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LMM: Large Middle Molecules

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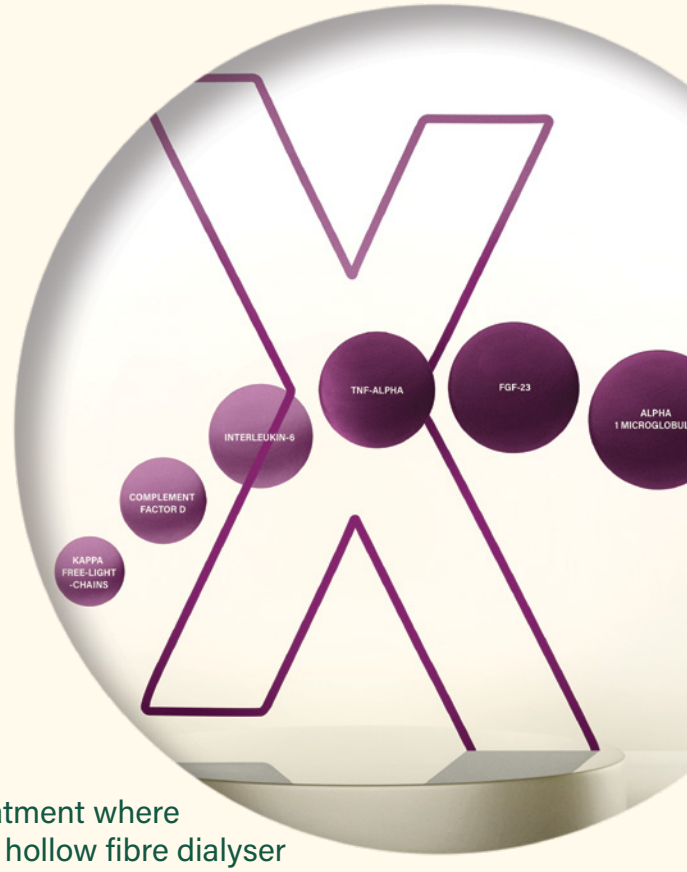


The Rise of Expanded Haemodialysis Therapy and the MCO membrane

HDx therapy enabled by **Theranova** dialyser is a dialysis treatment where diffusion and convection are conveniently combined inside a hollow fibre dialyser equipped with a High Retention Onset (HRO) membrane defined as **MCO** membrane, with no special requirement of a particular hardware, preparation of replacement fluid, or additional nursing skill, versus the necessary ones required to perform conventional haemodialysis (HD) in standard mode.[1,2]

Ronco, C.
The Rise of Expanded Haemodialysis. *Blood Purif.* 2017:44:I-VIII.

Hutchison CA and Wolley M.
The Rationale for Expanded Haemodialysis Therapy. *Contrib Nephrol.* 2017; 191:142-152.

Ronco C and Clark WR.
Haemodialysis Membranes. *Nat Rev Nephrol* 2018;14(6):394-410.

Lorenzin A, Golino G, de Cal M.
Flow Dynamic Analysis by Contrast-Enhanced Imaging Techniques of Medium Cutoff Dialyser. *Blood Purif.* 2022;51:138-146.

Boschetti-de-Fierro A, Voigt M, Storr M, Krause B.
MCO Membranes: Enhanced Selectivity in High-Flux Class. *Nature/Sci Rep.* 2015; 5:18448.

1. Ronco C, et al. The rise of Expanded Haemodialysis. Blood Purif. 2017;44:I–VIII.. Doi: 10.1159/000476012.
2. Zweigart C, et al. *Int J Artif Organs*. 2017; 40(7):328-334.

The Rise of Expanded Haemodialysis

Ronco, C. The Rise of Expanded Haemodialysis. *Blood Purif.* 2017:44:I-VIII. doi: 10.1159/000476012.

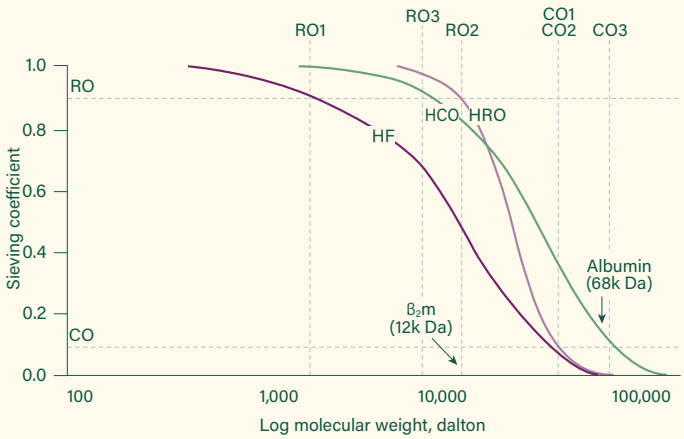
BACKGROUND

Significant improvements have been made to haemodialysis over the years leading to longer survival and improved quality of life in patients with end-stage kidney disease (ESKD). However, despite technological advances and improved patient care, long-term outcomes are still suboptimal with a high rate of hospitalisation and mortality. Important limitations of current dialysis techniques emerge clearly with several uraemic solutes inadequately removed.

The most recent evolution in the field of membranes is the development of a new class defined as "high retention onset" (HRO) due to the peculiar high sieving value in the middle to high molecular weight range. The introduction of HRO membranes in the clinical routine has enabled the development of a new concept therapy called "expanded haemodialysis." This new therapy is likely to modify the outcome of ESKD patients with the enhanced removal of molecules traditionally retained by current dialysis techniques. A careful analysis of the history of dialysis may help to avoid repeating mistakes of the past and project patient care toward more effective forms of treatments.

EVOLUTION OF DIALYSIS

Pore density and size distribution are the two main factors affecting the quantity and the spectrum of molecules that are removed by a dialysis membrane. The sieving coefficient curve is an empirically derived function that depends on these variables. However, the cutoff value is insufficient to fully characterise the shape of the sieving curve. A further parameter termed "retention onset" (RO) should also be determined for this purpose. This parameter describes the molecular weight/radius where the sieving value is 0.9. In an integrated transport model, a membrane with a tight pore size distribution will have a very steep sieving curve, while a membrane with a wide pore size distribution will have a flatter sieving curve possibly with a cutoff value beyond the molecular weight of albumin. This new approach helps the characterisation of new classes of membranes. See Figure 1.



New membranes with a relatively high cutoff value (HCO) have been recently introduced in clinical practice with the potential to remove toxins in the high molecular weight range that are increased in blood during sepsis, rhabdomyolysis, and haematological disorders. In such circumstances, high molecular weight solutes such as cytokines, myoglobin, or free light chains are the main targets for removal. The limit of these membranes, however, is the possibility of albumin leak due to the pore size opening and therefore their use is limited to a few sessions in diffusive mode until more information is available on clinical benefits and/or threats.

More recently, a great deal of effort has been made to produce membranes with a tight pore size distribution resulting in a steep sieving curve. The attempt is to keep molecular weight retention onset (MWRO) and molecular weight cutoff (MWCO) very close to each other, with a cutoff value close but lower than that of albumin. The result should be an improved removal of uraemia retention molecules in the middle-to-high weight range with marginal or no albumin leak. See Table 1.

Solute	MW, Da	Class	Action/effect
β-2 M	12,000		Amiloidosis CTS
Leptin	16,000	Middle*	Malnutrition
Myoglobin	17,000		Organ damage
κ-FLC	23,000		Toxicity
Prolactin	23,000		Infertility
Interleukin-6	25,000		Inflammation
Hepcidin	27,000	Large*	Anaemia
Bound P-cresol	33,500		CV toxicity
Pentraxin-3	43,000		Acute phase protein
λ-FLC	45,000		CV toxicity
TNF-α (trim)	51,000		Inflammation

TABLE 1. Uraemia Retention Solutes Inadequately Removed by Current Haemodialysis Techniques. *Value referred to the molecular weight interval between urea and albumin. MW: molecular weight; Da: daltons; β-2 M: beta-2 microglobulin; κ-FLC: kappa free light chain; λ-lambda free light chain; TNF-α: tumour necrosis factor-alpha; CTS: carpal tunnel syndrome; CV: cardiovascular. Table adapted from Ronco C.

FIGURE 1. Theoretical sieving curves for 3 different classes of membranes; high flux (HF), high retention onset (HRO) and high cutoff (HCO). The point in the curve where sieving coefficient is 0.1 determines the molecular weight cutoff (MWCO) value. The point in the curve where the sieving coefficient is 0.9 determines the molecular weight retention onset (RO) value. While the HRO membrane demonstrates a similar cutoff value of the HF membrane, it demonstrates a completely different behaviour. While the RO for the HF membrane is in the range of 1,200 Da (Vitamin B12), the RO for the HRO membrane is in the range of 12,000 Da (β-2 microglobulin). MWCO value can be different in the 2 membranes with small leakage of albumin in the HRO class, but this effect is neutralised after a few minutes from the beginning of the treatment due to protein deposition at the blood membrane interface. HCO membraned has a cut-off value beyond the molecular weight of albumin and determines some albumin loss. HF: high flux; HRO: high retention onset; MWCO: molecular weight cutoff; RO: retention onset. Figure adapted from Ronco C.

Based on these characteristics and the performance tests, it is proposed to define this new class of membranes as high RO (HRO) being the RO, the new dimension of characterisation. Please see Figure 2.

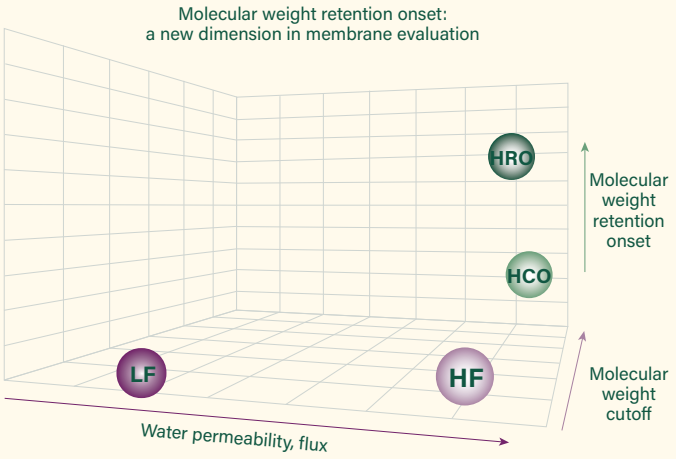


FIGURE 2. Molecular Weight Retention Onset: A New Dimension of Membrane Evaluation. The tridimensional graph describes the domain map of haemodialysis membranes. The main parameters describe the nature and performance of the membrane: water permeability or flux (increasing this parameter results in a move from low flux (LF) to high flux (HF) membranes), molecular weight cut off (MWCO) and molecular weight retention onset (MWRO). MWCO and MWRO characterise the steepness of the sieving coefficient curve and its location in terms of molecular weight range. LF: low flux; HF: high flux; HCO: high cutoff; MWCO: molecular weight cutoff; HRO: high retention onset; MWRO: molecular weight retention onset. Figure adapted from Ronco C.

HDx: A NEW THERAPY FOR A NEW MEMBRANE

The new term expanded Haemodialysis (**HDx** therapy) is the technique terminology for the application of HRO membranes in clinical dialysis. The term defines a treatment where diffusion and convection are conveniently combined inside a hollow fibre dialyser equipped with an HRO membrane. A dialysis machine with ultra filtration control is required, but no replacement solution or elevated ultra filtration rates are needed to perform the therapy. See Figure 3.

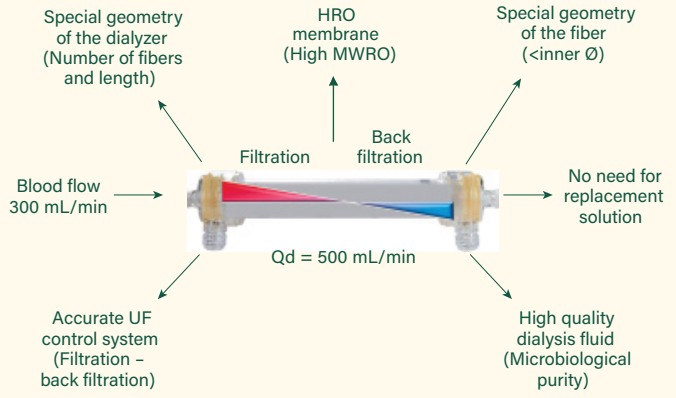


FIGURE 3. Expanded Haemodialysis and Related Operational Parameters. HRO: High retention onset; MWRO: molecular weight retention onset; UF: ultra filtration. Figure adapted from Ronco C.

The shape of the sieving curve of the HRO membrane is peculiar and optimised to perform expanded haemodialysis. Using a simple ultra filtration - controlled haemodialysis technique, solute clearances in the spectrum of molecular weights traditionally retained with other techniques and membranes appear enhanced. Large molecules have low diffusion coefficients, and their removal requires the contribution of convection. An increase in convection can be achieved either with high flux (HF) membranes in haemodiafiltration (HDF) or by HRO membranes in **HDx** therapy.

In HDF, the clearance (K) resulting from the product between ultra filtration rate (Quf) and sieving (S) coefficient, (K = Quf x S), is increased by Quf in the presence of a relatively low molecular S. In **HDx** therapy, the same clearance is achieved in the presence of a much lower Quf because of higher S. In HDF, large amounts of ultra filtration require replacement of volume by commercially prepared or fluids produced online. In **HDx** therapy, this is not needed. A significant amount of internal convection is present, but it is masked and balanced by an adequate amount of internal backfiltration. The mechanism of filtration-backfiltration is further enhanced using fibres with reduced inner diameter leading to a high pressure drop in the blood compartment at a given blood flow.

In the presence of enhanced sieving values for large molecules such as beta-2 microglobulin (β-2 M), (12,000 Da molecular weight) or free light chains, relatively high clearances are achieved even at lower levels of convective flux and without requiring the fluid exchange volumes normally required in HDF. The fibre length and inner diameter are essential elements to optimise internal filtration and the mechanism of filtration-backfiltration. This mechanism, although invisible, makes it possible to achieve a significant amount of convection inside the dialyser where filtration takes place in the proximal part and backfiltration compensates in the distal part. The ultra filtration control system of the dialysis machine regulates the process and provides the exact amount of net filtration required for the scheduled weight loss of the patient.

MULTIDIMENSIONAL MEMBRANE EVALUATION

The evaluation of a membrane should consider many different dimensions. The dimensions could include the composition, the sieving (S) for middle molecules such as β-2 M, the interaction with water molecules, the MWRO for different molecular weight solutes, the biocompatibility, the hydraulic conductance or permeability (Kf), the presence of electrical charges and potential, the molecular weight cut off (MWCO) for different molecular weight solutes, the thickness, and the diffusion coefficient (Ko).

A specific graph should be able to identify a membrane or a class of membranes and thus offer the needed information to clinicians for a correct application of the membrane in a defined technique and therapy. Please see Figure 4.

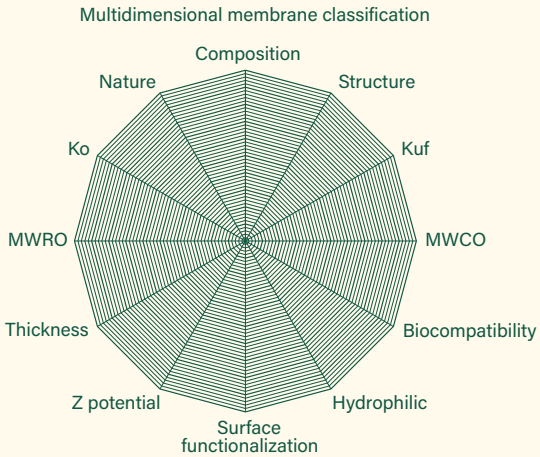


FIGURE 4. Membrane Class Domain Map. Multidimensional approach to membrane classification includes the composition, the sieving (S) for middle molecules such as β-2 M, the interaction with water molecules, the molecular weight retention onset (MWRO) for different molecular weight solutes, the biocompatibility, the hydraulic conductance or permeability (Kf), the presence of electrical charges and Z potential, the molecular weight cutoff (MWCO) for different molecular weight solutes, the thickness, and the diffusion coefficient (Ko). S: sieving; β-2 M: beta-2 microglobulin; MWRO: molecular weight retention onset; Kf: filtration coefficient; MWCO: molecular weight cutoff; Ko: diffusion coefficient. Figure adapted from Ronco C.

CONCLUSION

The analysis of outcomes in clinical dialysis demonstrates that haemodialysis is still far from effectively replacing the function of the native organ. The case of HRO membranes and **HDx** therapy is a typical example of progress and innovation in dialysis. It is unlikely that the efficacy of these membranes and this therapy will be proven in a large randomised controlled trial and their application will probably be based on application in clinical routine on criteria different from the classic evidence. It will be probably easier to perform simple pragmatic trials utilising registries and big data analysis derived from electronic medical records once **HDx** therapy is sufficiently adopted in clinical routine.

An important question remains to define the best utilisation of **HDx** therapy. Is it going to be a rescue therapy for patients with a high level of uraemia retention products, erythropoietin-resistant anaemia, malnutrition-inflammation syndrome; or an elective therapy for patients beginning haemodialysis and candidates for an early transplant? **HDx** therapy could be an ideal transition therapy for patients moving out from peritoneal dialysis and waiting for a transplant. This is an area where the application of precision medicine and treatment personalisation will be highly recommended and will be found useful. The interesting features of HRO membranes and the improved possibility to remove middle–high molecular weight solutes will spur new research in the field of haemodialysis and will constitute a new hope for ESKD patients of improved medium- to long-term clinical outcomes. The “rise of **HDx** therapy” is expected in the next few years, depending on the personal experience of users.

The Rationale for Expanded Haemodialysis Therapy

Hutchison CA and Wolley M. The rationale for expanded haemodialysis therapy. *Contrib Nephrol*. 2017; 191:142-152. doi: 10.1159/000479262.

BACKGROUND

To date, the class of uraemic toxins known as large middle-molecules has been classified as "difficult to remove" in dialysis membrane technologies. Expanded haemodialysis utilises a new generation of high-retention-onset haemodialysis 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 haemodialysis 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 β-2-microglobulin (16 kDa). Due to the non-uniformity of membranes' pores, molecules much larger than β-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 dialysers 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.

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

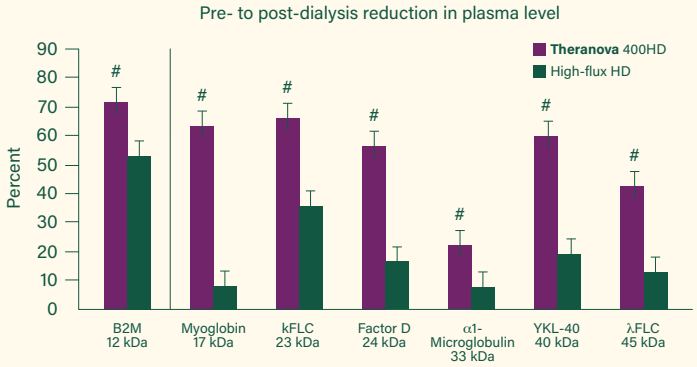


FIGURE 1. Reduction rates of large middle-molecules on high-flux and **Theranova** Dialysers. Nineteen patients, blood flow 301 ± 22 mL/min, treatment time 4 hours. High-flux haemodialysis undertaken on FX CorDiax 80 dialyser (polysulfone-PVP blend membrane). Dialysis with **Theranova** dialyser (polyarylethersulfone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

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)-1β (17.5 kDa), IL-6 (21-28 kDa), IL-18 (18 kDa), and tumour 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 uraemic 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 haemodialysis.

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.

Clinical Relevance of Large Middle-Molecules

To be classified as a uraemic 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 Haemodialysis Therapy

The high-retention-onset membranes provide clinicians the opportunity to increase the clearance of large middle-molecules beyond that provided with conventional haemodialysis 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 haemodialysis, the high-retention-onset membranes could be utilised for longer or more frequent dialysis treatments to further increase middle-molecule removal.

CONCLUSIONS

Expanded haemodialysis utilises a new generation of haemodialysis 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 haemodialysis 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 >15 kDa) 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 therapy utilises a new generation of high-retention-onset/MCO membranes that efficiently remove large middle-molecular uraemic 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.

	Class	Molecule	Molecular weight, kDa	Relative increase in dialysis	Clinical relevance
1	Cytokines	Interleukin-18	18	~2-fold higher	> Interleukins, 1β, 6, 18 and tumour 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		Tumour 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 uraemia
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.

Haemodialysis Membranes

Ronco C and Clark WR. Haemodialysis membranes. *Nat Rev Nephrol*. 2018; 14:394-410. doi: 10.1038/s41581-018-0002-x.

BACKGROUND

Haemodialysis is an extracorporeal process in which the blood is cleansed via removal of uraemic 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 dialysers, highlighting online haemodiafiltration and new therapies such as expanded haemodialysis, and considerations governing the clinical acceptable balance between large-solute clearance and albumin loss for extracorporeal therapies.

OVERVIEW

History of Dialysers

The availability of devices like the rotating drum kidney, coil dialyser, and Kiil dialyser 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 revolutionised 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 haemodialysers are utilised worldwide.

History of Hollow-fiber Membranes

Categorisation 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). Dialysers 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 (AN69), 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 haemofiltration. 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.

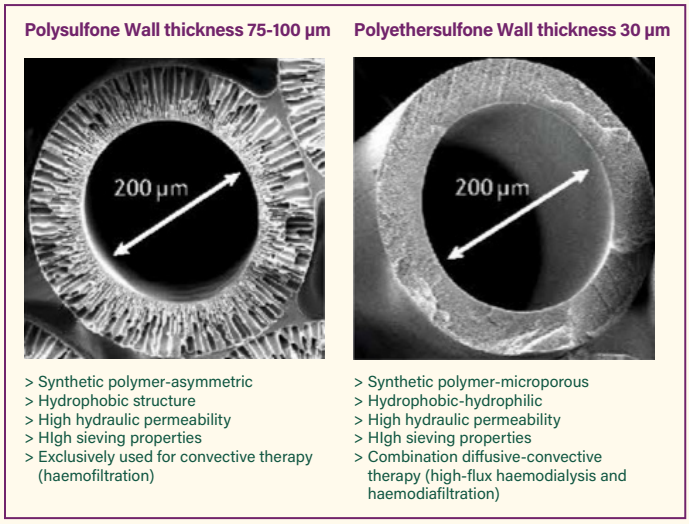


Figure 1. Physical Characteristics of Synthetic Membranes. Adapted from Ronco and Clark.

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 haemodialysis 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 haemodialysis operating conditions, the water permeability characteristics for a standard high-flux dialyser 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. Maximising 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 fibre 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 (β2m) (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 β2m SC of 0.7–0.8 and albumin loss of < 0.5 g (on the basis of a 4 h haemodialysis 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 and High Cut-Off Membranes
Two new membrane classes, (**MCO** membrane) and (**HCO** membrane), have been proposed, extending the earlier classification scheme.

The **HCO** class is characterised by a substantial increase in water permeability (relative to both high-flux and the protein-leaking classes) and a virgin β2m 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 high cut-off membranes, the medium cut-off class is intended to preserve the β2m 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. The **MCO** membrane represents the basis for a new diffusion-based therapy called 'expanded haemodialysis'.

Molecular Weight Retention Onset (MWRO)
A new solute removal parameter for the characterisation 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 rationalised 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 utilised in combination to define different dialyser 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 maximise the removal of large uraemic toxins while also maintaining albumin losses for long-term treatment of patients with ESRD. The updated classification system includes the previously mentioned **MCO** membrane that incorporates 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** membrane, and **HCO** membrane) reveal that as the separation between MWRO and MWCO decreases, the profile of the curve becomes steeper, resulting in increased removal of large uraemic toxins and decreased loss of albumin. As shown in Figure 2d, the **MCO** membrane curve is the steepest of the four curves, stopping before the molecular weight (MW) of albumin, while the **HCO** membrane 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** membrane class of dialysers relative to standard high-flux membranes. Although haemodialysis using this type of dialyser is technically diffusion-based, most large-solute removal still occurs by convection through the mechanism of internal filtration. For **MCO** membrane and other dialysers, the effect of this mechanism is intentionally augmented through increases in the mean pore size and reductions in the inner diameter of hollow fibres. Preliminary data suggest that this class of dialysers has depuration capabilities that approach those of online post-dilution haemodiafiltration without the need for (exogenous) substitution fluid administration.

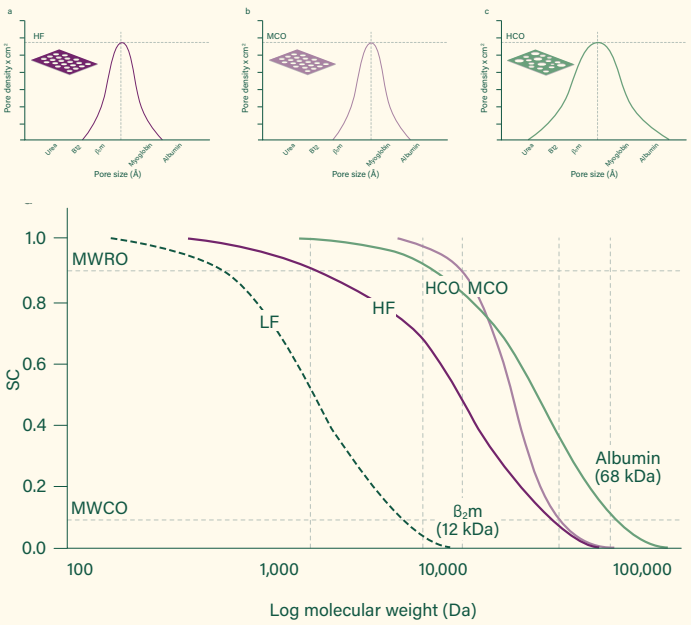


Figure 2. Performance characteristics of haemodialysis membranes derived from a suggested new classification system. Pore size distribution curves are schematically depicted for three classes of membranes, high flux (HF; part a), medium cut-off (**MCO** membrane; part b) and high cut-off (**HCO** membrane; 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 uraemic toxins (such as β2-microglobulin (β2m)) 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** membrane 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 haemodialysis.'

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 uraemic 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.

Flow Dynamic Analysis by Contrast-Enhanced Imaging Techniques of Medium Cutoff Membrane HaemoDIALYSER

Lorenzin A, Golino G, de Cal M, Pajarin G, Savastano S, Lupi A, Sandini A, Fiorin F, Ronco C.

Flow Dynamic Analysis by Contrast-Enhanced Imaging Techniques of Medium Cutoff Membrane HaemoDIALYSER. *Blood Purif*. 2021. doi: 10.1159/000516411.

BACKGROUND

The introduction of medium cutoff membrane has spurred new interest in potential improvements in medium- and long-term outcomes in patients undergoing chronic haemodialysis. **MCO** membrane presents increased solute permeability with improvement of molecular weight retention onset (MWRO) while maintaining cutoff values adequate to limit albumin losses. **MCO** membrane is utilised in a dialysis technique defined "expanded haemodialysis" that provides significant improvement in removal of large (PM > 25 and < 58 kDa) middle molecular weight solutes (LMM) responsible for symptoms and complications. While solute clearances, sieving coefficients, and hydraulic permeability have been extensively studied, flow dynamic characteristics of hollow fibre haemodialysers utilising such membranes have not been studied in detail. This information may be required to correctly prescribe **HDx** therapy and to optimise its operational parameters.

OBJECTIVE

To evaluate the flow dynamic and cross-filtration characteristics of the new **MCO** haemoDIALYSER (**Theranova** 400; Vantive, Deerfield, IL, USA), and aiming to gather specific information useful for the correct and safe delivery of expanded haemodialysis with the **MCO** membrane, specifically:

> Define the flow dynamic conditions inside the blood compartment and the flow distribution inside the dialysate compartment

> Define the hydraulic permeability and its sieving properties

> Analyse the segmental cross flow (direct filtration and backfiltration) along the length of the hollow fibre bundle

METHODOLOGY

Characteristics of Dialyser Evaluated

The **Theranova** 400 dialyser is a dialyser designed specifically for **HDx** therapy with a surface area of 1.7 m^2. The membrane has an asymmetric 3-layer structure composed of polyarylethersulfone and polyvinylpyrrolidone blend, BPA free. It is characterised by uniform pore distribution and specific sieving properties: high MWRO and molecular weight cutoff (MWCO) value lower than albumin.

Study Techniques

Blood and dialysate flow distribution and internal transmembrane cross-filtration were studied with two separate imaging techniques: CT helical scanning and sequential and static scintigraphic imaging. Two different experimental setups were used in the CT imaging technique for blood and dialysate flow dynamic analysis. See Figures 1 and 2.

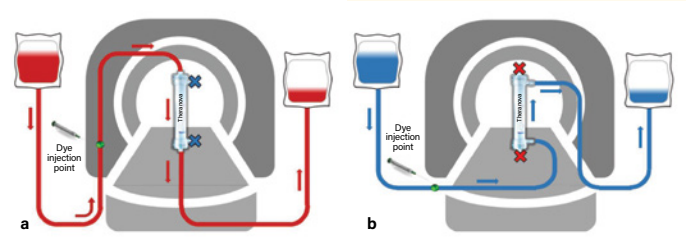


FIGURE 1. Schematic representation of the first experimental setup. The dialyser was held in vertical position and placed in the middle of the gantry. Blood and dialysate compartments were analysed separately. **a** Dye solution was injected in the blood inlet line, and flow was directed from the top to the bottom; the dialysate compartment was prefilled and sealed. **b** Dye solution was injected in the dialysate inlet line, and flow was directed from the bottom to the top; the blood compartment was prefilled and seal. Figure adapted from Lorenzin, et al.

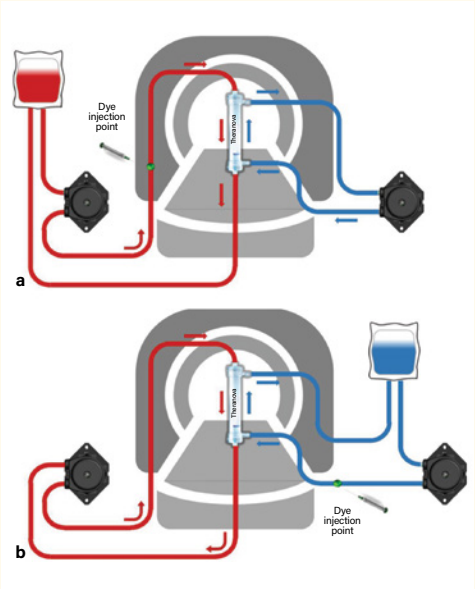


FIGURE 2. Schematic representation of the second experimental setup. The dialyser was held in vertical position and placed in the middle of the gantry. Two peristaltic pumps flowed blood and dialysate in counter-current at 300 and 500 mL/min, respectively, and no net filtration. **a** Dye solution was injected in the blood inlet line, and flow was directed from the top to the bottom; the dialysate circulated in a closed loop. **b** Dye solution was injected in the dialysate inlet line, and flow was directed from the bottom to the top; blood circulated in a closed loop. Figure adapted from Lorenzin, et al.

A third analysis was conducted employing a scintigraphic method to assess the internal filtration/backfiltration. See Figure 3.

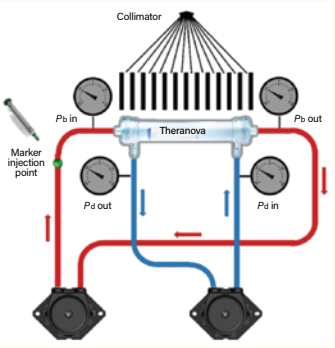


FIGURE 3. The scintigraphic experimental setup. Dialyser was laid on the gamma camera. Blood (300 mL/min) and dialysate (500 mL/min) were circulated in a closed loop configuration ensuring zero net filtration. The tracer was injected in the blood line, upstream the inlet of the dialyser. Pressures at inlet and outlet of the 2 compartments were monitored. Pb: pressure blood; Pd: pressure dialysate. Figure adapted from Lorenzin, et al.

RESULTS

Figure 4 displays the dye distribution pattern in blood and dialysate compartments during the first experimental setup: blue/green areas are free from dye solution, pink areas point out the presence of the dye solution, and red/yellow areas are transition zones. In the blood compartment (300 mL/min), flow distribution appears homogeneous. Minimal difference in velocity was observed between peripheral and central fibres. In the dialysate compartment (500 mL/min), no dead space or irregularities are noticed from the images of the filled compartments, except for a black spot in the blood one, due to n air bubble artifact (see Figure 4c).

In the blood compartment, the velocity profile changes its shape along the length of the dialyser. In the inlet blood port, the dye solution displays a homogeneous progression featuring a kind of plug flow configuration with an average velocity of 1.4 cm/s (See Figure 4a). A small increase in differential velocity among the fibres was observed as the dye proceeds toward the outlet port. Peaks of velocity are displayed in the central (1.1 cm/s) and in the extreme peripheral regions (1.5 cm/s) of the dialyser, while velocities as low as 0.6 cm/s are observed in rare groups of intermediate fibres.

The wall shear rates are 458, 666, and 276 s−1, respectively. These differences are absolutely acceptable and compatible with a well-designed blood compartment and a good flow distribution in the fibre bundle. Furthermore, the wall shear rate values are always above the limit where blood viscosity starts to increase significantly. The very short length of the Mass Transfer Zone (MTZ) (1.5 cm) in the proximal part of the dialyser (See Figure 5a) confirms the plug flow; at half length of the dialyser, MTZ is slightly longer but still within optimal values (3 cm) confirming the excellent utilisation of all fibres of the bundle and a good distribution of flow with optimised flow velocity per single fibre (See Figure 5b).

In the dialysate compartment, dye solution flows initially along the case and through the outlying fibres with a velocity of 1.8 cm/s. When the solution begins to seep in the centre of the fibre bundle, the velocity in the central region rises to 1.7 cm/s. Proceeding to the outlet port, the front of the velocity profile becomes more homogeneous with the optimal utilisation of the whole cross-sectional area available for the flow (See Figure 4f). The dialysate MTZ presents a value of 8.1 cm. While a moderate dispersion of local velocities is observed, the value is far below half of the length of the dialyser confirming optimal flow distribution of the dialysate (See Figure 5c).

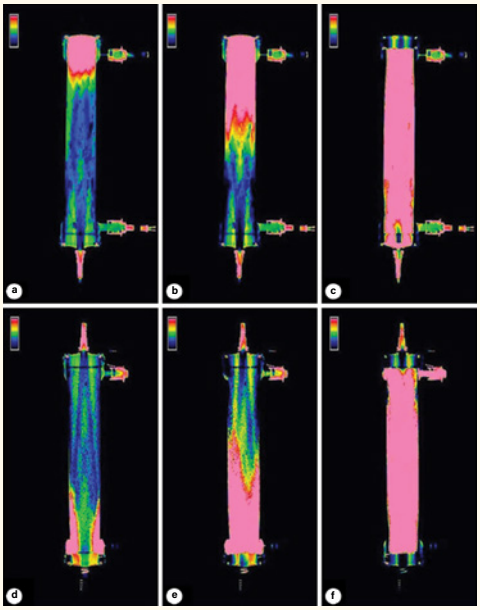


FIGURE 4. Dye progression in blood (a, b, c) and dialysate (d, e, f) compartments after 4 and 12 seconds and at total filling (33 seconds for blood and 28 seconds for dialysate), for the first experimental setup. Blue/green areas are free from dye solution, pink areas point out the presence of the dye solution, and red/yellow areas are transition zones. Figure adapted from Lorenzin, et al.

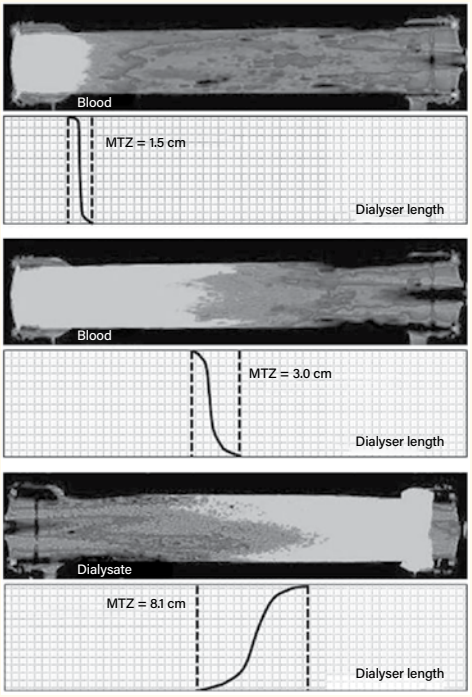


FIGURE 5. MTZ in blood (a, b) and dialysate (c) compartments obtained from the first acquisition. MTZ represents the distance between the point of maximal dye saturation and the point of absolute absence of dye. MTZ: mass transfer zone. Figure adapted from Lorenzin, et al.

Figures 6a and 6c report the images obtained from the second experimental setup, in which blood and dialysate circulated counter-current, simultaneously. Compared with the first experiment (Figures 6b, 6d), images display a perturbation of the dialyser perfusion likely induced by the local transmembrane cross flow of dialysate and plasma water. The blood flow distribution (blood flows from the top to the bottom) is homogeneous in the proximal half of the dialyser while in the distal part, yellow areas describe the effect of backfiltration from the dialysate compartment into the hollow fibres. Dialysate compartment describes an analog effect (dialysate flows from bottom to top in counter-current with blood). Dye solution saturates the portion of the dialyser close to the inlet (pink), while toward the middle of the dialyser, the flow distribution pattern becomes slightly dishomogeneous due to ultra filtration (plasma water cross flow from the blood into the dialysate compartment).

This complementary behaviour testifies the internal filtration and backfiltration phenomena. Considering the orientation of blood stream, in the proximal part of the dialyser, plasma water crosses the membrane, and the effect of the internal filtration causes a dilution of the dye solution in the dialysate compartment (See Figure 6c); in the distal part, instead, the reduction in dye intensity in the blood compartment is a consequence of backfiltration, the cross flow of the dialysate through the membrane into the fibres (see Figure 6a).

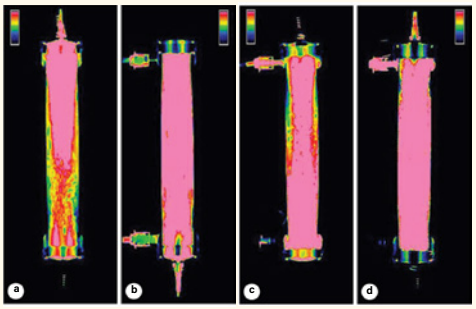


FIGURE 6. On the left, CT images acquired after reaching the total filling in the blood **(a)** and dialysate **(c)** compartment in the countercurrent flow experiment. Visible differences are noticed if compared with the same images acquired in the first experiment **(b, d)**. The reduction of dye intensity in the images on the left is the consequence of the internal/backfiltration phenomenon that occurs in counter-current flow configuration. Figure adapted from Lorenzin, et al.

Internal Filtration/Backfiltration

The kinetics of transmembrane cross flow are confirmed in the third experiment. Scintigraphic images (See Figure 7) display significant variation in radioactive count along the length of the dialyser, due to local transmembrane cross flow of plasma water through the membrane in both directions. The increase in concentration of the marker molecule in the proximal part is ascribed to the direct filtration of plasma water from blood into the dialysate while the dilution in the distal part to backfiltration of the dialysate into blood. The turning point is observed at 53% of the dialyser length. At steady state, the concentrations at inlet and outlet of the fibre bundle are identical, confirming the condition of zero net filtration. The calculation of relative changes of the marker molecule allows for the cumulative estimation of the amount of filtration and backfiltration inside the dialyser under the selected experimental conditions.

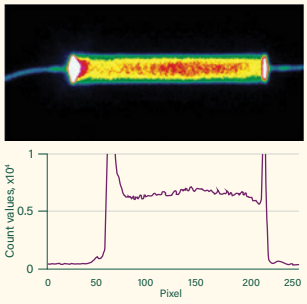


FIGURE 7. Scintigraphic image of the dialyser at the steady state and radioactivity count. The change in color corresponds to the relative variation in radio labeled marker molecule activity in a numerical scale. Radioactive count is proportional to the marker concentration in blood. In the proximal part, the internal filtration causes the increase in marker counts while the backfiltration in the distal part brings back the radioactive counts to the inlet value, restoring the zero net filtration condition. Figure adapted from Lorenzin, et al.

The concentration of the marker molecule increases reaching a peak value (Cmax) at half length of the filter and then decreases until the end of the fibre bundle reaching the same concentration observed at the inlet. This behaviour demonstrates a proximal direct filtration (IF) and a distal backfiltration (BF). Calculated IF was 32.75 mL/min while BF was 32.46 mL/min. The slight difference is compatible with the error of the method. Filtration and backfiltration rates can increase or decrease depending on blood and dialysate flows and dialyser surface area.

DISCUSSION

The clinical performance of **Theranova** dialyser as used in **HDx** therapy is based on a signficant convective exchange by enhanced mechanism of internal filtration/backfiltration. This study demonstrated the flow dynamics leading to the dialyser performance. The homogeneous pattern of blood distribution at the inlet port and along the fibre bundle represents a proof of an optimal design of the distributor and the entire blood compartment of the dialyser. This ensures a good performance of the filter in the diffusion mode and guarantees the optimal utilisation of the available surface area. Adequate flow distribution and high wall shear rates contribute to minimise the formation of a protein layer at the blood membrane interface. The occurrence of a significant concentration polarisation phenomenon with consequent reduction of membrane permeability is mitigated by the utilisation of the dialyser in the haemodialysis mode (**HDx** therapy).

Excessive convective rates such as those achieved in the haemodiafiltration (HDF) mode would contribute to impair membrane permeability by the formation of a protein cake onto the internal surface of the fibres. This phenomenon leads to the formation of a new contact surface whose thickness is added to that of the original membrane and interferes with the final permeability of the membrane in vivo. This unwanted effect is prevented by high wall shear rates in all fibres which contribute to reduce the thickness of the protein boundary layer and improve membrane hydraulic and sieving permeability.

The distribution of the dialysate flow outside the fibres is also optimised as demonstrated by the dynamic images where the dye appears to be homogeneously distributed in the entire cross-sectional area of the dialysate compartment. Thus, any blood-to-dialysate flow mismatch is avoided, and the surface area available for the exchange is maximised.

Considering the cross flow kinetic results obtained with the nuclear scintigraphic method, these observations are important. Results confirm a significant amount of filtration and backfiltration inside the dialyser at zero net filtration > 30 mL/min. This means that in presence of higher blood flows, higher surface areas, and higher net filtration rates (15–20 mL/min), direct fluid cross flow may reach values around 50 mL/minute. This is achieved in presence of a wall shear rate in all fibres sufficiently high to maintain blood viscosity at minimal levels and a significant cleaning effect at the blood membrane interface with negligible concentration polarisation of plasma proteins. Such effect results in a maintenance of the sieving properties of the membranes throughout the dialysis session. Internal filtration between 30 and 50 mL/min combined with the sieving characteristics of **MCO** membrane allows for convective clearance values of medium-large molecules equal or even superior to those achieved in high-volume online HDF, without need of fluid replacement and very high filtration fractions inside the haemoDIALYSER.

CONCLUSION

This study demonstrates the basis for the use of **MCO** membrane in expanded haemodialysis maximising the benefits of internal filtration while maintaining the simplicity and safety of high-flux dialysis configuration and blood flows in the range of 300 mL/min.

MCO Membranes: Enhanced Selectivity in High-Flux Class

Boschetti-de-Fierro A, Voigt M, Storr M, 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 haemodialysis is the adequate removal of uraemic toxins over a broad molecular weight range. As synthetic membranes are less selective than the glomerular membrane, current haemodialysis membranes do not remove higher molecular weight toxins appropriately. Consequently, patients on haemodialysis have higher levels of middle and large molecular solutes in plasma.

Membrane innovation is currently directed towards enhanced removal of uraemic 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 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 characterisation 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. haemodialysis treatment) was compared to the size of uraemic 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** membrane 1 to **MCO** membrane 4, which differ in permeability, were investigated. As reference, a HCO device (**Theralite** dialyser) and a conventional high flux membrane (**Revaclear** dialyser) were also tested. All devices were manufactured by Gambro Dialysatoren GmbH, Hechingen, Germany. The membrane material was a polyarylethersulfone/polyvinylpyrrolidone blend.

Membranes were characterised 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 characterisation 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** membrane 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

Characterisation 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** membrane 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.

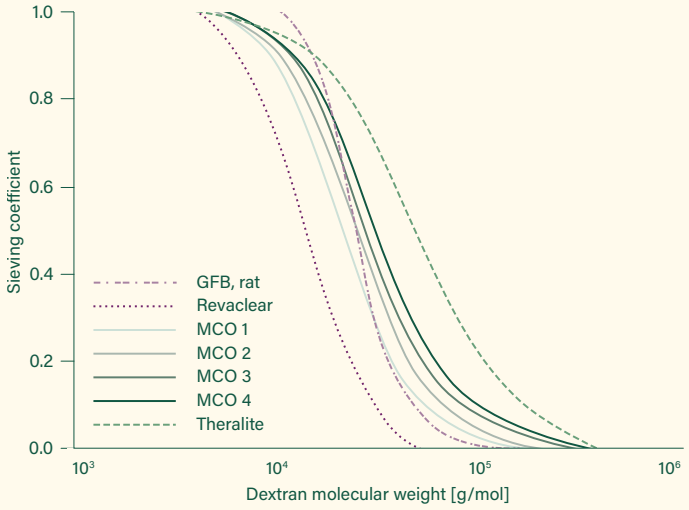


FIGURE 1. Characteristic in vitro dextran sieving curves measured in aqueous solution (pristine/before blood exposure) for different types of blood purification membranes: high-flux (Revaclear, MCO membranes 1-4), HCO (Theralite). Data for glomerural membrane added for comparison (rat specimen, ficoll filtration, measured in vivo).

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.

Membrane	Before blood exposure			After blood exposure		
	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. Characterisation of MCO haemodialysis 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** membrane 4 included one dextran fraction of 150 kDa.

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.

Membrane	Before blood exposure		After blood exposure	
	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 membranes, 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 haemodialysis 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 haemodialysis 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.

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 uraemic 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 (Rh) for albumin and representative middle and large uraemic toxins.

Molecule	R_h [nm]	Comments	Ref.
β2 microglobulin	1.7	calculated from the diffusion coefficient in free solution	16
Tumour 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 (Rh) for albumin and some representative middle and large toxins. Adapted from Boschetti-de-Fierro et al.

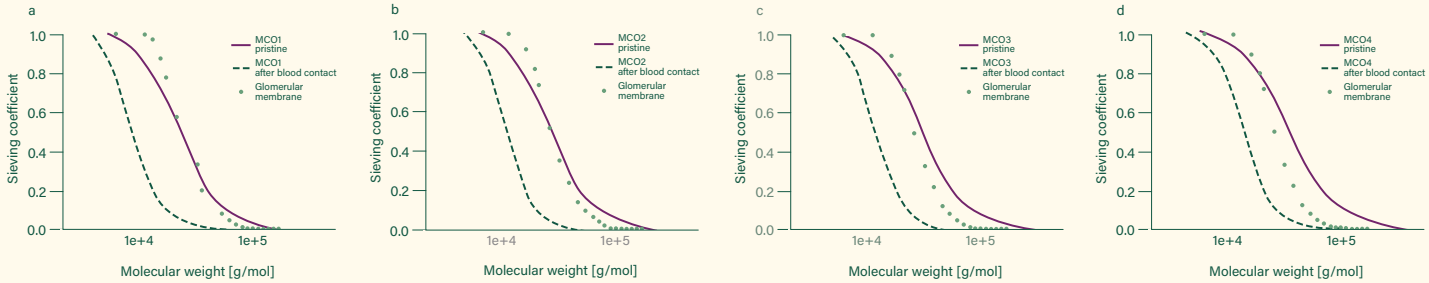


Figure 2. Characteristics of sieving curves for MCO high flux membranes before (solid line) and after blood contact (dashed line), for (a) MCO 1, (b) MCO 2, (c) MCO 3, (d) MCO 4. The data for the glomerular membrane has been added for comparison (rat specimen, ficoll filtration, measured in vivo).

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 haemodialysis treatments, they allow for removal of an expanded range of uraemic toxins compared to conventional high-flux membranes. A formation of a protein layer on top of the synthetic membrane during haemodialysis restricts the removal of molecules above 3.5 nm in radius, ensuring the retention of albumin during a treatment, while still optimising removal of large uraemic toxins.

Tailored pore sizes of MCO membrane promote removal of an expanded range of uraemic toxins, while ensuring retention of albumin. The unique design of the MCO membrane 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 Ultra filtration, *Kidney International*. 1995; 47:1242-1251.

Vantive

HDx therapy:
A world of difference

The Unmet Need that HDx therapy can address: Large Middle Molecules (LMM) Removal

A new classification links uraemic solutes with traditional clinical outcomes and QoL measures including pruritus, RLS, and recovery time.
Water soluble protein bound proteins generated by endogenous metabolism can be divided in five uraemic classes: Small water-soluble molecules (<500Da), Small-Middle Molecular weight solutes (0.5-15kDa), Medium-middle molecular weight solutes (>15-25 kDa), Large-middle molecular weight solutes (>25kDa-58kDa), and protein bond solutes.[1]

Rosner MH, Reis T, Husain-Syed F, et al.
Classification of Uraemic Toxins and Their Role in Kidney Failure. *Clin J Am Soc Nephrol* 2021;161(12):1918-1928.

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.

Kandi M, Brignardello-Petersen R, Couban R, Wu C, Nesrallah G.
Effects of Medium Cut-Off Versus High-Flux Haemodialysis Membranes on Biomarkers: A Systematic Review and Meta-Analysis. *Can J Kid Health Dis.* 2022;9:1-15.

Zweigart C, Boschetti-de-Fierro A, Hulko M, et al.
Medium cut-off membranes - closer to the natural kidney removal function
Int Artif Organs. 2017;40(7):328-334.

1. Rosner MH, Reis T, Husain-Syed F, et al. Classification of Uraemic Toxins and Their Role in Kidney Failure [published online ahead of print, 2021 Jul 7]. *Clin J Am Soc Nephrol*. 2021;16(12):1918-1928.

Classification of Uraemic Toxins and Their Role in Kidney Failure

Rosner MH, Reis T, Husain-Syed F, et al. Classification of Uraemic Toxins and Their Role in Kidney Failure. *CJASN* Dec 2021:16(12):1918-1928. doi:10.2215/CJN.02660221.

BACKGROUND

Uraemia is the build-up of metabolic waste products such as urea that occurs when kidney function is impaired. Along with retention of metabolic waste products, patients with advanced kidney disease typically experience symptoms that may include nausea, vomiting, fatigue, anorexia, muscle cramps, pruritus, mental status changes that lead to a reduced quality of life as well as excess morbidity and mortality.

Dialysis techniques are used to remove these metabolic waste products, with the hope that symptoms and outcomes will also improve. However, this goal has only been partially achieved, and outcomes for patients with kidney dysfunction remain suboptimal. While knowledge of solutes that build-up with uraemia has increased, there is a growing recognition that current dialysis prescriptions may not be effective in their removal. Technological advances such as the development of new medium cut-off haemodialysis membranes, and the ability to perform high efficiency haemodiafiltration enable the removal of molecules up to ~50 kDa.

OBJECTIVE

An expert conference was convened to identify limitations in the current definition and classification of uraemic retention solutes/toxins. Experts in the field of uraemia and uraemic toxins were tasked with a comprehensive review of the current definition and classification of uraemic retention solutes, and posed several critical questions and recommendations to define these toxins better and map future studies for improving outcomes.

METHODOLOGY

Experts in the field of uraemia and uraemic toxins were invited to participate in a consensus conference, held virtually November 31 – December 2, 2020. A modified Delphi method was used to achieve consensus.

A pre-conference literature search and appraisal of the scientific evidence was completed. Key themes were identified and workgroups were created to address the following themes: Critical appraisal of limitations in the current definition/classification of uraemic retention solutes; Rationale for updating definition and classification of uraemic retention solutes and molecules of interest in the field of maintenance haemodialysis and; proposal of a new classification of solutes of interest in uraemia and haemodialysis. Individual workgroups presented the output to conference participants for debate, discussion, suggested revisions, and recommendations for research were formulated. The final product was then assessed and aggregated in a videoconference session attended by all attendees, who approved the consensus recommendations.

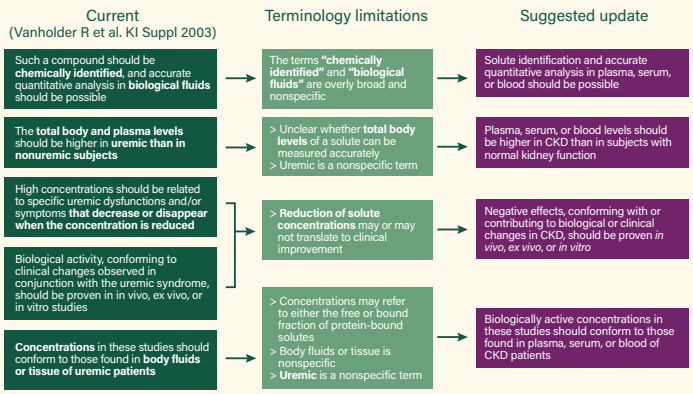
DISCUSSION

Redefining uraemic toxins

Rationale

In 2003, the European Uraemic Toxin Work Group (EUTox) proposed five criteria for an organic solute to be classified as a uraemic toxin (Figure 1). The panel identified limitations of the current definition and concluded that modifications to the definition are necessary to incorporate new advances in haemodialysis (Figure 1).

FIGURE 1. Definition of uraemic toxins



Panel Recommendations

1. We suggest that the current definition of uraemic toxins should be adapted in terminology to account for the growth in knowledge in the field (Figure 1).
2. We suggest that the scope of the definition should remain limited to organic solutes.

Physicochemical classification of uraemic toxins

Rationale

In 2003, EUTox categorised uraemic toxins according to their physicochemical characteristics that affect clearance during haemodialysis, which came from the need to simplify and organise uraemic toxicity concepts within a framework of therapeutic removal approaches, mainly by haemodialysis.

Panel Statement 1: The current physicochemical classification of uraemic toxins does not adequately address or reflect how current/modern haemodialysis technologies (mechanisms of adsorption, convection, and diffusion) remove toxins.

There is a continuum in the molecular weight of uraemic solutes, and any cut-off based on molecular weight is arbitrary, plus the degree of protein binding for uraemic solutes is variable and complicates classification based solely on molecular weight. The most practical classification approach is based upon the principles of removal patterns by standard haemodialysis schedules.

Newer haemodialysis membranes are likely to change the ability to remove higher molecular weight solutes that may be toxic. Currently this can be achieved mainly through convection. The high-flux dialyser has a molecular weight cut-off of 25 kDa in haemodialysis mode, boosted up to 30 kDa when in haemodiafiltration mode. A new class of membranes is the medium cut-off membrane with a cut-off of 56 kDa, a mean pore radius of 5 nm, and a fibre inner diameter of 180 μm. As a comparison, the high-flux membrane has a mean pore radius of 3.9 nm and an inner diameter of ~200 μm. Clearance is more efficient for larger molecules (25–58 kDa) with medium cut-off membranes than high-flux membranes. Clinical trials have consistently demonstrated increased clearance of larger molecular weight molecules such as complement factor D, free κ light chains, TNF-α, and β2-microglobulin.

Panel Recommendations

1. We suggest that the current definition of uraemic toxins should be based on haemodialysis strategies, membranes, and removal patterns acknowledging that any classification based on cut-off values and/or molecular spatial configuration or charge would be arbitrary and likely will need to be changed as technological development changes solute removal patterns.

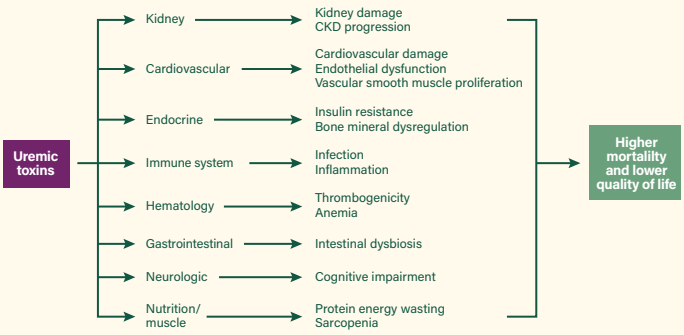
Classification based on toxicity

Rationale

Uraemic toxicity negatively affects multiple organ systems and metabolic pathways (Figure 2); cardiovascular damage, increased susceptibility to infection and neurologic manifestations are major factors affecting mortality and quality of life of patients with chronic kidney disease (CKD). The current classification does not link benefits of increased clearance or impact of inadequate clearance of uraemic toxins on the body.

Panel Statement 2: The current physicochemical classification of uraemic toxins does not adequately reflect the biological consequences of the toxins and is not able to identify which toxins possess the most clinical relevance.

FIGURE 2. Uraemic toxins and related systemic disorders



Panel Recommendations

1. We suggest using the 2018 classification system (Vanholder et al, *Toxins*) that reflects the degree of known toxicity based upon published peer-reviewed literature to define the pathophysiologic impact of each uraemic retention solute. Periodic updates will be required as new evidence of the toxicity of solutes becomes available, and new solutes are identified.
2. We suggest that the pathophysiologic impact of each uraemic toxins (e.g., inflammatory, cardiovascular) and solute origin (e.g., intestinal generation, posttranslational modification) should be stated wherever available.
3. We suggest focusing on a limited number of key body system effects that are the most prominent in uraemia, such as cardiovascular damage, susceptibility to infection, and neurologic manifestations for pathophysiologic classification.

Classification based on patient outcomes

Rationale

Patients with kidney failure have a high symptom burden and studies have shown that this is more important to patients than survival. The current classification does not guide clinicians in how to prescribe dialysis to improve for instance restless leg syndrome, pruritus or dialysis recovery time. There is a need to consider the role of a range of different solutes including those of larger molecular weight in causing specific clinical scenarios.

Panel Statement 3: The current physicochemical classification of uraemic toxins does not adequately address patient experience or outcomes and does not reflect personal patient characteristics by which the dialysis prescription should be made (e.g., targeting the prevention of cardiovascular disease, loss of residual kidney function, deterioration of vascular access, or quality of life).

Panel Recommendations:

1. Future studies should focus on correlating solute concentrations or the effect of interventions on solute concentrations with clinically relevant outcomes and outcomes of importance to patients.
2. Ideally, dialysis prescriptions would be tailored to improve these symptoms and quality of life based upon removal patterns of uraemic solutes linked to symptoms and outcomes.

Assessment of toxin measurement and removal capacity

Rationale

A marker of solute removal should be linked to its toxicity (and improvement of symptoms with removal) and be representative of other toxins with comparable characteristics. Pre-dialysis serum levels after implementation of an intervention are more appropriate measures of the effectiveness of a new technology.

Panel Recommendations:

1. For assessment of toxin removal by extracorporeal treatmet, we recommend measuring the pre-haemodialysis concentration of a toxin after a period of equilibration (≥4 weeks).
2. For comparability reasons, we suggest using the same equilibration time (4 weeks) to study any other strategy than extracorporeal removal to decrease toxin concentration (e.g., medication, dietary intervention, xenobiotics, and others).

Proposal for a new classification system of uraemic solutes

Rationale

The panel emphasised that decreased uraemic toxin clearance due to low glomerular filtration rate is not the only reason for toxin accumulation; for example, excessive production of cytokines and soluble receptors due to local tissue inflammation

is a major contributor to middle molecule accumulation. Therefore a broader view of uraemic solutes is needed.

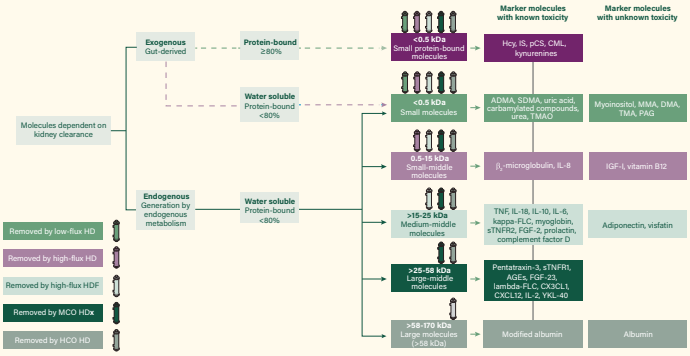
Panel Statement 4: New measurement tools for uraemic toxins are needed in each class that goes beyond physicochemical classification.

Panel Recommendation:

1. The new classification schema must link uraemic solutes to traditional clinical outcomes and quality of life measures, including pruritus, restless legs syndrome, and recovery time after dialysis.

New validated biomarkers are needed that are ideally inexpensive, easy to measure, globally available, correlate with severity of disease, and be sensitive to early subclinical disease, recovery, and response to therapy. A panel of biomarkers representing each uraemic toxin class was proposed; for estimation of medium middle (>15–25 kDa) and large-middle (>25–58 kDa) molecular weight clearance, the panel recommended analyses of κ (22.5 kDa) and βαξ (45 kDa) free light chains, respectively. The proposed new definition and classification of uraemic toxins is outlined in Figure 3.

FIGURE 3. New definition and classification of uraemic toxins



Panel Recommendation:

1. Candidate biomarkers representing different types of uraemic retention solutes should be identified and used as proxies to study various dialytic and non-dialytic removal strategies.

Panel Statement 5: Available and newer dialysis technology (including membranes) must be measured for its effective removal of uraemic toxins in each class.

New dialysis methods and new membranes with the ability to clear an extended range of uraemic toxins or with specific characteristics lead to the need of a new classification. Characteristics that should be considered are new permeability indices, the hydrophilic or hydrophobic nature of membranes, adsorption capacity, and electrical potential. Molecular weight retention onset, molecular weight cut-off, and the mass transfer area coefficient should be measured. Clinicians should consider molecular radius, electrical charges, protein binding solute characteristics, high vs. low molecular weight, hydrophilic vs. hydrophobic, endogenous vs. exogenous, secretion by kidney tubules, and different volumes of distribution.

Panel Statement 6: The panel stated that prototype uraemic biomarkers should be validated as new measurement tools of uraemic toxicity.

Identifying prototype biomarkers to optimise patient management is critical. These biomarkers should be linked to improving clinical outcomes and need to predict uraemic manifestations, inform about mechanisms and prognosis, improve safety of interventions for uraemia, or be a surrogate marker of a uraemic toxin or clinical outcome.

Continued research is critical to link biomarkers to improved clinical outcomes and quality of life, and to assess the cost of using novel biomarkers. A multidimensional approach including big data methodologies will be needed to understand the complex pathophysiology of uraemia.

CONCLUSIONS

Advances in the understanding of uraemic toxins and the availability of new haemodialysis membranes and techniques have led to a reappraisal of the definition and classification of uraemic toxins. The consensus panel recommended a more holistic classification of uraemic toxins that includes physicochemical characteristics and correlation to clinical symptoms and outcomes. In addition, the identification of representative biomarkers that correlate with removal patterns and are clinically relevant to toxicity may enable more personalised and targeted dialysis prescriptions. Validation of the novel classification will require big data methodologies, validation in external cohorts and experimental evidence of toxicity. Of note, new data on uraemic toxins and removal techniques are continuously being published and these recommendations may therefore require modifications as new results become available.

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. doi: 10.2215/CJN.10110917.

BACKGROUND

End stage kidney disease (ESKD) is accompanied by the retention of uraemic toxins, molecules that accumulate in kidney impairment and have an adverse biologic effect. Uraemic 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 β2-microglobulin (11.8 kDa).

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

OBJECTIVES

The 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** membrane, 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 Haemodialysis Membranes to Remove Large Middle Molecules

Hollow fibre haemodialysis 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 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** dialyser uses this new distribution of pores, which should provide in clinical practice the effective clearance of large molecules without excessive albumin loss.

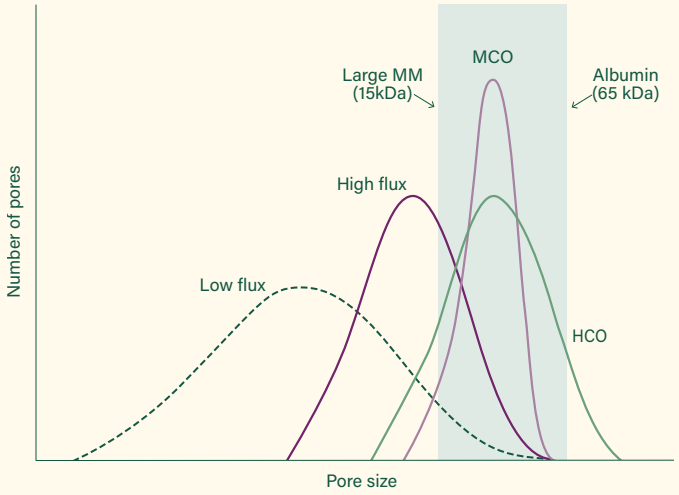


Figure 1. Medium cut-off membranes provide clearance of large middle molecules without albumin loss. Schematic of pore size distribution in dialysis membranes. As membranes have been developed to allow the removal of large middle molecules (MMs) without albumin loss, the distribution of the pore sizes 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. Adapted from Wolley M, et al.

Identification of Large Middle-Molecules

A list of uraemic 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 the **MCO** membrane. Protein-bound solutes are clinically relevant but were not assessed because they cannot be removed by the **MCO** membrane.

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 categorised 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 uraemia, and relative increase in dialysis or advanced CKD are described for each molecule.

Clinical Relevance of Large Middle Molecules as Uraemic 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-1β (17.5 kDa) and tumour 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-1β 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	β-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-1β	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). aDegranulation inhabiting protein corresponds to angiogenin. Abbreviations: HDF: haemodiafiltration; 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. β-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 chaemotaxis. 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 chaemotactic 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 neutralising antibody toward FGF-23. α-1 acid glycoprotein (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-1β (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-1β (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 uraemia, along with the increase of cytokine IL-1β (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

Molecule (Alternative Names)	Group	Size, kD	Usual Biologic Function	Possible Adverse Effects in Excess or Uraemia	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 chaemotaxis 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 chaemotaxis, 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	Opsonisation 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 chaemotaxis, 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	Neovascularisation; upregulates inflammatory cytokines and chaemokines	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, chaemotaxis, and IL-2 secretion	Approximately 9x higher
β-Trace protein (L-prostaglandin D2 synthase)	Protein	26	Catalyses isomerisation 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.

increased in CKD, which further contribute to the chronic inflammation of ESKD.

*Clinical Evaluation of the **MCO** Dialyser*

With increasingly porous membranes, such as those in the **MCO** dialyser, 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 the **MCO** dialyser compared with high-flux dialysers. Haemodialysis 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 dialysis with the **MCO** membrane, 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 dialysis with the **MCO** membrane for the removal of large middle molecules, the **MCO** membrane 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 whichhave biologic pathways through which they can contribute to cardiovascular disease, secondary immune deficiency, protein energy wasting and chronic inflammation. Robust clinical trials are now required to determine if increasing their removal by dialysis can improve clinical outcomes.

Effects of Medium Cut-Off Versus High-Flux Haemodialysis Membranes on Biomarkers: A Systematic Review and Meta-Analysis

Kandi M, Brignardello-Petersen R, Couban R, Wu C, Nesrallah G. Effects of Medium Cut-Off Versus High-Flux Haemodialysis Membranes on Biomarkers: A Systematic Review and Meta-Analysis. Can J Kidney Health Dis. 2022:9;1-15. doi: 10.1177/20543581211067090.

BACKGROUND

Uraemic toxins have a range of physiochemical properties associated with diverse effects that contribute to morbidity and mortality in patients with end-stage renal disease. Earlier membrane technologies provide minimal diffusive clearance above 15 kDa. A novel medium cut-off membrane (Theranova 400/500, Vantive) removes large middle-molecules while selectively retaining molecules >45kDa.

OBJECTIVE

Compare the effects of the **MCO** membrane versus high-flux membranes on biomarkers falling within an expanded range of molecular weights through a systematic review and meta-analysis.

METHODOLOGY

A search was conducted of the MEDLINE, Embase, CINAHL, Cochrane Library, and Web of Science from January 2015 to July 2020, and gray literature sources from 2017. Randomised (RS) and nonrandomised studies (NRS) comparing the **MCO** membrane and high-flux membranes in adults (>18 years) receiving maintenance haemodialysis were included. Study selection, data extraction, and quality appraisals were performed in duplicate and used the Grading of Recommendations Assessment, Development, and Evaluation framework. Outcomes included solute removal (plasma clearance or dialysate quantitation), reduction ratios, and predialysis serum concentrations for albumin, and a range of prespecified large middle molecules and inflammatory markers.

RESULTS

The search identified 26 eligible studies (10 randomised and 16 nonrandomised; N = 1883 patients; patient-years = 1366.3). All studies used the **MCO Theranova** dialysers.

Albumin

The mean difference (MD) for albumin removal was 2.31 g per session (95% confidence interval [CI], 2.79 to 1.83; high certainty), with a reduction in predialysis albumin of −0.12 g/dl (95% CI, −0.16 to −0.07; I2 = 0%; high certainty) in the first 24 weeks, returning to normal (MD = −0.02 g/dl, 95% CI, −0.07 to −0.03; I2 = 56%; high certainty) after 24 weeks. No studies reported hypoalbuminemia that required albumin infusion or discontinuation of treatment with the **MCO** membrane. A summary of albumin results across studies is shown in Table 1.

TABLE 1. Summary of findings - Albumin related measures.

Outcome No. of participants (studies)	Anticipated absolute effects (95% CI) Without MCO-HD	Difference	Certainty	What happens
Albumin loss (g) Follow-up: 2 weeks No. of participants: 230 (5 RS)	The mean albumin loss ranged from 0.2 to 0.56 g	MD 2.31 g higher (2.79 higher to 1.83 higher)	⊕⊕⊕⊕ High	MCO-HD increases albumin loss slightly.
Albumin reduction ratio (%) Follow-up: range, 2-26 weeks No. of participants: 162 (3 RS)	The mean albumin reduction ratio ranged from 7% to 11%	MD 2.39% higher (3.68 higher to 1.11 higher)	⊕⊕⊕⊕ High	MCO-HD increases albumin reduction ratio slightly.
Predialysis serum albumin (g/dl) Subgroup with <24-week follow-up Follow-up: range, 8-13 weeks No. of participants: 305 (5 RS)	The mean predialysis serum albumin ranged from 3.79 to 3.94 g/dl	MD 0.12 g/dl lower (0.17 lower to 0.07 lower)	⊕⊕⊕⊕ High	MCO-HD reduces predialysis serum albumin slightly over the short term (<24 weeks).
Predialysis serum albumin (g/dl) Subgroup with ≥24-week follow-up Follow-up: 24 weeks No. of participants: 129 (1 RS)	The mean predialysis serum albumin was 4.1 g/dl	MD 0 g/dl (0.1 lower to 0.1 higher)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely esults in little to no difference in predialysis serum albumin after 24 weeks of treatment.
Predialysis serum albumin (g/dl) Subgroup with ≥24-week follow-up Follow-up: range, 24-52 weeks No. of participants: 2010 (7 NRS)	The mean predialysis serum albumin (g/dl)—subgroup with ≥4-month follow-up ranged from 3.1 to 4.05 g/dl	MD 0.02 g/dl lower (0.08 lower to 0.04 higher)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely results in little to no difference in predialysis serum albumin after 24 weeks of treatment.
Predialysis serum albumin (g/dl) Subgroup analysis—RS and NRS with <24-week follow-up Follow-up: range, 8-13 weeks No. of participants: 325 (5 RS, 1 NRS	The mean predialysis serum albumin ranged from 3.76 to 3.94 g/dl	MD 0.12 g/dl lower (0.16 lower to 0.07 lower)	⊕⊕⊕⊕ High	MCO-HD reduces predialysis serum albumin slightly within the first 24 weeks of follow-up.
Predialysis serum albumin (g/dl) Subgroup analysis—RS and NRS with ≥24-week follow-up Follow-up: range, 24-52 weeks No. of participants: 2139 (1 RS, 7 NRS)	The mean predialysis serum albumin ranged from 3.10 to 4.05 g/dl	MD 0.02 g/dl lower (0.07 lower to 0.03 higher)	⊕⊕⊕⊕ High	MCO-HD results in little to no difference in predialysis serum albumin after 24 weeks of follow-up.

Note. CI = confidence interval; MCO-HD = haemodialysis with **MCO** membrane; MD = mean difference; NRS = nonrandomised study; RS = randomised study.

[a]Estimate prone to risk of bias due to patient attrition.

Middle Molecules

The review found with high certainty that dialysis with the **MCO** membrane resulted in a large increase (standardised mean difference [SMD]> 2.0 for all) in β2-microglobulin, κ- and λ-free light chains, and myoglobin removal, resulting in moderate (SMD > 0.5) to large (SMD > 0.8) reductions in predialysis concentrations for all of these solutes.

Table 2. Summary of findings - Middle Molecules

Outcome No. of participants (studies)	Anticipated absolute effects (95% CI) Without MCO-HD	 Difference	Certainty	What happens
β2M removal (mg) Follow-up: range, 2-8 weeks No. of participants: 152 (4 RS)	-	SMD 1.83 SD higher (0.02 higher to 3.64 higher)	⊕⊕⊕⊕ High[a]	MCO-HD results in a large increase in β2M removal.
β2M removal (mg) No. of participants: 16 (1 NRS)	-	SMD 1.4 SD higher (0.42 higher to 2.38 higher)	⊕⊕⊕⊕ High[b,c]	MCO-HD results in a large increase in β2M removal.
β2M reduction ratio (%) Follow-up: range, 2-26 weeks No. of participants: 323 (7 RS)	The mean β2M reduction ratio (%) ranged from 46% to 77%	MD 8.0% higher (2.8 higher to 13.2 higher)	⊕⊕⊕⊕ High[d]	MCO-HD increases β2M reduction ratio.
Predialysisβ2M Subgroup with <12-week follow-up Follow-up: 8 weeks No. of participants: 32 (1 RS	-	SMD 0.36 SD higher (0.33 lower to 1.06 higher)	⊕⊕◯◯ Low[e]	MCO-HD likely esults in little to no difference in predialysis serum albumin after 24 weeks of treatment.
Predialysis β2M Subgroup with ≥12-week follow-up Follow-up: range, 12-26 weeks No. of participants: 403 (5 RS)	-	SMD 0.54 SD lower (1 lower to 0.08 lower)	⊕⊕⊕◯ Moderate[f]	MCO-HD likely reduces predialysis β2M after 3 months of treatment.
Predialysis β2M Follow-up: range, 24-52 weeks No. of participants: 438 (6 NRS)	-	SMD 0.43 SD lower (0.84 lower to 0.002 lower)	⊕⊕⊕⊕ High	MCO-HD reduces predialysis β2M slightly.
Myoglobin removal Follow-up: 2 weeks No. of participants: 120 (3 RS)	-	SMD 2.9 SD higher (1.31 higher to 4.49 higher)	⊕⊕⊕⊕ High	MCO-HD likely results in a large increase in myoglobin removal.
Myoglobin reduction ratio (%) Follow-up: range, 2-26 weeks No. of participants: 242 (5 RS)	The mean myoglobin reduction ratio (%) ranged from 8% to 45%	MD 30.26% higher (15.5 higher to 45.03 higher)	⊕⊕⊕⊕ High[g]	MCO-HD results in large increase in myoglobin reduction ratio.
Myoglobin reduction ratio (%) Follow-up: range, 2-52 weeks No. of participants: 118 (6 NRS)	The mean myoglobin reduction ratio (%) ranged from 12% to 44%	MD 27.62% higher (24.29 higher to 30.95 higher)	⊕⊕⊕⊕ High	MCO-HD results in large increase in myoglobin reduction ratio.
Predialysis myoglobin Follow-up: 26 weeks No. of participants: 130 (2 RS)	-	SMD 0.51 SD lower (0.85 lower to 0.16 lower)	⊕⊕⊕◯ Moderate[h]	MCO-HD likely reduces predialysis myoglobin.
Predialysis myoglobin Follow-up: 26 weeks No. of participants: 82 (1 NRS)	-	SMD 0.12 SD lower (0.55 lower to 0.31 lower)	⊕⊕⊕◯ Moderate[b]	MCO-HD likely reduces myoglobin prehaemo-dialysis slightly.

Table 2. *continued*

Outcome No. of participants (studies)	Anticipated absolute effects (95% CI) Without MCO-HD	 Difference	Certainty	What happens
Kappa FLC removal Follow-up: 2 weeks No. of participants: 78 (2 RS)	-	SMD 3.89 SD higher (3.45 higher to 4.33 higher)	⊕⊕⊕⊕ High	MCO-HD results in large increase in kappa FLC removal.
Kappa FLC reduction r atio (%) Follow-up: range, 2-26 weeks No. of participants: 249 (5 RS)	The mean kappa FLC reduction ratio (%) ranged from 53% to 72%	MD 14.85% higher (8.27 higher to 21.43 higher)	⊕⊕⊕⊕ High[g]	MCO-HD results in large increase in kappa FLC reduction ratio.
Predialysis kappa-FLC Follow-up: range, 12-26 weeks No. of participants: 403 (5 RS)	-	SMD 0.39 SD lower (0.61 lower to 0.16 lower)	⊕⊕⊕⊕ High	MCO-HD reduces predialysis kappa FLC slightly.
Lambda FLC removal Follow-up: 2 weeks No. of participants: 118 (3 RS)	-	SMD 2.16 SD higher (1.8 higher to 2.52 higher	⊕⊕⊕⊕ High	MCO-HD results in large increase in lambda FLC removal.
Lambda FLC removal Follow-up: range, 2-3 weeks No. of participants: 130 (2 NRS)	-	SMD 3.71 SD higher (2.97 higher to 4.45 higher)	⊕⊕⊕⊕ High	MCO-HD results in large increase in lambda free light chain removal.
Lambda FLC reduction ratio (%) Follow-up: range, 2-26 weeks No. of participants: 450 (7 RS)	The mean lambda FLC reduction ratio (%) ranged from 13% to 41%	MD 20.85% higher (15.53 higher to 26.16 higher)	⊕⊕⊕⊕ High	MCO-HD increases lambda-FLC reduction ratio.
Predialysis lambda-FLC Follow-up: range, 12-26 weeks No. of participants: 402 (5 RS)	-	SMD 0.53 SD lower (0.9 lower to 0.17 lower)	⊕⊕⊕⊕ High[i]	MCO-HD reduces predialysis lambda-FLC.
Predialysis lambda-FLC Follow-up: range, 24-52 weeks No. of participants: 398 (4 NRS)	-	SMD 0.34 SD lower (0.54 lower to 0.14 lower)	⊕⊕⊕⊕ High	MCO-HD reduces ambda free light chain prehaemo-dialysis slightly.

Note. CI = confidence interval; MCO-HD = haemodialysis with **MCO** membrane; β2M = β2-microglobulin; RS = randomised study; SMD = standardised mean difference; SD = standard deviation; NRS = nonrandomised study; MD = mean difference; FLC = free light chains.

[a]I2 = 97%, but fully explained by measurement method—removal was higher when measured by plasma clearance versus dialysate quantitation.

[b]Small overall sample size; optimal information size criterion not met.

[c]SMD >0.8 considered a large treatment effect. Rated up 1 level.

[d]Although I2 was 99%, heterogeneity was explained by baseline removal ratio (larger effect if removal ratio was <70%), and was further explained by study duration (effect was attenuated with long-term treatment).

[e]Downgraded 2 levels for imprecision with only very small sample size and confidence interval crossing no effect.

[f]I2 > 50% and confidence intervals do not overlap.

[g]Inconsistency explained by baseline removal ratio such that studies with lower baseline RR had larger effects with **MCO** membrane-HD.

[h]I2 = 82% with opposite directions of effect.

[i]Inconsistency explained by duration of follow-up with a larger treatment effect with long-term treatment.

Cytokines and Inflammatory Markers

Use of the **MCO** membrane increased the reduction ratio for tumour necrosis factor-alpha (TNF-α) by 7.7% (95% CI, 4.7 to 10.6; moderate certainty), and reduced predialysis TNF-α by standardised mean difference (SMD) −0.48 (95% CI, −0.91 to −0.04; moderate certainty). This review showed with moderate certainty that dialysis with the **MCO** membrane had little to no effect on predialysis interleukin-6 (IL-6) plasma concentrations. Medium cut-off dialysis reduced mRNA expression of TNF-α and IL-6 in peripheral leukocytes by mean difference (MD) −15% (95% CI, −19.6 to −10.4; moderate certainty) and −8.8% (95% CI, −10.2 to −7.4; moderate certainty), respectively. There was little or no effect on C-reactive protein.

TABLE 3. Summary of findings - Inflammatory Markers and Cytokines

Outcome No. of participants (studies)	Anticipated absolute effects (95% CI) Without MCO-HD	 Difference	Certainty	What happens
IL-6 reduction ratio (%) Follow-up: 26 weeks No. of participants: 80 (1 RS)	The mean IL-6 reduction ratio (%) was 9.5%	MD 0.2% lower (3.44 lower to 3.04 higher)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely results in little to no difference in IL-6 reduction ratio (%).
Predialysis IL-6 Follow-up: range, 12-26 weeks No. of participants: 354 (4 RS)	-	SMD 0.04 SD higher (0.17 lower to 0.25 higher)	⊕⊕⊕◯ Moderate[b]	MCO-HD likely results in little to no difference in predialysis IL-6.
IL-6 mRNA expression Follow-up: 12 weeks No. of participants: 46 (1 RS)	The mean IL-6 expression was 100%	MD 8.8 % lower (10.2 lower to 7.4 lower)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely reduces IL-6 expression
TNF-α reduction ratio (%) Follow-up: 26 weeks No. of participants: 80 (1 RS)	The mean TNF-αreduction ratio (%) was 26%	MD 7.67% higher (4.7 higher to 10.64 higher)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely increases TNF-αreduction ratio.
TNF-α predialysis Follow-up: range, 12-26 weeks No. of participants: 304 (3 RS)	-	SMD 0.48 SD lower (0.91 lower to 0.04 lower)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely reduces predialysis TNF-α.
TNF-α mRNA expression Follow-up: 12 weeks No. of participants: 46 (1 RS)	The mean TNF-α expression was 100%	MD 15% lower (19.6 lower to 10.4 lower)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely reduces TNF-α expression.
C-reactive protein Follow-up: 12 weeks No. of participants: 145 (2 RS)	-	SMD 0.04 SD higher (0.37 lower to 0.29 higher)	⊕⊕⊕◯ Moderate[a]	MCO-HD likely results in little to no difference in C-reactive protein.
C-reactive protein Follow-up: range, 26-52 weeks No. of participants: 1940 (5 NRS)	-	SMD 0 SD (0.23 lower to 0.22 higher)	⊕⊕⊕⊕ High	MCO-HD results in little to no difference in C-reactive protein.

Note. CI = confidence interval; MCO-HD = haemodialysis with **MCO** membrane; IL-6 = interleukin-6; RS = randomised study; MD = mean difference; SMD = standardised mean difference; SD = standard deviation; TNF-α = tumour necrosis factor-alpha; NRS = nonrandomised study.
[a]Small overall sample size; optimal information size criterion not met.
[b]Small overall sample size and the confidence interval includes no effect.

DISCUSSION AND CONCLUSIONS

This review showed with high certainty that dialysis with the **MCO** membrane removes approximately 2 g of albumin per 4-hour conventional haemodialysis session, resulting in a transient decreased serum albumin level of 0.12 g/dl over the short term (<24 weeks), which then returned to baseline. It was shown with moderate to high certainty that compared with high-flux membranes, the **MCO** membrane increases middle-molecule clearance of β2 microglobulin, κ-FLC, λ-FLC, and myoglobin—solutes representing the full spectrum of large middle molecular weights. Little to no effect was seen on IL-6 removal or predialysis levels, while IL-6 mRNA expression was reduced by 8.8% in peripheral leukocytes. Medium cut-off dialysis increased the reduction ratio of TNF-α with a moderate reduction in predialysis levels and reduced peripheral leukocyte mRNA expression by 15%.

Taken together, these findings are consistent with the anticipated effects of the **MCO** membrane, and may account for improved clinical outcomes that have been associated with the **MCO** membrane including reduced symptom burden, recovery time, infection, hospital length of stay, and quality of life.

Strengths and Limitations

Strengths of this systematic review and meta-analysis comparing the **MCO** membrane with high-flux membranes include adherence to a rigorous registered protocol, a sensitive search strategy, performing study procedures in duplicate, and the use of GRADE methods. The lack of validation of the included biomarkers as surrogate outcomes is a major limitation in this review. Although they are associated with important physiological processes and clinical outcomes, none of the included biomarkers meet regulatory or statistical criteria for surrogacy.

Therefore, despite their familiarity and frequent use in dialysis trials and guidelines, the authors caution against the sole use of biomarkers in clinical or other decision-making. Further limitations of this review include the exclusion of small solutes and lack of direct comparisons with convective therapies.

Conclusion

Compared with high-flux membranes, the **MCO** membrane increases the elimination of large middle molecules, resulting in decreased predialysis solute concentrations of solutes ranging between 11.8 and 45 kDa. Although dialysis with the **MCO** membrane did not normalise serum concentrations of these solutes, the net effect of enhanced clearance within the large middle-molecule spectrum could explain the range of beneficial clinical effects reported to date.

Evidence generated to date supports that the MCO Theranova membrane increases the clearance of a wide range of large middle molecules and likely reduces inflammatory mediators, with a minimal transient reduction in serum albumin concentration.

Medium cut-off membranes—closer to the natural kidney removal function

Zweigart C, Boschetti-de-Fierro A, Hulko M, Nilsson L-G, Beck W, Storr M, Krause B.
Medium cut-off membranes—closer to the natural kidney removal function. *Int J Artif Organs*. 2017;40(7):328-334. doi: 10.5301/ijao.5000603.

BACKGROUND

Renal replacement therapy uses dialysis membranes that have improved over the decades. Cellulose membranes have been largely replaced by more biocompatible synthetic polymeric membranes. Newer membranes more efficiently remove different sizes of uraemic toxins. Membranes with larger pore size have been developed to filter higher-molecular weight substances, although these high cut-off membranes unfortunately allow the loss of essential plasma proteins such as albumin.

SUMMARY

Dialysis membranes with different permeabilities have been developed

Haemodialysis (HD) is a process whereby blood is removed from the patient, passed through a dialyser membrane, then returned to the patient's blood stream. From the 1940s through the 1970s, cellulose membranes were used. Cellulose membranes had several medical disadvantages: they could activate the complement system and induce adverse biological reactions (e.g., leucopenia), inhibit granulocyte metabolism, and release enzymes from granulocytes and monocytes. To mitigate these drawbacks of cellulose membranes, the cellulose' hydroxyl groups were modified in one of the following ways: acetylation (yielding cellulose acetate, diacetate, or triacetate membranes) or substitution using diethylamino groups or benzyl groups. Additionally, surface coating of the membrane with polyethylene glycol (PEG), polyacrylonitrile (PAN-REC) or vitamin E may improve biocompatibility.

Regardless of modifications, cellulose dialisers are "low-flux" membranes, meaning that they have low permeability to solutes, and they function through diffusion alone rather than convection. As a result, small toxins (up to 5000 Da) may be eliminated, but larger ones (like β2-microglobulin) are not. For haemofiltration (HF) and haemodiafiltration (HDF), membranes with higher permeability are required, so synthetic polymeric membranes were developed.

Synthetic polymeric membranes are generally hydrophobic (mainly polysulfone or polyethersulfone) or hydrophobic/hydrophilic polymer blends that are considered high flux. High-flux membranes are permeable to β2-microglobulin and allow passage of "middle molecules" (i.e., up to 20,000 Da) but still prevent significant protein loss; these may be used for HD, HF, HDF.

Additionally, high cut-off membranes have been developed for use in specific clinical conditions. These have pore sizes that allow permeability of substances >15,000 Da, including inflammatory cytokines and light chains of immunoglobulins. While these may be used to treat patients with sepsis, severe inflammation, or multiple myeloma, albumin loss may reach 9 to 23 g per treatment, so these membranes are used only for acute applications, not chronic care. Clearing protein-bound uraemic toxins without removing excessive amounts of protein has been an unmet need.

The newest generation of highly selective and permeable medium cut-off membranes aims to meet this need by removing substances up to 45,000 Da while providing low albumin loss. These membranes offer improved clearance of uraemic toxins in the 15,000 to 45,000 Da range as compared with older high-flux membranes used in HD. They may be equivalent to older high-flux membranes set in HDF mode.

Different dialysis therapies may affect uraemic toxin removal

In patients receiving HD maintenance therapy for endstage kidney disease, insufficient removal of mid-sized and protein-bound uraemic toxins has been associated with endothelial injury and chronic inflammation, which contribute to cardiovascular disease, coordination disturbances, or polyneuritis. Uraemic toxins are generally considered in three groups, as shown in Table 1: small water-soluble compounds, middle molecules, and protein-bound solutes. Numerous toxins exist; select examples are shown in Table 1.

TABLE 1. Examples of uraemic solutes and their molecular weights

Molecule	Molecular Weight	Examples
Small water-soluble compounds	<500 Da	asymmetric dimethylarginine, guanidine, uric acid, oxalate, ethylamine, methylguanidine, neopterin, phenylacetic acid
Middle molecules	>500 Da	β2-microglobulin, adiponectin, α1-acid glycoprotein, cystatin C, prolactin, osteocalcin, vascular endothelial growth factor
Protein-bound solutes	Variable	p-cresylsulfate, indoxyl sulfate, phenol, indol-3-acetic acid, hippuric acid, homocysteine, carboxymethyllysine, acrolein

Table adapted from Zweigart et al.

Small water-soluble compounds may be easily removed, even across a diffusive membrane using conventional HD. As molecular size and weight increase, however, the diffusion coefficient decreases, which is why middle molecules such as β2-microglobulin are not easily removed with HD, even when high-flux membranes are used.

In contrast to diffusion methods, convective transport mechanisms are driven by a pressure gradient across the

membrane, which leads to ultra filtration (UF), in which molecules are pulled from the dialysate. Increased UF leads to increased removal of both low- and middle-molecular weight solutes. Treatments such as HF and HDF include convective transport mechanisms and are thus associated with increased removal of low- and middle-molecular weight solutes. Note that the UF rate is limited by the blood flow rate; only 25% to 30% of the total volume may be filtered before blood cell concentrations may cause cellular damage and dialyser clotting.

Online HDF post-dilution is generally considered the safest and most effective dialysis treatment because of its superior removal of uraemic toxins and because it is associated with a lower incidence of cardiovascular events. Set up for online HDF is complex, and high exchange volume rates (up to 24L/treatment) are required. Automation optimises the filtration fraction but convection volume is still determined by blood flow rate and treatment time. HDF removes more protein-bound uraemic solutes than other dialysis methods. Compared to non-protein-bound solutes of similar weight, however, protein-bound solutes are not removed as efficiently; total removal of protein-bound toxins by HDF depends on how rapidly the toxins may be freed from their protein carrier.

To enhance removal of some protein-bound toxins, adsorptive treatments have been developed, but adsorption of protein-bound toxins is ultimately associated with loss of blood proteins. Protein-leaking membranes for HD offer greater clearance of protein-bound toxins than conventional high-flux membranes, but some albumin loss still occurs.

Limits of albumin removal are unclear

Low serum albumin is associated with mortality in patients with end-stage renal disease (ESRD). Conventional high-flux membranes are associated with a loss of 0 to 2 g albumin per 4-hour treatment. Albumin loss varies with treatment modality, though, and with treatment parameters. The amount of albumin lost with online HDF treatments varies with dilution mode, degree of flux across a membrane, type of membrane, and other treatment parameters. Reuse of dialysers after bleaching results in excessive protein removal levels (10 to 12 g), though an albumin removal rate of 4.3 g/session has been reported when bleach reprocessing was limited. Typical albumin loss per online HDF treatment is 1 to 4 g, but it can be > 5 g in some instances. It is unclear how much albumin loss is tolerated in ESRD patients. A summary of middle molecule results across studies is shown in Table 2.

Dialysis membranes with different permeabilities have been developed

Higher-molecular weight uraemic toxins are associated with dialysis comorbidities including chronic inflammation and related cardiovascular disease, immune dysfunction, anaemia, and EPO hyper responsiveness. As discussed, high cut-off membranes remove more of these toxins than high-flux membranes, but also remove more essential proteins, which is why the **MCO** membrane is needed.

For the new generation **MCO** membrane, the manufacturer optimises polymers, spinning, and dialyser production to make safer, improved products. Improvements such as fibre undulation, high package density, improved flow distribution of dialysate fluid, and decreased fibre diameter have improved dialyser performance. Removing middle-molecular weight toxins while retaining blood proteins requires an extremely narrow pore-size distribution in the dialysis membrane that can only be made using newer, well-controlled spinning technology. Ideally, **MCO** membrane properties are intended to be closer to those of the natural kidney than are existing dialysers.

Table 2 describes the basic categories of membranes in comparison with the **MCO** membrane. In terms of sieving coefficients, the **MCO** membrane resembles HCO and protein-leaking membranes in their ability to capture β2-microglobulin but they resemble low-flux and high-flux membranes in terms of protein retention.

TABLE 2. General classifications and typical performance of membranes used in dialysis

Dialyser type	Water permeability[a] (mL/(m²*h*mmHg))	Sieving coefficient	
		β2 Microglobulin	Albumin
Low-flux	10-20	—	<0.01
High-flux	200-400	0.7-0.8	<0.01
Protein-leaking	50-500	0.9-1.0	0.02-0.03
High cut-off	1100	1.0	0.2
Medium cut-off	600-850	1.0	0.008

[a] with 0.9% wt.-% sodium chloride at 37°C ± 1°C and QB 100-500 mL/min.
[b] according to EN1283.

In vitro data support the use of the **MCO** membrane in conventional treatment schedules and treatment modes, such as 4-hour treatments thrice weekly.

At the time of this review, two clinical trials had compared **MCO** dialysers with the recent generation of high-flux dialysers. In the first trial, two centres in Austria compared three prototype **MCO** dialysers (called **MCO** AA, BB, CC) with the high-flux dialyser FX CorDiax 80 in 19 patients with ESRD. The primary outcome measure was clearance of λ-free light chains (λ -FLCs) with a molecular weight of ~45,000 Da. Secondary outcomes included removal of other medium-sized solutes (κ–Ig free light chains, α1- and β2-microglobulins, complement factor D) and small solutes. All three prototypes achieved significantly higher clearances of λ -FLCs than did the high-flux membrane (**MCO** AA, 8.5 ±0.54; BB, 11.3 ± 0.51; CC, 15.0 ±0.53 vs. FX CorDiax 80, 3.6 ±0.51 mL/min). The removal of other medium-sized solutes was significantly greater with **MCO** membranes (including κ-FLC). The total mass of albumin removed was moderate with **MCO** membranes (medians [range]: **MCO** membrane AA, 2.9 g [1.5 – 3.9]; BB, 4.8 g [2.2 – 6.7]; CC, 7.3 g [1.9 – 9.7] vs. FX CorDiax, < 0.3 g [< 0.3 – < 0.3]).

In the second study from Germany, 2 **MCO** prototype dialysers (called **MCO** AA and BB), used in HD mode were compared to two high-flux dialysers (FX CorDiax 80 and FX CorDiax 800) used in HD and high-volume HDF modes (post-dilution volume-controlled mode with a target convective UF volume of ≥23 L). The primary outcome measure was overall clearance of λ -FLC. There was greater overall clearance of λ -FLC by both of the **MCO** dialysers in HD mode than by either of the high-flux dialysers (least squares mean (standard error)): **MCO** dialyser AA, 10.0 (0.57); **MCO** dialyser BB, 12.5 (0.57); vs. high-flux HD 4.4 (0.57) and HDF 6.2 (0.58) mL/min). The clearances of α1-microglobulin, complement factor D, κ-FLC, and myoglobin were generally greater for the **MCO** dialyser than for high-flux HD and similar or greater than in HDF treatments,

whereas the albumin removal was moderate with the **MCO** dialyser but greater than that of high-flux HD and HDF (medians [range]: **MCO** dialyser AA, 3.2 g [1.9 – 3.9]; **MCO** dialyser BB, 4.9 g [1.1 – 7.2] vs. FX CorDiax 80, 0.2 g [0.2 – 0.9]; FX CorDiax 800, 0.4 g [0.3-0.8]).

These studies indicate that **MCO** membranes effectively remove a wide range of middle molecules, substantially better than high-flux HD or even HDF, with moderate removal of albumin. Albumin removal in these studies is similar to what has been reported in the HDF literature. Thus, use of the **MCO** dialyser in routine HD mode would be expected to be safe.

CONCLUSIONS

According to this editorial review, the newest generation of highly selective and permeable **MCO** membrane:

> Provides efficient removal of large middle-molecules (up to 45,000 Da) while providing moderate or low removal of albumin

> Offers improved clearance of 15,000 to 45,000 Da size molecules compared with high-flux membranes used in HD mode

> Offers equivalent clearance of middle molecules compared with high-flux membranes in high-volume HDF mode

> May potentially offer similar benefits as high-volume HDF without the need for high volumes of fluid and the vascular access required for high blood flow rates

> Offers a simpler means of achieving high-removal therapy for ESRD patients

> Has potential to achieve HDF benefits using the regular HD setup with lesser volume of high-quality fluid

The **MCO** membrane has the potential to raise the standard of care for chronic HD patients, potentially decrease inflammatory responses, and generally improve patient outcomes.

Vantive

HDx therapy: A world of difference

Performance of HDx therapy: combination of diffusion and convection inside a hollow fibre dialyser to achieve LMM removal

HDx therapy targets the efficient removal of large-middle molecules, many of which have been linked to the development of inflammation, cardiovascular disease, and other co-morbidities in dialysis patients[1,2], while maintaining stable serum albumin levels over the long term.[3,4] **HDx** therapy enabled by **Theranova** dialyser provides superior removal of large middle molecules compared with HD and HDF modalities and it can do so using regular HD workflow and infrastructure.[5]

Kirsch AH, Lyko R, Nilsson LG, et al.
Performance of Haemodialysis with Novel Medium Cut-Off Dialysers. *Nephrol Dial Transplant* 2017:32:165-172.

Krishnasamy R, Hawley CM, Jardine MJ, et al.
A tRial Evaluating Mid Cut-Off Value Membrane Clearance of Albumin and Light Chains in HaemoDialysis Patients (REMOVAL-HD): A Safety Device Study. *Blood Purif.* 2020;49(4):468-478.

Bunch A, Sanchez R, Nilsson LG, et al.
Medium Cut-Off Dialysers in a Large Population of Haemodialysis Patients in Colombia: COREXH Registry. *Ther Apher Dial.* 2021;25(1):33-43.

Belmouaz M, Diolez J, Bauwens M, al.
Comparison of Haemodialysis with Medium Cut-Off Dialyser and On-Line haemodiafiltration on the Removal of Small and Middle-Sized Molecules. *Clin Nephrol.* 2018;89(1)50-56.

Weiner DE, Falzon L, Skoufos L, et al.
Efficacy and Safety of Expanded Haemodialysis with the Theranova 400 Dialyser: A Randomised Controlled Trial. *CJASN.* 2020;15(9):1310-1319.

Hagemann F, Linkhorst J, Roth H, Wessling M.
On the balance between albumin loss and removal of middle molecules in Dialysers. *Journal of Membrane Science Letters.* 2023;3(1):100044.

1. Ronco C, et al. The rise of Expanded Haemodialysis. *Blood Purif.* 2017;44:I–VIII.. Doi: 10.1159/000476012.
2. Hutchinson CA, et al. The Rationale for Expanded Haemodialysis Therapy (HDx): *Contrib Nephrol*. 2017;191:142-152.
3. Weiner D.E., Falzon L., Skoufos L., et al. Efficacy and safety of expanded haemodialysis with the Theranova 400 dialyser: a randomised controlled trial. *Clin J Am Soc Nephrol.* 2020; 15: 1310-1319.
4. Molano AP, Hutchison CA, Sanchez R, et al. Medium Cutoff Versus High-Flux Haemodialysis Membranes and Clinical Outcomes: A Cohort Study Using Inverse Probability Treatment Weighting. *Kidney Med*. 2022;4(4):100431.
5. Kirsch AH, Lyko R, Nisson LG, et al. Performance of Haemodialysis with Novel Medium Cut-Off Dialysers. *Nephrol Dial Transplant* 2017:32:165-172.

Performance of Haemodialysis with Novel Medium Cut-Off dialysers

Kirsch AH Lyko R, Nilsson LG, et al. Performance of haemodialysis with novel medium cut-off dialysers. *Nephrol Dial Transplant*. 2017; 32:165-172. doi:10.1093/ndt/gfw310.

BACKGROUND

End-stage renal disease (ESRD) results in the retention of uraemic toxins, which is associated with high mortality. Uraemic toxins are classified into small (< 500 Da) and middle molecular (500 Da–60 kDa) water-soluble solutes and protein-bound substances. While conventional haemodialysis (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 uraemia, 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 β2-microglobulin (12 kDa) and increasing convection with haemodiafiltration (HDF) considerably enhanced middle molecule clearance. However, high flux dialysers 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 dialysers utilise 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 membranes, are intended for routine use in maintenance HD patients.

OBJECTIVES

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

METHODOLOGY

Study Design

The two studies were prospective, open-label, 4-arm, randomised, active-control, crossover pilot studies comparing **Theranova** 400 dialyser (MCO AA; Gambro Dialysatoren GmbH, Hechingen, Germany, a subsidiary of Vantive Health LLC) and two **MCO** dialyser prototypes (**MCO** BB and CC) with high-flux dialysers 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** dialysers had increased permeability (AA < BB < CC). **MCO** membranes 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** membrane AA (**Theranova** 400 dialyser), BB, and CC with high-flux dialysers (FX CorDiax 80), while study 2 compared **MCO** membrane AA (**Theranova** 400 dialyser) and BB with high-flux dialysers (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 centre in Germany. The dialysis sessions in study 1 were 4 hours with a dialysate flow (QD) of 500 mL/min and a blood flow (QB) 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 enrolment, had a κFLC/λFLC ratio of > 0.37 and < 3.1, and no history of monoclonal gammopathy were enrolled and randomised to each study dialyser 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** dialyser prototypes in HD mode vs. K_{ovr} using high-flux dialysers 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** membrane HD.

RESULTS

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

Overall Clearance

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

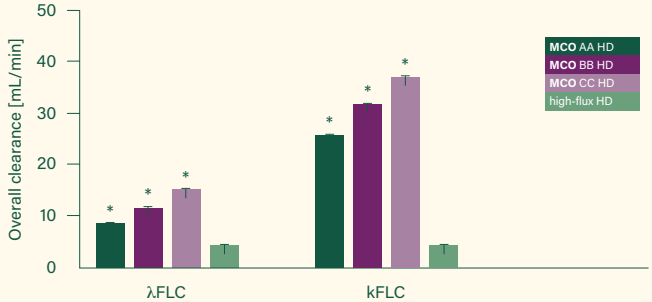


Figure 1A. Overall Clearance. Free immunoglobulin light chain removal during haemodialysis with mediums cut-off Dialysers and high-flux dialyser in study 1. Data are least square mean + standard error. *p < 0.001 compared to high-flux dialyser. Abbreviations: FLC, free light chain; **MCO** membrane,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

Test Dialyser	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 mean mL/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 haemodialysis with medium cut-off dialysers and high-flux dialysers in study 1. Data are least square mean + standard error. Abbreviations: FLC, free light chain; **MCO** membrane, medium cut-off dialyser; HD, haemodialysis. Adapted from Kirsch et al.

Reduction Ratios

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

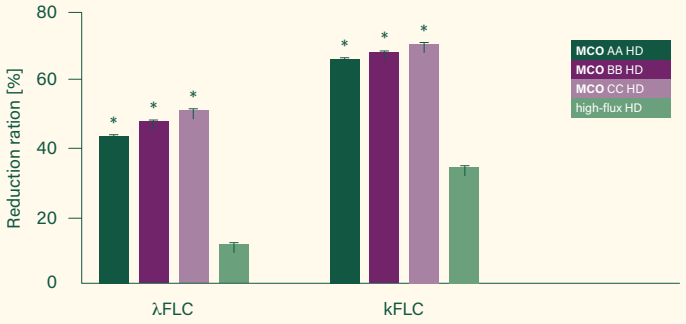


Figure 1B. Reduction Ratio. Free immunoglobulin light chain removal during haemodialysis with medium cut-off dialysers and high-flux dialyser in study 1. Data are least square mean + standard error. P < 0.001 compared to high-flux dialyser. Abbreviations: FLC, free light chain; **MCO** membrane, medium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

Test Dialyser	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)	

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

Study 2: Free Light Chain Removal During HD Using MCO dialysers 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** membrane 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.

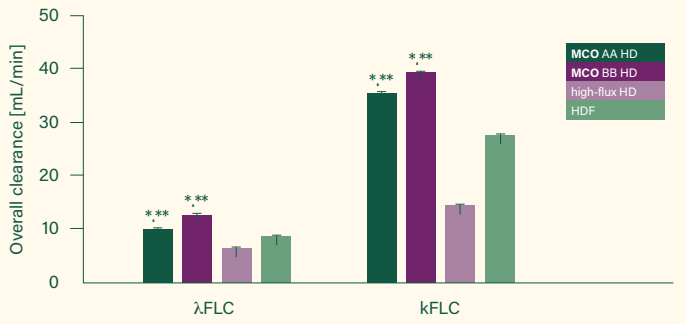


Figure 2A. Overall Clearance. Free immunoglobulin light chain removal during haemodialysis with medium cut-off dialysers and high-flux dialyser and haemodiafiltration in study 2. Data are least square mean + standard error. *p <0.001 compared to high-flux HD; **p < 0.001 compared to HDF. Abbreviations: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

Test Dialyser	λFLC K_{ovr} Overall Clearance		κFLC K_{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 haemodialysis with medium cut-off dialysers and haemodiafiltration in study 2. Data are least square mean + standard error. Abbreviations: FLC, free light chain; **MCO** membrane, medium cut-off dialyser; HDF, haemodiafiltration. Adapted from Kirsch et al.

Reduction Ratios

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

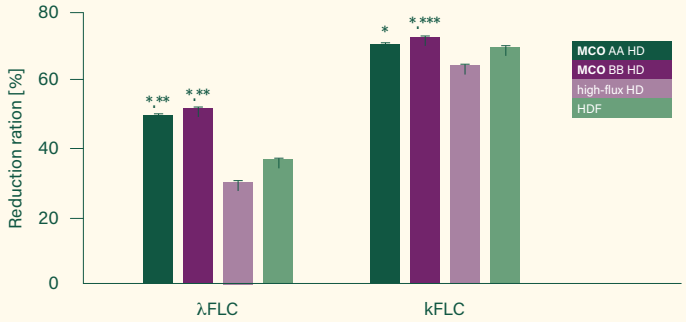


Figure 2B. Reduction Ratio. Free immunoglobulin light chain removal during haemodialysis with medium cut-off dialysers and high-flux dialyser and haemodiafiltration in study 2. Data are least square mean + standard error*p <0.001 compared to high-flux HD; **p < 0.001 compared to HDF; ***p p=0.01, compared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

Test Dialyser	λFLC K_{ovr} Reduction Ratio		κFLC_{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 haemodialysis with medium cut-off dialysers and high-flux dialysers and haemodiafiltration in study 2. Data are least square mean + standard error. Abbreviations: FLC, free light chain; **MCO** membrane, medium cut-off dialyser; HD, haemodialysis; HDF, haemodiafiltration. 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** membrane HD (MCO AA/ **Theranova** 400 dialyser) 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** membrane 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.

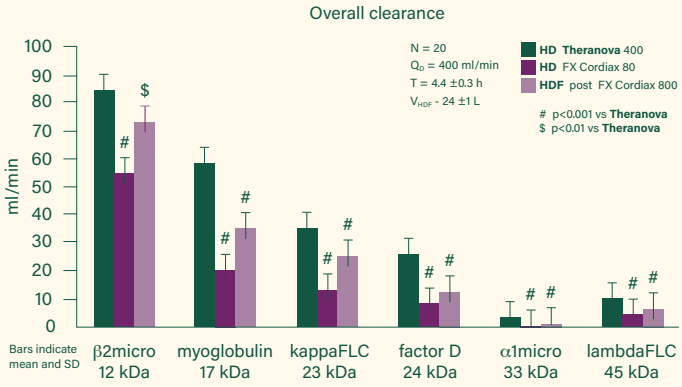


Figure 3A. Overall clearances of medium-sized and small molecules. Comparisons are based on a mixed model with fixed effects of period and study dialyser type, and the random effect of subject. Abbreviations: HD, haemodialysis; HDF, haemodiafiltration, SD: standard deviation.

The reduction ratios were significantly greater for **MCO** membrane AA HD (**Theranova** 400 dialyser) 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** membrane 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** membrane HD more efficiently removes larger middle molecules. See Figure 3B.

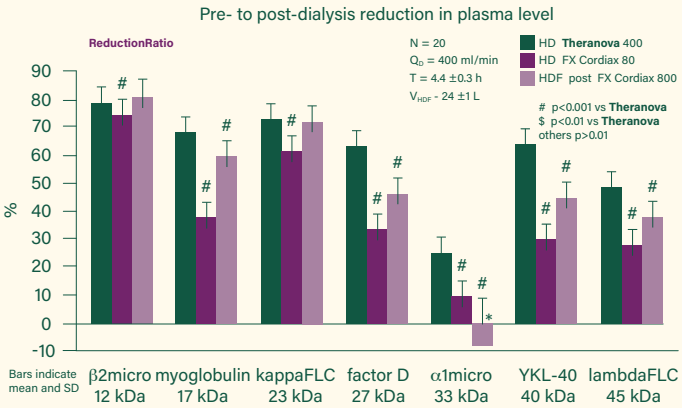


Figure 3B. Reduction ratios of medium-sized and small molecules. Comparisons are based on a mixed model with fixed effects of period and study dialyser type, and the random effect of subject. Abbreviations: HD, haemodialysis; HDF, haemodiafiltration, SD: standard deviation.

Albumin Removal During MCO membrane HD

Increasing pore sizes, while permitting clearance of larger uraemic toxins, bears the trade-off of the leaking of larger molecules such as albumin (65 kDa)[1]. In both studies, **MCO** dialysers resulted in larger albumin removal than high-flux HD and HDF. However, for **MCO** membrane 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 dialyser was within range observed in HDF treatment with high flux dialysers, below transperitoneal albumin losses seen in peritoneal analysis, and less than a third of what has been reported for HD with High Cut-off membranes. See Table 3.

Test Dialyser	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** membrane, medium cut-off dialyser; HD, haemodialysis; HDF, haemodiafiltration; nv, no value. Table adapted from Kirsch et al.

Safety

In study 1, the adverse events (AEs) between the study dialysers 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 hospitalisation 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 dialyser and one in FX CorDiax 80 dialyser (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 dialyser 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 characterisation of HD membrane clearance for an extensive size range of middle molecules (11.6 – 45 kDa). **MCO** membrane 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** membrane 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 dialyser specifications. Importantly, **MCO** membrane HD/**Theranova** dialyser can be applied to maintenance HD patients, in whom high volume HDF may not be used or is not available.

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

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

Krishnasamy R, Hawley CM, Jardein MJ,. A trial evaluating mid out-off value membrane clearance of albumin and light chains in haemodialysis patients (REMOVAL-HD): a safety device study. *Blood Purif.* 2020; 1-11. doi: 10.1159/000505567.

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 hospitalisation, reduced quality of life and increased risk of death. Retention of uraemic 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 dialysers and haemodiafiltration (HDF), the efficiency of middle molecule clearance by chronic haemodialysis (HD) has continually increased; however, the clearance of almost a third of the larger middle molecules (>25kDa) is yet to be optimised. 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 dialyser were associated with substantial albumin loss (molecular weight (MW) (65kDa[1]), requiring albumin supplementation following dialysis treatment. This resulted in the view that high cut-off 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 dialyser. This membrane has a pore size between that of a standard high flux and an high cut-off membrane with narrowly distributed pores to enhance membrane permeability and selectivity. However, the safety and efficiency data for the **MCO** dialyser 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 Haemodialysis Patients (REMOVAL-HD) study the was to determine the safety of HD using a **MCO** dialyser (**Theranova** dialyser; Vantive Health, 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-randomised, cross over, longitudinal device study conducted across 9 centres 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-centre HD for at least 12 weeks.

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 dialyser (**Revaclear** R400; Vantive Health, Sydney, Australia).

> **Intervention period (week 4-28)**
Participants then received 24 weeks of treatment with the investigational device, the **MCO** dialyser (**Theranova** 400 dialyser; Vantive Health, Sydney, Australia).

> **Wash-out period (week 28-32)**
Participants then received a 4-week wash-out period using the **Revaclear** high flux dialyser again.

Centres 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** dialyser. 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.

Study Procedures

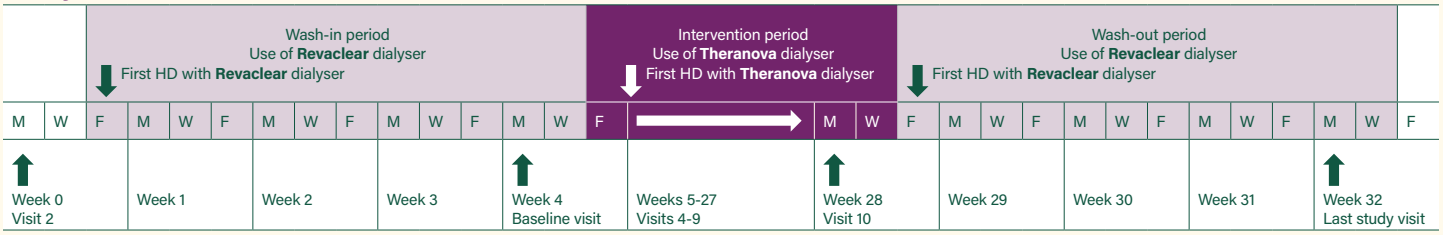


FIGURE 1. REMOVAL-HD study schema. Adapted from Krishnasamy et al. Abbreviations: HD, haemodialysis.

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 β2-microglobulin (11.8 kDa)).

RESULTS

Eighty-nine (89) participants started the **MCO** dialyser HD intervention and provided analysable data and 87 were sufficiently compliant with the intervention (at least 80% use of **MCO** dialyser) 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** dialyser 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.

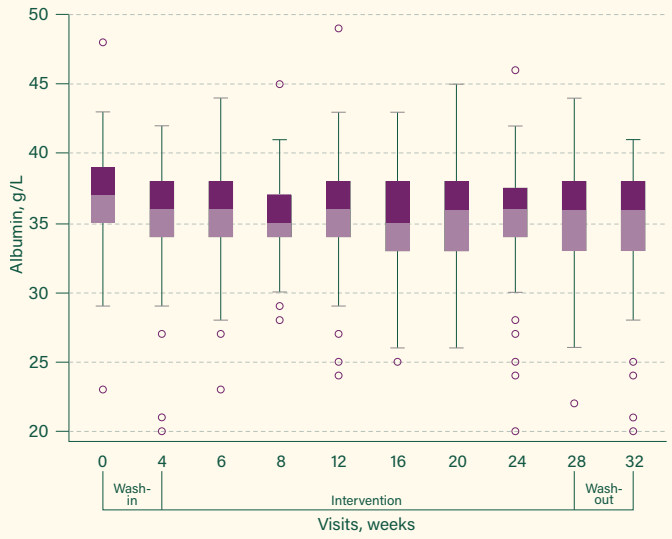


FIGURE 2. Measurement of serum albumin and reduction. Adapted from Krishnasamy, et al. Abbreviations: CI, confidence interval.

Middle Molecules

A reduction in FLC was observed 2 weeks into **MCO** dialyser 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** dialyser during the wash-out of high-flux HD phase. (see Table 2). The rebound supports the hypothesis that this dialyser 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** dialyser HD is a promising therapy to enhance removal of other large middle molecules such as soluble tumour 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 dialyser (week 28-32) respectively. Adapted from Krishnasamy et. al. Abbreviations: CI, confidence interval; FLC, free light chains.

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

Adverse Events

There were no reported SAE's related to the **MCO** dialyser 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** dialyser on serum albumin.

CONCLUSIONS

REMOVAL-HD demonstrated that regular use of **MCO** dialyser 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** dialyser in chronic HD. In addition, the significant rebound of FLC levels following cessation of HD with the **MCO** dialyser supports the hypothesis that this dialyser 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 DIALYSER'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.

Medium Cut-Off dialysers in a Large Population of Haemodialysis Patients in Colombia: COREXH Registry

Bunch A, Sanchez R, Nilsson LG, et al. Medium cut-off dialysers in a large population of haemodialysis 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 haemodialysis utilising medium cut-off membranes with high retention onset membranes. The **MCO** membrane easily clears 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 haemodialysis (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 Vantive 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 the **MCO** membrane, 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** therapy utilising an **MCO** membrane and document the long-term safety.

METHODOLOGY

Expanded Haemodialysis Registry Protocol in Colombia (COREXH) is a prospective, observational, multicentre cohort study of patients undergoing **HDx** therapy 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** therapy for a minimum of 4 hours, 3 times per week using an **MCO** membrane (**Theranova** dialyser, Vantive, 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** therapy 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 enrolment 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 enrolment 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** therapy. The changes in serum albumin levels was less pronounced, with an accumulated change of -1.2%. See Table 2.

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).

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 (PPa). Abbreviation: PP, per=protocol defined as patients who received all treatments with the **MCO** membrane during the follow-up period or until hospitalisation that involved >12 dialysis sessions without the **MCO** membrane or death. [a]Only patients in the PP population who had baseline and all six scheduled serum albumin measurements during **HDx** therapy 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 hospitalisation 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, hospitalisation rate, and number of hospital days were lower than previous experiences with the **RTS Network** in Colombia.

Dialysis Parameters

HDx therapy 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** therapy 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** therapy, 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** therapy using an **MCO** membrane maintains serum albumin levels within the normal range among patients undergoing expanded haemodialysis with nonoccurrence of dialyser related adverse effects.

The MCO membrane is safe and preserves serum albumin levels within the normal range among patients undergoing HDx therapy.

Comparison of Haemodialysis with Medium Cut-Off Dialyser and On-Line haemodiafiltration on the Removal of Small and Middle-Sized Molecules

Belmouaz M, Diolez J, Bauwens M, et al. Comparison of haemodialysis with medium cut-off dialyser and on-line haemodiafiltration on the removal of small and middle-sized molecules. *Clin Nephrol*. 2018;1:50-56. doi: 10.5414/CN109133.

BACKGROUND

Recent data suggest that the use of medium cut-off dialysers in haemodialysis (HD) promotes greater clearance and reduction ratio (RR) for myoglobin (17 kDa) and other large-sized molecules than on-line haemodiafiltration (ol-HDF), but its effects on β2-microglobulin (11.8 kDa) are not clear.

The association of high serum levels of middle-sized toxins, particularlyβ2-microglobulin, with inflammation, immune dysfunction, and patient survival has been established in several studies. The removal of middle-sized toxins such as β2-microglobulin (11.8 kDa) and myoglobin (17 kDa) depends on both dialyser permeability and treatment modalities. Ol-HDF, combining the use of a high-flux dialyser, 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 dialyser (polyarylethersulfone/ polyvinylpyrrolidone, Gambro Dialysatoren GmbH, Hechingen, Germany) is a novel-generation **MCO** dialyser designed to remove molecules over 25 kDa. Recent clinical data on the use of **MCO** dialyser in HD patients have shown efficient removal of β2-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 **MCO** dialyser 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 dialyser for at least 6 months. These patients were then switched to HD with the **Theranova**-500™ **MCO** dialyser (Gambro Dialysatoren GmbH, Hechingen, Germany) for a 6-month period.

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

All patients had negligible residual renal function. Pre-and postdialysis serum levels of small molecules (urea (60.055 Da2), creatinine (113.12 Da3)) and middle-sized molecules (β2-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 the **MCO** membrane-HD. A total of 28 sessions for each treatment period were available for analysis.

Limitations

Limitations of the study included its retrospective design without randomisation, small number of analysed 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 inter-dialytic hypotensive episodes requiring fluid volume expansion between high-flux ol-HDF and the **MCO** membrane-HD period. There were no clinically relevant adverse events reported with use of the **MCO** membrane-HD.

Renal Replacement Therapy Characteristics

There was no significant change between the high-flux ol-HDF and the the **MCO** membrane-HD period regarding blood flow rate, ultra filtration 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
Ultra filtration (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 haemodiafiltration; **MCO** membrane-HD, medium-cut off haemodialysis; NA, not applicable. Adapted from Belmouaz et al.

Biological, Nutritional and Inflammatory Parameters

Median serum albumin (65 kDa)1, serum prealbumin, normalised protein catabolic rate (nPCR), c-reactive protein (CRP) levels did not change significantly between the high-flux ol-HDF and the **MCO** membrane-HD period. Median serum β2-microglobulin and myoglobin levels before and after dialysis also did not change significantly between the high-flux ol-HDF and the the **MCO** membrane-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 (014)	.025

	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
β_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, normalised protein catabolic rate; CRP, c-reactive protein; ol-HDF, on-line hemodiafiltration; **MCO** membrane -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 the **MCO** membrane-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 the **MCO** membrane-HD. See Table 1.

There was no significant difference between the high-flux ol-HDF and the **MCO** membrane-HD for mean β2-microglobulin and myoglobin RR, as well as mean β2-microglobulin and myoglobin Kd. See Table 3. The similar efficacy of the **MCO** membrane-HD for β2-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/*V**	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
β_2-microglobulin	81 ± 5	81 ± 6	0.72
Myoglobin*	60 ± 9	61 ± 7	0.59
Overall clearances (mL/min)			
β_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 haemodiafiltration; **MCO** membrane-HD, medium-cut off haemodialysis. Adapted from Belmouaz et al.

CONCLUSION

This study is the first evaluating efficacy and safety of the **MCO** membrane-HD with the new-generation **Theranova**-500 dialyser in HD over a 6-month period, showing similar removal for small molecules, β-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 the **MCO** membrane-HD period.

Although the study has several limitations, the **MCO** membrane-HD may provide a useful alternative to high-flux ol-HDF for middle-sized molecule removal. The **MCO** membrane-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 dialyser produced similar removal of urea, creatinine, β2-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.

Efficacy and Safety of Expanded Haemodialysis with the Theranova 400 Dialyser: A Randomised Controlled Trial

Weiner DE, Falzon L, Skoufos L, et al. Efficacy and safety of expanded haemodialysis with the Theranova 400 dialyser: A randomised 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 uraemic 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 β2-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.

Haemodialysis (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 dialysers, allow some middle molecules like β2-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 has been proposed to define a treatment where diffusion and convection are technically integrated inside a hollow-fiber dialyser equipped with a medium cut-off membrane, enabling removal of small, conventional middle molecules and large middle molecular uraemic toxins. The **Theranova** dialsyer provides expanded haemodialysis using a hollow-fiber single-use dialyser, 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 dialysers are based on short-term, non-randomised clinical trials. In contrast, this study was a randomised, longer-term (6 months) study.

OBJECTIVE

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

METHODOLOGY

The multicentre open-label, randomised controlled trial was conducted in 21 centres in the US between September 2017 and October 2018.

Participants

Patients receiving 3X/week in-centre maintenance haemodialysis, 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 dialyser for at least 3 months prior
- Expected to maintain an acceptable urea clearance (Kt/V) with a dialyser of an approximate surface area of 1.7 m2
- Stable functioning vascular access

Key exclusion criteria included: history of acute infection ≤ 4 weeks prior to randomisation 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 randomised with 86 in each group.

Methods

The study was an open-label study without concealment of the dialyser used; the allocation was concealed to the central laboratory and study sponsor. Patients were randomised to receive treatment with either **Theranova** 400 dialyser (Vantive Healthcare International) or Elisio-17H (Nipro Corporation). Randomisation to **Theranova** 400 dialyser or Elisio-17H, a similar surface area high-flux dialyser (1.7 m2), 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 Centres for Medicare and Medicaid Services regulations for dialysis water and conventional dialysate were required. Haemodialysis 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 centre'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 λFLCs (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); Symbol FLC (23 kDa); interleukin 6 (IL-6) (25 kDa); tumour 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 centres participated in this clinical study. Of 282 patients meeting the inclusion criteria, 172 participants were randomised, with 86 in each group. A total of 130 participants completed the study; 65 in the **Theranova** 400 dialyser 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 dialyser 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 dialyser group and 4.1 ± 0.4 g/dL in the Elisio-17H group, demonstrating non-inferiority of **Theranova** 400* dialyser in maintaining serum albumin levels. See Table 1.

Parameter	Dialyser	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 dialyser 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 dialyser 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 dialyser 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 dialyser showed significantly larger removal of λ FLCs at 24 weeks of treatment than the Elisio-17H dialyser (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 the **Theranova** 400 dialyser at 4 weeks of treatment than with the Elisio-17H dialyser (mean RR of 39% ± 14% vs 20% ± 11% [p <0.001]). See Table 3, Figure 1.

Secondary Efficacy Outcomes
Theranova 400 dialyser 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 β2-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 dialyser 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.

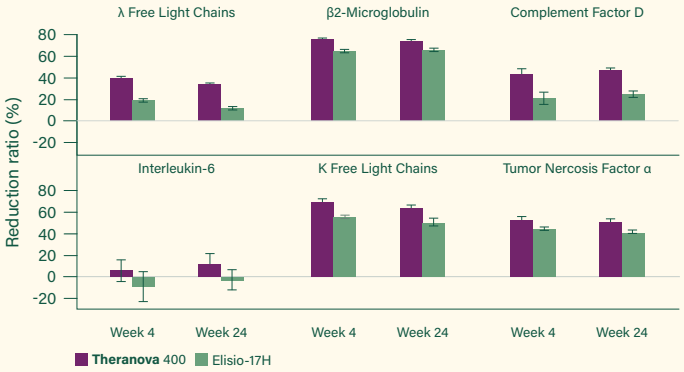


FIGURE 1. Reduction ratios of middle molecules at 4 weeks and 24 weeks. Adapted from Weiner et al.

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 dialyser 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 dialyser and the Elisio-17H groups in incidence

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	Dialyser	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 λ 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 β2-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.

(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 dialyser 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 & 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 randomised 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 analysed.

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 dialyser showed significantly greater (p <0.001) removal of conventional and larger middle molecules λ FLC (45 kDa), complement factor D (24kDa), λ 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 haemodialysis patients. Given the potential role of these uraemic 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** therapy 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 'uraemic 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 uraemic 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** therapy with the **Theranova** 400 dialyser is safe and efficacious, providing superior removal of larger middle molecules including several putative uraemic toxins as compared to a similar size high-flux dialyser while maintaining serum albumin. While this study demonstrated greater removal of large middle molecules among prevalent haemodialysis 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.

The Theranova dialyser provides significant and superior removal of larger 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.

On the Balance Between Albumin Loss and Removal of Middle Molecules in dialysers

Hagemann F, Linkhorst J, Roth H, Wessling M. On the balance between albumin loss and removal of middle molecules in dialysers. *Journal of Membrane Science Letters* 2023. 3(1) 100044. doi: https://doi.org/10.1016/j.memlet.2023.100044.

BACKGROUND

Uraemic toxins can be divided into small water-soluble molecules (< 0.5 kD), middle molecules (≥0.5 kD) and protein-bound solutes. Middle molecules have been classified according to weight as small-middle molecules (0.5 - 15 kDa), medium-middle molecules (> 15 - 25 kDa), and large-middle molecules (> 25 - 58 kDa). Retention of middle molecules may lead to negative outcomes, and it is believed that removing them leads to improved outcomes in patients on dialysis.

In addition to dialyser mode, the dialyser hollow fibre membranes play an extensive role in toxin removal. Membranes differ in mean pore radius, pore size distribution, and pure water permeability. The pore size distribution of haemodialysis membranes is essential for selectivity. Tailoring the membrane's molecular weight cut-off appropriately balances the removal of middle-molecular weight uraemic toxins while avoiding albumin loss.

Low and high flux membranes are used to remove small water-soluble molecules, but do not effectively eliminate larger toxins. High cut-off membranes with larger pores than low and high flux membranes remove toxins up to 50 kDa, but due to their large pore size distribution also allow unwanted albumin loss, which could lead to hypoalbuminemia. Medium cut-off membranes are designed to remove middle molecules up to 50 kDa, but due to their sharper pore size distribution, they can offer lower albumin loss.

Use of medium cut-off membranes with haemodialysis or expanded haemodialysis therapy has been investigated in numerous clinical studies with the overall consensus that expanded haemodialysis therapy improves the clearance of a wide range of middle molecules, and can improve quality of life, morbidity, and mortality. Various medium cut-off membranes are available from different manufacturers with potentially different properties.

OBJECTIVE

The current study aims to determine the plasma clearance of middle molecules (12 - 52 kDa) and the albumin loss in four commercially available dialysers:

- **Theranova** 500 dialyser (Gambro Dialysatoren GmbH)
- Phylther HF20SD from Bellco Mirandola
- VitE 21 X (Asahi Kasei Medical Co., Ltd)
- Elisio 19 HX (Nipro Medical Corp.)

Five dialysers from each type were tested.

METHODS

This study was a comprehensive ex-vivo investigation of performance characteristics of four different commercial dialysers. Membrane pore characteristics were analysed, ex-vivo experiments were performed to evaluate the clearances of significant middle molecules, and experiments at different blood flow rates quantified the influence on middle molecule clearance and albumin loss.

Dextran sieving experiments

Dextran sieving experiments were done to characterise the membranes. From the resulting sieving curves, the retention and the pore size distribution can be derived. In order to provide a comparable result, measurements were performed according to Boschetti-de Fierro et al. (2013)[1] using different dextran fractions. Dextran concentrations were measured with an Agilent HPLC 1200 equipped with a TSK gel column from Tosoh Bioscience. The pore size distribution was then calculated from the sieving curves.

Plasma clearance

Anti-coagulated (citrate) human plasma Octaplas from Octapharma with a protein concentration of 55 ± 10 g/L was used, and the following extrinsic marker substances were spiked in the plasma to measure clearance: 5 mg/L of LEE Biosolutions' β2 microglobulin (β2m) (M_w = 12 kDa) and Myoglobin (Myo) (M_w = 17 kDa). YKL-40 (MW = 40 kDa) is an intrinsic marker, therefore the initial concentration might vary for different plasma charges. The plasma volume loss due to ultra filtration was compensated by substituting dialysate into the plasma pool, and the temperature was set to 37 °C to mimic the human body. Set-up is shown in Figure 1.

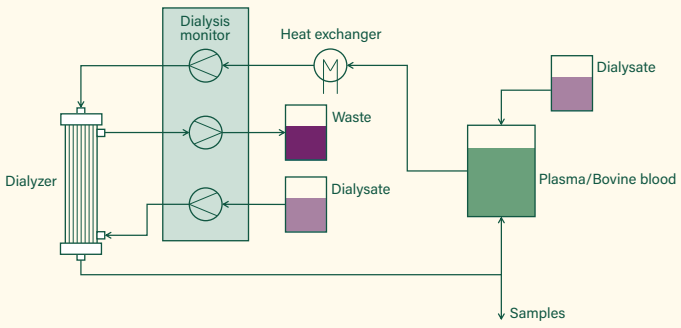


FIGURE 1. Experimental setup for the plasma clearance and albumin loss measurements.

The dialyser was rinsed and vented with 0.9 % saline solution (blood side: 5 min, dialysate side: 1 min). After replacing the saline solution with plasma and dialysate, the clearance tests started, beginning with a recirculation phase during which the membrane was saturated and adsorption of intrinsic plasma components took place. After 55 min, the extrinsic markers, β2m, and Myo were added, and the recirculation was continued for another 5 min. After that, the 60 min measuring phase started.

Clearance tests were performed at three different flow rate combinations, consisting of the plasma flow rate (QB), the dialysate flow rate (QD) and the flux over the membrane (UF):

(1) QB = 200 mL/min, QD = 500 mL/min and UF = 10 mL/min;

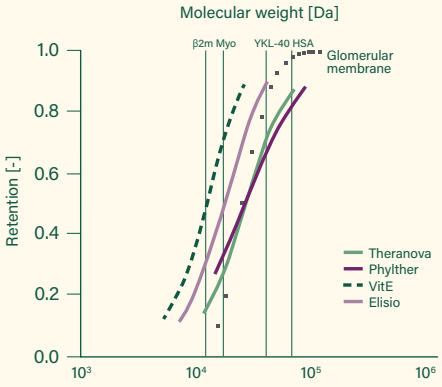
(2) QB = 300 mL/min, QD = 500 mL/min and UF = 10 mL/min;

(3) QB = 400 mL/min, QD = 600 127 mL/min and UF = 10 mL/min.

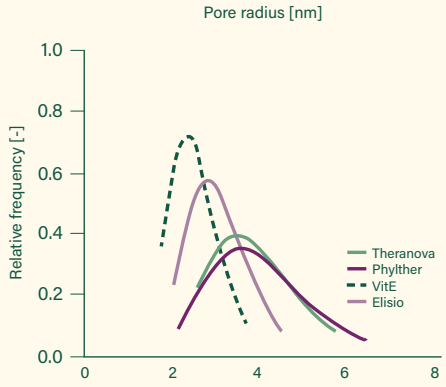
Marker concentrations were determined and clearance for each tracer was calculated.

Albumin loss

Experiments were run with bovine whole blood with protein concentration 60 ± 5 g/L, hematocrit 32 ± 3%, and viscosity 1.50 to 1.64 mm/s^2. The concentration of bovine serum albumin (BSA) in the blood and the dialysate was monitored. The experimental set-up was similar to Figure 1 with the addition of a sampling device installed in the dialysate drain line to collect spent dialysate continuously. The rinsing procedure for the dialyser was the same as described for clearance testing. The albumin loss was analysed for 240 min at a flow rate combination of QB = 300 mL/min, QD = 500 mL/min, and UF = 10 mL/min.

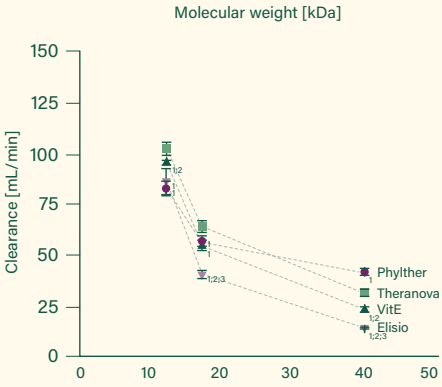


(a) Experimental dextran retention curve.

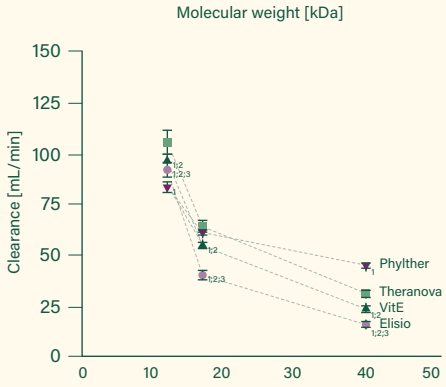


(b) Pore size distributions estimated from the retention curves using Equation SI.1.

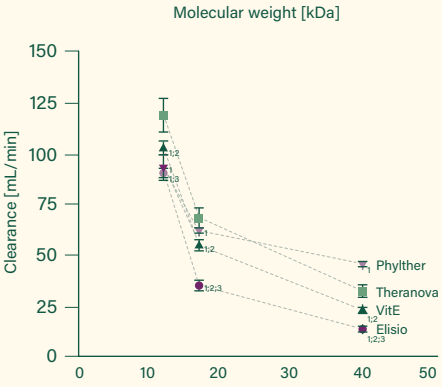
FIGURE 2. Solute transport characteristics of the 4 investigated dialysers. Retention of the glomerular membrane taken from Axelsson et al. (2009). The VitE membrane results need to be taken with care as membrane drying might have affected the pore structure.



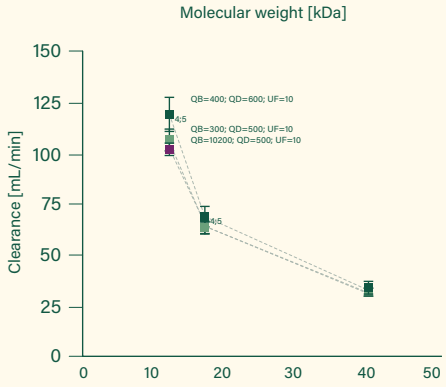
(a) Flow rates [mL/min]: QB=200 QD=500 UF=10.



(b) Flow rates [mL/min]: QB=300 QD=500 UF=10.



(c) Flow rates [mL/min]: QB=400 QD=600 UF=10.



(d) Impact of the flow rate, shown for Theranova.

FIGURE 3. Plasma clearance data for different middle molecules (β2 microglobulin (12 kDa), myoglobin (17 kDa) and YKL-40 (40 kDa)), dialyser types and flow rate configurations. (1) is tested vs. **Theranova**, (2) vs. Phylther, (3) vs. ViE21X with p<0.05. (4) is tested vs. QB=200; QD=500; UF=10 and (5) vs.QB=300; QD=500; UF=10 with p<0.05.

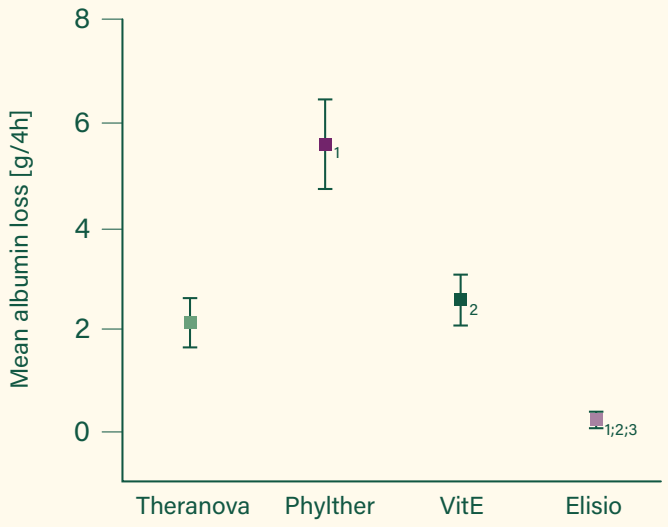


FIGURE 4. The mean albumin loss during a 4 h simulated treatment with bovine blood. The flow rate was set to QB=300 mL/min, QD = 500 mL/min, and UF = 10 mL/min. (1) is tested vs. Theranova, (2) vs. Phylther, (3) vs. VitE with $p<0.05$.

RESULTS

Dextran sieving experiments

Dextran retention curves along with the glomerular membrane retention curve are shown in Figure 2a, and pore size distributions in Figure 2b.

The Elisio retention curve is to the left of the glomerular membrane, meaning it retained smaller dextrans than the kidney's retention curve. The **Theranova** dialyser and Phylther curves cross the glomerular membrane, indicating there are pores large enough to let albumin pass. Compared to the **Theranova** dialyser and Phylther, the retention curve for Elisio is sharper, which results in a more precise cut-off, however the Elisio curve is not suitable for removing molecules larger than 30 kDa. The membranes in the VitE dialyser were dried to ensure potting adhesion in preparation of the mini-modules for sieving coefficient experiments, and it is assumed that this procedure significantly changed the pore structure therefore conclusions cannot be drawn from the results.

Plasma clearance

The clearance for the different molecules across dialyser types and flow rates are shown in Figures 3a-c. The **Theranova** 500 dialyser showed the highest clearance for β2 microglobulin (12 kDa) and myoglobin (17 kDa), and the Phylther dialyser showed the highest clearance for YKL-40 marker (40 kDa).

Figure 3d shows the impact of flow rate on clearance for the **Theranova** dialyser. It shows that the flow rate influences the clearance of smaller middle molecules, but for molecules larger than 40 kDa the impact of the flow rate is negligible.

Albumin loss

Literature reports that the acceptable threshold of albumin loss is less than 5 g per dialysis session.[3,4] The Phylther dialyser exceeded this limit (5.62 g/4h). The **Theranova** dialyser and VitE have significantly lower albumin loss compared to Phylther, and the Elisio dialyser had the lowest albumin loss (Figure 4).

DISCUSSION AND CONCLUSION

The current study investigated the plasma clearance of middle molecules and the albumin loss in four commercially available dialysers under carefully controlled and identical conditions. A previous study investigating four medium cut-off dialyser membranes reported reduction ratios for various middle molecules and found no significant difference between the dialysers.[2] In the current study, similar results were found by calculating reduction ratios. However, clearance should be used rather than reduction ratios to characterise the removal capacity of a dialyser as clearance takes into account efficiency and blood flow rate. The reduction ratio neglects the filtration duration, thus a long filtration time will result in a high reduction ratio value, impairing the comparison across literature.

The Elisio dialyser membrane has the sharpest distribution but the lowest mean pore radius. It showed lower clearance values for all tested markers and barely any albumin loss. The **Theranova** dialyser and Phylther membranes have larger mean pore sizes. For clearance of small-middle molecules, the **Theranova** dialyser was superior to Phylther, whereas, for clearance of large-middle molecules, Phylther was superior to the **Theranova** dialyser. However, this higher clearance of large-middle molecules by the Phylther dialyser comes with albumin loss that exceeds the recommended limit of 5 g per treatment, which could expose patients to the risk of hypoalbuminemia.

The **Theranova** dialyser demonstrates the best compromise between low albumin loss and good clearance of middle molecules.

Limitations

This was an ex-vivo study, and additional research is needed to compare the study results to real dialysis conditions.

Among four dialyser membranes, the Theranova dialyser offers the best compromise and good clearance of middle molecules and low albumin loss.

1. Boschetti-de-Fierro A, Voigt M, Storr M, Krause B. *Int J Artif Organs*. 2013;36(7):455-463. doi:10.5301/ijao.5000220.
2. Maduell F, Broseta JJ, Rodríguez-Espinosa D, et al. *Clin Kidney J*. 2022;15(12):2292-2299. Published 2022 Jul 13. doi:10.1093/ckj/sfac167
3. Boschetti-de-Fierro, A., Voigt, M., Storr, M. et al. *Sci Rep* 5, 18448 (2015). https://doi.org/10.1038/srep18448
4. Potier J, Queffeulou G, Bouet J. *Int J Artif Organs*. 2016;39(9):460-470. doi:10.5301/ijao.5000525

Vantive

HDx therapy: A world of difference

Clinical Effectiveness of the HDx Therapy

HDx therapy may decrease the pro-calcifying effect of uraemic serum compared with HD. Studying pathogenetic processes involved in high Pi–induced calcium deposition found that uraemic serum of patients treated with **HDx** therapy induced less VSMC necrosis compared with uraemic serum of HD patients.[1]

Ciceri P, Tettamanti G, Galassi A, et al.
Pro-calcifying analysis of uraemic serum from patients treated with medium cut-off membrane in a prospective, cross-over study. *Clinical Kidney Journal* 2021;14(7):1798-1807.

Willy K, Girndt M, Voelkl J, et al.
Expanded Haemodialysis Therapy of Chronic Haemodialysis Patients Prevents Calcification and Apoptosis of Vascular Smooth Muscle Cells in vitro. *Blood Purif* 2018;45:131-138.

Korucu B, Yeter H, Gonen S, Kursat Derici M, Ronco C, Derici U.
Impact of medium cut-off membranes on S100A12 and soluble receptor for advanced glycation end products. *Semin Dial.* 2023;36(3):193-200.

Kim YG, Lee SH, Jung SW, et al.
The Medium Cut-Off Membrane Does Not Lower Protein-Bound Uraemic Toxins. *Toxins* 2022;14:779.

1. Ciceri P, Tettamanti G, Galassi A, Magagnoli L, Fabresse N, Alvarez JC, Massy Z, Messa P, Cozzolino M. Pro-calcifying analysis of uraemic serum from patients treated with medium cut-off membrane in a prospective, cross-over study. *Clinical Kidney Journal*. 2021;14(7):1798-1807.

Pro-calcifying analysis of uraemic serum from patients treated with medium cut-off membrane in a prospective, cross-over study

Ciceri P, et al. Pro-calcifying analysis of uraemic serum from patients treated with medium cut-off membrane in a prospective, cross-over study. *Clinical Kidney Journal*. 2020:1-10. doi: 10.1093/ckj/sfaa216.

BACKGROUND

The retention of a large number of solutes that are normally excreted or metabolised by the kidney is responsible for the symptoms typical in uraemic patients. These molecules are defined as uraemic toxins and can be classified into three groups: small water-soluble molecules, middle molecules and protein-bound toxins. Recently, efforts were put towards developing dialysis membranes that allow the removal of large middle molecules without clinically relevant albumin loss. These membranes are the medium cut-off membranes that allow the removal of middle molecules up to ~50,000 Da.

Patients affected by ESRD have an increased morbidity and mortality due to many complications, mainly represented by cardiovascular diseases. In all the causes that induce cardiovascular diseases, an important role is played by vascular calcification (VC), which modifies arterial pulse wave velocity and pressure, leading to hypertension, left ventricular hypertrophy and heart failure. In end stage renal disease (ESRD) patients, VC affects principally the tunica media and vascular smooth muscle cells (VSMCs) and is due to different factors, among which the main ones are high phosphate levels and uraemic toxins.

Phosphate stimulates VSMCs to deposit calcium in the extracellular matrix. In this process, VSMCs loose muscular markers and start to express an osteoblastic phenotype, behaving as simil-osteoblasts. Besides the trans-differentiation, high phosphate promotes calcification by causing apoptosis, with the release of apoptotic bodies loaded with calcium that work as VSMC calcification machinery. Another process that induces calcification exacerbation is necrosis, as the massive release of calcium after rupture of the cellular membrane triggers and participates in calcium deposition.

OBJECTIVE

> To investigate whether different dialysis treatments, e.g., **MCO** membrane or high flux dialysis membranes, could have an impact on calcium deposition.

> To characterise uraemic serum composition to find which components are differentially removed by dialysis treatment that could play a role in modulation of the serum pro-calcifying effect.

METHODOLOGY

Study Design

Twenty prevalent haemodialysis (HD) patients participated in this prospective, open-label, controlled, cross-over pilot study. The study was undertaken at the dialysis unit at the University of Milan (Italy) from 1 October 2017 to 31 December 2018. Consecutively unselected male and female adult patients with ESRD on HD were eligible for participation in the study. Patients were divided into two groups (A and B) with similar mean age, male:female ratio and dialytic vintage. As a cross-over design, patients in Group A were treated with **HDx** therapy with the **Theranova** dialyser for the first three months of the study then switched to traditional bicarbonate dialysis for the remaining three months. Patients in Group B were treated with bicarbonate dialysis for the first three months and then switched to **HDx** therapy for the remaining three months. See Figure 1. Sera samples from both groups were collected at 1, 2, and 3 months. The pro-calcifying effect of uraemic serum in a well-established in vitro model of high-phosphate (Pi)-induced calcification in VSMCs was then analysed.

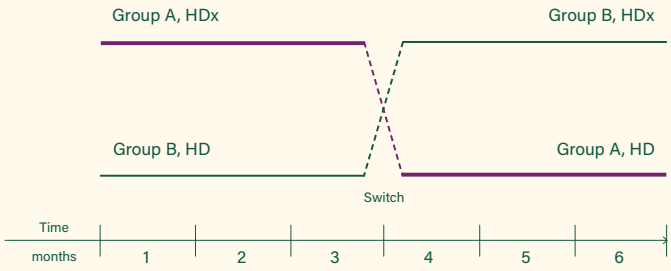


FIGURE 1. Study Design. **HDx** therapy: expanded haemodialysis. HD: haemodialysis. Figure adapted from Ciceri, et al.

Induction of Calcification/Quantification of Calcium Deposition

Rat VSMCs were obtained by enzymatic digestion, and were routinely subcultured in a growth medium. Quantification of calcium deposition was performed by staining, followed by perchloric acid destaining after high propidium iodide (Pi) treatment. Calcium content was determined colorimetrically at a wavelength of 450 mn. Protein content was assessed by a bicinchoninic acid (BCA) protein assay kit and calcium deposition was normalised to protein content and expressed as absorbance (optical density (OD)) per milligram of protein.

Detection of Apoptosis and Necrosis

Cytoplasmic histone-associated deoxyribonucleic acid (DNA) fragments were detected with a cell death detection Enzyme-Linked Immunosorbent Assay (ELISA) plus kit as a quantitative index of apoptosis 4 days after high Pi treatment. Necrosis was detected with the same kit.

VSMC Osteochondrogenic Shift

Seven different molecules, either inducers or inhibitors of calcification, involved in VSMC simil-osteoblast transformation were tested.

Uraemic Serum Characterisation

> *Uraemic Toxins and Precursors (Protein-Bound Uraemic Toxins)* – the levels of ten uraemic toxins and three precursors in patients' serum from either the **HDx** therapy or HD period were measured using a ultra-performance liquid chromatography-tandem mass spectrometer (ULPC-MS/MS)

> *Alpha 1 acid glycoprotein* - alpha-1 acid was detected by ELISA in patients' serum following the kit protocol

> *Micro ribonucleic acid (miRNA)* – content of miRNA was measured in serum-isolated exosomes

RESULTS

Effect of HDx therapy on vascular calcification

Patients in Group A showed a tendency to a slight reduction of calcium deposition during **HDx** therapy treatment with a following slight increase after treatment switch [6.6 ±1.1, 5.6 ±0.6 and 6.7 ±1.0 OD/mg protein for baseline, 3 months (**HDx** therapy) and 6 months (HD), respectively). Patients in Group B showed no modification in calcification in the first 3 months, whereas after treatment switching to **HDx** therapy we observed a significant reduction in calcification (4.7 ±0.6 versus 3.2 ±0.2 OD/mg protein at 3 versus 6 months, respectively; P <0.05). See Figure 2.

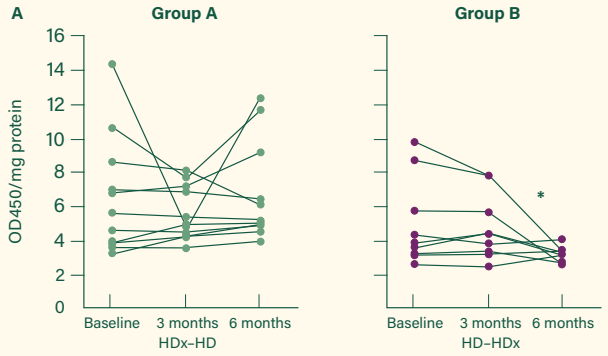


FIGURE 2. Effect of **HDx** therapy on human uraemic serum apoptotic potential in high Pi-simulated VSMCs. Data are presented as mean ± standard error. **HDx** therapy: expanded haemodialysis. HD: haemodialysis. Pi: propidium iodide; VSMC: vascular smooth muscle cells. Figure adapted from Ciceri, et al.

Effects of HDx therapy on VSMC Apoptosis and Necrosis

In an analysis of apoptosis, no statistically significant modulation by **HDx** therapy was found in Group A [1.85 ±0.36 versus 1.38 ±0.22, enrichment factor, baseline versus 3 months (**HDx** therapy)) respectively; or in Group B [1.38 ±0.17 versus 1.27 ±0.13, enrichment factor, 3 months (HD) versus 6 months (**HDx** therapy) respectively). See Figure 3.

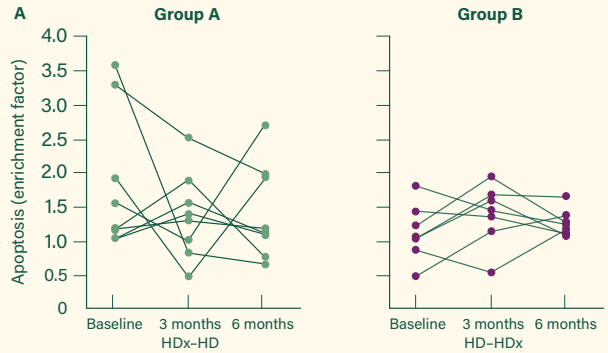


FIGURE 3. Effect of **HDx** therapy on human uraemic serum apoptotic potential in high Pi-simulated VSMCs. Data are presented as mean ± standard error. **HDx** therapy: expanded haemodialysis. HD: haemodialysis. Pi: propidium iodide; VSMC: vascular smooth muscle cells. Figure adapted from Ciceri, et al.

An analysis of necrosis found a decreasing positive effect of **HDx** therapy on this process. In Group A there were no statistically significant differences between **HDx** therapy and HD treatment [2.99 ±0.99, 3.69 ±0.95 and 5.94 ±2.85, enrichment factor, baseline, 3 months (**HDx** therapy) and 6 months (HD), respectively). In contrast, in Group B, **HDx** therapy induced a significant amelioration of the necrotic process [4.47 ±1.52 versus 1.86 ±1.10, enrichment factor, 3 months (HD) versus 6 months (**HDx** therapy); respectively; P <0.05). See Figure 4.

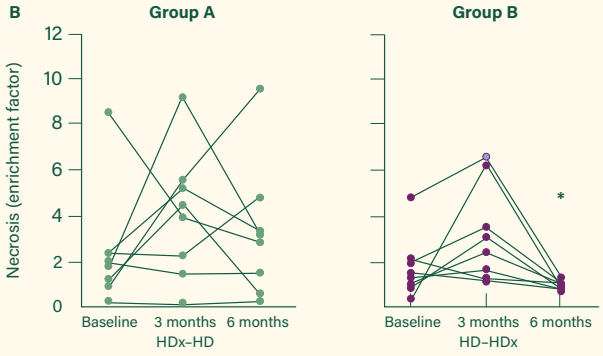


FIGURE 4. Effect of **HDx** therapy on human uraemic serum necrotic potential in high Pi-simulated VSMCs. Data are presented as mean ± standard error. (*P < 0.05). **HDx** therapy: expanded haemodialysis. HD: haemodialysis. Pi: propidium iodide; VSMC: vascular smooth muscle cells. Figure adapted from Ciceri, et al.

Effect of HDx therapy on Osteochondrogenic Shift

The uraemic serum from the **HDx** therapy period did not significantly induce a shift to an osteochondrogenic phenotype with high Pi induction or modify the levels of any inducers or inhibitors of calcification compared with the HD period.

Uraemic Toxin Characterisation

Uraemic Toxins and Precursors Profile

Among the 13 protein-bound uraemic toxins tested, a significant decrease was induced during the **HDx** therapy period in Group B for the following four:

> tryptophan: 51.7 ±14.2 versus -2.8 ±5.8 for HD versus **HDx** therapy; P <0.05 (Figure 5/A)

> kynurenine: 44.2 ±13.0 versus -0.1 ±6.8 for HD versus **HDx** therapy; P<0.05 (Figure 5/B)

> indole-3-acetic acid (IAA): 43.1 ±16.9 versus -2.4 ±11.0 for HD versus **HDx** therapy; P <0.05 (Figure 5/C)

> 3-Carboxy-4-methyl-5-propyl-2-furanpropionate (CMPF): 61.8 ±27.6 versus -16.1 ±9.6 for HD versus **HDx** therapy; P <0.05 (Figure 5/E)

For 3-idoxyl sulfate (IS) the percentage of variation decreased during the **HDx** therapy period both in Group A, with a rebound effect after the switch to HD (-21.1 ±13.0 versus 54.4 ±33.2 for **HDx** therapy versus HD; P <0.05), and in Group B (30.6 ±13.0 versus -17.1 ±10.2 for **HDx** therapy versus HD; P <0.05). (Figure 5/D)

No significant variation was found for the other protein-bound uraemic toxins tested.

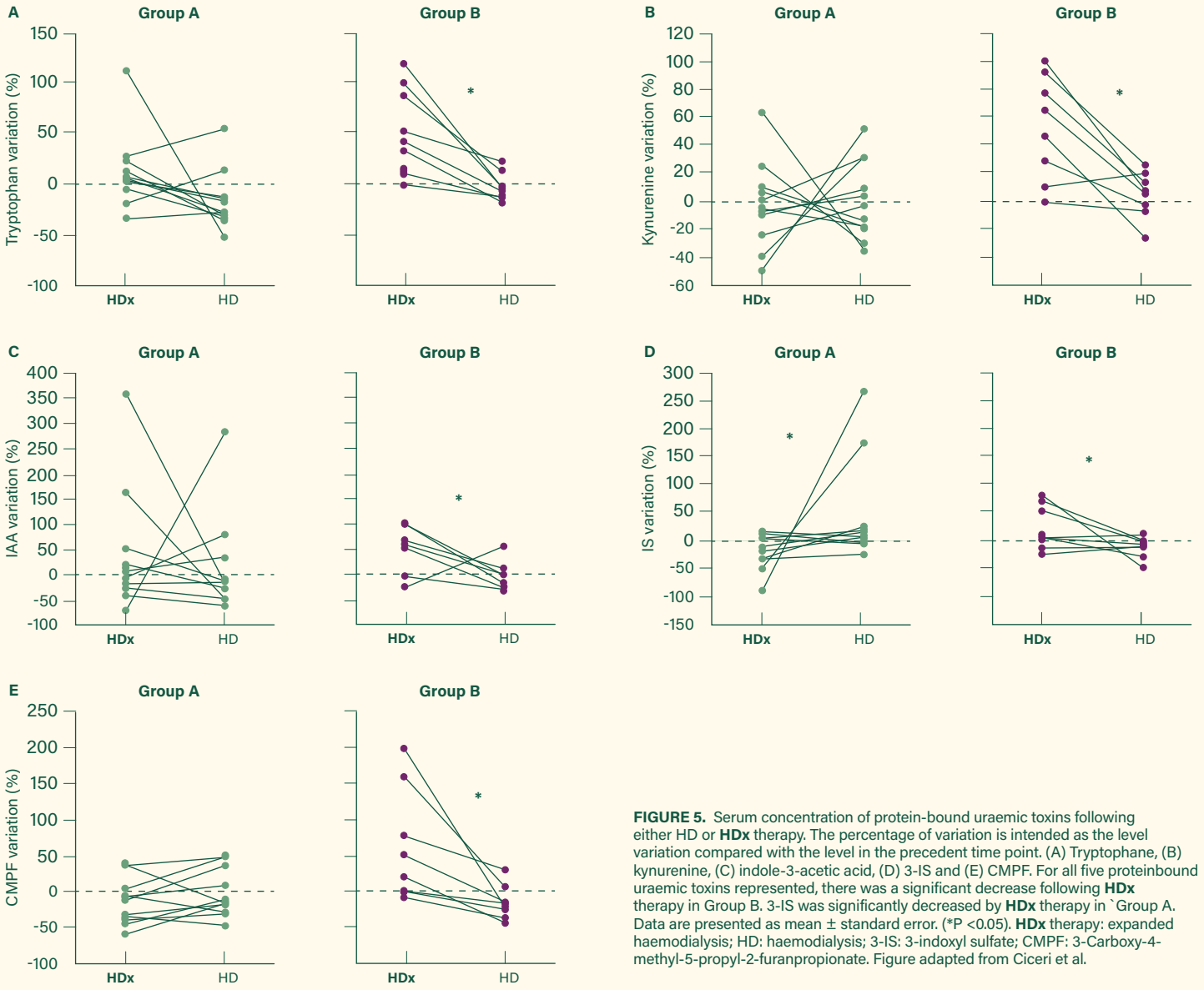


FIGURE 5. Serum concentration of protein-bound uraemic toxins following either HD or **HDx** therapy. The percentage of variation is intended as the level variation compared with the level in the precedent time point. (A) Tryptophane, (B) kynurenine, (C) indole-3-acetic acid, (D) 3-IS and (E) CMPF. For all five proteinbound uraemic toxins represented, there was a significant decrease following **HDx** therapy in Group B. 3-IS was significantly decreased by **HDx** therapy in `Group A. Data are presented as mean ± standard error. (*P <0.05). **HDx** therapy: expanded haemodialysis; HD: haemodialysis; 3-IS: 3-indoxyl sulfate; CMPF: 3-Carboxy-4-methyl-5-propyl-2-furanpropionate. Figure adapted from Ciceri et al.

Alpha 1 Acid Glycoprotein Levels

In Group A, **HDx** therapy and HD had almost the same effect, with a dispersion of data that resulted in no difference between the two dialysis treatments. In Group B, there was a non-significant tendency towards a decrease from HD to **HDx** therapy.

miRNA Levels

Due to the high variability between patients, no statistically significant results could be obtained.

CONCLUSIONS

This pilot study indicates that **HDx** therapy may decrease the pro-calcifying effect of uraemic serum. This reduction is important from a clinical point of view since necrosis is one of the driving mechanisms in calcium deposition. In this study **HDx** therapy reduction of uraemic serum toxicity is potentially one of the main mechanisms. This beneficial effect is due in part to a partial removal of tryptophan, some of its metabolites, such as IS, and CMPF. The effect of the **MCO** membrane on the removal of protein-bound uraemic toxins is probably related to albumin loss, the main carrier of these toxins. The partial albumin loss during **HDx** therapy might be beneficial, allowing a decrease in protein-bound uraemic toxin levels that have been difficult to eliminate with extracorporeal strategies until now. The **MCO** membrane might thus decrease uraemic serum pro-calcifying and necrotic effects through albumin loss without any clinical signs of hypoalbuminaemia. This hypothesis deserves deep investigation in larger clinical trials.

Expanded Haemodialysis Therapy of Chronic Haemodialysis Patients Prevents Calcification and Apoptosis of Vascular Smooth Muscle Cells in vitro

Willy K, Girndt M, Voelkl J, et al. Expanded Haemodialysis Therapy of Chronic Haemodialysis Patients Prevents Calcification and Apoptosis of Vascular Smooth Muscle Cells in vitro. *Blood Purif* 2018;45:131–138. DOI: 10.1159/000484925

BACKGROUND

Vascular calcification is common in patients with chronic kidney disease and is associated with cardiovascular mortality. Vascular calcification is an active process promoted by hyperphosphatemia. This process is mediated in part by inflammatory processes in vascular smooth muscle cells (VSMC) and inhibited by anti-calcific proteins such as matrix Gla protein (MGP) and Fetuin-A under normal conditions. The increased risk of mortality is associated with increased levels of the proinflammatory cytokine growth differentiation factor 15 (GDF-15). Insufficient removal of certain cytokines using conventional haemodialysis may contribute to vascular calcification.

High cut-off (**HCO**) membranes showed a reduction in the serum levels of soluble TNF receptors, vascular cell adhesion molecule (VCAM) and other markers of inflammation, but this is accompanied by marked loss of albumin.

High retention onset (HRO) dialysis membranes, or middle cut-off, with a steeper cutoff were developed and have been shown to provide clearance for middle-sized molecules while limiting albumin filtration. The potential impact of expanded haemodialysis therapy with an HRO membrane on vascular calcification has not yet been reported.

OBJECTIVE

The study examined the impact of HRO dialysis membrane on the process of the in vitro calcification of VSMC in a well-established cell culture model.

METHODS

The study used serum samples from the Permeability Enhancement to Reduce Chronic Inflammation (PERCI II) clinical trial,[1] which included 45 patients. Every patient was dialysed 3x/week using medium cut-off membrane (MCO-Ci 400) and high-flux (HF) membranes (Revaclear 400 dialyser) for 4 weeks in a randomised order. After the second phase, an extension period of 8 weeks was added to assess the long-term effects. No dialysis performed. In a consecutive manner, half of the patients who were randomised to the HRO dialysis membrane group in the second phase were dialysed using HRO membrane for 12 weeks.

Serum samples were collected before every first dialysis session of the week, centrifuged, frozen and then thawed for cell culture experiments. No freeze-thaw-refreeze cycles existed before the samples were used in the experiments.

Human VSMC were purchased from LifeLine Technology (Frederick, MD, USA). All cells used in these experiments came from the same donor. Cells were characterised as VSMC with alpha-SMA antibodies and passaged up to a maximum of 6 passages. During incubation, VSMC were stored in a humidified incubator at 37°C and 5% CO_2. Cells were cultured in 25-mL cell culture flasks until they were confluent. Cell number and viability were determined in a Neubauer counting chamber using Trypan blue, and cells were seeded at 100,000 cells/well on 24-well plates.

Induction and Determination of Calcification

An osteogenic medium was used to induce calcification in VSMC, and 5% of the serum samples collected from the 45 patients during the clinical trial was added. Calcification was evaluated using alkaline phosphatase (ALP) and alizarin red (AZR) after 7 and 10 days of incubation, respectively.

The water-soluble terazolium salt (WST-8) assay was used to measure the marker activity of viable cells, which served to normalise the activity of the calcification markers (ALP and AZR) of living cells.

Cell culture supernatants were collected at days 3 and 7 of incubation and later pooled, and the concentrations of calcification-associated proteins were measured.

Apoptosis rates caused by the different sera were measured via fluorescence spectroscopy after 7 days of incubation.

Results are presented as mean (standard error of the mean).

RESULTS

Calcification

ALP activity assay (Figure 1a):

- After 4 weeks of dialysis: HRO dialysis membrane induced 24% less calcification than HF dialysis (HF 2.91 (0.11) vs. HRO 2.21 (0.088), $p < 0.0001$)
- After 12 weeks of dialysis: HRO dialysis membrane induced 38% less calcification than HF dialysis (HF 3.14 (0.1) vs. HRO 1.96 (0.081), $p < 0.0001$)

AZR staining (Figure 1b)

- After 4 weeks of dialysis: HRO dialysis membrane induced 36% less calcification than HF dialysis (HF 0.31 (0.01) vs. HRO 0.19 (0.008), respectively, $p < 0.0001$)
- After 12 weeks of dialysis: HRO dialysis membrane induced 48% less calcification than HF dialysis (HF 0.30 (0.01) vs. HRO 12 weeks 0.16 (0.007), $p < 0.0001$)

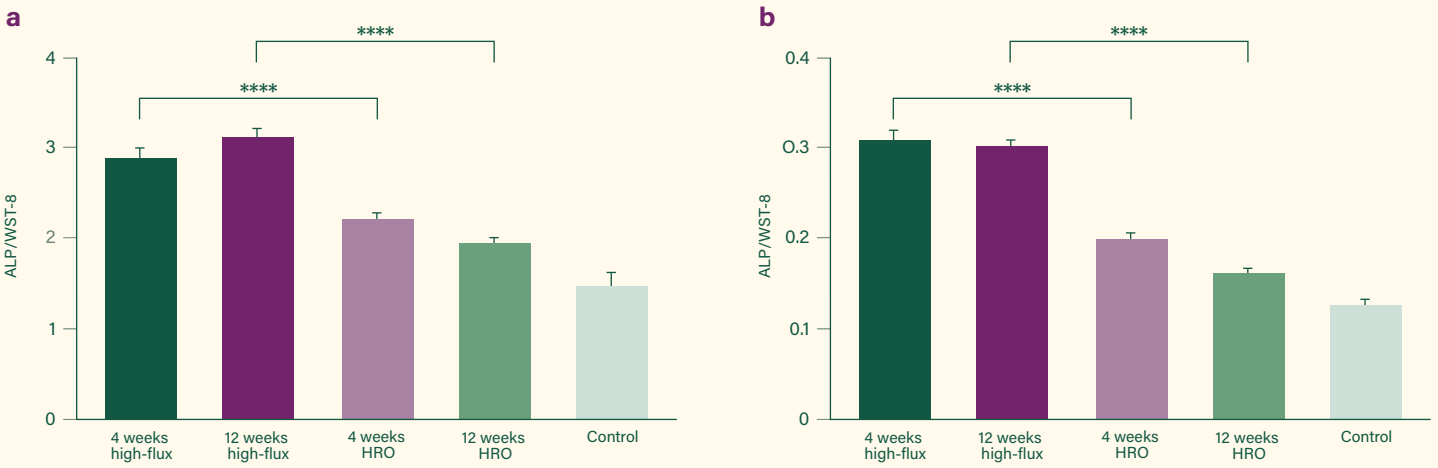


FIGURE 1. Ratio of ALP/WST-8 after 7 days of incubation **(a)** and AZR/WST-8 after 10 days of incubation **(b)**; n = 100 for 4 weeks High-Flux, 4 weeks HRO and 12 weeks HRO. n = 80 for 12 weeks High-Flux. n = 20 for control. **** p < 0.0001.

Calcification-Associated Proteins in Supernatants

MGP concentration (Figure 2a)

> MGP was 42% higher in the HF supernatants than HRO supernatants and 39% higher than the healthy control supernatants. MGP levels were comparable in the HRO and the healthy control supernatants.
> (HF 0.956 (0.045) vs. HRO 0.554 (0.018), $p < 0.0001$; HF 0.956 (0.045) vs. healthy serum 0.586 (0.01148), $p < 0.0001$)

OPN concentration (Figure 2b)

> OPN was 2.5 times higher in the HF supernatants than HRO supernatants and 4 times higher than the healthy serum group. (HF 0.238 (0.005) vs. HRO 0.083 (0.002), $p < 0.0001$; HF 0.238 (0.005) vs. healthy serum 0.060 (0.001), $p < 0.0001$

GDF-15 concentration collected at days 3 and 7 of incubation (Figure 3a and b respectively)

> GDF-15 was 27% lower in the HRO supernatant than HF supernatant at day 3 (HF 1,894.583 (158.4) vs. HRO 1,375.667 (48.68), $p < 0.01$)

> The absolute concentrations decreased towards day 7 in both groups, but the difference between HF and HRO groups remained stable at 32%
> (HF 402.5 (33.13) vs. 593.888 (34.30), $p < 0.05$)

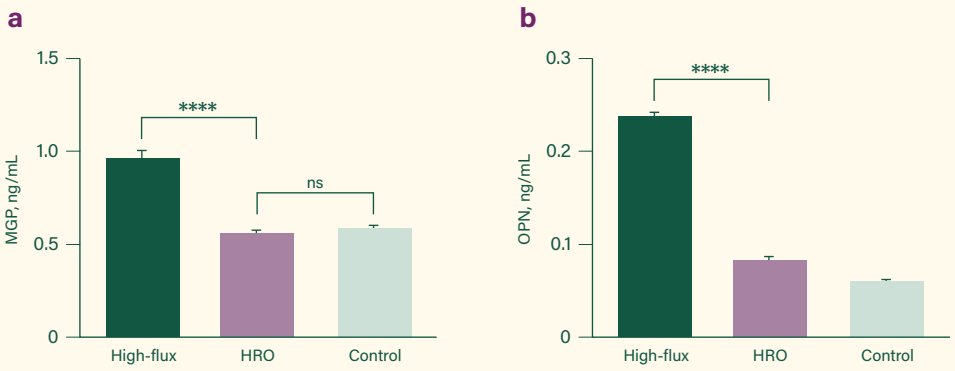


FIGURE 2. Production of Matrix-Gla protein **(a)** and OPN **(b)** by VSMC after incubation with HRO-serum, High-Flux-serum or serum from controls; $n = 18$ for High-Flux and HRO, $n = 12$ for control. **** $p < 0.0001$. ns, non significant.

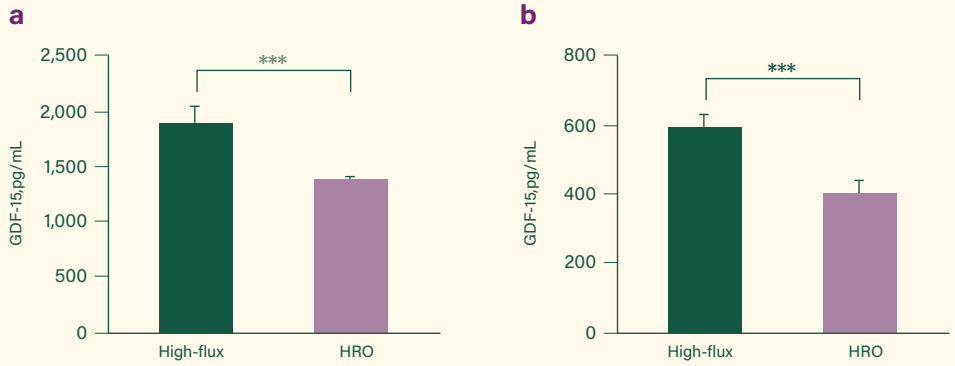


FIGURE 3. Concentration of GDF-15 in cell culture supernatants after 3 days (left, **a**) and after 7 days (right, **b**) of incubation with HRO-serum or High-Flux-serum; $n = 12$ (3 days), $n = 18$ (7 days). *** $p < 0.001$.

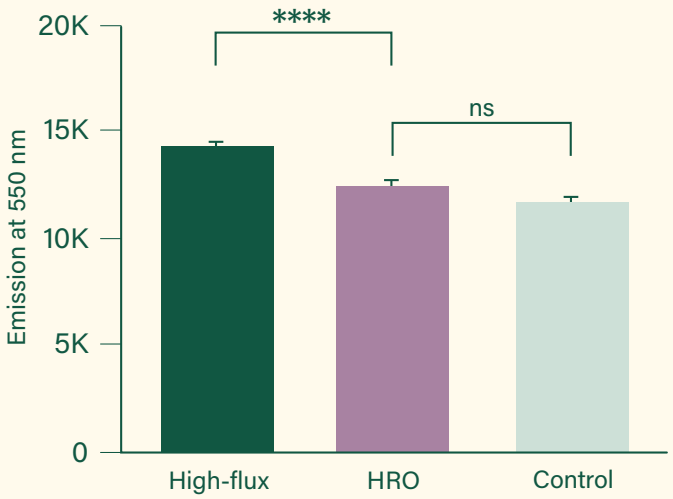


FIGURE 4. Apoptosis of VSMC after incubation with HRO-serum, Revaclear-serum or serum from controls. Apoptosis was determined by in situ cell death kit®; $n = 20$ for High-Flux and HRO, $n = 8$ for control. **** $p < 0.0001$. ns, non significant.

Apoptosis

The apoptosis rate of cells incubated with HRO was 15% lower than in HF-incubated cells (HF 14,410.15 (127.9) vs. HRO 12,452.9 (244.3), $p < 0.0001$). Apoptosis in the control group was similar to the HRO group (control 11,740.12 (225.5) vs. HRO 12,452.9 (244.3), p=ns) and 19% lower than the HF group ($p < 0.0001$). (Figure 4)

DISCUSSION

HCO dialysers can reduce inflammatory markers and improve vitro vascular calcification, however the relevant loss of albumin prohibits long-term use in chronic dialysis patients. The current in vitro study using samples from the PERCI II trial showed that HRO dialyser membranes maintained the beneficial effect on vitro calcification, while albumin loss was limited.

Vascular calcification in vitro was significantly reduced by 24% (ALP) and 36% (AZR) after 4 weeks of HRO dialysis membrane and by 33% (ALP) and 48% (AZR) after 12 weeks of dialysis using HRO membranes compared to HF dialysis. This observation suggests that the effects of HRO dialysis could involve long-term regulation of the pro-calcific properties inherent in dialysis patients.

The concentrations of MGP and OPN were significantly elevated after incubation with HF serum compared to HRO serum and healthy controls. OPN has been shown to help prevent several consequences of chronic kidney disease, including uraemia, calcium deposition and especially vascular calcification. MGP might interfere with both calcification signalling pathways and mineralisation and act as an inhibitor of cardiovascular calcification. The production of these molecules upon exposure to serum from dialysis patients could be a compensatory mechanism in response to inflammatory signals and enhanced calcification.

The release of GDF-15 in culture supernatants was significantly decreased after incubation with HRO serum. GDF-15 has been identified as a biomarker for cardiovascular events and risk, several vascular diseases, as well as renal and cardiac damage in general. It may also have an active role in atherosclerosis and other vascular pathologies. Whether the decrease in GDF-15 associated with HRO is causally involved in vascular calcification is unknown at this point.

Apoptosis was significantly lower in the HRO group. Apoptosis is important in the early process of vascular calcification, and VSMC apoptosis is known to be promoted by TNF-α and IL-6. The reduction of these molecules using HRO dialysis membrane may have led to the lower apoptosis rate that was comparable to healthy serum.

Limitations

First, we did not assess in vivo calcification but only the influence of serum samples on calcifying VSMC. The effects of reduced calcification were observed under cell culture conditions that were somewhat extreme with very high concentrations of procalcifying substances. Whether a comparable reduction can be observed in vivo under less extreme conditions has to be examined in future trials that include clinical end points. Second, even though we noted clear effects on in vitro calcification, we fail to provide a clear mechanism that explains the observed reduction in calcification. As discussed in previous publications, molecules of the TNF superfamily and other proinflammatory molecules are indeed reduced with HRO and provide a possible explanation. Finally, the HRO membranes used in our trial still allow relevant filtration of albumin, with a significant decline in serum albumin concentrations after 4 weeks of HRO dialysis. There was however an increase of serum albumin again after 12 weeks. Hence, in future trials, a further reduction in cut-off to reduce albumin filtration has to be considered to make these filters a treatment option for chronic dialysis patients.

CONCLUSION

The study results suggest that expanded haemodialysis therapy has beneficial effects on the calcific potential of uraemic serum. This indicates that dialysis with HRO membranes is a possible option to modify vascular calcification in end-stage renal disease. With a markedly reduced albumin filtration compared to high cut-off dialysis, use of the HRO dialysis membrane may provide a treatment option for chronic dialysis patients to reduce the progression of vascular calcification.

Expanded haemodialysis therapy reduced in vitro markers of calcification, which could mean a reduction in vascular calcification and corresponding cardiovascular mortality in patients with chronic kidney disease.

1. Zickler D, Schindler R, Willy K, et al. Medium Cut-Off (MCO) Membranes Reduce Inflammation in Chronic Dialysis Patients-A Randomised Controlled Clinical Trial. *PLoS One*. 2017;12(1):e0169024. Published 2017 Jan 13. doi:10.1371/journal.pone.0169024

Impact of Medium Cut-Off Membranes on S100A12 and Soluble Receptor for Advanced Glycation End Products

Korucu B, Yeter H, Gonen S, Kursat Derici M, Ronco C, Derici U. Impact of medium cut-off membranes on S100A12 and soluble receptor for advanced glycation end products. *Semin Dial*. 2023;36(3):193-200. doi: 10.1111/sdi.13107.

BACKGROUND

Cardiovascular diseases are the most common causes of morbidity and mortality in haemodialysis (HD) patients. Systemic inflammation, oxidative stress, and vascular calcification are important causes, therefore there is interest in atherosclerogenic inflammatory molecules and markers that can predict mortality. One such group of molecules is S100A12, also known as extracellular newly identified receptor for advanced glycation end products binding protein (EN-RAGE) and soluble receptor for advanced glycation end products (sRAGE). These molecules are produced under hyperglycemic and increased oxidative stress conditions.

RAGE is an immunoglobulin expressed on cell surfaces that induces secretion of proinflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α). RAGE may be a central regulator of vascular inflammation and is associated with vascular calcification, atherosclerosis and mortality in patients using HD.

sRAGE acts as an anti-inflammatory agent, neutralising RAGE ligands including S100A12. Higher levels of S100A12, lower levels of sRAGE, and a higher S100A12/sRAGE ratio are associated with chronic inflammatory conditions, vascular calcification, atherosclerosis, and high cardiovascular morbidity and mortality in both the general population and patients using HD.

Most uraemic toxins, including S100A12, have molecular weights (MW) above 10 kDa and cannot be eliminated by a standard HD treatment. **MCO** membranes have enhanced permeability, selectivity, and very high MW retention onset and cut-off close to the MW of albumin.

OBJECTIVE

This study investigated the effect of **MCO** dialyser membrane on the S100A12, sRAGE, and S100A12/sRAGE ratio.

METHODS

Study Design

This was a single-site, prospective, observational study comprising age and sex-matched three HD groups (low-flux, high-flux, and MCO dialyser).

Participants

Eligible patients had been undergoing dialysis for at least one year. Patients with central venous catheters, chronic inflammatory diseases, active infection, an active or recent malignancy history, and more than four weeks of **MCO** membrane use were excluded.

Fifteen HD patients previously using low-flux dialysers were switched to a **MCO** dialyser membrane. This **MCO** membrane group was compared with two groups, each consisting of 15 age and sex-matched patients treated with low-flux or high-flux dialysers.

Treatments

dialysers used were:

> Low-flux: NIPRO Polynephron Synthetic Hollow Fibre, Osaka, Japan

> High-flux: Fresenius CorDiax 800 HDFR, Hamburg, Germany

> **MCO** membrane: **Theranova** 400R dialyser, Vantive, Hechingen, Germany

All patients received treatment 3x/week and 4 h per session replacement therapy via arteriovenous fistulae or graft using bicarbonate-containing ultrapure dialysate. The blood flow rate ranged from 300 to 350 ml/min, and the dialysate flow rate was 500 ml/min.

Outcomes

The study assessed S100A12 and sRAGE levels, and S100A12/sRAGE ratio. Blood samples for S100A12 and sRAGE were drawn in the mid-week haemodialysis sessions at baseline (predialysis and postdialysis) and the sixth month (predialysis).

RESULTS

The groups had similar mean age, sex distribution, mean BMI, and frequency of diabetic patients. The baseline median C-Reactive Protein (CRP) test of the **MCO** membrane group was higher than the low-flux and high-flux groups with a borderline significance [8.2 (4.2–22.4), 5.0 (1–26.2), and 3.8 (0.8–18.1), respectively; $p = 0.05$]. At month 6, the groups' median CRP levels were similar ($p = 0.31$).

Groups had similar serum haemoglobin, Blood Urea Nitrogen (BUN) test, creatinine, albumin, electrolytes, parathormone, Kt/V and Urea Reduction Ration (URR) at baseline and the sixth-month evaluation.

Figure 1 shows the baseline and 6 month values for S100A12 and sRAGE levels, and S100A12/sRAGE ratio.

> **S100A12:** Baseline mean levels were similar across the low-flux, high-flux, and **MCO** membrane groups, and the sixth month mean levels of the groups had a trend toward significance, with the lowest level in the **MCO** membrane group. Compared to baseline, the 6 month mean level was similar in the low-flux and high-flux groups and significantly lower in the **MCO** membrane group (p=0.0004).

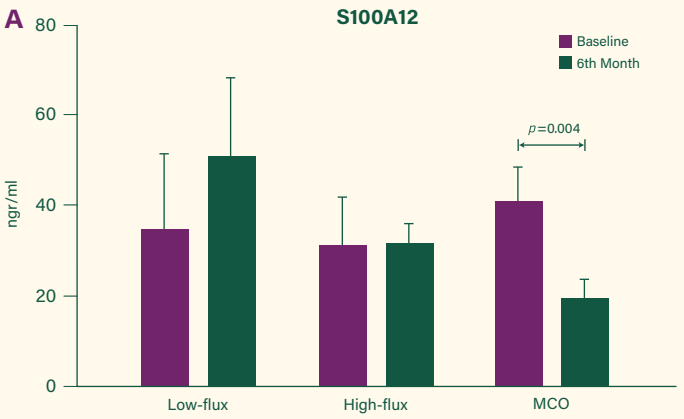
- **sRAGE:** Mean levels were similar at baseline at 6 months across the low-flux, high-flux, and **MCO** membrane groups. Compared to baseline, the 6 month mean levels remained constant in all groups.
- **S100A12/sRAGE:** Mean ratio at baseline and the sixth month was constant in the low-flux group and the high-flux group, and the ratio decreased significantly in the **MCO** membrane group (p=0.03).

Reduction ratios (RR) were also calculated. S100A12 had a mean RR of 19.8% in the low-flux group, 29.0% in the high-flux group, and 37.7% in the **MCO** membrane group. sRAGE had a mean RR of 4.2% in the low-flux group, 10.1% in the high-flux group, and 18.9% in the **MCO** membrane group.

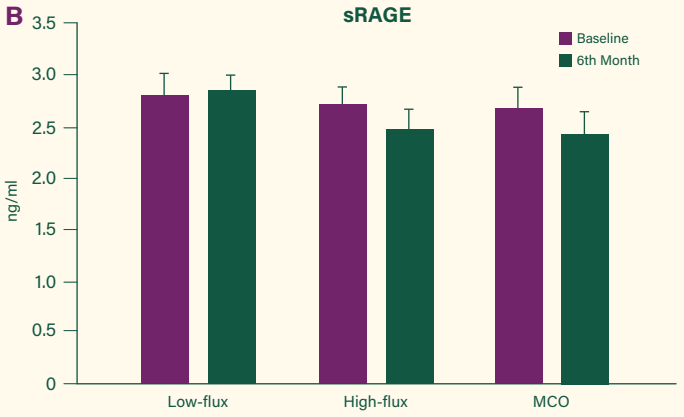
DISCUSSION AND CONCLUSION

This study showed an explicit decrease in predialysis S100A12 levels and S100A12/sRAGE ratio after 6 months of treatment with **MCO** dialyser membrane, but not with low-flux or high-flux dialysers. The median CRP levels were borderline significantly higher in the **MCO** membrane group at baseline, and this systemic inflammation status may have influenced the clinicians' decision to switch to **MCO** membrane dialyser. Despite this slight disadvantage in the **MCO** membrane group's baseline systemic inflammatory status, they were the only group to have a significant improvement in the S100A12-sRAGE profile. sRAGE, which neutralises activator ligands such as S100A12, and acts as a competitive inhibitor of cellular RAGE, remained constant in all 3 study groups.

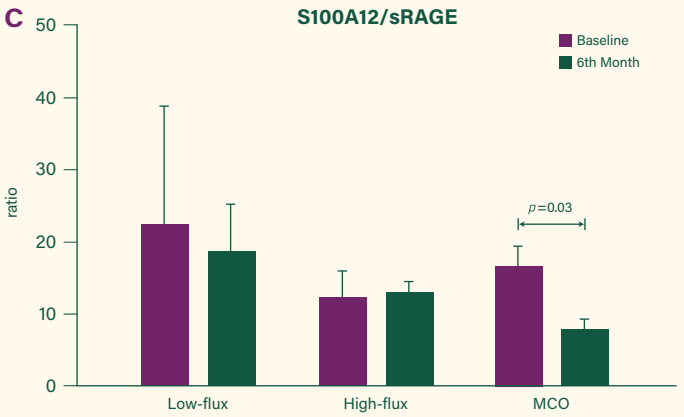
It has been previously reported that intermittent prolonged **HDx** therapy treatment may be associated with a decrease in systemic inflammation and oxidative stress molecules. The study found a significant improvement in the S100A12-sRAGE profile in the **MCO** membrane group at the end of month six is somewhat related to the membrane's pore size, and the authors state that the most important reason may be reducing systemic inflammation. Additional research is needed to determine whether the **MCO** membrane can decelerate the progression of atherosclerosis, vascular calcification, and decrease mortality in HD patients.



		Low-flux	High-flux	MCO	*p*-value
S100A12 ng/ml, mean[a]	Baseline	34.3 (±66.5)	30.9 (±42.7)	40.6 (±29.6)	0.13
	6th month	50.8 (±67.7)	31.4 (±16.9)	19.3 (±16.1)	0.08
	p value	0.16	0.33	**0.004**	



		Low-flux	High-flux	MCO	*p*-value
sRAGE ng/ml, mean[b]	Baseline	2.8 (±0.8)	2.7 (±0.6)	2.6 (±0.7)	0.65
	6th month	2.9 (±0.5)	2.4 (±0.7)	2.4 (±0.8)	0.24
	p value	0.84	0.13	0.39	



		Low-flux	High-flux	MCO	*p*-value
S100A12/ sRAGE, mean[a]	Baseline	22.3 (±63.7)	11.9 (±15.3)	16.5 (±11.6)	0.06
	6th month	18.1 (±24.8)	13.1 (±5.8)	7.8 (±5.5)	0.08
	p value	0.17	0.26	**0.03**	

FIGURE 1. Groups' mean predialysis S100A12 and sRAGE levels and ratios at baseline and sixth month. Abbreviations: MCO, medium cut-off dialyser; sRAGE, soluble receptor for advanced glycation end products. *Data are presented as mean values with standard errors.

Note: Statistically significant values are presented in bold.

Abbreviations: MCO, medium cut-off dialyser; sRAGE, soluble receptor for advanced glycation end products.

[a]Wilcoxon test was used for baseline and sixth-month values, and Kruskal–Wallis was used for independent groups.

[b]Paired sample t-test was used for baseline and sixth-month values, and ANOVA test was used for independent groups.

Limitations

This study's limitations are the small sample size, the lack of comparison of findings with sensitive systemic inflammatory molecules and oxidative stress markers, its retrospective design, and the inability to associate the results with patients' clinical outcomes due to the 6-month follow-up period of the study.

This study suggests that prolonged use of MCO membrane dialyser is associated with better S100A12-sRAGE profiles than low-flux and high-flux dialysers. Long-term studies with larger samples are needed to understand the effects of a better S100A12-sRAGE profile.

The Medium Cut-Off Membrane Does Not Lower Protein-Bound Uraemic Toxins

Kim YG, Lee SH, Jung SW, et al. The Medium Cut-Off Membrane Does Not Lower Protein-Bound Uraemic Toxins. *Toxins* 2022;14:779. https://doi.org/10.3390/toxins14110779.

BACKGROUND

The concentration of gut-derived protein-bound uraemic toxins (PBUTs) is increased in the circulation of patients with chronic kidney disease (CKD) due to alterations caused by uraemic conditions. Some PBUTs such as indoxyl sulfate (IS) and p-cresyl sulfate (pCS) are associated with increased cardiovascular disease in CKD.

Even though PBUTs are small (<500 Da), they tend to bind to larger-sized proteins, particularly albumin, and this bound form is difficult to remove by diffusive dialysis. IS and pCS are classified as PBUTs that are strongly bound to albumin (>90%), with a very small fraction as free form.

The kidney primarily removes PBUTs by tubular secretion via organic anion or cation transporters. Conventional haemodialysis (HD) partially replaces glomerular filtration but cannot reproduce tubular function, therefore PBUTs build up in the plasma of patients on HD despite HD therapy. Free forms of PBUTs are easily removed by HD, however they represent a small fraction. Various HD strategies have been investigated to remove PBUTs, for example, prolonged HD duration using nocturnal dialysis eliminated PBUTs to a greater extent. Adding a convective method with haemodiafiltration (HDF) enhanced the removal of middle molecular uraemic toxins, but did not consistently remove PBUTs. A recently developed medium cut-off (**MCO** dialyser) selectively removes larger-sized uraemic toxins, but the efficacy of PBUT removal remains unclear.

OBJECTIVE

This study was performed to test the effectiveness of high-flux HD (HF-HD), high-volume post-dilution online HDF (post-OL-HDF), and **MCO** membrane-HD dialysis for removing PBUTs.

METHODS

Study Design

This was a prospective, randomised, cross-over study conducted at three tertiary dialysis centres in Korea.

Participants

The study included adult patients who were anuric, had permanent vascular access, and received maintenance HDF 3x/week for >3 months. Patients were excluded if they had residual urine of more than 100 cc/day, were pregnant, received dialysis with a catheter, had malignancy, congestive heart failure, or haemodynamic instability.

Treatments

Patients received all dialysis treatments: HF-HD, post-OLHDF, and **MCO** membrane-HD 3x/week for three consecutive weeks each. The order in which patients received the treatments was randomly assigned. Plasma and dialysate samples were collected during the mid-week treatment in the third week. The dialysis duration was 4 h, with a dialysate flow of 500 mL/min and a blood flow of 250–320 mL/min. HDF was performed in post-dilution mode with a target convective volume of >23 L.

Characteristics of the dialysers tested are shown in Table 1. Please note the difference in surface area of the membranes used with the **MCO** membrane having the smallest surface area.

Outcomes

The following plasma levels were measured before and after dialysis:

- Blood urea nitrogen (BUN, 60 Da)
- λ-free light chain (λ -FLC, 45,000 Da)
- β2-microglobulin (β2MG, 11,800 Da)
- Albumin and protein levels

Pre-dialysis serum concentration, post-dialysis concentration, reduction rate (RR), dialysate mass removal and clearance were measured for IS and pCS.

Sample Collection and Analysis

Blood samples were taken before the dialysis onset and immediately after the dialysis session in a mid-week treatment in the 3rd week. Dialysate mixtures were collected from the

	HF-HD	Post-OL-HDF	MCO-HD
Dialyser	Fx CorDiax 80	Fx CorDiax 800	**Theranova** 400 dialyser
Inner diameter (μm)	185	210	180
Wall thickness (μm)	35	35	35
Membrane polymer	polysulphone-PVP blend	polysulphone-PVP blend	polyarylethersulphone-PVP blend
Effective surface area (m2)	1.8	2.0	1.7
Sieving coefficients of albumin	<0.001	<0.001	0.008
Clearance of inulin	135	178	183
UF coefficient (mL/h/mmHg)	64	62	48

PVP, polyvinylpyrrolidone; UF, ultra filtration.

TABLE 1. The characteristics of the dialysers

inverse pump at 60, 120, 180, and 240 min of the dialysis session. A total of 10 mL of the dialysate sample from the mixture was obtained and stored at -80° C until further use.

Simultaneous quantitative analyses of the total IS (212 Da, protein-bound ~90–95%) and pCS (187 Da, protein-bound ~95%) in the plasma and dialysate were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Briefly, 25 µL of human plasma or 125 µL of dialysate was precipitated with acetonitrile, including internal standards (pCS-d7), and vortexed for 30 s, followed by centrifugation for 10 min at 19,500 g at 4° C. Next, the supernatant (200 µL plasma sample and 300 µL dialysate sample) from each tube was transferred to a new microcentrifuge tube and evaporated using a Speed-Vac (HyperVac, VC2200, Gyrozen Co., LTD, Deajeon, Korea). Subsequently, 50 µL and 25 µL of solvent A (5 mmol/L ammonium acetate solution) were used for the dried plasma and dialysate samples, respectively. For quantification of target uraemic toxins, LC-MS analysis was performed using a triple quadrupole mass spectrometer (6490 series, Agilent Technologies, Wilmington, DE, USA) coupled to a 1200 series high-performance liquid chromatography system (Agilent Technologies, Wilmington, DE, USA) with a Hypersil GOLD column (2.1 100 mm ID; 1.9 µm, Thermo Fisher Scientific, Waltham, MA, USA). The injection volume was 2 µL for serum and 3 µL for dialysate analyses. The total run time was 14.5 min for each analysis and the LC gradient system was performed as follows: 0–1 min, 20% solvent B (100% methanol); 1–2.5 min, 20–60% solvent B; 2.5–3 min, 60–95% solvent B; 3–5 min, 95% solvent B; 5–5.5 min, 95-20% solvent B; and 5.5–14.5 min, 20% solvent B with a fixed LC flow rate of 0.15 mL/min. IS and pCS were eluted at 2.8 min and 4.3 min, respectively. The electrospray (ESI) MS method was used to analyse IS and pCS, and all acquisition method parameters were set as follows: capillary voltage: 3000 V in negative mode, drying gas flow: 12 L/min at 290° C, sheath gas flow: 12 L/min at 400° C, and nebuliser gas flow at 30 psi. Multiple reaction monitoring (MRM) conditions, including MS/MS collision energy and computed transitions, were optimized for each molecule to analyse the target uraemic toxins in individual samples. The MRM transition was selected at the following transitions: *m/z* 212.04→80.14, 132.05 for IS, *m/z* 186.98→80.02, 107.03 for pCS, and *m/z* 194.04→80.02, 114.04 for pCS-d7. The charcoal-stripped human plasma and dialysate before dialysis served as blank matrices for constructing the calibration curves. All experiments were performed in triplicate.

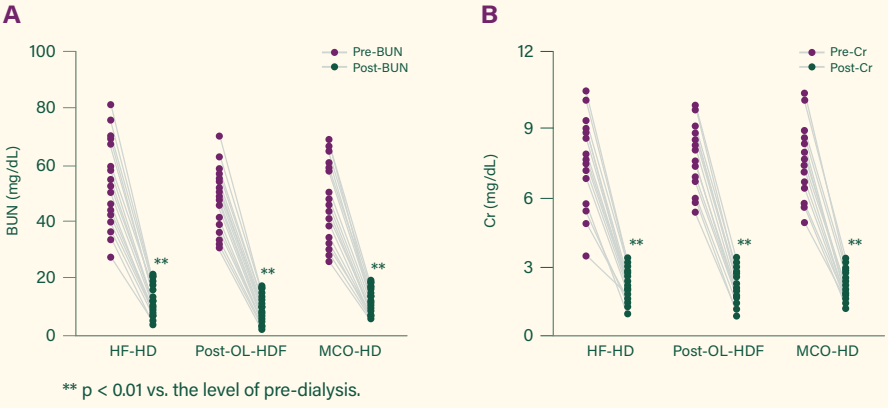
The reduction rate (RR) of the solutes was defined as ($RR = [1 - (C_{post}/C_{pre})] \times 100$, C_{post} = post-dialysis plasma concentration, C_{pre} = pre-dialysis plasma concentration). C_{post} was calculated using a reference formula reflecting haemoconcentration.

For the middle-molecular-weight toxins, C_{post} was replaced by $C_{post\text{-}corr}$ ($C_{post\text{-}corr} = C_{post} / [1 + (BW_{pre} - BW_{post})/(0.2 \times BW_{post})]$, BW_{pre} = body weight before dialysis, BW_{post} = body weight after dialysis).

Total solute removal (TSR) was calculated by multiplying the solute concentrations in the 10 mL dialysate by the effluent volume (dialysate, ultra filtration, and substitution volume). Dialytic clearance was attained by dividing the TSR by dialysis duration. Single-pool Kt/V(spKt/V) was assessed using the reference method.

RESULTS

The mean patient age was 62.18 ± 11.42 and the most common causes of ESRD were diabetes (45.5%) and hypertension (40.9%). Blood pressure and body weight were similar, and there were no differences in baseline dialysis parameters across the three methods. Average blood flow was 302.73 ± 6.92 mL/min. Mean blood and dialysate flow rates were approximately 300 and 530 mL/min, respectively. Mean convection volume was 21.50 ± 1.90 L in post-OL-HDF. The mean ultra filtration volume was 1.8–2.0 L/session.



** p < 0.01 vs. the level of pre-dialysis.

FIGURE 1. Plasma concentrations of blood urea nitrogen (BUN) and creatinine (Cr) before and after dialysis.

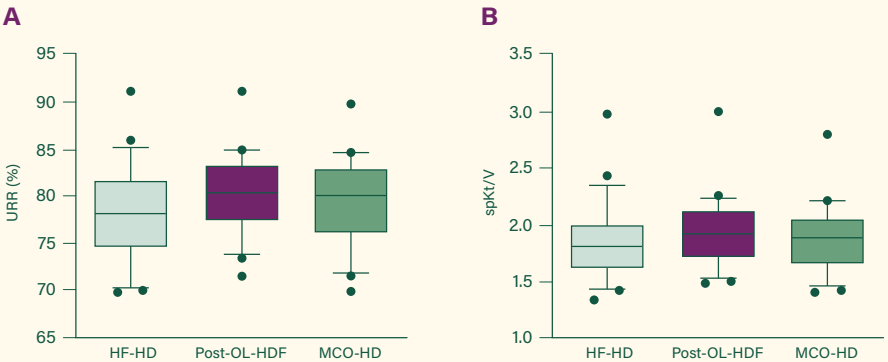


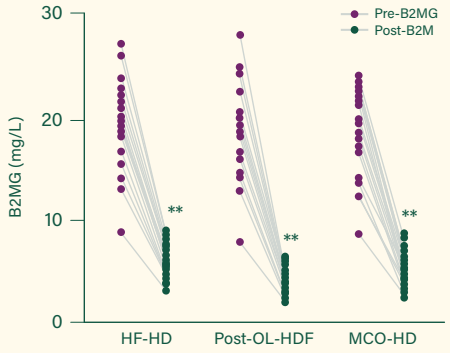
FIGURE 2. The reduction rates of urea reduction rate (URR) and single pool Kt/v (spKt/v).

Clearance of Small and Middle Molecular Weight Toxins

Blood urea nitrogen and creatinine were significantly reduced after dialysis, but there was no difference in post-dialysis levels across the different dialysis methods (Figures 1A and 1B).

URR and spKt/V were similar across dialysis methods (Figures 2A and 2B).

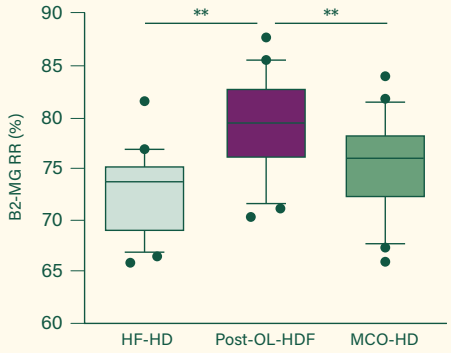
Post-dialysis β2MG level was lowest in post-OL-HDF (4.32 ± 1.21 mg/L, $p < 0.001$), followed by MCO-HD (5.27 ± 1.45 mg/L) and HF-HD (6.27 ± 1.70 mg/L) (Figure 3).



** p < 0.01 vs. the level of pre-dialysis.

FIGURE 3. Plasma concentrations of β2-microglobulin (β2MG) before and after dialysis.

RR of B2MG was higher in post-OL-HDF (79.54 ± 4.72%; $p < 0.001$) than in HF-HD (72.87 ± 3.98%) and MCO-HD (75.32 ± 4.72%). There was no statistical difference between HF-HD and MCO-HD groups (Figure 4).

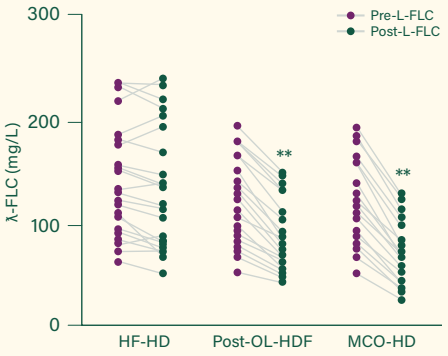


** p < 0.01 vs. other dialysis methods.

FIGURE 4. The reduction rates of β2-microglobulin (β2MG).

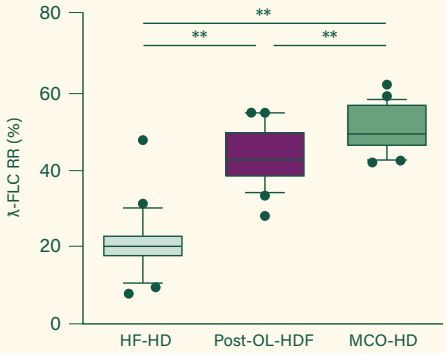
Post-dialysis λ -FLC level was lower in post-OL-HDF (89.23 ± 34.09 mg/L, $p < 0.001$), and MCO-HD (71.59 ± 29.61 mg/L) than in HF-HD (137.73 ± 60.71 mg/L) (Figure 5).

RR of λ -FLC was highest in MCO-HD (51.52 ± 6.08%; $p < 0.001$) followed by post-OL-HDF (43.48 ± 7.41%) and HF-HD (20.8% ± 8.14%) (Figure 6).



** p < 0.01 vs. the level of pre-dialysis.

FIGURE 5. Plasma concentrations of λ-free light chain (λ -FLC) before and after dialysis.



** p < 0.01 vs. other dialysis methods.

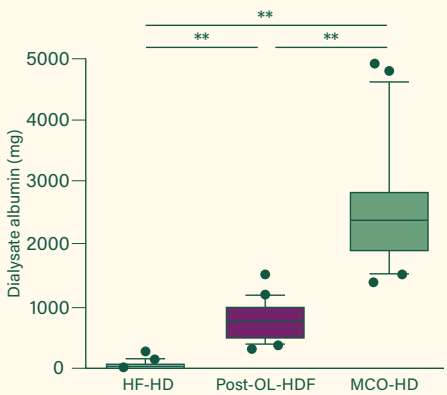
FIGURE 6. The reduction rates of λ-free light chain (λ -FLC).

Dialysate Albumin Removal

There was no significant difference across dialysis methods in:

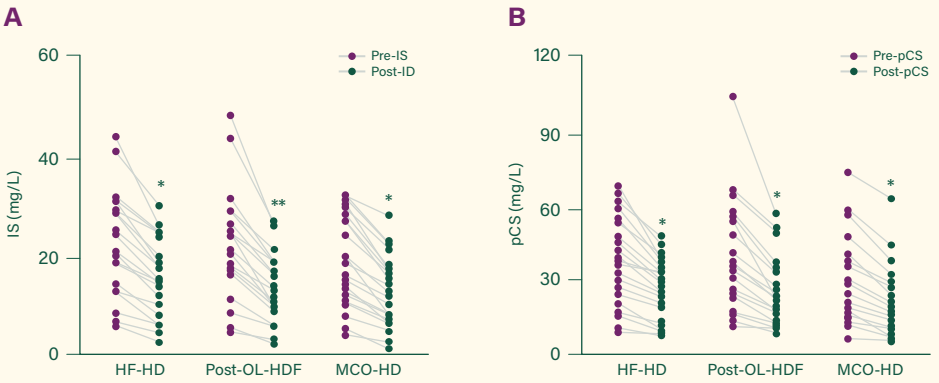
- Pre-dialysis plasma albumin (HD: 3.95 ± 0.21 g/dL, post-OL-HDF: 4.09 ± 0.29 g/dL, and MCO-HD: 3.91 ± 0.29 g/dL)
- Post-dialysis plasma albumin (HF-HD: 4.18 ± 0.50 g/dL, post-OL-HDF: 4.18 ± 0.50 g/dL, and MCO-HD: 4.09 ± 0.43 g/dL)
- RRs of albumin (HF-HD: −9.77 ± 11.09%, post-OL-HDF: −5.26 ± 7.73%, and MCO-HD: −5.50 ± 9.17%)

However, the dialysate albumin mass was highest in MCO-HD (2547.32 ± 968.31 mg/session, $p < 0.001$), followed by post-OL-HDF (778.32 ± 313.17 mg/session) and HF-HD (59.91 + 79.82 mg/session) (Figure 7).



** p < 0.01 vs.other dialysis methods.

FIGURE 7. Dialysate albumin.



* p < 0.05 vs. the level of pre-dialysis, ** p < 0.01 vs. the level of pre-dialysis.

FIGURE 8. Plasma concentrations of indoxyl sulfate (IS) and p-cresyl sulfate (pCS) before and after dialysis.

Clearance of Protein-Bound Uraemic Toxins

The post-dialysis IS and pCS were significantly reduced compared to pre-dialysis in the three different dialysis methods (Figure 8).

The plasma concentrations of IS and pCS were similar across the three methods both pre-dialysis and post-dialysis (Table 2). There was no statistical difference in the RR, dialysate mass removal, and dialytic clearance of IS and pCS among the three different dialysis methods (Table 2 and Figure 9).

There was no correlation between the dialysate mass of IS or pCS and dialysate albumin concentration (IS R=-0.126, p=0.242; pCS R=-0.169, p=0.174).

	HF-HD	Post-OL-HDF	MCO-HD
IS (mg/dL)			
Pre-dialysis plasma concentration (mg/dL)	22.59 ±10.41	21.39 ±11.05	20.08 ± 9.46
Post-dialysis plasma concentration (mg/dL)	15.27 ± 7.60	12.90 ± 6.83	13.12 ± 7.12
RR (%)	33.50 ± 11.48	40.03 ± 10.16	36.31 ± 12.75
Dialysate mass (mg)	94.98 ± 96.01	83.63 ± 100.50	74.31 ± 66.79
Clearance (mL/min)	21.12 ± 19.91	19.50 ± 17.22	19.49 ± 15.81
PCS (mg/dL)			
Pre-dialysis plasma concentration (mg/dL)	37.79 ±18.45	38.70 ± 22.65	33.09 ± 17.47
Post-dialysis plasma concentration (mg/dL)	26.60 ± 12.32	24.80 ± 14.08	23.39 ± 13.68
RR (%)	27.06 ± 10.74	34.44 ± 10.00	29.49 ± 10.28
Dialysate mass (mg)	114.56 ± 12.39	126.19 ± 69.54	101.71 ± 57.43
Clearance (mL/min)	13.58 ± 2.76	15.33 ± 3.17	14.10 ± 3.94

TABLE 2. IS and pCS results

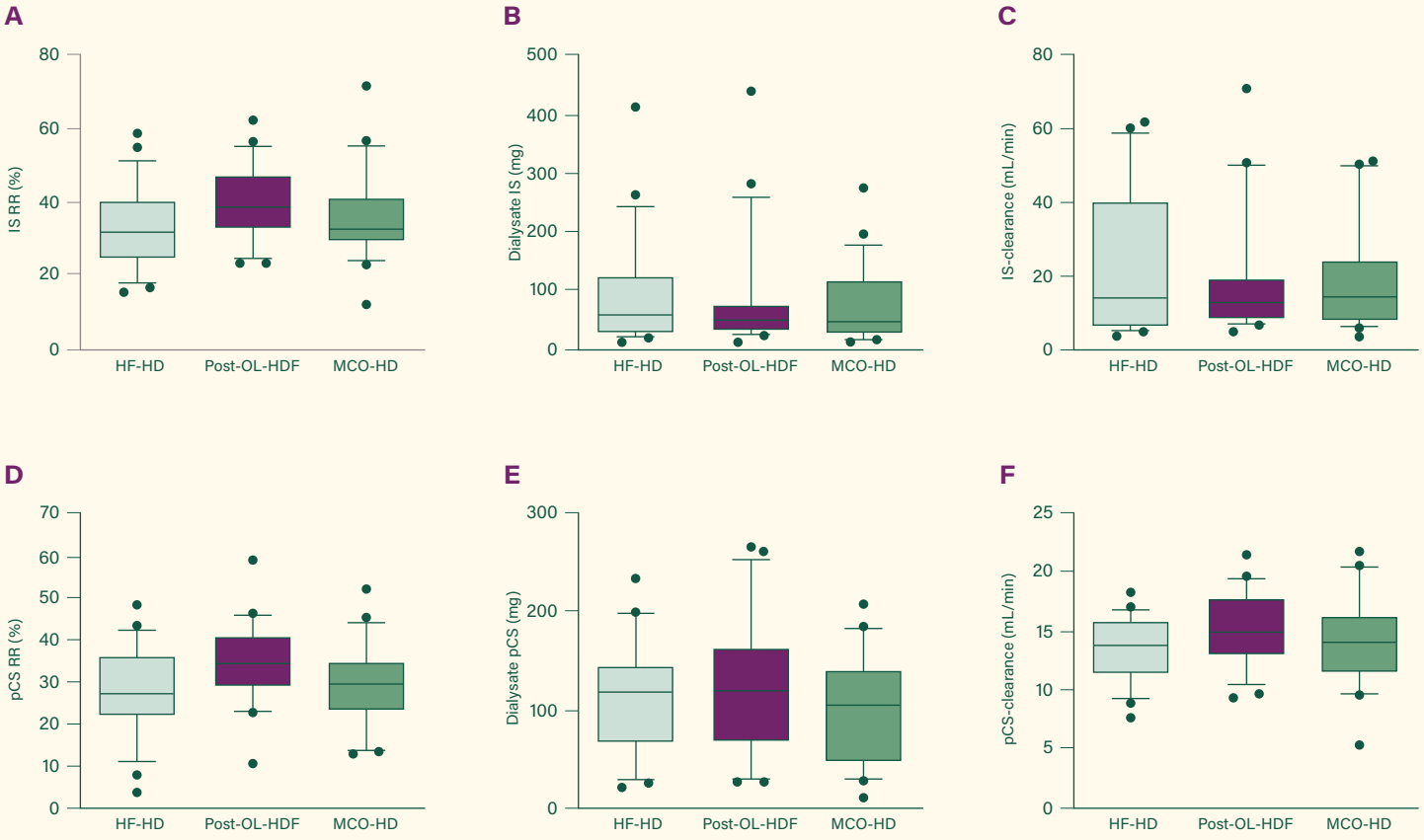


FIGURE 9. The clearing efficacy of IS and pCS. (**A,D**): Reduction rate (RR) of Indoxyl sulfate (IS) and p-cresyl sulfate (pCS); (**B,E**): Dialysate removed IS and pCS; (**C,F**): Dialytic clearance of IS and pCS mass in high-flux haemodialysis (HF-HD), post-dilution online haemodiafiltration(post-OL-HDF), and medium cut-off haemodialysis (MCO-HD).

DISCUSSION AND CONCLUSION

High-efficiency HD, such as **MCO** membrane-HD and high-volume HDF, was expected to have better performance in clearing protein-bound toxins by greater removal of albumin during a dialysis session. Despite higher albumin removal, **MCO** membrane-HD and post-OL-HDF did not show better performance in eliminating IS and pCS compared to that of HF-HD.

IS and pCS are known to be highly bound to albumin, with their free forms accounting for only approximately 2% in the circulation of normal individuals. Because HD cannot replace renal tubular secretion, the plasma levels of IS and pCS are 50–100 times higher in patients on dialysis than in those with normal kidney function.

The native kidney removes PBUTs mainly as free forms, and the clearance of total forms of IS and pCS are only 2.0 and 1.7%, respectively. Dialytic clearance of total forms was slightly lower or similar to kidney clearance, but dialytic clearance of the free forms was only 20–30% of that of the native kidney. The current study confirmed no correlation between dialysate albumin and IS or pCS, supporting that removal of albumin in HDF may not help eliminate PBUTs.

Limitations

The study did not measure the free forms of IS and pCS. Since the solute kinetics of bound and free PBUTs are different, more precise dialytic clearance might be obtained if the free forms are measured. However, dialytic clearance was mainly accomplished with free PBUTs and their blood level was low, thus the effect of their changes compared with total ones might be subtle. In addition, the clearance of the free forms of IS and pCS might be similar since their removal pattern was similar to those of total forms even after dialysis methods (HD vs. HDF) or time (4 vs. 8 h) were changed. Second, dialysate was collected four times every hour. Albumin elimination is higher in the first 90 min of the HD session. Considering albumin kinetics, the actual albumin loss via dialysis might be larger than was measured in the study.

Clearance of the protein-bound uraemic toxins (PBUTs) indoxyl sulfate (IS) and p-cresyl sulfate (pCS) was not different across the three different dialysis methods (HF-HD, post-OL-HDF, MCO-HD).

Vantive

HDx therapy: A world of difference

Patient Reported Measures Reported by Switching to HDx Therapy

HDx therapy may improve patient-reported quality of life aspects of kidney disease, including symptom burden, restless legs syndrome criteria, pruritus and dialysis recovery time.[1-3]

Lim JH, Park Y, Yook JM, et al.
Randomised Controlled Trial of Medium Cut-Off versus High-Flux Dialysers on Quality of Life Outcomes in Maintenance Haemodialysis Patients. *Nature/Sci Rep.* 2020;10:7780.

Alarcon JC, Bunch A, Ardila F, et al.
Impact of Medium Cut-Off Dialysers on Patient-Reported Outcomes (PROs): COREXH Registry. *Blood Purif* 2021;50:110-118.

Bolton S, Gair R, Nilsson LG, et al.
Clinical Assessment of Dialysis Recovery Time and Symptom Burden: Impact of Switching Haemodialysis Therapy Mode. *Patient Relat Outcome Meas* 2021;12:315-321.

Penny JD, Jarosz P, Salerno FR, et al.
Impact of Expanded Haemodialysis Using Medium Cut-off Dialyser on Quality of Life: Application of Dynamic Patient-Reported Outcome Measurement Tool. *Kidney Med.* 2021;3(6):992-1002.

1. Lim JH, Park Y, Yook JM, Choi SY, Jung HY, Choi JY, Park SH, Kim CD, Kim YL, Cho JH. Randomised Controlled Trial of Medium Cut-Off versus High-Flux Dialysers on Quality of Life Outcomes in Maintenance Haemodialysis Patients. *Nature/Sci Rep*. 2020; 10:7780.
2. Alarcon JC, Bunch A, Ardila F, Zuñiga E, Vesga JI, Rivera A, Sánchez R, Sanabria RM: Impact of Medium Cut-Off Dialysers on Patient-Reported Outcomes (PROs): COREXH Registry. *Blood Purif* 2020.
3. Bolton S, Gair R, Nilsson LG, Matthews M, Stewart L, McCullagh N. Clinical Assessment of Dialysis Recovery Time and Symptom Burden: Impact of Switching Haemodialysis Therapy Mode. *Patient Related Outcome Measures* 2021:12 315–321.

Randomised Controlled Trial of Medium Cut-Off versus High-Flux dialysers on Quality of Life Outcomes in Maintenance Haemodialysis Patients

Lim JH, Park Y, Yook JM, et al. Randomised controlled trial of medium cut-off versus high-flux dialysers on quality of life outcomes in maintenance haemodialysis patients. ***Nature/Sci Rep.*** 2020;10:7780.
doi: 10.1038/s41598-020-64622-z

BACKGROUND

Patients on maintenance haemodialysis suffer from symptoms such as fatigue, generalised weakness, and pruritus. These subjective conditions are assumed to be related to the accumulation of middle molecules that are not cleared by conventional haemodialysis (HD). Middle molecules have molecular weights (MWs) ranging between 500 and 60,000 Daltons, and their size is a barrier to removal with dialysers. 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 dialysers and haemodiafiltration (HDF), medium cut-off dialysers may improve the removal of middle molecules due to their higher permeability and increased convective transport, but clinical data on the effects of **MCO** dialysers on patient-reported outcomes are lacking.

OBJECTIVE

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

METHODOLOGY

Study Design

This study was a randomised, 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** dialyser and high-flux groups at 1:1 ratio. Patients and physicians were unblinded to the assignment. The **MCO** dialyser group switched from a high-flux membrane (Fx CorDiax 60 or 80; Fresenius Medical Care Deutschland, Bad Homburg, Germany) to a **Theranova** 400 dialyser (Vantive Health LLC., 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. Uraemic 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** dialyser 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 randomisation. Analysis identified differences between the **MCO** dialyser and high flux groups, pre-and post-dialysis, in levels of three middle molecules: β2-microglobulin (molecular weight (MW) 11.8 kDa[1]), a small middle molecule, and kappa free light chain [κ FLC] (22.5 kDa[1]) and lambda free light chain [λ FLC](45 kDa[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 dialyser, which has a greater surface area (2.0 m^2) than the **Theranova** 400 dialyser (1.7 m^2), was not applied in the **MCO** dialyser group because the **Theranova** 500 dialyser 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 uraemic 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** dialyser 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 dialyser, and dialysis vintage were observed.

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** dialyser group than in the high-flux group and the role-physical function domain score was also higher in the **MCO** dialyser group. See Table 1. The effect of the **MCO** dialyser on QOL is likely related to the better removal of middle molecules compared to high flux dialysers. The improvements in the physical components of the QOL questionnaire over a relatively short exposure period occurred concurrently with the change of the dialyser.

Reduction ratio (%)	Baseline			12 weeks		
	MCO dialyser (*n* = 24)	High-flux (*n* = 25)	*P*	MCO dialyser (*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 encouragement	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 Pruritus Scores

The morning pruritus intensity was worse in the **MCO** dialyser 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** dialyser group than in the high-flux group. The **MCO** dialyser group also had less frequent sleep disturbances caused by pruritus-related scratching. See Table 2.

Comparison of Middle Molecule Concentrations and Reduction Ratios

The serum pre-dialysis and post-dialysis levels of the of three middle molecules (β2-microglobulin, κFLC, and λFLC) did not differ between the **MCO** dialyser and high-flux groups at baseline or at 12 weeks. However, the **MCO** dialyser displayed better removal of κFLC and λFLC compared with the high-flux dialyser. The removal of λFLC was significant, $p < 0.001$. See Table 2.

Comparison of Laboratory Data, Ultra filtration Volume, and Dialysis Adequacy

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

Reduction ratio (%)	Baseline			12 weeks		
	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 uraemic pruritus at baseline and 12 weeks. Abbreviations: **MCO** dialyser, medium cut-off; VAS, visual analog scale. Adapted from Lim et al.

Adverse Events

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

CONCLUSION

This is the first randomised controlled prospective trial comparing the effects of the **MCO** dialyser and high-flux dialysers on QOL in patients receiving maintenance HD. The higher physical functioning and role-physical scores with **MCO** dialyser 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** dialyser group than in the high-flux group. The **MCO** dialyser group also had less frequent sleep disturbances caused by pruritus-related scratching. The new **MCO** dialyser may improve self-reported QOL, particularly in the physical domains and uraemic pruritus, in patients receiving maintenance HD who use permanent dialysis access. The **MCO** dialyser also had a non-significant effect on the serum albumin concentration over 12 weeks of treatment.

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

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.

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

Alarcon JC, Bunch A, Ardila F, et al. Impact of Medium Cut-Off dialysers on Patient-Reported Outcomes (PROs): COREXH Registry. *Blood Purif* 2020. doi: 10.1159/000508803.

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 haemodialysis (HD) therapy removes small solutes, the removal of larger molecules >25 kDa (often termed large middle molecules) is limited. haemodiafiltration (HDF) therapy can remove middle molecules more effectively than HD. However, the effect of an improved uraemic environment resulting from the clearance of middle molecules remains unclear based on randomised studies.

Advances in membrane technology have led to the development of novel medium cut-off 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 haemodialysis 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 haemofilter 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 Haemodialysis Registry Protocol in Colombia (COREXH).

METHODOLOGY

Study Design and Patients

The study was a prospective, multicentre, observational cohort study of 992 patients undergoing dialysis from 12 renal clinics in Colombia who were switched from high-flux HD to **HDx** therapy with **MCO** membrane 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** dialyser (**Theranova**, Vantive, 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 enrolment.

Assessments

Baseline (before switching to therapy with the **MCO** dialyser) 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 enrolment.

Kidney Disease Quality of Life 36-Item Short Form Survey

After 12 months of therapy with the **MCO** dialyser, 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 uraemic 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.

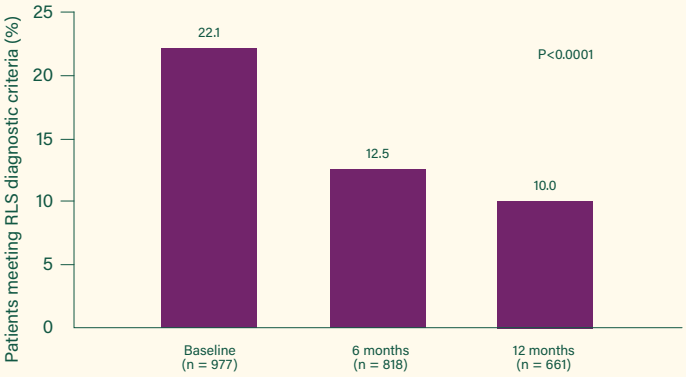


Figure 1. Longitudinal Changes in Patients Meeting Restless Legs Syndrome (RLS) Diagnosis over 12 Months of Follow Up. Abbreviation: RLS, restless leg syndrome. Adapted from Alarcon et al.

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 randomisation 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	0.1a
	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	0.0009b
	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 large-middle molecular uraemic toxins with the MCO membrane may improve patient reported kidney disease quality of life outcomes including symptom burden, and Restless Leg Syndrome (RLS) criteria.

Clinical Assessment of Dialysis Recovery Time and Symptom Burden: Impact of Switching Haemodialysis Therapy Mode

Bolton S, Gair R, Nilsson LG, Matthews M, Stewart L, McCullagh N. Clinical Assessment of Dialysis Recovery Time and Symptom Burden: Impact of Switching Haemodialysis Therapy Mode Patient Related Outcome Measures 2021:12 315–321. doi: 10.2147/PROM.S325016

BACKGROUND

People with end-stage renal disease on haemodialysis (HD) report a high symptom burden that impacts quality of life. Fatigue and lack of energy are common, which interfere with daily life and are associated with poor outcomes. Prolonged recovery time after each dialysis treatment is also common, with patients on conventional HD typically reporting recovery time of 2-4 hours, with approximately 25% reporting recovery time over 6 hours.

It has been proposed that the retention of large middle molecule (MM) uraemic toxins in people with chronic kidney disease could influence the symptom burden. A medium cut-off membrane was recently introduced, allowing for expanded haemodialysis to achieve more effective clearance of large MMs, even when compared to HDF. Further studies are needed to assess the impact of enhanced dialytic removal of large MMs on the dialysis-related symptom burden.

OBJECTIVE

The aim of this clinical assessment was to evaluate the impact of **HDx** therapy on patient reported recovery time and symptom burden.

METHODOLOGY

This pilot retrospective analysis reports on the initial 12-month experience at an in-centre renal unit after implementing **HDx** therapy, focusing on the patient-reported symptom burden during this period.

In 2018 a patient-reported outcome measures (PROM) program was implemented to capture HD patients' symptom burden as part of routine clinical care. At the same time, an evidence-based decision was made to implement **HDx** therapy, using the **MCO** membrane (**Theranova** dialyser; Vantive Health LLC), as the preferred in-centre haemodialysis therapy to achieve effective MM clearance.

PROM Data Collection

PROM data collection started in March/April 2018 and was thereafter performed quarterly. PROM assessments were typically administered to people while on dialysis at a mid-week session. Individuals were asked about their post-dialysis recovery time using the question "How long does it take to get back to normal, after dialysis?". Data on symptom prevalence and severity were collected using the 17-item version of the Palliative Care Outcome Scale–Symptom module for renal patients (POS-S Renal), which asks how 17 predefined symptoms had affected patients in the past week using a 5-point scale ("not at all"= 0, "slightly"= 1,"moderately"= 2,"severely"= 3,"overwhelmingly"= 4).

Dialysis Treatments

At the start of the PROM data collection individuals were on conventional thrice weekly haemodialysis at the in-centre dialysis unit and treated with regular high-flux membranes (Revaclear dialyser in HD and Polyflux H dialyser in HDF; Vantive Health LLC). Following the implementation of PROM assessments, the renal team made an evidence-based decision to implement **HDx** therapy using the **MCO** membrane (**Theranova** dialyser; Vantive Health LLC). Treatment prescription factors such as frequency, blood flow rate (median 300 mL/min), dialysate flow (500 mL/min), and treatment time (median 4 hours) were not affected by the therapy change.

RESULTS

Participants

Overall 90 patients agreed to provide PROM data. Mean age was 73 years, and 62% of participants had received haemodialysis treatments for 3 years or less, and 9% for 10 years or more. Prior to **HDx** therapy, 25 (28%) of participants received HDF and 65 (72%) received HD.

Participant numbers providing data at 3, 6, 9, and 12 months were 80, 72, 68, and 59 respectively.

Safety of Transition to HDx therapy

The **MCO** membrane was introduced without incident over a 2-week period to all individuals on HD at the unit, and it has remained the standard of care. All individuals tolerated the new membrane well. No clinically significant changes in albumin, C-reactive protein, and haemoglobin levels were noted. No signs of increased infection rate were observed.

Post-dialysis Recovery Time

Overall, the median self-reported recovery time at baseline was 240 min (IQR: 60–720; N = 89). At follow-up, the recovery time was shorter:

- > 120 min (22–435) at 3 months,
- > 60 min (0–240) at 6 months ($p < 0.01$),
- > 60 min (0– 240) at 9 months ($p < 0.01$), and
- > 105 min (0–180) at 12 months ($p < 0.01$).

The subgroup of participants who provided recovery time data throughout the 12-month period (N = 58) reported a similarly decreased recovery time (See Figure 1). In this subgroup, the percentage of people reporting a recovery time greater than 360 minutes decreased from 36% at baseline to 26%, 14%, 14%, and 9% at 3, 6, 9, and 12 months, respectively.

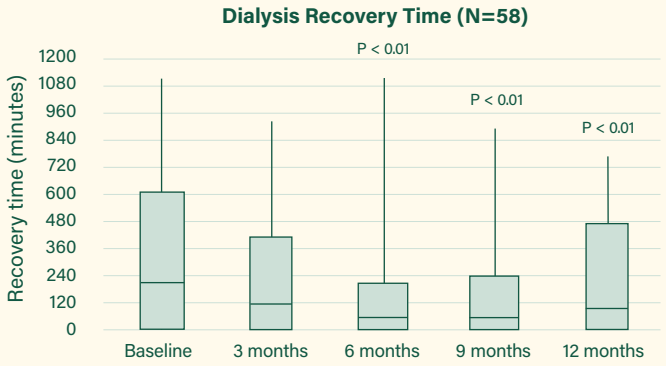


FIGURE 1. Reported post-dialysis recovery times for individuals who completed the 12-months observation period (N = 58). Notes: Boxes show medians and 25th/75th percentiles and whiskers show 95th percentiles #Denotes P < 0.01 vs baseline.

Symptom Burden

In the overall population, at baseline the median number of symptoms per participant was 7 (IQR 4–10; N = 90) and the total symptom score varied between individuals from 1 to 42, with a median value of 13 (IQR 7–18.8). At the 3- and 6-month follow-ups, the total symptom score showed a decrease to 11 (5–16) at 3 months (p = 0.03) and 10.5 (5–19) at 6 months (p = 0.005), while subsequent follow-ups were not different from baseline.

The subgroup of the population who completed the 12-month observation period showed a baseline total score of 12 (7–17.3; N = 56), with a significant decrease at 6 months (See Table 1).

Table 1. POS-S Renal Total Symptom Scores (Median, IQR for Participants Who Provided Ratings Up to 12 Months (N = 56)

Baseline	3 Months	6 Months	9 Months	12 Months
12 (7-17.3)	10 (4.5-16) P = 0.06	8 (5-19) P = 0.003	11 (5.8-20.3) P = 0.8	12 (5-22) P = 0.8

Notes: P-values are in comparison to baseline.

"Weakness or lack of energy" and "Poor mobility" were reported as the most bothersome symptoms. The percentage of patients reporting that "Weakness or lack of energy" affected them "severely" or "overwhelmingly" in the past week decreased from 28% at baseline to 16%, 15%, 20%, and 16% at 3, 6, 9, and 12 months, respectively.

Limitations

As this was not a formal study with a control group, we cannot exclude that improvements seen in recovery time and fatigue were unrelated to the membrane and therapy change. It should be considered that PROM data were not returned anonymously so the identification of specific symptoms at the initial assessment could have led to improved symptom management resulting in reduced symptom severity at later stages. These findings reflect a real-life assessment of PROMs in about 80% of the in-centre HD population, however, being a single-centre experience, these results may not necessarily be applicable to populations in other dialysis centres with other dialysis practices.

CONCLUSIONS

Sustained improvements in patient-reported post-dialysis recovery time and POS-S Renal fatigue score were observed over a 12-month period after a switch from regular HD/HDF using high-flux membranes to **HDx** therapy using the **MCO** membrane. Quarterly application of PROM tools to an in-centre HD population was feasible and well accepted by patients. These results provide indications that enhanced clearance of large middle molecules, as achieved by **HDx** therapy, may have a positive impact on HD individuals' symptom burden.

Switching to HDx therapy with MCO membrane led to a sustained, clinically relevant decrease in patient-reported recovery time after dialysis and a decrease in fatigue levels.

Impact of expanded haemodialysis using medium cut-off dialyser on quality of life: application of dynamic patient-reported outcome measurement tool

Penny JD, Jarosz P, Salerno FR, Lemoine S, McIntyre CW. Impact of expanded haemodialysis using medium cut-off dialyser on quality of life: application of dynamic patient-reported outcome measurement tool. *Kidney Med*. 2021; 3(6):992-1002.e1. doi: 10.1016/j.xkme.2021.05.010.

BACKGROUND

Many patients with chronic kidney disease (CKD) have retained toxins, particularly larger molecular weight toxins, despite maintenance haemodialysis (HD). These toxins have been associated with cardiovascular disease, chronic systemic inflammation, and increased mortality. Not surprisingly, patients receiving maintenance HD often report significant symptom burden and impaired health-related quality of life (HRQoL). To address this need, medium cut-off dialysers have been developed that offer the opportunity to remove middle-molecular-weight molecules without removing essential proteins such as albumin and without the need for high-flux haemodiafiltration and its added requirements of infrastructure, costs, and patient selection criteria. Patient-reported outcome measures (PROMs) may help guide clinicians in determining when traditional HD has not sufficiently managed patient symptoms and improved their HRQoL. Theoretically, the use of a rapid, relevant, and repeated PROM tool could guide clinical decisions about the most appropriate dialyser for a specific patient. The London Evaluation of Illness (LEVIL), is such a PROM instrument. Developed with user input, this tool measures well-being, energy level, sleep quality, bodily pain, appetite, and shortness of breath using visual analog scales. LEVIL takes only seconds to complete, provides real-time monitoring, allows a 24-hour recall period, and is intended for repeated use. An initial study has proven that LEVIL is easy to use, acceptable to patients, and sensitive to clinical changes in the short- and long-term.

OBJECTIVE

This pilot study's main purpose was to establish whether expanded haemodialysis utilising medium cut-off dialysers may be associated with changes in HRQoL/symptom burden, whether there may be a dose-dependent response, and whether effects were durable over time, as assessed using LEVIL.

METHODOLOGY

This single-centre, unblinded, exploratory pilot study was conducted in the prevalent adult HD population within the London Health Sciences Centre Renal Program in Ontario, Canada. All patients had been receiving thrice-weekly HD for > 3 months. During the 2-week baseline period, patients completed the app-based LEVIL assessment during each of their usual high-flux dialyser sessions. During the 12-week test period, patients completed LEVIL while receiving HD with a medium cut-off dialyser that maintained the surface area of the membrane that had been used during the baseline period (i.e., smaller-surface-area dialysers converted to **Theranova** 400 dialyser; larger surface dialysers to **Theranova** 500 dialyser). Blood work included complete blood cell count, electrolytes, C-reactive protein, β2-microglobulin (B2M), κ- and λ-free light chains (K-FLC, L-FLC), and the free light chain ratio.

A 24-week extension was planned to include a washout phase and a return to high-flux HD for 8 more weeks.

Dialysis treatments were delivered using Fresenius 5008 dialysis monitors, with treatment times between 3.5 and 4 hours. Net ultra filtration was calculated on an individual basis according to each patient's ideal dry weight. Dialysis prescriptions were unchanged except for the switch between high-flux polysulfone dialysers and **HDx** therapy. Patients answered 6-question LEVIL surveys via iPad app during each dialysis session. Each participant's LEVIL scores during the first two weeks (i.e., baseline) were averaged to create a collective baseline score that was used to stratify patients into those with high- or low-HRQoL scores. High HRQoL scores were those with an overall average score ≥70; determination of an "acceptable" HRQoL score was based on a survey of 11 study patients.

Primary outcomes were changes in HRQoL and symptoms when patients were treated with **HDx** therapy vs baseline conventional high-flux HD. Secondary outcomes included middle-molecule biomarkers and middle-molecule reduction ratios.

RESULTS

Study Population

Twenty-eight patients consented to participate. One died before study initiation, another died of overwhelming sepsis during the study, one patient was removed due to poor dialysis attendance, and three patients withdrew consent, leaving 22 patients to be analysed over 12 weeks. Due to limited patient access during the COVID-19 pandemic, only 6 patients were able to complete the 24-week extension program.

Participants' mean age was 65.6 ±14.6 years, with a median time on HD of 55 months. Half of the participants were men, 41% had diabetes mellitus type 2, and 41% of patients had some degree of residual kidney function. Half of the population was treated with **Theranova** 400 dialyser, half with **Theranova** 500 dialyser.

Stratification

Sixteen of 22 patients (73%) had a low overall HRQoL baseline. Figure 1A shows how individual participants' HRQoL fell on what survey participants deemed "acceptable" or "unacceptable" for HRQoL scores. Figure 1C shows, at baseline, how many participants had "low" vs "high" HRQoL scores for each domain. Note, for example, that none of the participants ranked energy levels as being at a high HRQoL level. When domain sub-analyses are shown, high and low QoL classifications refer to baseline rankings specific to that domain.

HR-QoL Changes

For the overall HRQoL, the 16 patients with "low" initial overall HRQoL scores and the 6 patients with high initial scores (as shown in Figure 1C) are tracked in Figure 1B and in Table 1 as they received 12 weeks of **HDx** therapy:

> **Low HRQoL group:** The average HRQoL among those with low initial HRQoL increased significantly from baseline to week 8 (P = 0.001) and week 12 (P = 0.001).

> **High HRQoL group:** Patients who had high initial HRQoL saw no significant changes in HRQoL throughout the study.

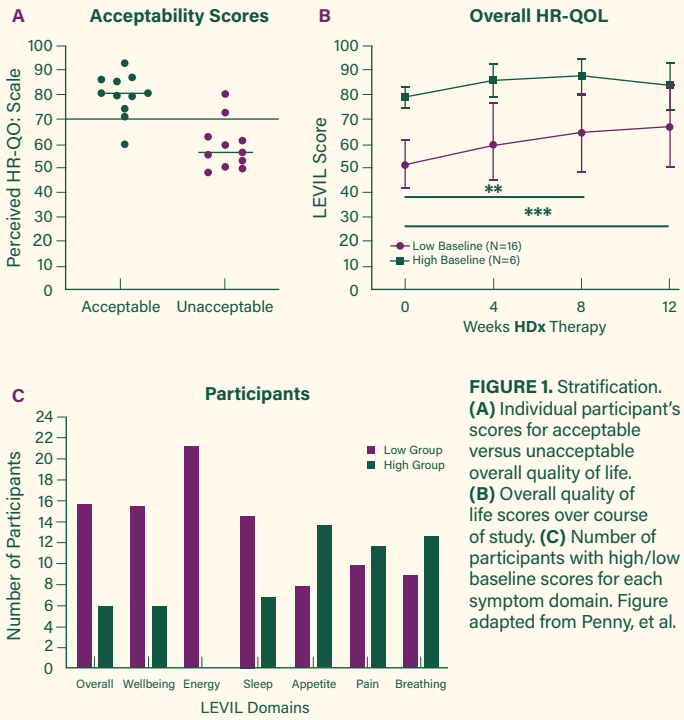


FIGURE 1. Stratification. **(A)** Individual participant's scores for acceptable versus unacceptable overall quality of life. **(B)** Overall quality of life scores over course of study. **(C)** Number of participants with high/low baseline scores for each symptom domain. Figure adapted from Penny, et al.

TABLE 1. LEVIL scores at baseline and 4, 8, and 12 weeks of **HDx** therapy; Total population and stratified groups. Adapted from Penny, et al.

	Total Population							
Initial Study	**N**	**Baseline**	**4-wk HDx**	***P***	**8-wk HDx**	***P***	**12-wk HDx**	***P***
Overall HRQoL	22	59.1±14.4	66.8±17.5	0.12	70.9±17.6	<0.001	71.9±16.8	<0.001
Subgroup analysis								
General well-being	22	52.2±19.6	60.9±23	0.28	69±21.1	0.001	71±17.9	0.002
Energy	22	40.3±20.5	53.4±23.3	0.16	59.9±22.8	0.001	64.7±19.6	<0.001
Sleep quality	22	49.4±26.8	62.2±27.9	<0.001	65.6±24.2	<0.001	68.9±24.5	<0.001
Bodily pain	22	67.3±25.5	68±26.8	>0.99	72.5±25.2	>0.99	71.5±22.1	>0.99
Appetite	22	70.3±21.8	77.9±21.6	>0.99	81.1±21.2	0.28	78.0±22.5	>0.99
Breathing	22	78.2±27.5	77.4±25.8	>0.99	75.9±22.9	>0.99	49.6±22.2	>0.99
Scores < 70 at Baseline: Low								
Initial Study	**N**	**Baseline**	**4-wk HDx**	***P***	**8-wk HDx**	***P***	**12-wk HDx**	***P***
Overall HRQoL	16	51.5±10.2	59.5±14.4	0.33	64.6±16.2	0.001	67.2±16.9	<0.001
Subgroup analysis								
General well-being	16	43±14.1	52.9±21.4	>0.99	65.2±21.9	<0.001	66.3±17.7	0.002
Energy	22	40.3±20.5	53.4±23.3	0.16	59.9±22.8	0.001	64.7±19.6	<0.001
Sleep quality	16	37.2±20.1	52.8±26.7	0.01	57±22.2	0.002	61.7±24.5	<0.001
Bodily pain	10	43.2±12.3	47.4±24	>0.99	56.2±25.7	0.23	57.3±20.5	>0.99
Appetite	8	46.1±14.8	63.8±28	>0.99	67±30.8	0.05	66.9±31.8	>0.99
Breathing	9	49.6±22.2	53.7±27.3	>0.99	53.7±23.5	>0.99	61.6±24.6	>0.99
Scores ≥ 70 at Baseline: High								
Initial Study	**N**	**Baseline**	**4-wk HDx**	***P***	**8-wk HDx**	***P***	**12-wk HDx**	***P***
Overall HRQoL	6	79.2±4.3	86.1±6.8	>0.99	70.9±17.6	0.15	83.6±9.6	>0.99
Subgroup analysis								
General well-being	6	76.6±5.6	82.1±9.7	.71	69±21.1	>0.99	83.5±12.2	>0.99
Energy	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Sleep quality	6	81.8±8.3	87.3±10.4	.15	65.6±24.2	<0.01	89.2±6.3	0.04
Bodily pain	12	87.4±12.1	85.2±13.8	>0.99	72.5±25.2	>0.99	82.9±16.1	0.68
Appetite	14	84.3±8.8	85.9±11.9	>0.99	81.1±21.2	>0.99	84.4±12.4	>0.99
Breathing	13	92±9	95.2±8.4	>0.99	75.9±22.9	>0.99	85.8±16	>0.99

Laboratory Values

Table 2 shows laboratory values for the total population and for the two HRQoL subpopulations at baseline and after 12 weeks of **HDx** therapy:

> **Proteins:** Circulating levels of albumin did not change during therapy (*P* = 0.73); this was also true of the subpopulations (low HRQoL, *P* = 0.096; high HRQoL, *P* = 0.69).

> **B2M:** There was no significant change in serum B2M level, but there was significance in the reduction ratio of B2M between high-flux HD and **HDx** therapy (P < 0.001), and this was true of the subpopulations (low HRQoL, P < 0.001; high HRQoL, P = 0.03).

> **Free light chains:** A significant reduction in serum levels of K-FLC was noted in the overall population (P < 0.001) and the low HRQoL subpopulation (P = 0.02) but not the high HRQoL population (P = 0.16). For L-FLC, the overall population showed a significant decrease over 12 weeks (P = 0.02) even though subpopulations did not reach statistical significance (low HRQoL, P = 0.07; high HRQoL, P = 0.22). Reduction ratios were consistently significant higher with **HDx** therapy for K-FLC and L-FLC in the total population and the subpopulations.

TABLE 2. Laboratory values at baseline compared with 12-Week HDx. Table adapted from Penny et al.

	Baseline	12-wk HDx	Baseline to 12-wk HDx
Total Population Overall HRQoL (N=22)			
Alb, g/L	41±3.8	40.8±2.8	0.73
Alb RR, %	3.9±6.4	4.3±7.1	0.78
B2M, mg/L	28.8±6.8	28.6±5.9	0.91
B2M RR, %	54.2±9.6	70.6±6.3	<0.001
K-FLC, mg/L	183.6±126.7	164.1±100.4	0.002
K-FLC RR, %	27±22.1	53.3±12.7	<0.001
L-FLC, mg/L	119.2±40.1	111.6±36.8	0.02
L-FLC RR, %	3±9.1	29.5±10	<0.001
FLC-R	1.7±1.3	1.6±1.1	0.15
FLC-R RR, %	24.9±21.1	34.1±13.3	0.05
Low Overall HRQoL Group (N=16)			
Alb, g/L	40.6±2.9	40.6±2.9	0.96
Alb RR, %	3.1±6.3	3.8±8	0.88
B2M, mg/L	29.4±7.4	29±6.4	0.63
B2M RR, %	55.3±10.1	71.5±6.4	<0.001
K-FLC, mg/L	198.9±145.1	178.2±113.3	0.02
K-FLC RR, %	25.8±25.9	54.2±14.1	<0.001
L-FLC, mg/L	118.6±36.5	111.7±36.1	0.07
L-FLC RR, %	3.6±8	32.5±10.1	<0.001
FLC-R	1.8±1.5	1.7±1.2	0.37
FLC-R RR, %	23.5±24.6	32.8±14.9	0.23
High Overall HRQoL Group (N=6)			
Alb, g/L	41.8±4.6	41.2±2.9	0.69
Alb RR, %	6.6±6.7	6±2.3	>0.99
B2M, mg/L	27.3±5.2	27.6±4.4	0.44
B2M RR, %	51.1±8.1	68.3±5.9	0.03
K-FLC, mg/L	142.8±38.5	126.6±38	0.16
K-FLC RR, %	30±8	50.9±9.1	0.03
L-FLC, mg/L	120.8±52.6	111.3±42.2	0.22
L-FLC RR, %	1.7±12.1	22.1±4	0.03
FLC-R	1.3±0.3	1.2±0.2	0.22
FLC-R RR, %	28.4±7.9	37.1±8	0.31

Note: Values are represented as mean ± standard deviation.

Domain-specific Subgroup Analysis

Both Table 1 and Figure 2 illustrate changes in individual domains over time:

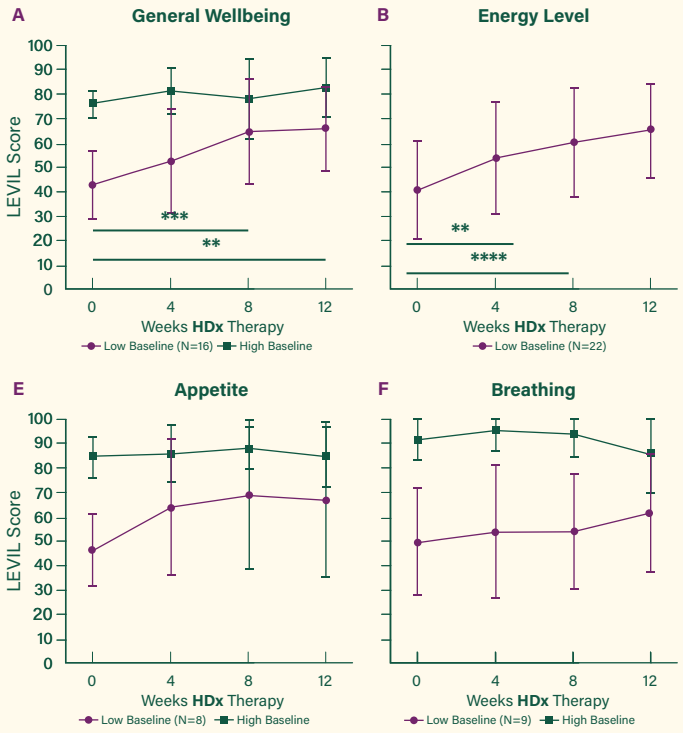
> General well being improved significantly at 8 and 12 weeks (P < 0.001, P = 0.002, respectively) for those in the low HRQoL group at baseline and did not change among those with a high baseline score.

> Energy level was poor for all patients at baseline, but improved significantly at 8 and 12 weeks (P = 0.001, P < 0.001, respectively).

> Sleep quality improved significantly among those with low baseline scores at 4, 8, and 12 weeks (P = 0.01, P = 0.002, P < 0.001, respectively). Those with acceptable sleep quality at baseline reported additional benefit after 8 and 12 weeks of **HDx** therapy (P = 0.001, P = 0.04, respectively).

> Bodily pain, appetite, and difficulty breathing/shortness of breath were not significantly affected by **HDx** therapy over 12 weeks.

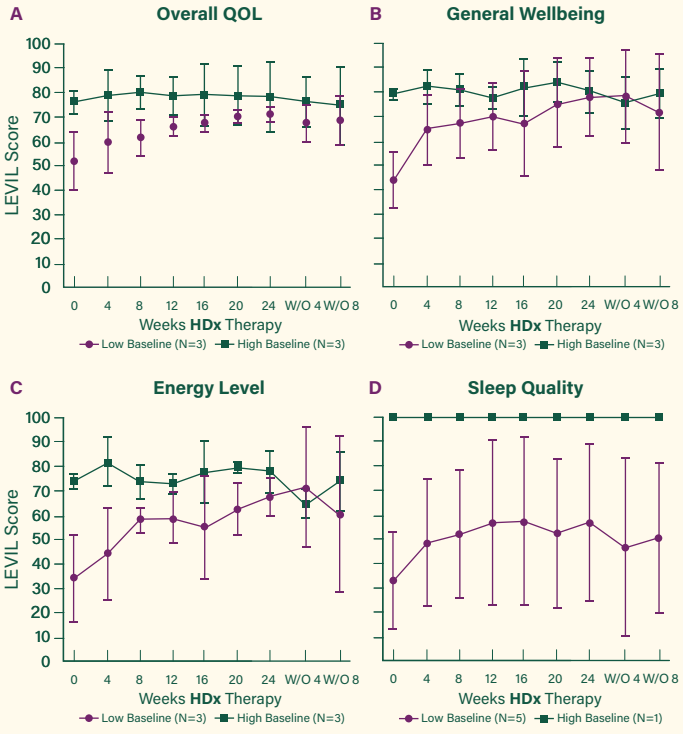
FIGURE 2. Subgroup analysis: domain specific analysis. (A) General well-being, (B) energy, (C) sleep, (D) pain, (E) appetite, and (F) breathing. Figure adapted from Penny, et al.



Extension Evaluation

The COVID-19 pandemic hindered data collection in all but 6 patients, so significance cannot be accurately assessed; Figure 3 is included simply to illustrate that these 6 patient profiles followed a profile consistent with that of the main study. Overall HRQoL, general well-being, and energy level seemed to follow different trends during the 8-week washout period.

FIGURE 3. Extension phase; 24 weeks of **HDx** therapy with 8 weeks of washout (W/O). (A) Overall and (B, C, D) domain-specific quality of life. Figure adapted from Penny, et al.



Study Limitations

Study limitations included small sample size in a single-centre setting and nonrandomised unblinded design.

CONCLUSIONS

In this study, expanded haemodialysis using medium cut-off dialysers over 12 weeks was shown to reduce symptom burden and improve overall HRQoL, particularly among those with poorer HRQoL at baseline. Specific findings include the following:

> Overall HRQoL improved significantly at 8 and 12 weeks among those with low HRQoL at baseline and did not change for those with higher baseline scores.

> Improvements in overall HRQoL seemed to be driven mainly by scores in the domains of general well being, energy level, and sleep quality, which all improved for the group with low scores in those domains at baseline. Interestingly, sleep quality improved with **HDx** therapy even for those with "acceptable" sleep quality at baseline.

> Laboratory values show no significant change in albumin levels for the total population nor for the subpopulations after 12 weeks of **HDx** therapy.

> B2M levels in serum were not significantly changed over 12 weeks, but the reduction ratio of B2M was significantly higher in **HDx** therapy versus high-flux HD .

> A significant reduction in serum levels of κ-FLC was noted in the overall population and the low HRQoL subpopulation but not the high HRQoL population. For L-FLC, the overall population showed a significant decrease over 12 weeks (P = 0.02) even though subpopulations did not reach statistical significance. Reduction ratios were consistently significantly higher for K-FLC and L-FLC in **HDx** therapy versus high flux HD in the total population and the subpopulations.

Vantive

HDx therapy: A world of difference

Clinical Outcomes Impacted by HDx Therapy

Patients receiving **HDx** therapy enabled by **Theranova** dialyser may have a lower rate of infection and fewer infection-related hospitalisation days compared with patients receiving HD.[1-3]

Molano AP, Hutchison CA, Sanchez R, et al.
Medium Cutoff Versus High-Flux Haemodialysis Membranes and Clinical Outcomes: A Cohort Study Using Inverse Probability Treatment Weighting. *Kidney Med.* 2022;4(4):1-10.

Kandi M, Brignardello-Petersen R, Couban R, et al.
Clinical Outcomes With Medium Cut-Off Versus High-Flux Haemodialysis Membranes: A Systematic Review and Meta-Analysis. *Can J Kid Health Dis.* 2022;9:1-16.

Cozzolino M, Magagnoli L, Ciceri P, et al.
Medium Cut-Off (Theranova) Dialyser on Haemodialysis Patients: A Prospective, Cross-over Study. *Clinical Kidney Journal* 2019:1-8.

Lee Y, Jang Mj, Jeon J, et al.
Cardiovascular Risk Comparison between Expanded Haemodialysis Using Theranova and Online haemodiafiltration (CARTOON): A Multicentre Randomised Controlled Trial. *Sci Rep* 2020;11:10807.

1. Molano AP, Hutchison CA, Sanchez R, et al. Medium Cut-Off Versus High-Flux Haemodialysis Membranes and Clinical Outcomes: A Cohort Study Using Inverse Probability Treatment Weighting *Kidney Med*. 2022;4(4):1-10.
2. Kandi M, Brignardello-Petersen R, Couban R, Wu C, Nesrallah G. Clinical Outcomes With Medium Cut-Off Versus High-Flux Haemodialysis Membranes: A Systematic Review and Meta-Analysis. *Can J Kid Health Dis*, 2022:9:1-16.
3. Cozzolino M, Magagnoli L, Ciceri P, Conte F, Galassi A. Effects of a Medium Cut-Off (Theranova®) Dialyser on Haemodialysis Patients: A Prospective, Cross-over Study. *Clinical Kidney Journal*. 2019;1-8.

Medium Cut-Off Versus High-Flux Haemodialysis Membranes and Clinical Outcomes: A Cohort Study Using Inverse Probability Treatment Weighting

Molano AP, Hutchison CA, Sanchez R, et al. Medium Cut-Off Versus High-Flux Haemodialysis Membranes and Clinical Outcomes: A Cohort Study Using Inverse Probability Treatment Weighting. Kidney Medicine. 2022. In Press. doi: https://doi.org/10.1016/j.xkme.2022.100431.

BACKGROUND

Despite advances in the field of chronic haemodialysis (HD) and improvements in morbidity and mortality, outcomes for chronic HD patients are far from optimal. Improved clearance of uraemic toxins may reduce their adverse effects on biological systems and lead to improved outcomes. Recent data suggest that increased removal of large middle molecules may improve clinical outcomes through impact on inflammation and atherosclerosis. Advances in bioengineering have allowed development of a novel medium cut-off membrane (**Theranova** 400/500, Vantive) that improves clearance of large middle-molecules 25 kDa and above.

OBJECTIVE

Determine whether there are differences in clinical outcomes when chronic HD patients are treated with **MCO** membranes compared to high-flux (HF) membranes.

METHODOLOGY

This was a retrospective, observational, multicentre, cohort study of patients undergoing HD (defined as received HD for 90 days) in Colombia. Patients were included from Vantive Renal Care Services network of renal between September 1, 2017 to November 30, 2017, with follow up to November 30, 2019. Enrolment occurred immediately for patients in the **MCO** membrane cohort once switched to this new membrane type. Inclusion criteria included: age >18 years; receiving expanded haemodialysis using an **MCO** membrane (**Theranova** dialyser, Vantive, Deerfield, IL, USA) or conventional HD using HF membrane for a minimum of 4 hours 3 times per week. Patients in both cohorts were included from the same dialysis clinics and membrane allocation was not done by predetermined methods but rather by decisions by each individual clinician. Standard dialysers available in renal clinics were used. Exclusion criteria were life expectancy of less than six months, active infection, metastatic disease, or a Charlson comorbidity index greater than eight.

Patients were divided into two cohorts according to the membrane used at the time of enrolment: 1) **MCO** membrane group or 2) HF group. Once a patient had been included in one of the two cohorts, the clinical teams were instructed not to change the membrane type unless determined by a medical decision or patient request.

OUTCOMES AND ANALYSIS

Primary outcome: hospitalisation rate from any cause and hospitalisation days per patient-year.

Secondary outcomes: non-fatal cardiovascular events (any hospitalisation event for causes predefined in the International Classification of Diseases 10 as cardiovascular); time to death survival analysis from any cause; changes in serum albumin levels during follow-up.

Inverse Probability of Treatment Weighting (IPTW) using a propensity score was used to control differences between groups. The propensity score for each subject was calculated from a logistic regression model that included clinical and demographic variables as predictors of the exposure status such as age, sex, race, dialysis duration, CKD cause, hypertension history, diabetes history, cardiovascular disease history, Charlson comorbidity index, Karnofsky scale, urine output ml/day, BMI, serum albumin, haemoglobin, parathormone intact, phosphorous, potassium, Kt/V single pool, and normalised protein catabolic rate and the research site location. The IPTW for each individual subject was then calculated and the balance between exposed and unexposed groups in the weighted sample was evaluated based on descriptive methods (standardised differences, with a target value of < 0.1, and variance ratios) and inferential methods (over identification test for covariate balance), and the effect of the exposure on each outcome was estimated using robust standard errors. For the outcome hospitalisation rate and days, negative binomial regression models were used for violation of the over dispersion assumption and Akaike and Bayesian Information criteria were used to compare the models; the association was estimated with Incidence Rate Ratio (IRR). Survival time was estimated in the full sample, and the weighed population according to membrane use; the log-rank test was used to compare the equality of the survival functions in the full sample; for comparison in the weighed population, we used Cox regression. To confirm the direction of the effect, negative binomial regression models in full sample were performed.

RESULTS

Patients

A total of 1098 patients were enrolled (564 in the **MCO** membrane group and 534 in the HF group); and 711 completed the total follow-up time (391 in the **MCO** membrane group and 320 in the HF). Mean age was 60.3 ±14.9 years in the **MCO** membrane group, and 60.8 ±15.0 years in the HF group, and 65.2% were male in the HF group and 59.6% in the HF group. The proportion of patients with diabetes mellitus was similar in the two groups, 43.6% for **MCO** membrane vs. 40.1% for HF, p = 0.23. Baseline albumin levels were 4.0 g/dl for **MCO** membrane vs. 4.0 g/dl for HF. After weighting using IPW, no statistically significant difference between groups in baseline characteristics was observed.

Clinical Outcomes

In the weighted sample, **MCO** membrane was associated with fewer all-cause hospitalisation events rate per patient-year, IR = 0.93 [95% CI 0.82–1.03] versus 1.13 [95% CI 0.96–1.30] for HF, corresponding to a significant IRR **MCO** membrane/HF of 0.82 [95% CI 0.68-0.99]; p = 0.04.

There was no significant difference in hospital days; p = 0.73. Across both groups, cardiovascular events were the most frequent cause of hospitalisation (28.5% **MCO** membrane vs 31.1% HF).

The non-fatal cardiovascular event rate per patient-year was lower in the **MCO** membrane group, IR = 0.18 [95% CI 0.14-0.22] versus IR = 0.28 [95% CI 0.19-0.36] for HF, corresponding to a significant IRR **MCO** membrane/HF of 0.66 [95% CI 0.46-0.96]; p = 0.03.

There was no significant difference in survival between **MCO** membrane vs HF dialysers, HR = 0.88 [95% CI 0.64 to 1.23]; p = 0.48.

Table 1 shows clinical outcomes, and Figures 1 and 2 show survival curves.

Table 1. Clinical outcomes in full simple and weighted samples.

Clinical Outcomes	Crude Estimate (95% CI)	*P* value	Adjusted Estimate With IPTW (95% CI)	*P* value
Hospitalisation events[a]				
HF membrane	1.07 (0.99-1.14)	—	1.13 (0.96-1.30)	—
MCO membrane	0.79 (0.73-0.84)	—	0.93 (0.82-1.03)	—
IRR	0.74 (0.67-0.82)	<0.01	0.82 (0.68-0.99)	0.04
Hospital days[a]				
HF membrane	10.18 (9.96-10.4)	—	13.21 (10.47-15.95)	—
MCO membrane	6.45 (6.29-6.62)	—	12.47 (9.36-15.59)	—
IRR	0.63 (0.61-0.66)	<0.01	0.94 (0.68-1.30)	0.73
Nonfatal cardiovascular events[a]				
HF membrane	0.25 (0.21-0.28)	—	0.28 (0.19-0.36)	—
MCO membrane	0.16 (0.13-0.18)	—	0.18 (0.14-0.22)	—
IRR	0.64 (0.52-0.80)	<0.01	0.66 (0.46-0.96)	0.03
Time to death[b]				
HR **MCO**/HF membrane	0.66 (0.50-0.89)	0.01	0.88 (0.64-1.23)	0.48

Note: The IRR was defined as **MCO** membrane/HF.
Abbreviations: CI, confidence interval; HR, hazard ratio; IPTW, inverse probability of treated weighting; IRR, incidence rate ratio.
aNegative binomial regression and expressed as hospital days per patient-years.
bCox proportional regression.

FIGURES 1 and 2. Survival curves according to the type of dialyser membrane. Figure 1 compares the survival functions in the full sample (crude), finding a statistically significant difference in favor of the **MCO** membrane and Figure 2 compares the survival functions in weighted samples, where no difference is observed statistically significant.

FIGURE 1

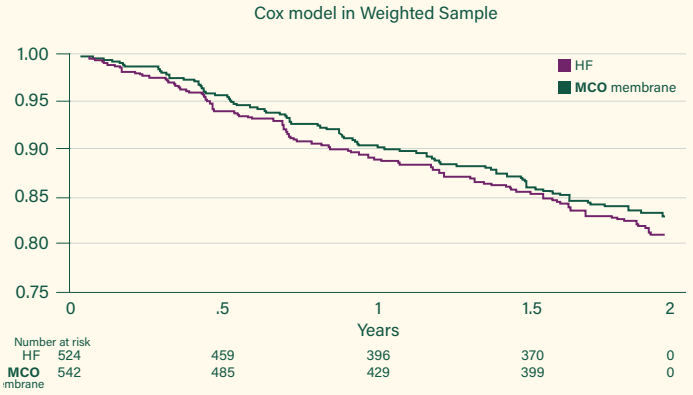
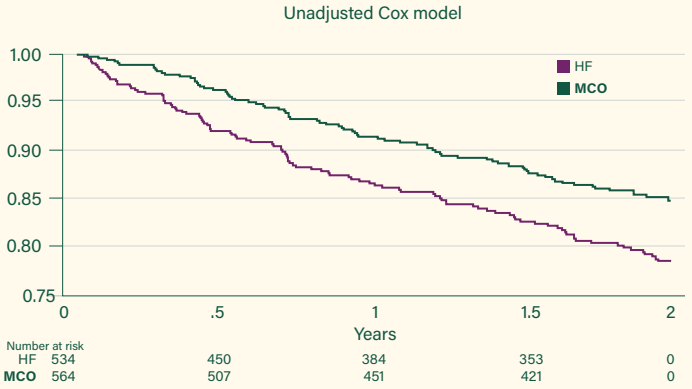


FIGURE 2

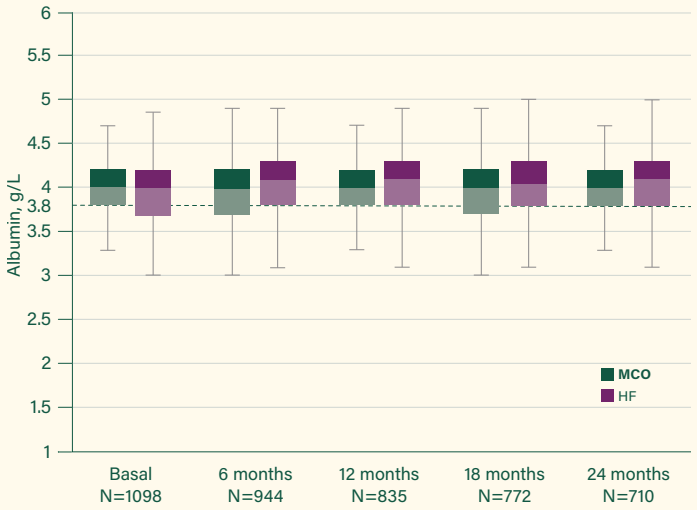


Laboratory Results

There were no differences between the two groups in albumin, phosphate, potassium, or parathyroid hormone evaluated with the split-plot ANOVA test at baseline or at 6, 12, 18, and 24 months of follow-up. Values for haemoglobin and single-pool Kt/V were higher in the **MCO** membrane group at baseline, which continued during follow-up (p < 0.01).

Serum albumin levels remained stable during the 24-month period, see Figure 3.

FIGURE 3. Serum albumin levels



DISCUSSION AND CONCLUSIONS

This cohort study showed that patients treated with **MCO** membranes had lower rates of hospitalisation and cardiovascular events compared with patients treated with HF membranes. These data support the hypothesis that increased clearance of pro-inflammatory middle-molecules could reduce the occurrence of chronic inflammation, atherosclerosis, structural heart disease and immunodeficiency, thereby reducing the frequency of hospitalisation events, particularly for cardiovascular causes.

No difference was found in all-cause mortality or cardiovascular mortality, which could be explained by the short period of follow-up of two years.

There were no differences in serum albumin levels between groups over 2 years of follow-up, demonstrating that stable serum albumin levels are maintained with **MCO** membranes.

Strengths and Limitations

This study's strength lies in the large number of patients included, standardised data collection with robust quality controls and statistical analysis methods used to deal with the confounding inherent in this type of observational study.

The main weakness of the study is its observational nature. Despite the robust statistical analysis there remains the possibility that unmeasured variables may still generate residual imbalance, skewing the results.

CONCLUSION

This large cohort study demonstrated that there is a lower incidence of hospitalisation events and non-fatal cardiovascular events in chronic HD patients dialysed with **MCO** membranes compared to those treated with HF membranes. These results should be corroborated with randomised controlled trials. These results should be corroborated with randomised controlled trials.

HDx therapy with the MCO membrane is associated with fewer hospitalisation events and non-fatal cardiovascular events, and no change in serum albumin compared with HF HD over 2 years follow-up.

Clinical Outcomes with Medium Cut-Off Versus High-Flux Haemodialysis Membranes: A Systematic Review and Meta-Analysis

Kandi M, Brignardello-Petersen R, Couban R, Wu C, Nesrallah G. Clinical Outcomes with Medium Cut-Off Versus High-Flux Haemodialysis Membranes: A Systematic Review and Meta-Analysis. *Can J Kidney Health Dis*. 2022:9;1-16. doi: 10.1177/20543581211067087.

BACKGROUND

Earlier membrane technologies provide minimal diffusive clearance for solutes with molecular weight above 15 kDa. Convective options have been difficult to scale, and high cut-off membranes designed to filter out larger middle molecules unfortunately remove essential blood proteins such as albumin. A novel medium cut-off membrane (**Theranova** 400/500 dialyser, Vantive) removes large middle-molecules while selectively retaining molecules > 45kDa.

OBJECTIVE

Compare the effects of **MCO** membrane versus high-flux membranes used for maintenance haemodialysis (HD) through a systemic review and meta-analysis.

METHODOLOGY

A search was conducted of the MEDLINE, Embase, CINAHL, Cochrane Library, and Web of Science from January 2015 to July 2020, with gray literature that included abstracts from pre-specified conferences. Randomised (RS) and non-randomised studies (NRS) comparing the **MCO** membrane and high-flux membranes in adult outpatients receiving maintenance haemodialysis were included. Study selection, data extraction, and quality appraisals were performed in duplicate and used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework. Outcomes included major clinical events, patient-reported outcomes, safety outcomes, and medication utilisation.

RESULTS

The search identified 22 eligible studies that reported clinical outcomes, including six randomised studies. Among the 16 non-randomised studies, two were cohort studies and the remainder used before-after or crossover designs. The **Theranova** dialyser was the only **MCO** membrane identified in the search. Analysis results are listed by the certainty of evidence per GRADE.

Table 1. Characteristics of Included Studies, Populations, and Interventions.

Author	Publication type	Country (number of centres)	Number of participants enrolled (number analysed)	Follow-up (weeks)	Mean age ± SD (years)	% Male	Interventions	Reported outcomes
Randomised controlled trials **Parallel arm studies**								
Lim et al[20,22]	Full text	Korea (1)	50 (49)	12	I: 62.2 ± 13.7 C: 63.8 ± 15.2	66	I: Theranova 400 C: FxCordiax 80 or 60	Survival, QoL (KDQO)L, pruritus, adverse events, ERI, iron use, albumin (predialysis), MM
Weiner et al[21]	Full text	USA (21)	172 (130)	24	59 ± 13	39	I: Theranova 400 C: Elisio-17H	Survival, hospitalisation, QoL (KDQOL, EQ-5D-5L), albumin (predialysis), MM
Randomised crossover trials								
Belmouaz et al[23]	Full text	France (1)	40 (40)	26	75.5 ± 9.9	70	I: Theranova 500 C: Elisio 21H	Survival, ERI, iron utilisation, albumin RR, albumin (predialysis), MM
Santos et al[24]	Full text	Spain (1)	13 (13)	2	60.1 ± 4.6	92	I: Theranova 500 C: FxCordiax 80VR	Bleeding, extracorporeal circuit clotting, aPTT, anti-Xa
Sevinc et al[25]	Full text	Turkey (2)	52 (42)	26	56.4 (median)	58	I: Theranova 500 C: FxCordiax 80	Adverse events, albumin (predialysis), MM, inflammatory marker
Zickler et al[26]	Full text	Germany (2)	50 (47)	4 (+8 week extension study)	I: 58.1 ± 16.6 C: 59.8 ± 16	38	I: MCO-Ci400 C: Revaclear 400	Survival, adverse events, CRP, albumin (predialysis), MM, inflammatory mark
Non-randomised studies **Cohort studies**								
Cho et al[27]	Full text	Korea (1)	57 (57)	52	I: 53.7 ± 10.9 C: 56.4 ± 10.4	58	I: Theranova 400 C: FxCordiax 80	Survival, ERI, MM, cell-free haemoglobin
Yeter et al[28]	Full text	Turkey (1)	47 (42)	26	52.9 ± 16	63	I: Theranova 400 C: CorDiax 800	Survival, ERI, iron utilisation, CRP, albumin (predialysis)

Table 1. *continued*

Author	Publication type	Country (number of centres)	Number of participants enrolled (number analysed)	Follow-up (weeks)	Mean age ± SD (years)	% Male	Interventions	Reported outcomes
Before-after studies								
Alarcon et al[16]	Full text	Colombia (12)	992 (661)	52	60.5 ± 15.1	62	I: Theranova C: HF	QoL (KDQOL), dialysis symptoms index, restless legs syndrome
Albrizio et al[29]	Abstract	Italy (1)	8 (8)	2	78 ± 14	25	I: Theranova 400 C: HF	Adverse events, myoglobin
Ariza et al (Sanabria, 2020)[17]	Full text	Colombia (3)	81 (81)	104	61.1 ± 12.6	52	I: Theranova 400, 500 C: Polyflux 140, Revaclear 300, 400	Hospitalisation, hospitalisation days, ERI, iron utilisation
Baharani, 2017[a30]	Full text	England (1)	8 (8)	9	71 ± 11.8	75	I: Theranova 400 C: FxCordiax 60, 80	Adverse events, MM
Baharani et al[30]	Abstract	England (1)	18 (18)	8	73 ± 16.7	78	I: Theranova 400 C: Revaclear 300	Adverse events, MM
Bolton et al[31]	Full text (pre-print)	UK (1)	89 (58)	52	73 ± 1	61	I: Theranova C: Revaclear	Minutes to recover, symptoms (POS-S renal) and symptom severity
Bunch et al[a18]	Full text	Colombia (1)	992 (638)	52	60 ± 15	62	I: Theranova C: HF	Survival, hospitalisation, safety, albumin (predialysis)
D'Achiardi et al[32]	Abstract	Colombia (multiple)	52 (41)	24	61 ± 13	65	I: MCO C: HF	Adverse events, albumin (predialysis), MM inflammatory markers, CRP
García-Prieto et al[33]	Full text	Spain (1)	18 (18)	3	65 ± 13	50	I: Theranova 500 C: FxCordiax 80VR	Adverse events, albumin loss and RR, MM
Gernone et al[34]	Abstract	Italy (1)	11 (11)	52	70.8 ± 9	73	I: Theranova C: HF	PCS, MCS, ERI, albumin (predialysis), MM
Kim et al[35]	Full text	Korea (1)	6 (6)	3	66.1 ± 9.1	100	I: Theranova 400 C: Rexeed-21A	Adverse events, albumin loss, albumin RR, MM
Krishnasamy et al[36]	Full text	Australia and New Zealand (9)	89 (79)	32	66 ± 14	62	I: Theranova 400 C: Revaclear 400	Adverse events, QoL, RLS, ERI, CRP, albumin (predialysis)
Penny et al[37]	Abstract	Canada (2)	28 (23)	12	65.8 ± 14.3	52	I: Theranova C: HF	QoL (LEVIL)
Crossover studies								
Cozzolino et al[19]	Full text	Italy (1)	21 (20)	26	71 ± 13	76	I: Theranova 400 C: FX8, FX10, FX80, FX100, BK1.6, BG2.1	Survival, hospitalisation, infection, albumin (predialysis), inflammatory markers

Note. QoL = quality of life; KDQOL = Kidney Disease Quality of Life Instrument; ERI = erythropoiesis resistance index; MM = middle-molecules (removal, reduction ratios, or predialysis serum levels); aPTT = activated partial thromboplastin time; EQ-5D-5L = EuroQol 5-Dimension Questionnaire; RR = risk ratio; **MCO** membrane = medium cut-off membrane; CRP = C-reactive protein; HF = highflux (not otherwise specified); NR = not reported; PMMA = polymethylmethacrylate; POS-S Renal = Palliative Care Outcome Scale–Symptom module for renal patients; PS = polysulfone.
[a]Single-arm **MCO** membrane-HD data without comparator group for survival and hospitalisation outcomes; not amenable to meta-analysis

High Certainty

Recovery Time: A non-randomised study (n=89) found that a year of treatment with **MCO** membrane dialysis reduced recovery time by 420 minutes (95% CI, −540 to −299) shown in Table 1.

Moderate Certainty

Infection: Two non-randomised studies with 68.8 patient years found that **MCO** membrane dialysis likely reduces infection with a rate ratio of 0.38 (95% CI, 0.17 to 0.85; I^2 = 0%).

Hospital Length of Stay: One non-randomised study with 162 patient-years found that **MCO** membrane dialysis likely reduced mean length of stay by a mean difference of −1.5 days (95% CI, −2.22 to −0.78).

Quality of Life: Small non-randomised study reported an improvement of 16.7/100 points on a novel instrument (the London Evaluation of Illness [LEVIL] questionnaire).

Effects of Kidney Disease: Two randomised studies found little to no difference on KDQOL effects (low certainty). A non-randomised study with 993 subjects demonstrated an improvement of 5.4 points (95% CI, 3.2 to 7.6) after 1 year of treatment with **MCO** membrane dialysis.

Pruritus: A randomised study (n=49) found that **MCO** membrane dialysis likely reduces pruritus with MD −4.4 points on a 45-point scale (95% CI, −7.1 to −1.66).

Restless Leg Syndrome: One large non-randomised study measured a reduction in the prevalence of restless legs syndrome from 22.1% at baseline to 10.0%, 1 year after converting to **MCO** membrane dialysis with odds ratio 0.39 (95% CI, 0.29 to 0.53).

Symptom Severity: One non-randomised study measured the proportion of patients with 1 or more symptom rated as "severe" or "overwhelming" at baseline and 1 year. The odds ratio for a reduction in symptom severity with **MCO** membrane dialysis was 0.81 (95% CI, 0.76 to 0.86).

Erythropoiesis Resistance Index (ERI): In 2 randomised studies the pooled mean difference for ERI was −2.92 U/kg/week/g/L achieved haemoglobin (95% CI, −4.25 to −1.6; I^2 = 0%) with **MCO** membrane dialysis.

Low Certainty

KDQOL symptoms/problem list, physical health: The study found that **MCO** membrane dialysis had little to no effect on KDQOL symptoms/problem list, and physical health.

Mortality: Per existing data, **MCO** membrane dialysis may have little to no effect on mortality. Four randomised studies and four non-randomised studies treating a total of 597 patients for 12-52 weeks found an all-cause mortality rate of 3.3/100 person-years for the **MCO** membrane group versus 4.4/100 person-years for the high-flux group, yielding a conclusion that there is little to no difference in mortality.

Hospitalisation for any cause: One randomised study with 78.7 person-years provided low certainty evidence that **MCO** membrane dialysis may result in a reduction in hospitalisation, with a rate-ratio of 0.48 (95% CI, 0.27 to 0.84); two non-randomised studies showed similar effects.

Safety Concerns

Iron Utilisation: In 2 randomised studies (n=90), the pooled mean difference in cumulative intravenous iron use over 12 weeks was −293 mg (95% CI, −368 to −218; I^2=93%), with **MCO** membrane dialysis.

Mental Health Composite Score: **MCO** membrane dialysis had no effect on the KDQOL mental health composite.

Serious adverse events: Four trials that reported SAEs provided low certainty of little to no difference in the rate of fatal or life-threatening adverse events leading to hospitalisation, with seven additional non-randomised studies stating that there were no dialysis-related complications related to the **MCO** membrane.

Other safety outcomes: One study of 130,601 haemodialysis sessions reported no Type A or Type B dialyser reactions with **MCO** dialysis.

Strengths and Limitations

Strengths of this systematic review and meta-analysis include adherence to a registered protocol, a sensitive search strategy, independent screening, data extraction and quality appraisal in duplicate. It used GRADE in all aspects of the review and used a rigorous risk of bias assessment tools. Bias in the meta-analysis of PRO measures was ensured by enlisting a blinded collaborator for groupings. Most outcomes were based on a small number of studies, many non-randomised and studies were relatively small resulting in downgrading for imprecision.

CONCLUSION

The **MCO** dialyser improved a range of outcomes with concordant signals of benefit, and in a manner consistent with its anticipated mechanism of effect. While the current available evidence for **MCO** membrane dialysis is of predominantly moderate certainty, promising innovations in dialysis care are scarce and thus likely to generate interest as the evidence base evolves. The notion that patient-important outcomes can be improved by simply substituting a dialysis membrane is appealing and could by virtue of its scalability, impact patient care, and by its novelty stimulate further innovation. Although larger studies would be needed to further quantify any effects of **MCO** membrane dialysis on major clinical events, to date, there are no signals in the published literature to suggest risk or harm with this device. Given the very low event rates in trials to date, future studies powered for mortality and other major outcomes could be impracticably large; hence, alternate designs such as registry-based cluster randomised trials, prospective cohort studies, and ongoing surveillance might help fill these evidence gaps.

Evidence generated supports that MCO membrane dialysis with the Theranova dialyser may improve patient outcomes.

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

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

BACKGROUND

Current haemodialysis (HD) techniques have important limitations in adequately removing some of the uraemic solutes such as middle molecules and protein-bound uraemic toxins. Middle molecules are organic compounds characterised 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 haemodiafiltration 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 membrane provides diffusive and to some extent convective removal of solutes of molecular weight up to 45 kDa, with only marginal albumin leak. **MCO** membrane has 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 uraemic toxins that can be removed by HD, therefore called 'expanded HD.'

OBJECTIVE

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

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** dialyser (**HDx** therapy) 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.

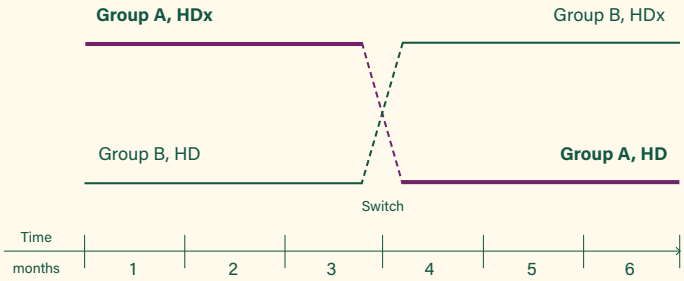


FIGURE 1. Study Design. **HDx** therapy: expanded haemodialysis. HD: haemodialysis. Figure adapted from Ciceri, et al.

There were four dropouts from the study: one patient was relocated to another dialysis centre; one patient was allergic to polysulphone; one patient died; and one patient discontinued treatment with **HDx** therapy. With incidence and timing of dropouts and enrolment 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 dialyser (Vantive USA) was used for patients undergoing **HDx** therapy, 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** therapy treatments.

Haematological Parameters

Haematological 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** therapy 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** therapy 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.

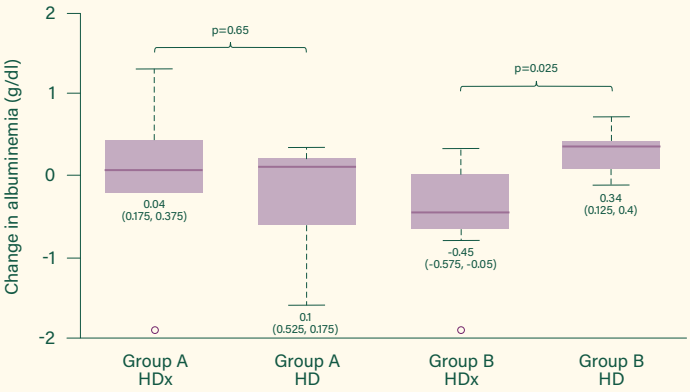


FIGURE 2. Change in albuminemia in Groups A and B during treatment with HDx therapy and HD. Values are presented as median (IQR). Abbreviations: **HDx** therapy, expanded haemodialysis; HD, haemodialysis; IQR, interquartile range. Adapted from Cozzolino et al.

Inflammatory Cytokines

Levels of two inflammatory cytokines, middle molecules interleukin (IL)-1β (17.5 kDa[1]) and IL-6 (21-28 kDa[1]) were measured. While not statistically significant, levels of sera IL-1β were higher under Group A under HD compared with **HDx** therapy, and IL-1β levels were reduced under **HDx** therapy versus HD in Group B. IL-6 levels slightly increased following HD compared with patients under **HDx** therapy in Group A and reduced in **HDx** therapy 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-1β 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-1β 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** therapy 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** therapy 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 categorised into two classes: none and one or more infections per patient. The total number of infections was lower during treatment with **HDx** therapy than with HD (n=7/19 vs n=14/20; p=0.03). See Figure 3.

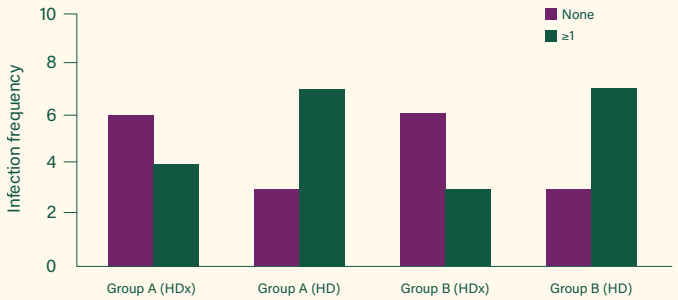


FIGURE 3. Frequency of infections in Groups A and B following HD and **HDx** therapy treatments. Abbreviations: **HDx** therapy, expanded haemodialysis; HD, haemodialysis. Adapted from Cozzolino et al.

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 hospitalisation 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 uraemic toxins and chronic inflammation.

Hospitalisation

The frequency of hospitalisations was categorised into two classes: none or one or more episode per patient. Data revealed a non-significant trend toward higher risk of hospitalisation with HD; the total number of hospitalisations was higher during treatment with HD than **HDx** therapy (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 **Theranova** dialyser with the **MCO** membrance appears to be safe and well-tolerated, without serious side effects or hypoalbuminemia. Importantly, the total number of infections was lower with **HDx** therapy 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 membranes' ability to reduce serum concentration of soluble inflammatory mediators, including cytokines.

Cardiovascular Risk Comparison between Expanded Haemodialysis Using Theranova and Online haemodiafiltration (CARTOON): A Multicentre Randomised Controlled Trial

Lee Y, Jang Mj, Jeon J, et al. Cardiovascular Risk Comparison between Expanded Haemodialysis Using Theranova and Online haemodiafiltration (CARTOON): A Multicentre Randomised Controlled Trial. *Sci Rep* 2020;11:10807 https://doi.org/10.1038/s41598-021-90311-6.

BACKGROUND

Patients with end-stage renal disease are at risk of cardiovascular disease, which is a leading cause of mortality and morbidity. Uraemic toxins contribute to this risk by inducing oxidative stress and vascular inflammation, and inducing platelet activation and aggregation which leads to thrombus formation.

Diffusion-based haemodialysis (HD) in the conventional mode has limited ability to completely remove uraemic toxins, including middle to large uraemic molecules that potentially contribute to the risk of cardiovascular disease. Online haemodialfiltration (online-HDF) is a great option for removing middle-to-large molecules using ultra filtration and subsequent convection. However, online-HDF is not widely used due to the high cost, technical burden, and risk of albumin loss.

HDx therapy integrates diffusion and convection inside a dialyser equipped with a **MCO** membrane. It has shown greater removal of middle-to-large molecules than conventional HD.

OBJECTIVE

The aim of this study was to assess cardiovascular outcomes with the use of **HDx** therapy with an **MCO** membrane versus online-HDF. Online-HDF was associated with improved cardiovascular survival compared with a high-flux dialyser in some pooled analyses, therefore this study assessed noninferiority of **HDx** therapy with an **MCO** membrane to online-HDF.

METHODS

Study Design

This was a multicentre, prospective, open-label, randomised trial conducted at four tertiary referral hospitals in South Korea.

Participants

Adults who underwent in-centre maintenance HD 3x/week for > 3 months, with use of a high-flux dialyser for at least 1 month prior to enrolment were eligible for enrolment. Patients were not eligible if they received online-HDF or used **MCO** membrane before study enrolment, underwent HD more or less than 3x/week, used concurrent peritoneal dialysis, planned kidney transplantation within 1 year, had advanced or active cancer, had monoclonal and polyclonal gammopathies, were pregnant or planning to become pregnant, or were enrolled in another clinical trial.

Of the 86 patients screened, 80 were enrolled and randomly assigned to receive either:

- **HDx** therapy with a **Theranova** membrane (n = 43) (**Theranova** 400 dialyser; Vantive Health LLC, Hechingen, Germany), or
- Online-HDF (n = 37) (**Artis Physio** system; Vantive Health LLC) with a high-flux dialyser (**Polyflux** 170H or 210H dialyser; Vantive Health LLC)

Randomisation was stratified by centre and patient age (≥ 65 or < 65 years old). HD was conducted in three 4-h sessions per week, and the postdilution volume-controlled mode with a target convective ultra filtration volume of ≥ 19 L and a dialysate flow rate of ≥ 500 mL/min was used in online-HDF.

Study Outcomes

The primary outcomes were changes in cardiovascular outcomes after 12 months: brachial-ankle pulse wave velocity (baPWV), echocardiographic parameters, and coronary artery calcium (CAC) scores.

Secondary outcomes included:

- Blood biomarkers including troponin I and T, brain natriuretic peptide (BNP), N-terminal prohormone of BNP (NT-proBNP), plasma interleukin (IL)-6, albumin, calcium, phosphate, and high-sensitivity C-reactive protein
- Patient-reported outcomes including the Dialysis Symptom Index (DSI), the degree of fatigue, and the recovery time after dialysis
- Mortality and its causes (evaluated based on prospective monitoring)

Adverse events were reported throughout the study.

Data collection and Analysis

Baseline information including cardiovascular risk factors were collected. All cardiovascular parameters were recorded at baseline, 6 months and 12 months after enrolment. BaPWV was measured using a noninvasive vascular testing device (VP-1000 plus; Colin Co. Ltd., Japan), obtained from the arm contralateral to the patient's vascular access. Transthoracic two-dimensional echocardiography was conducted to estimate the left ventricular ejection fraction (LVEF) and left ventricular mass index (LVMI). Using pulsed-wave spectral Doppler tissue images, the early transmitral inflow (E) and early diastolic mitral annular peak (e') velocities were calculated to display E/e' as an indicator of LV diastolic function. Noncontrast cardiac computed tomography was performed to obtain the CAC score. The Agatston score was used to calculate the CAC scores, which ranged from 0 to several thousand, indicating extensive coronary atherosclerosis.

The Dialysis Symptom Index (DSI) contained 30 items targeting specific and common physical and emotional symptoms of HD patients. The degree of fatigue after dialysis ranged from 0 to 10, with higher scores indicating worse fatigue. Patients were asked a single open-ended question "How long does it take you to recover from a dialysis session?" The recovery time was scored as 1, within minutes; 2, upon arriving home; 3, by bedtime; 4, by the next morning; and 5, by the next dialysis session.

The sample size was determined by expected differences in baPWV. A comparison of baseline characteristics was performed with the unpaired t-test or Mann–Whitney *U* test for continuous variables and with the chi-square test for categorical variables. Linear mixed-effects models were used to assess changes from baseline and differences between groups in primary and secondary endpoints with treatment assignment and time as fixed effects and patients as random effects. Because there was no prespecified plan to adjust for multiple comparisons, P values and their corresponding 95% confidence intervals were not adjusted for multiple tests, and a Bonferroni-adjusted significance of 0.025 (i.e., 0.05 divided by two) was applied to account for two tests for between-group differences at 6 and 12 months. Kaplan–Meier survival curves were drawn to evaluate the all-cause and cardiovascular mortalities. The log-rank test was used to compare survival curves. Cox proportional hazard ratio models were conducted to calculate hazard ratios of the mortality risk.

RESULTS

Of the 80 patients enrolled, 65 were followed to the end of the study. Five (5) patients in the **HDx** therapy group withdrew from the study, and 3 patients in each group died before the end of the 12 month follow-up. The mean age of all patients was 62 ± 14 years old, and 58.8% were men. The mean Kt/V value was 1.71 ± 0.33. The mean value of achieved convective volume adjusted by weight was 0.3 ± 0.1 L/kg/session in the online-HDF group. There were no differences in baseline characteristics between the groups.

Cardiovascular parameters

There were no differences between group changes in baPWV and echocardiographic parameters such as LVEF, LVMI, and E/e'. See Table 2. The CAC scores remained stable in the online-HDF group, whereas an increasing trend was shown in the **HDx** therapy group. Overall results were similar in the adjusted analysis.

Blood biomarkers

Changes in blood biomarkers did not differ between the two groups. See Table 3. Overall results were similar in the adjusted analysis. The change in albumin levels did not differ between the two groups, with between-group differences of − 0.06 (− 0.20 to 0.09) at 6 months and − 0.10 (− 0.25 to 0.05) at 12 months (both P > 0.05).

Variables	Baseline values	Change from the baseline (mean and 95% confidence intervals)			
		6 months	*P* value	12 months	*P* value
baPWV (m/s)					
HDx therapy	1.8 ± 0.7	0.1 (0 to 0.2)	0.176	0 (− 0.1 to 0.2)	0.518
Online-HDF	1.9 ± 0.7	− 0.1 (− 0.2 to 0)	0.221	0.1 (0 to 0.3)	0.046
Between-group difference		0.2 (0 to 0.3)	0.066	− 0.1 (− 0.3 to 0.1)	0.317
LVEF (%)					
HDx therapy	63.0 (57.0–69.0)	− 0.2 (− 2.5 to 2.1)	0.860	− 0.7 (− 3.1 to 1.7)	0.561
Online-HDF	63.0 (56.0–66.0)	0.6 (–1.8 to 3.0)	0.622	− 0.6 (− 3.1 to 1.9)	0.660
Between-group difference		− 0.8 (− 4.1 to 2.6)	0.648	− 0.1 (− 3.5 to 3.4)	0.966
LVMI (g/m²)					
HDx therapy	111.3 (88.7–138.9)	− 36.1 (− 79.9 to 7.6)	0.106	− 39.2 (− 84.0 to 5.5)	0.086
Online-HDF	114.7 (102.2–142.9)	− 22.5 (− 69.8 to 24.8)	0.351	− 54.7 (− 103.2 to − 6.1)	0.027
Between-group difference		− 13.4 (− 77.7 to 50.8)	0.682	14.5 (− 51.4 to 80.4)	0.666
E/e'					
HDx therapy	12.0 (10.0–16.0)	0.3 (− 1.0 to 1.7)	0.628	0.4 (− 1.0 to 1.8)	0.536
Online-HDF	12.8 (10.0–14.8)	− 0.8 (− 2.3 to 0.6)	0.274	− 0.2 (− 1.7 to 1.3)	0.815
Between-group difference		1.2 (− 0.8 to 3.2)	0.240	0.7 (− 1.4 to 2.7)	0.516
Coronary artery calcium score*					
HDx therapy	295 (19–799)	64.6 (0.7 to 128.5)	0.048	154.9 (91.0 to 218.8)	< 0.001
Online-HDF	268 (16–678)	23.0 (− 43.1 to 89.1)	0.496	36.6 (− 29.5 to 102.7)	0.277
Between-group difference		41.6 (− 50.3 to 133.6)	0.375	118.3 (26.3 to 210.2)	0.012

TABLE 2. Linear mixed effects model for cardiovascular biomarkers.

Variables	Baseline values	Change from the baseline (mean and 95% confidence intervals)			
		6 months	*P* value	12 months	*P* value
BNP (pg/mL)					
HDx therapy	369.1 (185.0–910.0)	51.4 (– 267.9 to 370.7)	0.752	127.4 (– 206.1 to 460.9)	0.454
Online-HDF	287.2 (139.5–907.0)	– 140.7 (– 487.4 to 205.9)	0.426	– 111.7 (– 470.0 to 246.7	0.541
Between-group difference		192.2 (– 279.2 to 663.5)	0.424	239.1 (– 250.4 to 728.6)	0.338
NT-proBNP (ng/mL)					
HDx therapy	3.95 (2.16–6.83)	– 0.31 (– 4.19 to 3.56)	0.874	1.42 (– 2.64 to 5.48)	0.493
Online-HDF	4.27 (2.19–16.82)	– 3.24 (– 7.40 to 0.92)	0.127	– 3.32 (– 7.61 to 0.98	0.130
Between-group difference		2.96 (– 2.72 to 8.64)	0.307	4.66 (– 1.25 to 10.57)	0.122
Troponin-I (ng/mL)					
HDx therapy	0.06 (0.04–0.09)	0 (0 to 0.01)	0.538	0 (– 0.01 to 0.01)	0.550
Online-HDF	0.06 (0.03–0.07)	0 (– 0.01 to 0.01)	0.901	0 (0 to 0.01)	0.189
Between-group difference		0 (– 0.03 to 0.01)	0.224	0 (– 0.02 to 0.02)	0.926
Troponin-T (ng/mL)					
HDx therapy	0.06 (0.04–0.09)	0 (– 0.01 to 0)	0.362	0 (– 0.01 to 0.01)	0.437
Online-HDF	0.06 (0.03–0.07)	– 0.01 (– 0.02 to 0)	0.130	– 0.01 (– 0.02 to 0)	0.139
Between-group difference		0 (– 0.01 to 0.02)	0.595	0 (– 0.01 to 0.02)	0.526
C-reactive protein (mg/dL)					
HDx therapy	0.07 (0.04–0.23)	0.21 (– 0.30 to 0.72)	0.421	0.10 (– 0.43 to 0.62)	0.722
Online-HDF	0.09 (0.04–0.23)	0.47 (– 0.08 to 1.01)	0.095	– 0.02 (– 0.54 to 0.58)	0.951
Between-group difference		– 0.25 (– 1.00 to 0.50)	0.511	0.08 (– 0.69 to 0.74)	0.835
Interleukin-6					
HDx therapy	9.55 (7.78–11.48)	4.09 (– 2.28 to 10.47)	0.208	1.85 (– 4.76 to 8.47)	0.582
Online-HDF	8.40 (7.02–11.94)	0.55 (– 6.17 to 7.27)	0.872	– 1.88 (– 8.77 to 5.00)	0.592
Between-group difference		3.54 (– 5.72 to 12.80)	0.453	3.74 (– 5.81 to 13.28)	0.443

TABLE 3. Linear mixed effects model for blood biomarkers.

Mortality

There were 6 deaths (7.5%; 3 in the **HDx** therapy group and 3 in the online-HDF group) during the study, with 2 of the deaths due to cardiovascular events. Survival curves for cardiovascular and all-cause mortality did not differ between the two groups (*P*=0.868 all-cause; *P*=0.928 cardiovascular).

When the Cox models were applied, the **HDx** therapy and online-HDF groups had similar risks of cardiovascular and all-cause mortality.

Patient-reported outcomes

DSI scores, degree of fatigue, and recovery time after dialysis were not different between the two groups. See Table 4.

Variables	Baseline values	Change from the baseline (mean and 95% confidence intervals)			
		6 months	*P* value	12 months	*P* value
Number of symptoms in DSI					
HDx therapy	12 (5–20)	– 2.0 (– 3.8 to – 0.3)	0.024	– 1.4 (– 3.3 to 0.5)	0.140
Online-HDF	11 (4–16)	– 0.5 (– 2.3 to 1.4)	0.634	– 1.0 (– 2.9 to 1.0)	0.321
Between-group difference		– 1.6 (– 4.2 to 1.0)	0.231	– 0.4 (– 3.1 to 2.3)	0.765
Overall symptom severity score in DSI					
HDx therapy	17 (10–37)	– 2.1 (–6.1 to 1.9)	0.299	– 2.7 (– 6.8 to 1.5)	0.208
Online-HDF	18 (6–27)	– 1.4 (–5.6 to 2.8)	0.517	– 1.3 (– 5.7 to 3.1)	0.567
Between-group difference		– 0.7 (– 6.5 to 5.1)	0.814	– 1.4 (– 7.4 to 4.6)	0.651
Degree of fatigue after dialysis					
HDx therapy	6 (5–7)	0.1 (– 0.6 to 0.9)	0.702	– 0.6 (– 1.3 to 0.2)	0.164
Online-HDF	5 (3–6)	– 0.3 (– 1.1 to 0.5)	0.513	0.1 (– 0.8 to 0.9)	0.880
Between-group difference		0.4 (– 0.7 to 1.5)	0.459	– 0.6 (– 1.8 to 0.5)	0.287
Recovery time after dialysis					
HDx therapy	3 (2–3)	0 (– 0.3 to 0.3)	0.975	0 (– 0.3 to 0.4)	0.786
Online-HDF	2 (1–3)	– 0.1 (– 0.4 to 0.3)	0.730	– 0.1 (– 0.4 to 0.3)	0.713
Between-group difference		0 (– 0.4 to 0.5)	0.912	0.1 (– 0.4 to 0.6)	0.644

TABLE 4. Linear mixed effects model for patient-reported outcomes

DISCUSSION AND CONCLUSION

This trial indicated that **HDx** therapy with **MCO** membrane was not inferior to online-HDF in terms of cardiovascular risk according to the change trends in the values of baPWV, LVEF, LVMI, E/e', and other blood biomarkers. Additionally, the changes in patient-reported outcomes did not differ between the two groups.

The CAC, a marker of vascular remodeling in ESRD patients, seemed to be increasing in the **HDx** therapy group compared with the online-HDF group. However, these results were based on subgroup analysis because of missing data in some patients. In addition, the normal aging process might mask the changes caused by uraemia-related calcification, particularly in elderly patients.

Meta-analyses reported survival and cardiovascular benefits for online-HDF, and some studies have shown that patients on online-HDF had better quality of life than patients on conventional HD. This clinical trial was the first to compare cardiovascular parameters between **HDx** therapy and online-HDF, and study results may support the interchangeability of **HDx** therapy with **MCO** membrane when online-HDF is recommended in terms of cardiovascular benefit.

Limitations

This trial was conducted only in Korean patients, and the dialysis settings or the risk of cardiovascular disease may be different in other populations. The study duration and sample size were relatively short and low, respectively. Most of the measurements were conducted in individual hospitals, not in a central laboratory, which might increase the measurement variability, although the same protocol was used. CAC analysis was based on subgroup analysis because of missing data in some patients. Other important parameters were not examined, such as residual kidney function, intradialytic haemodynamic stability, and nutritional status.

HDx therapy with **Theranova** membrane was similar to online-HDF in most cardiovascular parameters, blood markers, patient-reported outcomes and in both all-cause mortality and cardiovascular mortality.

Vantive

HDx therapy: A world of difference

Economic Outcomes Impacted by HDx Therapy

HDx therapy enabled by **Theranova** dialyser in chronic dialysis patients may reduce hospitalisation rates and cardiovascular events compared to conventional HD.[1,2]

Blackowicz MJ, Falzon L, Beck W, et al.
Economic evaluation of expanded haemodialysis with the Theranova 400 dialyser: A post hoc evaluation of a randomised clinical trial in the United States. [published online ahead of print, 2022 Apr 19]. *Haemodial Int.* 2022.

Ariza JG, Walton SM, Suarez AM, et al.
An initial evaluation of expanded haemodialysis on hospitalisations, drug utilisation, costs, and patient utility in Colombia. *Ther Apher Dial.* 2021;25(5):621-627.

Sanabria R, Hutchison C, Vesga J, et al.
ExpandedHaemodialysis and Its Effects on Hospitalisations and Medication Usage: A Cohort Study. *Nephron* 2021;145:179-187.

Lim JH, Jeon Y, Yook JM, et al.
Medium Cut-Off Dialyser Improves Erythropoiesis Stimulating Agent Resistance in a Hepcidin-Independent Manner in Maintenance Haemodialysis Patients: Results from a Randomised Controlled Trial. *Nature/Sci Rep* 2020;10:16062.

Hadad-Arrascue F, Nilsson L-G, Rivera AS, et al.
Expanded haemodialysis as effective alternative to on-line haemodiafiltration: A randomised mid-term clinical trial. *Ther Apher Dial. 2022;26:37-44.*

1. Blackowicz MJ, Falzon L, Beck W, Tran H., Weiner DE, Economic evaluation of expanded haemodialysis with the Theranova 400 dialyser: A post hoc evaluation of a randomised clinical trial in the United States. [published online ahead of print, 2022 Apr 19]. *haemodial Int*. 2022.
2. Molano AP, Hutchison CA, Sanchez R, et al. Medium Cut-Off Versus High-Flux Haemodialysis Membranes and Clinical Outcomes: A Cohort Study Using Inverse Probability Treatment Weighting, *Kidney Med*. 2022;4(4):1-10.

Economic evaluation of expanded haemodialysis with the Theranova 400 dialyser: A post hoc evaluation of a randomised clinical trial in the United States

Blackowicz MJ, Falzon L, Beck W, Tran H, Weiner DE et al. Economic evaluation of expanded haemodialysis with the Theranova 400 dialyser: A post hoc evaluation of a randomised clinical trial in the United States *haemodial Int*. 2022 Online ahead of print. doi: 10.1111/hdi.13015.

BACKGROUND

In 2018, approximately 485,000 people in the US received maintenance dialysis for kidney failure, a number that has doubled in the last 20 years. Medicare funds about three fourths of patients requiring dialysis; although dialysis is required by <1% of Medicare enrollees, these patients account for 7.2% of paid Medicare claims.

Despite dialysis, uraemic toxins may accumulate over time and increase patient morbidity and mortality. Conventional dialysis modalities such as high-flux haemodialysis (HD) typically remove small molecules (<0.5 kDa) and smaller middle molecules (0.5-25 kDa), but not larger middle molecules (25-60 kDa). To address this unmet need, the **Theranova** 400 dialyser (Vantive Health LLC, Deerfield, IL) has been developed and was approved by the FDA in August 2020. The **Theranova** 400 has an expanded solute removal profile up to 45 kDa and may be used in expanded haemodialysis, a technique that combines diffusion and convection in a hollow-fiber dialyser with a medium cut-off membrane. In the original analysis, this randomised, controlled trial has shown **HDx** therapy with **Theranova** to be superior to high-flux HD in terms of the removal of larger middle molecules such as lambda-free light chains, while maintaining adequate serum albumin levels. This secondary analysis aimed to determine if use of **HDx** therapy could also reduce healthcare costs for this patient population, particularly if, as expected, removal of larger middle molecules is associated with improved clinical outcomes.

OBJECTIVE

This post-hoc analysis therefore reviewed trial data regarding clinical outcomes in particular hospitalisation rates, and healthcare costs of patients treated with either **HDx** therapy with **Theranova** or high-flux HD.

METHODOLOGY

This prospective, randomised, open-label, parallel study (NCT03257410) in patients receiving maintenance dialysis treatment compared results of those treated with **HDx** therapy with the **Theranova** 400 dialyser to those dialysed with a high-flux HD dialyser of the same size, with thrice-weekly sessions over 24 weeks. Patients were clinically stable adults who had received HD with a high-flux dialyser for ≥3 months; all had stable vascular access and maintained an acceptable urea clearance. Patients were excluded if they had had an acute infection in the previous 4 weeks or if they had certain chronic diseases (i.e., cancer, HIV, hepatitis, chronic liver disease, paraprotein-associated disease, monoclonal/polyclonal gammopathy).

- Hospitalisation – any SAE which contained a hospitalisation admission date. Hospitalisation rate - total number of hospitalisations/total person years of follow up during the trial period.
- Erythropoietic stimulating agents (ESA) use was captured in the study but data were incomplete so use and dose were assumed to be equal between 2 arms.
- Cost of hospitalisation – taken from Kaiser Family Foundation USA average in 2018.

This analysis examined hospitalisation, hospitalisation rate (number of hospitalisations divided by total person-years of follow up), hospital length of stay, total cost of hospitalisation, and cost of dialysers. The cost per high-flux dialyser was assumed to be $6.50, and the cost per **Theranova** dialyser was $15.00; other costs associated with dialysis were assumed to be equal and were excluded from the model, as were patient medications and medication doses.

Statistical Analysis

Mean length of hospitalisation was estimated using a Poison (log-count) general linear model. A univariate sensitivity analysis was included to evaluate the impact of observed variability on cost differences between treatments. Itemised and total costs per group were calculated through random sampling of all input parameters based on the closest approximations of their observed distributions; repetition over 10,000 simulations generated the mean and 95% confidence intervals (CI).

RESULTS

Study Population

A total of 172 patients were randomised, and 171 were treated at 21 US centres between September 2017 and October 2018; 86 were treated with **HDx** therapy using **Theranova** (389 patient-months), and 85 with a high-flux dialyser (366 patient-months). Baseline demographics and clinical characteristics were similar between groups; 39% of patients were female, and the mean age was 59±13 years.

Clinical outcomes: hospitalisation and length of stay

Clinical outcomes are shown in Table 1. Hospitalisation was 45% lower in patients treated with **HDx** therapy with **Theranova** compared with high-flux HD (IRR=0.55; 95% CI: 0.30, 1.00; P=0.042). There was no significant difference in length of hospital stay.

TABLE 1. Clinical Outcomes

Health resource utilisation	Theranova (n=86)	High-flux HD (n=85)	P-value
Hospitalisation events	18	31	--
Total hospital days	74	139	--
Total patient-years (PY)	32.4	30.5	--
Hospitalisation rate per PY	0.56 (0.13)	1.02 (0.12)	0.042
Hospital length of stay (mean days [SE])	4.11 (0.57)	4.63 (0.58)	0.406

HD, haemodialysis; PY, patient year; SE, standard error.

Adapted from Blackowicz et al.

Economic evaluation

Table 2 shows the economic evaluation of **HDx** therapy with **Theranova** compared with high-flux HD. While the trial lasted 6 months, the economic model calculates values over one year of treatment. Although **HDx** therapy with **Theranova** is associated with a higher dialyser cost, its lower all-cause hospitalisation rate makes it a cost-efficient alternative. Compared with high-flux HD, **Theranova** offered an average annual estimated cost savings of $4772. Hospitalisation rate and length of stay were the main drivers of cumulative cost.

TABLE 2. Economic Evaluation

		Per Patient Cost		
Item	**Unit cost**	**Theranova**	**High-flux HD**	**Difference**
All-cause hospitalisation[a]	$2518 per day	$5756	$11,853	-$6097
Dialyser cost[b]	$15.00 ea/ $6.50 ea	$2340	$1014	$1326
Cumulative		$8096	$12,867	-$4771

[a]All-cause hospitalisation was defined as any serious adverse event that resulted in hospitalisation. [b]**Theranova** dialyser was priced at $15 in the United States and high-flux dialyser was assumed to cost $6.50.
HD, haemodialysis.

Adapted from Blackowicz et al.

Probabilistic sensitivity analysis

A univariate sensitivity analysis (Figure 1) accounted for the observed variability in each model separately. Hospitalisation rates were the main drivers of cost difference, particularly in the high-flux HD group. Results favoured **Theranova** at the upper and lower thresholds for all inputs.

FIGURE 1. Univariate sensitivity analysis

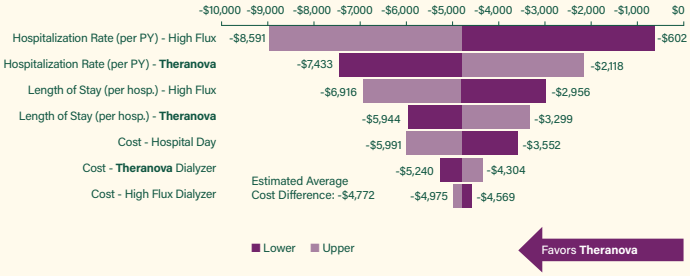


Figure adapted from Blackowicz et al.

Summary estimates were run with >10,000 simulations of costs to verify the mean difference in cost between treatments. Table 4 shows the probabilistic analysis, which determined that **HDx** therapy with **Theranova** was associated with lower costs in 96% of the simulations.

TABLE 3. Simulated summary of methods of mean cost difference

	Per Patient Cost Difference	
Item	**Mean**	**(95% CI)**
All-cause hospitalisation (per day)	-$6103	(-$11,604 to -$601)
Dialyser cost	$1326	--
Cumulative	-$4777	(-$10,278 to $725)
Proportion of simulations demonstrating Theranova cost-saving over high-flux HD	95.7%	

HD, haemodialysis

Adapted froackowicz et al.

Study Limitations

The 6-month trial had too few hospitalisation events to draw sound statistical conclusions between categories (e.g., to eliminate trauma cases or assess infection- or cardiovascular-related events). Cost assumptions had to be made to complete the analysis; assuming that medications would be equivalent between arms was one such assumption. Also, study records on erythropoietin-stimulating agents and iron dosing were incomplete, and authors would have liked to incorporate that variable. Other potential study confounders seem to balance across treatment arms and are unlikely to affect study results.

DISCUSSION

There are few studies on health economics and patient outcomes regarding **HDx** therapy with **Theranova**, but this study supports the findings of two other studies, one showing that **HDx** therapy with **Theranova** was associated with lower hospitalisation costs and the second (a retrospective, observational study) that showed that **HDx** therapy with **Theranova** was associated with a lower risk of hospitalisation.

Authors add that the study entry criteria required that dialysis patients be medically stable so the study group tends to be younger than the average dialysis patient age in the USA. Since hospitalisation costs drove healthcare costs in this relatively young and healthy population, cost differences could be greater still in a real-world setting, where patients may be older and less medically stable.

Findings are of potential importance in upcoming US payment models in kidney care, particularly the Comprehensive End-Stage Renal Disease Care Model (ESCO) and Kidney Care Choices, which consider hospitalisation costs and the number of hospitalisations.

CONCLUSIONS

In this post-hoc analysis of a randomised clinical trial, the **HDx** therapy with **Theranova** dialyser was associated with lower healthcare costs than standard high-flux HD. In addition to its superior removal of large middle molecules, the **Theranova** dialyser is associated with a reduced hospitalisation rate per patient year and is expected to be a cost-saving therapy, based on this significant reduction in hospitalisation events.

An Initial Evaluation of Expanded Haemodialysis on Hospitalisations, Drug Utilisation, Costs, and Patient Utility in Colombia

Ariza JG, Walton SM, Suarez AM, et al. An initial evaluation of expanded haemodialysis on hospitalisations, drug utilisation, costs, and patient utility in Colombia. *Ther Apher Dial*. 2021;25(5): 621-627. doi: 10.1111/1744-9987.13620

BACKGROUND

An innovation called expanded haemodialysis with a middle cut-off dialyser membrane allows for superior removal of potentially toxic medium-size molecules compared with traditional high flux (HF) haemodialysis (HD). Early clinical evidence suggests that **MCO** dialyser membrane has the potential to improve patient outcomes and Quality of Life (QoL).

OBJECTIVE

The purpose of this retrospective, before and after study was to provide an initial health economic assessment of the impact of switching patients from HF HD to the **MCO** dialyser membrane, on hospitalisations, hospital days, medication use, hospitalisation and drug related costs, and patient utilities.

METHODS

Study Design

This retrospective study was undertaken in the Renal Therapy Services (RTS)-Colombia network to track and compare outcomes of a subset of patients over the age of 18 prior to, and after switching from HF-HD to the **MCO** dialyser membrane. The patient's prescriptions (QD, QB, target Kt/V, duration, and dialysate) were not changed as part of this study. The study included clinical surveillance and periodic survey data related to quality of life between 2017 - 2019. The data for this analysis came from clinics in the RTS database in Colombia that had high-quality electronic medical record data with complete data for every patient. In addition, clinics included had switched all of their patients from HF-HD to **HDx** therapy, and had at least a year of data prior to, as well as after the switch to **HDx** therapy.

Eighty-one (81) patients qualified for inclusion in the study, and data in both the HF-HD phase and the **HDx** therapy phase of the study were collected.

Data Collection and Analysis

The annual count of hospitalisations, hospital length of stay, use of selected medications and QoL as measured by the Kidney Quality of Life (KDQoL)-36 were collected during the 1 year prior to initiation of the **MCO** dialyser membrane, while patients were receiving HF HD, and during the 1 year after initiation of the **MCO** dialyser membrane. Detailed information on comorbidities was used to calculate severity using a modified Charlson comorbidity index validated in End Stage Kidney Disease (ESKD) patients.

Costs were estimated for hospitalisation and medications. Hospital days were monetised based on a recent estimate of cost per day for dialysis-related hospitalisations conduc greater ted by RTS. Drug costs were estimated based on published prices, in Colombia. The hospital and drug-related cost estimates were then converted to US dollars based on an average of the US Colombian exchange rate from March to September 2019 (3,338.27 pesos per US dollar). To examine patient utility associated with the treatment change, a published algorithm based on results from a population in Spain was used to convert the KDQoL results into EQ-5D utility scores. As a sensitivity analysis, a related method for generating utility scores based solely on the Short Form (SF)-12 results of the KDQoL-36 was also used; a patient utility score of 1 signifies perfect health.

A generalised linear multivariable model was conducted to assess the effect of the **MCO** dialyser membrane on hospitalisation days, which were controlled for some demographic and clinical confounding variables. In addition, annual cost estimates for a patient on HF-HD and **HDx** therapy were calculated along with percent changes over time. All analyses were performed using Stata version 14.

Study Limitations

> With a before and after design, time trends in medication use and hospitalisations may confound the treatment effect. The main analysis controlled for statistically relevant patient characteristics, which helps alleviate the potential for bias related to time-varying factors, but bias may still be present.

> The results are from three clinics from one network in Colombia. Practices may vary to other settings; generalising results should be performed with care.

> The cost results reflect Colombian rates for hospitalisations and medications that are unlikely to match those in other countries.

> The EQ-5D utilities mapped from the KDQoL are based on a scoring algorithm developed in Spain and may not adequately reflect Colombian preferences.

> This analysis has focused solely on estimating the impact of the **MCO** dialyser membrane, in reducing the cost of hospitalisations and medications. The potential cost savings/cost-avoidances of this technology will depend on pricing dynamics and the contracting models for such treatments, over time.

RESULTS

Patient Characteristics

Baseline characteristics of the 81 patients enrolled in the study included:

> Average age: 61.1 years

> 64.2% male, 35.8% female

> 98.8% from urban areas

> Primary cause of Chronic Kidney Disease (CKD): 39.5% diabetes, 28.4% hypertension

- Median dialysis vintage: 3.8 years
- 25.9% with a modified Charlson comorbidity index of 3 or greater

Hospitalisations

- Hospitalisation rate per patient year: 0.77 with HF-HD and 0.71 in **HDx** therapy
- Hospitalisation days showed a sizable and statistically significant reduction of ~1.53 days; 5.94 hospital days per patient year on HF-HD to 4.41 on **HDx** therapy

Medication Utilisation

- While the proportion of patients on Erythropoietin Stimulating Agents (ESA), iron, insulin, and hypertension-related medications was roughly the same for patients on HF-HD and **HDx** therapy, doses per patient were significantly lower on **HDx** therapy (See Table 2)
  - Intravenous (IV) In-Centre Medications - mean dosage per patient, per year
    - ESA: significantly reduced by 13,194 IU; p=<0.01
    - Iron: significantly reduced by 200 mg; p=<0.01
  - Other Medications
    - Insulin: mean dosage per patient, per year significantly reduced by 1,949 IU; p=<0.01
    - Hypertension medications: mean number of tablets, per patient year significantly reduced by 452 tablets; p=<0.01

Patient Utilities

- The average utility of the patients was estimated, with patient utility remaining stable
- Using a KDQoL-36 based estimate, patient utility was 0.70 on HF-HD and 0.72 on **HDx** therapy
- Using the SF-12 based estimate, patient utility was 0.83 during both the HF-HD and **HDx** therapy phases

Please see Table 2.

Outcome	HD HF mean (95% CI) N=81	HDx mean (95% CI) N=81
Yearly Hospitalisation Rate	0.77 (0.60–0.98)	0.71 (0.55–0.92)
Yearly Hospitalisation Days	5.94 (5.41–6.50)	4.41 (3.97-4.90)a
Proportion Using ESA	0.85 (0.77–0.93)	0.88 (0.80–0.94)
Dosage Per Patient Per Year of ESA (IU)	181 318 (151 647–210 988)	168 124 (138 452–197 794)a
Proportion of Patients using Iron	0.81 (0.70–0.90)	0.78 (0.69–0.87)
Dosage Per Patient Per Year of Iron (mg)	959 (760–1158)	759 (560–958)a
Proportion of Patients Using Insulin	0.35 (0.24–0.45)	0.35 (0.24–045)
Dosage Per Patient Per Year of Insulin (IU)	5383 (3274–7490)	3434 (1327–5543)a
Proportion using Hypertension Medications	0.78 (0.69–0.87)	0.74 (0.65–0.84)
No. Tablets Patient Per Year of Hypertension Medications	1183 (970–1394)	731 (518–943)a
KDQOL-36 based EQ-5O Utility Score	0.70 (0.65–0.75)	0.72 (0.67–0.77)
SF-12 based EQ-50 Utility Score	0.83 (0.80–0.86)	0.83 (0.80–0.86)

Table 2. Hospitalisations, Medication Utilisation, and Patient Utilities with HD or HDx therapy. Table adapted from Ariza et al. Statistically significant difference found in corresponding univariate GLM analysis of outcome on **HDx** therapy. aAll had a P-value <0.01. HD HF: high flux haemodialysis; **HDx** therapy: expanded haemodialysis; ESA: erythropoietin stimulating agents; KDQOL: Kidney disease quality of life; EQ-5D: EuroQol-5 Dimension; GLM: Generalised linear Poisson.

Annual Costs

Estimates for annual average costs of hospitalisation were nearly 24% lower with **HDx** therapy and sizably lower for many of the medication-related estimates of costs, per patient year.

- Hospitalisations: average annual costs reduced by $428 with **HDx** therapy; 23.9% decrease
- IV in-centre Medications – average annual costs:
  - ESA costs reduced by $28 (7.27%) with **HDx** therapy
  - Iron costs reduced by $0.90 (20.83%) with **HDx** therapy
- Other Medications – average annual costs:
  - Insulin: costs reduced by $79 (32.64%) with **HDx** therapy
  - Antihypertensives: costs decreased by $57 (30.16%) with **HDx** therapy

Please see Table 3.

Annual Per Patient Cost Category	Average Annual Costs with HD HF	Average Annual Costs with HDx	Percent change HDx vs. HD HF
Hospitalisations	$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 3. Annual Costs with HD HF and with HDx therapy. Adapted from Ariza et al. HD HF: high flux haemodialysis; HDx: expanded haemodialysis; ESA: erythropoietin stimulating agents.

DISCUSSION AND CONCLUSIONS

The **MCO** dialyser membrane has shown promise as an important advancement in chronic dialysis-related care, with efficient removal of conventional/large middle molecules and the potential to improve patient outcomes while maintaining QoL. This analysis shows that **HDx** therapy statistically and significantly reduces hospitalisation days and lowers doses of medications (including those used in-centre), in a real-world setting over a period of two years. These reductions suggest a potential cost savings of $593 USD per patient, per year with the **MCO** dialyser membrane. In addition, the results on utility projections provided initial evidence that **HDx** therapy was associated with stable EQ-5D utility scores, over time.

A switch to HDx therapy was associated with a significant reduction in hospital days and medication usage (including ESA and iron), suggesting potential savings of $593 per patient, per year (or ~$4/treatment) with the MCO dialyser.

Expanded Haemodialysis and Its Effects on Hospitalisations and Medication Usage: A Cohort Study

Sanabria RM, Hutchison CA, Vesga JI, et al. Expanded haemodialysis and its effects on hospitalisations and medication usage: a cohort study. *Nephron*. 2021;145(2):179-187 doi:10.1159/000513328.

BACKGROUND

End stage kidney disease (ESKD) places a substantial burden on healthcare systems and patients, due in part to retention of large middle molecule uraemic toxins. Recently, expanded haemodialysis with medium cut-off dialyser membrane has been developed, which expands the capacity of haemodialysis (HD) to remove large middle molecules, while maintaining stable albumin. Clinical trials have shown that clearances of middle molecules during **HDx** therapy exceeded the clearances provided by high-flux (HF) membranes in HD and haemodiafiltration (HDF) mode. The introduction of **HDx** therapy enabled by **MCO** membrane to Colombia in 2017 has provided the ability to report real-life comparative data of the effects of **HDx** therapy on outcomes.

OBJECTIVE

The purpose was to compare laboratory parameters, hospitalisation rates, medication usage and adverse events in a cohort of patients switched from high-flux HD (HF-HD) to **HDx** therapy.

METHODS

Study Design

This historical, multicentre, observational cohort study of chronic HD patients was undertaken in the Renal Therapy Services (RTS) network in Colombia. Dialysis clinic inclusion was based on standardised processes and electronic medical records, and only centres where 100% of patients switched from HF-HD to **HDx** therapy were included.

Patients who had received HF-HD (**Polyflux** 140 dialyser; **Revaclear** 300 or 400 dialyser, Vantive, Deerfield, IL, USA) three times weekly for 4 hours, for at least 12 months, were switched to receive **HDx** therapy enabled by **Theranova** dialyser (Vantive, Deerfield, IL, USA). The patient's prescriptions (QD, QB, target Kt/V, duration, and dialysate) were not changed as part of this study.

Eligible ESKD patients aged 18 years or greater treated for chronic HD, received HF HD for at least 1 year prior to conversion to **HDx** therapy, and were then maintained on **HDx** therapy, for at least 1 year. Patients were required to receive both HF-HD and **HDx** therapy treatments at the same centre. Patients were excluded if they discontinued therapy, changed provider, underwent kidney transplant, recovered kidney function, went to another renal clinic, or changed to peritoneal dialysis or to another dialyser.

Eighty-one (81) patients from 3 clinics qualified for inclusion in the study, and data from both the HF-HD phase and the **HDx** therapy phase of the study were collected.

Study Outcomes

Outcomes studied were laboratory parameters, annual hospitalisation rates, hospitalisation rates by causes, annual hospital stay, 30-day readmission rates, adverse events related to haemodialysis procedures, and medication use. All outcomes were assessed throughout year 1, while on HF-HD and during year 2, after switching to **HDx** therapy.

Data Collection and Analysis:

Data collected included patient demographic characteristics, haemodialysis treatment parameters, laboratory parameters, and monthly data on medication prescriptions of IV (intravenous) ESA/iron. The Wilcoxon signed-rank test was used to evaluate any differences before and after switching to **HDx** therapy, to account for the distribution of continuous variables. Rates of hospitalisation, hospital days, and hospital readmission were estimated where the numerator constituted the number of events and the denominator was the time contributed by each patient, within the study. These rates were presented with their respective 95% confidence intervals. The incidence rates pre- and post-**HDx** therapy were compared using the incidence rate ratio. A hospitalisation event was counted if the duration was 1 day or more. A readmission event was counted when a new hospitalisation occurred between the fourth and thirtieth day of hospital discharge, immediately before. Stata Statistical Software: Release 14 (StataCorp LP, College Station, TX, USA) was used to perform statistical analyses. Two-tailed tests were used, and a p value of <0.05 was considered significant.

STUDY LIMITATIONS

While the study was a multicentre study, only 81 patients met full study criteria for analysis. The "before and after" design introduces the potential for bias that is secondary to the changes in dialysis care after switching from HF-HD to **HDx** therapy. In addition, this was a single-arm observational study.

RESULTS

Patients for Analysis

Of the potential 175 patients receiving HF-HD at the three study clinics, a total of 81 patients were included in the analysis. Twenty-three did not meet eligibility criteria, 48 were lost to follow-up during the HF-HD period, and 23 were lost during the **HDx** therapy study period, leaving 81 patients for analysis.

Laboratory Parameters

Anaemia profile after 12 months of **HDx** therapy showed a reduction in median erythropoietin resistance, but no significant change in median ferritin levels, haemoglobin levels, and transferrin saturation (TSAT).

> Erythropoietin resistance was reduced significantly on **HDx** therapy (3.37 (IQR 7.28)) vs. HF-HD (4.16 (IQR 8.03)), p=0.016
> Ferritin level was 482.70 ng/mL (IQR 934.9) on HF-HD and 530.20 (IQR 825.40) on **HDx** therapy (p=0.855)
> Median TSAT was 27.73% (IQR 14.28) on HF-HD vs. 31.01% (IQR 16.27) on **HDx** therapy (p=0.454)
> Median haemoglobin level was 11.90 g/dL (IQR 2.3) on HF-HD and 11.80 g/dL (IQR 2.2) on **HDx** therapy (p=0.397)

The median levels of the studied markers of inflammation did not change significantly.

> Albumin: 4.01 g/dL (IQR 0.43) during HF-HD vs. 3.99 g/dL (IQR 0.43) after switching to **HDx** therapy (p=0.82)
> High-sensitivity C-reactive protein (hsCRP) (66 measurements in 20 patients): 0.58 mg/dL (IQR 0.92) on HF-HD and 0.43 mg/dL (IQR 0.56) with **HDx** therapy (p=0.122)

Hospitalisation Rates See Table 1.

> The overall rate of hospitalisation events per patient-year was 0.77 on HF-HD vs. 0.71 on **HDx** therapy (p = 0.698), with no significant differences in hospitalisation rate per patient-year due to cardiovascular events, events related to dialysis, or infections
> The rate of hospital days per patient-year reduced significantly by 1.53 days (25.8%) on **HDx** therapy (5.94 HF-HD vs 4.41 with **HDx** therapy; p < 0.001)
> The 30-days readmission rate per patient-year was 0.15 on HF-HD vs. 0.04 on **HDx** therapy (p = 0.259)

Characteristic	Events	Rate	95%	CI	*p* value
Global hospitalisation rate (events/patient-year)					
Before	61	0.77	0.60	0.98	0.698
After	57	0.71	0.55	0.92	
Hospitalisation rate for cardiovascular causes					
Before	17	0.21	0.13	0.34	0.589
After	14	0.18	0.10	0.30	
Hospitalisation rate for causes related with dialysis					
Before	17	0.21	0.12	0.34	0.356
After	12	0.15	0.08	0.26	
Hospitalisation rate for infectious causes per patient-year					
Before	8	0.10	0.04	0.20	0.653
After	10	0.13	0.06	0.23	
Hospitalisation rate for other causes per patient-year					
Before	19	0.24	0.14	0.37	0.764
After	21	0.26	0.16	0.40	
Hospital days per patient-year					
Before	**473**	**5.94**	**5.41**	**6.50**	**<0.001**
After	**353**	**4.41**	**3.97**	**4.90**	
30-day readmission rates per patient-year					
Before	12	0.15	0.09	0.27	0.259
After	7	0.09	0.04	0.18	

Table 1. Hospitalisation Rates. CI, confidence interval; rate is defined as events per person year. Person-year is the sum of each person's individual time in the population by one year at risk to the event hospitalisation; Events are defined as hospitalisation events with a duration longer than 24 hours. Adapted from Sanabria et al.

Medication Use See Table 2.

Significant reductions in medication usage were seen after switching to **HDx** therapy.

> In-centre IV medication – monthly-median doses:
  > ESA: significantly decreased from 12,000 IU on HF-HD to 10,000 IU on **HDx** therapy (p = 0.036)
  > Iron: significantly decreased from 73.46 mg on HF-HD to 66.36 mg on **HDx** therapy (p = 0.003)
> Phosphate binders - median dose of aluminum hydroxide was not significantly different and change in median dose of calcium carbonate was not clinically important due to variability

Adverse Events

A reduction in adverse events related to haemodialysis procedures was observed after switching to **HDx** therapy (18 events with HF-HD vs 5 events with **HDx** therapy). There were no adverse events related to the dialyser membrane on **HDx** therapy. A total of 172 patients were randomised, and 171 were treated at 21 US centres between September 2017 and October 2018; 86 were treated with **HDx** therapy (389 patient-months), and 85 with a high-flux dialyser (366 patient-months). Baseline demographics and clinical characteristics were similar between groups; 39% of patients were female, and the mean age was 59±13 years.

Characteristic	Mean	SD	P_{25}	Median	P_{75}	IQR	*p* value
ESA (epoetin α), IU/month							
Before	**15,109.82**	**15,564.73**	**0.00**	**12,000.00**	**24,000.00**	**24,000.00**	**0.036**
After	**14,010.29**	**15,864.38**	**0.00**	**10,000.00**	**22,000.00**	**22,000.00**	
IV iron, mg/month							
Before	**73.46**	**142.13**	**0.00**	**0.00**	**100.00**	**100.00**	**<0.001**
After	**66.36**	**167.34**	**0.00**	**0.00**	**100.00**	**100.00**	
Calcium carbonate, mg/month							
Before	**749.28**	**1,001.60**	**0.00**	**600.00**	**1,200.00**	**1,200.00**	**<0.001**
After	**989.51**	**167.34**	**0.00**	**600.00**	**1,800.00**	**1,800.00**	
Aluminum hydroxide, mg/month							
Before	410.33	1,056.54	0.00	0.00	0.00	0.00	0.471
After	319.17	788.36	0.00	0.00	0.00	0.00	

Table 2. Medication Use. ESA, erythropoiesis-stimulating agents; IU, international unit; IV, intravenous; IQR, interquartile range; SD, standard deviation; P25, 25th percentile, P75, 75th percentile. * Wilcoxon signed-rank test. Adapted from Sanabria et al.

Clinical outcomes: hospitalisation and length of stay

Clinical outcomes are shown in Table 1. Hospitalisation was 45% lower in patients treated with **HDx** therapy compared with high-flux HD (IRR=0.55; 95% CI: 0.30, 1.00; P=0.042). There was no significant difference in length of hospital stay.

DISCUSSION AND CONCLUSIONS

In this cohort of chronic HD patients, switching from HF-HD to **HDx** therapy enabled by **Theranova** dialyser membrane was associated with a ~26% reduction in number of hospital days per patient-year, and a trend towards reduction in hospitalisation rate, which can ease the burden of ESKD on patients and healthcare systems.

During the 12-month period of **HDx** therapy, in-centre IV medication doses of ESA and iron were significantly reduced, in comparison to the 12 months on HF-HD, and were accompanied by a reduction in erythropoietin resistance, while maintaining stable haemoglobin levels. Albumin levels also remained stable with **HDx** therapy, further supporting **Theranova** DIALYSER's ability to efficiently remove large middle molecules, while retaining larger proteins and essential substances.

Although not significant, a trend toward reduction in an inflammatory marker, hsCRP, was observed after switching to **HDx** therapy. While the reduction in the rate of hospitalisations related to cardiovascular events with **HDx** therapy was non-significant, the trend aligns with observations from the general population that show an association of elevated hsCRP levels with cardiac events.

A switch to HDx therapy enabled by Theranova dialyser was associated with significant reduction in hospital days, IV ESA/iron doses, and improvement in erythropoietin resistance, which can inform treatment modality decisions and reduce the impact of ESKD on patients and healthcare systems.

Medium Cut-Off Dialyser Improves Erythropoiesis Stimulating Agent Resistance in a Hepcidin-Independent Manner in Maintenance Haemodialysis Patients: Results from a Randomised Controlled Trial

Lim JH, Jeon Y, Yook JM, et al. Medium cut-off dialyser improves erythropoiesis stimulating agent resistance in a hepcidin-independent manner in maintenance haemodialysis patients: results from a randomised controlled trials *Sci Rep*. 2020 2020;10(1):16062. doi: 10.1038/s41598-020-73124-x.

BACKGROUND

Anaemia is a frequent complication of end-stage kidney disease (ESKD) and is associated with increased morbidity and mortality rates. Anaemia in ESKD has multiple causes, including erythropoietin deficiency, uraemia-related inhibition of erythropoiesis, and inflammation. Erythropoiesis stimulating agents (ESAs) and iron are used to treat anaemia in ESKD patients. However, the responses to ESA vary greatly due to iron deficiency, poor nutritional state, and chronic inflammation. Patients who have been on maintenance haemodialysis regularly exhibit this resistance, which also has a cumulative effect over time.

Uraemic toxins are associated with chronic inflammation and are known to affect iron metabolism in ESKD patients by interfering with the response to ESAs. There are uraemic substances of various sizes that cause ESA resistance, including hepcidin and inflammatory cytokines. Traditional haemodialysis (HD) effectively removes small molecular uraemic toxins but has limited capacity to remove large middle molecules, which is believed to improve ESA response.

Newly introduced medium cut-off dialysers have uniformly distributed larger pores and have a greater capacity to remove conventional/large middle molecules and inflammatory cytokines compared to traditional high-flux (HF) dialysers. However, there is no clear evidence that demonstrates the effect of **MCO** dialyser membranes on ESA resistance in maintenance HD patients.

OBJECTIVE

This study aimed to evaluate whether the **MCO** dialyser membrane can improve the ESA resistance in chronic HD patient.

METHODS

Study Design

This is a post-hoc analysis of a prospective, randomised, controlled, open-label trial in patients treated with maintenance HD, at a national university hospital in South Korea, for a study period of 12 weeks. The original trial evaluated the impact of the **MCO** dialyser membrane on quality of life compared to HD with high flux dialyser.* The post-hoc analysis evaluated the impact of the **MCO** dialyser membrane on ESA resistance.

*Lim et al. Randomised Controlled Trial of Medium Cut-Off versus High-Flux dialysers on Quality of Life Outcomes in Maintenance Haemodialysis Patients. Nature/Sci Rep. 2020; 10:7780. A summary of this study can be found in this compendium.

In the original trial, patients were randomly allocated in a 1:1 ratio to the **MCO** dialyser membrane (**MCO** dialyser group) and high-flux dialyser (HF HD group). The HDx-MCO group changed their dialysis membrane from a high-flux dialyser (FX CorDiax 80 or 60; Fresenius Medical Care Deutschland, Bad Homburg, Germany) to a **MCO** dialyser (**Theranova** 400; Vantive Health LLC, Hechingen, Germany). The HF HD group continued their treatments with the high-flux dialyser. There was no change in the dialysis prescription for dialysis time per session, dialysis frequency per week, blood flow rate, and dialysate flow rate during the study period.

Data Collection and Analysis

Data collected in the original trial was used for this post-hoc analysis. Baseline demographics, comorbid diseases, biochemical data, and dialysis information were collected at the time of enrolment. The erythropoietin resistance index (ERI), calculated as the mean weekly weight-adjusted ESA dose divided by the haemoglobin level, was used to evaluate the ESA resistance; the level was measured every 4 weeks. Blood samples for the measurement of biochemical markers were obtained at the start of a midweek dialysis session. All the baseline samples were collected while patients were being dialysed with high-flux HD.

Study Outcomes

The primary outcome was ESA resistance change, defined as the change in ERI after 12 weeks of treatment with either HF HD or **MCO** dialyser therapy. The secondary outcomes were iron- and anaemia-related markers, reduction ratios of the iron regulator (hepcidin), and the inflammatory cytokine tumour necrosis factor-alpha (TNF-α), after 12 weeks of treatment with either HF HD or **MCO** dialyser therapy.

Study Limitations

The number of registered patients was small (n=49), and the study duration (12 weeks) was not long enough to get definite results. Although anaemia-related parameters such as iron, transferrin saturation (TSAT), and TNF-α, were significantly different at 12 weeks, the within-group differences were not significant for these parameters. The study was not blinded to clinicians and could have affected the prescription of ESA and iron supplementation. Finally, the detailed mechanism regarding how ESA response was improved by increased removal of conventional/large middle molecules remains unclear.

RESULTS

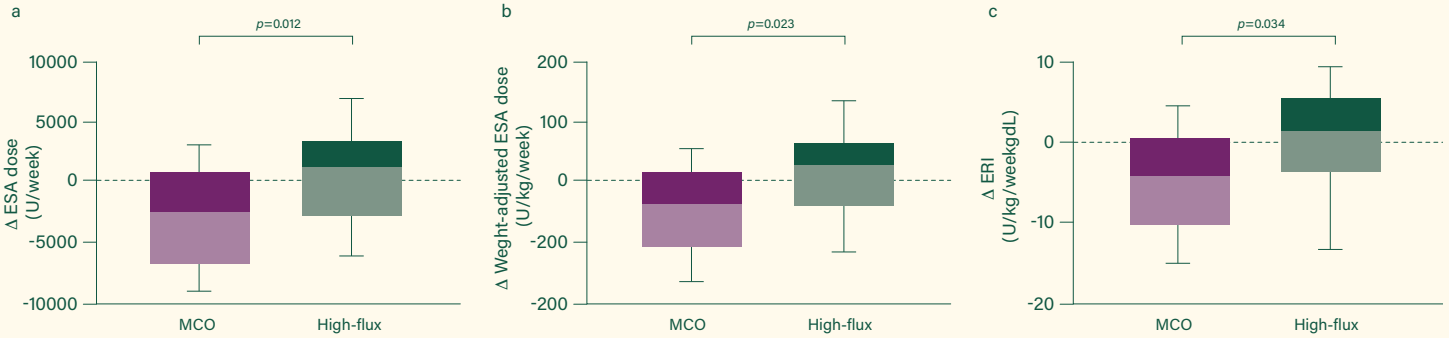
Patient Characteristics

All the enrolled patients (n=49) completed the study except one patient who withdrew consent in the **MCO** dialyser group. The age, sex, residual renal function, type of dialyser, dialysis method, comorbidities and ESA treatment were well balanced between the two groups.

Primary Outcome: Change in ERI (Reflecting ESA Resistance)

MCO dialyser membrane reduced ESA resistance compared to high flux HD. A comparison of differences (Δ) in the baseline and 12 weeks' values of ESA dose and weight-adjusted ESA dose showed significantly lower values in the **MCO** dialyser group, than in the high flux group (Δ ESA dose, p=0.012; Δ

Figure 1. Comparison of ESA (a), weight-adjusted ESA (b), and ERI (c) difference. Abbreviations: ESA, erythropoiesis stimulating agent; ERI, erythropoietin index. Figure adapted from Lim et al.



weight-adjusted ESA dose, p=0.023). The difference in ERI was significantly lower in the **MCO** dialyser group than in the high-flux group (Δ ERI, p=0.034). See Figure 1.

Secondary Outcome: Iron Metabolism and Anaemia-Related Markers

- The serum iron level and TSAT after 12 weeks of therapy were significantly higher in the **MCO** dialyser group than the HF HD group, despite the comparable use of intravenous (IV) iron (iron, p=0.029; TSAT, p=0.031)
- Other parameters of iron metabolism such as erythroferrone (ERFE), erythropoietin (EPO), and soluble transferrin receptor (sTfR) were not significantly different between the HF HD and the **MCO** dialyser groups, at baseline or after 12 weeks of therapy
- Serum hepcidin level was not different between groups at baseline or after 12 weeks of therapy
- TNF-α level was significantly lower in the **MCO** dialyser group compared to the HF HD group, after 12 weeks of therapy
- Biochemical data for serum haemoglobin, albumin, and high-sensitivity C-reactive protein (hsCRP) levels were similar between groups at the start and end of the study, and the changes during the study were not significant. See Table 1.
- The changes in these iron metabolism and biochemical parameters did not show significant differences between groups

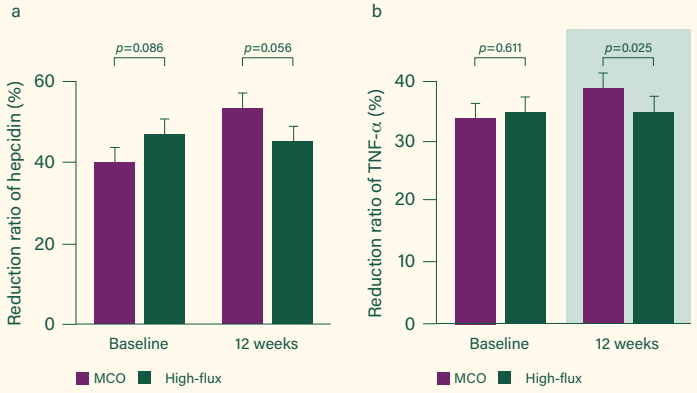


Figure 2. Reduction ratio of serum hepcidin (a) and TNF-α (b) at baseline and 12 weeks. Abbreviation: TNF-α, tumour necrosis factor-alpha. Figures adapted from Lim et al.

Secondary Outcome: Comparison of the Reduction Ratio (RR) of Serum Hepcidin and TNF-α

The RR of serum hepcidin was not significantly different between the HF HD and **MCO** dialyser groups at baseline or after 12 weeks of therapy. The RR of TNF-α was similar at baseline for the HF HD and **MCO** dialyser groups; however, after 12 weeks, it was higher in the **MCO** dialyser group than in the HF HD group (p = 0.025). See Figure 2.

	Baseline			12 weeks			Difference (Δ) between baseline and 12 weeks		p for difference (Δ) between groups
	MCO	High-flux	*p*	MCO	High-flux	*p*	MCO	High-flux	
Haemoglobin (g/dL)	10.6 ± 0.9	10.7 ± 1.1	0.859	10.9 ± 0.9	11.0 ± 1.0	0.697	0.2 (-0.5, 0.0)	0.0 (-0.3, 0.9)	0.841
Albumin (g/dL)	4.11 ± 0.38	4.06 ± 0.33	0.635	3.98 ± 0.27	4.04 ± 0.33	0.450	-0.05 (-0.30, 0.00)	-0.10 (-0.20, 0.15)	0.252
hs-CRP (mg/dL)	0.11 (0.03,0.26)	0.18 (0.05, 0.71)	0.704	0.13 (0.04, 0.46)	0.22 (0.06, 1.30)	0.250	0.00 (-0.10, 0.17)	0.06 (-0.03, 0.78)	0.161
Ferritin (ng/mL)	161.1 (70.1, 305.3)	90.3 (38.6, 205.9)	0.156	123.9 (57.9, 312.2)	158.1 (59.5, 284.2)	0.904	19.2 ± 173.6	19.1 ± 151.7	0.998
Iron (μg/dL)	66.1 ± 25.0	59.6 ± 29.8	0.410	72.1 ± 25.4	55.9 ± 25.0	**0.029**	2.49 (-5.9, 15.8)	-1.6 (-15.8, 4.0)	0.131
TIBC (μg/dL)	221.4 ± 37.8	234.8 ± 51.7	0.309	221. 1 ± 46.3	227.1 ± 33.9	0.607	-0.3 ± 36.3	-7.7 ± 32.2	0.455
TSAT (%)	30.6 ± 12.3	26.1 ± 11.9	0.196	34.0 ± 15.0	25.3 ± 11.9	**0.031**	-0.6 (-4.4, 8.1)	0.8 (-8.5, 3.7)	0.325
ERFE (pg/ml)	402.5 ± 122.3	360.3 ± 136.0	0.259	483.3 ± 123.0	386.0 ± 116.2	0.133	53.0 (-38.3, 95.8)	44.6 (0.8, 73.6)	0.660
EPO (mU/mL)	9.5 (6.7,16.0)	11.9 (5.0,18.5)	0.818	10.1 (4.6, 19.9)	7.9 (5.3, 15.3)	0.741	-2.3 (-6.5, 6.2)	0.5 (-9.5, 3.2)	0.889
sTfR (nmol/L)	16.7 (12.8, 23.5)	17.8 (13.6, 23.7)	0.617	16.4 (11.3, 21.9)	18.5 (11.1, 23.6)	0.660	-1.4 ± 8.3	-1.5 ± 10.1	0.981
Hepcidin (ng/mL)	46.8 ± 36.9	32.4 ± 27.3	0.128	42.1 ± 23.8	44.9 ± 26.3	0.688	-3.5 ± 31.1	12.3 ± 27.0	0.063
TNF-α (pg/mL)	17.9 ± 5.0	18.0 ± 4.7	0.915	16.3 ± 3.4	19.0 ± 4.8	**0.027**	-1.6 ± 4.3	1.0 ± 5.7	0.079

Table 1. Comparisons of the iron metabolism and biochemical parameters. Data are shown as mean ± standard deviation or median (interquartile range). Values in bold indicate statistically significant results. Abbreviations: TSAT, transferrin saturation; ERFE, erythroferrone; EPO, erythropoietin; sTfR, soluble transferrin receptor; TIBC, total iron binding capacity; TNF-α, tumour necrosis factor-alpha, hs-CRP, high sensitivity C-reactive protein. Table adapted from Lim et al.

DISCUSSION AND CONCLUSIONS

Changing from HD with high-flux dialyser to HD with the **MCO** dialyser membrane improved the ESA resistance as compared to continuing HD with high-flux dialyser. The importance of ESA resistance in HD patients has been emphasised in several reports; ESA hyporesponsive HD patients have shown increased all-cause mortality and cardiovascular complications. Although the impact of improved ESA resistance on mortality and cardiovascular events was not evaluated in this study, it might have a positive effect on the prognosis in the long-term.

The improvement in ESA resistance using the **MCO** dialyser membrane may be attributable to the efficient removal of conventional/large middle molecules including TNF-α. Under uraemic conditions, chronic inflammation may induce an enhanced state of T-cell activation that leads to ESA resistance. TNF-α (molecular weight (MW) 17.3 kDa) is a representative pro-inflammatory cytokine that is usually elevated with chronic kidney disease. TNF-α causes anaemia by inhibiting erythroid-precursor proliferation and promoting hypoferremia. The study confirmed that switching to the **MCO** dialyser membrane resulted in improved TNF-α removal, as shown by lower TNF-α level after 12 weeks in the **MCO** dialyser group, as compared to patients who continued with HF HD. Changes in erythropoiesis and iron metabolism-related parameters, such as hepcidin, erythroferrone, erythropoietin were analysed to investigate the association between TNF-α and ESA resistance. TNF-α induces hypoferremia through both hepcidin-independent and hepcidin-dependent mechanisms. Hepcidin is a relatively small-sized (~27 kDa) large middle molecular uraemic toxin that has an effect on ESA resistance. The serum hepcidin level is typically controlled by inflammation and erythropoietin. While the RR and difference in hepcidin at baseline and after 12 weeks of therapy did not vary significantly between groups, after 12 weeks of the **MCO** dialyser group hepcidin levels decreased by 3.5 ng/mL vs. an increase of 12.3 ng/mL in the HF HD group (p=0.063). There was no significant difference between groups in serum level of erythropoietin (30.4 kDa), a regulator of hepcidin, with a decrease of 2.3 mU/mL in the **MCO** dialyser group, vs. an increase of 0.5 mU/mL in the HF HD group. Erythroferrone (52 kDa), also a regulator of hepcidin, remained unchanged after switching to the **MCO** dialyser, which further supports the **Theranova** DIALYSER's ability to retain larger (>45 kDa) proteins and essential substances. Considering these results, it is possible that the hepcidin-independent pathway played a dominant role in improving iron status, in this study.

In conclusion, the **MCO** dialyser membrane achieved greater improvement in ESA resistance than HD with high-flux dialyser. The **MCO** dialyser membrane efficiently removed the conventional/large middle molecule TNF-α, an inflammatory cytokine, potentially influencing iron metabolism.

MCO dialyser membrane improves ESA resistance over time compared to high-flux HD in maintenance HD patients. MCO dialyser membrane provides superior removal of large middle molecules, reducing inflammatory cytokines potentially improving iron metabolism.

Expanded haemodialysis as effective alternative to on-line haemodiafiltration: A randomised mid-term clinical trial

Hadad-Arrascue F, Nilsson L-G, Rivera AS, et al. Expanded haemodialysis as effective alternative to on-line haemodiafiltration: A randomised mid-term clinical trial. *Ther Apher Dial.* 2022;26:37-44. doi: 10.1111/1744-9987.13700.

BACKGROUND

Innovation in dialysis membrane design has recently provided a medium cut-off membrane that has higher retention onset than conventional high-flux membranes with selectively limited permeability for albumin. This membrane is designed for use in expanded haemodialysis, a therapy option that enhances clearance of middle molecules but does not require external replacement fluid, as does on-line haemodiafiltration (HDF). Short-term studies have found that **HDx** therapy with this filter provides similar clearance of beta2-microglobulins (β2m) and other large middle molecules as on-line HDF with the potential to outperform HDF in clearing some large molecules. Over a 6-month period, an observational study found noninferiority between the two therapies, but long-term data from a randomised trial are needed.

OBJECTIVE

To assess long-term utility and safety of **HDx** therapy relative to HDF, this trial randomised patients already receiving post-dilutional online HDF three times weekly for chronic kidney disease in Spain to receive either **HDx** therapy or HDF over a 24-week period.

METHODS

Study Design

Patients receiving HDF in the Renal Therapy Services dialysis centre, Murcia, Spain, were included in an open-label, prospective, 1:1 randomised, parallel-group study over 24 weeks. Patients were clinically stable and between the ages of 18 and 80 years. They were stratified by residual renal function prior to randomisation.

HDx therapy treatments used the **Theranova** 500 dialyser, while HDF treatments used the Polyflux 170H dialyser (2.0 m2 vs 1.7 m2 surface area; both from Vantive, Hechingen, Germany). Treatments were delivered via Artis Physio dialysis systems (Vantive, Medolla, Italy). Treatment duration, blood flow rate targets (350 mL/min), dialysis fluid composition, and temperature were maintained as before study initiation. Low-molecular-weight heparin was provided.

The primary outcome measure of this study was the reduction ratio (RR) for middle molecules, calculated by comparing pre-study values with values after 12 weeks of treatment. RR were measured for the following molecules: β2m, fibroblast growth factor 23 (FGF-23), chitinase-3-like protein 1 (YKL-40), and kappa and lambda free light chains (κ and λ FLC).

Secondary outcome measures included change from baseline at 12 and 24 weeks of treatment for various inflammatory markers and middle molecules. Changes in key protein and iron levels were also assessed, as was single pool Kt/v urea and weekly dosing of erythropoiesis-stimulating agents (ESA) and intravenous iron required to manage anaemia.

The study was registered as NCT03499691, and its protocol was approved by the ethics committee of Reina Sofia General University Hospital, Murcia.

Statistical Analysis

A sample size of 40 was possible in the study site and was determined sufficient to assess the primary outcome. Differences between study arms were analysed using the ANCOVA model. Differences between therapies in change over time were analysed using mixed-effect repeated measurement (MMRM) models. Both models used pre-dialysis concentration and baseline urine output as covariates. Wilcoxon rank-sum test used to analyse change over time for variables with non-normal distribution of data.

RESULTS

Patients

In April 2018, 43 patients were enrolled, with the characteristics shown in Table 1. Baseline characteristics were similar between groups. One patient in the HDF arm died during the study. Two were excluded from analysis due to severe adverse events not related to dialysis, one in each arm of the study. Nine patients were excluded from analysis for leaving dialysis for more than 2 weeks; most were in the HDF arm. Only one of these patients left prior to the 12-week analysis.

Table 1. Patient demographics at study baseline.

	HDx Arm N=21	HDF Arm N=22
Age (years)	60.7 ± 14.3	61.8 ± 9.4
Gender (% males)	57%	73%
Body weight (kg)	76.6 ± 13.1	75.9 ±16.0
Dialysis vintage (months; median/range)	30 /6-224	35/ 5-375
Urine production		
Anuric (<100 mL/24 hr)	48%	45%
Oliguric (100-500 mL/24 hr)	29%	32%
Non-oliguric (>500 mL/24 hr)	23%	23%
ESRD comorbidity index	2.5 ± 1.7	1.9 ±1.8
Malnutrition inflammation score	3.4 ± 2.1	3.5 ±1.4

ESRD, end-stage renal disease. Adapted from Hadad-Arrascue et al.

Treatments

Treatment characteristics at week 12 are shown in Table 2.

TABLE 2. Study treatment characteristics at week 12.

	HDx Arm N=21	HDF Arm N=19
Treatment duration (min)	241 ± 4	239 ± 7
Blood flow rate (mL/min)	400 ± 12	396 ± 8
Dialysis fluid flow rate (mL/min)	500	600
Ultra filtration volume (L)	2.5 ± 0.8	2.1 ± 3.2
Substitution fluid volume (L)	N/A	24.4 ± 3.2

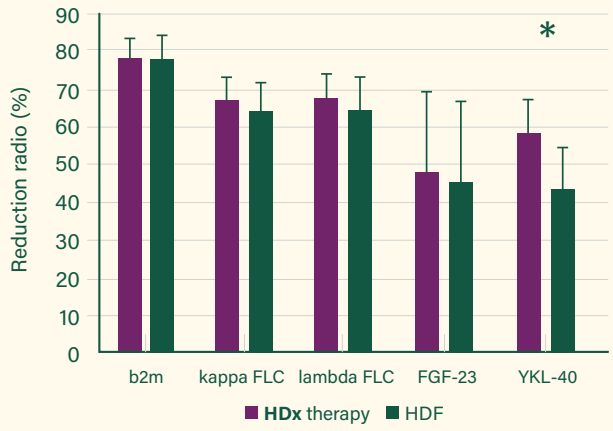
Adapted from Hadad-Arrascue et al.

Primary and Secondary Outcome Measures

Figure 1 shows the primary outcome measure, the 12-week pre- to post-dialysis RR. The RR for YKL-40 was significantly greater for the **HDx** therapy group compared to the HDF group. RR for the other middle molecules and for key inflammatory markers were comparable for **HDx** therapy vs HDF:

- Chitinase-3-like protein 1 (YKL-40): 58.1 ± 9.5 vs 42.4 ± 12.5%; P=0.0001
- Beta2 microglobulin (β2M): 76.6 ± 5.6 vs 77.2 ± 5.6%; P=0.47
- Fibroblast growth factor 23 (FGF-23): 48.1 ± 21.3 vs 45.1 ± 20.8%; P=0.63
- Kappa free light chains (κ-FLC): 67.0 ± 5.9 vs 64.9 ± 6.9%; P=0.40
- Lambda free light chains (λ-FLC): 67.7 ± 6.2 vs 65.9 ± 8.2%; P=0.31
- Interleukin-6 (IL-6): -13.7 ± 13.2 vs -16.9 ± 14.7%; P=0.29
- C-reactive protein (CRP): -7.2 ± 12.1 vs -8.8 ± 10.6%; P=0.62

FIGURE 1. Middle molecule pre- to post-dialysis reduction ratios (RR), using week 12 measures.



*p<0.0001

Note: FLC concentrations were measured by the N Latex assay; the lambda FLC RR likely reflect only the removal of lambda monomers. B2m, beta2-microglobulin; FGF-23, fibroblast growth factor 23; FLC, free light chains; YKL-40, chitinase-3-like protein 1. Adapted from Hadad-Arrascue et al.

Predialysis biomarkers (both middle molecules and inflammatory markers) were present in similar levels at baseline and showed comparable changes between groups at weeks 12 and 24. This includes FLC, YKL-40, CRP, IL-6, and pentraxin-3 (PTX3). In the same way plasma levels of albumin, fibrinogen, haemoglobin, parathyroid hormone (PTH), and phosphorous did not differ between the study arms.

Table 3 shows data for the only two biomarkers for which there were significant differences between groups for either time point. At week 24, **HDx** therapy had lower β2m and FGF-23 than did HDF, but this as a "borderline difference." Overall, the two therapies are otherwise comparable in terms of biomarker changes.

Table 3. Baseline pre-dialysis levels and changes from baseline for the studied biomarkers for those with significant differences between groups at either time point.

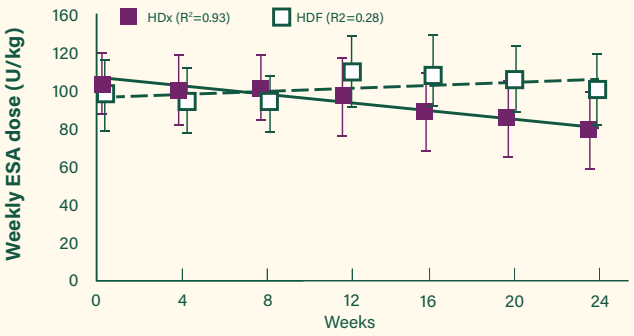
		Baseline	Change, Wk 12	Change, Wk 24
B2m (mg/L)	HDx	25.4 ± 7.6	-0.6 ± 3.6	-0.6 ± 3.9
	HDF	24.3 ± 7.5	-1.0 ± 4.5	+3.3 ± 6.1
	P-value	0.62	0.55	0.046
FGF-23 (pg/mL)	HDx	1153 (402, 1979)	-20 (-597, +512)	-24 (-623, +202)
	HDF	825 (277, 1438)	+208 (-537, +494)	+343 (+44, +1152)
	P-value	0.28	0.45	0.039

B2m, beta2-microglobulin; FGF-23, fibroblast growth factor 23. Adapted from Hadad-Arrascue et al

Exploratory Outcome Measure

Anaemia management was analysed all patients in the HDF arm and 20 of 21 patients in the **HDx** therapy arm received ESA during the study. Those in the HDF study arm received a stable mean weekly dose of ESA over time, while those in the **HDx** therapy study arm showed a trend toward a decrease in weekly ESA dose from week 8 onward (Figure 2). Erythropoietin resistance index (ERI) in ESA-treated patients showed a similar trend but was not significantly different between groups, and haemoglobin levels were stable over time in both groups.

FIGURE 2. Weekly ESA dose per kg body weight



ESA, erythropoiesis-stimulating agent. Adapted from Hadad-Arrascue et al.

Adverse Events

A total of 37 patients (86%) experienced an adverse event (AE) during the study, with 18 subjects reporting 79 AE in the **HDx** therapy group and 19 subjects reporting 55 AE in the HDF group. Most common AE were hypotension, muscle cramps, and hypertension. Eight patients (HDx, n=3; HDF, n=5) reported serious AE, but none were related to the dialysis procedure.

DISCUSSION

In the first randomised controlled study comparing **HDx** therapy to HDF treatment over 24 weeks, **HDx** therapy provided similar RR as on-line HDF for many middle molecules, with greater reduction for YKL-40. Based on the comparable biomarker levels, a **HDx** therapy was non-inferior to HDF in clinical effectiveness in this trial.

The online HDF achieved in this study was comparable to recommended HDF therapies with a average HDF convective volume of approximately 26 l/treatment and a convective flow rate close to 28% of blood flow rate.

The large middle molecule clearance data are comparable to previous studies but no significant change over time in pre-dialysis plasma levels. Caution is needed in the interpretation of the B2m and FGF-23 differences that were noted. The stability of pre-dialysis albumin levels is important to note.

There was a trend for reduced ESA dose over time with **HDx** therapy alongside a steady haemoglobin level indicating an improved ESA response. However the low sample size means firm conclusions cannot be drawn.

CONCLUSIONS

This randomised, controlled study comparing **HDx** therapy with online HDF over 24 weeks found the following:

- **HDx** therapy was noninferior to HDF in terms of middle molecule RR at 12 weeks, and **HDx** therapy produced a greater RR for YKL-40 over 12 weeks
- **HDx** therapy was noninferior to HDF in terms of 12- and 24-week effects on middle molecules, markers of inflammation, and plasma proteins. In particular albumin levels were stable
- **HDx** therapy was associated with a trend for reduced ESA requirement
- Neither **HDx** therapy nor HDF was judged to have serious AE associated with the dialysis procedure
- **HDx** therapy may be an attractive option for long-term dialysis, particularly since it is easy to implement and not dependent on on-line HDF equipment

Vantive

HDx therapy:
A world of difference

Proven Safety when creating internal filtration through HDx therapy and MCO membrane

The permeability of the **Theranova** membrane for endotoxins is not significantly different when comparing low flux, high-flux, medium cut-off, and high cut-off membranes.[1]

Schepers E, Glorieux G, Eloot S, et al.
Assessment of the Association Between Increasing Membrane Pore Size and Endotoxin Permeability Using a Novel Experimental Dialysis Simulation Set-Up. *BMC Nephrol.* 2018;19:1

1. Schepers E, Glorieux G, Eloot S, Hulko M, Boschetti-de-Fierro A, Beck W, Krause, B, Van Biesen W. Assessment of the Association Between Increasing Membrane Pore Size and Endotoxin Permeability Using a Novel Experimental Dialysis Simulation Set-Up. *BMC Nephrol*. 2018; 19:1.

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

Schepers E, Glorieux G, Eloot S, et al. Assessment of the association between increasing membrane pore size and endotoxin permeability using a novel experimental dialysis simulation 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 uraemic toxins in the larger molecular weight range even when used in dialysis mode. High cut-off dialysers 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 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 haemodialysis (HD) patients to low levels of cytokine-inducing microbial components can potentially contribute to the micro-inflammatory status of these patients, thus neutralising the potential positive effect induced by their capacity of enhanced removal of pro-inflammatory uraemic toxins. Therefore, the request for ultrapure dialysate might become a more important concern as membrane pore size becomes larger, even when applied in haemodialysis 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 dialysers 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 dialysers 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 dialyser (low flux); **Revaclear** 400 dialyser (high flux); **Theranova** 400 dialyser; and **Theralite** 2100 dialyser; Vantive Health LLC, Hechingen, Germany. See Table 1.

Dialyser	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 dialysers. [a] effective Stokes-Einstein radius; calculated from molecular weight cut-off measured with polydisperse Dextran. Abbreviations: PAES: polyarylethersulfone; PVP, polyvinylpyrrolidone; PA, polyamide, UF, ultra filtration. 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 Ca2+, 0.50 mM Mg2+ 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 mm2/s, to mimic the viscosity of whole blood. Compatibility of PVP dissolved in PBS (PVPPBS) 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 haemodialysis (Gambro) and the dialysers were connected. The four different membranes were tested in random order; for each membrane type, 6 different dialysers 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 (QB) of 400 mL/min while the PVP pool was continuously mixed.

The dialysate flow (QD) 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 dialyser 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 dialyser 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 (PVPstart) and at the end (PVPend) of the experiment. For the PVPpost 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.

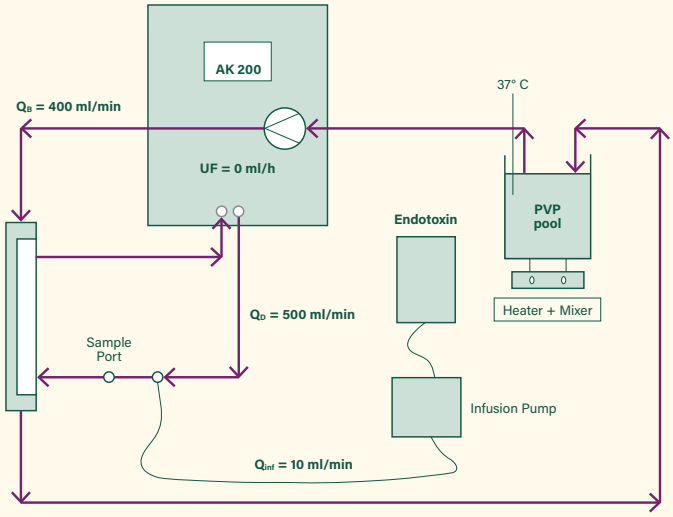


FIGURE 1. Schematic overview of the dialysis set-up. After priming both blood and dialysate circuit and after coating the test membrane with plasma, 3 L of PVP solution, continuously mixed, was recirculated at 37° C at the blood side at a blood flow rate (Q_B) of 400 mL/min. Samples of the PVP were taken 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. Ultra filtration 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 dialyser, 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, categorised 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 PVPpost solutions.

LAL activity in the PVP solution at the blood side of the dialyser was below the limit of detection (limit of detection (LOD)=0.005 EU/mL) both before (PVPpre) and after the experiment (PVPpost) for 12 out of 24 tested dialysers. Endotoxin levels were below LOD in all but three (High-flux 2, high cut-off 1) PVPpre solutions. This potentially indicates contamination occurred already before the start of the experiment in these three experiments. Positive PVPpost reading higher than the corresponding PVPpre 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** dialyser 3/6; **HCO** dialyser: 4/6). While more open membranes (**MCO** dialyser and **HCO** dialyser) 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-1β (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-1β expression, whereas none of the PVP solutions used in the different experiments induced IL-1β expression neither before or at the end of the experiments. Moreover, no significant difference in induction of IL-1β 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 25 EU/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-1β 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 dialyser inlet.

The four different membranes were tested in random order; for each membrane type, six different dialysers 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 dialyser and with it, realistic filtration profiles.

> Full size dialysers 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, ultra filtration 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.

Limitations Study

dialysers of comparable membrane composition from a single manufacturer were chosen to focus the investigation in pore size. Subsequently, the results can not likely be generalised 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 neutralise 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-1β 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 PVPpost solutions induced IL-1β 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** dialyser and **HCO** dialyser, is likely safe from that regard. Indeed, in none of the experiments, biological activation of the inflammatory system was observed.

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

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.

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Do not use **Theranova** devices for HDF (haemodiafiltration) or HF (haemofiltration) due to higher permeability of larger molecular weight proteins such as albumin.

Theranova dialysers are indicated for treatment of chronic and acute renal failure by Haemodialysis. For single use only.

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

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