high-flux HD, with the trade-off of increased albumin removal, compared to high-flux HD and HDF. MCO HD also exceeds the performance of high-volume HDF for removal of larger middle molecules, particularly λFLC. However, for MCO AA, the tightest of the three membranes, the per treatment albumin loss was about 3 g on average, and the controlled loss was between 1 and 4 g.

The MCO AA prototype, the most restrictive of the three prototypes tested and the most attractive benefit-risk profile, became the basis for the **Theranova** 400 specifications. Importantly, MCO HD/ **Theranova** can be applied to maintenance HD patients, in whom high volume HDF may not be used or is not available.

MCO (Theranova) membrane provides superior removal of conventional/large middle molecular uremic toxins (up to 45,000 Da), compared to traditional high flux membranes, with controlled albumin loss between 1 and 4g, per treatment.

References: 1 Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

TABLE OF CONTENTS

Comparison of Hemodialysis with Medium Cut-Off z

Dialyzer and On-Line Hemodiafiltration on the Removal of Small and Medium-Sized Molecules

Belmouaz M et al. Comparison of hemodialysis with medium cut-off dialyzer and on-line hemodiafiltration on the removal of small and medium-sized molecules. *Clin Nephrol*. 2018; 1:50-56.

BACKGROUND

Recent data suggest that the use of medium cut-off (MCO) dialyzers in hemodialysis (HD) promotes greater clearance and reduction ratio (RR) for myoglobin (17k Da) and other large-sized molecules than on-line hemodiafiltration (ol-HDF), but its effects on $ß_2$-microglobulin (11.8k Da) are not clear.

The association of high serum levels of middle-sized toxins, particularly $ß_2$-microglobulin, with inflammation, immune dysfunction, and patient survival has been established in several studies. The removal of middle-sized toxins such as $ß_2$-microglobulin (11.8 kDa) and myoglobin (17 kDa) depends on both dialyzer permeability and treatment modalities. Ol-HDF, combining the use of a high-flux dialyzer, ultrapure dialysis fluid and extensive convective fluid exchange is currently considered as the new standard for highly efficient renal replacement therapy (RRT), achieving the best extraction of small and middle-sized molecules.

Theranova (polyarylethersulfone/polyvinylpyrrolidone, Gambro Dialysatoren GmbH, Hechingen, Germany) is a novel-generation MCO dialyzer designed to remove molecules over 25 kDa. Recent clinical data on the use of MCO dialyzer in HD patients have shown efficient removal of $ß_2$-microglobulin (11.8 kDa) , myoglobin (17 kDa), k free light chains (FLC) (22.5 kDa)1, λFLCs (45 kDa)[1] complement factor D (24 kDa)[1], and α1-microglobulin (33kDa)[1].

OBJECTIVE

The aim of the study was to compare high-flux ol-HDF with the **Theranova** MCO dialyzer with respect to removal of small (<500 Da) and medium-sized molecules (>500 Da) and nutritional parameters.

METHODOLOGY

The study was a retrospective analysis of ten stable patients on post-dilution ol-HDF using high-flux dialyzer for at least 6 months. These patients were then switched to HD with the **Theranova**-500™ MCO dialyzer (Gambro Dialysatoren GmbH, Hechingen, Germany) for a 6-month period.

Before switching to MCO-HD, all patients were on ol-HDF using a Polyflux-210H (Gambro Dialysatoren GmbH, Hechingen, Germany) or an Elisio-21H (Nipro Europe, Zaventem, Belgium) dialyzer.

All patients had negligible residual renal function. Pre-and postdialysis serum levels of small molecules (urea (60.055 Da[2]), creatinine (113.12 Da[3])) and middle-sized molecules (ß2-microglobulin (11.8 kDa) and myoglobin (17 kDa)) measured during the first mid-week session on 2-month intervals, were compared in each patient during treatments with ol-HDF and MCO-HD. A total of 28 sessions for each treatment period were available for analysis.

Limitations

Limitations of the study included its retrospective design without randomization, small number of analyzed patients, calculation methods without dialysate concentration measures, and the use of slow-flow methods in patients with catheters.

RESULTS

Safety

There was no statistical significance for mean number of interdialytic hypotensive episodes requiring fluid volume expansion between high-flux ol-HDF and MCO-HD period. There were no clinically relevant adverse events reported with use of MCO-HD.

Renal Replacement Therapy Characteristics

There was no significant change between the high-flux ol-HDF and the MCO-HD period regarding blood flow rate, ultrafiltration rate, session length, ionic dialysance, or KT/V monitor. See Table 1.

	High-flux ol-HDF	MCO-HD	p
Blood flow rate (mL/min)*	346 ± 27	338 ± 23	.097
Dialysate flow rate (mL/min)	600	500	NA
Ultrafiltration (L)*	1.79 ± 0.66	1.66 ± 0.71	0.58
Session length (min)*	231 ± 6	233 ± 7	0.52
Ionic dialysance* (mL/min)	217 ± 26	218 ± 30	0.52
KT/V monitor*	1.41 ± 0.2	1.40 ± 0.2	0.165
Convection volume (L)*	2.44 ± 0.2.38	NA	NA

Table 1. RRT characteristics. *Data are expressed as mean ± standard deviation (SD). Abbreviations: RRT: renal replacement therapy; ol-HDF, on-line hemodiafiltration; MCO-HD, medium-cut off hemodialysis; NA, not applicable. Adapted from Belmouaz et al.

Biological, Nutritional and Inflammatory Parameters

Median serum albumin (65 kDa)[1], serum prealbumin, normalized protein catabolic rate (nPCR), c-reactive protein (CRP) levels did not change significantly between the high-flux ol-HDF and MCO-HD period. Median serum ß2-microglobulin and myoglobin levels before and after dialysis also did not change significantly between the high-flux ol-HDF and the MCO-HD period. See Table 2.

	High-flux ol-HDF	MCO-HD	p
Albumin (g/L)*	37.8 (5)	38 (6.4)	.029
Prealbumin (mg/L)*	0.28 (0.08)	0.26 (0.14)	.025
nPCR*	0.9 (0.3)	1 (0.4)	0.95
CRP (mg/L)*	8 (9.0)	7 (6.5)	0.35
$ß_2$-microglobulin			
Before*	27.5 (4)	28 (3.0)	0.63
After*	5.6 (1.6)	6.2 (0.9)	0.56
Myoglobin (µg/L)			
Before*	164 (81)	184 (151)	0.67
After*	79 (51)	76 (64)	0.72

Table 2. Biological, nutritional, and inflammatory parameters. *Data are expressed as median interquartile rate (IQR). Abbreviations: nPCR, normalized protein catabolic rate; CRP, c-reactive protein; ol-HDF, on-line hemodiafiltration; MCO-HD, medium cut-off hemodialysis. Adapted from Belmouaz et al.

Small and Middle-Sized Molecule Removal

Similar urea and creatinine reduction ratios (RRs) of 79% and 71% respectively, were found using both techniques. Other parameters of removal of these small molecules (KT/V, eKT/V, and ionic dialysance) were similar during the high-flux ol-HDF and MCO-HD periods (see Table 3) despite a difference in dialysis flow rate (600 mL/min for high-flux ol-HDF vs 500 mL/min. for MCO-HD. See Table 1.

There was no significant difference between the high-flux ol-HDF and MCO-HD for mean ß2-microglobulin and myoglobin RR, as well as mean ß2-microglobulin and myoglobin Kd. See Table 3. The similar efficacy of MCO-HD for ß2-microglobulin and myoglobin RR are probably related to diffusive transfer, supplemented by uncontrolled convection arising from the process of internal filtration and backfiltration.

	High-flux ol-HDF	MCO-HD	p
eKT*N**	1.49 ± 0.19	1.52 ± 0.19	0.06
Overall reduction ratio (%)			
Urea*	79 ± 4	79 ± 3	0.09
Creatinine*	71 ± 5	71 ± 3	0.26
$ß_2$-microglobulin	81 ± 5	81 ± 6	0.72
Myoglobin*	60 ± 9	61 ± 7	0.59
Overall clearances (mL/min)			
$ß_2$-microglobulin	91 ± 11	84 ± 10	0.24
Myoglobin*	51 ± 10	50 ± 10	0.92

Table 3. Small and middle-sized molecule removal. *Data are expressed as mean ± standard deviation (SD). Abbreviations: ol-HDF, on-line hemodiafiltration; MCO-HD, medium-cut off hemodialysis. Adapted from Belmouaz et al.

CONCLUSION

This study is the first evaluating efficacy and safety of MCO-HD with the new-generation **Theranova**-500 dialyzer in HD over a 6-month period, showing similar removal for small molecules, ß-2 microglobulin and myoglobin when compared to ol-HDF, with good tolerance profile and without modification of nutritional status. In addition, median serum albumin levels did not change significantly between the high-flux ol-HDF and MCO-HD period.

Although the study has several limitations, MCO-HD may provide a useful alternative to high-flux ol-HDF for middle-sized molecule removal. MCO-HD has been suggested to more efficiently remove k and λFLCs than high-flux HD and HDF, which have little impact on the removal of molecules beyond 30 kDa. However, the efficacy of this strategy compared to online high efficiency ol-HDF remains to be assessed by clinical trial.

The use of MCO (Theranova) dialyzer produced similar removal of urea, creatinine, ß2-microglobulin and myoglobin as ol-HDF with good tolerance profile and without modification of nutritional status.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

2. https://biocyc.org/compound?orgid=META&id=UREA Accessed 6/15/20.

3. https://biocyc.org/compound?orgid=META&id=CREATININE Accessed 6/15/20.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Effects of a Medium Cut-Off (Theranova®) Dialyser on Haemodialysis Patients: A Prospective Cross-Over Study

Cozzolino M et al. Effects of a medium cut-off Theranova®) dialyzer on haemodialysis patients: a prospective, cross-over study. *Clinical Kidney Journal* 2019; 1-8. doi: 10.1093/ckj/sfz155.

BACKGROUND

Current hemodialysis (HD) techniques have important limitations in adequately removing some of the uremic solutes such as middle molecules and protein-bound uremic toxins. Middle molecules are organic compounds characterized by a molecular weight > 500 Da which can accumulate in end stage renal disease (ESRD) and exert many toxic effects. The retention of middle molecules is associated with the development of cardiovascular disease, chronic inflammatory disease, chronic kidney disease-mineral and bone disorder, and other conditions. Better clearance of these toxins would lead to improved long-term outcomes in patients with ESRD.

Online hemodiafiltration provides a good clearance of middle molecules, but the use of this technique is limited by the need for high blood flows and accurate monitoring of devices. Also, high-cut off membranes can be used to remove efficiently middle molecules from the bloodstream, but this treatment is also associated with protein loss, and its chronic use leads to hypoalbuminemia.

The new medium cut-off (MCO) membranes provide diffusive and to some extent convective removal of solutes of molecular weight up to 45 kDa, with only marginal albumin leak. MCO membranes have a tight pore size distribution resulting in a steep sieving curve, with the values of molecular weight retention onset and molecular weight cut-off close to but lower than albumin (65 kDa[1]). Due to these novel membrane characteristics, the treatment with MCO membranes 'expands' the spectrum of uremic toxins that can be removed by HD, therefore called 'expanded HD' (HDx).

OBJECTIVE

The aim of this study was to compare HDx using the new MCO membrane **Theranova** 400 (Baxter, USA) and bicarbonate dialysis in prevalent HD patients based on hematochemical values, inflammatory markers, parameters of dialysis adequacy, incidence of adverse events, incidence of infections, number and causes of hospitalization.

METHODOLOGY

Twenty (20) prevalent HD patients participated in this prospective, open-label, controlled, cross-over pilot study. The study was undertaken from October 1, 2017 to December 31, 2018. Consecutively, unselected male and female patients with end stage renal disease (ESRD) on HD were eligible for participation in the study. Participation in the study was voluntary. Patients presenting with cachexia or cancer were excluded.

Patients were discretionally divided into two groups (A and B), with similar mean-age, male-female ratio, and dialytic vintage. In the cross-over design, patients in Group A were treated with **Theranova** dialyzer (HDx) for the first 3 months of the study, and then switched to conventional bicarbonate dialysis (HD) for the remaining 3 months. Patients in Group B were treated with HD dialysis for the first three months of the study, and then switched to HDx for the next three months. There was no wash-out period prior to switching treatment. Sera samples from both groups were collected at 1, 2 and 3 months. See Figure 1.

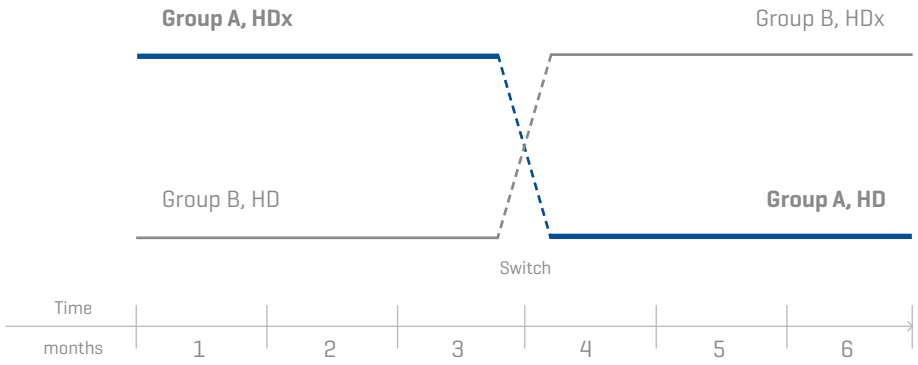


Figure 1. Study design. Abbreviations: HDx: Expanded hemodialysis: HD, hemodialysis. Adapted from Cozzolino et al.

There were four dropouts from the study: one patient was relocated to another dialysis center; one patient was allergic to polysulphone; one patient died; and one patient discontinued treatment with HDx. With incidence and timing of dropouts and enrollment of one replacement patient, biochemical parameters were available at the beginning and end of each study period in 10 patients in Group A. For Group B, biochemical parameters were available for 10 patients for the entire first study period and beginning of the second study period, and for 8 patients during the second study period.

All treatments were provided by Fresenius 5008 (Fresenius 5008, Fresenius Medical Care, Bad Homburg, Germany). A novel membrane, **Theranova** 400 (Baxter USA) was used for patients undergoing HDx, while various other membranes (FX8, FX10, FX80, FX100, BK1.6, BG2.1) were used in patients undergoing HD dialysis based on clinical needs.

RESULTS

Dialytic Parameters

No significant differences between the two groups were observed during the study, except for higher Kt/V values in Group A which achieved statistical significance only within the third month of the first study period.

Blood Pressure

A non-significant trend toward higher systolic blood pressure (SBP) was detected in Group B prior to treatment, without further variations throughout the study by both HD and HDx treatments.

Hematological Parameters

Hematological parameters did not significantly change during the study and did not differ between the study groups.

Serum Albumin Levels

Serum albumin levels were maintained in patients in Group A during both treatments, while patients in Group B showed a decrease in albumin concentration following treatment with HDx compared to HD. A median interquartile range (IQR) reduction in circulating albumin of -0.45 g/dl (-0.575 to -0.05) was observed within Group B during the HDx period compared with an increase of 0.34 g/dL (0.125-0.40) under HD (p=0.025) after exclusion of two patients without available albumin levels at the end of the study. See Figure 2. However, median albumin levels were ≥ 3.5 g d/L and no patients had clinical symptoms of hypoalbuminemia or needed intravenous albumin administration.

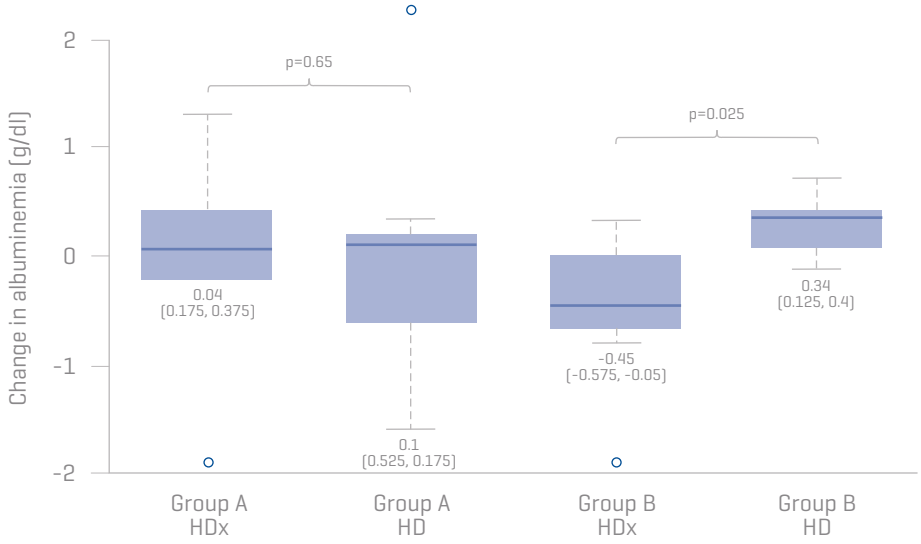


Figure 2. Change in albuminemia in Groups A and B during treatment with HDx and HD. Values are presented as median (IQR). Abbreviations: HDx, expanded hemodialysis; HD, hemodialysis; IQR, interquartile range. Adapted from Cozzolino et al.

Inflammatory Cytokines

Levels of two inflammatory cytokines, middle molecules interleukin (IL)-1ß (17.5 kDa[1]) and IL-6 (21-28 kDa[1]) were measured. While not statistically significant, levels of sera IL-1ß were higher under Group A under HD compared with HDx, and IL-1ß levels were reduced under HDx versus HD in Group B. IL-6 levels slightly increased following HD compared with patients under HDx in Group A and reduced in HDx patients versus those under HD in Group B, but this difference did not achieve statistical significance.

Lower concentrations of inflammatory cytokines induced by treatment with MCO membranes may lead to beneficial effect in patients with ESRD. IL-1ß seems to be associated with left ventricular hypertrophy in dialysis patients and with the progression of atherosclerosis in patients with ischemic heart disease, in whom serum concentrations of IL-1ß correlate with plaque severity. Elevated levels of IL-6 are also associated with cardiovascular mortality and left ventricular hypertrophy in HD patients.

Hypotension

Although there was a slightly higher incidence of symptomatic hypotensive events in the HDx group (8 events vs 5 events), the total number of absent/low hypotensive events was 11 in the HD group versus 9 in the HDx group, and both groups had the same number of moderate or high hypotensive events (n=9). A larger sample size will be needed to verify whether hypotension rate could be different among dialysis methods.

Infections

The frequency of infection per patient was categorized into two classes: none and one or more infections per patient. The total number of infections was lower during treatment with HDx than with HD (n=7/19 vs n=14/20; *p*=0.03). See Figure 3.

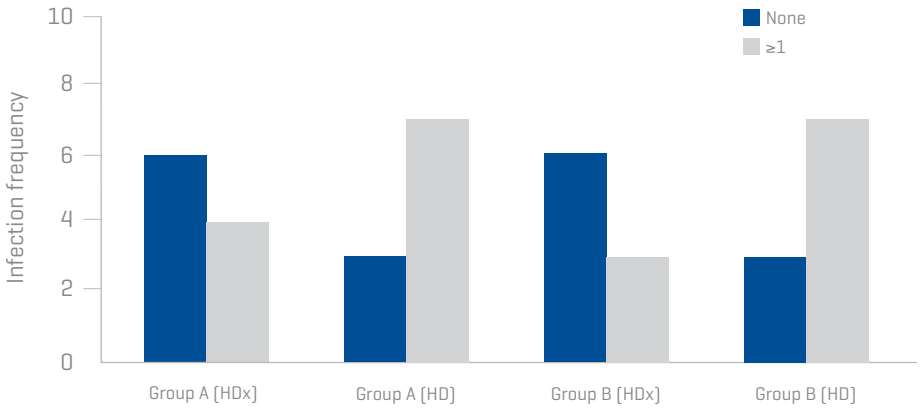


Figure 3. Frequency of infections in Groups A and B following HD and HDx treatments. Abbreviations: HDx, expanded hemodialysis; HD, hemodialysis. Adapted from Cozzolino et al.

Although difficult to interpret because of small sample size and potential bias, the difference in incidence in of infections during the two treatments is very encouraging since infectious diseases are the most common cause of hospitalization and second most common cause of death among HD patients. These patients are more prone to infections then the general population, mainly because of the high prevalence of an indwelling catheter and a condition of acquired immune dysfunction due to the retention of uremic toxins and chronic inflammation.

Hospitalization

The frequency of hospitalizations was categorized into two classes: none or one or more episode per patient. Data revealed a non-significant trend toward higher risk of hospitalization with HD; the total number of hospitalizations was higher during treatment with HD than HDx (n=11/19 versus n=8/19; *p*=0.53).

LIMITATIONS

Limitations of this study included small sample size, high number of dropouts, absence of wash-out period prior to switching treatment, and suggested under-treatment for selected conditions based on dialysis adequacy.

CONCLUSION

This study demonstrates that the chronic use of the novel MCO dialyzer **Theranova** appears to be safe and well-tolerated, without serious side effects or hypoalbuminemia. Importantly, the total number of infections was lower with HDx than with HD (p=0.03). Furthermore, it validated the ability of these new membranes to reduce the serum concentration of soluble inflammatory mediators. These results encourage further trials with longer treatment periods and larger sample sizes.

HDx therapy showed a statistically significant decrease in the rate of infections. Additionally, this study validated MCO (Theranova) membranes' ability to reduce serum concentration of soluble inflammatory mediators, including cytokines.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

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In Vitro Cytokine Removal: Comparison of Conventional High-Flux Dialyzers and Middle-Cut-Off Dialyzer (Theranova HDx)

Koball S et al. *In Vitro* Cytokine Removal: Comparison of conventional high-flux dialyzers and middle-cut-off dialyzer (Theranova HDx). ASN 2019. **Abstract FR-P0465**.

BACKGROUND

The removal of inflammatory mediators is important for the treatment of acute (ARF) or chronic renal failure (CRF). In ARF and sepsis, attempts is made to achieve removal through the use of high-volume treatments or adsorbers. In CRF, this has so far not been sufficiently addressed, but is important for mortality (MIA syndrome). By using new MCO filters (Baxter **Theranova** (HDx), an improvement of cytokine status seems to be possible in both areas. The effectiveness of HDx in the removal of interleukins (interleukin 6, interleukin 10 and TNFalpha in hemodialysis treatments will be assessed.

METHODS

The efficacy of HDx was compared to a conventional high-flux dialyzer (Fresenius FX80). The measurements were performed in vitro in a 3l pool of fresh frozen plasma (citrate and heparin anticoagulation). IL6 (24.5 kDa) IL10 (18.6 kDa, dimer)) and TNFalpha (17.4 kDa, trimer) were added to plasma (1.5 µg/l each). Samples were taken before and after the dialyzer (after 5, 15, 30, 60, 120 and 180 minutes). In addition to cytokines, albumin and total serum protein concentrations were measured (LEGEND MAX Human IL-6/IL-10/TNF-α; Cobas Mira Plus; Roche LT-AB0103, LT-TP0253). Every test was repeated 5 times.

RESULTS

Theranova HDx showed significantly higher removal rates of all tested cytokines over a period of 180 minutes. A comparison of the concentrations at the beginning and end of the measurements showed:

- IL-6 reduction - HDx about 80% / FX80 about 40%.
- IL-10 reduction - HDx about 50% and FX80 about 10%.
- TNF-αReduction - HDx about 25%; FX80 no reduction.

The concentration of albumin and total serum protein was not significant different during the treatment in both groups.

CONCLUSION

Hemodialysis therapy with **Theranova** HDx appears to be a superior therapy option for the removal of cytokines. This opens up new treatment options for both acute and chronic dialysis patients. However, clinical studies are still necessary to assess the significance in patient treatment.

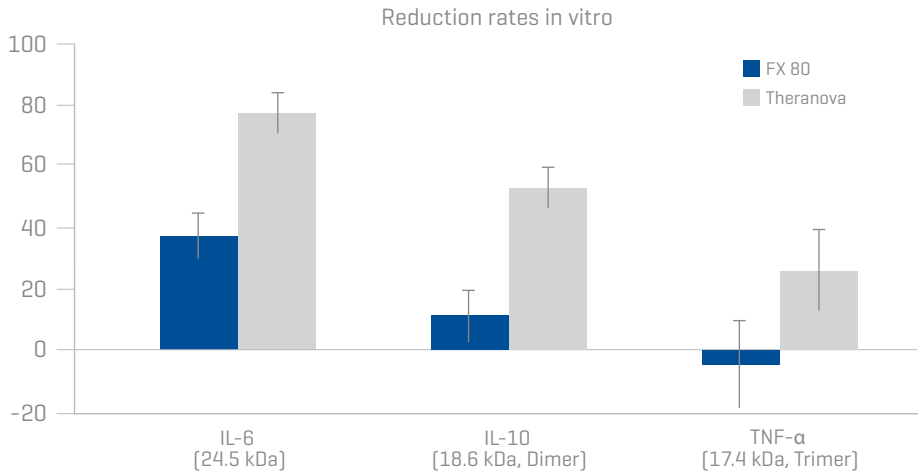

Figure adapted from Koball et al.

Terms highlighted in Blue: refer to Glossary of Terms for explanation.

Efficacy and Safety of Expanded Hemodialysis with the Theranova 400 Dialyzer: A Randomized Controlled Trial

Weiner DE et al. Efficacy and safety of expanded hemodialysis with the Theranova 400 dialyzer: A randomized controlled trial. *CJASN* ePress 2020. doi:10.2215/CJN.01210120.

BACKGROUND

The loss of kidney function in patients with kidney failure causes accumulation of solutes termed uremic toxins due to their negative impact on patient health. These toxins can be grouped into small molecular weight water-soluble molecules, middle molecules, and protein-bound solutes. While the smaller molecules with a molecular mass <0.5 kilodaltons (kDa) are effectively removed by dialysis, conventional dialysis has more difficulty in clearing middle molecules ranging from 0.5 to 60 kDa. Middle molecules can be further subdivided into two groups based on their molecular weight: conventional middle molecules of 0.5-25 kDa and larger middle molecules of >25 kDa. The former group includes ß2-microglobulin (11.8 kDa), historically considered the standard representative of a middle molecule, while the latter includes free immunoglobulin light chains including λ free light chains (FLCs) (45 kDa). Larger middle molecules are associated with inflammation, cardiovascular events, and other dialysis-related comorbidities in patients with comorbid cardiovascular disease, mineral and bone disorders, and infectious diseases.

Hemodialysis (HD) removes solutes, including small molecules (<0.5 kDa) and conventional middle molecules (0.5-25 kDa), primarily by diffusion, with very limited convection. Highly porous membranes, such as those featured in high-flux dialyzers, allow some middle molecules like ß2-microglobulin to pass through the membrane, but these membranes do not readily clear larger solutes. Larger middle molecules (>25 kDa) need to be removed either by convection or by highly permeable membranes.

The term expanded HD (HDx) has been proposed to define a treatment where diffusion and convection are technically integrated inside a hollow-fiber dialyzer equipped with a medium cut-off membrane, enabling removal of small, conventional middle molecules and large middle molecular uremic toxins. **Theranova** provides expanded hemodialysis using a hollow-fiber single-use dialyzer, with improved removal of large proteins >25 kDa while selectively maintaining essential proteins such as albumin.

Existing data on the performance of medium cut-off dialyzers are based on short-term, non-randomized clinical trials. In contrast, this study was a randomized, longer-term (6 months) study.

OBJECTIVE

To evaluate the efficacy of HDx with the **Theranova** 400 membrane for larger middle molecule removal with acceptable serum albumin loss and safety profile over a 6-month period.

METHODOLGY

The multicenter open-label, randomized controlled trial was conducted in 21 centers in the US between September 2017 and October 2018.

Participants

Patients receiving 3X/week in-center maintenance hemodialysis, ages 22 years and older, who met the following criteria were included in the study:

- Clinically stable without acute medical events in the past 30 days
- Receiving HD with a high-flux dialyzer for at least 3 months prior
- Expected to maintain an acceptable urea clearance (Kt/V) with a dialyzer of an approximate surface area of 1.7 m^2
- Stable functioning vascular access

Key exclusion criteria included: history of acute infection ≤ 4 weeks prior to randomization and patients with chronic liver disease, paraprotein-associated disease, hepatitis, HIV, bleeding disorders, active cancer, monoclonal or polyclonal gammopathy. Patients with known serum κ/λ FLC ratio less than 0.37 or greater than 3.1 suggestive of monoclonal plasma diseases were also excluded.

Of 282 patients meeting the inclusion criteria, 172 participants were randomized with 86 in each group.

Methods

The study was an open-label study without concealment of the dialyzer used; the allocation was concealed to the central laboratory and study sponsor. Patients were randomized to receive treatment with either **Theranova** 400 (Baxter Healthcare International) or Elisio-17H (Nipro Corporation). Randomization to **Theranova** 400 or Elisio-17H, a similar surface area high-flux dialyzer (1.7 m^2), was stratified by site with dynamic allocation. Dialysis prescription and management were performed per institutional practice. Monthly microbiological water/dialysate quality testing according to current Centers for Medicare and Medicaid Services regulations for dialysis water and conventional dialysate were required. Hemodialysis treatment duration per session for each individual varied based on clinical requirements determined by the clinician, based on the participants' needs. Medications were administered according to each center's standard practice.

Outcomes

Primary Safety and Efficacy Outcomes

The primary safety endpoint was the level of pre-dialysis serum albumin (65 kDa)[1] measured after 24 weeks of treatment, and the primary efficacy endpoint was the removal of λ FLCs (45 kDa) measured at 24 weeks of treatment expressed as a reduction ratio (RR).

Secondary Safety and Efficacy Outcomes

Secondary safety endpoints were change in serum albumin from baseline at weeks 4 and 8. Secondary efficacy endpoints included the RRs of λ FLCs at 4 weeks and other middle to large molecules: complement factor D (24 kDa); κ FLC (23 kDa); interleukin 6 (IL-6) (25 kDa); tumor necrosis factor alpha (TNFα) (17 kDa); and β2-microglobulin (11.8 kDa) at 4 weeks and at 24 weeks of treatment. Single pool Kt/V was also assessed.

Adverse Outcomes

Adverse events were monitored through study completion.

Exploratory Outcomes

Exploratory endpoints consisted of patient reported quality of life using the Kidney Disease Quality of Life (KDQoL-36) instrument and the EuroQol (EQ-5D-5L) instrument as well as inflammation assessment by highly sensitive C-reactive protein (hsCRP).

RESULTS

Patient Population

Twenty-one centers participated in this clinical study. Of 282 patients meeting the inclusion criteria, 172 participants were randomized, with 86 in each group. A total of 130 participants completed the study; 65 in the **Theranova** 400 group; 65 in the Elisio-17H group. Sensitivity analyses via multiple imputation and last observation carried forward for participants who did not complete the study demonstrated similar results to participants who completed the study.

Safety Outcomes

Primary Safety Outcome

At baseline, the mean pre-dialysis level of serum albumin in the **Theranova** 400 group (4.0 ± 0.3 g/dL) was comparable to the Elisio-17H group (4.0 ± 0.3 g/dL). Likewise, after 24 weeks of treatment, the mean pre-dialysis serum albumin level was 4.0 ± 0.3 g/dL in the **Theranova** 400 group and 4.1 ± 0.4 g/dL in the Elisio-17H group, demonstrating non-inferiority of **Theranova** 400* in maintaining serum albumin levels. See Table 1.

Parameter	Dialyzer	n	Mean (SD)	Median	Min, Max	Two-Sided 95% Confidence Interval*
Pre-dialysis serum albumin after 24 weeks (g/dL)	Theranova 400	64	4.0 (0.3)	4.0	3.5, 4.7	-0.12 to 0.05
	Control	65	4.1 (0.4)	4.0	3.2, 4.9	

Table 1. Primary Safety Outcome: Pre-dialysis Serum Albumin Assessment after 24 Weeks. Abbreviations: SD: standard deviation. Table adapted from Weiner et al.* If the lower bound of the two-sided 95% confidence interval around the mean estimated treatment difference between Theranova 400 and the control is > -0.1765 then non-inferiority can be claimed. If the lower bound of the two-sided 95% confidence interval is > 0, then superiority may be concluded.

Secondary Safety Outcomes

The change in serum albumin from baseline was significantly different between the two groups only after weeks 4 and 8. After week 4, the mean level was 4.0 ± 0.3 g/dL with a -0.1 ± 0.2 mean change from baseline in the **Theranova** 400 group, whereas in the Elisio-17H group, the mean level was 4.0 ± 0.3 with a 0.0 ± 0.2 mean change from baseline (p=0.03). After week 8, the mean level was 3.9 ± 0.3 g/dL with a -0.1 ± 0.3 mean change from baseline in the **Theranova** 400 group, whereas in the Elisio-17H group, the mean level was 4.0 ± 0.3 g/dL with a 0.0 ± 0.2 mean change from baseline (p=0.004). Although the differences in change from baseline between the two groups after weeks 4 and 8 were statistically significant, the observed changes were well below 5%, and the mean levels were still within normal lab ranges. See Table 2.

Efficacy Outcomes

Primary Efficacy Outcome

Theranova 400 showed significantly larger removal of λ FLCs at 24 weeks of treatment than the Elisio-17H dialyzer (mean RR of 33% ± 11.0% vs 17% ±13% at 24 weeks [p<0.001]. Significantly larger removal of λ FLC was also observed with **Theranova** at 4 weeks of treatment than with the Elisio-17H dialyzer (mean RR of 39% ± 14% vs 20% ± 11% [p<0.001]). See Table 3, Figure 1.

Secondary Efficacy Outcomes

Theranova 400 demonstrated superior removal of middle to large molecules as demonstrated by reduction ratios measured at 4 and 24 weeks: complement factor D, κ FLCs, TNFα, and ß2-microglobulin (p<0.001 for all).The level of IL-6 at the end of the study was lower than at the start of treatment for the **Theranova** 400 group. The RR of IL-6 was negative for the control at both 4 (5% ± 46% vs -9% ± 61%; p=0.09) and 24 weeks of treatment (11% ± 38% vs -3% ± 39%; p=0.05); these differences were not statistically significant. See Table 3, Figure 1.

Parameter	Timepoint	Theranova 400					Control					
		(n)	Mean (SD)	Median	Min, Max	95% Confidence Interval	(n)	Mean (SD)	Median	Min, Max	95% Confidence Interval	p-value
Pre-dialysis serum albumin (g/dL)	Baseline	86	4.0 (0.3)	4.0	3.4, 4.9	NA	86	4.0(0.3)	4.0	3.3, 4.7	NA	NA
Change in pre-dialysis serum albumin from baseline (g/dL)	4 weeks	80	-0.1 (0.2)	-0.1	-0.8, 0.6	-0.14 to -0.03	77	0.0 (0.2)	0.0	-0.7, 0.5	-0.04 to 0.05	0.03
	8 weeks	78	-0.1 (0.3)	-0.1	-0.8, 0.5	-0.17 to -0.05	77	0.0 (0.2)	0.0	-0.6, 0.5	-0.05 to 0.05	0.004
	12 weeks	77	-0.1 (0.3)	-0.1	-1.2, 0.6	-0.19 to -0.06	72	-0.0 (0.2)	0.0	-0.8, 0.5	-0.10 to 0.01	0.13
	16 weeks	72	-0.1 (0.3)	-0.1	-1.3, 0.7	-0.21 to -0.05	71	-0.0 (0.3)	0.0	-1.6, 0.5	-0.10 to 0.05	0.11
	20 weeks	66	-0.1 (0.3)	-0.1	-0.7, 0.5	-0.15 to -0.02	69	0.0 (0.3)	0.0	-0.9, 0.5	-0.05, to 0.08	0.07
	24 weeks	64	0.0 (0.3)	0.0	-0.6, 0.4	-0.06 to 0.07	65	0.0 (0.3)	0.1	-0.6, 0.8	-0.02 to 0.11	0.61

Table 2. Secondary Safety Outcomes: Baseline and Change from Baseline of Pre-dialysis Serum Albumin. Abbreviations: SD: standard deviation. Table adapted from Weiner et al.

Parameter	Dialyzer	Week 4 (n)	Mean (SD)	Median	Min, Max	p-value	Week 24(n)	Mean (SD)	Median	Min, Max	p value
Primary Efficacy Outcome											
RR of λ FLCs* (45 kDa)	Theranova 400	80	39.3 (14.5)	42.4	-46.2, 71.1	-<0.001	63	33.3 (11.0]	32.8	8.1, 54.1	<0.001
	Control	75	19.9 (11.4)	18.9	-4.5, 41.6		65	17.2 (12.9)	15.9	-10.7, 74.2	
Secondary Efficacy Outcomes											
RR of complement factor D (24 kDa)	Theranova 400	83	43.0 (23.9)	48.0	-58.1, 78.5	<0.001	62	45.0 (10.4)	46.0	11.0, 68.0	<0.001
	Control	76	20.9 (23.7)	22.5	-110.9, 77.8		65	23.6 (12.1)	23.9	-47.0, 45.5	
RR of κ FLC (23 kDa)	Theranova 400	80	68.8 (17.3)	72.1	-57.9, 94.6	<0.001	63	63.8 (11.8)	65.8	27.8, 87.4	<0.001
	Control	75	54.8 (14.5)	56.0	-29.1, 77.6		65	50.0 (13.2)	49.4	2.3, 74.1	
RR of IL-6 (25 kDa)	Theranova 400	80	5.5 (45.9)	19.6	-155.4, 66.1	0.09	63	11.0 (37.8)	20.8	-128.5, 66.2	0.05
	Control	78	-9.2 (60.6)	3.9	-341.2, 55.6		65	-2.6 (39.4)	7.8	-162.2, 46.2	
RRof TNFα (17 kDa)	Theranova 400	80	52.5 (9.4)	54.3	16.3, 72.4	<0.001	63	50.7 (9.3)	52.2	23.8, 68.5	<0.001
	Control	78	44.1 (9.3)	45.3	11.0, 58.1		65	41.5 (10.2)	41.9	-0.9, 57.9	
RR of ß2-microglobulin (11.8 kDa)	Theranova 400	78	75.7 (8.2)	77.2	46.6, 98.9	<0.001	63	73.6 (10.4)	75.9	30.3, 96.7	<0.001
	Control	76	64.9 (8.9)	65.6	24.1, 83.2		65	65.4 (9.4)	65.9	36.8, 90.0	

Table 3. Primary and Secondary Efficacy Outcomes: Reduction Ratios (RR) (%) of Middle Molecules at 4 Weeks and 24 Weeks.

*The reduction ratio of λ free light chains at 4 weeks of treatment was a secondary efficacy outcome. Abbreviations. RR: reduction ratio; FLC: free light chain. Table adapted from Weiner et al.

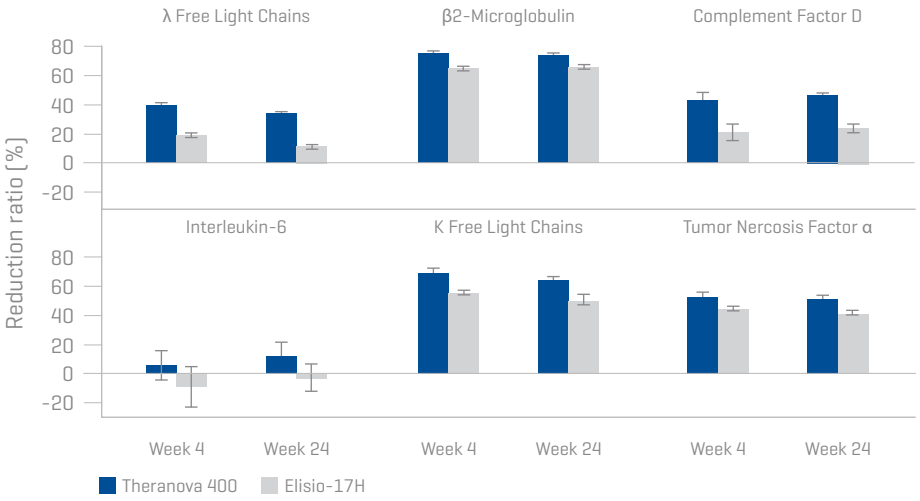

Figure 1. Reduction ratios of middle molecules at 4 weeks and 24 weeks. Adapted from Weiner et al.

Single pool Kt/V was assessed after every 4 weeks of treatment. There were no significant differences in the mean single pool (sp) Kt/V values at all measured time points, except for week 8, where the spKt/V for **Theranova** 400 was 1.62 ± 0.29 and the value for the Elisio-17H was 1.51 ± 0.32 (p=0.02). The value of spKt/V was within adequacy standards at all measured time points.

Adverse Outcomes

No significant differences were observed between the **Theranova** 400 and the Elisio-17H groups in incidence (p=0.87) and incidence rate (p=0.32) of adverse events (AEs). There were 19 serious adverse events (SAEs) in 15 participants in the **Theranova** 400 group, and 39 SAEs in 23 participants in the Elisio-17H group. This difference was not statistically significant. There were no SAEs associated with either device. None of the AEs were unanticipated; all were AEs typically seen in maintenance HD patients. Six patients died during

the study, 3 in each group, with 1 death in the Elisio-17H group occurring after participant withdrawal from the study. None of the deaths were assessed as related to either device.

Exploratory Outcomes
There were no significant differences in the mean hs-CRP at all trial time points. Additionally, no significant differences were observed in the KDQOL-36 survey and EQ-5D-5L questionnaire results between the two groups.

Strengths and Limitations
Study limitations included conservative exclusion criteria and associated high screening failure rates, study completion rate, insufficient sample size/duration to comment on clinical outcomes such as cardiovascular events and mortality.

The trial had multiple strengths, including the randomized design and longer length of study (6 months) vs previous studies, high rates of adherence with minimal loss to follow-up and consistent results across solutes analyzed.

DISCUSSION AND CONCLUSIONS

Multiple middle molecules are present at higher levels in dialysis patients and have been associated with adverse outcomes. Specifically, larger middle molecules are associated with inflammation, cardiovascular events, and other dialysis-related comorbidities in patients with comorbid cardiovascular disease, mineral and bone disorders, and infectious diseases. In this study, **Theranova** 400 showed significantly greater (p <0.001) removal of conventional and larger middle molecules λFLC (45 kDa), complement factor D (24kDa), κ FLC (23 kDa), TNF-α (17 kDa), and β-2 microglobulin (11.8 kDa) than Elisio 17-H at 4 weeks and 24 weeks. In parallel, no sustained reduction was seen in serum albumin levels (65 kDa), an important finding with the association between higher serum albumin concentration and better outcomes in hemodialysis patients. Given the potential role of these uremic toxins in cardiovascular disease and inflammation in kidney failure patients, newer technologies enhancing clearance of middle molecules while limiting loss of important proteins such as albumin may have an important role in improving health of dialysis patients.

Study results suggest that there may be a role for HDx to improve clinical outcomes. In this trial, λ FLCs (45 kDa) were studied as a representative large middle molecule that is easily measured rather than as a presumptive 'uremic toxin', with the current study critically focusing on both clearance of these molecules as well as pre-dialysis levels of large middle molecules. The latter is notable; if there is toxicity associated with retained uremic solutes, therapeutic management will require sustained reduction in the levels of these solutes.

In conclusion, primary and secondary endpoints for safety and efficacy were met. HDx with the **Theranova** 400 dialyzer is safe and efficacious, providing superior removal of larger middle molecules including several putative uremic toxins as compared to a similar size high-flux dialyzer while maintaining serum albumin. While this study demonstrated greater removal of large middle molecules among prevalent hemodialysis patients, larger studies of longer duration are needed to assess long term potential beneficial effects related to more effective removal of these middle molecules, including improvements in cardiovascular disease, inflammation, mortality, and key patient-reported outcomes.

Theranova provides significant and superior removal of conventional/large middle molecules while retaining stable albumin levels.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Removal of Large-Middle Molecules, Inhibition of Neutrophil Activation and Modulation of Inflammation-Related Endothelial Dysfunction During Expanded Hemodialysis (HDx)

Cantaluppi V et al. Removal of large-middle molecules, inhibition of neutrophil activation and modulation of inflammation-telated endothelial dysfunction during expanded hemodialysis (HDx). *Nephrol Dial Transplant* 2019. **Abstract FO048**.

BACKGROUND

HemoDialysis expanded (HDx) represents an innovative strategy to remove uremic toxins of Large-Medium Molecular weight (LMMs, ≤ equal 45 Kda) thanks to the membrane medium cut-off (MCO) and internal convection. LMMs (in particular Free Light Chains-FLCs) are involved in the inhibition of neutrophil apoptosis leading to persistent inflammation, endothelial dysfunction and increased cardiovascular risk of hemodialysis patients.

Aims of the study were to evaluate: 1) the efficacy of HDx on LMM removal (in particular kappa and lambda FLCs) and inflammation inhibition during an observational multicentric study of 6 months; 2) the in vitro effects of plasma drawn from HDx-treated patients (T0 and T6 months) on neutrophil activation and endothelial dysfunction.

METHODS

Forty-one (41) HD patients (age 67,6±13,4) in standard high flux HD were shift to HDx using **Theranova**® 400 (1.7 m^2, Baxter). Each patient was studied at baseline HD (T0), 3 months (T3) and 6 months (T6) after HDx. We evaluated the following predialysis parameters: Urea, Creatinine (Creat), Phosphate (P), Beta2-microglobulin (ß2m), Myoglobin (Myo), Free Light Chains (FLC-k, FLC-λ), Hemoglobin (Hb), Albumin and C Reactive Protein (CRP). For in vitro studies, T0 and T6 plasma were used to evaluate neutrophil activation (ROS generation, apoptosis, adhesion) and endothelial dysfunction/senescence.

RESULTS

HDx therapy was well tolerated without evidence of major adverse side effects. After 3months of HDx (T3), we observed a significant decrease of urea (p=0,008), ß2m (p=0,003), FLC-k (p=0,026) and FLC-λ (p=0,001). No significant differences of the other uremic toxins between the periods (T3 vs. T6) were observed. Albumin levels remained stable during all the study period. A significant decrease of CRP was observed at T3 and T6, suggesting a positive effect of HDx on inflammatory parameters correlated with a worse outcome. *In vitro* studies confirmed that LMM removal by HDx associated with a limitation of neutrophil activation (decrease of ROS, TNF-alpha and IL6 production, increase of apoptosis assessed by TUNEL and caspase- 3 ELISA) and of endothelial dysfunction (significant decrease of neutrophil adhesion, increased NO bioavailability and angiogenesis, inhibition of vascular senescence with increased expression of the anti-oxidant and anti-aging factor Nrf2). Preliminary data revealed a potential role of HDx in the modulation of the microRNA content of circulating extracellular vesicles isolated from patients' plasma.

CONCLUSION

HDx therapy provided high removal of different LMMs, leading to a significant reduction of molecules involved in uremia-associated inflammation and organ dysfunction (in particular FLC kappa and lambda). Long-term studies with a larger sample size are needed to evaluate the clinical impact of HDx. However, our preliminary clinical data and in vitro studies suggested that by improving LMM removal, HDx may limit neutrophil activation and endothelial dysfunction, key factors of the increased cardiovascular risk of hemodialysis patients. A new randomized multicenter clinical trial in Italy is currently ongoing to confirm these data and to evaluate the effect of HDx on microRNA content of circulating extracellular vesicles, microparticles involved in cell-to-cell communication and recently identified as new mechanisms of vascular senescence.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

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Removal of Large-Middle Molecules on Expanded Hemodialysis (HDx): A Multicentric Observational Study of 6 Months Follow-Up

Cantaluppi V et al. Removal of large-middle molecules on expanded hemodialysis (HDx): A multicentric observational study of 6 months follow-up. *J Am Soc Nephrol.* 2018. **Poster TH-PO357**.

BACKGROUND

HemoDialysis expanded (HDx) may potentially represent an innovative way to remove uremic toxins of Large-Medium Molecular weight (LMMs, ≤45 Kda) thanks to the membrane medium cut-off (MCO, **Theranova**®, Baxter). LMMs are involved in the pathogenic mechanisms of organ dysfunction associated with uremia including inflammation, malnutrition and atherosclerosis. The aim of this study was to evaluate the efficacy of LMMs removal in HDx during an observational multicentric study of 6 months follow-up.

METHODS

Forty-one (41) HD stable patients (age 67,6±13,4) were dialyzed in HDx with **Theranova**® 400 (1.7 m^2). Each patient was evaluated at baseline with standard HD (T0), 3 months (T3) and 6 months (T6) after HDx. In the first session of the week (for each period), we evaluated the following pre-dialysis parameters: Urea, Creatinine (Creat), Phosphate (P), Beta2-microglobulin (B2m), Myoglobin (Myo), Free Light Chains (FLC-k, FLC-λ), Hemoglobin (Hb), Albumin and C Reactive Protein (CRP). Data are reported as mean ± standard deviation (SD).

RESULTS

THDx therapy was well tolerated without evidence of major adverse side effects. The main results of the study are summarized in Figure 1. After 3 months of HDx (T3), we observed a significant decrease of pre-dialysis levels of urea (p=0,008), ß2m (p=0,003), FLC-k (p=0,026), FLC-λ (p=0,001). No significant differences of the other uremic toxins between the periods (T3 vs. T6) were observed. Albumin levels remained stable during all the study period. A significant decrease of CRP was observed at T3 and T6, suggesting a positive effect of HDx on inflammatory parameters correlated with a worse outcome.

CONCLUSIONS

HDx therapy provided high removal of different LMMs, leading to a significant reduction of molecules involved in uremia-associated organ dysfunction in the first 3 months of treatment (T0-T3). Long-term studies with a larger sample size are needed to evaluate the clinical impact of HDx. However, our preliminary data suggest that HDx may improve LMMs removal and inflammatory parameters.

	Urea (mg/dl)	Creat (mg/dl)	P (mg/dl)	ß2 m (mg/l)	Myo (ng/mL)	FLC-λ (mg/l)	FLC-κ (mg/l)	CRP (mg/l)	Hb(mg/l)	Albumin (g/dl)
T0	130.8 ± 41.7	9.8 ± 2.5	4.6 ± 1.4	29.2 ± 7.7	206.6 ± 84.8	111.6 ± 106.5	84.3 ± 72.7	5.09 ± 9.16	10.9 ± 1.3	3.5 ± 0.5
T3	117.1 ± 31.9	9.2 ± 2.1	4.6 ± 1.4	26.5 ± 5.8	206.8 ± 80.3	101.9 ± 95.9	78.1 ± 65.2	3.65 ± 4.52	11.4 ± 1.4	3.4 ± 0.5
T6	120.3 ± 34.1	9.6 ± 2.0	4.8 ± 1.2	26.4 ± 7.2	196.4 ± 85.5	106.6 ± 102.7	79.5 ± 67.8	2.69 ± 3.62	11.3 ± 1.2	3.5 ± 0.4

Figure 1: Pre-dialysis levels of different uremic toxins at study start (T0) and after 3 (T3) and 6 (T6) months of HDx. Adapted from Cantaluppi et al.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Randomized Controlled Trial of Medium Cut-Off versus High-Flux Dialyzers on Quality of Life Outcomes in Maintenance Hemodialysis Patients

Lim JH et al. Randomized controlled trial of medium cut-off versus high-flux dialyzers on quality of life outcomes in maintenance hemodialysis patients. *Nature/Sci Rep*. 2020; 10:7780. doi: 10.1038/s41598-020-64622-z.

BACKGROUND

Patients on maintenance hemodialysis suffer from symptoms such as fatigue, generalized weakness, and pruritus. These subjective conditions are assumed to be related to the accumulation of middle molecules that are not cleared by conventional hemodialysis (HD). Middle molecules have molecular weights (MWs) ranging between 500 and 60,000 daltons, and their size is a barrier to removal with dialyzers. The accumulation of middle molecules is associated with specific complications such as amyloidosis, inflammatory reactions, oxidative stress, and endothelial dysfunction. Consequently, middle molecules contribute to morbidity and mortality and poor quality of life (QOL) in in patients with end-stage renal disease (ESRD).

Compared with high-flux dialyzers and hemodiafiltration (HDF), medium cut-off (MCO) dialyzers may improve the removal of middle molecules due to their higher permeability and increased convective transport, but clinical data on the effects of MCO dialyzers on patient-reported outcomes are lacking.

OBJECTIVES

This study aimed to investigate potential QOL improvement using MCO dialyzers in patients undergoing maintenance HD with a high-flux dialyzer. This study also sought to evaluate the effect of MCO dialyzers on the removal of middle molecules and pre-dialysis plasma concentrations.

METHODS

Study Design

This study was a randomized, prospective, controlled, open-label, phase 4 trial in patients treated with maintenance HD at a national university hospital in South Korea. Patients aged 18 years or older, had been receiving maintenance high-flux membrane HD for more than three months, had vascular access by arteriovenous fistula/graft and adequate dialysis were enrolled.

Patients were randomLy assigned into MCO and high-flux groups at 1:1 ratio. Patients and physicians were unblinded to the assignment. The MCO group switched from a high-flux membrane (Fx CorDiax 60 or 80; Fresenius Medical Care Deutschland, Bad Homburg, Germany) to a **Theranova** 400 dialyzer (Baxter International Inc., Hechingen, Germany) and the high-flux group continued with a high-flux membrane.

Data Collection and Analysis

Patients completed the Kidney Disease Quality of Life-Short Form (KDQOL-SF) questionnaire. Uremic pruritus was assessed using the modified scoring questionnaire consisting of severity, distribution, and sleep disturbance categories. Questionnaires about QOL and pruritus were completed at baseline and at 12 weeks. Blood samples to identify middle molecule removal were obtained before and at the end of dialysis.

Study Outcomes

The primary outcomes were the KDQOL-SF and pruritus assessment. For the KDQOL-SF, analysis identified differences between the MCO and high-flux groups, pre- and post-dialysis, in the questionnaire's 26 categories. For pruritus assessment, analysis identified differences in questionnaire responses between the two groups, pre- and post-dialysis, on severity and distribution by time of day (morning, afternoon), sleep disturbance, and scoring of responses to a visual analog scale.

The secondary outcomes were pre-dialysis plasma concentrations and reduction ratios (RRs) of middle molecules at baseline and 12 weeks after randomization. Analysis identified differences between the MCO and high flux groups, pre-and post-dialysis, in levels of three middle molecules: ß2-microglobulin (molecular weight (MW) 11.8kDa[1]), a small middle molecule, and kappa free light chain [κ FLC] (22.5kDa[1]) and lambda free light chain [λ FLC](45kDa[1]), larger middle molecules.

Study Limitations

This study has several limitations. The sample size was small, and the study duration was insufficient to evaluate definite effects of the MCO membrane. The **Theranova** 500 dialyzer, which has a greater surface area (2.0 m^2) than the **Theranova** 400 dialyzer (1.7 m^2), was not applied in the MCO group because the **Theranova** 500 has not yet been introduced in South Korea. The actual extent of solute removal could not be estimated, or the exact pathophysiologic correlations proven between middle molecules and the physical components of QOL and uremic pruritus.

RESULTS

Patient Characteristics

A total of 50 patients were enrolled and one patient withdrew consent, resulting in 49 patients who completed the study. Twenty-four patients were in the MCO group and 25 were in the high-flux group. No significant between-group differences in age, sex, body mass index, dry weight, daily urine volume, vascular access, baseline dialyzer, and dialysis vintage were observed.

	Baseline			12 weeks		
Reduction ratio (%)	MCO (*n* = 24)	High-flux (*n* = 25)	*P*	MCO (*n* = 24)	High-flux (*n* = 25)	*P*
Total score	63.7 ± 13.8	57.0 ± 16.4	0.134	63.9 ± 14.4	59.0 ± 17.3	0.283
Kidney disease targeted items	67.9 ± 11.4	62.9 ± 12.3	0.142	66.2 ± 13.3	66.2 ± 12.9	0.995
Symptoms	81.9 ± 13.8	75.4 ± 14.0	0.107	81.3 ± 14.9	78.3 ± 14.6	0.471
Effects of kidney disease	67.6 ± 14.9	60.7 ± 18.9	0.163	65.1 ± 20.3	67.6 ± 18.9	0.654
Burden of kidney disease	40.9 ± 24.4	31.5 ± 26.1	0.200	39.3 ± 27.2	30.8 ± 23.5	0.244
Work status	14.6 ± 27.5	14.0 ± 30.7	0.945	12.5 ± 26.6	18.0 ± 35.0	0.540
Cognitive function	82.5 ± 19.0	83.7 ± 13.6	0.795	78.1 ± 24.1	84.0 ± 17.6	0.328
Quality of social interaction	67.8 ± 18.3	60.5 ± 15.0	0.136	68.1 ± 22.7	67.5 ± 20.3	0.927
Sexual function	57.5 ± 28.8	40.6 ± 42.5	0.500	45.8 ± 35.9	50.0 ± 70.7	0.911
Sleep	64.1 ± 19.3	60.9 ± 17.7	0.553	62.6 ± 15.1	61.6 ± 18.6	0.837
Social support	66.0 ± 22.2	66.0 ± 23.3	0.997	61.8 ± 23.3	73.3 ± 22.1	0.082
Dialysis staff encouragment	87.0 ± 14.0	85.5 ± 16.4	0.736	85.9 ± 15.3	85.5 ± 17.9	0.927
Patient satisfaction	61.8 ± 23.8	60.7 ± 23.0	0.866	61.1 ± 20.1	59.3 ± 22.6	0.773
Short form 36 items	58.9 ± 18.7	50.4 ± 22.6	0.158	61.5 ± 17.7	51.0 ± 24.1	0.088
PCS	61.4 ± 21.7	51.4 ± 25.8	0.150	62.8 ± 20.5	51.7 ± 25.8	0.100
Physical functioning	**72.1 ± 23.7**	**59.4 ± 28.3**	**0.096**	**75.2 ± 20.8**	**59.8 ± 30.1**	**0.042**
Role-physical	**56.3 ± 39.2**	**44.0 ± 40.4**	**0.287**	**61.5 ± 37.6**	**39.0 ± 39.6**	**0.047**
Pain	70.9 ± 22.9	65.0 ± 28.2	0.424	72.2 ± 24.9	69.3 ± 24.1	0.682
General health	37.9 ± 18.7	36.0 ± 26.0	0.768	35.4 ± 20.1	38.4 ± 27.3	0.666
MCS	55.8 ± 18.1	49.2 ± 21.1	0.249	60.2 ± 16.4	50.5 ± 23.8	0.104
Emotional well-being	54.7 ± 16.0	57.9 ± 18.6	0.515	61.7 ± 16.1	53.4 ± 21.8	0.141
Role-emotional	61.1 ± 40.1	38.7 ± 44.8	0.071	62.5 ± 38.5	45.3 ± 45.0	0.159
Social function	70.3 ± 21.1	62.0 ± 28.1	0.249	69.8 ± 23.6	64.0 ± 26.6	0.425
Energy/fatigue	45.8 ± 20.7	39.8 ± 18.6	0.289	51.7 ± 17.9	43.8 ± 21.6	0.173
Health status compared to one year ago	51.0 ± 21.5	46.0 ± 25.7	0.461	53.1 ± 23.7	46.0 ± 24.7	0.308
Overall health rate	57.9 ± 22.1	56.4 ± 25.2	0.824	58.8 ± 22.5	50.0 ± 26.3	0.218

Table 1. Quality of life questionnaire scores at baseline and 12 weeks. Values are shown as the ± standard deviation. Abbreviations: PCS, physical composite summary; MCS, mental composite summary. Adapted from Lim et al.

Comparison of QOL Scores

The baseline perceptions of QOL assessed by the KDQOL-SF were similar in both groups. After 12 weeks, the physical function domain score was better in the MCO group than in the high-flux group and the role-physical function domain score was also higher in the MCO group. See Table 1. The effect of the MCO dialyzer on QOL is likely related to the better removal of middle molecules compared to high flux dialyzers. The improvements in the physical components of the QOL questionnaire over a relatively short exposure period occurred concurrently with the change of the dialyzer

Comparison of Pruritus Scores

The morning pruritus intensity was worse in the MCO group than in the high-flux group at baseline, but this difference was not observed at 12 weeks. After 12 weeks, the pruritus distribution in the morning was smaller in the MCO group than in the high-flux group. The MCO group also had less frequent sleep disturbances caused by pruritus-related scratching. See Table 2.

	Baseline			12 weeks		
Reduction ratio (%)	**MCO (*n* = 24)**	**High-flux (*n* = 25)**	***P***	**MCO (*n* = 24)**	**High-flux (*n* = 25)**	***P***
Severity						
Morning	1.92 ± 1.06	1.40 ± 0.50	0.033	1.54 ± 0.72	1.64 ±0.86	0.667
Afternoon	2.00 ± 1.14	1.72 ± 0.84	0.332	1.88 ± 0.95	1.84 ± 1.07	0.904
Distribution						
Morning	1.42 ± 0.58	1.48 ± 0.71	0.736	1.29 ± 0.46	1.64 ± 0.64	0.034
Afternoon	1.46 ± 0.59	1.56 ± 0.96	0.659	1.38 ± 0.65	1.56 ± 0.71	0.347
Sleep disturbance						
Frequency of waking from sleep	**0.83 ± 1.05**	**0.68 ± 1.28**	**0.650**	**0.75 ± 0.85**	**1.32 ± 1.60**	**0.126**
Frequency of scratching during sleep	**0.38 ± 0.92**	**0.24 ± 0.72**	**0.571**	**0.25 ± 0.53**	**1.00 ± 1.47**	**0.023**
Total score by measuring system	8.58 ± 7.74	7.20 ± 7.58	0.530	6.92 ± 5.98	9.92 ± 8.23	0.152
VAS scoring system						
Morning	2.58 ± 2.24	2.14 ± 2.28	0.496	2.50 ± 1.93	3.34 ± 2.82	0.232
Afternoon	3.04 ± 2.57	2.74 ± 2.53	0.680	3.46 ± 2.32	4.24 ± 3.18	0.333
Average	2.81 ± 2.19	2.44 ± 2.31	0.565	2.98 ± 1.98	3.79 ± 2.91	0.262

Table 2. Assessment of uremic pruritus at baseline and 12 weeks. Abbreviations: MCO, medium cut-off; VAS, visual analog scale. Adapted from Lim et al.

Comparison of Middle Molecule Concentrations and Reduction Ratios

The serum pre-dialysis and post-dialysis levels of the of three middle molecules (ß 2-microglobulin, κFLC, and λFLC) did not differ between the MCO and high-flux groups at baseline or at 12 weeks. However, the MCO dialyzer displayed better removal of κFLC and λFLC compared with the high-flux dialyzer. The removal of λFLC was significant, $p<0.001$. See Table 3.

Reduction ratio (%)	Baseline			12 weeks		
	MCO	High-flux	*P*	MCO	High-flux	*P*
β_2-microglobulin	82.1 ± 7.8	77.8 ± 16.2	0.265	79.8 ± 12.2	72.3 ± 18.2	0.109
κFLC	46.5 ± 15.7	45.5 ± 21.0	0.851	55.8 ± 13.7	44.6 ±18.9	0.022
λFLC	48.3 ± 11.6	47.7 ± 14.8	0.865	56.1 ± 11.4	40.9 ± 9.0	<0.001

Table 3. Reduction ratios of uremic retention solutes. Abbreviations: MCO, medium cut-off; κFLC, kappa free light chain; λFLC, lambda free light chain. Adapted from Lim et al.

Comparison of Laboratory Data, Ultrafiltration Volume, and Dialysis Adequacy

No significant differences in biochemical markers including serum albumin (65kDa[1]), ultrafiltration volume, and dialysis adequacy between the MCO and high-flux groups at baseline and at 12 weeks were found.

Adverse Events

No serious adverse events including cardiovascular events, death, or blood pressure decline that required dialyzer changes were observed.

CONCLUSION

This is the first randomized controlled prospective trial comparing the effects of MCO and high-flux dialyzers on QOL in patients receiving maintenance HD. The higher physical functioning and role-physical scores with MCO dialyzer than with high-flux membrane found in this study were consistent with prior studies and is likely related to the better removal rate of middle molecules in the MCO group than in the high-flux group. The MCO group also had less frequent sleep disturbances caused by pruritus-related scratching. The new MCO dialyzer may improve self-reported QOL, particularly in the physical domains and uremic pruritus, in patients receiving maintenance HD who use permanent dialysis access. The MCO dialyzer also had a non-significant effect on the serum albumin concentration over 12 weeks of treatment.

MCO (Theranova) membrane may improve patient-reported outcomes, particularly in the physical domains of QOL and uremic pruritus, through efficient removal of middle molecules, in stable maintenance HD patients.

References: 1. Wolley M, Jardine M, Hutchison CA. Exploring the clinical relevance of providing increased removal of large middle molecules. *Clin J Am Soc Nephrol*. 2018; 13:805-814.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Impact of Medium Cut-Off Dialyzers on Patient-Reported Outcomes (PROs): COREXH Registry

Alarcon JC et al. Impact of medium cut-off dialyzers on patient-reported outcomes (PROs): COREXH Registry. *Blood Purif*. In press.

BACKGROUND

Health-related quality of life (HRQoL) is a patient reported outcome (PRO) that considers the subjective point of view of the patient and supports the evaluation of outcomes and healthcare quality. Patients on dialysis experience poor HRQoL due to the symptoms of end-stage renal disease (ESRD) and the physical and psychosocial burdens of their treatments.

The impact of contemporary renal replacement therapies on a patient's perceived HRQoL is critical to treatment success. While hemodialysis (HD) therapy removes small solutes, the removal of larger molecules >25 kDa (often termed large middle molecules) is limited. Hemodiafiltration (HDF) therapy can remove middle molecules more effectively than HD. However, the effect of an improved uremic environment resulting from the clearance of middle molecules remains unclear based on randomized studies.

Advances in membrane technology have led to the development of novel medium cut-off (MCO) membranes that have enhanced selectivity and increased permeability to conventional and large middle molecules. This results in a steep sieving curve in which the molecular weight retention onset and molecular weight cut-off are very close to each other while remaining lower than that of albumin, mimicking the filtration profile of the native kidney. The application of these membranes in clinical dialysis is known as expanded hemodialysis (HDx) therapy due to the enhanced clearance of large middle molecules, which are associated with cardiovascular disease, immune modulation, and protein-energy wasting. Initial studies have demonstrated that the MCO membrane removes toxins at least as effectively as a hemofilter used in HDF mode. The goal is that this enhanced removal will improve PROs and QoL for dialysis patients.

OBJECTIVE

The goal of this study was to determine the impact of the MCO membranes on PROs, including HRQoL, presence and severity of symptoms, as well as diagnostic criteria of restless legs syndrome (RLS) in a large multicentric cohort of patients in the Expanded Hemodialysis Registry Protocol in Colombia (COREXH).

METHODOLOGY

Study Design and Patients

The study was a prospective, multicenter, observational cohort study of 992 patients undergoing dialysis from 12 renal clinics in Colombia who were switched from high-flux HD to MCO therapy and observed for 12 months. Patients with chronic kidney disease (CKD) aged 18 or older who had been undergoing HD therapy for more than 90 days at a **Renal Therapy Services** network clinic were invited to participate. Patients received HD therapy using the MCO dialyzer (**Theranova**, Baxter, Deerfield, IL, USA) three times a week for a minimum of 4 hours. Patients diagnosed with an active infection within the last 4 weeks or had a life expectancy less than 6 months were excluded. Eligible patients were prospectively followed for 12 months from enrollment.

Assessments

Baseline (before switching to therapy with the MCO dialyzer) demographic and disease characteristics were collected. HD treatment parameters, including session duration, number of sessions per week, blood flow, dialysate flow, and type of vascular access were recorded. Baseline values of Kidney Disease Quality of Life 36-Item Short Form Survey (KDQoL-SF36), Dialysis Symptom Index (DSI), and diagnostic criteria for RLS were captured and repeated at 6 and 12 months.

RESULTS

Patient Profile

A total of 992 patients from 12 clinics were included in the baseline, with 638 remaining at 12-month follow-up. Patients had been receiving high-flux HD for a median of 3.7 years at the time of enrollment.

Kidney Disease Quality of Life 36-Item Short Form Survey

After 12 months of therapy with the MCO dialyzer, three of five KDQoL domains improved compared with baseline, with the most pronounced improvements found in the kidney disease effects domain. Significant increases in KDQoL-36 mean scores from baseline were also observed for symptoms/problems and burden of kidney disease. No significant changes in scores for mental and physical domains were found (see Table 1). The effect size was modest but consistent across the full 12-month follow up period, suggesting that the expanded clearance of large molecules may be associated with improvements in QoL. In addition, contrary to the expected outcomes for patients receiving chronic dialysis, QoL trended towards improvement over the course of follow-up.

Decreases in the physical and kidney disease components of KDQoL-36 had been associated with increased adjusted mortality risk. The Convective Transport Study (CONTRAST) demonstrated that decreases in physical function, emotional health, and social functioning were significantly associated with mortality over 2 years and were independent of age. Thus, the positive impact of the expanded removal of large middle molecules on QoL measures observed in this study is encouraging.

KDQoL-36 Domain	Statistic	Baseline n = 971	6 months n = 808	12 months n = 642	*P* value*
Symptoms/problems	**Mean**	**78.6**	**81.0**	**81.5**	**<0.0001**
	SD	**15.8**	**15.4**	**14.9**	
Effects of kidney disease	**Mean**	**69.7**	**72.8**	**75.1**	**<0.0001**
	SD	**22.3**	**22.0**	**21.0**	
Burden of kidney disease	**Mean**	**46.2**	**48.9**	**50.2**	**<0.0001**
	SD	**27.5**	**29.9**	**32.3**	
SF-12 Physical	Mean	41.1	41.0	41.7	0.3
	SD	11.1	11.2	10.5	
SF-Mental	Mean	51.1	51.9	52.3	0.02
	SD	11.6	11.3	11.1	

Table 1. Change of Kidney Disease Quality of Life 36-Item Short Form Survey (KDQoL-36) Score Over 12 Months of Follow Up. *For hypothesis testing, type-1 error/p value significance was set at p=0.01.
Abbreviation: SD, standard deviation. Adapted from Alarcon et al.

Restless Legs Syndrome Diagnostic Criteria

The proportion of patients meeting RLS diagnostic criteria significantly decreased (54.6%) over the follow-up period. See Figure 1. Combined with the difficulties in correlating uremic toxin removal with RLS occurrence as well as the consistent, yet limited, data indicating HD has minimal impact on RLS, results suggest that the expanded clearance of large middle molecules with the MCO membrane comparable with the natural kidney) may alleviate the development and impact of RLS.

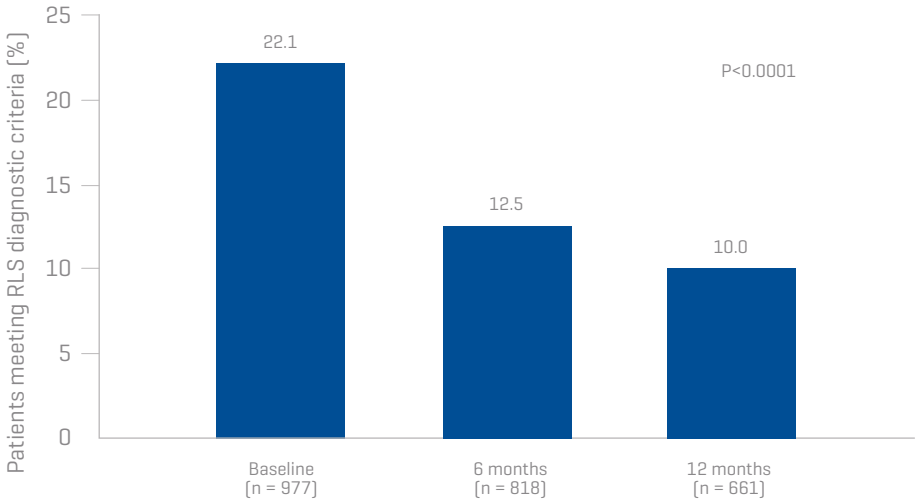

Figure 1. Longitudinal Changes in Patients Meeting Restless Legs Syndrome (RLS) Diagnosis over 12 Months of Follow Up. Abbreviation: RLS, restless leg syndrome. Adapted from Alarcon et al.

Dialysis Symptom Index

No significant differences in the mean number of symptoms from baseline were observed at 6- and 12-month follow up. However, a significant decrease in mean severity scores from baseline were observed at 6 and 12 months. See Table 2. All 30 DSI items, each of which target a specific physical or emotional symptom, were reported at a lower frequency at 6 and 12 months than at baseline, with marginally significant reductions in shortness of breath, dizziness/light-headedness, and difficulty falling asleep. The decrease in the proportion of patients with difficulties falling asleep, as well as in the presence of dizziness/light headedness was likely related to improvements in RLS and sleep pattern, which has been previously linked to the clearance of middle molecules in literature.

Strengths and Limitations

The study strengths included its prospective observation, enabling the longitudinal measurement of outcomes and comparison with historical baseline values, and its large cohort of patients (*n*=992) from multiple clinics. The study limitations included no randomization as a registry, or blinding to the intervention, as well as no comparator group.

DSI Domain	Statistic	Baseline n = 977	6 months n = 813	12 months n = 642	*P* value
Number of symptoms	Mean	10.3	10.3	10.0	
	SD	6.6	6.7	6.6	0.1[a]
	Mean	9	9	9	
	IQR	10	10	9	NA
Symptom severity score	Mean	30.7	29.9	28.5	
	SD	22.3	32.0	21.7	0.0009[b]
	Mean	26	26	23	
	IQR	32	30	31	NA

Table 2. Changes in Dialysis Symptom Index (DSI) Over 12 Months of Follow Up. a. by Friedman's test. b. by ANOVA. Abbreviations: ANVOA, analysis of variance; DSI: dialysis symptom index; IQR, interquartile range; SD, standard deviation. Adapted from Alarcon et al.

CONCLUSION

Significant improvements were observed in three of the five HRQoL domains measured by KDQol: symptoms/problems; effects of kidney disease; and burden of kidney disease. A significant decrease was also shown for the percentage of patients meeting the diagnostic criteria for RLS at 12 months. Expanded clearance of large middle molecules provided by the MCO membrane (closer to the natural kidney) may be associated with improvements in patient's QoL and may alleviate the development and impact of RLS.

Expanded clearance of middle molecular uremic toxins with MCO membranes (Theranova) may improve patient reported kidney disease quality of life outcomes including symptom burden, and Restless Leg Syndrome (RLS) criteria.

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Patient-Reported Outcome Measures (PROMs) and Expanded Hemodialysis (HDx) with Medium Cut-Off Dialyzers in a Large Cohort of Patients in Colombia: The COREXH Study

Sanabria M et al. Patient-reported outcome measures (PROMs) and expanded hemodialysis (HDx) with medium cut-off dialyzers in a large cohort of patients in Colombia: The COREXH study. ASN 2019. **Abstract FR-PO493**.

BACKGROUND

Increasing importance and focus have been directed towards quality of life measures (QoL) and patient experience in end stage kidney patients on hemodialysis (HD). A new therapy, expanded hemodialysis (HDx) with **Theranova** membane improved clearance of middle molecular uremic toxins but to date its effects of QoL are lacking.

METHODS

Historical cohort, multicenter study in prevalent patients older than 18 years under the HDx therapy with MCO membrane that complete the twelve months of follow up in the COREXH Registry, in **Renal Therapy Services** (RTS) Colombia network. PROMs were assessed by KDQOL-36, Dialysis Symptoms Index (DSI), and diagnostic criteria for Restless Legs Syndrome (RLS) tools. The ANOVA and Cochran's Q test was used.

RESULTS

Out of 992, 619 were men (62.4%) with mean age 60.4±15.7 years. For KDQoL 36 domains, symptoms, burden and effects of kidney disease and mental component, significant increase in score at 6 and 12 mos was noted (Table 1). ANOVA for DSI shows statistically significant differences in mean severity scores over the follow-up with improvement from 30.7, 29.9 and 28.5 at baseline, six months and one year respectively, (F (2, 1450) = 6.92, p = 0.0087)). The proportion of patients with RLS scores improved from 22.11% at baseline, to 12.47% at 6 m and 9.98 at 12 m (Cochran's Q, 2 df) = 145.42, p < 0.0001.

CONCLUSION

In this large multicenter study, HDx with **Theranova** resulted in improved patients' related outcomes.

KDQoL–36 domains	Baseline (N=971) Mean (SD)	At Six Months (N=808) Mean (SD)	At Twelve Months (N=642) Mean (SD)	p-Value
Sympton/Problem Domain	78.6 (15.8)	81.0 (15.4)	81.5 (14.9)	< 0.0001*
Effect of Kidney Disease	69.7 (22.03)	72.8 (22)	75.1 (21)	< 0.0001*
Burden of Kidney Disease	46.2 (27.5)	48.9 (29.9)	50.2 (32.3)	<0.0012*
SF-12 Physical	41.1 (11.1)	41.0 (11.2)	41.7 (10.5)	0.27*
SF-Mental	51.1 (11.6)	51.9 (11.3)	52.3 (11.1)	<0.0016*
DSI	30.7 (22.3)	29.9 (32)	28.5 (21.7)	0.0087 *
RLS				
% of patients with RLS	22.1	12.47	9.98	<0.0001**

* Anova test ** Cochran's Q test Abbreviations KDQOL-36: Kidney Disease Quality of Life-36 DSI: Dialysis Symptoms Index RLS: Diagnostic criteria for Restless Legs Syndrome, SD: Standard Deviation

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

A Randomised Study Investigating the Effect of Medium Cut-Off Haemodialysis on Markers of Vascular Health Compared with On-Line Haemodiafiltration (MoDal Study)

Kharbanda K et al. A randomised study investigating the effect of medium cut-off haemodialysis on markers of vascular health compared with on-line haemodiafiltration (MoDal study). Manchester University NHS Foundation Trust. **Poster clinicaltrials.gov (NCT03510520).**

BACKGROUND

- Current haemodialysis treatment offers poor clearance of larger "middle" molecules ie. those between 20 kDa and 60 kDa in size
- Cardiovascular disease remains the leading cause for death in this patient group and is intimately linked to inflammation and endothelial dysfunction (ED)
- Retention of larger middle molecules may contribute to these poor outcomes and therefore improving their removal during treatment is of significant interest
- Medium Cut-Off (MCO) haemodialysis membranes are a novel class of membrane with a larger pore size and a more uniform pore size distribution compared with high flux membranes[1]
- Haemodialysis using an MCO membrane (HDx) provides improved solute clearance (up to 45kDa) compared with high-flux haemodialysis (HFHD)[2]
- Clearance is similar to and the case of some solutes, increased when compared with haemodiafiltration (HDF)[2]
- The primary aim of this study was to compare the effect of 6 months treatment with HDx on EMV (endothelial microvesicles) compared with HDF

METHODS

- This was a 6 month open-label RCT comparing HFHD with HDx
- Inclusion Criteria : 1. Aged >18 years, 2. Established HDF > 12 weeks with treatment 3x/week, 3. Ability to consent
- Exclusion Criteria: 1. Planned live donor transplant, 2. Planned switch in dialysis modality, 3. Predicted clinical prognosis <6 months
- 1:1 simple randomization, Target blood flow rate>300mL/min
- Recruitment target: 64, 25 each group, 80 % power to detect difference 80 events/ul between 2 groups based on Ariza et al 2013[6]
- **PRIMARY OUTCOME:** Change in EMV level at 6 months
- **SECONDARY OUTCOMES:**
  1. **PROM's:** POS-S Renal, Dialysis Recovery Time, Chalder Fatigue Score
  2. **Inflammatory Cytokines:** IL-6, IL-8, IL-10, TNFa, sVCAM, sICAM, p-selectin, VEGF A , C & R!, E-selectin
  3. **Middle Molecules:** ß2M, α-1M, Leptin, Beta Trace Protein, YKL-40, Pentraxin 3, vWF
  4. **OTHER:** Pulse wave velocity, Body Composition Bioimpedance, Urine urea clearance, ultrafiltration volume

RESULTS

- 63 patients underwent randomisation into the study with 50 participants completing the full study protocol (25 in each group)
- The mean substitution volume for the HDF group at the end of the study was 22.3 ± 2.8 litres
- There was a rise in EMV in the HDF group (change in mean EMV 0.145 logCD144+ EMV/mL at T12 [p>0.05], 0.269 at T24 [p<0.05]

- There was a fall in EMV in the MCO group (-0.18 T12 [p<0.05], -0.145 T24 [p<0.05])
- Self-reported dialysis recovery time was similar in both groups at the start of the study
- By the end of the study, a greater proportion of patients in the MCO group had a dialysis recovery time <6 hours (86% MCO vs 66% HDF, p = 0.0524)
- Mean albumin change in the MCO group was -1.8±2.93 g/l vs 0 ±1.89g/l in the HDF group (p<0.05)

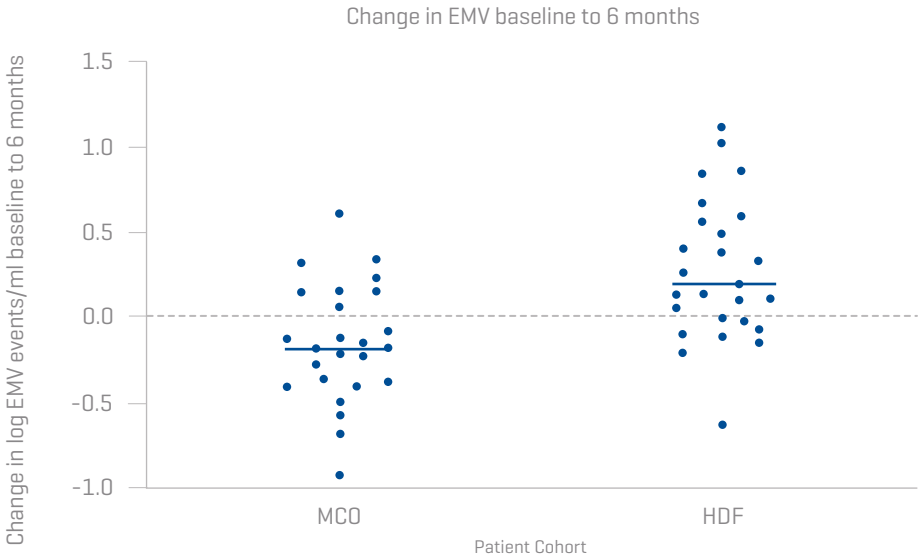

Figure 1: Change in log EMV events/mL in the two groups at 6 months compared to baseline. Adapted from Kharbanda et al.

References: 1. Boschetti-de-Fierro, A., Voigt, M., Storr, M., & Krause, B. (2015). MCO Membranes: Enhanced Selectivity in High-Flux Class. Nature Publishing Group, 1–7. http://doi.org/10.1038/srep18448
2. Kirsch, A. H., Lyko, R., Nilsson, L.-G. R., Beck, W., Amdahl, M., Lechner, P., et al. (2016). Performance of hemodialysis with novel medium cut-off dialyzers. Nephrology Dialysis Transplantation, gfw310–8. http://doi.org/10.1093/ndt/gfw310

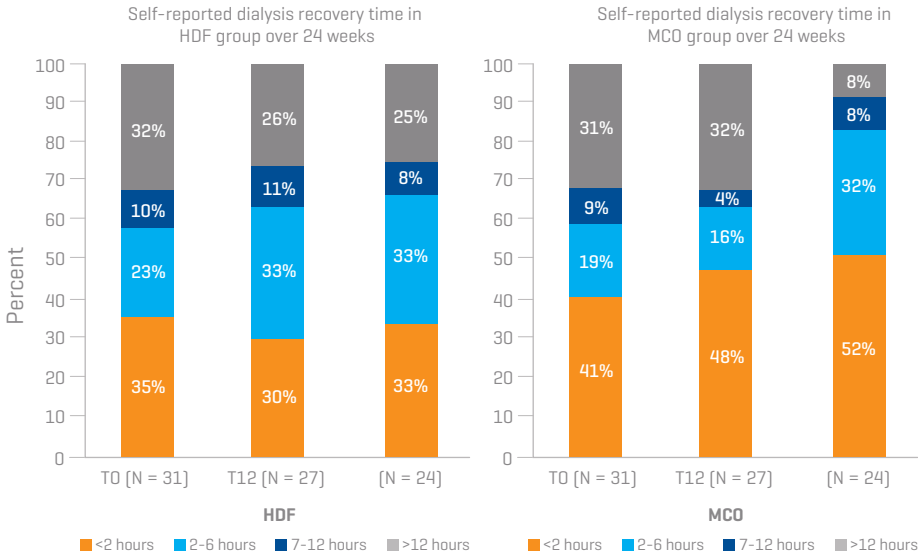

Figure 2: Change in self-reported dialysis recovery time during study period. Adapted from Kharbanda et al.

CONCLUSION AND DISCUSSION

- This study is one of the first pilot RCT's to compare high volume HDF therapy with MCO **Theranova** dialysis (HDx)
- Switching from HDF to HDx therapy is associated with a reduction in plasma EMV levels at 3 months with a sustained reduction at 6 months
- This is in contrast with a rise in plasma EMV levels seen within the same time period in those remaining on HDF
- Switching from HDF therapy to HDx therapy may lead to a reduction in dialysis recovery time
- A fall in serum albumin is seen with HDx treatment within the limits expected
- In an era where equipoise still exists between diffusive and convective treatment modalities, HDx could be an important future direction
- This is an interim analysis, further correlation with solute removal and inflammatory cytokines is required to explore the biological plausibility for the benefit of this membrane on endothelial health- full study publication planned for 2020

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

Measuring the Association of Switching Patients from Hemodialysis to Expanded Hemodialysis, with Hospitalizations, Medication Use, Costs and Patient Utility.

Ariza JG et al. Measuring the association of switching patients from hemodialysis to expanded hemodialysis, with hospitalizations, medication use, costs and patient utility. Virtual International ISPOR 2020. **Poster PUK15**.

BACKGROUND

- An innovation called expanded hemodialysis (HDx) – using the Medium Cut-Off membrane **Theranova** – allows for improved removal of molecules with a size of 25 kDa and above, including larger uremic toxins in comparison with conventional high flux hemodialysis (HD)[1].
- Early clinical evidence suggests that using HDx has the potential to improve patient outcomes and quality of life[2].

OBJECTIVE

- To provide an initial assessment of the impact of switching patients to HDx on hospitalizations, hospital days, medication use, hospitalization and drug related costs, and patient utilities.

METHODS

The data for this before and after retrospective analysis came from the Renal Therapy Services (RTS) medical records database in Colombia. All of the patients included switched from HD to HDx and had at least a year of data on HD and HDx (see inclusion/exclusion criteria in figure 1). For these patients, it was possible to capture clinical and demographic covariates; annual counts of hospitalizations, total length of stay, use of medications, and quality of life as measured by the KDQOoL-36 and the KDQOoL-SF at the start of HDx, and 1 year after HDx.

Hospital days were monetized based on a Colombian cost study in dialysis[3]. Drug costs were also estimated based on published prices in Colombia[4,5]. The hospital and drug related cost estimates were then converted to US dollars (3,338.27 Pesos per US dollar). To examine patient utility associated with the treatment type, published algorithms were used to convert the KDQOL results into EQ-5D utility scores[6,7].

Generalized linear models were used to examine the univariate relationship between HDx and hospitalizations, hospital days, the proportion of patients taking ESA, iron, insulin, and hypertension related medications, and measures of dosing of those medications. In the univariate analyses, the functional form was selected based on the best fit with the data as measured by the Akaike Information Criteria (AIC) score. All analyses were performed using Stata version 13. In addition, annual cost estimates for a patient on HD and HDx were calculated along with percent changes across time.

RESULTS

Of 175 patients in the three clinics with complete data and that had switched all their patients to HDx, 23 did not meet the eligibility criteria, 48 were lost to follow-up while receiving HD and 23 were lost to follow-up while receiving HDx. At baseline, the 81 patients included in the study had an average age of 61.1 years, 64.2% were male, 98.8% were from urban areas, 98.8% had an education level of high school or less, and 80.2% had a middle or low socioeconomic status. In addition, the patients on average had been on dialysis for 6.23 years, 25.9% of them had a modified Charlson comorbidity index of 3 or greater.

CONCLUSION

HDx has shown promise as an important advancement in dialysis-related care and this analysis has provided evidence that HDx was statistically significantly related to reduced hospitalization days and lower doses of medications in a real-world setting. Further, the cost valuation of hospitalizations and medications was suggestive of a potential savings related with HDx (USD $593 per patient per year in Colombia). In addition, the results on utility projections provided initial evidence that HDx was not negatively associated with EQ-5D utility scores for patients over time. Future research should examine more rigorously the potential of HDx to be considered as a dominant treatment.

There are limitations to consider in interpreting the results as the analyses rely on a before and after design in a single provider. Time trends in medication use and hospitalizations may confound the treatment effect. Future work should incorporate cohorts of control patients that remain on HD during the same time period. Generalizing the results to other settings should be done with care, especially for costs and utility scores. This analysis has focused solely on estimating the impact of HDx in reducing cost of hospitalizations and medications. In this sense, the potential cost savings/cost-avoidances of this technology will depend on the dynamics of prices and the contracting models for these therapies over time.

Annual Per Patient Cost Category*	Average Annual Costs with HD	Average Annual Costs with HDx	Average Annual Costs with HDx
Hospitalizations	$1,822	$1,394	-23.9%
ESA	$385	$357	-7.27%
Iron	$4.32	$3.42	-20.83%
Insulin	$242	$163	-32.64%
Antihypertensives	$189	$132	-30.16%

Table 2. Annual hospitalization and medication costs (USD) Adapted from Ariza et al.

*Cost savings may not be transferable to other markets.

Outcome	HD Mean (SE)	HDx Mean (SE)
Yearly Hospitalization Rate	0.75 (0.129)	0.73 (0.129)
Yearly Hospitalization Days	5.84 (1.066)	4.47 (1.066)[a]
Proportion Using ESA	0.85 (0.0395)	0.88 (0.366)
Dosage Per Patient Per Year of ESA (IU)	181,318 (15,024)	168,124 (15,024)[a]
Proportion of Patients using Iron	0.81 (0.432)	0.78 (0.046)
Dosage Per Patient Per Year of Iron (mg)	959 (101)	759 (101)[a]
Proportion of Patients Using Insulin	0.35 (0.528)	0.35 (0.528)
Dosage Per Patient Per Year of Insulin (IU)	5,383 (1,067)	3,434 (1,067)[a]
Proportion using Hypertension Medications	0.78 (0.046)	0.74 (0.049)
No. Tablets Patient Per Year of Hypertension Medications	1,183 (108)	731 (108)[a]
KDQOL-36 based EQ-50 Utility Score	0. 70 (0.026)	0.72 (0.026)
SF-12 based EQ-50 Utility Score	0.83 (0.015)	0.83 (0.015)

Table 1. Hospitalizations, Medication Utilization, and Patient Utilities at Baseline and with HDx (N = 81). Adapted from Ariza et al.

[a]Statistically significant difference {P value< .05) found in corresponding univariate GLM analysis of outcome on HDx IU = International units

mg= milligrams

ESA = Erythropoietin stimulating agents

References: 1.Kirsch AH, et al. *Nephrol Dial Transplant*. 2017;32(1):165-172.

2. Teatini U, et al. *Blood Purif*. 2016;41 (1-3):80-86.

3. Ariza J, et a l. *Value in Health*. 2017;20:A380.

4. CN P;yDM. Circular 01 de 2018. https:/ /www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDENS/MET /circular-04-de-2018.pdf. Accessed.

5. SISM ED 2017. https://web.sispro.gov.co/WebPublico/Consultas/ConsultarCNPMCadenaComercializacionCircu2yPA_028_2_2.aspx. Accessed December 3, 2019.

6. Yang F, et al. *The European Journal of Health Economics* (2019) 20:1195-1206

7. Sullivan P, et al. *Med Decis Making*. 2006; 26(4): 401-409

Terms Highlighted in Blue: refer to Glossary of Terms for explanation

GLOSSARY OF TERMS

Back filtration: fluid movement inside a dialyzer of dialysate and plasma water moving back and forth across the membrane. This movement of fluid may happen multiple times as blood travels from the start to the end of the hollow fibers of the dialyzer. Back filtration is also known as internal filtration.

Basement/glomerular membrane: the extracellular matrix component of the selectively permeable glomerular filtration barrier (GFB) that separates the vasculature from the urinary space.

COREXH (Colombian Registry of Expanded Hemodialysis): a registry of chronic hemodialysis patients in Colombia specifically founded to explore the **Theranova** membrane and expanded hemodialysis (HDx) therapy on a large, in-center cohort.

Dialysis Symptom Index (DSI): a survey created by the University of Pittsburgh to analyze the physical and emotional symptoms that people on dialysis may have.

Expanded Hemodialysis (HDx): a therapy where diffusion and convection are conveniently combined inside a hollow-fiber dialyzer manufactured with a MCO/**Theranova** membrane.

Health-related quality of life (HRQoL)/ Kidney Disease Quality of Life 36-Item Short Form Survey (KDQoL-SF36): a survey taken by patients with chronic kidney disease (CKD) to assess their quality of life at a given time.

Hemodiafiltration/On-Line Hemodiafiltration (ol-HDF): extracorporeal therapy that uses both convection and diffusion simultaneously across a dialyzer membrane. Both the dialysate and the replacement fluids are made on-line by the dialysis machine. Twenty liters or more of replacement fluid may be exchanged in one treatment, having the capability to remove large conventional middle molecules.

Hemodialysis (HD) therapy (conventional): extracorporeal therapy that uses diffusion alone to remove molecules. Uses concentration levels to dictate which molecules are retained, which are removed, and which are added to the patient's blood.

high-flux dialyzers (also referred to as standard/conventional high-flux): non cellulosic dialyzers that can remove molecules of size 25 kDa or less.

HCO High Cut-Off dialyzer: non cellulosic dialyzers that have a significant reduction ratio of molecules 50 kDa or less. These dialyzers are much more porous than high-flux dialyzers and usually lose significant amounts of albumin, rendering them unusable in chronic dialysis treatments.

high retention onset membrane: membranes which have significant reduction ratio of molecules 45 kDa or less, without demonstrating clinically significant amounts of albumin loss. Also known as MCO or mid cut-off membranes.

internal filtration: fluid movement inside a dialyzer of dialysate and plasma water moving back and forth across the membrane. This movement of fluid may happen multiple times as blood travels from the start to the end of the hollow fibers of the dialyzer. Internal filtration is known as back filtration.

ionic dialysance: effective ionic dialysance can be defined as the ratio of a Fresenius Medical Care 4008B machine equipped with meters to measure the ionic flux (easily derivable from dialysate inlet and dialysate outlet conductivity (Cdi and Cdo)).

KT/V: a number used to quantify hemodialysis and peritoneal dialysis treatment adequacy. **K**=dialyzer clearance of urea; **T**= dialysis time; **V**=volume of distribution of urea, approximately equal to patient's total body water.

Limulus Amebocyte Lysate (LAL): an aqueous extract of blood cells (amoebocytes) from the Atlantic horseshoe crab *Limulus* polyphemus. LAL reacts with bacterial endotoxin lipopolysaccharide (LPS), which is a membrane component of gram-negative bacteria.

MCO Mid Cut-Off membrane: membranes which have a significant reduction ratio of molecules 45 kDa or less, without demonstrating clinically significant amounts of albumin loss. Also known as high retention onset membranes.

membrane fouling: a condition that occurs soon after a membrane is exposed to blood or other proteinaceous matter during extracorporeal treatment. Proteins are absorbed and adsorbed to the surface of the dialyzer's membrane, usually making the dialyzer less permeable. Also known as protein caking.

Molecular Weight Retention Onset (MWRO): molecular weight at which the sieving coefficient value first reaches 0.9.

Molecular Weight Cut-Off (MWCO): method of characterization used in filtration to describe pore size distribution and retention capabilities of membranes. It is defined as the lowest molecular weight (in Daltons) at which greater than 90% of a solute with a known molecular weight is retained by the membrane.

Normalized Protein Catabolic Rate (nPCR): a formula commonly used to assess dietary protein intake in dialysis patients as a means towards determining nutritional adequacy.

Patient-Reported Outcome: report of the status of a patient's health condition that comes directly from the patient, without interpretation of the patient's response by a clinician or anyone else.

Polysulfone: any of several classes of thermoplastic polymers whose repeat units contain sulfone.

polyarylethersulfone-PVP blend membrane polymer: layered membranes made of Polyvinylpyrrolidone (PVP), also commonly called polyvidone or povidone, a water-soluble polymer made from the monomer N-vinylpyrrolidone and Polyarylethersulfone.

poly (methyl methacrylate) (PMMA): a chemical compound $C_5O_2H_8$, mostly used as a monomer for the production of plastic polymethyl methacrylate (PMMA).

Reduction Ratios (RR): the amount of change in the level of a molecule or substance before and after a blood purification treatment. Usually expressed as a percentage.

Sieving Curve/Coefficient/ (SC): measure of equilibration between the concentrations of two mass transfer streams. It is defined as the mean pre- and post-contact concentration of the mass receiving stream divided by the pre- and post-contact concentration of the mass donating stream.

sulfonated polyacrylonitrile (AN69): a negatively charged synthetic membrane, which is composed of a copolymer of acrylonitrile and an aryl sulfonate**.**

THP-1 assay/cell line: a human monocytic cell line derived from an acute monocytic leukemia patient. It is used to test leukemia cell lines in immunocytochemical analysis of protein-protein interactions, and immunohistochemistry.

trans peritoneal albumin losses: losses of albumin across the patient's peritoneum during a treatment of peritoneal dialysis.

uremic solutes: compounds which accumulate in blood and tissues during the development of end stage kidney disease and have an impact on biological functions.

uremic toxins: compounds that are usually filtered and excreted by the kidneys. In the setting of CKD, these compounds may accumulate and exert their uremic effects on various systems, including the immune system.

Baxter

The **Theranova** Dialyzer is indicated for patients with chronic kidney failure who are prescribed intermittent hemodialysis. It provides an expanded solute removal profile with increased removal of various middle and large molecules (up to 45 kDa) that may play a pathologic role in the uremic clinical syndrome. The **Theranova** Dialyzer is not intended for hemofiltration or hemodiafiltration therapy. The total extracorporeal blood volume for the **Theranova** Dialyzer and the set should represent less than 10% of the patient's blood volume. For single use only.

Rx Only. For safe and proper use of these devices refer to the Instructions for Use.

USMP/MG209/20-0011 08/20