PERITONEAL HISTOMORPHOLOGY, BIOMARKERS AND CLINICAL OUTCOMES IN PATIENTS WITH CHRONIC KIDNEY DISEASE ON PERITONEAL DIALYSIS

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Doctoral Degree in Medicine at Faculdade de Ciências Médicas |
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September, 2022

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September, 2022

Funding

Sociedade Portuguesa de Nefrologia (SPN)
SPN funded a project and Ana Rita Martins, MD, Nephrology fellow, for a residence at Jiménez Díaz Foundation University Hospital, Madrid under the scope of novel serum biomarkers of CKD

The present dissertation originated the following scientific outcomes:

Peer-reviewed papers

- **Branco, Patricia**; Calça, Rita; Martins, Ana Rita; Mateus, Ana; Jervis, Maria João; Pinto, Daniel; Lopes, Sofia; Melo-Júnior, Sousa Cátia; Civantos, Ester; Mas-Fontao, Sebastian; Gaspar, Maria Augusta; Ramos, Sância; Morello, Judit; Nolasco, Fernando; Rodrigues, Anabela*; Pereira, Sofia*.Fibrosis of peritoneal membrane, molecular indicators of aging and frailty unveil vulnerable patients in long-term peritoneal dialysis. *International Journal of Molecular Sciences*, 2023 (accepted)

- **Branco, Patricia**; Martins, Ana Rita; Calça, Rita; Mateus, Ana; Jervis, Maria João; Rodrigues, Anabela; Lopes, Sofia, A; Civantos, Ester; Mas-Fontao, Sebastian; Gaspar, Maria Augusta; Ramos, Sância; Morello, Judit; Pinto, Daniel*; Pereira, Sofia*. Alpha-klotho is an assessment tool of peritoneal membrane status. *European Journal of Clinical Investigation*, 2023, 53(3): e13903.

- Gomes da Silva, Francisca; Calça, Rita; Martins, Ana Rita; Araújo, Inês; Aguiar, Carlos: Fonseca, Candida; **Branco, Patrícia**. Diuretic resistant heart failure and the role of ultrafiltration: a proposed protocol. 2022 Accepted at *Revista Portuguesa de Cardiologia*

- Gomes da Silva Francisca, Calça Rita, Martins Ana Rita, Araújo Inês, Aguiar Carlos, Fonseca Cândida **Branco Patrícia**. Is peritoneal dialysis an effective treatment for cardiorenal syndrome with decompensated heart failure? A single center experience. Med Clin Res, 2022 Volume 7 | Issue 1 | 05

- Querido S, **Quadros Branco P**, Silva Sousa H, Adragão T, Araújo Gonçalves P, Gaspar MA, Barata JD. Hypervolemia, hypoalbuminemia and mitral calcification as markers of cardiovascular risk in peritoneal dialysis patients. Rev Port Cardiol. 2017 Sep;36(9):599-604.

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ACKNOWLEDGMENTS

À Profª Doutora Sofia Azeredo Pereira, com a sua enorme capacidade de transformar dificuldades em possibilidades, na constante procura de conhecimento, intui que nada existe em estado puro, é preciso trabalhar. Esta tese nunca teria sido possível sem ela. Não sei como lhe agradecer. Foi certamente por amizade. Seguimos juntas.

À Profª Doutora Anabela Rodrigues, sabe tudo de diálise peritoneal, ajudou de forma incondicional e generosa, corrigiu erros e perdoou alguns. Se não fosse ela não teria persistido. A sua curiosidade foi um incentivo. Obrigada pelo entusiasmo e confiança neste caminho de humildade e reflexão. Estamos sempre a começar.

Ao Prof Doutor Fernando Nolasco, Obrigada pelo futuro que me indicou. Consegue agregar no entusiasmo que imprime pessoas diferentes e ver o melhor de cada um. Deixa na nossa escola a sua audácia, criação e exemplo.

À Profª Doutora Teresa Adragão, obrigada porque acreditou que havia um sentido mesmo quando ele escasseava.

Rita Calça, Obrigada pelas leituras, releituras e pelo carinho sem fim em todos os detalhes! Por seres parceira em comunhão e trabalharmos com propósito comum. E pela Amizade.

Ana Rita Martins, Obrigada por tudo e por tanto, pela ideia da escala de fragilidade, pela tua preocupação com o humanismo, pelo que nos ensinas a juntar à ciência. Ter sido tua tutora foi uma honra e um privilégio.

Agradeço à Equipa da Farmacologia Cardiovascular e Metabólica, Judith Morello, António Júnior, Cátia Sousa, Luísa Santos e Catarina Sequeira pelo envolvimento e toda a empenhada colaboração. Sem a vossa ajuda teria sido impossível. Este foi só o pretexto que nos uniu, cumpre-nos continuar.

À Sofia Azeredo Lopes, do Departamento de Bioestatística, o meu louvor por toda a paciência e rigor no apoio estatístico e na procura de convergência de linguagens.

Ao Dr José Diogo Barata, meu mestre, que me ajudou com paciência e amizade a pensar Nefrologia por cujo saber tenho tanta admiração.

À Drª Augusta Gaspar, obrigada pelos desafios e confiança. Foi sua a ideia de avaliar o índice tornozelo braço. Acompanhou com paciência todas as fases.

À Drª Helena Boquinhas, o seu envolvimento e sentido de Missão, são referências. Aceitei os desafios que me propôs e da minha parte pode esperar sempre compromisso e dedicação. Agradeço-lhe o estímulo e a confiança.

Aos colegas do Serviço de Anatomia Patológica, Drs Sância Ramos e Daniel Pinto, pela inexcedível colaboração, rigor na análise e ensino da histomorfologia peritoneal.

Destaco naturalmente, os Drs António Martinho e Belarmino Clemente, cuja influência se fez sentir em toda a minha vida profissional. Sublinho, nomeadamente, a sua colaboração nos procedimentos cirúrgicos.

Às Enfª Sara Pereira e Enfª Elisabete Costa, obrigada queridas Enfermeiras com quem trabalho na Unidade de DP. Pela partilha de vivências, angústias, empenho e confiança.

A actividade no Departamento de Investigação e inovação clínica do CHLO tem sido uma oportunidade única de crescimento, realço o impulso dado pela Doutora Sara Maia e pelo Prof Doutor Miguel Viana Baptista ao DIIC, também extrapolado neste projecto.

À Drª Maria João Pais, fundadora da Unidade de DP do HSC, uma referência pelo coorte histórico dos estudos histológicos que por sua iniciativa existem no CHLO.

Obrigada às técnicas Lilia Oliveira e Leonor Jacinto por terem aceite ajudar-me neste projecto.

Agradeço á equipa da Fundação Jimenez Diaz, Esther Cirvantes e Sebastian Más, e ao Prof Dr Jesus Egidio, o acolhimento à ideia, integração da Ana Rita Martins, a identificação dos marcadores e que este seja apenas o início de uma parceria ibérica.

Aos meus colegas que nos referenciam os seus doentes, da Nefrologia, Cardiologia e Cardiologia Pediátrica, com quem partilho a vida hospitalar, na partilha de angústias, dúvidas, confrontos, tudo contribui para a minha aprendizagem diária e se reflecte neste trabalho e a quem agradeço a experiência que me transmitem. A todos nefrologistas que prescrevem Diálise peritoneal, destaco o Manuel Amoedo e a Anabela Malho, pelo entusiasmo e sentido de projecto.

Aos colegas que escolhem a nossa Unidade para a sua formação especifica e dão forma aos desafios.

Aos doentes com quem aprendo diariamente e me esforço por continuar a merecer a sua confiança.

Prof Doutor Nuno Neuparth porque me chamou de volta à escola e me integrou na sua equipa.

Profª Doutora Cândida Fonseca, por ser uma visionária e pela capacidade de criar pontes e alavancar vontades.

Henrique Silva Sousa, com lucidez afinada e o coração no sítio certo. Obrigada por teres sido suporte. Sabes bem a gratidão e amizade que te tenho mas gosto de repetir.

Nuno Fonseca e Rui Barata que são motor para pensar e agir e parceiros diários de trabalho cúmplice.

Rita Birne, há amizades na Medicina. Não se encontra muitas vezes tanta inteligência, sensibilidade e coragem na mesma pessoa. Obrigada por nunca me deixares desistir.

Graça Almeida, uma das minhas rochas há 25 anos. Obrigada pela leitura, correcções inglês e por todas as vezes em que me "acertas os ponteiros".

Sofia Corredoura e Joana Paiva, As irmãs que um dia Deus pôs no meu caminho para tornar a minha vida (muito) mais feliz. Obrigada amigas, pelo tudo e pelo nada.... Nós somos da vida toda. E para sempre.

Catai, A nossa amizade (e os teus gelados) sustiveram este projecto.

Paula Paiva, não há palavras que cheguem para te agradecer. Salvaste-me aos 10a e desde aí todos os dias. És amparo de todos.

P José Manuel Pereira de Almeida, agradeço-lhe a marca que deixa em nós e a ternura que contagia quem o rodeia. Agradeço sempre ao Senhor tê-lo na minha vida.

Berta, uma amizade que os meus pais me deixaram. A minha geração deve-vos muito. Obrigada pelo tanto por que lutaram, as oportunidades que criaram e por me ensinares a focar a energia no essencial.

Zeferino, defensor da Escola Pública, de uma Saúde para todos, da Igualdade, e que sempre me incentivou a continuar.

Zé Miguel, obrigada por tudo, por teres sido sempre incondicional. McWhites, obrigada pelas horas de conversa, os momentos de confidências, o transcendente com o nascimento de cada filho, as nossas famílias que se entrelaçam e esta eternidade que garantimos quando os nossos 4 filhos se unem numa amizade sem tempo. É agradecer, agradecer e aproveitarmos sermos rede uns dos outros.

Vera, somos uma da outra. Agradeço-te por todas as razões.

Aos meus sogros pela compreensão (e esforço associado), por serem modelo, força e união. Aos meus cunhados e sobrinhos, todos diferentes, a alegria de cada um.

Tio Narciso, porque valoriza o estudo, nos ensinou a importância das raízes e por ser a árvore do Til da família Branco. Saibamos nós estar à altura de todos os que nos precederam.

Avó, cuja memória me testemunha uma eternidade risonha, gloriosa e que continuamente me interpela a nunca cair na tentação do desalento.

Mãe e Pai, caríssima memória, de quem sinto a falta em cada instante, que me ensinaram a gostar de aprender ("Aprender, aprender, aprender sempre"), que me transmitiram as suas convicções com a mesma liberdade de as podermos questionar.

Mateus e Meg, as maiores Bençãos da minha vida, a realidade a ser melhor do que fui capaz de sonhar. A Meg, serena, terna, assertiva, segura e certa, no colo de quem descanso, que me reduz o ritmo para alcançar a sua paz, que cuida de todos, todos os dias. Mateus, que me obriga a fazer o pino, entrar ao contrário nos

lados certos e incertos da vida, esta tese foi pensada e repensada com o que aprendo contigo. Obrigada aos dois.

Pedro, a razão de tudo. Qualquer que seja o caminho o seu Amor vence o temor, anula o cansaço, ilumina a mente, dá-me calma, prudência e ânimo. Todos os dias seguem perfeitos.

ABSTRACT

Chronic kidney disease (CKD) is a major health problem because of its high prevalence: up to 10% of the general population suffers from CKD and 30% are considered at risk of developing CKD. The mortality rates are higher than other chronic diseases, including most types of cancer.

Even though there is a relative lack of innovative solutions in CKD management, which is holding CKD outcomes back. This scenario calls for new tools of risk stratification and tailored peritoneal dialysis PD prescriptions.

Advances in the current knowledge might necessarily include the identification of biomarkers, potential targets and mechanisms of toxicity of uremia. Together with better knowledge on the impact of peritoneal dialysis on these mechanisms will definitely improve patient-oriented outcomes. The unravelling of reasons for the extremely high burden of cardiovascular disease in uremia is also a medical unmet need.

On the other hand, it is consensual that PD negatively impacts the integrity of the peritoneal membrane, and that fibrosis is a key pathophysiological path of functional loss, menacing both patient and technique survival. However, evidence was missing the impact of uremia in membrane fibrosis and the impact of fibrosis of the peritoneal membrane before the start of PD in PD long-term outcomes.

In chapter III, we hypothesized that uremia has different forms of presentation among patients and that fibrosis is already present in the peritoneal membrane in pre-PD for some patients. To follow our hypothesis, we performed a histomorphological characterization of the membrane status in pre-PD aiming to investigate if fibrosis was already present in some patients. In addition, we investigated the link between uremia and fibrosis in peritoneal membrane to identify a biopsy-free marker of the status of the peritoneal membrane (Chapter I).

A clinical-mechanistic, transversal, single-center study was conducted in adult incident PD patients with baseline peritoneal biopsies: 58 biopsies were scored considering the submesothelial compact zone thickness (STM), vasculopathy and inflammation. We investigated if the membrane status could be inferred from a panel of blood proteins (TNFα, α-Klotho, Galectin-3, FGF21, FGF23, TWEAK, TNFα, hsPCR).

In Chapter IV, we assumed that membrane integrity at pre-PD was a predictor of membrane survival in DP and that the combination of uremic factors, pre-existent cardiovascular disease and other age-related indicators might be predictive indicators of the global cardiovascular disease during PD. For that, in a 5-year follow-up, the membrane status, aging and fibrosis related indicators (age, frailty score and panel of blood biomarkers of chapter 1) were characterized at the baseline and investigated as predictors of the endpoints: a) PD failure and time until PD failure and b) a major cardiovascular event (MACE) or time until MACE and c) all causes mortality.

These studies provided evidence that support strategies for tailored prescriptions patients:

1- While there is a high person-to-person variability, fibrosis might be already present in the membrane at pre-dialysis (Chapter III).
2- Membrane fibrosis in pre-PD is related to the individual´s uremic fingerprint in the blood (Chapter III).
3- Low α-Klotho circulating levels can infer fibrosis in membrane (Chapter III).
4- Fibrosis of peritoneal membrane before the start of PD is not related to the peritoneal transport and is not an indicator of membrane survival in PD (Chapter IV).
5- Galectine-3, a key driver of the aging process is predictive of the survival of the peritoneal membrane (Chapter IV).
6- Fibrosis of peritoneal membrane before the start of PD is an indicator of cardiovascular vulnerability (Chapter IV).

7- Klotho level is related to fibrosis of the membrane and a predictive of major cardiovascular events (Chapter IV).
8- Galectine-3 and Klotho are putative minimally invasive tools guide nephrologists in PD (Chapter IV).

Overall, outputs of chapter III and IV contribute to novelty in PD field: highlighted the histopathology of the peritoneal membrane in the uremic milieu and unveiled the status of the peritoneal membrane pre- PD as a window to the systemic cardiovascular risk. Two putative tools for precision PD emerged from our research: a predictor of membrane survival (galectine-3) and a predictor of cardiovascular risk in PD patients (α-Klotho). Within a non-invasive assessment at the beginning of the technique and a capability to infer the longitudinal evolution and outcomes, they are candidates to support tailored prescriptions.

In chapters V to VII we analyzed the repercussion of PD in different cardiovascular clinical contexts:

a) demonstrating its ability to preserve residual renal function and mitigate cardiovascular disease (Chapter V and VI),
b) documenting the benefits of PD in cardiovascular disease with resistance to diuretics resistance (Chapter VII)
c) producing a protocol proposing the value of ultrafiltration in the Diuretic resistant heart failure ((Chapter VII)

In chapters V-VII we contributed to support the reasons why PD is a better therapeutic option for patients with cardiovascular disease (Chapter IV - Chapter VII). Our data support that even patients with vulnerable phenotype of biological accelerated ageing could take advantage of this home-based modality of renal replacement therapy, with no differences in mortality and had good survival in the technique, despite a higher risk of CV events (Chapter IV).

It offers gentle ultrafiltration with minimal impact on hemodynamics that results in a lower degree of neurohumoral stimulation and in slower decline of renal function, factors known to be associated with survival. Peritoneal ultrafiltration leads to effective continuous solute clearance, such as potassium, allowing better up-titration of risk-modifying pharmacological treatment (Chapter VII).

This technique is also not associated with myocardial stunning and seems to achieve a reduction in inflammatory burden. These advantages are confirmed when treating patients with cardiovascular established disease (Chapter V and Chapter VII) and for congenital heart disease in children undergoing cardiac surgery (Chapter V).

RESUMO

A doença renal crónica, com uma prevalência de 10% na população e 30% sob risco, é um grave problema de saúde pública.

As taxas de mortalidade são superiores à de outras doenças crónicas, incluindo a maioria das doenças oncológicas. Há falta de progresso na inovação pelo que os resultados na doença renal crónica são insatisfatórios. Este panorama exige novas ferramentas de estratificação de risco e prescrições de diálise e terapêuticas individualizadas.

Os avanços no conhecimento atual podem incluir a identificação de biomarcadores, de potenciais alvos e aumentar o conhecimento dos mecanismos de toxicidade da uremia. O aumento do conhecimento do papel da diálise peritoneal nestes mecanismos pode, definitivamente, conseguir melhorar os resultados do doente.

O desvendar de razões para o fardo extremamente elevado das doenças cardiovasculares na uremia é uma necessidade médica não satisfeita. Por outro lado, é consensual que a diálise peritoneal (DP) tem um impacto negativo na integridade da membrana peritoneal e que a fibrose é um caminho fisiopatológico de perda funcional, ameaçando tanto a sobrevivência do doente como a técnica. No entanto, falta evidência sobre o impacto da urémia no estabelecimento de fibrose da membrana antes da diálise peritoneal e no impacto dessa fibrose nos resultados a longo prazo dos doentes sob diálise peritoneal.

No capítulo III, colocámos a hipótese que a uremia tem diferentes formas de apresentação entre os doentes e que a fibrose já está presente na membrana peritoneal na pré-dialise em alguns doentes. Na sequência desta hipótese, nós fizemos uma caracterização histomorfológica para caracterização da membrana na pré diálise e assim estudar a possibilidade da existência de fibrose prévia ao início da técnica.

Além disso, investigámos a ligação entre a uremia e a fibrose na membrana peritoneal, com o objetivo de identificar uma ferramenta, não invasiva,

relacionada com a uremia para inferir alterações histológicas, como um instrumento de precisão na DP: um marcador sem biópsia para avaliar o estado da membrana peritoneal (Capítulo I).

Foi realizado um estudo clínico-mecanicista, transversal e monocêntrico em doentes adultos incidentes em DP com biópsias peritoneais no início da técnica: 58 biópsias foram pontuadas considerando a espessura da zona submesotelial compacta (STM), vasculopatia e inflamação.

Investigámos se o estado da membrana poderia ser inferido a partir de um painel de proteínas (TNFα, α-klotho, galectina-3, FGF21, FGF23, TWEAK, TNFα, hsPCR) sanguíneas. Avaliamos se a histomorfologia da membrana peritoneal e indicadores relacionados com o envelhecimento e fibrose poderiam ser preditores dos resultados finais num estudo com 5 anos de seguimento: a) tempo até à falha técnica da DP, b) tempo até ao primeiro grande evento cardiovascular (MACE) ou morte cardiovascular. A avaliação da fragilidade (escala de Edmonton) foi incluída nos instrumentos de estratificação do risco.

No capítulo IV, avaliamos se a histomorfologia da membrana peritoneal seria predictora de sobrevida na técnica e se a combinação de factores urémicos, indicadores relacionados com o envelhecimento e doença cardiovascular prévia poderia ser preditores de doença cardiovascular global durante a DP. Para isso, num estudo com 5 anos de seguimento, o estado da membrana, indicadores de fibrose e de envelhecimento e o painel de biomarcadores do capítulo 1 foram caracterizados no início e avaliados como preditores de resultados: a) falência da técnica e tempo até à falência b) primeiro grande evento cardiovascular (MACE) e tempo até ao primeiro MACE e c) Todas as causas de morte.

Estes estudos forneceram provas que apoiam estratégias para estratégias terapêuticas personalizadas:

9- A fibrose peritoneal já está presente na membrana na pré-dialise, há variabilidade individual (Capítulo III).

10- A fibrose da membrana peritoneal na pré diálise está relacionado com a impressão digital urémica (Capítulo III)
11- Alfa-Klotho consegue inferir a fibrose da membrana (Capítulo III).
12- A Fibrose da membrana na pré-dialise não foi preditiva do transporte peritoneal no início da diálise nem da resposta a longo prazo à DP (Capítulo IV).
13- Galectina-3, uma toxina urémica relacionada com o processo de envelhecimento é predictora de sobrevida da membrana peritoneal (Capítulo IV).
14- A Fibrose da membrana peritoneal antes do início da técnica foi indicadora de vulnerabilidade cardiovascular (Capítulo IV).
15- Nível de alfa-Klotho está relacionada com a fibrose da membrana e um preditor de MACE (Capítulo IV).
16- Galectina-3 e alfa-Klotho são promissores instrumentos, minimamente invasivos, úteis aos nefrologistas, para estratificação de risco (Capítulo IV).

Nos capítulos V, VI e VII analisámos as repercussões da diálise peritoneal em diferentes contextos clínicos de doenças cardiovasculares:

d) Demonstrámos a utilidade da diálise peritoneal na doença cardiovascular, crónica e aguda, e na sua preservação da função renal residual (Capítulos IV, V, VI),
e) Documentámos o benefício da DP nas doenças cardiovasculares com resistência aos diuréticos (Capítulo VII)
f) Concebemos um protocolo que propõe o valor da ultrafiltração na insuficiência cardíaca resistente aos diuréticos (Capítulo VII)

Nos capítulos V-VII contribuímos para fundamentar as razões pelas quais a DP é uma boa opção terapêutica para doentes com doença cardiovascular (Capítulo IV - Capítulo VII). Os nossos dados validam que mesmo doentes com fenótipo vulnerável de envelhecimento biológico acelerado podem tirar partido desta modalidade de substituição da renal substitutiva domiciliar, sem diferenças na mortalidade e com boas sobrevidas na técnica, apesar de maior risco de eventos CV (Capítulo IV).

A Diálise peritoneal oferece uma ultrafiltração suave, com um impacto mínimo na hemodinâmica, com menor grau de estimulação neuro-humoral e um declínio mais lento da função renal, fatores solidamente associados à sobrevida. A ultrafiltração peritoneal leva a uma depuração contínua de solutos, como o potássio, permitindo assim uma melhor titulação do tratamento farmacológico modificador de risco com segurança (Capítulo VII).

Esta técnica também não está associada ao *stunning* miocárdico e parece conseguir uma redução da inflamação. Essas vantagens são confirmadas no tratamento de doentes com doença cardiovascular estabelecida (Capítulo V e Capítulo VII) e na lesão renal aguda nas crianças submetidas à cirurgia cardíaca para correcção de cardiopatia congénita (Capítulo V).

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CHAPTER VII, PART 2.

Figure 1: Variation of NYHA class and LVEF with PD treatment. LVEF: Left Ventricular Ejection Fraction; NYHA: New York Heart Association; PD: Peritoneal dialysis.

LIST OF ACRONYMS AND ABBREVIATIONS

CKD	Chronic kidney disease
PD	Peritoneal dialysis
ESKD	End stage kidney disease
AAKH	Advancement of American Kidney Health
DCOR103	Non-calcium-based phosphate binders
RRF	Residual renal function
APDT	Automated PD treatment
QoL	Quality of life
CRS	cardiorenal syndrome
CHULN	Centro Hospitalar Universitário de Lisboa Norte
ESRD	End Stage Renal Disease
CAPD	Continuous ambulatory peritoneal dialysis
APD	Automated peritoneal dialysis
GDPs	Glucose degradation products
ICO	Icodextrin
MW	Molecular weight
IgG	Immunoglobulin G
EPS	Encapsulating peritoneal sclerosis
SGLT-2	Glucose transporter - sodium-glucose transport-2
GLUT1	Glucose transporter 1
GLUT3	Glucose transporter
EMT	Epithelial-mesenchymal transition
ECM	Extracellular matrix
ZO-1	Zona occlusive-1
α -SMA	α-smooth muscle actin
VEGF	Vascular endothelial growth factor
TGF- β	Transforming growth factor β
EndMT	Endothelial-mesenchymal transition
TGF- β1	Transforming growth factor β1
TNF-α	Tumor necrosis factor α
IL-1β	Interleukin 1β
AGEs	Advanced glycation end-products
PET	Peritoneal equilibration test
HGF	Hepatocyte growth factor
RAAS	Renin-angiotensin-aldosterone system
D/P	dialysis to plasma ratio
D/P Cr	Creatinine dialysis to plasma ratio
GAGs	Glycosaminoglycans
LMWHs	Low molecular weight heparins
VDR	Vitamin D receptor

STM	Submesothelial compact zone thickness
HD	Hemodialysis
BMI	Body mass index
PCV	Packed Cell Volume
ELISA	Enzyme-Linked Immunosorbent Assay
FGF21	Fibroblast growth factor 21
FGF23	Fibroblast growth factor 23
TWEAK	Tumor necrosis factor-like weak inducer of apoptosis
TNFα	Tumor necrosis factor alfa
hr-CRP	High-sensitivity C-reactive Protein
GFR	Glomerular filtration rate
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance index
PCA	Principle Component Analysis
NFκB	Nuclear factor-κB
SD	standard deviation
IQR	interquartil range
AKI	Acute kidney injury
RRT	Renal Replacement Therapy
CVVHDF	Continuous veno-venous hemodiafiltration
ICU	Intensitive care unit
UF	Ultrafiltration
HF	Heart Failure
SPPA	Systolic pressure of the pulmonary artery
EF	Ejection fraction
ACE	Angiotensin Converting Enzyme
ARA	Angiotensin Receptor Antagonists
NYHA	New York Heart Association
PH	Pulmonary hypertension
eGFR	Estimated glomerular filtration rate
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
nPCR	normalized protein catabolic rate
Kt/V	Volume of urea distribution
EPO	Erythropoietin
ANP	Atrial natriuretic peptide
EUF	Extracorporeal ultrafiltration
DE	Doppler echocardiography
LVEF	Left ventricular ejection fraction

CHAPTER I

INTRODUCTION

CHAPTER I

1 INTRODUCTION

1.1 CKD prevalence

The global prevalence of chronic kidney disease (CKD) is estimated at more than 843 million people. Of these, a *minor* percentage progress to the final stage, requiring treatments to replace renal function. However, there are currently more than 2.5 million under these treatments and by 2030 it is estimated that there will be 5.4 million. Hemodialysis (HD) is the most common form of renal replacement therapy (International Society of Nefrology, no date; Vanholder *et al.*, 2021).

1.2 Economic impact

Dialysis has an important economic impact. Dialysis patients are often unemployed. In the US, >75% of patients are unemployed at the start of dialysis, compared to <20% in the general population. Unemployment affects individually and collectively. It reduces purchasing power, self-image, mental health and opportunities for new generations. The search for strategies to prevent kidney disease, control risk factors and adjust renal function replacement therapies, with flexibility, at home that allow productivity, family balance and less cardiovascular stress (Bikbov *et al.*, 2020).

1.3 Home dialysis

A 2020 review by a panel of nephrologists and ethicists appointed by three major nephrology societies outlined key ethical concerns associated with kidney care. With regard to the management of renal failure, equitable access to adequate treatment is probably the most important and relevant ethical issue, not only in the context of HD, but

also for other renal care modalities (including transplantation, peritoneal dialysis (PD) and conservative therapy (Lok *et al.*, 2020).

The new KDOQI Vascular Access Guideline 2019 makes a shift to a global, patient-centered strategy for accessing renal function replacement therapies. Introduces a new concept, the "End stage kidney disease (ESKD) Life Plan", an individualized set of renal replacement modalities (HD, PD, transplantation, conservative care) necessary to sustain the life of an ESKD patient that considers their medical and current and future life and preferences. The Life Plan should be reassessed regularly to adapt to changes in the patient's life circumstances. In July 2019, the President of the United States signed an Executive Order on the Advancement of American Kidney Health (AAKH), which promises to fundamentally change the clinical care of kidney disease in general and kidney failure. Components of the AAKH that are relevant to dialysis care include a guideline for education and support programs to promote awareness of kidney disease; a shift in focus from HD reimbursement initiatives at the center to home therapies, transplants and CKD care; a system that rewards physicians and dialysis facilities for providing a variety of treatments for kidney failure, with the aim of increasing acceptance of home dialysis and transplantation; and incentives for nephrology care teams to focus on reducing costs and improving outcomes by providing longitudinal care to patients with kidney disease. Worldwide, PD is less widely available than HD.

1.4 Imperiousness of improving results

Despite the many interventions that have been tested, including increasing the dose of dialysis (Depner *et al.*, 1999; Paniagua *et al.*, 2002), increasing hemoglobin, using non-calcium-based phosphate binders (St. Peter *et al.*, 2008), or reducing serum cholesterol level (Fellström *et al.*, 2009; SHARP Collaborative Group, 2010) no intervention clearly reduced all-cause or cardiovascular mortality for dialysis patients.

Many symptoms of uremic syndrome can be related to the persistence of uremic toxins bound to proteins and small peptides (so-called medium molecules) that are not effectively removed by current dialysis modalities, so the status quo of dialysis care is suboptimal. The burden of residual symptoms, morbidity, mortality, and economic cost are unacceptable, which challenges nephrology researchers to develop tools that allow valid and consistent measurement of these outcomes and identify interventions that ameliorates them (Himmelfarb *et al.*, 2020).

1.5 Peritoneal Dialysis

PD, a home dialysis, depends on the structure, physiology and characteristics of a specific membrane – the peritoneum. Comparing to HD, it does not require complex equipment, being simple to use and accessible in remote geographic locations. Due to its flexibility, PD allows maintaining working activity, preserves residual renal function (RRF) and has lower cardiovascular impact (Lameire and Van Biesen, 2010; Silva F *et al.*, 2022). There are few contraindications for PD. The absence of cognitive ability or a caregiver in this situation, or presence of peritoneal bands. In this regard, the degree of healing can be assessed laparoscopically from the peritoneal view at the time of attempted catheter placement and can be treated by adhesiolysis (Crabtree and Burchette, 2009). Other chronic contraindications related to peritoneal dialysis include inflammatory bowel disease, ventriculoperitoneal shunt, severe lung disease, or abdominal skin infections(Crabtree and Burchette, 2009; Mehrotra *et al.*, 2011; van de Luijtgaarden *et al.*, 2016).

1.6 Variations in PD prevalence

Worldwide, PD has a lower prevalence than HD. In 2017, in 125 countries, PD was available in 75% of countries, while HD was available in 96%. In 2018, it was estimated that 11% of the world's dialysis patients were on

PD. There is a large discrepancy between countries, in Hong Kong, for example, >80% of dialysis patients are on PD, while in Japan this proportion is <5%. This fact is, in part, determined by government policies and the density of HD facilities.

In Thailand, the government adopted a “PD-First” approach in 2008 as part of its universal health coverage scheme, as in Hong Kong, under the umbrella that dialysis services will be paid for only if the patient is treated with PD, given its lower cost (Choy and Li, 2015). Finally, China has been rapidly expanding access to renal replacement therapy for its population and has a policy that encourages the non-mandatory use of PD.

As mentioned earlier, access to such care is limited in many countries but inequalities in access to dialysis also depend on health education, literacy, and socioeconomic status. Inadequate patient education may result from a lack of confidence when choosing a home dialysis technique, and referral *bias* from physicians and nurses. Appropriate training of professionals is essential to ensure equity and shared decision-making in the accessibility of techniques (Mehrotra *et al.*, 2005; Lameire and Van Biesen, 2010; Tantivess *et al.*, 2013; Blake, Golper and Saxena, 2014; Cho *et al.*, 2021).

1.7 The history of peritoneal dialysis

Peritoneal dialysis has a long and tortuous history. It was first used in animals in the late 1800's and entered clinical practice in the early 1960's. Peritoneal access was firstly provided by intermittent abdominal puncture and, when silastic became available, Henry Tenckhoff created a technique for permanent access. Successful peritoneal dialysis was performed intermittently with infusion of 2 liters of balanced fluid followed by a dwell time of 30-45 min, which in turn was followed by drainage and re-infusion. The procedure was initially used almost exclusively in the setting of acute kidney injury in the critically ill patients but was not successful when applied in the long term.

Equilibration with dialysis fluid, five exchanges of 2 liters per 10 liters per day, would allow the removal of 7,000 mg of urea, the average amount generated in the diet of a 70 kg person ingesting 1 g of protein per kg of body weight per day. The procedure was originally called “balance peritoneal dialysis” but was later changed to “continuous ambulatory peritoneal dialysis”.

1.8 History of peritoneal dialysis in HSC/CHLO

This year we celebrate the 40th anniversary of the beginning of PD at Hospital de Santa Cruz, a pioneer institution in this technique, introduced by the initiative of Prof. Dr Jacinto Simões and Dr. Maria João Pais, belonging to the newly formed Nephrology Department of this hospital. The first patient was a 6-year-old child with ESRD, residing far from Lisbon, with difficulties in creating vascular access and with no expectation for an immediate transplant. Dialysis started with the availability of Dr. Humberto Messias, the surgeon who also placed, for the first time in Portugal, a definitive peritoneal catheter.

The patient remained on PD for one year and was subsequently transplanted. Forty years later, the Unit maintains a PD program of meaningful expression, well established for patients with ESRD, in a total of about 900 patients treated so far. Currently, 73 renal patients are being treated with this technique.

Despite the good clinical results and similar patient survival between HD and PD, this is still an underused modality. In Portugal, only 7% of patients in need of renal replacement therapy are on PD. Several factors can explain this paradox, such as lack of experience of health personnel, lack of infrastructure for training and monitoring of patients on PD, organizational issues, installed capacity of HD units and lack of economic incentive. In addition, perceived (correctly or incorrectly) contraindications for PD represent barriers to the use of this technique, which is often considered less effective and safer than HD.

In the following paragraphs, we present the way in which our Unit was organized and the measures that have been taken over the years to overcome these problems and become what is currently the largest program in the south of the country.

Our dialysis adequacy strategy is always patient-centered, in a broad multidimensional approach. From the beginning, there was a partnership with the Nursing team. Over the years with the involvement of the Social, Nutrition and Pharmaceutical Services, with the aim of improving adherence to treatment and preventing complications, namely infectious ones.

In 2010, the consultation on options was created to clarify available therapies for ESRD, in order to increase patient information and allow a free, informed and clarified choice about renal function replacement techniques, contributing to improve the penetration of PD.

It was also possible to provide an education program, which contributed to positioning PD as the first renal function replacement technique. Its clinical advantages are proven (protection of vascular territory, preservation of residual renal function and ability to maintain diuresis), making it necessary to demystify preconceived ideas. Thus, in the last 10 years a stabilized percentage of HSC patients has been opting for PD (10.8-11.4%), in line with world statistics and higher than the national average (6-7%).

In the Unit, protocols have been established for service improvement at all levels, as well as prevention and treatment of the most frequent problems, as listed below:

a) Problems with Peritoneal Dialysis Catheters

Permanent peritoneal access and the risk of peritonitis are major obstacles to larger scale use. Henry Tenckhoff designed a permanent peritoneal catheter in 1968, named after its inventor and which is still the most used peritoneal access today. Initially used in intermittent PD

techniques, it benefited from the breakthrough that took place in 1975, when Jack Moncrief and Robert Popovich introduced continuous ambulatory PD.

The skill of the surgeon who implants the catheter is the most important factor for success. In our hospital there is an excellent collaboration with the surgical team. The placement is fast and the resolution of problems with the catheter very efficient. Most of the time, the implantation of the peritoneal catheter is done by laparoscopy. A word of gratitude to the memory of Dr António Pina, whose premature and difficult to accept death did not prevent his legacy from persisting among those who worked with him.

b) Infectious complications

Peritonitis remains an important complication in PD and the main cause of morbidity in these patients. Touch associated contamination incidence has significantly decreased since 1980, with simple measures such as washing hands before filling and the Y system created by Buoncristiani. Currently, peritonitis incidence is of less than 1 episode/25 months of dialysis treatment and 70% of patients have never had this complication.

c) Problems with fluids of Peritoneal Dialysis

Over the years, sugar-containing dialysate solutions have remained the cornerstone in the treatment of PD. To provide these solutions with a more physiological pH, with greater biocompatibility, different buffers have been developed. The use of PD solution with neutral pH and low content of glucose degradation products led to a greater preservation of diuresis and residual renal function. In the 1990s, PD solutions with molecules alternative to glucose were developed, and are currently used in our unit for long periods of stay, or in patients with diabetes, in order to reduce the use of sugar.

d) Automated Peritoneal Dialysis

Cyclers have been developed for automated PD treatment (APDT). In the beginning, medical indications for this treatment modality prevailed, such as dialysis dose increase and volume optimization. Subsequently, social indications became more important. Patients who do not urinate may be properly treated with PD. There are no consistent differences between manual or automatic PD in the rate of loss of residual renal function, rate of peritonitis, maintenance of euvolemia, technique survival, mortality or health-related quality of life (QoL). We emphasize the tenacity of Drª Augusta Gaspar and Nurse Fátima Marques who started this modality at HSC.

e) Assisted Peritoneal Dialysis

In the last two decades, most developed countries have seen a continuous growth in the number of ESRD elderly patients with indication to initiate renal replacement therapy. PD is still underused in vulnerable, physically or mentally dependent patients that interfere with technique autonomy. Home care assistance from a family member and caregiver can overcome this problem. We pioneered the creation of assisted PD for patients with a high rate of comorbidity who are unable to perform exchanges alone. Technique failure rate, incidence of peritonitis and quality of life indices became, with this support, comparable to those of autonomous patients.

f) Benefits for other groups of patients

Chronic kidney and heart disease patients

PD has emerged as a therapeutic alternative for the control of fluid overload in patients with refractory heart failure, providing a slow and daily ultrafiltration in an outpatient setting. The Unit has the experience of 18 patients whose indication for starting the technique was cardio-renal syndrome (CRS) with congestion refractory to medical therapy.

CRS is a frequent problem in our clinical practice and it causes significant morbidity, due to disabling symptoms, recurrent hospitalizations, and increased mortality. Its approach is, therefore, a challenge, as there is scarce scientific evidence, since patients with advanced CKD are excluded from main randomized clinical trials in heart failure. Thus, the need for a coordinated and multidisciplinary approach to this syndrome between Cardiologists and Nephrologists arose, focusing on all aspects of the treatment of CRS.

Since the beginning of the multidisciplinary renal-cardiac consultation, we have increased the number of patients on PD with refractory CRS, with encouraging results regarding a decrease in congestive symptoms, need for hospitalization, improvement in quality of life and, and even improvement of laboratory and echocardiographic parameters.

g) Peritoneal Dialysis in pediatric patients with heart disease

PD has been used in acute renal injury in children hospitalized by the Pediatric Cardiology Service, during the perioperative period of cardiac surgery to correct congenital cardiac malformations. This modality of dialysis has contributed to the treatment of acute kidney injury and metabolic balance in this extremely fragile phase.

h) A Rare and Happy Case

It is extremely rare for PD patients to succeed in their legitimate dream of becoming mothers. Local and systemic complications are numerous and the risk is considered high for both mother and fetus. In 2021, the team had greater joy, one of our patients became pregnant and became a mother in June. The baby was born at term, healthy, and the mother also lived through the pregnancy successfully without major. Collaboration with the nephro-obstetrics consultation at Centro

Hospitalar Universitário de Lisboa Norte (CHULN) was essential throughout the process.

i) The Unit and the formation of young physicians

During these 40 years, the unit has benefited from the contribution of young interns, participated in the postgraduate training of more than 40 portuguese Nephrologists, both at our Hospital Center and at other National and Brazilian Hospitals. Dr. Margarida Bruges trained us in the austere taste for rigor.

j) Bridges with other Units

Chronic kidney patients must have the same opportunities as other citizens, including professional, family or leisure travel. Thus, our Unit has given support to patients from other national and international Units (Spain, France, Belgium, Angola, Mozambique, Cape Verde, Guinea-Bissau, South Africa, and Libya, among others), occasionally on vacation or in change of residence.

In summary, I am proud of the positive path taken by PD in HSC/CHLO, its mission and specific capabilities. Over the past 40 years, PD has proven to be an effective treatment for patients with ESKD. During this period, understanding of the physiology and pathology of the peritoneal membrane has increased. Due to improvements in dialysis systems, availability of cyclers for APD, development of new solutions, and efforts to reduce complications such as peritonitis, treatment has considerably improved. Currently, patient survival in the first 5 years is, at least, equal to conventional HD. As important as scientific and technological advances has been the people contribution, their generosity and ability to innovate, as well as everything we have learned from the courage of our patients and their families.

What is described in these pages is just a portion of the events that have taken place during these 40 years and the impact that this Unit has had on people's lives.

1.9 Peritoneal dialysis Procedure

Peritoneal dialysis is performed by instilling fluid (dialysate) into the peritoneal cavity. The fluid is allowed to dwell (being the dwell defined by the time the dialysate remains in the abdominal cavity) for a defined period, after which it is drained, and fresh fluid is instilled. The volume of fluid instilled is 2 L in most adults, although lower volumes are often used in smaller patients and higher volumes in larger patients. During the dwell period, solute diffusion and ultrafiltration occur across the peritoneal membrane; the used dialysate is then discarded, and the cycle is repeated. Peritoneal dialysis may be performed manually, usually four times daily, with the dialysate dwelling in the abdominal cavity between exchanges to equilibrate; this is called continuous ambulatory peritoneal dialysis (CAPD). Alternatively, a mechanical device, a “cycler”, may be used to perform a few exchanges over a period of several hours in a procedure called automated peritoneal dialysis (APD).

1.10 The peritoneum

The peritoneum is a serous membrane that lines the peritoneal cavity. Its area is proportional to the body surface in size and normally varies from 1 to 2 m^2 in an adult. Anatomically, it is composed of two layers: the visceral peritoneum, which covers the abdominal organs and represents 80% of the total surface area, and the parietal peritoneum, which lines the lower surface of the diaphragm and the inner surface of the anterior abdominal wall (Blake PG and Daugirdas JT, 2015).
During peritoneal dialysis, the main player in peritoneal transport is the parietal peritoneum, as only approximately one third of the visceral

peritoneum is in contact with the dialysis solution during the treatment of PD (Flessner, 1996).

The peritoneal cavity contains the omentum, ligaments, mesentery, and intraperitoneal organs, including the stomach, spleen, liver, parts of the small intestine, and sigmoid colon. The retroperitoneal organs include the aorta, esophagus, parts of the small intestine and colon, pancreas, kidneys, ureters, and adrenal glands. The visceral peritoneum is supplied from the superior mesenteric artery and the parietal peritoneum from the lumbar, intercostal and epigastric arteries.

The visceral peritoneum provides venous drainage to the portal system and the parietal to the inferior vena cava. Total peritoneal blood flow ranges from 250 to 150 mL/min. The main lymphatic drainage of the peritoneum and peritoneal cavity is the sub-diaphragmatic lymphatic system, which drains into the right lymphatic duct through large collecting ducts. Additional lymphatic drainage occurs through smaller lymphatic vessels in the visceral and parietal peritoneum.

Histologically, the peritoneum consists of a single layer of mesothelial cells resting on sub-mesothelial interstitial tissue, a gelatinous matrix containing fibroblasts, adipocytes, collagen fibers, nerves, lymphatic vessels, and capillaries (Di Paolo N and Sacchi G, 2000).

1.11 Peritoneal Transport

During peritoneal dialysis, the transport of solutes and water occurs across the peritoneal membrane, by diffusion, ultrafiltration and absorption, between the peritoneal capillaries and the dialysis solution in the peritoneal cavity. The dialysis solution normally contains sodium, chloride and lactate or bicarbonate and is made hyperosmolar by a high concentration of glucose. In PD, fluid (dialysate) is instilled into the peritoneal cavity and solutes diffuse from the blood in the peritoneal capillaries into the dialysate. Likewise, the imposition of a transmembrane osmotic pressure gradient creates the driving force for ultrafiltration of fluid from the capillaries into the dialysate. Osmotic

strength is increased with the infusion of hypertonic dialysate in the form of 1.5, 2.5 and 4.5% glucose or glucose polymer.

The three-pore model of peritoneal transport treats the capillary membrane as a primary barrier determining the amount of solute that transports to the interstitium and the peritoneal cavity.

Large pores have a radius 20-40 nm, are formed by clefts between endothelial cells and are in small number. Macromolecules can be transported by convection through these pores. Small pores have a radius 4-6 nm, they are also formed by clefts between endothelial cells. This type of pore is the most abundant type, accounting for more than 90% of the transport of small solutes (e.g., urea, creatinine, sodium, and potassium), which is associated with water removal.

Ultrapores have a radius of less than 0.8 nm and are comprised of aquaporin channels in the endothelial cell membrane. Only water is transported through these ultrapores. The transport of water via aquaporin-1 contributes to around 50% of ultrafiltration in PD. The rate of solute transfer across the peritoneum depends on the concentration gradient. The rate of solute transfer across the peritoneum depends on the concentration gradient and the degree of peritoneal vascularity (Davies *et al.*, 2011), which varies from person to person. Thus, peritoneal transport is dependent on the surface area of the peritoneal capillaries rather than on the total peritoneal surface area. The distance of each capillary from the mesothelium determines its relative contribution. The term “effective peritoneal surface area” refers to the area of the peritoneal surface that is sufficiently close to the peritoneal to play a role in peritoneal transport.

In patients with less peritoneal vascularity, solutes diffuse slowly in both directions. Waste products transfer slowly into the dialysate, and the glucose gradient that is driving ultrafiltration dissipates slowly. Conversely, in patients with greater peritoneal vascularity, solutes diffuse more rapidly, also in both directions. Waste products

accumulate in the dialysate more rapidly, and the glucose gradient dissipates more rapidly.

Some patients have poor, sometimes even negative ultrafiltration, especially during long dwells. The use of a non-glucose-based fluid such as icodextrin during long dwells may be beneficial in these patients (Finkelstein *et al.*, 2005). Icodextrin is a colloid osmotic agent that does not diffuse across the peritoneum; its effect on ultrafiltration is sustained for 12 to 16 h (Wilkie *et al.*, 1997). Icodextrin has been shown to improve ultrafiltration and volume status in PD patients (Davies *et al.*, 2003). It has also been shown to improve glycemic control, decrease weight gain, and lessen glucose-induced lipid abnormalities. There is some evidence of better long-term preservation of peritoneal membrane function (Davies *et al.*, 2005). Other types of dialysate fluids include an amino acid-based fluid and fluids that are low in glucose degradation products (GDPs). So-called “biocompatible” solutions are two-bag system solutions that have a physiological pH after mixing. They contain reduced amounts of GDPs and they are theoretically more biocompatible than standard solutions where the pH is 5.5. However, there is a lack of consistent evidence supporting superiority of those solutions compared to conventional PD solutions, in preservation of membrane function and in long-term survival of patients or technique. Several factors affect the rate of transport of solutes across the peritoneal membrane, of which the molecular weight (MW) of solutes is probably the most important factor affecting the rate of transport (Renkin EM, 1979). Peritoneal transport of larger molecules occurs at a much slower rate compared to small solutes. Thus, creatinine (PM 113 g/mol) is slower than urea (PM 56 g/mol), inulin (PM 5200 g/mol) is slower than creatinine, and larger proteins cross the peritoneum very slowly. Higher molecular weight proteins such as albumin, transferrin and immunoglobulin G (IgG) use the large pores described above for transport across the peritoneal membrane and into the peritoneal cavity. Regardless of the mechanism, this process is slow enough that

serum proteins are present in low concentration in the dialysate and equilibrium with plasma does not occur at clinically used residence times. The transport of these solutes out of the peritoneal cavity occurs mainly through the subdiaphragmatic lymphatics and, to a lesser extent, through the peritoneal interstitium (Krediet RT *et al.*, 1990; Struijk DG *et al.*, 1990).This process is independent of molecular size peritoneal interstitium (Struijk DG *et al.*, 1990).Even through the slow transport of serum proteins with higher molecular weights, such as albumin, the daily loss of peritoneal protein with PD can be substantial. Peritoneal clearances of large uremic toxins such as beta2-microglobulin are significantly lower compared to clearances of urea nitrogen and creatinine (Bammens *et al.*, 2003). However, molecular weight cannot fully explain the peritoneal permeability characteristics of all molecules (Asano *et al.*, 2019). Other factors, including load and rate of protein binding, can affect peritoneal transport rates. As the tissue mass of the peritoneal cavity is smaller compared to the whole body, serum concentrations of cytokines, growth factors, cardiac markers and adipokines are generally higher than in dialysis fluid.

1.12 Molecular and Genetic Studies

Among patients starting treatment with peritoneal dialysis, there is wide variability in the transport of water and solute across the peritoneal membrane, which influences dialysis prescriptions and outcomes (Brimble *et al.*, 2006).

A recent genomics study showed that the rate of peritoneal transport of small solutes was associated with a polygenic risk score and 17% heritability; these findings support a genetic influence on solute transport across the peritoneal membrane (Morelle, Stachowska-Pietka, *et al.*, 2021).

A common promoter variant rs2075574 in AQP1 is shown to be associated with decreased ultrafiltration and an increased risk of death or lower survival in the technique (Devuyst, 2021).

Another recent study analyzed peritoneal biopsies from uremic patients, treated with PD and with encapsulating peritoneal sclerosis (EPS) and found the expression of the glucose transporter - sodium-glucose transport-2 (SGLT-2), glucose transporter 1 (GLUT1), and glucose transporter 3 (GLUT3) – in the peritoneal membrane. SGLT-2 protein expression increased with duration of PD and was significantly increased among patients with EPS (Morelle, Stachowska-Pietka, *et al.*, 2021).

Preclinical studies in animals showed that SGLT-2 inhibitors or downregulation of SGLT-2 reversed pathological changes in the peritoneum. SGLT-2 inhibitors can reduce glucose uptake across the peritoneal membrane and delay the effects of glucose on peritoneal membrane transport function, and perhaps preventing fibrosis and EPS (Schricker *et al.*, 2022).

The translation of genetic and molecular insights into precision medicine would help to better understand the dialysis process and improve care (Devuyst, 2021; Mehrotra *et al.*, 2021).

1.13 Peritoneal Fibrosis

Peritoneal fibrosis is a common complication of PD that can lead to failure of ultrafiltration and discontinuation of PD.

Research in the last two decades has shown that peritoneal fibrosis is strongly associated with inflammation, angiogenesis and epithelial-mesenchymal transition (EMT), and that these processes interact (Zhou *et al.*, 2016). These are the final destructive changes along a spectrum of different other harmful processes that may occur in parallel with or precede fibrotic changes. In the epithelial-mesenchymal transition, epithelial cells undergo a transition in which they lose their cell-cell contacts, cell-matrix interaction, cell polarity and, consequently, their epithelial markers and gain a mobile mesenchymal phenotype (Balzer, 2020). TMS plays an important role in both organ development and wound healing, but also in diseases including cancer and tissue fibrosis.

In the peritoneum, this has also been referred to as the mesothelial-mesenchymal transition. During this transition process, mesothelial cells migrate from the superficial mesothelial layer towards the sub-mesothelium, where they produce extracellular matrix (ECM), that contributes to fibrosis (Yáñez-Mó *et al.*, 2003). Initially it was shown that mesothelial cells lose their epithelial markers such as E-cadherin, cytokeratin and zona occlusive-1 (ZO-1) and adopt a myofibroblast-like phenotype expressing N-cadherin, vimentin and α-smooth muscle actin (α -SMA) (Aroeira *et al.*, 2007; Wang *et al.*, 2016). However, this hypothesis has been questioned in animal models, showing that type I collagen-producing sub-mesothelial fibroblasts are the progenitors of α-SMA-positive myo-fibroblasts during peritoneal injury (Chen *et al.*, 2014).

The individual contribution of TMS-derived myofibroblasts to the set of sub-mesothelial myofibroblasts or activated resident stromal fibroblasts has not yet been defined (Liu *et al.*, 2015).

In this context, several studies have shown that, as EMT is closely linked to angiogenesis, stimulation with Transforming growth factor β (TGF-β) induces Vascular endothelial growth factor (VEGF) production in human peritoneal mesothelial cells and a fibroblast lineage (Kariya *et al.*, 2018). The authors also showed that pharmacological inhibition of TGF-β in a rat model of PD decreased not only peritoneal fibrosis but also VEGF production, suggesting a close relationship between TGF-β and VEGF signaling.

Angiogenesis and fibrosis are therefore intimately interconnected through common early growth factors and inflammatory cytokines, as well as through the EMT process, which has made a challenging task to identify specific mechanisms that contribute to peritoneal membrane failure. There is, however, a growing body of evidence that show the importance of growing and differentiation of resident stromal fibroblasts fibroblasts as a main cause of peritoneal fibrosis and inflammation (Liu *et al.*, 2015). Furthermore, the contribution of other

processes, such as the endothelial-mesenchymal transition (EndMT), is unclear and deserves further investigation, especially as the vasculature within the sub-mesothelial zona compact is an important determinant of peritoneal solute and water exchange success during PD. Vascular endothelial growth factor mediated signaling in angiogenesis has been shown to be implicated in increasing the effective vascular surface area of PD patients, resulting in a decrease in osmotic pressure and ultrafiltration failure (Kariya *et al.*, 2018). Furthermore, AGEs are known to upregulate VEGF (Yamagishi *et al.*, 1997).

Studies in PD patients have demonstrated a correlation between glucose-based PD time, increased VEGF production, and failure of ultrafiltration.

It has been demonstrated that a number of cytokines are related to the pathogenesis of PF, including TGF- β1, tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) (Hung, Wu and Tsai, 2007). TGF-β/Smad signaling is considered the major factor in the regulation of fibrosis. In addition, previous studies have provided insights into the treatment of PF via targeting of TGF-β/Smad signaling (Li *et al.*, 2014).

Recent studies have reported that epigenetic modifications play an important role in PD-associated peritoneal fibrosis. The main epigenetic modifications associated with peritoneal fibrosis include DNA methylation, histone modification and non-coding RNAs. The mechanisms of epigenetic regulation in peritoneal fibrosis predominantly involve the modification of signaling molecules, transcriptional factors and genes (Kariya *et al.*, 2018).

1.14 Peritoneal Membrane Preservation

The peritoneum is a specialized endogenous membrane with unique structural and physiological characteristics. Preservation of the integrity and transport function of the peritoneal membrane is critical to the long-term success of PD treatment.

Repeated exposure of the peritoneal membrane to high and non-physiological concentrations of glucose through peritoneal dialysis solutions conditions or worsens peritoneal fibrosis, leading to a progressive and irreversible loss of its ultrafiltration capacity (Holmes and Mujais, 2006).It is possible to assess the transport function of the peritoneal membrane in patients on peritoneal dialysis with the peritoneal balance test (PET) (Twardowski *et al.*, 1987; Pannekeet *et al.*, 1995). Solute transport rates are assessed by the equilibrium rates between peritoneal capillary and dialysate blood. The ratio of solute concentrations in dialysate and plasma (D/P ratio) at specific times during the pause defines the extent of solute equilibrium. Changes in the D/P ratio can be used to monitor peritoneal membrane function over time. The creatinine D/P ratio has been shown to be a strong predictor of outcomes (mortality and hospitalization) in patients on peritoneal dialysis (Mehrotra *et al.*, 2015).

Preservation of intact mesothelial cells should be the first target of protection. Several strategies have been proposed to preserve the peritoneal membrane.
To decrease peritoneal membrane changes related to the use of conventional glucose-based PD solutions, a new generation of PD solutions was developed, with neutral pH, low content of GDPs and bicarbonate-lactate as buffers. Other strategies include the use of non-glycemic osmotic agents, but only two of them, icodextrin and amino acids, have been successfully used in clinical practice.

1.15 Neutral low-GDP pH solutions

Several studies, both in vitro vivo and in vivo, have shown that low pH neutral solutions have a positive impact on preserving peritoneal morphology, lowering EMT induction and improving peritoneal defense mechanisms.

There is evidence of less fibrosis and vasculopathy in patients treated with biocompatible solutions for 51.9 months compared with a group of patients using conventional solutions (Kawanishi *et al.*, 2013). A Spanish group has published a larger series of peritoneal biopsies in patients treated with biocompatible solutions that confirm significantly better preservation of the mesothelial cell layer and a lower prevalence of Hyalinizing vasculopathy in patients using low PD solutions (del Peso *et al.*, 2016).

1.16 Glucose-Sparing Strategies

General strategies to decrease the need for hypertonic glucose use include interventions to preserve residual renal function as well as glucose-free dialysis solutions containing icodextrin or amino acids (Paniagua R *et al.*, 2009; Li *et al.*, 2013). Innovative UF strategies have highlighted the potential variety of combination of a crystalloid osmotic agent (glucose) and a colloid (icodextrin), a combination that goes beyond the individual ultrafiltration of each of the components (Freida *et al.*, 2008). A new peritoneal dialysis solution containing L-carnitine and xylitol was recently reported (Bonomini *et al.*, 2021). These two molecules have a molecular weight that is similar to glucose, high solubility in water, chemical stability in aqueous solutions, and osmotic properties, which make them suitable for use in PD fluids (Bonomini *et al.*, 2021). Studies on the biocompatibility of a DP solution containing carnitine or xylitol showed a better profile than the glucose base (Rago *et al.*, 2021).

1.17 Icodextrin and low-glucose PD regimens

Icodextrin has been used as an agent to increase UF. Its effect is slow but prolonged, and therefore it is especially useful for long stays. There is robust evidence that low glucose PD regimens may be associated with better peritoneal membrane preservation. The use of icodextrin may be official for reducing the glucose load (Holmes CJ and Shockley

TR, 2000). In anuric patients with PD treated with automated peritoneal dialysis, Davies et al confirmed that ultrafiltration improved (Davies *et al.*, 2005).

A low glucose PD regimen, including icodextrin and amino acids as osmotic agents, has been proposed as an alternative in PD. In a randomized study in 80 patients, comparing two daily glucose-based solutions with two daily exchanges of icodextrin and amino acid solutions, the levels of CA125, decorin, hepatocyte growth factor (HGF), IL-6, adiponectin and adhesion molecules were significantly higher in the low glucose group compared to the control. These data suggest that a low-glucose PD regimen may be associated with better preservation of membrane integrity (Yung *et al.*, 2015).

1.18 Peritoneal Resting

Peritoneal Resting, with temporary transfer to HD, has been shown to be a useful tool for the treatment of patients with acquired elevated transport status. Rodrigues et al confirmed this fact and suggested that the pause be initiated soon after the transition to high transport status in order to obtain better results (Rodrigues A *et al.*, 2002). Peritoneal rest has been shown to induce functional changes in patients with ultrafiltration failure due to rapid transport state acquisition but has no effect in patients with normal peritoneal function (De Sousa *et al.*, 2014).The potential role of intermittent heparin administration during the peritoneal rest period is unknown. In animal models, it was confirmed that with peritoneal rest structural changes caused by PD may be reversible. A reduction in glucose hyperpermeability, a significant reduction in blood vessel density, in the degree of fibrosis with recovery of the mesothelial layer was demonstrated (Kim YL *et al.*, 1999)

1.19 Renin-Angiotensin-Aldosterone System Blockade Agents

One of the main mechanisms involved in the pathogenesis of peritoneal fibrosis is the activation of the local renin-angiotensin-aldosterone system (RAAS). RAAS components are expressed in peritoneal mesothelial cells and are regulated in the presence of inflammation and chronic exposure to PD fluids. All these factors contribute to the accumulation of extracellular matrix and neo-angiogenesis, components of progressive fibrosis of the peritoneum (Nessim, Perl and Bargman, 2010).The effect of blocking RAAS in preventing MP damage has been studied in vitro, in animal models and in humans. Both losartan and captopril inhibit glucose-induced TGF-β and fibronectin expression in cultured human peritoneal MCs, attenuating VEGF overproduction (Noh H *et al.*, 2005; Sauter M *et al.*, 2007).

These same effects were confirmed in patients on angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, with a slower decrease in UF capacity, reduction of fibronectin, TGF-β1 and VEGF in the dialysate (Duman S *et al.*, 2001).

Beneficial effects of RAAS blockade have been observed in human beings as well Jing et al (JING *et al.*, 2010)observed a slower rate of decrease in UF capacity in a group of patients using angiotensin-converting enzyme inhibitors or angiotensin II–receptor blockers relative to patients not using these drugs. They also observed that the non-treated group showed higher peritoneal effluent levels of fibronectin, TGF-β1, and VEGF than the treated patients, suggesting that these agents could protect against peritoneal fibrosis (Ersoy R *et al.*, 2007; Nakamoto H *et al.*, 2008). Similarly, a group from the Netherlands validated the use of angiotensin converting enzyme inhibitors/angiotensin I receptor blockers by preventing the increase in mass transfer area coefficients that occurs in patients with long-term PD. Although this was not a randomized study, these results are

promising enough that many professionals advocate the routine use of RAAS inhibitors in patients with PD whenever blood pressure allows (Kolesnyk I *et al.*, 2007).

1.20 Heparin and other glycosaminoglycans

Glycosaminoglycans (GAGs) are long, unbranched polysaccharides classified according to their central disaccharide structures. GAGs are classified into several groups: heparin/heparin sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and HA.

The anti-fibrotic effects of heparin and its derivatives are beneficial for the peritoneum. Fibrin provides a matrix for the initiation of peritoneal fibrotic processes, which heparin can modulate. In addition, the pleiotropic, immunomodulatory, antiangiogenic, anti-inflammatory, antiproliferative effects of heparin on the extracellular matrix are documented (De Vriese AS, Mortier S and Lameire NH, 2001; Margetts, 2009).

Low molecular weight heparins (LMWHs) inhibit the activity of VEGF and fibroblast growth factor (Ludwig, 2009). Despite the described properties, there is no evidence to support the use of heparin or its derivatives to prevent or treat peritoneal membrane dysfunction in PD as studies have been contradictory.

Hyaluronic acid, a component of the mesothelial glycocalyx, is essential for adhesion. Cell culture studies can show that peritoneal MCs and fibroblasts can synthesize HA and confirm their importance in maintaining the epithelial cell phenotype and tissue integrity by contributing to the structural support of the peritoneum. Its role in clinical studies is still uncertain (Jones *et al.*, 2001).

Sulodexide is a mixture of GAGs, anticoagulant and antithrombotic, which in an animal model of exposure to PDFs confirmed to reduce neovascularization, submesothelial thickening and mesenchymal transition (Pletinck *et al.*, 2012).

1.21 Additives to Peritoneal Dialysis Solution

An innovative method to preserve the integrity of the peritoneal membrane is to add pharmacological agents to PDFs.
Addition of alanyl-glutamine (Ala-Gln) to cell cultures of mesothelial cells has been shown to preserve the mesothelium and modulate inflammation (Herzog *et al.*, 2014)

1.22 Alternative Osmotic Agents

L-carnitine has been used to reduce intradialytic hypotension and treat resistant anemia in HD patients. Because it is highly soluble in water and stable at physiological pH, it has been considered as an alternative osmotic agent for peritoneal dialysis. Studies in cell cultures, animal models or patients have been promising. Addition of a dialysate solution with carnitine to human peritoneal mesothelial cells resulted in superior cell growth, increased prostaglandin E2 secretion, less lactate dehydrogenase release, less apoptosis, and better conservation of cell morphology (Gaggiotti *et al.*, 2005). In 4 patients long night dialysis with 1.5% glucose and 0.25% L-carnitine was well tolerated, with superior ultrafiltration compared to dialysate with 2.5% glucose (Bajo, del Peso and Teitelbaum, 2017)

1.23 Other Therapeutic Agents

Tamoxifen is a regulator of the estrogen receptor because of its effect on TGF-β (Huang JW, Blood Purif. 2011;31: 252-8). In an animal model Loureiro showed that tamoxifen blocks EMT induced by TGF-β, significantly reduced peritoneal thickness and angiogenesis, and improved peritoneal function(Loureiro *et al.*, 2013). Clinically, tamoxifen has been used to treat EPS associated with PD (De Sousa–Amorim *et al.*, 2014). A Dutch study showed a decrease in mortality among patients with EPS after treatment.

Statins are potent inhibitors of cholesterol biosynthesis that are often used in patients with PD. They have pleiotropic effects and may be a promising therapeutic strategy for preserving the integrity of the peritoneal membrane in patients undergoing peritoneal dialysis.

An in vitro and animal study showed that statin treatment inhibits mesenchymal transition changes in glucose-treated MCs and in mice instilled with PDFs (Chang *et al.*, 2014). Simvastatin is an effective stimulator of mesothelial fibrinolytic capacity and suppresses procoagulant activity under normal and inflammatory conditions, but randomized studies are lacking.

Paricalcitol is a selective vitamin D receptor (VDR) agonist. The vitamin D hormonal system has classically been implicated in the regulation of calcium homeostasis and bone metabolism. However, it also modulates inflammation, fibrosis, angiogenesis and immune responses. In a mouse model of PD paricalcitol prevents MP deterioration, reducing fibrosis and UF failure (González-Mateo *et al.*, 2014). A preliminary study reported an increase in UF capacity with a decrease in peritoneal protein losses in 23 PD patients (Coronel *et al.*, 2012).

Rosiglitazone is a peroxisome proliferator-activated γ receptor agonist used in the treatment of diabetes. The diabetic environment in the peritoneum created by PD provides justification for the use of this class of drugs that reduce the effects of GDPs on the formation of AGEs. In addition, these drugs have anti-inflammatory effects not yet studied in clinical practice (Sandoval *et al.*, 2010).

SGLT-2 inhibitors can reduce blood glucose levels by enhancing urinary glucose excretion (Cherney *et al.*, 2014; Ahmed-Sarwar *et al.*, 2017). SGLT-2 was confirmed to be expressed in peritoneal mesothelial cells and exert a glucose-lowering effect in the peritoneum exposed to peritoneal dialysis solution (Zhou *et al.*, 2019). It has been reported that SGLT-2 inhibitors ameliorate renal (Zhang *et al.*, 2018; Ali BH *et al.*, 2019), myocardial (Li *et al.*, 2019) and liver fibrosis (Raj *et al.*, 2019). In addition, Li et al. (Li *et al.*, 2019) found that SGLT-2 inhibition with empagliflozin

suppressed myocardial fibrosis through inhibition of the TGF-β/Smad signaling pathway in the hearts of diabetic mice. However, the effect of SGLT-2 inhibitors on PF via attenuation of the TGF-β/Smad signaling pathway has not yet been reported. In summary, our findings demonstrate that the secretion of pro- inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, is increased in mice with PF. In addition, SGLT-2 inhibition with empagliflozin reduced the levels of Col-I, α-SMA, and pro-inflammatory cytokines and increased the expression of E-cadherin in mice with PF, eventually leading to delayed progression of PF. We equally established that the downregulation of SGLT-2 also elicits the same effect. These findings suggest that empagliflozin has the potential to exert a protective effect on high-glucose peritoneal dialysis solution-induced PF by suppressing TGF-β/Smad (Shentu *et al.*, 2021).

1.24 Predictors of membrane fibrosis and link with systemic cardiovascular disease

The first attempt to use the human peritoneum to dialyze uremic molecules was made in 1923 (Mehrotra *et al.*, 2016).

Recent studies have expanded our understanding of solute and water transport processes, increasing our understanding of peritoneal physiology and pathophysiology, with the identification of aquaporins and mechanisms of peritoneal inflammation. With the increase in our understanding of the mechanisms involved in the structural alterations of the peritoneal membrane, whether associated with fibrosis, angiogenesis, inflammation, or with the improvement of knowledge of cellular mechanisms, we can seek to individualize therapies and interventions to overcome barriers. Specifically in the choice of solutions, new osmotic agents, combination of different types of osmotic agents. The search for non-invasive biomarkers, at the beginning of the technique and throughout the PD, are details of areas that need further investigation. The clinical relevance of uremia leads to

the research for biochemical targets to predict and improve patient outcome in several retrospective and prospective cohort studies. In PD patients this goal wasn't achieved (Gamba *et al.*, 1993).

Individual differences in peritoneal membrane function are documented to influence clinical outcomes in PD. Fast transporters of solutes have, specifically, worse survival and more cardiovascular events. This basal velocity of transport is a manifestation of the systemic inflammation, common in advanced kidney disease.

In order to understand high-risk cardiovascular risk phenotypes, including interactions with membrane fibrosis (Mehrotra *et al.*, 2016) and whether this translates into a reduced development of uremic vascular complications, further investigation is required.

There is evidence related to the inflammatory nature of many diseases associated with ageing, including atherosclerosis, vascular calcification, diabetes and CKD. Ageing itself results in chronic low-grade inflammation that promotes fibrosis and tissue damage.

α-Klotho, a protein secreted by the kidney, plays an important role in this aging-inflammation interface. Klotho downregulation can be induced by specific cytokines. The potential relationship between fibrosis and inflammation requires further clarification.

The pathogenesis of cardiovascular disease in uremia has not yet been elucidated. Ageing is highly associated with the progression of fibrosis. Fibrosis is the final hallmark of pathological remodeling, which is a major contributor to the pathogenesis of several chronic diseases involving multiple anatomical territories, vessels, kidney, lung, liver, heart, and peritoneal membrane (Vasko *et al.*, 2014; Hu *et al.*, 2020).

In uremia, the factors that contribute to, initiate or maintain, chronic inflammation are alterations in mineral metabolism, cellular senescence, intestinal dysbiosis, acid-base imbalance, oxidative stress and accumulated non-enzymatic glycation (Kooman *et al.*, 2017; Ebert *et al.*, 2020).

Galectin-3 is a β-galactoside binding protein that is critical in several cellular functions, including cell adhesion, proliferation, apoptosis, signal transduction, and regulation of the immune and inflammatory response (Tsai *et al.*, 2021). This protein is highly expressed in different types of human cells, such as immune and inflammatory cells, fibroblasts, endothelium, and epithelium. It is increased in kidney disease and involved in tissue inflammation and fibrosis and associated with increased risks of incident CKD, progressive loss of kidney function and adverse cardiovascular events (Drechsler *et al.*, 2015).

Furthermore, galectin-3 may be a robust biomarker for cardiovascular complications and all-cause mortality in patients with non-dialysis-dependent CKD. However, it is less reliable in predicting the prognosis of patients with ESRD (Zhang *et al.*, 2019).

These data are important because healthcare delivery needs to adjust to your individual biology to improve treatment efficiency, reduce cardiovascular risk, and better apply lessons learned from research in clinical practice in managing patient expectations.

1.25 Residual Kidney Function

Residual renal function is associated with better survival in PD (Marrón *et al.*, 2008). In the Canada-US study, each 250 ml greater urinary volume per day resulted in a 36% reduction in mortality at 2 years (BARGMAN, THORPE and CHURCHILL, 2001). PD is associated with better preservation of residual renal function, with monthly clearance losses of 0.25–0.28 less than HD 0.30–0.40 ml/min/1.73 m^2 (MOIST *et al.*, 2000; Fernández-Lucas M *et al.*, 2012; Seo *et al.*, 2014).The causes avoid the intravascular volume depletion that occurs more frequently with HD (van Biesen *et al.*, 2013). Cohort studies and controlled trials find, in PD patients, that lower rate of loss of renal function are associated with the absence of intermittent volume depletion, while using renin-

angiotensin-aldosterone system blockers and diuretics (Medcalf, Harris and Walls, 2001; Cho *et al.*, 2014; Ha *et al.*, 2015).

One of the most studied interventions to maintain residual renal function is the use of biocompatible solutions. Biocompatible solutions avoid the need to sterilize glucose at a higher pH, limiting the formation of glucose breakdown products and avoiding associated toxicity, delay the time to anuria, and decrease the rate of clearance loss from 0.28 to 0.22 ml/min/1.73 m^2 per month (Johnson *et al.*, 2012). Subsequent meta-analyses confirmed this observation (Cho *et al.*, 2014; Seo *et al.*, 2014).

1.26 Accelerated aging and frailty

Frailty, prevalent in age and in chronic kidney disease, is a state of greater vulnerability to external and internal aggressors and a risk factor for adverse events. CKD accelerates the ageing process, reducing life expectancy and being associated with cardiovascular and musculoskeletal disorders. There are several descriptions of frailty prevalence in dialysis patients, extrapolated from the five-item, Fried frailty phenotype (Bao *et al.*, 2012). These criteria are based on the presence of unintentional weight loss, exhaustion, reduced grip strength, slow walking speed, or low physical activity (Fried *et al.*, 2001). The incremental severity of frailty is not captured using these measurement tools. It has been shown in the population at the start of dialysis that even small changes in the degree of frailty are associated with worse outcomes (Alfaadhel *et al.*, 2015).

1.27 Morbidity and Morbidity

CKD is a growing cause of morbidity and mortality. It is predicted to be the fifth most frequent cause of death by 2040 and the second before the end of the 21st century in countries with long life expectancy (Ortiz *et al.*, 2022).

Despite multiple technical advances and improved knowledge, mortality remains unacceptably high in dialysis patients for cardiovascular causes and infections. A 2019 study showed that cardiovascular mortality among young adults aged 22 to 29 years with incident renal failure was 143 to 500 times higher than that of comparable individuals without renal failure, due to a very high burden of cardiovascular risk factors (Mehrotra *et al.*, 2016). Several studies have shown that survival on HD or PD is similar between patients (Teitelbaum, 2021).

1.28 World Health Organization sustainable development objectives and *Laudato si*

In the last century, humanity has achieved important targets, increased accessibility to education, purchase power, technological advances, and health gains. These goals have brought collective benefits, still without an equitable distribution, resulting in increases in life expectancy, a reduction in infant mortality, a reduction in the percentage of people in poverty and in extreme poverty. However, this progress came at a considerable environmental cost. Health systems contribute substantially to greenhouse gas emissions and consume the planet's resources. If we are to maintain the health gains made over the last century, urgent action is needed in all sectors of the economy, including health, to preserve natural resources and sustainability of health-care systems.

Peritoneal dialysis also contributes to the consumption of resources and waste generation, PVC bags, cardboard boxes, electricity and water costs, and material transport costs. In line with the objectives of the World Health Organization for sustainable development, there is also a need for more ecological packaging materials in the PD, bio-based or compostable plastics or plastics with superior recyclability with less carbon generation into the atmosphere in its destruction. To reduce all

this environmental impact, the hypothesis of generating dialysate at home is being studied Ellen Medical Devices (Ellen Medical Devices, no date; Barraclough and Agar, 2020).

In 2015, the Holy Father Francisco wrote in the Encyclical letter, *Laudato si,* about the care of the Common Home. The publication of *Laudato si* in 2015 was the first social encyclical to focus on the environment, wanting to contribute to dialogue, improve the Catholic contribution to the discussion of public policy issues in this area.

CHAPTER II

RATIONALE, HYPOTHESIS AND AIMS

CHAPTER II

2 RATIONALE, HYPOTHESIS AND AIMS

2.1 Rationale

CKD is a major health problem because of its high prevalence, around 10% of the general population suffers from CKD and 30% are considered at risk of develop CKD.

CKD contributes to significant physical limitations, loss of quality of life, emotional and cognitive disorders, social isolation, and premature death. In addition, CKD imposes financial burden, in direct healthcare costs and inability to work.

Comfort is only rarely restored with dialysis treatment, either peritoneal dialysis or HD, which corroborates the ominous impact of CKD on the lives of the affected patients. This negative effect continues to worsen over time and CKD associated complications like cardiovascular disease and infection, cause multiple hospitalizations, surgical interventions, cardiovascular events, and premature death. Even more, those reaching kidney replacement therapy, especially people living on dialysis, have similar or even worse survival chances than most people diagnosed with cancer.

Mortality rates of CKD are increasing alarmingly and will continue to increase. Additionally, the presence of CKD further increases mortality risk associated with other diseases like diabetes. Over the last 20 years mortality from CKD has not improved, in contrast to most other chronic diseases. The increase in CKD as a cause of death may reflect the rising prevalence of CKD globally related to population aging as well as improving access to diagnosis in lower income settings. Nonetheless, it might worryingly also reflect the relative lack of progress in innovation that is holding CKD back compared with other chronic diseases.

The uremic syndrome is a complex clinical condition that develops in the advanced stages of CKD, resulting in a multitude of complications

and high early mortality. This clinical picture is largely defined by the retention of metabolites, peptides and proteins, which are not excreted or degraded by the kidney or that have an increased production. This profile of uremic molecules has a negative biological impact. The unravelling of reasons for the extremely high burden of cardiovascular disease in uremia is a medical unmet need. Advances in the current knowledge might necessarily include the identification of biomarkers, potential targets and mechanisms of toxicity of uremia as well as the role of extracorporeal removal and ultimately, improved patient-oriented outcomes.

The mean age and number of comorbidities of the patient population with ESRD have increased over recent decades. Therefore, a home-based therapy may be a straightforward option for such frail patients and this group might benefit most from spending more time in their home environment, avoiding the burden of thrice-weekly transfer to a dialysis unit and post-dialysis hangover. Thus, it might also be useful to increase PD use in frail and comorbid patients

However, several unmet needs in PD might limit its optimal use. For example, it is generally accepted that PD negatively impacts the integrity of the peritoneal membrane by well-known reasons. On the contrary, the dependence of the effectiveness of PD on the structural and functional integrity of the peritoneum in the uremic patient is still debatable, which was one of the focuses of the current thesis.

2.2 Hypothesis

We hypothesize that uremia has different forms of presentation among patients and that fibrosis is already present in the peritoneal membrane in pre-dialysis for some patients. There is a link between uremia and fibrosis in peritoneal membrane, which allows to identify a uremia-related tool to infer histological changes, representing a way to find out a precision tool in PD: a biopsy-free marker to assess the status of the peritoneal membrane. In addition, basal membrane fibrosis impacts

patients' outcomes. But most of all, the combination of the uremic factors related to ageing/fibrosis and basal peritoneal membrane are predictive indicators of the global cardiovascular disease during PD. PD, while preserving renal function, is a good option in cardiovascular disease.

2.3 General and specific Aims

The global aim of this thesis was to provide evidence that support strategies for the optimization of PD outcomes.

Specifically, this work aims at:

1- Investigate if fibrosis is already present in the membrane at pre-dialysis (Chapter III). Investigate individual variability in membrane status (Chapter III).

2- Investigate if membrane status in pre-dialysis is related to the individual´s uremic fingerprint in the blood (Chapter III), using a panel of uremic toxins known to be related to ageing and fibrosis in other tissues.

3- Investigate if the membrane status in pre-dialysis is predictive of long-term response to PD, i.e., outcomes related to the technique (effectiveness) and to the patient (cardiovascular events and loss of renal residual function and mortality) (Chapter IV)

4- Ascertain the impact of the selected ageing and fibrosis related uremic toxins before start PD in the long-term global response to PD (Chapter IV)

5- Using a frailty scale, investigate the impact of vulnerability of patients before start PD in in the long-term global response to PD (Chapter IV)

6- Analyze the repercussion of peritoneal dialysis, with its ability to preserve residual renal function, on cardiovascular disease (Chapter V)

7- Describe the use of peritoneal dialysis in cardiovascular disease with resistance to diuretics resistance (Chapter VI)

8- Design a protocol proposing the value of ultrafiltration in the Diuretic resistant heart failure (Chapter VII)
9- Individualize peritoneal dialysis, with a patient-centered strategy, according to outcome predictors (Chapter VIII).

2.4 Global considerations

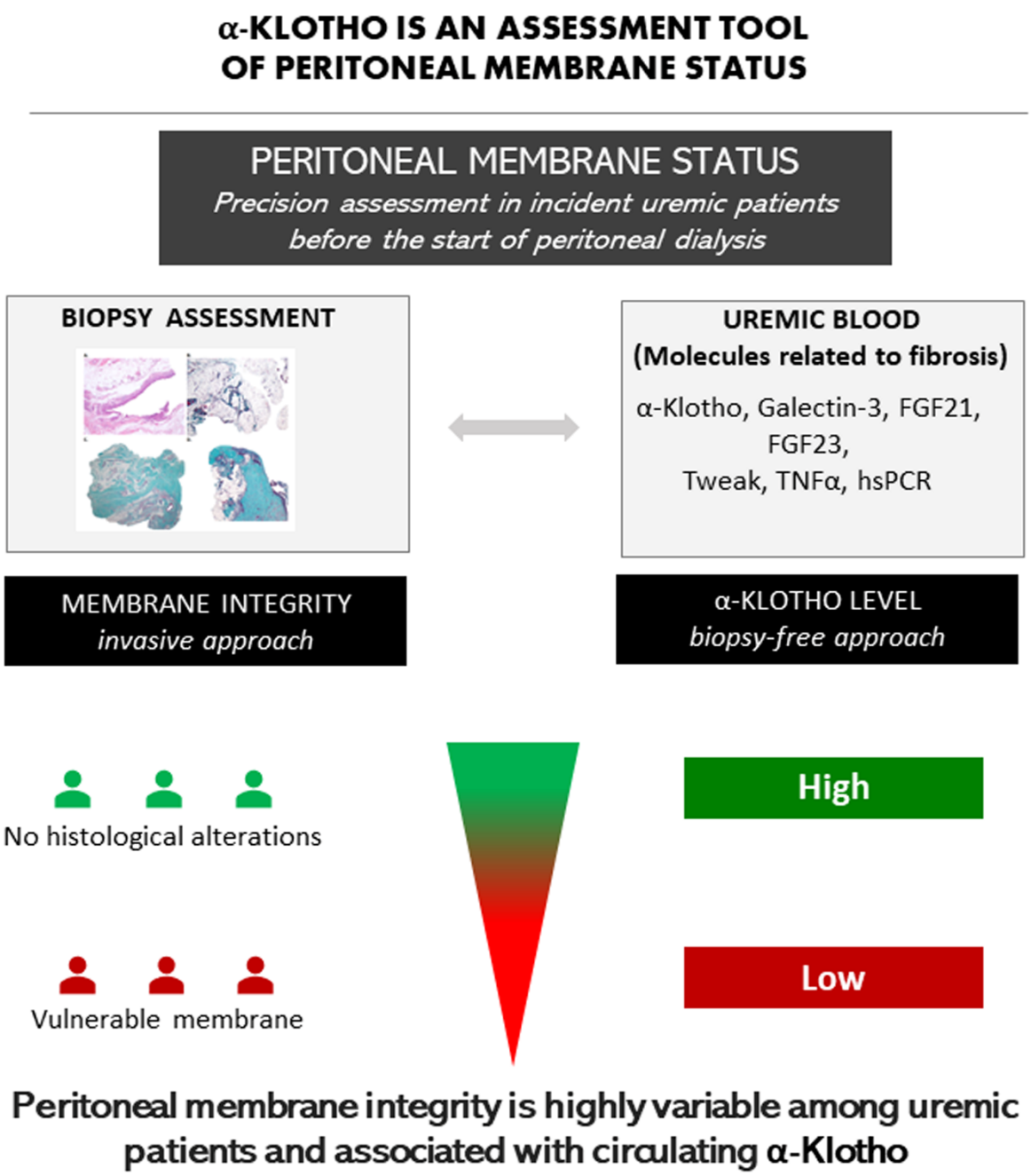
The pathophysiology of peritoneal fibrosis involves chronic uremic inflammation and the fibrotic process itself. Inflammation often precedes the development of membrane fibrosis, although there is a bidirectional relationship of one inducing the other. An increase in fibrinogenesis and in endothelial permeability causes fibrin deposition on the peritoneum and cardiovascular disease.

This work aims to highlight the histopathology of the peritoneal membrane and the identification of the existence of fibrosis before the beginning of the technique, outlining the interaction of fibrosis and uremic cytokines, associated with important fibrogenic pathways of premature ageing, involved in the manifestations of cardiovascular disease, technical failure and reduced residual renal function. In addition, we sought to know the predictive capacity of this assessment at the beginning of the technique to infer the longitudinal evolution and these outcomes.

CHAPTER III

ALPHA-KLOTHO AND PERITONEAL MEMBRANE STATUS: A HYPOTHESIS GENERATING STUDY

GRAPHICAL ABSTRACT



CHAPTER III

ALPHA-KLOTHO AND PERITONEAL MEMBRANE STATUS: A HYPOTHESIS GENERATING STUDY.

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Abstract

Background: Long-term success of peritoneal dialysis (PD) relies on the integrity of the peritoneal membrane. This proof-of-concept study addressed the hypothesis that fibrosis is already present in the membrane at pre-dialysis and that the membrane status is related to the individual´s uremic fingerprint.

Methods: A clinical-mechanistic, transversal, single-center study was conducted. Pre-dialysis peritoneal biopsies were scored considering the submesothelial compact zone thickness (STM), vasculopathy and inflammation. We investigated if the membrane status could be inferred from a panel of proteins (α-Klotho, Galectin-3, FGF21, FGF23, TWEAK, TNFα, hsPCR) in blood.

Results: A total 58 incident patients aged 56 ± 15 years old were included, 31% female, 55% hypertension, 29% diabetic, 24% obese. Person-to-person STM was found to be highly variable and 38% of patients were fibrosis positive. Both α-Klotho (Spearman r=-0.7491, p<0.001) and FGF21 (Spearman r=-0.5102, p<0.001) were negatively associated with STM. α-Klotho, but not FGF21, was able to discriminate fibrosis from non-fibrosis with/without inflammation and vasculopathy. PLS models identified α-Klotho as the protein most relevant for fibrosis. α-Klotho was independently associated with fibrosis of the peritoneal membrane (OR=0.991 (0.896 - 0.997), p=0.002).

Conclusion: In uremic patients at pre-dialysis the status of the peritoneal membrane was highly variable among patients. More vulnerable membranes were associated with low circulating α-Klotho, which might be a minimally invasive biomarker to guide clinical decisions in PD.

Keywords

Peritoneal membrane Fibrosis; Cytokines; Uremic toxins; Precision medicine; Diabetes, Biopsy.

3.1 Introduction

Peritoneal dialysis (PD) is a home-based modality of renal replacement therapy, based on the dialytic properties of the peritoneal membrane. When compared with hemodialysis (HD), PD is more cost-effective, it allows better preservation of residual kidney function, and it has less impact on hemodynamics. In addition, the risks related to vascular access are avoided, while the hospitalization rates, the functional status and the quality of life are improved, maintaining similar survival rates(Yeates *et al.*, 2012; Escoli *et al.*, 2019; Cho *et al.*, 2021; Kunin and Beckerman, 2022; Silva F *et al.*, 2022).Furthermore, PD is easier to use, has less need for expert medical staff and technical support, and is accessible in remote geographical locations. PD gives patients more flexibility, allowing them to continue working(Theofilou, 2011)

The world prevalence of PD is of approximately 11%, with variations from country to country (Li *et al.*, 2017).

Despite the advantages, some patients will eventually progress into fibrosis and the loss of integrity of the peritoneal membrane might impact the long-term success of PD and it often leads to PD discontinuation. The rationale underlying the present study relies on a novel paradigm in PD. We hypothesized that the systemic uremic profile of the patients with indication to renal replacement therapy impacts the integrity of the peritoneal membrane. Therefore, the risk of progress into fibrosis upon PD treatment is not equal for all individuals and it is related to the uremic fingerprint of the individual.

This systemic uremic context, an imbalance of circulating protective and injurious molecules (Ebert *et al.*, 2020), might promote inflammation and cardiac or renal fibrosis (Elewa *et al.*, 2012; Kunin *et al.*, 2015). Therefore, it is reasonable to expect that the peritoneal membrane might be compromised even before the onset of PD, at least for some individuals (Dobbie, Lloyd and Gall, 1990; Williams *et al.*, 2002; Honda *et al.*, 2008; Devuyst, Margetts and Topley, 2010). However,

the factors known to impact the peritoneal membrane have almost exclusively been identified in studies wherein PD was already ongoing (Zimmeck *et al.*, 2002; Erixon *et al.*, 2004; Lui *et al.*, 2012; Morelle *et al.*, 2018; Balzer, 2020). For instance, and among other factors (Lui *et al.*, 2012; Balzer, 2020), the constant exposure of the peritoneum to bio-incompatible glucose-based PD fluids (and the related carbonyl stress and advanced glycation end-products) is well known to be deleterious for the membrane (Zimmeck *et al.*, 2002; Erixon *et al.*, 2004; Morelle *et al.*, 2018). This also support that the diabetic individuals might be at increased risk of membrane fibrosis at pre-PD that non-diabetics (Mizumasa *et al.*, 2013). Altogether, these data suggest that the membrane status before PD is already highly variable among individuals.

As the assessment of the membrane integrity requires biopsy, which is not feasible in every patient, the development of biopsy-independent strategies would push the field forward and benefit the efficiency of this renal replacement therapy.

As learned from PD studies, events underlying membrane injury include denudation of mesothelial cells, proliferation of fibroblasts, thickening of sclerotic lesions in the sub-mesothelial connective tissue, and vasculopathy (neovascularization, vascular hyperplasia, lumen narrowing and wall thickening) (Lui *et al.*, 2012; Balzer, 2020). The mechanisms behind these effects are multifactorial and poorly understood, possibly including epigenetic, immune, hemodynamic, apoptotic or inflammatory changes. Preventive or therapeutic measures (Lui *et al.*, 2012; Zhou *et al.*, 2016; Balzer, 2020) based on these mechanisms are currently not available and the uremic profile associated with membrane fibrosis still to be unveiled.

Presently, there are no alternatives to biopsy in the clinical practice. This might be related to the gaps in the current knowledge at pre-dialysis about the status of the membrane, its variability among patients and the resultant clinical implications. Therefore, and in the context of pre-

dialysis, we herein defined two general research goals. First, to unveil the variability among patients in the status of the peritoneal membrane. Second, to identify relationships between accessible and minimally invasive molecules and fibrosis of the peritoneal membrane. For that and resorting to biopsy, we assessed integrity of the membrane at pre-PD. According to our mechanistic-driven hypothesis, we have also investigated the putative association between uremic blood proteins and the status of the membrane. The panel of proteins selected was based on the existing knowledge in aging-related diseases that links these proteins to inflammation and fibrosis in other organs/tissues and that demonstrates that these proteins are modified in uremia (Moreno *et al.*, 2011; de Boer *et al.*, 2012; Moe, 2012; Pedersen *et al.*, 2013; Grabner and Faul, 2016; Castillo-Rodríguez *et al.*, 2017; Takenaka *et al.*, 2019; Salgado, Goes and Salgado Filho, 2021).

3.2 Materials and Methods

3.2.1 Study Design and Participants

This was a single center, transversal study that included incident patients at the PD Unit of Santa Cruz Hospital, Centro Hospitalar de Lisboa Ocidental, Portugal and that was approved by the Ethics Committee of the NOVA Medical school, Faculdade de Ciências Médicas University of Lisbon (Approval number 50/2019). The study was conducted according to the Declaration of Helsinki and Good Clinical Practices and complied with the European Union GDPR Legislation.

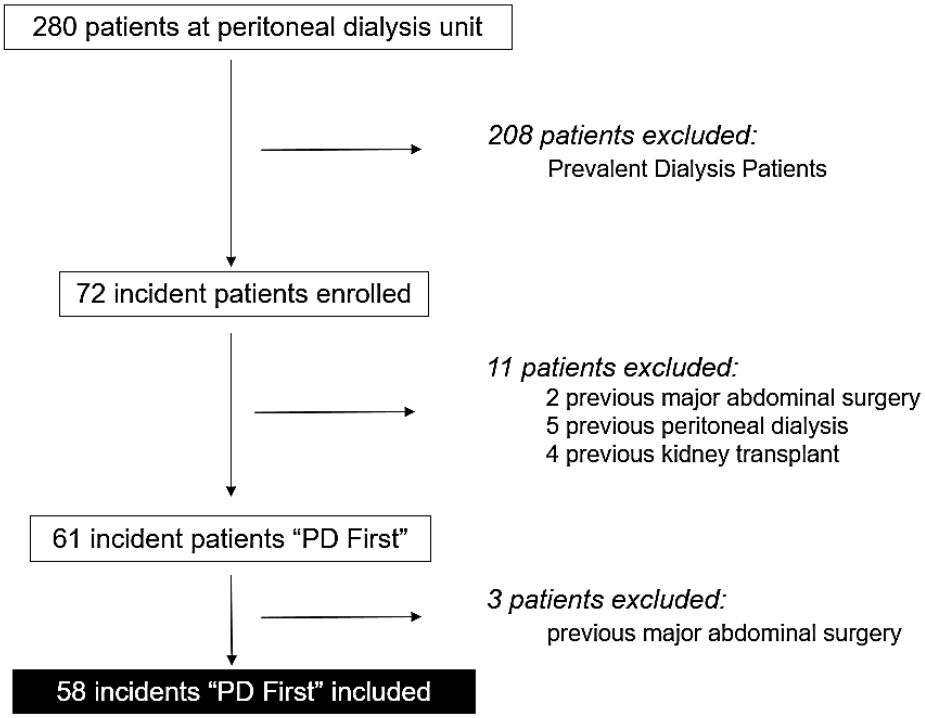
Patients were referred from the Nephrology consultation or from the hospital for an information consultation. This consultation included the information transmitted by a multidisciplinary team including doctor, nurse, nutritionist, and social worker. The enrollment of the patients was in a consecutive manner, and it followed the patient´s flow bellow.

The main purposes of the consultation were to assess the eligibility criteria for the renal substitution technique and to provide information that allows an informed choice.

Inclusion criteria were being over 18 years of age in the moment of choice of renal replacement therapy and having a stable clinical condition, defined by the absence of serious abdominal infections (diverticulitis, pancreatitis, and cholecystitis) or active neoplasm. Non-autonomous patients were included for assisted PD when there is a helper-person. Patients signed an informed consent.

The exclusion criteria were related to the presence of confounding factors, which reflected previous aggressions to the peritoneal membrane (such as surgeries or peritonitis). Therefore, only incident patients (PD first) were included.

Reporting of the study conforms to broad EQUATOR guidelines (Simera et al. A catalogue of reporting guidelines for health research. Eur J Clin Invest. 2010 Jan;40(1):35-53).



3.2.2 Clinical and Anthropometric data

Clinical and anthropometric data gathered included age; sex; glomerular filtration rate, residual diuresis, co-morbidities including arterial hypertension, congestive heart failure, ischemic cardiomyopathy, diabetes mellitus, obesity (defined as body mass index (BMI) of 30.0 kg/m2 or higher) and overweight (defined as BMI between 25.0 to 29.9 kg/m2) and medical therapy. Twenty-nine patients were on calcium channel blockers, 12 on bisoprolol, 7 on clonidine, 17 on pentoxifylline, 17 under anti-aggregation, 17 on statins and 34 were medicated with erythropoiesis-stimulating agents.

3.2.3 Peritoneal Biopsy

Briefly, all surgical procedures were performed by two surgeons with expertise in insertion of PD catheter and in parietal peritoneum biopsy under anesthesia. Through a paramedian incision, at a point twice the width of a 3-4 cm left lateral to the umbilicus, the rectus abdominis muscle anterior sheath was incised and separated to expose the posterior sheath and peritoneum. Parietal peritoneum (5×2 mm) was collected at a point 3 cm below and before the PD catheter insertion site. No electrocautery was applied, and the peritoneum was sampled without direct contact to avoid mechanical injury. The time between sampling and fixation was minimized, as long-term exposure of the peritoneum to air causes degeneration of the mesothelial cells.20 Samples were fixed at room temperature for 24 hours in 10% phosphate-buffered formalin, embedded with paraffin, cut into 3-µm sections, and fixed on a glass slide. Hematoxylin and eosin staining, azan staining, and elastic-van Gieson staining were performed. Samples were evaluated using an Olympus light microscope (Bx41: Olympus, Tokyo, Japan).

3.2.4 Histologic Analyses

Two examiners evaluated the samples, a nephrologist and a pathologist. The peritoneal membrane was evaluated to determine the presence or the absence of mesothelial cell denudation, acellular sclerotic changes in submesothelial connective tissue thickness, vasculopathy and inflammation.

The submesothelial compact zone thickness (STM) was analyzed immediately before the mesothelial cell layer to the fat tissue layer. The mean of this parameter at five randomly selected sites was used in the analysis. Fibrosis was defined as STM larger than 150 µm (Morelle *et al.*, 2018).

From our best knowledge, no method has been previously standardized for the evaluation of peritoneal vasculopathy. In the present study, vasculopathy was evaluated semi-quantitatively using a grading system based on vascular wall thickening and vascular lumen stenosis at the Packed Cell Volume (PCV) level.7 Blood vessels with the largest changes were evaluated on a 4-grade scale: grade 0 (no change), grade 1 (wall thickening and less than 50% luminal stenosis), grade 2 (wall thickening and more than 50% luminal stenosis) and grade 3 (complete obstruction)(Mizumasa *et al.*, 2013).

Inflammation was characterized as acute (defined as the presence of neutrophils, eosinophils, or mast cells) or as chronic (defined as increased numbers of lymphocytes or mononuclear cells).

The description of the unique characteristics of the peritoneal membrane before the start of PD were further scored considering STM, vasculopathy and inflammation: Score 0 represents no fibrosis, no vasculopathy, nor inflammation; Score 1: no fibrosis, but vasculopathy and/or inflammation; Score 2: fibrosis with/without vasculopathy and/or inflammatory changes.

3.2.5 Quantification of a blood panel of markers related to fibrosis and inflammation

To investigate if the biopsy score of the peritoneal membrane could be inferred by a unique signature of uremic toxins in the blood, a panel of serum proteins was analyzed by Enzyme-Linked Immunosorbent Assay (ELISA), including α-Klotho (Bionava assay), Galectin-3 and FGF21 (Fibroblast growth factor 21) (Quantikine ELISAS, R&D systems), FGF23 (Fibroblast growth factor 23 c-terminal ELISA, Immunotopics), TWEAK (Tumor necrosis factor-like weak inducer of apoptosis) (Preprotech), TNFα (Tumor necrosis factor alfa) (Preprotech) and hr-CRP (High-sensitivity C-reactive Protein) (protein assay using a Cobas c702 analyzer, Roche Diagnostics).

3.2.6 Statistical analyses

Statistical analyses were performed using the SPSS Software program. Categorical variables are presented as absolute (n) and relative frequencies (%), continuous normally distributed data are expressed as mean ± standard deviation (SD), and continuous non normally distributed data are expressed as median ± interquartil range (IQR). Normality of distributions was assessed using the Shapiro-Wilk test. The Mann–Whitney-U-test was used to compare unpaired data. Potential associations between categorical data were analyzed using the chi-square test. Logistic regressions were used to evaluate which parameters were independently associated with peritoneal fibrosis. Multivariate logistic regression analyses were performed using the method “enter” with those variables having a p-value < 0.1 after univariate logistic regressions. Multivariate analyses using Principal Component Analysis and Partial Least Square Discriminant Analysis were performed with SIMCA software (Umetrics, version 16). The dataset, containing the seven serum biomarkers, was centered and unit

variance scaled before multivariate analyses. A p-value less than 0.05 was considered statistically significant.

3.3 Results

3.3.1 Baseline characteristics of study population

This cohort consisted of 58 patients, aged 56±15 years old, of whom 31% were women. The glomerular filtration rate (GFR) was 6.45 (3.80-10.0) ml/min/1.73m2 and residual diuresis 1600 (1000-2400) mL/day. BMI was 24.80 (22.5-28.4) Kg/m2 and 29% were overweight.

Comorbidities were present in more than 85% of the individuals; arterial hypertension (55%), diabetes (29%) and obesity (24%) were the most common. Underlying renal disease included diabetic renal disease (24%), chronic glomerulonephritis (21%), hypertensive nephrosclerosis (12%), autosomal dominant polycystic kidney disease (9%) and chronic pyelonephritis (7%).

3.3.2 Biopsy score

No foreign material or granulomatous inflammation were identified in any of the biopsies examined. All patients were free of acellular sclerotic changes of the peritoneum. Mesothelial cells were retained in all cases. Representative images are presented in Figure 1. Surface mesothelium was absent in 8 of 58 biopsies (14%).

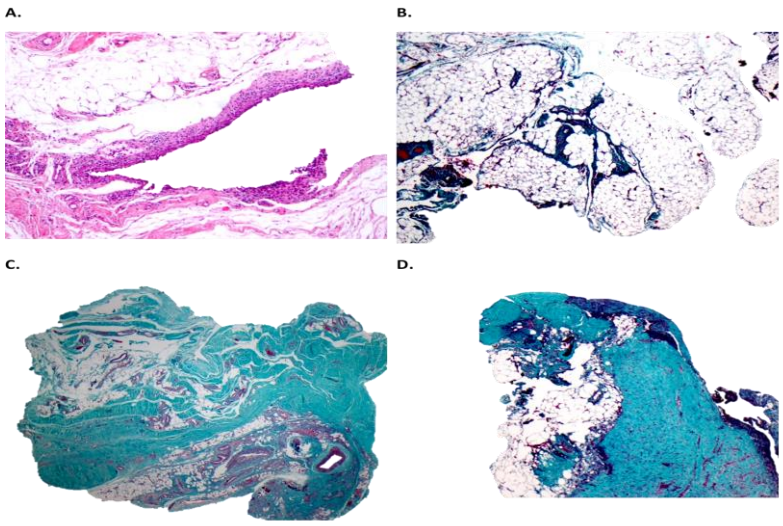


Figure 1. Panel of representative images of the peritoneal membrane biopsies. A. Hematoxylin & Eosin (H&E) – Medium amplification – Visceral peritoneum, showing submesothelial acute inflammation, in a patient with no prior history of abdominal disease. B. Masson's Trichrome (MT) – Low magnification – Obliterated vessels (vasculopathy – center) amidst sub-mesothelial adipose tissue. C. Masson's Trichrome (MT) – Low magnification – Subperitoneal and deep perivascular fibrosis. D. Masson's Trichrome (MT) – Low magnification – Marked sub-mesothelial fibrosis and underlying, hypovascularized, adipose tissue.

SMT was highly variable among patients (coefficient of variation of 72%) with a median of 90 (33.75-190.0) µm (Figure 2). Twenty-two patients (38%) presented a STM higher than 150 µm, fulfilling the definition of peritoneal fibrosis12. Diabetes mellitus prevalence was superior in the fibrosis group: 10/22 (45%) versus 7/36 (19%) (p=0.036). The number of metabolic comorbidities (among arterial hypertension, diabetes and/or obesity) was higher in the fibrosis group (1 comoborbidity (0-1.25) for non-fibrosis vs. 1.5 co-morbidities (1-2) for fibrosis, p=0.04. STM was not associated with glucose, insulin blood levels or HOMA-IR (Homeostatic Model Assessment for Insulin Resistance index).

Vasculopathy was rated as grade 0 in 30 (52%) cases, grade 1 in 22 (38%) cases and grade 2 in 6 (10%) cases. There was no biopsy with grade 3 vasculopathy. Diabetes mellitus prevalence was similar in the three groups (Grade 0 = 20%, Grade 1 = 36%, Grade 2 = 5%).

Inflammatory changes were present in 21 (36.2%) patients. In this group, 47.6% were diabetic. In fact, only 18% of diabetic patients had no inflammatory changes (p=0.023).

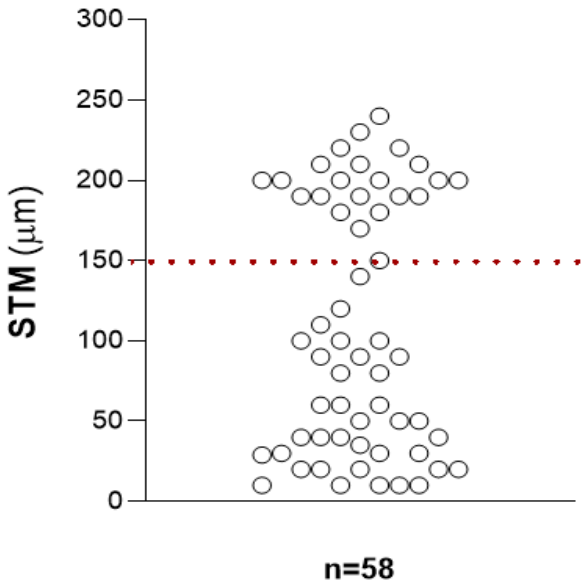


Figure 2. Interindividual variability among the 58 participants for submesothelial compact zone thickness (STM). Fibrosis is defined as an STM above 150 µm12.

In Table 1 is presented the patient distribution according to the biopsy score that combines STM, vasculopathy and inflammation. Differences among the three groups were observed for diabetes (p=0.034). Moreover, the proportion of patients with 2 or 3 metabolic comorbidities - among arterial hypertension, diabetes and/or obesity - was higher in those with S2 biopsy score: two patients (11%) in score 0; five patients (42%) in score 1 and nine (50%) patients in score 2 (p=0.036). Except for diabetes, the membrane status was not associate with easily available variables (age, sex, comorbidities, BMI, laboratorial data - Table 1). In multivariate analysis the association with the status of the membrane and diabetes was lost.

Table 1. Clinical and anthropometric data, comorbidities and etiology of kidney disease according to Biopsy Score.

Biopsy Score	S0 (n=24)	S1 (n=12)	S2 (n=22)	P
Male (n)	14	8	18	ns
Age (years) *Median (IQR)*	59 (43-68)	61 (35-69)	54 (44-72)	ns
BMI (kg/m²) *Median (IQR)*	23 (21-27)	26 (23-30)	25 (24-29)	ns
GFR mL/min/1.73m² *Median (IQR)*	6.2 (3.5-10)	5.8 (0.6-11)	7.2 (5.912.5)	ns
Residual Diuresis (L/day) *Median (IQR)*	1.7 (1.3-2.4)	1.4 (0.28-1.7)	1.7 (1.3-2.6)	ns
Glucose (mg/dL) *Median (IQR)*	107 (98-141)	102 (91-153)	120 (103-105)	ns
Insulin (µUI/mL) *Median (IQR)*	15.1 (8.1-26.6)	14.0 (6.6-19.6)	14.9 (9.7-29.7)	ns
HOMA-IR *Median (IQR)*	3.9 (1.9 - 9.3)	3.2 (1.7-4.6)	4.4 (2.6-9.3)	ns
Diabetes mellitus n (%)	4 (17%)	3 (25%)	10 (45%)	0.034
Arterial hypertension (n, %)	15 (63%)	6 (50%)	14 (64%)	ns
Obesity (n, %)	2 (8%)	4 (33%)	5 (24%)	ns
Diabetic nephropathy (n, %)	4 (29%)	3 (21%)	7 (50%)	ns
Chronic glomerulonephritis (n, %)	4 (17%)	1 (8%)	3 (14%)	ns
Hypertensive nephrosclerosis (n, %)	3 (43%)	0 (0%)	4 (57%)	ns

BMI – Body max index **GFR**- glomerular filtration rate, **IQR** interquartile range **ns –** non-significant *

3.3.3 Serum biomarkers

Both α-Klotho (Spearman r=-0.7491 p<0.001) and FGF21 (Spearman r=-0.5102, p<0.001) were negatively associated with STM.

The Table 2 presents the obtained concentrations of serum markers according to the biopsy score. α-Klotho discriminated the fibrosis group S2 from both groups without fibrosis (Figure 3).

Table 2. Serum biomarkers according to Biopsy Score.

Parameter	*Biopsy Score*			*p* value	CV (%)
	S0	S1	S2		
Biopsies (n)	24	12	22		
α-Klotho (pg/mL)	959.0 (670-1631)	864.9 (740-1178)	576.6 (430-719)	< 0.001 (S2 *vs.* S0) 0.002 (S2 *vs.* S1)	60
Galectin-3 (ng/mL)	9.51 (7.9-10.7)	10.68 (9.6-11.5)	9.87 (8.5-10.9)	ns	23
FGF21 (pg/mL)	1438 (976.3-3135)	1936 (1204-3270)	1133 (423.0-1661)	0.021 (S2 *vs* S1)	64
FGF23 (pg/mL)	668.9 (550.2-835.1)	822.1 (676.0-864.2)	791.2 (527.8-858.5)	ns	28
TWEAK (pg/mL)	125.5 (69.40-525.5)	149.0 (50.75-301.2)	105.1 (54.60-231.8)	ns	99
TNF-α (pg/mL)	169.0 (130.3-200.0)	190.0 (130.0-250.1)	170.0 (150.0-210.2)	ns	47
hs-CRP (µg/mL)	0.27 (0.16-0.54)	1.1 (0.36-1.5)	0.36 (0.2-0.51)	ns	103

Data is present as median (IQR) **biopsy score**: **S0 –** no fibrosis, no inflammation, no vasculopathy **S1** - no fibrosis, with inflammation and/or vasculopathy; **S2** fibrosis. **ns**-non significant. **CV –** coefficient of variation **FGF**-Fibroblast growth factor. **Hs-CRP** High sensitivity C reactive protein**. TNF-α** Tumor necrosis factor alfa **TWEAK** Tumor necrosis factor-like weak inducer of apoptosis.

To ascertain if the decreased α-Klotho in score S2 was related to diabetes, we compared α-Klotho concentrations between diabetic (722.0 (556.9-975.3 pg/mL) and non-diabetic (758.0 (630.0-1166) pg/mL) patients ($p>0.05$). Thus, the association of α-Klotho and fibrosis was independent of diabetes. In addition, patients with none or with only one metabolic comorbidity had similar α-Klotho levels than patients with 2 or 3 metabolic comorbidities (793.9 (611.7 - 1191) pg/mL vs 723.8 (571.7 - 957.3) pg/mL). α-Klotho was not associated with glucose or insulin levels, or with HOMA-IR index.

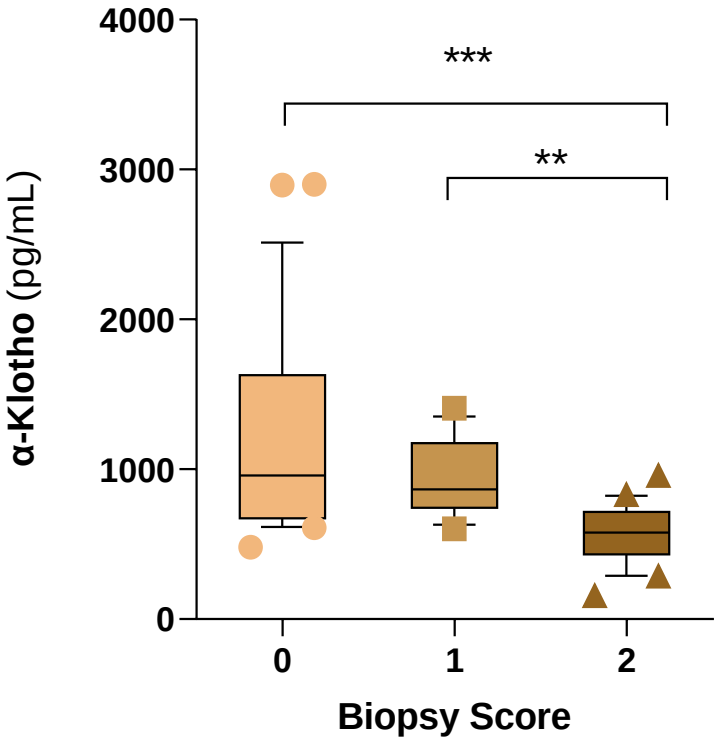


Figure 3. α-Klotho levels according to Biopsy Score.

α-Klotho was also among the serum biomarkers that were more variable among individuals (CV = 60%), different from Galectin-3 and FGF23 (CV = 23 and 28%, respectively).

FGF21 (CV = 64%) was positively associated with α-Klotho (Spearman r=0.4748, p=0.0017) and negatively associated with STM (Spearman r= -0.5102, p<0.001). FGF21 was higher in S1 than in S2 (Table 2) but did not distinguish S0 from S2. Different from α-Klotho, FGF21 levels were decreased in diabetes (1031.0 (548.5-1492.0) pg/mL vs 1580.0 (1109.0-2744.0) pg/mL, p=0.037). FG21 levels were not related to glucose, insulin or HOMA-IR index.

To ascertain if fibrosis could be inferred from a unique signature of the studied proteins, a Principal Component Analysis (PCA) was performed with the 7 serum biomarkers (α-Klotho, Galectin-3, FGF21, FGF23, TWEAK, TNFα, hr-CRP). The first two components of the model explained 44% of the variance of the data. Fibrosis had a clear influence

on the PCA (Figure 4A). The first component had the biggest influence on the separation of the samples according to fibrosis. Klotho was the parameter with the greatest weight on the first component. A PLS-DA model was further built to identify the most relevant parameters associated with fibrosis (R2(X)=0.212, R2(Y)=0.289, Q2=0.154, p=0.009).

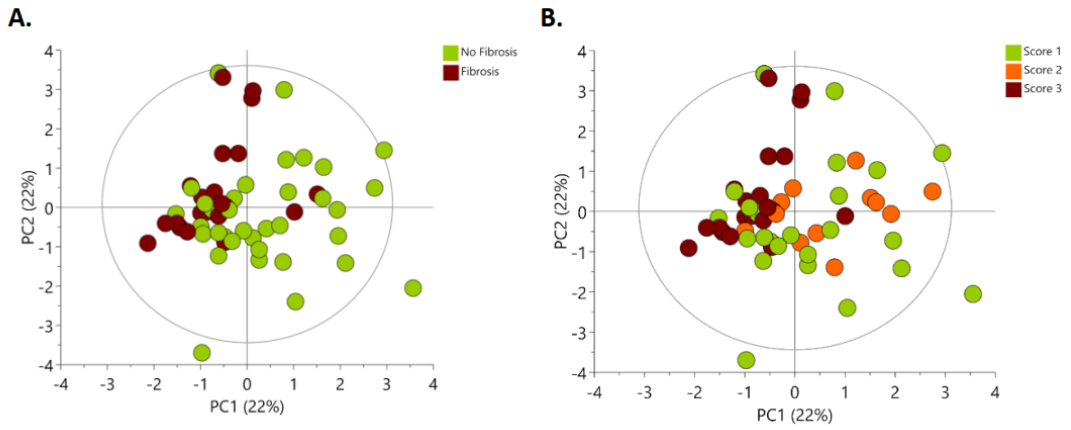


Figure 4. Score plot of the PCA model for serum biomarkers. Samples are colored according to fibrosis (**A**) or to Biopsy Score (**B**) that considered inflammation, vasculopathy and fibrosis.

According to the Variable Importance on the Projection (VIP) value and correlation coefficient (p(corr)), and confirming the PCA result, the most relevant blood marker was α-Klotho (VIP value >1.5 and p(corr)>|0.8|). Alternatively, a PLS model was built for the variable STM (R2(X)=0.212, R2(Y)=0.475, Q2=0.369, p=3.15817E-06). Again, the most important parameter associated with STM (VIP>1.5 and p(corr)>|0.8|) was α-Klotho. The same was observed for the biopsy analysis (Figure 4B PLS-DA model (R2(X)=0.273, R2(Y)=0.558, Q2=0.495). Neither vasculopathy, nor inflammation or diabetes influenced the distribution of the samples of the PCA.

To address confounding factors in the association between α-Klotho and fibrosis, logistic regressions were performed. α-Klotho (OR=0.991 (0.896 - 0.997), p=0.002) was related to peritoneal membrane fibrosis

(defined by STM >150 μm)17, independently of patients' sex, age, BMI, diabetes status and FGF21 levels.

3.4 Discussion

This work contributes to filling the gap in the current knowledge about the impact of uremia in the peritoneal membrane. It is clearly concluded that fibrosis is already present in the peritoneal membrane in pre-dialysis. Furthermore, the data supports α-Klotho as a precision tool in PD: a biopsy-free marker to assess the status of the peritoneal membrane.

We were not able to find literature about markers of the status of peritoneal membrane in incident PD patients. Therefore, we tried to provide evidence that uremia has different forms of presentation among patients, and we searched for a uremia-related tool to infer histological alterations.

While the integrity of the membrane has a central role for long-term success of PD3,4 the evidence on the membrane status before PD, as herein provided, can barely be found in literature (Dobbie, Lloyd and Gall, 1990; Honda *et al.*, 2008; Mizumasa *et al.*, 2013). On the other hand, these are stage 5 chronic kidney disease (CKD) patients, i.e. uremic patients. Classically, uremia was seen as an accumulation of toxins due to impaired renal excretory function. A more integrated and comprehensive view of uremia is gaining ground, agreeing that each molecule which is disrupted in uremia might exacerbate on-target effects, lose selectivity for its targets, present off-target effects, regulate its own levels or the levels of other molecules, which might have either offensive or protective roles (Moe, 2012). In fact, some molecules increased in uremia might also be adaptive rather than pathophysiologic (Moe, 2012; Castillo-Rodríguez *et al.*, 2017). Thus, the net effect of this complex state with disruption of the levels of signaling, metabolic or inflammatory molecules (Elewa *et al.*, 2012; Moe, 2012; Kunin *et al.*, 2015; Castillo-Rodríguez *et al.*, 2017; Balzer, 2020; Ebert *et*

al., 2020), together with the lifestyle and clinical features, will define the phenotype of uremia of the individual.

STM showed a clear bimodal distribution (fibrosis, no fibrosis, Figure 2). This means the existence of fibrosis prior to start of the PD for some patients, which was unrelated to the consensual iatrogenic nature of this technique. While the underlying mechanisms are not known, in our hypothesis “uremia” is an umbrella for different blood fingerprints which can have different impacts on the peritoneal membrane.

This study is a pioneer in linking α-Klotho, uremia and the integrity of peritoneal membrane. α-Klotho was demonstrated to be a molecule in uremic profile associated with membrane integrity. If α-Klotho has a role as a biomarker and/or a pathophysiologic role needs further investigation. For instance, α-Klotho has antiaging properties and is a protective factor for aging-related diseases, such as arterial hypertension and CKD. The pleiotropic functions of this molecule include anti-inflammatory role and inhibition of apoptosis, fibrosis, and cell senescence(Takenaka *et al.*, 2019).

In addition, α-Klotho differentiated the membranes without fibrosis, even in the presence of inflammation or vasculopathy. The biopsy scores S0-S1 (non-fibrosis) (Table 2) presented much higher α-Klotho levels than the described for healthy volunteers, 472 pg/mL (204-741)25 and therefore patients present uremic levels for this molecule.

There was a negative relation between α-Klotho and STM. This is suggestive that non-fibrotic membranes are present in patients with higher α-Klotho. However, patients with biopsy scores S2 presented the lower α-Klotho values, which were near those described for healthy controls (Table 2). This might support the idea that higher α-Klotho levels in CKD are adaptive and allow membrane protection. Likewise, patients with lower α-Klotho levels in their uremic profile miss these adaptive mechanisms. In alternative, α-Klotho is a retention molecule in CKD, but some uremic profiles are disrupting α-Klotho availability.

For instance, the TNFα, a cytokine associated with inflammation in chronic renal disease might decrease the expression of α-Klotho (Moreno *et al.*, 2011). We found no relation between α-Klotho and TNFα, but these complex interactions among uremic molecules are difficult to ascertain individually. Like TNFα, other cytokines might have changed circulating levels in these patients, who suffer from chronic inflammation because of impaired renal function and accumulation of uremic toxins (Castillo-Rodríguez *et al.*, 2017) For example, the v26 impairs the renal expression of α-Klotho through Nuclear factor-κB (NFκB) activation.

Another possibility is that a lower α-Klotho, which is a co-receptor for FGF23, facilitates off-target effects of FGF2322. In fact, increased FGF23 and decreased α-Klotho have been implicated in cardiac remodeling and progression of uremic cardiomyopathy (Grabner and Faul, 2016). Like FGF23, Galectin-328 have also been associated with fibrosis, but none of the molecules herein investigated were studied in peritoneal membrane context.

The deposition of plasma containing fibrin and profibrotic factors in diabetes promote submesothelial connective thickening and diabetes has been indicated as a risk factor for fibrosis of the peritoneal membrane (Mizumasa *et al.*, 2013). We found that diabetic patients have similar α-Klotho and decreased FGF21 concentrations in comparison to non-diabetics. FGF21 has been described as an anti-diabetes and anti-fibrotic hormone (Salgado, Goes and Salgado Filho, 2021) and it was positively associated with α-Klotho in our study. However, multivariate models showed the superiority of α-Klotho to discriminate fibrosis. We found no association between diabetes and α-Klotho, highlighting that this molecule is an independent marker of fibrosis and diabetes a risk factor.

A putative mechanistic rationale for our clinical findings was recently obtained in an animal model of PD (Kadoya *et al.*, 2020). This work (Kadoya *et al.*, 2020) showed the attenuation of peritoneal fibrosis by α-

Klotho, through the inactivation of Wnt/β-catenin signaling pathway. The authors pointed out the recombinant α-Klotho as a potential therapeutic target in peritoneal fibrosis associated to PD, which is somehow supported by our data.

Finally, our work would benefit from the inclusion of a control group with normal renal function to support the link of fibrosis with uremia. However, that is difficult to achieve due to ethical reasons. Likewise, the cross-sectional nature and sample dimension are inherent limitations to be considered, but the authors would like to highlight that this is a proof-of-concept mechanist-driven study that brings novel perspectives for research in the PD field. In the future, longitudinal studies in the same group of patients would be mandatory to prove the expected high relevance of our findings and evaluate if alpha-klotho and interventions that modify its levels have benefits in the clinical practice and outcomes of PD.

CHAPTER IV

FIBROSIS OF PERITONEAL MEMBRANE, MOLECULAR INDICATORS OF AGING AND FRAILTY UNVEIL VULNERABLE PATIENTS IN LONG-TERM PERITONEAL DIALYSIS

CHAPTER IV

4 FIBROSIS OF PERITONEAL MEMBRANE, MOLECULAR INDICATORS OF AGING AND FRAILTY UNVEIL VULNERABLE PATIENTS IN LONG-TERM PERITONEAL DIALYSIS

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Acknowledgements

The authors would like to thank Lilia Oliveira (Clinical Pathology laboratory, CHLO) and Leonor Jacinto (Pathology Department, CHLO) for providing and to the nurses at the peritoneal dialysis team at HSC/CHLO, Sara Pereira e Elisabete Costa, for their laboratory technical assistance and logistical support.

Sociedade Portuguesa de Nefrologia (SPN) SPN funded a project and Ana Rita Martins, MD, Nephrology fellow, for a residence at Jiménez Díaz Foundation University Hospital, Madrid under the scope of novel serum biomarkers of CKD.

Abstract

Peritoneal membrane status, clinical data and aging-related molecules were investigated as predictors of long-term peritoneal dialysis (PD) outcomes. A 5-year prospective study was conducted with the following endpoints: (a) PD failure and time until PD failure, (b) major cardiovascular event (MACE) and time until MACE. A total of 58 incident patients with peritoneal biopsy at study baseline were included. Peritoneal membrane histomorphology and aging-related indicators were assessed before the start of PD and investigated as predictors of study endpoints. Fibrosis of the peritoneal membrane was associated with MACE occurrence and earlier MACE, but not with the patient or membrane survival. Serum α-Klotho bellow 742 pg/mL was related to the submesothelial thickness of the peritoneal membrane. This cutoff stratified the patients according to the risk of MACE and time until MACE. Uremic levels of galectin-3 were associated with PD failure and time until PD failure. This work unveils peritoneal membrane fibrosis as a window to the vulnerability of the cardiovascular system, whose mechanisms and links to biological aging need to be better investigated. Galectin-3 and α-Klotho are putative tools to tailor patient management in this home-based renal replacement therapy.

Keywords: Klotho, galectin-3, uremic toxins, cardiovascular toxicity, chronic kidney disease

4.1 Introduction

Peritoneal dialysis (PD) is a home-based modality of renal replacement therapy and a good option for patients with chronic kidney disease (CKD). Independently of the chronological age, this population commonly presents an accelerated aging process affecting skeletal, immune, renal, and cardiovascular systems (Dai *et al.*, 2019). Therefore, the risk of mortality in CKD patients is increased by 10- to 20-fold in comparison to individuals with normal renal function (Lanzani, Citterio and Vezzoli, 2020). Moreover, cardiovascular toxicity caused by uremia represents a major factor for the increased mortality in dialysis programs (Lozier *et al.*, 2019).

The dialytic capacity of the peritoneal membrane is pivotal in PD, but the integrity of the membrane in the uremic patient might have been overlooked. The existence of a high person-to-person variability in the status of the membrane before the start of PD was recently reported and related to the anti-aging molecule α-Klotho (Branco *et al.*, 2023).

Deficiency of α-Klotho is well known to be involved in damage of the cardiovascular system, atherosclerosis, skin atrophy and osteoporosis, traits commonly associated with human aging (Kuro-o, 2019; Bi *et al.*, 2020). Those traits also overlap with the manifestations of CKD (Kadoya *et al.*, 2020), suggesting that α-Klotho might be an important player in PD outcomes. α-Klotho is changed in uremia (Bao *et al.*, 2020), which is recognized as a disbalance between protective and harmful molecules (Zoccali *et al.*, 2017). In uremia, the concentrations of proteins associated with mechanisms underlying early aging can be affected, such as those related to inflammation and fibrosis in multiple organs/tissues (Roh *et al.*, 2021).

In fact, α-Klotho was shown to be a uremic molecule implicated in the vulnerability of the peritoneal membrane, expressed as submesothelial fibrosis (Branco *et al.*, 2023). As more vulnerable peritoneal membranes were associated with low circulating α-Klotho, we herein hypothesized

that α-Klotho might represent a multifaceted marker of both the survival of the membrane and the survival of the patient. Therefore, we conducted a prospective longitudinal observational study in a cohort of incident PD patients to investigate the impact of uremic toxins related to aging, peritoneal membrane status and the patient's frailty in long-term PD outcomes.

4.2 Methods

4.2.1 Study design and participants

This was a single center, prospective study with 60 months of follow-up that included incident patients at the PD Unit of Santa Cruz Hospital, Centro Hospitalar de Lisboa Ocidental, Portugal. The study was approved by the Ethics Committee of the NOVA Medical school, Faculdade de Ciências Médicas, NOVA University of Lisbon (Approval number 50/2019). The study was conducted according to the Declaration of Helsinki and Good Clinical Practices and complied with the European Union GDPR Legislation.

At the enrollment, patients were referred from the Nephrology consultation inside or outside the hospital for an information consultation. Patients were enrolled in a consecutive manner. The main purposes of the consultation were to assess the eligibility criteria for the renal substitution technique and to provide information to allow an informed choice. This consultation included a multidisciplinary team composed of a doctor, nurse, nutritionist, and social worker. Inclusion criteria were being over 18 years of age and having a stable clinical condition, defined by the absence of serious abdominal infections (diverticulitis, pancreatitis and cholecystitis) or active neoplasia, and on PD with biopsy of the peritoneal membrane. The exclusion criteria were having had previous aggressions to the peritoneal membrane (such as surgeries or peritonitis).

Non-autonomous patients were included for assisted PD whenever there was a caretaker. All patients signed informed consent.

4.2.2 PD prescription

PD was started within 30 (21–44) days after the implantation of the catheter. All patients started on continuous ambulatory peritoneal dialysis (CAPD) and after the first year, 30% of patients switched to automated peritoneal dialysis (APD) and remained stable over the observation period. All patients were treated with dialysis solutions with a reduced content of glucose degradation products and a normal pH (Baxter®, Deerfield, MA, USA and Fresenius®, Bad Homburg, Germany). At baseline, no hypertonic solutions were used and a total of 38% of the patients received polyglucose. The major reasons to include polyglucose in the prescription was hydration status (41%), diabetes and the presence of basal peritoneal membrane fibrosis (30%). Prescriptions of amino-acid-containing solutions for PD were exclusive for diabetic patients. The daily quantity of administered glucose was maintained for CAPD, but increased in APD patients over time to achieve adequate ultrafiltration and fluid balance.

4.2.3 Baseline variables

The following variables were assessed at study baseline

a) *Anthropometric, clinical and therapeutic variables*
b) *pre-PD histomorphology score* (Biopsy score)
c) *Dialysis Efficacy*. The dialysis efficacy was defined by the calculation of the urea clearance index (Kt/Vurea). This index considers the concentration of urea in blood, in the urine (renal clearance) and dialysate (peritoneal clearance) and the body surface. This was obtained from the collection of the PD effluent and of the patient´s urine for 24 h, prior to the scheduled visit at the PD unit. Weekly Kt/V

values were calculated according to the recommendations of the kidney disease outcomes quality initiative ('II. NKF-K/DOQI Clinical Practice Guidelines for Peritoneal Dialysis Adequacy: Update 2000', 2001) (DOQI). According to K/DOQI guidelines, the cut-off value of Kt/V ≥1.7 was set to define Dialysis Efficacy (Blake *et al.*, 2011).

d) *Profile of Peritoneal Transport*. The category of Peritoneal Membrane Transport was determined using the peritoneal equilibration test (PET). The PET and dialysis efficacy were evaluated at the same time. In the night before the PET test, at home, a long overnight dwell of PD was performed using a 1.36% glucose dialysis fluid (isotonic). The next morning at the Hospital, the PETs were performed using a 2 L dialysis solution with 3.86% or 4.25% glucose (hypertonic). The dialysate/plasma (D/P) creatinine ratio was measured and used to identify the patients as low (D/P creatinine 0.34-0.50, L), low average (D/P creatinine 0.50 to 0.65, LA), high average (D/P creatinine 0.65 to 0.80, HA) or high (D/P creatinine 0.81-1.03, H) transporters, according to previously defined (Twardowski Z, 1987).

e) *Fluid removal by the peritoneal membrane*. The permeability of the membrane to fluid is defined by the ultrafiltration test, which compares the amount of drained dialysate with the 2L of dialysis fluid instilled at start of the test. *Ultrafiltration failure* is defined as failure when the target of at least 400 mL of net ultrafiltration during a 4-hours period of PD using 3.86% or 4.25% glucose solutions is not achieved (in absence of catheter malposition or mechanical dysfunction).

f) *Residual renal function* (RRF). The RRF was obtained through the creatinine clearance, which was calculated by collecting 24 h urine before blood sampling and using conventional formulas and correcting the result for a body surface area of 1.73 m^2/Kg

g) *Daily protein intake*. Nutritional status is an important adequacy parameter in patients on dialysis. The normalized protein catabolic rate (nPCR) was calculated from the urea eliminated in urine and in

dialysate and normalized to body weight. The recommended standard value of this parameter is ≥ 1 g/Kg/day.

h) *Effluent CA125 levels*. Effluent levels were measured using an electrochemiluminescence (Elecsys, Roche diagnostics).

i) *Levels of serum biomarkers*. A panel of proteins related to aging and fibrosis was quantified by Enzyme-Linked Immunosorbent Assay (ELISA) at the baseline, ie. before the start of PD, which consisted of α-Klotho (Bionava assay), Galectin-3 and FGF21 (Fibroblast growth factor 21) (Quantikine ELISAS, R&D systems), FGF23 (FGF23 c-terminal ELISA, Immunotopics), Tweak (Tumor necrosis factor-like weak inducer of apoptosis, Preprotech), TNFα (Tumor necrosis factor alfa, Preprotech) and hr-CRP (ultra-sensitive C-reactive protein assay using a Cobas c702 analyzer, Roche Diagnostics).

j) *Frailty assessment*. The Edmonton Frailty scale FS was selected as a simple assessment tool comprising eleven items focusing on different frailty dimensions (Rolfson *et al.*, 2006).

Peritoneal and renal Kt/V urea and creatinine clearances, glomerular filtration rate (GFR), body surface area (BSA), and protein catabolic rate were calculated using Patient onLine (POL) software version 6.3 (Fresenius®, Bad Homburg, Germany). These variables were investigated as factors with impact on PD outcomes. All patients were followed up until death, PD drop-out, or 30 June 2019.

4.2.4 Study outcomes

The primary outcomes were PD related Outcomes:

- **PD Technique failure** was referred to ultrafiltration failure, peritonitis, or dialysis inefficacy. Patients were considered with no technical failure when achieving 60 months of follow-up.
- **Time for Technique failure** is the time on PD of each patient in the study until technical failure. Participants dropping-out PD for

reasons other than technical failure (switching to hemodialysis by option, kidney transplantation, transference to other PD centers or loss to follow-up) were censored.

The secondary outcomes were Cardiovascular Outcomes:

a) all-cause mortality

b) Major Cardiovascular event

- **To have a Major Cardiovascular event *(*MACE)** after 3 months on PD. MACEs were defined according to validated clinical criteria and included coronary heart disease (CHD), congestive heart failure (HF), acute myocardial infarction (AMI), and acute cerebral infarction (ACI) and cardiac death caused by AMI, arrhythmias or HF. CHD was defined as ≥50% diameter stenosis of coronary arteries by either coronary angiography or CT angiography (Fihn *et al.*, 2014). HF was diagnosed according to ESC Guidelines for the diagnosis and treatment of chronic heart failure (McDonagh *et al.*, 2018). AMI was diagnosed according to ESC Guidelines for the management of acute coronary syndromes (Collet *et al.*, 2021). ACI was defined as an acute neurological event lasting more than 24 h associated with the clinical evidence of ischemic focus of the brain (Smith, English and Jonhston, 2012). Cardiac death was defined as death caused by AMI, arrhythmias, or CHF.
- **Time for MACE**, which was defined for each patient as the time in the study until a MACE. Censored data defined for those dropping out the study without MACE or those achieving the end of the study without MACE.

4.2.5 Statistical analyses

Categorical variables are presented as absolute (n) and relative frequencies (%); continuous non-normally distributed data are expressed as median (interquartile range). The Kruskal–Wallis test was used to assess differences between three or more independent groups.

The Mann–Whitney U-test was used to assess differences between two independent groups. Potential associations between categorical data were analyzed using the Chi-Squared test. ROC curves were also used to identify cut-offs for potential blood biomarkers. Multiple Cox proportional hazards regression models were performed to assess potential predictors of survival, technique survival and the time to the occurrence of a cardiovascular event. The proportional hazards assumption was assessed through the Schoenfeld residual plots. All models were fit using the 'survival'R package (Therneau and Grambsch, 2000; Therneau *et al.*, 2022).

The optimal cutpoints were obtained through the maximally selected rank statistics method [see (Hothorn and Lausen, 2003) for more details], using the '*maxstat*' R package (Hothorn, 2017), and considering the time until PD failure with the censor variable, indicating whether the patient suffered a technique failure or not.

Survival curves were generated using the Kaplan–Meier technique and tested using the log-rank test.

A confidence level alpha of 0.05 was considered throughout the study. Statistical analyses were performed with the R software, R version 4.2.0 and SPSS.

4.3 Results

4.3.1 Baseline characterization of study population

This observational prospective cohort study included 58 patients, followed for 60 months. A total of 31% were female. At baseline, patients were 56 (30–79) years old with a median renal residual function assessed by rGFR of 7 (4–10) mL/min/1.73 m^2. The underlying renal diseases were diabetic renal disease (20%), chronic glomerulonephritis (20%), hypertensive nephrosclerosis (23%), autosomal dominant polycystic kidney disease (11%) and chronic pyelonephritis (10%).

Twenty-two patients had fibrosis of the peritoneal membrane at the baseline of the study.

Concerning dialysis parameters, 2 and 32 patients were fast and average-fast transporters, respectively, and 94% of patients had good efficacy of dialysis.

Regarding therapeutics, patients with atherosclerosis artery diseases (40%) were treated with the highest tolerated dose of statins and antiplatelet therapy. In addition, all patients were on inhibitors of renin–angiotensin axis (IECA or ARA) and diuretic therapy. Eighteen patients (31%) were on spironolactone, which was mainly added in those with fibrosis of the peritoneal membrane before the start of PD. A total of 12 patients were on beta-blockers and 30% were on other antihypertensive drugs. The number of patients in treatment for mineral bone disease was low.

The normalized protein catabolic rate (nPCR) was 0.99 (0.79–1.09) g/Kg/day and 47% had proper nutrition. A total of 10 patients were vulnerable and 5 were frail according to the Edmonton scale.

The baseline variables of the study were analyzed according to the biopsy score of the membrane (Table 1), which considers submesothelial compact zone thickness (STM), vasculopathy and inflammation [4]: Score 0 represents no fibrosis, no vasculopathy, nor inflammation; Score 1: no fibrosis, but vasculopathy and/or inflammation; Score 2: fibrosis with/without vasculopathy and/or inflammatory changes.

Overall, at baseline, patients with membrane fibrosis received more spironolactone, antiplatelet and statins therapy. In the S2 group (fibrosis), more than half of the patients had peripheral arterial disease (PAD). While the biopsy score was not related to the age of the patients, the cutoff for the level of circulating α-Klotho (anti-aging molecule) that discriminated the existence of peritoneal membrane fibrosis before the start of PD was defined by performing a ROC curve (AUC = 0.860, p = 4

× 10^{-6}). This cutoff was established at 742 pg/mL, with 83% sensitivity (to detect fibrosis) and 71% specificity (to detect no fibrosis).

Table 1. Baseline variables of the study according to the biopsy score of the peritoneal membrane.

	Study Variable	Biopsy Score			
		S0 (n = 24)	S1 (n = 12)	S2 (n = 22)	*p*
Anthropometric & Clinical data	Women, n (%)	10 (42)	4 (33)	4 (18)	ns
	Age (years old)	59 (43–68)	61 (35–69)	54 (45–72)	ns
	Diabetes mellitus, n (%)	4 (17)	3 (25)	10 (46)	ns
	Arterial hypertension, n (%)	15 (63)	6 (50)	14 (74)	ns
	Coronaryischemic disease, n (%)	4 (17)	4 (33)	9 (43)	ns
	Cerebrovasculardisease, n (%)	2 (8)	1 (8)	1 (5)	ns
	Cardiac failure, n (%)	2 (8)	1 (8)	3 (14)	ns
	Peripheralarterialdisease, n (%)	3 (13)	2 (17)	12 (55)	0.004
PD Prescription & Therapeutics	Icodextrin, n (%)	6 (25)	5 (42)	8 (36)	ns
	Use of amino acid solution, n (%)	0 (0)	2 (17)	1 (5)	ns
	Glucose applied (g/day)	120 (114–137)	120 (98–120)	120 (90–120)	ns
	Spironolactone, n (%)	2 (8)	1 (8)	15 (68)	<0.001
	Beta-blockers, n (%)	2 (8)	3 (25)	7 (32)	ns
	Other antihypertensives, n (%)	13 (54)	7 (58)	10 (46)	ns
	Vitamin D analogues, n (%)	15 (63)	10 (83)	16 (73)	ns
	non-calcium Phosphate binders, n (%)	3 (13)	3 (25)	6 (27)	ns
	Vitamin D3 supplements, n (%)	3 (13)	3 (13)	5 (23)	ns
	Cinacalcet, n (%)	6 (25)	3 (25)	6 (27)	ns
	Antiplatelettherapy, n (%)	5 (21)	4 (33)	14 (64)	0.011
	Statins, n (%)	5 (21)	4 (33)	14 (64)	0.011
	Erythropoietin/darbepoetin, n (%)	13 (54)	5 (42)	16 (73)	ns
PD-parameters, Nutrition Status & Frailty	Peritoneal transport (H, HA, L, LA), n (%)	1/14/6/3 (4/58/25/12)	0/7/4/1 (0/58/33/8)	1/11/9/1 (5/50/41/5)	ns
	CA 125 (UI/L)	25.7 (10.8–33.0)	16.5 (11.0–37.3)	16.5 (11.7–23.9)	ns
	nPCR (g/Kg/day)	1.0 (0.76–1.1)	0.89 (0.79–1.1)	0.99 (0.79–1.1)	ns
	Kt/V	2.6 (2.1–3.2)	2.7 (1.8–3.2)	2.7(2.0–3.3)	ns
	rGFR (mL/min/1.73m²)	6.2 (3.6–9.8)	5.9 (1.1–10.7)	7.9 (5.9–11.2)	ns
	Residual Diuresis (mL)	1700 (1.25–2350)	1350 (280–1650)	1700 (1300–2600)	ns
	Non-Frail, n (%)	18 (75)	9 (75)	15 (68)	ns

Data are presented as median (IQR) or n (%). Biopsy score: S0—no fibrosis, no inflammation, no vasculopathy; S1—no fibrosis, with inflammation and/or vasculopathy; S2—fibrosis; PD—peritoneal dialysis; n—number of individuals; ns—non-significant; H—High, HA—high average, L—low, LA—low average; nPCR—normalized protein catabolic rate; Kt/V—Dialysis efficacy index; rGFR—residual glomerular filtration rate; CA125—cancer antigen 125.

4.3.2 Impact of the status of the peritoneal membrane and age-related indicators in PD-related outcomes

Regarding the long-term outcomes of the study, the minimum time on PD was 13 months and the median time was 42 (30–58) months. Technical failure during the follow-up period occurred in 41% of patients, with a median time until failure of 40 (26–56) months.

A total of 27 patients (47%) had a MACE during the study, with a minimum time for MACE of 8 months and a median time of 17 (12–31) months.

Next, we investigated the relation of study outcomes with the status of the membrane (biopsy score, STM, α-Klotho levels with a 742 pg/mL cutoff as a surrogate of fibrosis) and the age-related baseline indicators (age, serum biomarkers, frailty).

4.3.3 Status of peritoneal membrane, age-related indicators and technical failure of PD

Contrary to our initial hypothesis, the status of the membrane was not associated with technical failure (Table 2). Overall, the patients with PD failure, compared to those without, were older, had higher frailty scores, were more likely to be on calcium channel blockers (Table 2) and presented higher circulating galectin-3 at the study baseline. The use of icodextrin solutions, glucose applied, or diabetes were not associated with failure (Table 2) or time to PD failure (Table 3).

In addition, the galectin-3 was also related to the time until PD failure (Table 3). A cut-off of galectin-3 to discriminate PD failure was established at 8.88 ng/mL (sensitivity = 92% and specificity of 46%), which was also associated with the survival of the peritoneal membrane (Figure 1A). This cut-off was independently associated with PD failure in an adjusted model to age, PAD, and calcium channel blockers (CCB) (Figure 1B), wherein age, frailty score and icodextrin did not account for the prediction of time to PD failure.

Table 2. Baseline variables according to technical failure or MACE.

	Study Variable	PD Failure			MACE		
		No (n = 34)	Yes (n = 24)	*p*	No (n = 31)	Yes (n = 27)	*p*
Anthropometric and Clinical data	Women	10 (29)	8 (33)	ns	12 (39)	6 (22)	ns
	Age (years old)	51.0 (37.8–65.5)	65.0 (53.5–71.8)	0.006	51.0 (37.0–63.0)	67.0 (52.0–72.0)	0.003
	Diabetes mellitus	10 (29)	7 (29)	ns	3 (10)	14 (52)	<0.001
	Arterial hypertension	21 (66)	14 (61)	ns	19 (63)	16 (64)	ns
	Coronary ischemic disease	10 (30)	7 (29)	ns	0 (0)	17 (65)	<0.001
	Cerebrovascular disease	3 (9)	1 (4)	ns	0 (0)	4 (15)	0.038
	Cardiac failure	3 (9)	3 (12)	ns	0 (0)	6 (23)	0.006
	Peripheral arterial disease	7 (21)	10 (42)	ns	0 (0)	17 (63)	<0.001
	rGFR (mL/min/1.73 m^2)	7.9 (5.6–10.2)	6.1 (2.6–10.3)	ns	8.9 (5.3–10.4)	6.0 (2.6–9.3)	ns
	Residual Diuresis (mL)	1700 (1225–2350)	1500 (600–2500)	ns	1700 (1300–2400)	1500 (1000–2350)	ns
PD and Therapeutics Prescription	Icodextrin	14 (41)	5 (21)	ns	10 (32)	9 (33)	ns
	Use of amino acid solution	2 (6)	1 (4)	ns	1 (3)	2 (7)	ns
	Glucose applied	120.0 (90.0–120.0)	120.0 (120.0–143.8)	ns	120.0 (90.0–135.0)	120.0 (90.0–120.0)	ns
	Spironolactone	9 (27)	9 (38)	ns	8 (26)	10 (37)	ns
	Beta–blockers	7 (21)	5 (21)	ns	2 (6)	10 (37)	0.004
	Calcium channels blockers	12 (35)	18 (75)	0.003	16 (52)	14 (52)	ns
	Vitamin D analogues	26 (77)	18 (75)	ns	23 (74)	21 (78)	ns
	Non–calcium Phosphate binders	9 (27)	11 (46)	ns	9 (29)	11 (41)	ns
	Vitamin D3 supplements	24 (71)	17 (71)	ns	20 (64)	21 (78)	ns
	Cinacalcet	8 (24)	7 (29)	ns	9 (29)	6 (22)	ns
	Antiplatelet therapy	10 (30)	8 (33)	ns	0 (0)	18 (69)	<0.001
	Statins	10 (30)	8 (33)	ns	0 (0)	18 (69)	<0.001
	Erythropoietin/darbepoetin	17 (50)	17 (71)	ns	15 (48)	19 (70)	ns
PD-related parameters, Frailty	Peritoneal transport (High)	20 (59)	14 (58)	ns	19 (61)	15 (56)	ns
	CA 125 (UI/L)	14.3 (9.8–25.0)	25.2 (13.5–36.5)	ns	15.6 (10.5–32.3)	16.9 (12.0–36.9)	ns
	nPCR (g/Kg/day)	1.0 (0.81–1.1)	0.87 (0.77–1.1)	ns	1.1 (0.88–1.2)	0.83 (0.78–1.0)	0.023
	Kt/V	2.9 (2.1–3.3)	2.4 (1.9–2.9)	ns	2.9 (2.4–3.4)	2.4 (1.8–3.1)	0.017
	Frail	4 (12)	7 (29)	ns	3 (10)	8 (30)	ns
	Score de Edmonton	2 (2–3)	3.5 (2–6)	0.038	2 (2–3)	4 (2–6)	<0.001
Membrane Fibrosis and Serum biomarkers	α–Klotho (pg/mL)	779 (589–1016)	724 (626–1090)	ns	803 (640–1119)	698 (548–958)	ns
	Galectin–3 (ng/mL)	9.4 (7.50–10.9)	10.4 (9.2–11.0)	0.048	9.9 (8.6–10.8)	10.2 (8.6–11.1)	ns
	FGF21(pg/mL)	1324 (834–2226)	1435 (980–3135)	ns	1410 (967–2061)	1336 (921–2507)	ns
	FGF23 (pg/mL)	748.9 (525.9–862.4)	744.2 (649.2–848.6)	ns	685.9 (578.3–858.8)	761.9 (570.9–850.5)	ns
	TWEAK (pg/mL)	0.14 (0.06–0.49]	0.12 (0.05–0.28)	ns	0.11 (0.06–0.48)	0.16 (0.06–0.27)	ns
	TNF–α (pg/mL)	0.18 (0.13–0.24)	0.16 (0.14–0.20)	ns	0.17 (0.12–0.19)	0.17 (0.15–0.25)	ns
	hs–CRP (µg/mL)	0.35 (0.17–0.48)	0.39 (0.17–0.90)	ns	0.39 (0.16–0.54)	0.35 (0.19–0.63)	ns
	Peritoneal membrane fibrosis	13 (38)	9 (38)	ns	5 (16)	17 (63)	<0.001
	STM	95.0 (40.0–190.0)	60.0 (20.0–200.0)	ns	60.0 (30.0–110.0)	190.0 (50.0–200.0)	0.004
	α–Klotho < 742 pg/mL	16 (47)	13 (54)	ns	12 (39)	17 (63)	ns

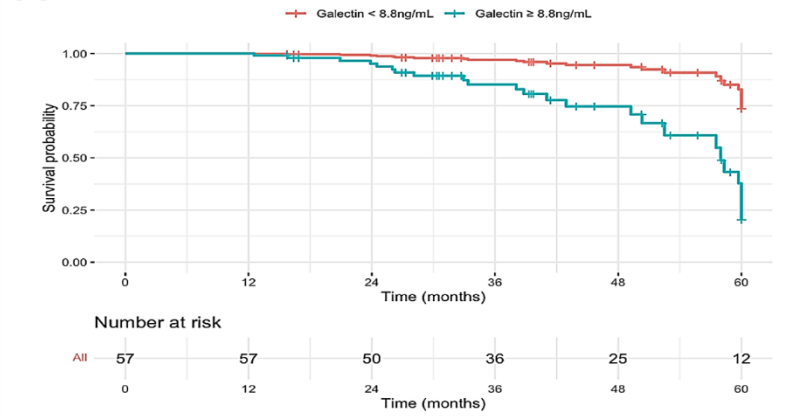
Data are present as median (IQR) or n (%). PD—peritoneal dialysis; n—number of individuals; ns—non-significant; nPCR—normalized protein catabolic rate; Kt/V—Dialysis efficacy index; rGFR—residual glomerular filtration rate; CA125—cancer antigen 125; FGF21—Fibroblast growth factor 21; FGF23—Fibroblast growth factor 23 c-terminal; TWEAK—Tumor necrosis factor-like weak inducer of apoptosis; TNFα—Tumor necrosis factor α; hs-CRP—ultra-sensitive C-reactive protein; STM—submesothelial compact zone thickness.

Table 3. Baseline variables as predictors of 5 years peritoneal dialysis outcomes

	Study Variables	Time until Event (Month) HR (95% CI), *p* Value	
		PD Failure	MACE
Anthropometric and Clinical data	Women	ns	ns
	Age (years old)	$p = 0.08$	1.044 (1.012–1.077), $p = 0.04$
	Diabetes mellitus	ns	3.717 (1.732–7.978), $p = 0.01$
	Arterial hypertension	ns	ns
	Coronary ischemic disease	ns	10.063 (4.239–23.894), $p = 0.001$
	Cerebrovascular disease	ns	4.206 (1.409–12.582), $p = 0.27$
	Cardiac failure	ns	$p = 0.059$
	Peripheral arterial disease	2.432 (1.066–5.552), $p = 0.035$	8.875 (3.890, 20.248), $p = 0.001$
	GFRr (mL/min/1.73 m^2)	ns	$p = 0.110$
	Residual Diuresis (mL)	ns	ns
PD and Therapeutics Prescription	Icodextrin	$p = 0.099$	ns
	Use of amino acid solution	ns	ns
	Glucose applied	ns	ns
	Spironolactone	ns	ns
	Beta-blockers	ns	3.518 (1.579–7.838), $p = 0.001$
	Calcium channels blockers	2.88 (1.139–7.236), $p = 0.017$	ns
	Vitamin D analogues	ns	ns
	Non-calcium Phosphate binders	ns	ns
	Vitamin D3 supplements	ns	ns
	Cinacalcet	ns	ns
	Antiplatelet therapy	ns	12.153 (4.982–29.745), $p = 0.001$
	Statins	ns	ns
	Erythropoietin/darbepoetin	ns	ns
PD-related parameters, Frailty	Peritoneal transport (High)	ns	ns
	CA 125 (UI/L)	ns	ns
	nPCR (g/Kg/day)	ns	0.093 (0.011–0.802), $p = 0.024$
	Kt/V	$p = 0.078$	0.475 (0.260–0.869), $p = 0.013$
	Frail	$p = 0.093$	$p = 0.073$
	Score de Edmonton	$p = 0.150$	$p = 0.051$
Membrane Fibrosis and Serum biomarkers	α-Klotho (pg/mL)	ns	$p = 0.055$
	Galectin-3 (ng/mL)	1.271 (0.988–1.635), $p = 0.042$	ns
	FGF21(pg/mL)	$p = 0.146$	ns
	FGF23 (pg/mL)	ns	ns
	TWEAK (pg/mL)	$p = 0.118$	ns
	TNF-α (pg/mL)	$p = 0.087$	ns
	hs-CRP (μg/mL)	ns	ns
	Peritoneal membrane fibrosis	ns	4.181 (1.905–9.175), $p = 0.001$
	STM	ns	1.009 (1.003–1.014), $p = 0.001$
	α-Klotho < 742 pg/mL	ns	$p = 0.055$

Data are present as hazard ratio (95% CI). PD—peritoneal dialysis; n—number of individuals; ns—non-significant; nPCR—normalized protein catabolic rate; Kt/V—Dialysis efficacy index; rGFR—residual glomerular filtration rate; CA125—cancer antigen 125; FGF21—Fibroblast growth factor 21; FGF23—Fibroblast growth factor 23 c-terminal; TWEAK—Tumor necrosis factor-like weak inducer of apoptosis; TNFα—Tumor necrosis factor α; hs-CRP—ultra-sensitive C-reactive protein; STM—submesothelial compact zone thickness.

A



B

Variable		N	Hazard ratio	p
Age		57	1.05 (1.00, 1.10)	0.03
Galectin.3	<8.8ng/mL	16	Reference	
	≥8.8ng/mL	41	5.17 (1.15, 23.21)	0.03
PAD	no	40	Reference	
	yes	17	2.61 (0.79, 8.64)	0.12
CCB	no	28	Reference	
	yes	29	2.37 (0.73, 7.67)	0.15
TWEAK		57	15.36 (1.48, 159.19)	0.02
FGF21		57	1.00 (1.00, 1.00)	0.03
Edmonton_Scale		57	0.87 (0.66, 1.16)	0.35
Icodextrin	no	39	Reference	
	yes	18	1.13 (0.27, 4.75)	0.87

Figure 1. Predictors of time until failure in peritoneal dialysis (PD). (**A**) Survival curve of the peritoneal membrane according to galectin-3 cutoff of 8.8 ng/mL. (**B**) Multivariate Cox regression model of peritoneal dialysis failure using galectin-3 cutoff of 8.8 ng/mL. PAD—peripheral arterial disease; CCB—calcium channel blocker; TWEAK—Tumor necrosis factor-like weak inducer of apoptosis; FGF21—Fibroblast growth factor 21.

4.3.4 Peritoneal membrane, age-related indicators and major cardiovascular event

While not related to PD failure, the presence of membrane fibrosis at the study baseline was associated with occurrence of MACE (Table 2) and time to MACE (Table 3, Figure 2A). Both endpoints were also related to age, frailty score, arterial atherosclerotic disease, use of statins, nPCR, beta-blockers and Kt/v (Tables 2 and 3).

The existence of fibrosis in the peritoneal membrane at the study baseline was independently associated with time to MACE in an adjusted model to age, nutritional status and PAD (Figure 2B), wherein the frailty score or heart failure did not account for the prediction of the time to PD failure. This multivariate association was maintained when the membrane status was inferred by the non-invasive surrogate α-Klotho, using the identified cut-off for α-Klotho of 742 pg/mL instead of biopsy score (Figure 2C). The association of time until the occurrence of MACE with atherosclerotic disease might be inferred by the use of antiplatelet therapy (Figure 2D), maintaining α-Klotho cutoff as an independent factor in the model, together with age and antiplatelet use at the study baseline. The estimated survival probability for time to MACE discriminated by α-Klotho levels in an adjusted model to age, frailty, nPCR, rGFR and use of antiplatelet drugs is represented in Figure 2E.

Overall, our results suggest a link between the vulnerability of a patient´s cardiovascular system and the status of the peritoneal membrane. In addition to age, lower α-Klotho and PAD were also predictors of cardiovascular risk over time in different multivariate models.

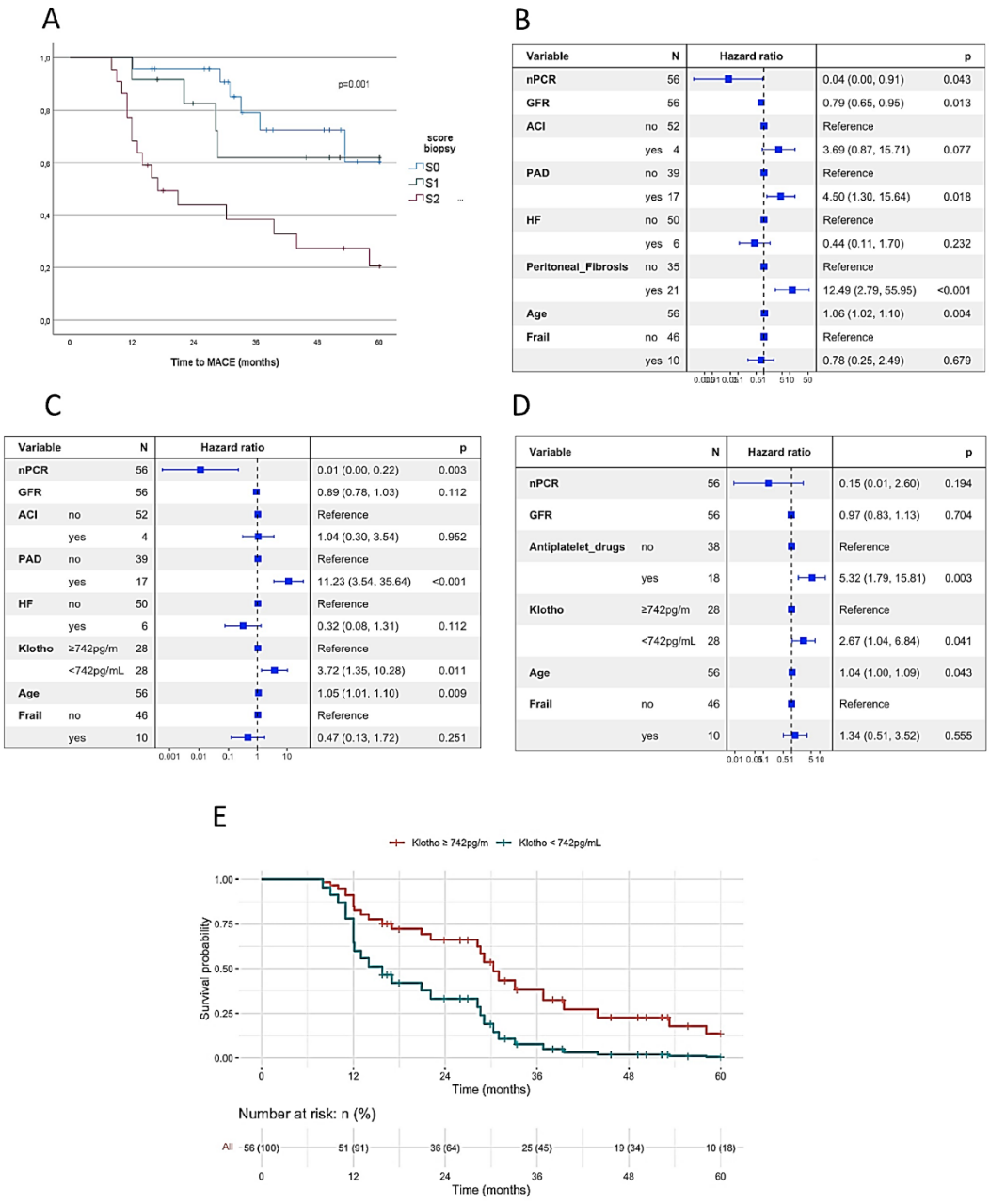


Figure 2. Predictors of time to major cardiovascular event (MACE). (**A**) Survival curve for time until MACE accordingly to peritoneal membrane status. (**B**) Multivariate Cox model that includes peritoneal fibrosis as an indicator of the membrane status adjusted for age, nutritional status, frailty and PAD. (**C**) Multivariate Cox model similar to B, but using α-Klotho cutoff as a surrogate of membrane fibrosis. (**D**) Multivariate Cox model using levels of circulating α-Klotho as surrogate of membrane fibrosis and using the antiplatelet therapy as an indicator of atherosclerotic arterial disease. (**E**) Survival curves for time until MACE according to α-Klotho cutoff of 742 pg/mL. GFR—glomerular filtration; PAD—peripheral arterial disease; CHD—coronary heart disease; HF—heart failure; nPCR—normalized protein catabolic rate.

4.3.5 Peritoneal membrane, age-related indicators and all-causes mortality

A total of six deaths occurred during the study, five related to cardiovascular disease and 1 to malignancy, which were not related to the biopsy score of the peritoneal membrane. Cardiovascular mortality and survivor groups had similar age, scores of biopsies and frailty as well as similar levels of serum biomarkers.

4.4 Discussion

Our data provides new information about the links between the peritoneal membrane, uremia and PD outcomes. We found that blood levels of galectin-3 represent a putative tool to identify patients at higher risk of PD failure. In addition, and contrary to our initial hypothesis, the baseline membrane fibrosis was not a predictor of technical failure, time to failure or all-causes mortality in PD. Instead, the status of the peritoneal membrane was related to MACE and time until occurrence of MACE, which can be inferred by circulating α-Klotho. The rationale for the choice of the pre-PD molecules was driven by the hypothesis that prematurely aged phenotypes of the peritoneal membrane could be associated with poorer long-term PD outcomes. These phenotypes are difficult to predict only from demographic characteristics, but could be favored by a uremic toxic environment, patients' frailty and aging. Therefore, a group of aging-related indicators was investigated as predictors of PD outcomes. PD outcomes were associated with uremic molecules, but not with the frailty test applied. This test was chosen due to its simplicity and ease for daily clinical practice and validation in Portuguese (Dent, Kowal and Hoogendijk, 2016; Perna *et al.*, 2017).

The person-to-person variability in membrane status and functions, even before the start of PD (Branco *et al.*, 2023), is likely to be driven by genetic and non-genetic factors (Rumpsfeld *et al.*, 2004; Gillerot *et al.*,

2005; Morelle, Marechal, *et al.*, 2021; Teitelbaum, 2021). The latter includes exposure to glucose, peritonitis, loss of residual renal function, inflammation and uremia (Mehrotra *et al.*, 2016; Zhou *et al.*, 2016; Branco et al., 2023). In this context, better knowledge about aging-related uremic molecules might fulfill clinicians' aims for accessible risk stratification tools for tailored prescriptions. Foreseeing a proof of concept that uremia-related mechanisms impact both membrane and patient survival, we selected a panel of proteins reported to be associated with aging, inflammation and fibrosis in other organs/tissues (Campbell, 2004; Blanco-Colio *et al.*, 2007; Winkles, 2008; Sanz, Sanchez-Niño and Ortiz, 2011; Moreno *et al.*, 2011; de Boer *et al.*, 2012; Moe, 2012; Pedersen *et al.*, 2013; Sanz *et al.*, 2014; Grabner and Faul, 2016; Castillo-Rodríguez *et al.*, 2017; Takenaka *et al.*, 2019; Ebert *et al.*, 2020; Salgado, Goes and Salgado Filho, 2021; Lu *et al.*, 2021).

We found that the status of the membrane (evaluated by histomorphology, STM and by a surrogate α-Klotho cutoff) was not associated with changes in the functions of the peritoneal transport. Moreover, the pre-PD membrane status was not predictive of long-term survival of both peritoneal membrane and patients.

As α-Klotho is associated with fibrosis of the peritoneal membrane (Branco et al., 2023), the absence of association between α-Klotho and PD failure was an unexpected finding. While it did not consider fibrosis, a previous study about the arteriolar structure concluded that membrane arteriolar frailty in CKD stage 5 patients follows with cardiovascular system damage (Donderski *et al.*, 2018). Therefore, as α-Klotho is associated with arteriosclerosis and aging, our results might suggest that the peritoneal biopsy score reflects a vascular vulnerability more than the integrity of the membrane. This novel and overlooked dimension might account for the shared mechanisms of persistent uremic phenotype, premature aging, and fibrosis of different tissues. In fact, the membrane might not represent a risk factor but a marker of a particular cardiovascular vulnerability profile.

Substantial cardiovascular risk persists in CKD patients, despite the treatment of established cardiovascular risk factors such as arterial hypertension and dyslipidemia. Knowledge about the uremia profiles that might be predictors of these risks will pave the way for personalized interventions. Moreover, this knowledge aligns with the need for novel drugs to control the unbalanced status of protective and deleterious molecules that constitutes uremia.

Our data might support that even older, frail, at higher cardiovascular risk and/or with a worsened status of peritoneal membrane patients might take advantage of this home-based modality of renal replacement therapy because we did not find any association between frailty or peritoneal membrane status and mortality or survival in the technique. Attention must be paid to the combination of atherosclerotic arterial disease, namely PAD and low α-Klotho levels. α-Klotho is an anti-aging molecule that exerts beneficial effects on the endothelium (Vila Cuenca, Hordijk and Vervloet, 2020). Moreover, α-Klotho-deficient mice show increased vascular calcification (Hu *et al.*, 2015; Kuro-o, 2021), further supporting a beneficial cardiovascular role of α-Klotho and putative relevance of recombinant α-Klotho to control the burden of comorbidities in PD patients.

Differently from α-Klotho, there was a clear association with galectin-3 and PD failure. Galectin-3, which is secreted by macrophages, has been associated with an inflammatory and fibrotic phenotype (Campbell, 2004; Moreno *et al.*, 2011; de Boer *et al.*, 2012; Pedersen *et al.*, 2013; Grabner and Faul, 2016; Takenaka *et al.*, 2019). Moreover, Béllon et al. (2011) showed that alternative activated macrophages or M2 phenotypes were present in the peritoneal effluent drained from patients, and were able to stimulate fibroblast proliferation and the loss of peritoneal function (Salgado, Goes and Salgado Filho, 2021).

α-Klotho and galectin-3 share common characteristics, e.g., both are uremic toxins and have been related to fibrosis and inflammation. However, unlike α-Klotho, galectin-3 was associated with PD failure.

Therefore, the differences found in PD outcomes between poor α-Klotho versus rich galectin-3 uremic profiles suggest different underlying mechanisms. Moreover, while low baseline α-Klotho was highly associated with cardiovascular disease, such an association was not found for galectin-3 (Table 2). Instead, our data indicated galectin-3 as a predictor of earlier PD failure. Further studies are necessary to validate this data, but a putative explanation for the galectin-3 result is that this molecule is a high-affinity binding protein for advanced glycation products (Vlassara *et al.*, 1995) whose relation to poor membrane efficiency and survival is well accepted (Roumeliotis *et al.*, 2020; Masola *et al.*, 2022). Of note, inhibitors of galectin-3 are currently investigated in clinical trials (NCT02800629, 2018; Lau *et al.*, 2021; NCT05240131, 2024), although in areas other than PD.

Our study has several strengths. All biomarker measurements were performed in the same laboratory to ensure measurement consistency across the pooled cohort, and we analyzed an anatomical territory with fibrosis and achieved a long follow-up period.

However, the study has some limitations. Firstly, the strict inclusion criteria from a single PD center implied that a rather small sample was studied; serum biomarkers were only measured at baseline, which might have hampered finding associations with time-dependent outcomes (PD and MACE). Secondly, other parameters of adequacy such as nutrition and volemia were neglected in our research, which may have influence data analysis and affect our prediction of long-term outcomes of patients. Moreover, our data only focused on clinical examinations and basic personal information, not including environmental conditions such as psychosocial and economic dimensions, which can affect their clinical outcomes.

Further research might focus on the putative role of galectin-3 and α-Klotho as tools to tailor patient management in this home-based renal replacement therapy.

CHAPTER V

USE OF PERITONEAL DIALYSIS IN CHILDREN AFTER CONGENITAL HEART DISEASE SURGERY

CHAPTER V

5 Use of peritoneal dialysis in children after congenital heart disease surgery

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Abstract

Acute kidney injury (AKI) is a common complication in children following congenital heart disease surgery and peritoneal dialysis (PD) is usually the renal replacement therapy (RRT) of choice, especially in very young children. The aim of this study is to describe our experience in the treatment of AKI using PD following cardiac surgery.

Retrospectively, we analysed children aged 1 week to 16 years old undergoing cardiac surgery from 2000 to 2008 and showed the incidence of AKI treated with PD was 2.3%. Of the 23 patients treated with PD, 13 were male, average age 29 ± 48.4 months and weight 9.1 ± 8.1 Kg. The indications for PD initiation were oliguria (13 patients), anuria (9 patients) and acidosis (1 patient). The average time between cardiac surgery and AKI was 4.8 ± 16.8 hours and between AKI and PD initiation 12 ± 16.8 hours. Patients were treated for a mean of 4.8 ± 3.8 days and 2 developed peritonitis and 1 patient had mechanical dysfunction of PD catheter. In-hospital mortality was 43.4%. Patients treated with PD weighed less (p=0.004), had longer bypass time (p=0.004), duration of inotropes use (p=0.000) and length of mechanical ventilation (p=0.000). However, in regression analysis, only cardiopulmonary bypass time (odds ratio=1.021; 95th confidence interval 0.998-1.027; p =0.032) remained predictive of the subsequent need of PD.

We conclude that PD is an efficacious RRT for AKI in children undergoing cardiac surgery and, in this setting, bypass time is the strongest predictor of the subsequent need of RRT.

Key words: peritoneal dialysis, acute kidney injury, cardiac surgery, prognosis.

5.1 Introduction

Children with congenital heart disease are at increased risk of acute kidney injury (AKI) after cardiac surgery. Several conditions increase the vulnerability to this complication, including the acute inflammatory response to cardiopulmonary bypass and post-operative hemodynamic instability, ischemia/reperfusion injury, circulating endotoxins and myoglobin, postoperative hemolysis, septic complications, congestive heart failure or baseline renal disease secondary to cyanotic congenital heart disease. The reported incidence ranges between 1 and 17% (Skippen and Krahn, 2005) depending largely on the criteria used to define AKI, and the associated mortality is high (between 21 and 70%) (JANDER *et al.*, 2007).

General considerations about the treatment of post-operative AKI include correction or removal of the precipitating factor and management of volume status. However, when volume overload and oliguria or anuria supervenes, renal replacement therapies (RRT) should be considered. In this setting, peritoneal dialysis (PD) has long been advocated as the preferred technique because of better hemodynamic stability, no vascular access requirement, simplicity and low cost (Pedersen *et al.*, 2008).

The aim of this work is to describe our experience using PD following congenital heart disease surgery in children under 16 years of age and to identify risk factors associated with poor outcome.

5.2 Methods

We retrospectively analyzed children who required PD for the treatment of AKI following congenital heart disease surgery from January 2000 to March 2008. Data were collected from patient's files and intensive care unit (ICU) registries and included first time and consecutive PD procedures. Demographic and clinical data included gestational age, weight and age at time of surgery, type of cardiac

disease, surgical procedure details and Risk Adjusted Classification of Congenital Heart Surgery (RACHS-1) score (Jenkins *et al.*, 2002).This score was developed to compare outcomes of congenital cardiac surgical care and is based on the complexity of the cardiac defects. It comprises six risk categories (risk category 1, the least severe, through risk category 6, the most severe) and is associated with in-hospital mortality.

Pre and postoperative renal function data, time until diagnosis of AKI and to PD initiation after surgery, cardiopulmonary and aorta cross-clamping time and type and duration of inotropic support were obtained. Paediatric scoring system using Paediatric Risk of Mortality (PRISM II) Score (POLLACK, RUTTIMANN and GETSON, 1988) was also calculated. This score employs data about the first 24 hours after admission to the ICU to predict the outcome of patients, with values above 15 being associated with high risk of mortality.

AKI was diagnosed according to the Acute Kidney Injury Network (AKIN) criteria (Mehta *et al.*, 2007), defined as a percentage increase in serum creatinine of more than or equal to 50% (1.5 fold from baseline) or a reduction in urine output with documented oliguria of less than 0.5 mL/Kg per hour for more than six hours. Two patients had renal failure before cardiac surgery and were preoperatively treated with PD.

Catheters for acute dialysis were implanted surgically by experienced surgeons and under general anaesthesia. Some patients had their PD catheter inserted during cardiac surgery because of reduced urinary output throughout surgical procedure or predictable development of AKI postoperatively. PD catheters were of Tenckhoff type (Fresenius Medical Care AG, Bad Homburg), paediatric size (25 cm), double-cuffed and tunnelled with a downward-oriented exit site.

PD was performed with a manual technique because of its simplicity and no requirements for more sophisticated equipment. To prevent leakage, the exchange volume of dialysate solution was restricted to 10

mL/Kg at the beginning of PD in all patients. It was subsequently increased according to body surface area and clinical and laboratory parameters, namely ultrafiltration and solute clearance requirements.

Standard, commercially available, bicarbonate-based dialysate solutions with dextrose concentrations of 1.5, 2.5 and 4.25 % (Fresenius Medical Care Deutschland GmbH) were used. Before the introduction of bicarbonate-based solutions, a 1.5% dextrose solution was prepared by the hospital pharmacy (700 mL of sodium chloride 0.9%, 30 mL of 30% dextrose, 30 mL of sodium bicarbonate 8.4% and distilled water until 1L of solution). Most of the patients were initially prescribed the lowest osmolarity solutions. When clinically appropriate, more hypertonic solutions were employed to treat fluid overload (in the solution prepared by the hospital pharmacy hypertonic solutions were achieved by adding 100 mL of 30% dextrose instead of the initial 30 mL).

Dialysate calcium concentration 1.75 mM was selected when commercial solutions were used and additives as potassium chloride, heparin or antibiotics added as necessary. None of the patients received intraperitoneal insulin.

Complications related to PD were reported, including major infectious and non-infectious problems.

Statistical analysis was performed using SPSS version 17. Quantitative variables were compared using Student's T-test and categorical variables $\chi 2$ test. Considering the small sample size, logistic regression analysis to identify for factors predictive of subsequent need of PD and mortality following cardiac surgery was performed using the whole cohort of patients that developed AKI (N=280). In the first model of logistic regression analysis, we included preoperative and intraoperative variables (age, weight, RACHS-1 score ≥ 4, aortic cross-clamp time, cardiopulmonary bypass time and urinary output) to identify the factors that would be predictive of need of PD. Subsequently, in the second model, we included preoperative,

intraoperative and postoperative variables (age, weight, RACHS-1 score ≥ 4, aortic cross-clamp time, cardiopulmonary bypass time, duration of inotropes uses, length of mechanical ventilation and treatment with PD (yes versus no)) to find the independent predictors of mortality. In both models of logistic regression analysis, the level of significance was defined to $p< 0.05$.

5.3 Results

Between January 2000 and March 2008, 998 patients under 16 years of age underwent cardiac surgery for congenital heart disease. The incidence of AKI in this period using AKIN criteria was 28.1% (280 patients), of which 63.6% were male, 67% less than one year old and 4.2% had already been subjected to a previous cardiac surgery. Twenty-three patients (2.3%) required PD for management of acute renal dysfunction. Of these, thirteen (56.5%) were male, average age 29 ± 48.4 months (ranging from 7 days to 165 months) and average weight 9.1 ± 8.1 Kg. Three infants (13%) were preterm (gestational ages of 30, 34 and 36 weeks). Table 1 presents cardiac disease diagnoses, type of surgical procedure and RACHS-1 classification. Nine patients (39%) had RACHS-1 score ≥4 and two patients could not be classified according to this system because they had a cardiac transplant. Average PRISM II score was 19.3 ± 6.

Table 1: Patient's age, cardiac disease diagnosis, type of surgery and RACHS-1 Score (TOF: Tetralogy of Fallot; VSD: ventricular septal defect; PDA: patent ductus arteriosus; ASD: atrial septal defect; RV: right ventricle). N/A: not applicable.

Patient	Age (months)	Cardiac diagnosis	Surgical procedure	RACHS score
1	3.6	Tetralogy of Fallot	Total repair TOF	2
2	14.1	VSD	VSD repair	4
3	0.26	PDA	PDA surgery	4
4	6.4	Tetralogy of Fallot	Total repair TOF	2
5	1.2	ASD, VSD, PDA	VSD repair, PDA surgery, arterial switch + VSD closure	4
6	12.1	ASD, VSD, pulmonary outflow stenosis	Atrial , VSD repair, pulmonary outflow tract augmentation	2
7	3.2	Perimembranous VSD	VSD repair, PDA surgery, subaortic stenosis ressection	2
8	0.23	PDA, VSD, pulmonary atresia	Modified Blalock-Taussig shunt, PDA surgery, arterial systemic to pulmonary artery shunt	3
9	17.8	Tetralogy Fallot	Repair of TOF with double outlet RV	3
10	1.8	PDA, VSD, aortic coartaction	Repair of coarctation, VSD repair and PDA surgery	3
11	1.4	Anomalous venous return, A-V septal defect	Anomalous venous return correction, septal defect repair	3
12	39.7	Ostium-primum ASD	ASD repair	2
13	113.4	Congenital dilated miocardiopaty	Cardiac transplant	N/A
14	126	VSD, intracardiac tumor	VSD repair, excision intracardiac tumor	3
15	0.6	Hypoplastic left heart syndrome, aortic atresia	Norwood operation	6
16	99.7	Congenital dilated myocardiopathy	Cardiac transplant	N/A
17	0.2	Hypoplastic left heart syndrome	Norwood operation	6
18	49.8	VSD, pulmonary atresia	Blalock-Taussig shunt, VSD repair, unifocalization for TOF	4
19	2.5	ASD, VSD, transposi-tion of great arteries	Blalock-Taussig shunt, pulmonary artery banding	3
20	0.4	Hypoplastic left heart syndrome	Norwood operation	6
21	0.36	Hypoplastic left heart syndrome	Norwood operation	6
22	7.7	Double-outlet RV, aortic coartaction, aortic arch hipoplasia	Double-outlet right ventricle repair	3
23	165.8	Transposition of great arteries, pulmonary vessels hipoplasia	Rastelli operation	4

Four patients had previous history of AKI. Two with hypoplastic left heart syndrome with severe hemodynamic compromise began PD preoperatively (2 days and 51 days before the surgery, respectively) and continued the technique after the surgery until resolution of AKI. Concerning the two other patients, one presented with AKI after

therapy with angiotensin converting enzyme inhibitor and the other AKI was secondary to low cardiac output syndrome, but in both complete recovery of renal function was achieved at least two months before surgery. Additionally, urinary tract congenital anomalies had been previously identified in three patients: bilateral pelviectasis, unilateral megaureter and horseshoe kidney, but in none of these patients' previous history of AKI was documented.

Fourteen patients had AKI in the first 12 to 24 hours after surgery. The mean time between surgery and AKI diagnosis was 4.8 ± 16.8 hours (ranging between 0 and 48 hours) and between AKI diagnosis and PD initiation 12 ± 16.8 hours (ranging between 0 and 72 hours and with a median value of 2 days between surgery and RRT). Eight patients (34.7%) began PD less than 24 hours after surgery, including all the preterm infants; in 5 a PD catheter had already been inserted during the cardiac surgery. The indications for starting dialysis were oliguria (13 patients), anuria (9 patients) and severe acidosis (1 patient).

The mean duration of dialysis censored by death was 115.2 ± 91.2 hours or 4.8 ± 3.8 days (ranging from 1 to 14 days). Among those that survived the mean duration of dialysis was longer, at 5.1 ± 4.2 days, ranging between 1 and 14 days.

The exchange volume of dialysate solution was 10 mL/Kg at the beginning of PD. In 18 patients this volume was progressively increased until 35 mL/Kg (minimum 25 mL and maximum 1000 mL). Dwell time was steadily increased in 20 patients to 20 to 60 minutes. The ultrafiltration rate with PD ranged between 0 and 165 mL/Kg/day with a mean value of 23 ± 20 mL/Kg/day. Thirteen patients (56.5%) required hypertonic solutions to optimize ultrafiltration and correct volume overload.

All patients who required PD, except one, received inotropic support after cardiac surgery with at least two drugs. Twelve (52%) required three inotropic agents. The average duration of inotropes use was 124.8 ± 40.8 hours (5.2 ± 1.7 days). Cardiopulmonary bypass was performed in

20 patients (86.9%). Mean time was 126.6 ± 78.4 minutes and aortic cross clamp time 55.7 ± 46.5 minutes. Nine patients (39%) required temporary pacing.

There were 3 complications related to PD: two patients developed peritonitis and another patient had mechanical dysfunction of the PD catheter. Episodes of peritonitis were diagnosed 2 and 6 days after PD initiation, respectively, and were both due to S. aureus. However, no systemic septic complications were evident and in none of the cases did this lead to the discontinuation of PD. Episodes of peritonitis were treated with intraperitoneal antibiotics while PD was maintained and, subsequently, a switch to systemic intravenous therapy was performed to complete the antimicrobial course. Catheter malposition was corrected by manipulation with a guide-wire and confirmed subsequently with an abdominal X-ray. None of the patients required catheter removal because of these complications.

In-hospital mortality in patients with AKI was 6.2% (16 patients) and 43.4% (10 patients) in those treated with PD. Eight patients (34.7%) died while on PD treatment. In-hospital mortality was attributable to cardiogenic shock in 4 patients (40%), cardio-respiratory arrest in 2 patients (20%), multi-organic failure in 2 patients (20%), disseminated intravascular coagulation in 1 patient (10%) and malignant arrhythmia also in 1 patient (10%). Three deaths were in preterm infants (30%), one had a previous episode of AKI not treated with PD prior to surgery and in another an episode of peritonitis was documented. In the long-term follow-up 3 patients died, two from cardiac failure and in the other patient death occurred five years after the surgery from undetermined causes. All patients who survived had complete recovery of renal function.

Regarding the cohort of patients that developed AKI, those who required PD had lower weight but longer bypass time, length of use of inotropic agents, mechanical ventilation and hospital stay (Table 2). In addition, a trend toward reduced urinary output immediately after

surgery was also observed but no differences were seen for RACHS-1 score. In those patients treated with PD but who died, we also observed a longer cardiopulmonary bypass time and duration of inotropes use (Table 2). Moreover, in this group, a longer interval time between AKI diagnosis and PD initiation was also documented (0.25 ± 0.45 versus 3.1 ± 7.9 days, respectively; p=0.089), albeit not statistically significant. No differences were seen for in-hospital mortality in patients treated with PD according to RACHS-1 score (p=0.456).

Table 2: Clinical characteristics of patients with AKI and of those treated with PD

	AKI (n=280)			PD (n=23)		
	NON-PD (n=257)	**PD (n=23)**	*p*	**SURVIVORS (n=13)**	***NON-SURVIVORS (n=10)**	*p*
Males	64%	56.5%	0.544	61.5%	40%	0.439
Age (months)	14.3 ± 24	29 ± 48.4	0.721	44.5 ± 59.9	17.2 ± 35.4	0.310
Weight (Kg)	15.6 ± 16.9	9.1 ± 8.1	0.004	11.7 ± 8.4	7.1 ± 7.6	0.193
RACHS-1 score ≥ 4	16%	30.4%	0.075	50%	30%	0.456
Cardiopulmonary bypass time (minutes)	66 ± 56.5	126.6 ± 78	0.004	76.3 ± 35	172 ± 72.1	0.003
Aortic cross-clamp time (minutes)	57.1 ± 33.8	55.1 ± 48.3	0.878	44 ± 52	63 ± 47.2	0.504
Duration of mechanical ventilation (hours)	106 ± 20.8	187 ± 124	0.000	156 ± 108.1	208.6 ± 136	0.336
Duration of inotropes use (hours)	60 ± 52.8	125 ± 40.8	0.000	64.5 ± 55.2	120 ± 35.2	0.001
Sepsis	4.9%	26%	0.000	15.3%	30%	0.335
Urinary output beginning PD (mL/Kg/24h)	71.1 ± 36.6	107 ± 175	0.092	129.3 ± 171.6	40.3 ± 61.9	0.061
Length of hospital stay (days)	14.2 ± 7	34 ± 32.4	0.003	15 ± 19.5	37.5 ± 68.5	0.242

* Non-survivors include the cases of in-hospital mortality

By logistic regression analysis, longer bypass time was the strongest predictor of subsequent need of PD after the cardiac surgery (Table 3). However, when considering prognosis, length of cardiopulmonary bypass time and of mechanical ventilation as well as duration of inotropes use were associated with mortality (Table 4).

Table 3: Logistic regression analysis for predictive factors for PD in patients with AKI (N=280).

Independent variables	Odds ratio	95 th confidence interval	*p*
Age (months)	0.960	0.617 – 1.494	0.856
Weight (Kg)	1.013	0.853 – 1.203	0.885
RACHS-1 score ≥ 4	0.987	0.652 – 1.085	0.581
Aortic cross clamp time (minutes)	0.976	0.931 – 1.024	0.321
CPB time (minutes)	1.021	0.998 – 1.027	0.032
Urinary output (mL/Kg/24h)	0.991	0.962 – 1.020	0.540

RACHS-1 score refers to Risk Adjusted Classification of Congenital Heart Surgery score (ranging between 1-6) and CPB to cardiopulmonary bypass time.

Table 4: Logistic regression analysis for predictive factors for mortality among patients with AKI (N=280).

Independent variables	Odds ratio	95 th confidence interval	*p*
Age (months)	0.175	0.033 – 0.936	0.142
Weight (Kg)	1.060	0.854 – 1.316	0.596
RACHS-1 score ≥ 4	1.173	0.409 – 2.300	0.553
Aortic cross clamp time (minutes)	0.925	0.746 – 0.998	0.722
CPB time (minutes)	1.022	1.007 – 1.037	0.004
PD (yes *versus* no)	0.845	0.652 – 1.075	0.573
Duration of inotropes use (hours)	0.595	0.363 – 0.973	0.039
Duration of MV (hours)	1.019	1.006 – 1.032	0.003

RACHS-1 score refers to Risk Adjusted Classification of Congenital Heart Surgery score (ranging between 1-6), CPB to cardiopulmonary bypass time and MV to mechanical ventilation.

5.4 Discussion

Children undergoing surgery for congenital heart disease are especially prone to AKI. However, it is difficult to appreciate its true incidence because, in the literature, we find great variability in the criteria to diagnose this complication. This has been defined as an increase in serum creatinine 30% above basal levels or a reduction in diuresis (Romão Jr *et al.*, 2000), 100% rise in serum creatinine or oliguria with levels of urinary output reduction according to age and weight (JANDER *et al.*, 2007) or tripling of baseline serum creatinine and/or

oliguria or anuria (Skippen and Krahn, 2005). One of the largest series published to date (Pedersen *et al.*, 2008), for example, considers only patients with AKI requiring PD, which inevitably underestimates the overall occurrence of this complication. In our patients we used the recently published AKIN criteria and obtained an incidence of 28.1%. This value is higher than that found in the literature, usually ranging between 1 to 10.8% (Skippen and Krahn, 2005), and might be related to the greater sensitivity of this criteria, especially for milder forms of AKI. It has been reported that 1 to 17% of patients with AKI require RRT (JANDER *et al.*, 2007). In this setting, peritoneal dialysis or continuous veno-venous hemodiafiltration (CVVHDF) can be offered to paediatric patients. Some may advocate that PD may not be as effective as CVVHDF in hypercatabolic patients or in those with severe fluid overload, requiring rapid net ultrafiltration. However, several prospective and retrospective studies have compared clinical outcomes with the different techniques of RRT, and none have been shown to be superior (JANDER *et al.*, 2007; Pedersen *et al.*, 2008). In smaller children, PD may have some advantages over CVVHDF because it obviates the need for a vascular catheter and is well tolerated in patients with hemodynamic instability (Bonilla-Félix, 2009). In most instances, however, the option for one technique relates more to the availability and experience of ICU staff and costs(Reznik *et al.*, 1993; Bonilla-Félix, 2009; Walters, Porter and Brophy, 2009). At our hospital only PD has been used in the treatment of severe AKI following congenital heart disease surgery. The percentage of patients with AKI treated with PD was 2.3%, a value in the range of others previously published (Romão Jr *et al.*, 2000; Plötz *et al.*, 2008). The indications to start RRT were similar to that described in other reports (Romão Jr *et al.*, 2000; Pedersen *et al.*, 2008), namely oliguria and anuria and we also included metabolic acidosis unresponsive to conservative measures, which is an indication usually not found in the literature.

It is recognized that AKI is an early complication following cardiac surgery. Some centres, anticipating this event, place dialysis catheter in the operating room, immediately after cardiac surgery (Skippen and Krahn, 2005) and RRT is usually prescribed in the first days after the operation. In our experience, we obtained a median value of 2 days between surgery and PD commencement and other series also report similar values (Romão Jr *et al.*, 2000; JANDER *et al.*, 2007). Nonetheless, pre-emptive placement of the dialysis catheter may impact on the time to initiation of dialysis, as in our series we observed that five of eight patients that already had a PD catheter began RRT on the day of surgery.

Different authors have demonstrated that the earlier initiation of dialysis is associated with a lower mortality rate (Werner *et al.*, 1997; Dittrich *et al.*, 1999; ELAHI, 2004; JANDER *et al.*, 2007). In our series, patients treated with PD but who died presented a trend towards longer time between AKI diagnosis and PD initiation, however this was not statistically significant, probably due to the small sample size. However, these results may suggest that in seriously ill children the rapid correction of metabolic disturbances and hypervolemia is associated with better outcomes. Moreover, an earlier start of RRT may also be related to recovery of AKI (JANDER *et al.*, 2007), although in our series we could not demonstrate this, since all surviving patients' recovered renal function.

Once correction of the low output syndrome is achieved, rapid improvement of renal function is usually the rule. In our series the rate of recovery of renal function among surviving patients was 100% but, in the literature, this value is variable, ranging from 29.5% (Picca *et al.*, 1995) to 50% (Baxter *et al.*, 1985; JANDER *et al.*, 2007) or higher values (94%) (Giuffre *et al.*, 1992). These differences are probably related to diverse patient populations described, with varying degrees of cardiac disease complexity, or in-center practices related to criteria to initiate RRT.

Concerning complications, PD is generally a safe method. Even though cardiothoracic surgery may constitute a relative contraindication to PD for the risk of peritoneo-pleural diaphragmatic communication, the technique is widely used with a low rate of complications. In our series, we reported only 3 major complications, although minor problems, like leakage, might be overlooked because of negligible clinical impact and the retrospective nature of our analyses. Nonetheless, minor complications are the most frequently reported problems, but difficult to evaluate in this context. One of the largest series on PD after cardiac congenital surgery (Pedersen *et al.*, 2008), found an overall rate of complications of 21%, of which 6.2% were major complications and the remaining minor problems. Complexity of cardiac lesions and duration of PD were among the factors associated with increased risk for PD problems(Pedersen *et al.*, 2008).

Overall mortality, both in-patient and in the long-term, was comparable to the values found in the literature (JANDER *et al.*, 2007). In this setting, mortality relates more to the primary underlying cardiac disease rather than to other medical conditions. Even if the presence of AKI is usually associated with increased rate of complications, longer hospital stay and worst prognosis, in this setting, the association between AKI and mortality must take into account patient's overall risk of death due to the underlying cardiac condition (Chan *et al.*, 2003). In those with low risk there is a strong correlation between AKI and mortality whilst in those with higher risk the association is weaker because death is attributable not only to AKI but also to other organ failure, especially cardiac failure (4). Like in other series (19), we also found longer bypass time as a predictive factor for the subsequent need of PD, although, in our cohort, the severity of the cardiac disease did not correlate with the likelihood of RRT or mortality.

Limitations of our retrospective analysis should also be addressed. Besides the shortcomings concerning minor problems, which might not be recorded because the clinical impact is perceived to be

negligible, we were also unable to determine indexes of dialysis efficacy in this setting and evaluate their impact in prognosis.

5.5 Conclusion

It is expected that the incidence of AKI following congenital heart disease surgery will continue to rise. Factors contributing to this increase include earlier and better accuracy in diagnosis, leading to more complex surgeries at younger ages. Thus, attention to the improvement of the technique of PD and the identification of factors associated with poor prognosis are crucial to improve the care of these patients.

CHAPTER VI

PART VI.1 PERITONEAL DIALYSIS IS A REASONABLE OPTION IN PATIENTS WITH CARDIOVASCULAR DISEASE

PART VI.2 HYPERVOLEMIA, HYPOALBUMINEMIA AND MITRAL CALCIFICATION AS MARKERS OF CARDIOVASCULAR RISK IN PERITONEAL DIALYSIS PATIENTS

CHAPTER VI, Part VI.1

PERITONEAL DIALYSIS IS A REASONABLE OPTION IN PATIENTS WITH CARDIOVASCULAR DISEASE

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Abstract

Introduction: Peritoneal dialysis (PD) has been proposed as a therapeutic option for patients with end-stage renal disease (ESRD) and cardiovascular (CV) disease. The study presented here aimed to compare incident PD patients with and without CV disease at baseline, in order to determine the impact of CV disease in the outcomes of long-term PD patients. **Methods:** This is a prospective cohort study performed at a single PD unit where 112 consecutive incident patients admitted to the PD program for 5 years were studied. The background of CV disease at PD initiation was defined as: presence of coronary artery disease, cerebrovascular disease, heart failure, or peripheral arterial disease. Laboratory measurements as well as PD adequacy were obtained at the beginning of PD and at the last evaluation. The outcomes examined were patient and technique survival, hospitalization and peritonitis rate. **Results:** Prevalence of diabetes was higher in patients with CV disease (53.3% vs. 31.7%, $p = 0.036$). Patients who suffered from CV disease were, on average, older (62.8 ± 13.1 vs. 49.7 ± 15.7 years, $p < 0.05$). There were no significant differences in other demographic or clinical variables, including hospital admissions (0.99

vs. 0.72 episodes/patient-year, p = 0.057) or peritonitis rates (0.69 vs. 0.61 episodes/ patient-year, p = 0.652). The overall rates of PD technique failure were similar between both groups (CV disease patients: 12.7 transfers to hemodialysis (HD)/100 patient years vs. control: 13.7 transfers to HD/100 patient-year; p = 0.54). Diabetes and age were independently associated with the presence of CV disease (p = 0.011), in a model adjusted for time on PD. The mortality rate was higher in CV disease patients (14.9 vs. 0.8 deaths/100 patient-years, p = 0.000) and 75% of all-cause mortality occurred in diabetic patients. In a multivariate analysis, diabetes (hazard ratio (HR): 5.5, confidence interval (CI): 0.84 – 36.29, p = 0.049) and age (HR: 1.07, CI: 1.0 – 1.13, p = 0.047) were independent predictors of death in a model adjusted for residual diuresis, body mass index, and time on PD.

Conclusions: This study compared incident PD patients with and without CV disease. CV disease patients were older but clinical and laboratorial targets, peritonitis rates, hospitalizations, and technique survival were similar between both groups, suggesting PD as an effective therapy for patients with CV comorbidities.

6.1 Introduction

Cardiovascular (CV) disease is the leading cause of morbidity and mortality in patients with chronic kidney disease (CKD), most notably in those with end-stage renal disease (ESRD) ('Patient mortality and survival. United States Renal Data System', 1998; Sarnak and Levey, 2000). CV disease presentation includes a number of different events, such as coronary artery disease, cerebrovascular disease, heart failure (HF), or peripheral arterial disease. HF is the most frequent presentation of CV disease in ESRD patients, as nearly one third of patients had HF at the initiation of dialysis (Harnett *et al.*, 1995). Additionally, HF when present on dialysis initiation is a strong and independent predictor of short (90 days) and long-term mortality (Soucie and McClellan, 1996).

Peritoneal dialysis (PD) has been proposed as a renal replacement therapy (RRT) for patients with ESRD and CV disease. Some studies were done in order to compare survival rates between PD and hemodialysis (HD) patients with CV disease (Stack *et al.*, 2003; Sens *et al.*, 2011). Despite the theoretical advantages of PD compared to HD (continuous, slow, and more physiologic removal of extracellular fluid with stable hemodynamic and preservation of residual renal function) in this specific group of patients, clinical results have been controversial, revealing that PD may be associated with greater mortality in patients with congestive HF (Stack *et al.*, 2003; Sens *et al.*, 2011).

There is scarce published literature referring, exclusively, to incident PD patients with CV disease. The study presented here aimed to compare incident PD patients with and without CV disease at baseline, in order to determine its prevalence and outcome in long-term PD patients, testing the hypothesis that PD is a valuable therapy to treat patients with CV disease.

6.2 Material and Methods

We performed a prospective, longitudinal, 5-year follow-up study, in a cohort of 112 incident PD patients. The study was performed at a single PD unit in Portugal between 2010 and 2014. The study protocol complies with the declaration of Helsinki and has obtained full approval from the local clinical research ethics committee. All patients provided informed consent before study entry. Patients were excluded if they were younger than 18 years of age at the time of PD onset, had an acute infection or were clinically unstable, had renal graft failure before starting PD, or their life expectancy was shorter than 6 months.

Patients' demographic characteristics (age, gender), etiology of CKD, and laboratorial results (albumin, C-reactive protein (CRP)) were recorded at baseline. Diabetes was a comorbidity collected in the registry. Diabetes was diagnosed if patients were using insulin or oral hypoglycemic agents, or if the fasting glucose concentration was > 126 mg/dL twice.

Background of cardiovascular disease at PD initiation was defined as: presence of coronary artery disease (history of angina, myocardial infarction with or without history of percutaneous coronary intervention or coronary artery bypass surgery), cerebrovascular disease (defined as previous cerebrovascular event), HF (diagnosed on the basis of the presence of all three clinical criteria: symptoms and signs of HF, including dyspnea, raised jugular venous pressure, and basal crepitation; radiographic evidence of pulmonary venous congestion or interstitial edema and resolution of symptoms, signs, and radiographic changes with hypertonic PD exchanges), or peripheral arterial disease (known by the evidence of intermittent claudication or restless leg pain associated with clinical signs of peripheral vascular disease with or without history of amputation or revascularization).

Anthropometric data and other laboratory measurements (adjusted calcium, phosphorus, intact parathyroid hormone (PTHi), bone alkaline

phosphatase) as well as PD adequacy were obtained at the beginning of PD and at the last evaluation.

PD adequacy was evaluated through peritoneal equilibration test (PET). Peritoneal transport characteristics were identified using D/P Cr reference values after a PET and PD adequacy was assessed using weekly Kt/V, residual creatinine clearance, and normalized protein catabolic rate (nPCR). Patients who were treated with icodextrin PD solution were recorded. Clinical events such as peritonitis and hospitalization were also recorded.

The examined outcomes were patient and technique survival, hospitalization and peritonitis rate. Technique failure was defined as a permanent cessation of PD due to PD-related complications. For the technique survival analysis, patients were censored at transplantation or death at the end of the study period. Technique survival status was censored at the end of the follow-up period.

For the patient survival analysis, subjects were censored at transplantation, transfer to HD or when completing the follow-up period. Survival status was censored on December 31, 2014.

6.2.1 Statistical analysis

Categorical variables were described as numbers or percentage of relative frequencies and quantitative variables as mean ± standard deviation (SD) for continuous normally distributed variables.

Differences between clinical data were assessed by Student's t-test for paired samples for normal variables, paired Wilcoxon test for continuous data with non-normal distribution.

Stepwise logistic regression was performed to evaluate the associations of different factors of background CV disease at study entry. Age and duration of dialysis were considered as important confounding factors and were included in all multivariate analysis.

Cumulative overall survival was generated by the Kaplan-Meier method, and between-group survival was compared using the log-rank test. Survival analyses were made with the Cox proportional hazard model.

The relative risk for mortality was determined by multivariate Cox regression analysis and presented as hazard ratios (hazards ratio (HR); 95% confidence intervals (CI)).

A p-value of < 0.05 was considered to be statistically significant.

All statistical tests were performed using the statistical package for the social sciences (SPSS) 14.0 software (SPSS, Inc., Chicago, IL, USA).

6.3 Results

A total of 112 patients were studied, of whom 65.2% were male with a mean age of 53.1 ± 16.1 (range 20 – 85) years. 38% of the patients had diabetes, and body mass index (BMI) at PD initiation was 25.7 ± 4.7 kg/m2. The underlying causes of CKD are shown in Table 1. The CV disease group included 30 patients (26.8% of all patients who started PD in this period); 82 patients did not have a history of CV comorbidities at baseline.

Table 1. Baseline characteristics and time under PD treatment of the study cohort for total group of patients, patients with CV disease and patients without CV disease.

	Total	CV disease	No CV disease	P value
Patients (n)	**112**	**30**	**82**	
Male (n,%)	73(65.2)	19(63.3)	54(65.9)	NS
Diabetes (n,%)	42(37.8)	16(53,3)	26(31.7)	0.036
Age, years (mean, SD)	53.1±16.1	62.8±13.1	49.7±15.7	<0.05
CKD etiologies (N/%)				
Diabetic nephropathy	35 (31.3)	22 (26.9)	13 (43.3)	
Unknown	22 (19.6)	18 (22)	4 (13.3)	
CGN	17 (15.2)	14 (17.1)	3 (10)	
Nephroangiosclerosis	12 (10,7)	8 (9.7)	4 (13.3)	
ADPKD	8 (7,1%)	8 (9.7)	0	
Chronic pyelonephritis	7 (6,3%)	5 (6.1)	2 (6.8)	
Other etiologies	11 (9,8%)	7 (8.5)	4 (13.3)	
BMI - Kg/m^2 (mean±SD)	25,7±4,7	24.8±8.4	23.6±7.9	NS
Time under PD - months (mean±SD)	22,1±15,7	21.4±13.6	22.4±16.5	NS
CAPD/APD (n/%)	76 (67.9)/36 (32.1)	20(66.7)/10(33.3)	56(68.3)/26(31.7)	NS
Icodextrin n/%	48(42.9)	17(56.6)	31(37.8)	NS
CPR - mg/dL (mean±SD)	1.17±1	1.5±1.4	1.1±0.9	NS
Albumin - g/dL (mean±SD)	3.4±0.6	3.3±0.8	3.5±0.5	NS
nPCR - g/Kg/day (mean±SD)	0.9±0.3	0.9±0.4	0.9±0.5	NS

Data shown as mean (standard deviation) or number (percentage). A p value of <0.05 was considered statistically significant.

ADPKD = autosomal dominant polycystic kidney disease; APD = automated peritoneal dialysis BMI= Body mass index, CAPD = continuous ambulatory peritoneal dialysis; CV = Cardiovascular, nPCR = normalized protein catabolic rate, CKD = chronic kidney disease, CPR= c-protein reactive; NS = not significant; CGN= chronic glomerulonephritis; PD = Peritoneal Dialysis; SD = standard deviation.

All patients were dialyzed using bicarbonate-buffered PD solution, and 48 (42.9%) patients were treated with icodextrin solutions. Patients were treated with PD for 22.1 ± 15.7 months and the cumulative follow-up time was 178.7 patient-years.

The total follow-up time of CV disease patients was 47.1 patient-years with a mean of 1.6 and a median of 2.2 years per patient (range: 0.14 – 4.34 years). In the non-CV disease group, total follow-up time was 131.6 patient-years with a mean of 1.6 and a median of 2.1 years per patient (range:0.05 – 4.78 years).

The prevalence of diabetes was higher in patients with CV disease (53.3% vs. 31.7%, p = 0.036).

Patients who suffered from CV disease were, on average, older (62.8 ± 13.1 vs. 49.7 ± 15.7 years, p < 0.05), but gender (63.3% vs. 65.9%, male, p = 0.469) and time under PD therapy (21.4 ± 13.6 vs. 22.4 ± 16.5 months, p = 0.773) were similar between both groups.

Residual diuresis (Δ-473.8 ± 844 vs.Δ-393.33 ± 953.9 mL/day, p = 0.763), eGFR (Δ-1.3 ± 4.2 vs. Δ-2.7 ± 2.9 mL/min/1.73 m^2, p = 0.149), Kt/v (Δ-1 ± –1.5 vs. –1.3 ± 1.4, p = 0.412) and nPCR (Δ-0.02 ± 0.3 vs. Δ-0.07 ± 0.2 mg/kg/day, p = 0.531) deteriorated through time. Nevertheless, no differences were found between both groups.

There were no significant differences in serum albumin levels (3.3 ± 0.8 vs. 3.5 ± 0.5 g/dL, p = 0.35), CRP (1.5 ± 1.4 vs. 1.1 ± 0.9 mg/dL, p = 0.086) and nPCR (0.9 ± 0.4 vs. 0.9 ± 0.5 g/kg/day, p = 0.953) or BMI (24.8 ± 8.4 vs. 23.6 ± 7.9 kg/m2, p = 0.469).

Table 1 presents baseline characteristics and time under PD treatment of the study cohort for the total group of patients, patients with CV disease, and patients without CV disease.

During the observation period (baseline and last evaluation), no differences were found in the following results between both groups: eGFR (5.9 ± 4.8 vs. 7 ± 4.4 mL/min/1.73 m2, p = 0.263, baseline; 4 ± 3.6 vs.

4.7 ± 3.7 mL/min/1.73 m^2, p = 0.559, last evaluation), Kt/V (2.34 ± 0.6 vs. 2.7 ± 0.8, p = 0.063, baseline; 1.3 ± 1.2 vs. 1.2 ± 1.2, p = 0.717, last evaluation), or peritoneal ultrafiltration (1,047 ± 814 vs. 1,456 ± 1,633 mL/day, p = 0.081, baseline; 1,073 ± 1,033 vs. 1,262 ± 1,136 mL/day, p = 0.94, last evaluation).

The prescription of icodextrin solution was similar between both groups (56.6% vs. 37.8%, p = 0.074). At the last evaluation, calcium (8.8 ± 0.7 vs. 9.1 ± 0.8 mg/dL, p = 0.038) and phosphorus (5.1 ± 1.3 vs. 5.8 ± 1.3 mg/dL, p = 0.012) serum levels were lower in patients with CV disease, but PTHi levels (558.4 ± 398.6 vs. 650.3 ± 517.3 pg/mL, p = 0.408) did not differ between both groups.

There were no significant differences in other parameters, including hospital admissions (0.99 vs. 0.72 episodes/patient, p = 0.057) or peritonitis rates (0.69 vs. 0.61 episodes/patient/year, p = 0.652).

During the 5-year observation period, 5 patients (2 patients with CV disease vs. 3 patients without CV disease) developed diabetes, and 9 patients were diagnosed with de-novo CV disease. Eight patients (7.1%) died (7 of them with CV disease at baseline), mainly due to infectious complications; only 4 patients died due to CV disease progression; 5 patients (4.5%) received a kidney allograft; and 24 patients (21.4%) were permanently transferred to HD due to ultrafiltration (UF) failure or peritonitis.

In the CV disease group, 6 out of the 30 (20%) patients required a permanent transfer to HD due to UF failure or peritonitis. Seven patients (23.3%) died, 4 of them (57.1%) due to CV diseases. Five (6.1%) patients without CV disease received a kidney allograft, 18 (22%) patients started HD, and 1 (1.2%) patient died due to sudden death. The overall rates of PD technique failure were similar between both groups (CV disease patients: 12.7 transfers to HD/100 patient-years vs. control: 13.7 transfers to HD/100 patient-years p = 0.54).

Diabetes and age were independently associated with the presence of CV disease (p = 0.011), in a model adjusted for time on PD. The mortality rate was higher in CV disease patients (14.9 vs. 0.8 deaths/100 patient-years, p = 0.000), and 75% of all-cause mortality occurred in diabetic patients. In a multivariate analysis, diabetes (HR: 5.5, CI: 0.84 – 36.29, p = 0.049) and age (HR: 1.07, CI: 1.0 – 1.13, p = 0.047) were independent predictors of death in a model adjusted for residual diuresis, BMI, and time on PD.

The cumulative survival of CV disease patients at 12 and 24 months was 86.7% and 83.3%, respectively, and for patients without CV disease it was 96.7% at 12 and 24 months. Cumulative survival of both groups is shown in Figure 1.

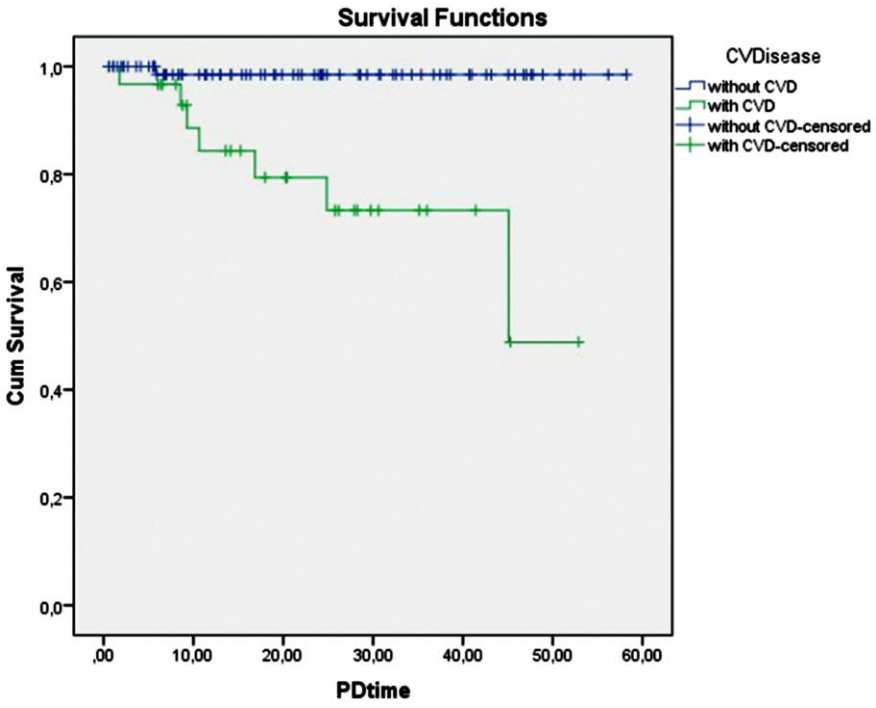

Figure 1. Cumulative survival of patients with and without CV disease.

6.4 Discussion

This study is the first study conducted exclusively in incident PD patients, where the prevalence of the spectrum of CV disease and its outcomes at PD initiation were assessed.

In the present study, it was shown that CV disease was present in 26.8% of patients at PD initiation. Despite the scarce literature concerning epidemiology of CV in PD patients, a previous report showed that the overall prevalence of CV disease in patients with CKD prior to dialysis is 46% (Levin *et al.*, 2001). A recent Portuguese study (Pego, Rodrigues and Ronco, 2012) prevalent PD patients showed that 18% of them had chronic heart disease as a baseline comorbidity. The prevalence of other CV events was not assessed in this study. Thus, our population seems to have a lower incidence of CV disease than expected. One reasonable explanation is that our study sample chose PD as the first option for renal replacement therapy and patients were younger than in other studies (Sens *et al.*, 2011). Age is a well-established traditional CV risk factor; accordingly, our patients with CV disease were older than non-CV disease patients (62.7 ± 13.1 vs. 49.7 ± 15.7 years, $p < 0.05$). As expected, increasing age was an independent predictor of CV disease and all-cause mortality.

Many reports revealed a high prevalence of CV disease in patients prior to dialysis, as well as the impact of the presence of CV disease on time to renal replacement therapy (Holland and Lam, 2000; Levin *et al.*, 2001). During the follow-up, no differences were observed between groups regarding achievement of clinical or laboratorial targets (albumin, PTHi, nPCR, Kt/v, peritoneal ultrafiltration, or loss of residual diuresis), except for calcium and phosphorus serum levels at the last evaluation, which were lower in CV disease patients. Our study showed, by means of adequacy parameters, that PD was a valuable renal replacement therapy in both groups.

In our cohort, overall technique failure rates were similar. However, patients in the CV disease group did not receive a kidney transplant. CV disease is recognized as an important risk factor for renal transplantation (RT), and in Portuguese transplant units these patients are not usually in the waiting list for RT.

Additionally, the presence of CKD in association with ischemic cardiac events is associated with poorer outcomes, including longer hospital stays and reduced survival (Al-Ahmad *et al.*, 2001). Indeed, the mortality rate was significantly higher in CV disease patients. However, 75% of all CV disease patients who died had diabetes, which was an independent predictor of all-cause mortality.

After controlling for confounding covariates, CV disease, by itself, did not confer an increased risk of all-cause mortality. There were no significant differences in other parameters, including hospital admissions or peritonitis rates.

Our study has some limitations. We found heterogeneous CKD etiologies and different management of CV comorbidities. Nevertheless, the encouraging results may suggest that PD is a safe, valid, and effective renal replacement therapy in this group of high-risk patients.

6.5 Conclusions

In conclusion, CV disease is the leading cause of morbidity and mortality in CKD patients. PD has been proposed as a therapeutic option for patients with ESRD and CV disease, but literature referring to incident PD patients with CV disease is scarce. This study compared incident PD patients with and without CV disease. CV disease patients were older, but clinical and laboratory targets, peritonitis rates, hospitalizations, and technique survival were similar between both groups, suggesting PD as an effective therapy for patients with CV comorbidities.

CHAPTER VI, Part VI.2

HYPERVOLEMIA, HYPOALBUMINEMIA AND MITRAL CALCIFICATION AS MARKERS OF CARDIOVASCULAR RISK IN PERITONEAL DIALYSIS PATIENTS

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ABSTRACT

Introduction: Mortality in patients with end-stage renal disease is higher than in the general population. This is linked to traditional and non-traditional cardiovascular (CV) risk factors, as well as with risk factors associated with end-stage renal disease itself. The aim of this study is to identify CV risk markers in patients beginning peritoneal dialysis (PD) and their association with CV events and CV mortality. **Methods:** This was a retrospective cohort study of 112 incident PD patients, in which demographic, clinical and laboratory parameters, valvular calcifications, types of PD solutions, hospitalizations, CV events and death were analyzed. Occurrence of CV events or death due to a CV event after PD initiation was defined as the primary endpoint. The use of icodextrin solution was taken as a marker of hypervolemia. **Results:** Mean age was 53.7±16.1 years. Patients were treated with PD for 29.3±17.4 months. Eighteen patients (16.1%) had valvular calcifications at baseline, 15 patients (13.4%) had major CV events and 11 patients (9.8%) died from CV-related causes. Cox proportional hazards analysis of CV events or CV-related mortality revealed that mitral calcification, use of icodextrin solution and low albumin were independent predictors of CV events or mortality. **Conclusions:** Traditional CV risk factors appear to have little impact on CV complications in PD patients. Nevertheless, hypervolemia, hypoalbuminemia and mitral calcifications were independent predictors of CV events or mortality in this group of patients.

6.1 Introduction

Chronic kidney disease is associated with an increased risk of stroke (Aguiar, 2012). Mortality in patients with end-stage renal disease (ESRD) is much higher than in the general population in spite of advances in dialysis treatment. Reasons for this higher incidence include greater risk of cardiovascular (CV) disease and associated higher comorbidity and mortality in ESRD patients, including peritoneal dialysis (PD) patients (Locatelli *et al.*, 2004; Wang, 2011).

In addition, CV mortality is up to 100 times higher in these patients than in the general same-age population, especially in younger groups (Foley, Parfrey and Sarnak, 1998; Go *et al.*, 2004). Worsening renal function also has an unquestionably negative impact on prognosis in patients with acute heart failure (Caetano *et al.*, 2014). Traditional risk factors for CV disease such as older age, diabetes, hypertension and hyperlipidemia, non-traditional CV risk factors related to PD therapy such as decreased residual renal function(Wang and Lai, 2006), ultrafiltration failure, peritoneal protein loss and use of glucose-based solutions (Holmes, 2007), and also novel risk factors such as chronic inflammation (Monfared *et al.*, 2013; van der Walt *et al.*, 2013), have all been established as CV risk factors in these patients (Perl *et al.*, 2009; Krediet and Balafa, 2010; Pérez-Fontán *et al.*, 2010).

Additionally, ESRD-related risk factors, such as fluid overload, anemia, mineral metabolism disorders and poor nutritional state play an important role in the prognosis of PD patients.

Although the effects of various CV risk factors on morbidity and mortality in PD patients are recognized, CV disease is still frequently under-diagnosed and under-treated in these patients. Up until recently, predictors of CV risk in PD patients have not been extensively investigated in incident PD patients.

The aim of this study is to identify potential attributes or characteristics that affect the occurrence of fatal and non-fatal cardiovascular events in patients beginning PD.

6.2 Methods

This is a retrospective cohort study performed at a single PD unit, based on 112 adult incident patients admitted to the PD program over a five-year period (2010-2014).

For the patient survival analysis, subjects were censored after the first CV event, at transplantation, transfer to hemodialysis (HD), transfer to another PD center or when completing the follow-up period. Survival status was censored on November 30, 2015.

Patients were excluded if they were under 18 years of age at the time of PD initiation, had acute infection or were clinically unstable, had previous renal graft failure, or had a life expectancy shorter than six months.

Demographic characteristics (age, gender), etiology of chronic kidney disease, previous comorbidities, and laboratory results (albumin, C-reactive protein, total cholesterol and triglycerides) were recorded at baseline. The presence of valvular calcification (mitral, aortic or both) at the start of dialysis was also assessed by Doppler echocardiography.

Anthropometric data and other laboratory measurements (adjusted calcium level, phosphorus, intact parathyroid hormone [iPTH], and bone alkaline phosphatase [BAP]) as well as adequacy of PD and nutrition parameters were obtained at the beginning of PD and also at the last assessment.

Peritoneal function was assessed through the peritoneal equilibration test (PET). Peritoneal transport characteristics were identified using dialysate/plasma creatinine reference values after a PET and PD adequacy was assessed using weekly Kt/V, peritoneal ultrafiltration,

residual creatinine clearance and normalized protein catabolic rate (nPCR). Patients who were treated with icodextrin solution were recorded.

Patients were classified as having a major CV event if the main cause of death or hospitalization was acute coronary syndrome, ischemic heart disease, congestive heart failure or stroke. Occurrence of a CV event or death due to a CV event after PD initiation was defined as the primary endpoint. Hypervolemia was diagnosed based on the presence of specific criteria: dyspnea, raised jugular venous pressure and basal crepitations; radiographic evidence of pulmonary venous congestion or interstitial edema; and resolution of symptoms, signs and radiographic changes with icodextrin exchanges. The use of icodextrin solution was thus taken as a marker of hypervolemia.

The study was approved by the institutional ethics committee and all participants gave their written consent.

6.2.1 Statistical analysis

Categorical variables were described as number or percentage of relative frequencies and quantitative variables as mean ± standard deviation (SD) for continuous normally distributed variables.

Differences between clinical data were assessed by the Student's t test for paired samples for continuous variables with normal distribution and the paired Wilcoxon test for continuous data with non-normal distribution. Survival curves were computed by the Kaplan-Meier method. Unadjusted and adjusted hazard ratios (HRs) for all-cause and CV events or mortality were estimated by multivariate regression analysis with Cox proportional regression and reported with 95% confidence intervals (CIs).

A p value of <0.05 was considered statistically significant. To identify patients at highest risk for the study endpoint, albumin values and the

corresponding endpoint rates were related via receiver operating characteristic (ROC) curves.

All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS), version 14.0 (SPSS Inc., Chicago, IL, USA).

6.3 Results

Table 1 presents clinical and biochemical parameters for the study group at PD initiation. All 112 patients studied were dialyzed using bicarbonate-buffered PD solution, and 48 (42.95%) patients were treated with icodextrin solutions. Mean age was 53.7±16.1 (range 20-85) years; 65.2% were male and 37.8% had diabetes. At baseline, 107 patients (95.6%) were treated with antihypertensive drugs (mean 3; standard deviation 1.1) and 20 patients (17.9%) were prescribed statins due to hyperlipidemia. Body mass index at PD initiation was 25.74±4.66 kg/m2. Patients were treated with PD for 29.3±17.4 months. The etiologies of ESRD are described in Table 1.

During the PD follow-up period, no changes were observed between initial and final assessments in the following results: adjusted calcium: 9.05 vs. 9.03 mg/dl (p=NS), nPCR: 0.95 vs. 0.92 g/kg/day (p=NS) and peritoneal ultrafiltration: 685.4 vs. 720.1 ml/day (p=NS). However, iPTH (p=0.001), BAP (p=0.011) and phosphorus levels (p<0.001) were higher in the final assessments (Table 2). Residual renal function (eGFR: 6.76 vs. 4.49 ml/min/1.73 m2, p<0.001) and dialysis adequacy (Kt/v: 2.48 vs. 2.26, p=0.017) deteriorated over time.

Table 1. Clinical and biochemical parameters for the study group at initiation of PD

Patients	**N= 112**
Male (%)	65.2
Diabetes (%)	37.8
Age (years)	53.7±16.1(20-85)
CKD etiology	
Diabetic nephropathy	35 (31.3%)
Unknown	22 (19.6%)
CGN	17 (15.2%)
Nefroangiosclerosis	12 (10.7%)
ADPKD	8 (7.1%)
Chronic pyelonephritis	7 (6.3%)
Other etiologies	11 (9.8%)
BMI - Kg/m^2	25.74±4.66
Comorbidities	
Ischemic cardiopathy	20 (17.9)
Stroke	11 (13.6)
Heart failure	18 (16.1)
CAPD/APD (n/%)	76 (67.9)/ 36 (32.1)
Icodextrin (n/%)	48 (42.9)
CRP (mg/dL)	1.17±1
Albumin (g/dL)	3.43±0.58
Phosphorus> 5.5 mg/dL (%)	26.4
iPTH> 500 pg/mL (%)	31.2
BAP <18 UI/l (%)	63.4
nPCR<1 g/Kg/day (%)	50

ADPKD: autosomal dominant polycystic kidney disease; APD: automated peritoneal dialysis; BAP: bone alkaline phosphatase; BMI: body mass index; CAPD: continuous ambulatory peritoneal dialysis; CGN: chronic glomerulonephritis; CKD: chronic kidney disease; CRP: C-reactive protein; iPTH: intact parathyroid hormone; nPCR: normalized protein catabolic rate.

Table 2. Changes in laboratory values at the initiation and at last follow-up of PD treatment.

	Beginning of PD	**Last follow-up**	**p**
Calcium(mg/dL)	9.05	9.03	p = NS
nPCR (mg/Kg/day)	0.95	0.92	P = NS
PTHi (pg/mL)	469.97	624.69	0.001
BAP (UI/L)	16.18	21,03	0.011
Phosphorus(mg/dL)	5.03	5.62	<0.001
eGFR (mL/min/1.73 m²)	6.76	4.49	<0.001

BAP: bone alkaline phosphatase; eGFR: estimated glomerular filtration rate; iPTH: intact parathyroid hormone; nPCR: normalized protein catabolic rate; PD: peritoneal dialysis. NS = non-significative

Valvular calcifications were assessed at baseline. Ninety patients (80.4%) were assessed at PD initiation by Doppler echocardiography, of whom 18 (16.1%) had valvular calcifications (two patients had exclusively aortic calcification, four had mitral calcification only and 12 had both). Besides these, no significant valvular dysfunction (stenosis or regurgitation) was found in these patients.

During the five-year observation period, 15 patients (13.4%) had major CV events and 11 (9.8%) died from CV-related causes. Time until the first CV event or death from CV-related causes was 26.3±17.8 months.

Twelve patients (10.7%) received a kidney allograft and 33 (29.4%) were transferred to hemodialysis.

Cox proportional hazards analysis of CV events or mortality from CV-related causes revealed that mitral calcification (HR: 3.25, 95% CI: 1.3-8.3, p=0.001), use of icodextrin solution (HR: 3.8, 95% CI: 1.13-12.8, p=0.003) and albumin <3.35 g/dl (HR: 2.84, 95% CI: 0.15-7.0, p=0.024) were

independent predictors of CV events or mortality, in a model adjusted to diabetes and age.

There was no increased risk of CV events or mortality with different PD solutions (calcium levels of 1.25 mmol/l vs. 1.75 mmol/l; p=NS). Also, elevated serum phosphorus, elevated calcium-phosphorus product and elevated C-reactive protein could not predict CV events or mortality (p=NS).

Kaplan-Meier survival curves revealed that time until first event (defined as a major CV event or death from CV-related causes) was shorter in patients treated with icodextrin (p=0.025) and in patients with mitral calcification (p<0.001) (Figures 1 and 2).

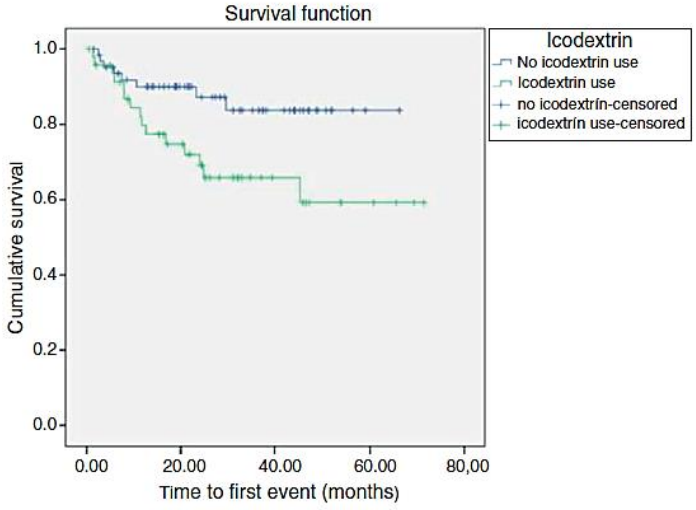

Figure 1 Kaplan-Meier survival curves relating occurrence of a non-fatal or fatal cardiovascular event to treatment with icodextrin (p=0.025).

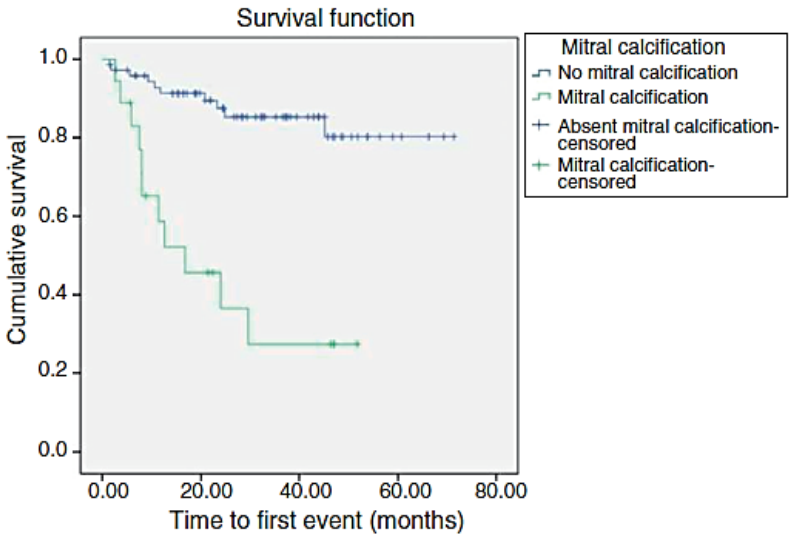

Figure 2 Kaplan-Meier survival curves relating occurrence of a non-fatal or fatal cardiovascular event to mitral calcification ($p<0.001$).

6.4 Discussion

Cardiovascular disease is common in renal failure and the causes of CV events in these patients are usually multifactorial. Their manifestations are multiple and heterogeneous and most aspects are not well studied in PD patients. Most information on CV complications in ESRD patients covers both HD and PD patients and few studies consider only incident patients. Additionally, published randomized controlled trials solely on PD patients had small population samples, inadequate study power and short follow-up periods, and rarely examined hard primary outcome measures such as mortality and CV events.

In our study, we determined that certain non-traditional risk factors were important predictors of major CV events and/or death in PD patients.

In our five-year observation period, 26 patients had CV events: 11 patients died (9.8%) from CV-related causes and 15 (13.4%) had major CV events. Some biases could explain the paucity of major CV events: the young age of the cohort and the study's inclusion criteria (considering only incident patients without acute infection or clinical instability, previous renal graft failure or life expectancy shorter than six months).

Besides, fluid overload (FO) is one of the most studied conditions in ESRD patients. FO is frequently associated with CV complications (Cheng *et al.*, 2008; Van Biesen *et al.*, 2011), inflammation (Demirci *et al.*, 2011) and mortality(Chen *et al.*, 2008; Paniagua *et al.*, 2010) in these patients, although few studies have shown a direct relation between clinically assessed FO and outcome in PD patients. A recent study from China (Guo *et al.*, 2015) demonstrated that overhydrated patients had significantly increased all-cause mortality and a trend for increased CV mortality and technique failure. Moreover, patients with FO had significantly more cardiac and cerebrovascular events.

Furthermore, PD patients experiencing difficulty in maintaining euvolemia due to insufficient peritoneal ultrafiltration are usually treated with icodextrin solution once daily as an alternative to hypertonic glucose PD solutions for long dwells. Use of icodextrin was accordingly taken in our study as a marker of hypervolemia.

In our population, 48 patients (42.9%) were treated with icodextrin and these patients had a higher incidence of CV events or death (HR: 3.8, 95% CI: 1.13-12.8, p=0.003). Icodextrin was mainly prescribed for patients with significant FO, in line with the addition of icodextrin to PD treatment in order to increase peritoneal ultrafiltration volume and control FO. So, as the use of icodextrin was heavily associated with FO, it can be used as a marker of vascular fragility, identifying PD patients at risk of CV events or death. Nevertheless, icodextrin was not associated with drop-out from the PD program (p=NS).

Furthermore, a study from Turkey (Demirci *et al.*, 2011) showed that FO was significantly correlated with malnutrition, inflammation and atherosclerosis. A PD patient developing malnutrition may gradually accumulate extracellular water to balance loss of body cell mass or body fat mass. Additionally, the existence of malnutrition, inflammation and atherosclerosis (MIA) syndrome is now established. Inflammation promotes neoangiogenesis, generation of profibrotic factors,

progressive increase in peritoneal permeability, loss of ultrafiltration and more fluid accumulation (Devereux *et al.*, 1984; Hassan *et al.*, 2005). Inflammation also contributes to the development of endothelial dysfunction, atherosclerosis and vascular calcification through the secretion of acute phase proteins and cytokines, complement activation and immune cell recruitment (Hassan *et al.*, 2005). Inflammation is also accompanied by malnutrition, hypoalbuminemia and reduction in oncotic pressure. In our study, albumin <3.35 g/dl (HR: 2.84, 95% CI: 0.15-7.0, p=0.024) was an independent predictor of CV events or mortality.

The third independent predictor of major CV event or death was mitral calcification (HR: 3.25, 95% CI: 1.3-8.3, p=0.001).

Surprisingly, only 14.4% of patients who were assessed by Doppler echocardiography at PD initiation had valvular calcifications (aortic, mitral or both), a significantly smaller percentage of patients than in recently published studies (Mohamed *et al.*, 2013; Takahashi *et al.*, 2013; Sánchez-Perales *et al.*, 2015). A Spanish study (Sánchez-Perales *et al.*, 2015) established the prevalence of valvular calcification at the start of dialysis and the relationship between valvular calcification and the presentation of composite endpoints of acute myocardial infarction, stroke or death from CV causes in the follow-up of incident dialysis patients. Vascular calcification had a prevalence of 50% at the beginning of dialysis, which differs significantly from our results. Once again, this may be due not only to the younger age of our patients (10 years younger comparing the mean age of both study populations), but also to the fact that our patients were exclusively treated by PD. Patients from the other series(Sánchez-Perales *et al.*, 2015) were treated by both PD and HD, which makes comparisons difficult. Other studies in the literature on valvular calcification in dialysis patients only refer to prevalent patients, describing high prevalence, between 30% and 70% in PD and HD patients.

Nevertheless, both studies obtained a similar result: valvular calcification was an independent predictor of CV events or death related to major CV events. In other studies on prevalent HD (Raggi *et al.*, 2011) and PD (Wang *et al.*, 2003) patients, valvular calcification was an independent predictor of all-cause mortality, and is accepted as a marker of CV disease.

Our study has limitations. It was performed in a single center and included a relatively small population, and valvular calcifications, CV events and deaths occurred in a relatively small number of patients.

Nevertheless, predictors of CV events or death were consistent with the published literature. This report is also one of the few in which only incident and exclusively PD patients were studied. The encouraging results (few CV events and few deaths) were probably related to the young age of this patient group. Randomized controlled trials are needed to confirm these results, in order to monitor traditional and non-traditional risk factors in PD patients. These results could provide significant prognostic information that will be useful in clinical practice for patient management, since these predictors are signaling variables of frail patients at the beginning of PD, indicating the need to adjust dialysate solutions to maintain euvolemia.

6.5 Conclusions

Traditional CV risk factors appear to have little impact on CV complications in PD patients. Nevertheless, hypervolemia, hypoalbuminemia and mitral calcifications were independent predictors of CV events or mortality in this group of patients.

CHAPTER VII

PART VII.1: PERITONEAL DIALYSIS AS A SUCCESSFUL TREATMENT IN PATIENTS WITH REFRACTORY CONGESTIVE HEART FAILURE: A ONE-CENTER EXPERIENCE

PART VII.2: IS PERITONEAL DIALYSIS AN EFFECTIVE TREATMENT FOR CARDIORENAL SYNDROME WITH DECOMPENSATED HEART FAILURE? A SINGLE CENTER EXPERIENCE

CHAPTER VII, Part 1

PERITONEAL DIALYSIS AS A SUCCESSFUL TREATMENT IN PATIENTS WITH REFRACTORY CONGESTIVE HEART FAILURE: A ONE-CENTER EXPERIENCE

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ABSTRACT

Introduction: Ultrafiltration (UF) technique is a valuable alternative to pharmacological therapy in the treatment of patients with refractory congestive heart failure (HF). The aim of this study was to describe a single-center experience in the treatment of refractory HF patients with peritoneal dialysis (PD). Methods: Retrospective study of 5 patients included in a single PD Unit, showing symptoms and signs of severe refractory congestive HF to optimal pharmacological therapy (NYHA class IV). Clinical and laboratory parameters, survival, hospitalization, and peritonitis rates were recorded. Results: Patients were followed for 9.36 (± 6.36) months; population mean age was 62 (± 16) years and Charlson's comorbidity index was 7.2 (± 2.1). After PD therapy, functional class of NYHA significantly improved (class IV to class II in 4 patients). Doppler-echocardiography improved in terms of ejection fraction (EF) or systolic pressure of the pulmonary artery (SPPA) in 3 patients. No patient was readmitted due to HF. Hospitalization days substantially de- creased in 4 patients. One patient presented with peritonitis episodes. Three patients died but the mean survival was higher than expected according to their comorbidity index. Conclusion: PD, applied to refractory HF in addition to optimal pharmacological therapy, improves quality of life and functional class and reduces hospitalization days due to HF.

7.1 Introduction

Chronic congestive heart failure (HF) is a chronic progressive disease affecting 1 – 10% of the adult population in developed countries. It is one of the major causes of morbidity and mortality in the world and it is also responsible for high rates of hospitalization and readmission (Khalifeh, Vychytil and Hörl, 2006).

In patients with HF, neurohormonal activation leads to fluid overload that can be treated by diuretics. The latest guidelines (Jessup *et al.*, 2009) recommend diuretics as the most useful tool for eliminating excess fluids. Furthermore, studies show that more than 20% of patients with HF did not have symptomatic improvement with diuretic therapy (Fonarow and ADHERE Scientific Advisory Committee, 2003). Other pharmacological treatments include intermittent intravenous inotropic drugs, vasodilators, calcium and β-blocking agents, angiotensin- converting enzyme (ACE) inhibitors, or angiotensin receptor antagonists (ARA).

For patients with refractory HF, heart transplantation represents the only rescue treatment to keep patients alive, however, it is not available for all patients. An alternative to conventional treatment in refractory HF is ultrafiltration (UF) technique (Agostoni and Marenzi, 2001; Cadnapaphornchai *et al.*, 2001; Khalifeh, Vychytil and Hörl, 2006).

Continuous renal replacement therapies (continuous veno-venous hemodiafiltration, slow continuous ultrafiltration, and slow daily ultrafiltration) have been mostly used in acute situations of overhydrated and oliguric patients. Application of these techniques as maintenance therapy has shown poor survival rates (Canaud, 1998).

The first case report (Schneierson, 1949) published using peritoneal dialysis (PD) to treat successfully a patient with severe HF dates from 1949. Available literature has revealed that PD can be useful in HF patients under some clinical conditions: concomitant renal disease, electrolyte imbalance, preparation for heart surgery, rapid deterioration

of a previous stable cardiac situation (Mailloux, 1967), and in patients with hemodynamic instability and significant systolic dysfunction (Swartz, 1999; Cnossen *et al.*, 2006).

The principal advantage of peritoneal UF is the continuous, slow, and more physiologic removal of extra-cellular fluid with stable hemodynamics while preserving residual renal function (Krishnan and Oreopoulos, 2007; Quirós-Ganga PL and Remón-Rodríguez C, 2012; Quirós-Ganga PL *et al.*, 2012). Peritoneal UF does not change the course of HF, but improves the congestive state by a renewed response to diuretics, a correction of electrolyte im- balance, weight loss as well as an overall clinical improvement (Mailloux, 1967). It also improves NYHA functional status, reduces hospitalization periods (Samoni *et al.*, 2015), and improves quality of life at a reasonable cost (Sánchez *et al.*, 2010).

Even though available reports include only small series of patients, it seems obvious that both automated peritoneal dialysis (APD) and continuous ambulatory peritoneal dialysis (CAPD) are effective in the treatment of patients with simultaneous HF and chronic kidney disease (CKD) (Ryckelynck, 1998).

The aim of this study is to describe 4 single-center experience in the treatment of refractory HF patients with PD, including analysis of mortality, rehabilitation, and hospitalization data.

7.2 Material and methods

This is an observational study of 5 patients included in a single-unit PD program in Portugal. These patients were admitted to PD from December 2008 to January 2012, showing symptoms and signs of severe congestive HF refractory to optimal pharmaco- logical therapy (NYHA class IV). The follow-up period started on the first day of PD treatment and finished when patients dropped out of the program due to transfer to hemodialysis (HD) or death.

Patients were evaluated through Doppler- echocardiography at the beginning of PD and at the 4th month. Left ventricular systolic function

was determined through ejection fraction (EF); Pulmonary hypertension was evaluated by systolic pressure of the pulmonary artery (SPPA). All patients fitted at the criteria list: pulmonary hypertension (PH), defined as SPPA > 35 mmHg while resting or severe systolic dysfunction, defined as EF < 35%.

Every patient had different degrees of CKD; estimated glomerular filtration rate (eGFR) was calculated by CKD-EPI formula. Demographic characteristics (age, gender), Charlson's co-morbidity index, etiology of HF, previous comorbidities (diabetes, hypertension), and laboratorial results (albumin, hemoglobin) were also recorded at baseline.

PD adequacy was evaluated through peritoneal equilibration test (PET). Peritoneal transport characteristics were identified using D/P Cr reference values after a PET, and PD adequacy was assessed using weekly Kt/V, residual creatinine clearance, and normalized protein catabolic rate (nPCR). The type of PD solutions was recorded, including icodextrin.

The clinical progression was evaluated according to: (i) the evolution of NYHA classification functional class, (ii) weight progression, (iii) Doppler-echocardiography evaluation, (iv) number of hospitalization days, and (v) peritonitis rate. Every measure was recorded before and after PD therapy.

Categorical variables were described as numbers or percentages of relative frequencies and quantitative variables were present- ed as mean ± standard deviation (SD).

7.3 Results

We followed a cohort of 5 patients, 2 women and 3 men, with HF (NYHA class IV) who started PD from December 2008 to January 2012. Three patients had a story of hypertension and 2 were diabetic. These patients were all excluded as candidates for heart transplantation. The etiology of HF was ischemic cardiopathy in 3 patients, congenital cardiopathy in 1 patient, and valvular cardiopathy in 1 patient. Patients were followed

for 9.36 ± 6.36 (range 1.7 – 17.3) months, resulting in a total of 46.8 months of therapy in 5 patients. The mean age of this patient sample was 62 ± 16 years. The initial CKD- EPI estimated a glomerular filtration rate of 23.4 ± 9.71 mL/min/1.73m2. One patient had been previously treated with continuous veno-venous hemodiafiltration, which was suspended due to hemodynamic instability; the other 4 patients started PD ab initium.

Table 1. Time on PD, PET results, PD modality, type of PD solutions, daily PD fluid volume, residual diuresis, and daily ultrafiltration of patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Time on PD (months)	1.7	8.7	14.1	17.3	4.9
Gender	M	F	F	M	M
Age (years)	62	77	78	39	55
PD modality	CAPD	APD	CAPD	APD	CAPD
Icodextrin	Yes	Yes	Yes	Yes	Yes
Type of solutions	ICO (1 night exchange)	ICO + glucose 2.27 + 2.27 + 1.36 + 1.36%	ICO + glucose 1.36 + 1.36 + 1.36%	ICO + glucose 1.36%	ICO + glucose 1.36 + 1.36 + 2.27 + 3.86%
Total volume PD solutions (mL)	2,000	1,800	6,800	10,000	8,000
Residual diuresis (mL)	2,600	400	500	110	625
Kt/v	–	–	3.83	1.32	3.53
nPCR (g/Kg/day)	–	–	1.52	0.74	1.05
Fluid removal (mL)	–	–	1420	1100	1270
D/P	–	–	0.66	0.75	0.6
UF 3.86% (mL)	–	–	550	500	100
eGFR (mL/min/1.73m^2)	–	–	6.3	0.1	13.6
Residual diuresis (mL)	2,600	400	500	110	625

APD = automated peritoneal dialysis; CAPD = continuous ambulatory peritoneal dialysis; eGFR = estimated glomerular filtration rate; F = female; ICO = icodextrin; M = male; PD = peritoneal dialysis.

Charlson's comorbidity index was 7.2 ± 2.1 (range 6 – 11). At the beginning of PD, all 5 patients had hypoalbuminemia (2.98 ± 0.70 g/dL), and 4 of them were receiving erythropoietin (EPO) due to anemia (hemoglobin: 10.0 ± 0.99 g/dL). All patients were treated with icodextrin solutions. PD adequacy was evaluated through PET results. PD modality (CAPD or APD), type of PD solutions, and daily PD fluid volume and residual diuresis are described in Table1. Daily ultrafiltration was 1,263 ± 160 mL. No patient had catheter dysfunction.

Three patients died. Except for 1 patient who suffered early sudden death after initiating PD, functional class of NYHA significantly

improved (class IV to class II in 4 patients). Four patients had a substantial weight loss (mean of 4.2 kg). Doppler-echo- cardiography evaluation showed significant improvements in terms of EF or SPPA in 3 patients. No patient was readmitted due to HF. Moreover, the number of hospitalization days substantially decreased in 4 patients; only 1 patient had more hospitalization days after beginning of PD, due to a peritonitis episode. One patient presented peritonitis episodes, which lead to HD transference.

Two patients were transferred to HD during the observation period. The mean survival time of patients who died was 9.24 months (range 1.73 – 17.26); the 2 patients who were transferred to HD had a mean survival of 9.53 months under PD therapy. Table 2 shows clinical progression and the causes of death for every patient.

Table 2. Clinical progression and causes of death of all patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Echocardiogram admission vs. postPD	SPPA: 58 – > 49 EF: 36 – > 42	SPPA: 65 – > absence of TI	EF: 34 – > 56	SPPA: 34 – > 60 EF: 33 – > 35	SPPA: 16 – > 16 EF: 80 – > 81
Functional class (NYHA) admissionvs. post PD	IV – > IV	IV – > II	IV – > II	IV – > II	IV – > II
Weight (kg) admission vs. post PD	70 – > 75.8	14.8 – > 14	47.8 – > 45.9	64 – > 62	77 – > 65
Peritonitis episodes on PD therapy	0	0	3	0	0
Hospitalization days admission vs.post PD	55 – > 0	117 – > 21	6 – > 22	128 – > 18	128 – > 86
Causes of death	Sudden death	Intestinal ischemia	–	Hepatocellular carcinoma	–

EF = ejection fraction; PD = peritoneal dialysis; SPPA = systolic pressure of the pulmonary artery; TI = tricuspid insufficiency.

7.4 Discussion

Renal dysfunction is a common pathology in HF patients, with a prevalence of 36 – 50% and is associated to decreased cardiac output and aggressive diuretic therapy. In addition, 25% of patients with CKD develop HF, a figure that rises to 64% among patients who start dialysis (Silverberg *et al.*, 2004; Rubinger, 2005; Arora and Dellsperger, 2007). Furthermore, episodes of acute deterioration of renal function are often

observed during the decompensation stages of HF (Gottlieb *et al.*, 2002).

Some published literature reports that PD in acute decompensated HF has resulted in reduction of pulmonary wedge pressure, restoration of response to diuretics, and enhanced glomerular filtration (Mehrotra and Kathuria, 2006). Mailloux et al. (Mailloux, 1967), studied a group of 15 patients and observed an improvement in the response to diuretics in 12 of the cases, as well as weight reduction and improved cardiac output. Shapira described his experience based in the study of 10 patients and reported an improvement in clinical symptoms, weight reduction, and increased diuresis with response to diuretics (Shapira J, Lang R and Jutrin I, 1983).

Nevertheless, the mechanism by which patients with refractory HF improve with PD therapy is unknown. Gotloib et al. (Gotloib *et al.*, 2005) proposed a theory of peritoneal clearance of myocardium-depressing substances, such as atrial natriuretic peptide (ANP), tumor necrosis factor-a, myocardial depressant factor, and interleukins 1 and 6. These sub- stances have been shown to induce apoptosis of cardiac myocytes and/or to have negative inotropic effects (Diwan *et al.*, 2003). Circulating levels of ANP seem to be associated with left ventricular mass and could predict mortality in dialysis patients (ZOCCALI *et al.*, 2001). Myocyte cell apoptosis induced by ANP is, probably, a fundamental step in the cascade of events that lead to HF. Therefore, by clearing ANP molecules, myocyte cells would not die.

At the moment, however, there is only indirect evidence supporting the beneficial mechanisms of PD in HF. Shilo et al. (Shilo, Slotki and Iaina, 1987) reported that ultrafiltration by PD improves renal hemodynamic, with a lowering of filtration fraction and amelioration of efferent glomerular vasoconstriction. These are indirect indicators that clearance of free water and sodium by PD leads to attenuated

activation of the renin-angiotensin-aldosterone system, when compared to hemodynamic changes following diuretic therapy.

There are no randomized controlled trials concerning the effect of chronic PD in survival and quality of life of HF patients. All experiences are based on small series with both CAPD and APD patients, and they are virtually unanimous: improved clinical symptoms, functional class and reduction in the frequency of hospitalization, reduction in the expected mortality by 1 year and improved CV parameters (EF or SPPA). Peritonitis is a frequent complication, however with no impact on mortality, being more frequent in CAPD patients (Kagan and Rapoport, 2005; Mehrotra and Kathuria, 2006; Krishnan and Oreopoulos, 2007).

In 2005, a prospective non-randomized study with 20 patients with HF NYHA class IV revealed regression to NYHA class I, recovery of systolic left ventricular function, first-year mortality lower than expected according to comorbidity index, and reduction of hospitalization days (Gotloib *et al.*, 2005). Another 2007 study de- scribed a single-center experience in treating HF patients in diverse stages of CKD with PD. They found a significant improvement in quality of life and reduction in morbidity and hospitalization rates in all 5 studied patients, with a low rate of peritonitis (Díez Ojea *et al.*, 2007).

Recently, a systematic review of 21 studies including a total of 673 HF patients treat- ed with PD, reported that PD was effective in reducing hospitalization days and in improving heart function, without worsening renal function. Additionally, it showed acceptable rates of peritonitis, similar to patients who underwent standard chronic PD (Lu *et al.*, 2015).

In terms of PD modality, results seem unclear (Ortiz *et al.*, 2003). Moreover, the use of icodextrin has been associated with reduced generation of glucose degradation products, less intra- peritoneal inflammation, smaller systemic glucose absorption, fewer carbohydrate and lipid metabolism alterations, and greater UF with

long stays. A single night exchange with icodextrin seems to be a cost-effective option, reducing morbidity, mortality, and being well tolerated by patients (Bertoli *et al.*, 2005; Khalifeh, Vychytil and Hörl, 2006; Mehrotra and Kathuria, 2006).

Most of our results are in accordance with literature data. We found improvement of the Doppler-echocardiography parameters (EF or SPPA) after PD in all patients, as well as a regression in NYHA from class IV to class II in 4 patients. Just 1 patient did not have an improvement due to early death. Additionally, the weight loss in the 4 prevalent patients was consistent (mean of 4.2 kg). The decrease of hospitalization days, the absence of readmissions due to HF, and the improvement in functional class could be important predictors of a better quality of life.

Four patients had anemia (hemoglobin: 10.0 ± 0.99 g/dL) at start of PD and were treated with EPO. Anemia due to multifactorial causes has a prevalence of 40 – 50% in HF patients and it is responsible for cardiac and renal deterioration, with a consequent fast progression to dialysis. EPO improves cardiac function and quality of life, decreases hospitalization rate (Silverberg *et al.*, 2003), protects the myocardium in ischemia-reperfusion, and inhibits apoptosis in myocardiocytes, improving the immune function in CKD (Cai and Semenza, 2004; Rubinger, 2005).

A previous systematic review of 14 studies with 10 or more HF patients treated with PD (Lu *et al.*, 2015) reported a mean incidence of peritonitis of 14.5% per year and a mean mortality of 20.3% per year. In our report, 3 peritonitis episodes were diagnosed in the same patient during a follow-up period of 46.8 months, leading to an extremely low peritonitis rate (0.013 episodes/patient/year). Nevertheless, this patient was transferred to HD due to re- current peritonitis.

According to Charlson's comorbidity index, a score > 5 implies an 85% mortality rate after the first year of follow-up. In our series, 3 patients

died in the first year after a mean survival of 9.24 months, leading to a mortality rate of 60%. However, 2 patients had a mean survival higher than expected (14 and 17 months).

Besides that, it is difficult to compare mortality and peritonitis rates between our study and the previous reports due to the different number of patients studied; nevertheless, considering that Charlson's comorbidity index implied an 85% mortality rate at the first follow-up year, we assume that a relative higher survival rate than the expected one, according to their comorbidity index, was achieved.

This study has some limitations: it included a small number of patients, with heterogeneous etiologies and management of CKD and HF. The indication for HD, hemofiltration, or PD for managing fluid overload in HF has not been clearly established (Sheppard *et al.*, 2004).

Nevertheless, our report still supports some published literature, showing that PD, applied to refractory HF in addition to optimal pharmacological therapy, is effective, since it improves quality of life and functional class and reduces hospitalization days due to HF.

However, given the morbidity and mortality of HF refractory to diuretics, a multi- center prospective study is needed, in order to definitively consider PD as a reasonable therapy for long-term ambulatory management of these patients.

CHAPTER VII, Part 2

IS PERITONEAL DIALYSIS AN EFFECTIVE TREATMENT FOR CARDIORENAL SYNDROME WITH DECOMPENSATED HEART FAILURE? A SINGLE CENTER EXPERIENCE

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Abstract

Introduction and Objectives: ultrafiltration techniques have shown promise in the treatment of diuretic-resistant heart failure (HF). The aim of this study was to describe a center experience in treating refractory HF with peritoneal dialysis (PD). Methods: we performed a retrospective study of 14 patients presenting symptoms and signs of refractory congestive HF despite optimal pharmacological therapy, all excluded as candidates for heart transplantation. Baseline characteristics, laboratory data, Charlson score, and transthoracic doppler-echocardiogram results were collected. PD adequacy was evaluated through peritoneal equilibrium test results. Results: 12 patients were males and 2 females, with a median age of 72.13 years. The mean following time was 52.5 months. Symptoms of HF improved in 5 patients, with an upgrade of New York Heart Association (NYHA) Functional Classification and improvement in ejection fraction. NHYA remained the same in 6 PD treated individuals, despite and improvement of absolute ejection fraction. At the beginning of PD, the mean Charlson Score value was 5.7 ± 2.3, which reduced to 5.3 ± 2.6 by the end of observation time ($p<0.01$; $r=0.984$). Six patients presented one episode of decompensated heart failure needing hospitalization, with a median length of stay of 2 days. During the observation period 2 patients died, 1 from an acute hemorrhagic stroke and the other with a septic shock. Discussion: PD treatment in refractory HF seems to be effective since it improves quality of life and functional class.

Keywords: Cardio-Renal Syndrome, Heart Failure, Peritoneal Dialysis, Diuretic Resistance.

7.1 Introduction

Heart failure (HF) is a public health problem which prevalence has been rising globally as a result of an increase in longevity. It is a deadly and costly disease with a high symptom burden and decreased quality of life (Kazory and Bargman, 2019). Fluid retention and congestion are hallmarks of decompensated HF, and diuretics are a cornerstone of the management of these patients. With chronic therapy diuretic resistance is common and it's defined by an attenuation or absence of the maximal diuretic effect that ultimately limits sodium and chloride excretion (Jardim *et al.*, 2018; Gupta, Testani and Collins, 2019).

Most patients with treatment-resistant HF have underlying cardiorenal syndrome (CRS). Numerous interventions have been tried to overcome diuretic resistance, including furosemide adjustment (higher dose, more frequent administration and change route to intravenous), sequential nephron blockage, intravenous inotropic therapy, dual chamber pacing or resynchronization therapy (Mehrotra and Kathuria, 2006; Jardim *et al.*, 2018).

Extracorporeal ultrafiltration (EUF) has been used for acute decompensated diuretic-resistant HF with a favorable outcome. However, it is not recommended as long-term treatment in CRS due to hemodynamic complications, high cost, and vascular access-related problems (Shao *et al.*, 2018). Peritoneal dialysis (PD) is a home-based therapy that achieves salt and water removal though two important physiologic processes: diffusion that leads to solute clearance and convection that removes water from the blood stream into the peritoneal cavity (Rippe, Stelin and Haraldsson, 1991).

The reasons why PD is a great therapeutic option for CRS are multiple. It offers gentle ultrafiltration with minimal impact on hemodynamics that result in a lower degree of neurohumoral stimulation and in slower decline of renal function, factors known to be associated with survival. Peritoneal ultrafiltration leads to effective continuous solute clearance,

such as potassium, allowing better up-titration of HF pharmacological treatment. This technique is also not associated with myocardial stunning and seems to achieve a reduction in inflammatory burden (Zemel *et al.*, 1994; Misra *et al.*, 2001; Núñez *et al.*, 2012).

The aim of this study was to describe a single-center experience in using PD to treat CRS patients with decompensated HF and analyze the safety of this technique in this group of patients, especially regarding infections.

7.2 Methods

Retrospective study of 14 patients with diuretic resistant HF, in a single-unit PD program. Patients started PD between November 2009 and November 2017 and the follow-up included the period from the first day of PD until May of 2019. Inclusion criteria were age ≥ 18 years old; symptoms and signs of severe refractory congestive HF despite optimal pharmacological therapy; non-end stage kidney disease; absence of criteria for a heart transplant; heart failure diagnosis by echocardiographic structural abnormality, systolic dysfunction, diastolic dysfunction, or a combination of these abnormalities in patients with resting or/ and exertional symptoms of heart failure. The following data were registered at the beginning and at the end of follow up: New York Heart Association (NYHA) functional classification, estimated glomerular filtration rate (eGFR) through CKD-EPI formula.

Charlson's Comorbidity Index; Doppler-echocardiography (DE) with determination of left ventricular ejection fraction (LVEF). Hospitalizations due to decompensated heart failure, their length of stay and peritonitis rates were also accounted during PD treatment. Demographic characteristics, previous comorbidities, etiology of HF and PD prescription were recorded at baseline.

PD adequacy was evaluated through peritoneal equilibration test (PET) with classification of peritoneal transport type by creatinine and urea dialysis-to-plasma (D/P) ratio, dialysis dosage by measurement of the ratio between dialyzer urea clearance over time and patient's volume of urea distribution (Kt/V), ultrafiltration by free water removal 1 hour after a 3.86% dextrose solution dwell (UF 3.86%), residual creatinine clearance, and normalized protein catabolic rate (nPCR).

Numerical data are presented with median and interquartile range (IQR) or mean and standard deviation (SD) according to its distribution. The level of significance used was $p<0.05$ (two- sided). All data was analysed using SPSS Statistics® version 23.0.

The study was approved by the ethical committee of the local hospital, and written consent was obtained from all patients. All personal information were analyzed according to data protection law.

7.3 Results

We followed a cohort of 14 patients with HF, 12 were males (85.7%) and 2 females (14.3%). Patients' characteristics are described on Table 1.

Referring to PD treatment itself, 11 patients started PD ab initium but 3 (21.4%) started treatment with EUF, which was suspended due to hemodynamic instability. PD prescription is summed on Table 2 and the type of solutions refers to patients last therapeutic scheme. Only one patient was on automated peritoneal dialysis and another one on assisted peritoneal dialysis. All patients were treated with icodextrin solutions (ICO).

Table 1: Patients baseline characteristics.

Characteristics	N (%)
Male Gender	12 (85.7)
Median age (years)	72.13 (IQR 42.5-75.38)
Mean following time (months)	52.5 (SD 18-95)
Comorbidities	
Hypertension	7 (50)
Diabetes mellitus	7 (50)
Myocardial infarction	3 (21.4)
Hepatitis C infection	2 (14.3)
Etiology of heart failure	
Arterial hypertension	7 (50)
Ischemic cardiopathy	3 (21.4)
Valvular cardiopathy	3 (21.4)
Congenital cardiopathy	1 (7.1)
IQR: Interquartil range; SD: Standard deviation	

Table 2: Peritoneal Dialysis prescription and efficacy.

PD precription	N (%)
PD first	11 (78.6)
PD modality	
CAPD	13 (92.9)
APD	1 (7.1)
Assisted Peritoneal Dialysis	1 (7.1)
Type of solutions	
ICO 1 night exchange	6 (42.9)
ICO + glucose 1.36 + 1.36 + 2.27 + 2.27%	5 (35.7)
ICO + glucose 1.36 + 1.36 + 1.36%	2 (14.3)
ICO + glucose 1.36 + 2.27 + 2.27+ 3.86%	1 (7.1)
Total volume PD solutions (L)	8 (IQR 6.72-8.64)
Residual diuresis (mL)	1364.3 (SD 849.1-1879.52)
Kt/v	2 (IQR 1.93-2.64)
nPCR (g/Kg/day)	0.93 (SD 0.8-1.06)
Fluid removal (mL)	1940 (SD 1456.7-2423.3)
D/P	0.66 (SD 0.60-0.72)
UF after a 3.86% glucose solution (mL)	709.2 (SD 524.6-893.8)
GFR (mL/min/1.73m^2)	4.71 (SD 3.3-6.1)
APD: Automated peritoneal dialysis; CAPD: Continuous ambulatory peritoneal dialysis; D/P: Four-hour dialysate/plasma creatinine; GFR: Glomerular filtration rate; ICO: Icodextrin solution; Npcr: normalized protein catabolic rate; PD: peritoneal dialysis; UF: ultrafiltration.	

There was a relieve in symptoms of HF in 5 patients (patient 8, 11, 12, 13 and 14), with an improvement of NYHA functional class and in LVEF (Figure 1).

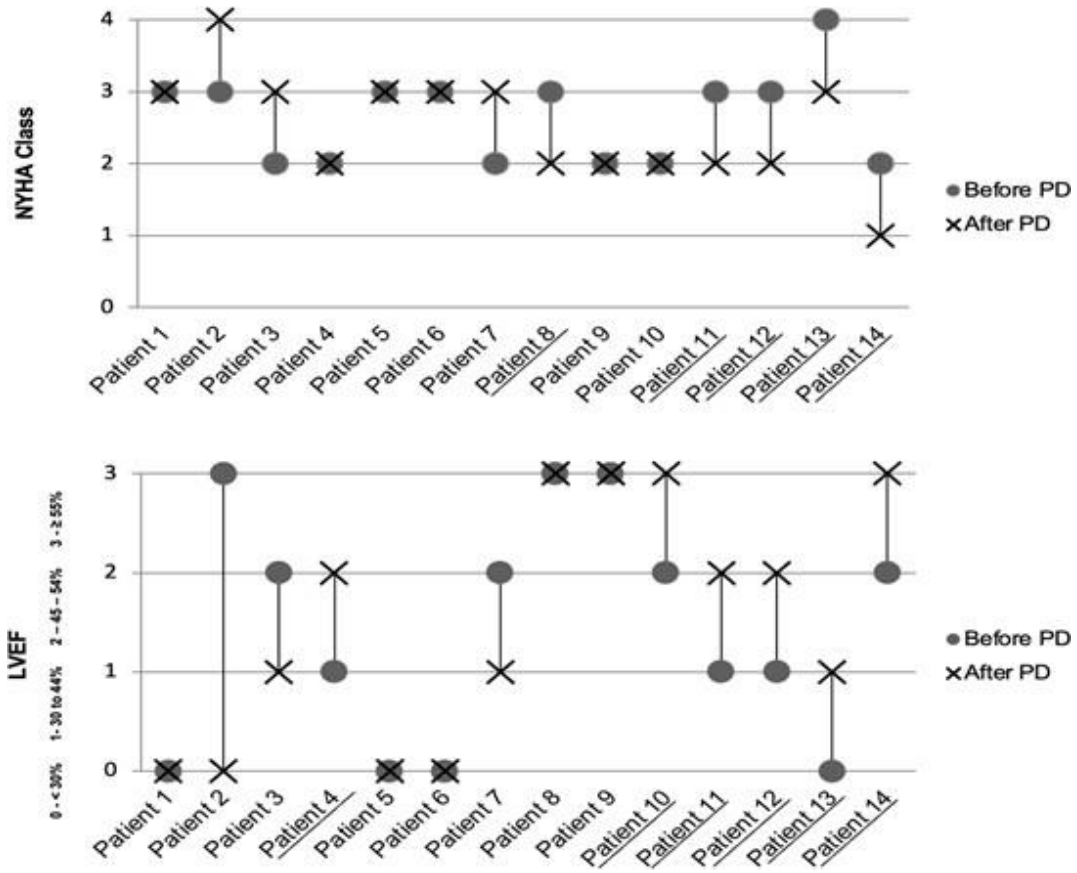

Figure 1: Variation of NYHA class and LVEF with PD treatment. LVEF: Left Ventricular Ejection Fraction; NYHA: New York Heart Association; PD: Peritoneal dialysis.

NHYA remained the same in 6 PD treated individuals, despite and improvement of absolute LVEF value (Table 3). HF progressed with worsening NYHA class and LVEF in 3 patients. However, there was a global significant increase in absolute LVEF value with PD treatment (p=0.016; r=0.63).

Table 3: Absolute left ventricular ejection fraction value.

Patient	Ejection Fraction (%)		Patient	Ejection Fraction (%)	
	Before PD	After PD		Before PD	After PD
1	<25	27	8	56	75
2	55	29	9	59	67
3	46	35	10	51	63
4	44	51	11	33	49
5	27	29	12	31	52
6	<25	28	13	<25	41
7	45	43	14	50	72
PD: Peritoneal dialysis.					

During the observation period 7 patients were transferred to HD. In 3 cases this was led by peritonitis episodes and in 4 by ultrafiltration failure. Six patients presented one episode of decompensated heart failure needing hospitalization with a median length of stay was 2 days. Two patients died, one from an acute hemorrhagic stroke and the other with septic shock. The mean survival time was 62 ± 14.1 months (range 52-72).

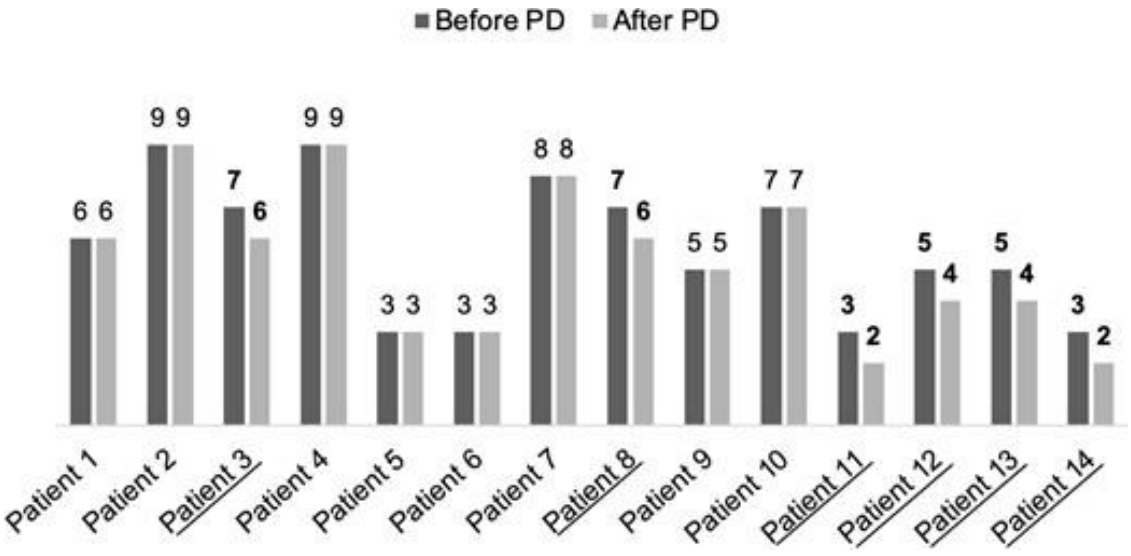

Figure 2: Variation of Charlson Comorbidity Index with PD treatment. PD: Peritoneal dialysis.

Estimated GFR (p=0.114) and diuresis (p=0.084) at baseline and at the end of follow-up were not significantly different. On the other hand, Charlson Comorbidity Index (Figure 2) a mean value of 5.7 at the beginning of PD which reduced to 5.3 by the end of observation time (p<0.001; r=0.98).

7.4 Discussion

Since the first use of PD in humans by Ganter, this technique has been used for several clinical conditions (Pratsinis, Devuyst and Leroux, 2018). The interest of PD for HF is original from the 1940s, when Scheierson used peritoneal irrigation to treat intractable edema of cardiac origin (Schneierson, 1949). In 1964 Mailloux et al. were pioneers in the successful treatment of nonuremic refractory congestive HF with peritoneal ultrafiltration (Mailloux, 1967). Since then, various studies have been carried out to evaluate the role of PD in refractory congestive HF. Whether PD improves survival is still a topic of dispute. Some studies demonstrated a remarkable survival advantage of PD, with 1-year survival rates as high as 85% and mean survival time of 24 ± 15 months (Bertoli *et al.*, 2014). However other trials have discrepant conclusions with a survival at the end of the first year with this technique as low as 12.5% (Cnossen *et al.*, 2012; Kunin *et al.*, 2013; Courivaud *et al.*, 2014).

In our study the survival was much higher than the ones reported, probably due to our inclusion criteria. We selected individuals with refractory congestive HF despite optimal pharmacological therapy which included patients with persistent symptoms during daily physical activity (NYHA class II), for less than ordinary physical activity and at rest (NYHA III and IV respectively). However, most studies analyzed patients with predominant NHYA classes III or IV and consequently worst prognosis (Paniagua *et al.*, 2008; Sanchez *et al.*,

2010; Koch *et al.*, 2012; François, Ronco and Bargman, 2015; Puttagunta and Holt, 2015; Kazory, Koratala and Ronco, 2019).

We also compared our patients Charlson Comorbidity index at beginning of PD and at the end of follow-up period. A significant reduction in this score after treatment with PD suggests a beneficial effect of this technique not only in short-time but also on 10-year predicted survival. There are limited data comparing the survival of refractory congestive HF patients treated with peritoneal dialysis to those with a conservative medical management and it is unknown whether a therapeutic strategy including PD improves the survival rate for this patient population.

In most of our surviving patients there was an improvement in functional status by a decrease in NYHA class. Several studies confirmed a symptomatic relieve with a reduction in NYHA grading after starting PD (Sanchez *et al.*, 2010; Cnossen *et al.*, 2012; Koch *et al.*, 2012; Núñez *et al.*, 2012; François, Ronco and Bargman, 2015). Weather this is really due to improvement in cardiac muscle function is still a question. We used LVEF as a parameter of cardiac function and our result was a value significantly higher with PD treatment. There are reports of amelioration in LVEF as high as from 29.3% to 48.5%, some even observed in patients with lower baseline LVEF(Mailloux, 1967; Courivaud *et al.*, 2014).

A low rate of hospital stays is a key to judge the success of any PD regimen for resistant HF. The majority of the PD studies have confirmed marked reduction in presentations and hospital bed days, for fluid overload and congestive symptoms, after institution of this therapy (Paniagua *et al.*, 2008; Bertoli *et al.*, 2014; Querido *et al.*, 2016). Similarly, our patients had a median length of hospital stay of 2 days. Results are variable but can be as impressive as a reduction of 90% in the number of days of hospitalization due to decompensated HF, after started PD therapy (Courivaud *et al.*, 2014). This achievement has a considerable

impact on quality of life but also on these patients cost of care (Sanchez *et al.*, 2010).

One major concern about the application of PD in decompensated HF is the potential high rates of technique failure and complications, such as peritonitis. Of the total 14 patients, 7 had at least 1 peritonitis episode during the mean following time of 52.5 ± 25.3 months. In 3 patients the recurrency of this infection led to PD drop out and 4 patients had to change to EUF due to peritoneal ultrafiltration failure. Like others, our results showed good technique survival without a higher peritonitis rate (Shao *et al.*, 2018).

In our group of patients, PD was a safe and effective technique in relieving resistant HF symptoms, with improvements in LVEF measured by doppler-echocardiography and in 10-years predicted survival, without worsening renal function. This study has several limitations as it is a prospective and nonrandomized trial with a small number of patients, including those with mild HF symptoms. Also, diagnosis of CRS was clinical which can lead to misclassification and/or underestimation of other etiologies for renal failure rather than heart disease.

Our study corroborates the accumulating evidence that points to PD as a beneficial adjunct to medical therapy in patients with chronic refractory HF without end-stage renal disease. This home- based therapy can efficiently extract sodium-rich fluid resulting in decongestion which provides a better functional status and quality of life with significant savings in health-care expenditure for CRS patients.

The retrospective analysis of data and the low number of patients analyzed are the main limitations of this research.

CHAPTER VIII

DISCUSSION

CHAPTER VIII

8. Discussion

These studies provided evidence that support strategies for tailored prescriptions in vulnerable patients.

1. Fibrosis is already present in the membrane at pre-dialysis with individual variability in membrane status and phenotypes (Chapter III).

Peritoneal dialysis (PD) is a life-sustaining renal replacement therapy used for around 10–15% of patients with ESRD worldwide (Cho *et al.*, 2021). It is equivalent to hemodialysis with respect to adequacy, mortality, has lower cardiovascular impact (Heaf and Wehberg, 2014), and provides better preservation of residual renal function, superior quality-of-life measures, and cost savings (Van Biesen, Verbeke and Vanholder, 2006; Tam, 2009). However, increasing survival and slowing the deterioration of the peritoneal membrane continue to be major problems in PD.

Classically, fibrosis is described as secondary to prolonged use of the peritoneal membrane as a dialyzer filter, a non-physiological situation. It is an important factor in patient morbidity and failure of the PD technique, resulting in transfer to hemodialysis or death. Pathophysiology of peritoneal fibrosis involves direct stimulation and lesions promoted mostly by bioincompatible solutions and infections, with consequent chronic inflammation.

Our hypothesis was that uremic toxicity has different impacts on the peritoneal membrane, conditioning histological changes during the progression of chronic kidney disease, starting in uremic stages that preceded initiation of PD. Unraveling the mechanisms of peritoneal fibrosis development is still at the heart of efforts to slow peritoneal membrane function decline and improve PD outcomes.

To understand the nature of progressive membrane injury, it is important to acknowledge the variability in membrane function and morphology at the beginning of the treatment and possible causes for the variation between patients as it is unlikely that the peritoneal membrane in uremic individuals is completely normal.

The opportunity to gather peritoneal tissue samples from the start of PD at catheter implantation was a cornerstone to evaluate vasculopathy, fibrosis and inflammation (Branco et al., 2023). A rigorous protocol excluded samples that have not accomplished the critical conditions. Very rare studies benefited from the availability of baseline peritoneal biopsies. Comparisons of membrane thickness among healthy individuals, hemodialysis patients and new PD patients undertaken by the Peritoneal Biopsy Registry and other biopsy studies (Davies, 2011) show that the uremic membrane is already thickened, typically to twice normal. However, the extent to which this reflects specific structural changes, such as collagen deposition or inflammatory edema, remained unclear (Davies, 2011).

In our study we were able to detail the different dimensions of structural changes and additionally evaluated its functional impact.

Future studies are needed, to further explain the intricate interplay between peritoneal fibrosis and other contributing processes such as chronic inflammation. A better understanding of the specific contribution of resident and invading cell types to peritoneal fibrosis progression may willingly allow development of novel treatment options for prolonging peritoneal membrane viability and translate this into clinical benefits for PD patients.

In our data, membrane morphology varied considerably between individuals but was not clearly different in diabetic patients nor in other sub-groups. In at pre-dialysis the membrane status was highly variable among patients. More vulnerable membranes were associated with low circulating α-Klotho, which might be a minimally invasive biomarker to

guide clinical decisions in peritoneal dialysis. A putative mechanistic rationale for our clinical findings was in an animal model of PD. This work showed the attenuation of peritoneal fibrosis by α- Klotho, through the inactivation of Wnt/β-catenin signaling pathway. The authors pointed out the recombinant α- Klotho as a potential therapeutic target in peritoneal fibrosis associated to PD, which is somehow supported by our data.

2. Membrane status in pre-dialysis was not predictive of baseline peritoneal transport neither of long-term response to PD, ie outcomes related to the technique (effectiveness) and to the patient (cardiovascular events and loss of renal residual function and mortality) (Chapter IV).

The data herein presented brings as novelty the baseline peritoneal membrane structural changes, as expressed by fibrosis, as the interface with the uremic patients, associated with humoral biomarkers of vulnerability of the cardiovascular system. On the contrary to our initial hypothesis, the fibrosis of peritoneal membrane was not a predictor of a deleterious peritoneal transport profile or other worsen outcomes of PD. Instead baseline biomarkers predicted cardiovascular morbidity and mortality in PD. Moreover, when combined with molecular and clinical indicators of aging, membrane fibrosis predictive of earlier technical failure and early MACE in PD patients.

The rationale for this investigation in pre-PD was that the association of a uremic environment toxicity and premature aging and senescence could favor prematurely aged phenotypes in peritoneal membrane, which could be associated with poorer long-term PD outcomes. These phenotypes are difficult to predict from demographic characteristics

and therefore a group of aging related indicators were investigated as baseline variables of this study and predictors of PD outcomes.

As we urge for non-invasive tools of risk stratification, selected biomarkers were investigated.

In our study the peritoneal membrane thickness at the baseline did not promote changes in peritoneal transport functions and did not negatively affect the long-term survival of both the peritoneal membrane and the patients. The properties of the peritoneal membrane are highly variable among patients and likely to be driven by genetic (Rumpsfeld *et al.*, 2004; Teitelbaum, 2021) and non-genetic factors, which might include uremia and inflammation, exposure to glucose, peritonitis, and loss of residual renal function (Mehrotra *et al.*, 2016; Zhou *et al.*, 2016; Branco et al., 2023). The mechanisms involved in this variability are poorly defined and the strategies to reduce complications and improve outcomes in patients treated with peritoneal dialysis are limited, which was a motivation for this long-term follow-up.

According to our data, the association of peritoneal membrane status and vascular aging might have been overlooked and constitute a novel link in the shared mechanisms of persistent uremic phenotype and premature ageing (Ebert *et al.*, 2020). In fact, the membrane might not represent a risk factor but a marker of a profile of vulnerability of a patient. Importantly, we found that even older patients and those with a diseased cardiovascular system at the baseline and a worsen peritoneal membrane might take advantage of this home-based modality of renal replacement therapy, with no differences in mortality, a good survival in the technique, despite a higher risk of cardiovascular events. Substantial cardiovascular risk persists in CKD patients despite treatment of established cardiovascular risk factors, such as hypertension and dyslipidemia, and there are uremia-profiles, which

might be predictors of this risks, paving the way for personalized interventions and highlighting the need for novel molecules to control the unbalance status of protective and deleterious that constitutes uremia

3. Investigate if membrane status in pre-dialysis is related to the individual´s uremic fingerprint in the blood (Chapter II), using a panel of uremic toxins known to be related to ageing and fibrosis in other tissues.

As the uremic environment is associated with premature aging and senescence, uremic toxin could signalize a prematurely aged phenotype in peritoneal membrane.

An impaired renal function leads to the accumulation of nitrogenous substances in the blood that would normally be excreted in the urine. At progressively increasing concentration, these substances exert toxic effects, which eventually become apparent as symptoms of *uremia*. The altering effects of the uremic milieu on the immune system has been described as *uremic inflammation* (Cobo, Lindholm and Stenvinkel, 2018; Ebert *et al.*, 2020) and include mechanisms of both immunoactivation and immunosuppression. Uremic inflammation resembles the premature ageing phenotype in many ways (Ebert *et al.*, 2020). The choice for this panel of uremia-modified proteins was based on the existing knowledge of their modification by uremia, their role in aging-related diseases and their connection to the mechanisms of inflammation and fibrosis in other organs/tissues (de Boer *et al.*, 2012; Moe, 2012; Pedersen *et al.*, 2013; Grabner and Faul, 2016; Takenaka *et al.*, 2019; Salgado, Goes and Salgado Filho, 2021).

While the underlying mechanisms are not known in our hypothesis, “uremia” is an umbrella for different blood fingerprints which can have

different impacts on the peritoneal membrane. This study is a pioneer linking α-Klotho with uremia, and the baseline fibrosis in peritoneal membrane. α-Kloto gained protagonism as a predictor of cardiovascular and membrane vulnerability as documented by our group (Branco et al., 2023).

4. Ascertain the impact of the selected ageing and fibrosis related uremic toxins before start PD in the long-term global response to PD (Chapter IV)

The data herein presented bring novelty about the impact of the status of the peritoneal membrane in uremic patients incident PD patients and before the PD start, in the long-term PD outcomes. A main discovery herein presented, and on the contrary to our initial hypothesis, was that fibrosis of peritoneal membrane before the start of PD was neither a predictor of technical failure in PD, earlier membrane failure or all-causes mortality. Instead, the status of peritoneal membrane was predictive of MACE and earlier MACE in PD and can be inferred by circulating klotho levels, ie a minimally invasive tool for risk stratification. Likewise, blood galectin-3 levels represent a putative tool to identify patients at higher risk of PD failure.

We still do not know a priori, which patients due to the genetic make-up of their peritoneum are optimal candidates for PD. Studies, even on smaller scales, that contributes to improve individual's risk assessment are lacking.

The rationale for the choice of the pre-PD variables was driven by the hypothesis that prematurely aged phenotypes of the peritoneal membrane could be associated with poorer long-term PD outcomes. These phenotypes could be favored by a uremic toxic environment,

patients' frailty, aging and senescence and are difficult to predict from demographic characteristics. Therefore, a group of aging-related indicators was investigated as predictors of PD outcomes. We found that the status of the membrane (evaluated by histomorphology, STM and by a surrogate klotho cutoff) was not associated with changes in the functions of the peritoneal transport. Moreover, the pre-PD membrane status was not predictive of long-term survival of both the peritoneal membrane and the patients.

The high variability in membrane status and functions found, even before start of PD, are likely to be driven by genetic (Rumpsfeld *et al.*, 2004; Teitelbaum, 2021) and non-genetic factors, which include uremia and inflammation, exposure to glucose, peritonitis, and loss of residual renal function (Mehrotra *et al.*, 2016; Zhou *et al.*, 2016; Branco et al., 2023). The underlying mechanisms of this variability are poorly defined and the strategies to reduce complications and improve outcomes in PD are limited, which constituted a motivation for this long-term follow-up.

We got inspired in uremia-related mechanisms and selected a panel of proteins reported to be associated with ageing-related diseases and that link these proteins to inflammation and fibrosis in other organs/tissues (Campbell, 2004; Blanco-Colio *et al.*, 2007; Winkles, 2008; Sanz, Sanchez-Niño and Ortiz, 2011; Sanz *et al.*, 2014; Ornitz and Itoh, 2015; Lu *et al.*, 2021). This profile of proteins might be modified in "uremia", an umbrella for different blood fingerprints that characterizes CKD patients and impacts peritoneal membrane (Branco et al, 2022). The screening of these molecules might help to identify patients at higher risk of poor PD outcomes. In fact, we herein identified Galectin-3 as a predictor of earlier PD failure.

Galectin-3 is a highly expressed protein in the heart and kidney (Sygitowicz, Maciejak-Jastrzębska and Sitkiewicz, 2021) is a promoter of fibrosis (Dong *et al.*, 2017) and has emerged as one of the strongest

predictors of cardiovascular disease (Hogas *et al.*, 2016; Suthahar *et al.*, 2018; Tuegel *et al.*, 2018). Although this is the first study about galectin in PD.

According to our data, there is a link between the status of the peritoneal membrane and the risk of major cardiovascular events. This novel and overlooked dimension might account for the shared mechanisms of persistent uremic phenotype, premature ageing, and fibrosis of different tissues. In fact, the membrane might not represent a risk factor but a marker of a profile of cardiovascular vulnerability. Importantly, even older patients, patients at higher cardiovascular risk and/or with a worsen status of peritoneal membrane might take advantage of this home-based modality of renal replacement therapy, with no differences in mortality, a good survival in the technique. Attention must be paid to the combination of PAD and low Klotho levels.

With aging, the vasculature undergoes structural and functional changes characterized by endothelial dysfunction, wall thickening, reduced distensibility, and arterial stiffening. Vascular stiffness results from fibrosis and extracellular matrix (ECM) remodeling, processes that are associated with aging (Harvey *et al.*, 2016). The relation of lower α-klotho and membrane fibrosis (Branco, et al, 2022) further supports the existence of shared mechanisms in aging, tissue fibrosis and cardiovascular system. The link between PAD and PD outcomes needs to be disclosed. Our study also highlights the need for more research to elucidate the fibrotic pathways reflected by reductions in Klotho and increased Galectin-3 specifically in the vulnerable CKD population.

PD outcomes were not associated with the frailty test applied, chosen due to its simplicity and easily for daily clinical practice and validation in Portuguese (Dent, Kowal and Hoogendijk, 2016; Perna *et al.*, 2017) that might highlighting the relevance of bring to the clinical practice risk assessment tools related to biological markers.

Substantial cardiovascular risk persists in CKD patients despite treatment of established cardiovascular risk factors, such as arterial hypertension and dyslipidemia. The knowledge about the uremia-profiles which might be predictors of these risks will pave the way for personalized interventions. It also, highlight the need for novel drugs to control the unbalance status of protective and deleterious molecules that constitutes uremia.

Due to the low number of mortality events we were unable to explore the impact of these biomarkers in mortality but found an association with worse combined survival and shorter time to CV event**.**

Since we applied strict inclusion criteria we faced limitations in these studies. First, the patient sample, gathering patients from a single Academic Hospital PD center, is modest, although empowered by the availability of baseline peritoneal biopsies. Important and clinically relevant associations of non-invasive tools with outcomes were evidenced although residual renal function and peritoneal transport as well as peritonitis events, as important modifiers of patient survival, should be further explored as time-dependent variables

Our study has several strengths: 1. inclusion criteria were highly rigorous concerning quality of biopsy samples, 2. biomarker measurements were performed in the same laboratory to ensure measurement consistency across the pooled cohort, 3. we analyzed an anatomical territory with fibrosis and achieved a long follow-up period 4. Investigation applied high standard statistical methodology under the scrutiny of experts

Finally, a larger scale cohort study with longitudinal follow-up is needed to further confirm our findings and provide comprehensive insight into the mechanism. Prospective studies will be needed to determine whether treatment of klotho deficiency may be a promising strategy to decrease the burden of comorbidity in peritoneal dialysis patients.

But the most relevant achievement was to document that those non-invasive tools are able to stratify vulnerable patients and peritoneal membrane, allowing tailored prescriptions.

5. Analyze the repercussion of peritoneal dialysis on cardiovascular disease (Chapter IV, V, VI and VIII)

The reasons why PD is a better therapeutic option for patients with cardiovascular disease are multiple. It offers gentle ultrafiltration with minimal impact on hemodynamics that result in a lower degree of neurohumoral stimulation and in slower decline of renal function, factors known to be associated with survival. Peritoneal ultrafiltration leads to effective continuous solute clearance, such as potassium for example, allowing better up-titration of risk-modifying pharmacological treatment. This technique is also not associated with myocardial stunning and seems to achieve a reduction in inflammatory burden. These advantages are confirmed when treating patients with established disease and for congenital heart disease in children undergoing cardiac surgery (Chapter V). We have also shown that even patients with vulnerable phenotype of biological accelerated ageing could take advantage of this home-based modality of renal replacement therapy, with no differences in mortality and had good survival in the technique, despite a higher risk of CV events (Chapter IV).

6. We documented the benefit of peritoneal dialysis in cardiovascular disease with resistance to diuretics resistance (Chapter VII). We Designed a protocol proposing the value of ultrafiltration in the Diuretic resistant heart failure

Comments:

The membrane score is not a risk factor but a marker of patient vulnerability.

We have shown that even patients with vulnerable phenotype of biological accelerated ageing could take advantage of this home-based modality of renal replacement therapy, with no differences in mortality and had good survival in the technique, despite a higher risk of CV events.

This work unveils a putative link of peritoneal membrane fibrosis and vulnerability of cardiovascular system. Patients with poor long-term outcomes in PD have an aging-related vulnerability at the baseline, which might have important implications to personalized therapeutic interventions in PD patients.

CHAPTER IX
REFERENCES

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