Study Title: **Impact of medium cut-off membrane on FGF-23 level in haemodialysis patients**

Short Title: **Impact of medium cut-off membrane on FGF-23 level in haemodialysis patients**

Sponsored by:

Northern Care Alliance NHS Foundation Trust

Salford I Oldham I Bury I Rochdale I

Study Protocol

Version 2.2
Date 11/01/2022

IRAS ID: 262813

Sponsor ID: S19REN08-S

*Sponsor's representative: Professor Steve Woby, Managing Director of R&I
Northern Care Alliance NHS Foundation Trust, Summerfield House, 544 Eccles New Road, Salford, M5 5AP
Tel: 0161 206 5235; email: Steve.Woby@nca.nhs.uk

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature:

Date:

Name:

Katie Doyle

Position: Research & Innovation Manager

Chief Investigator:

Signature: 

Date: 7/4/2022

Name:

Dimitrios Poulikakos.

Contents

1 Study summary

End stage renal disease leads to accumulation of different sized molecules and toxins. Dialysis patients routinely undergo haemodialysis (HD) treatment three times per week. Standard dialysis treatment utilizes traditional dialysis membranes which provide size specific clearance lead to accumulation of larger molecules {such as Fibroblast Growth Factor-23 (FGF-23) molecular weight: 32 KiloDaltons (KDa)}. Accumulation of FGF-23 is thought to be associated with increased cardiovascular events and overall mortality in dialysis patients. Newer dialysis membranes so called medium cut-off (MCO) membrane (e.g Theranova by Baxter Healthcare) can potentially clear relatively bigger sized molecules due to bigger pore size No data is available regarding clearance of FGF-23 on MCO membranes. The aim of this study is to investigate the new membranes can remove FGF 23.

This study will include adult patients with renal failure who are on long term HD at Salford HD Unit and Bolton HD Unit which is a satellite unit of Northern Care Alliance NHS Foundation Trust. During this study, patients will be randomized to 1 week monitored HD treatment with MCO membrane followed by 1 week monitored conventional HD or to receive 1 week monitored conventional HD followed by 1 week monitored MCO membrane treatment. Both options will include a 3-week interval between monitored sessions during which the patients will receive conventional HD. Blood samples will be collected before and after dialysis during monitored treatment week. Blood samples will be tested for FGF-23 levels but also for calcium, phosphate levels, Vitamin D and PTH levels which are known to affect FGF-23 levels. In addition to blood sample, dialysate generated during the monitored dialysis session will be collected and analyzed for solute levels including FGF-23 levels.

Generated data will be analysed to compare clearance of FGF-23 on conventional dialysis membranes versus clearance on MCO membrane and rate of re-accumulation of FGF-23 between dialysis sessions. If the new membrane is effective in removing FGF 23 further studies should explore the impact of the new membranes on cardiovascular profiles and cardiovascular outcomes

2 Study Team Roles and Responsibilities

2.1 Contributorship

1. Dr Dimitrios Poulikakos, conceptualisation, study design, refinement of protocol, supervision, consent
2. Dr Smeeta Sinha, conceptualisation, study design, refinement of protocol
3. Dr Darren Green, conceptualisation, study design,
4. Professor Phil Kalra, conceptualisation, study design, refinement of protocol
5. Dr Sarah Withers, conceptualisation, study design, analysis of samples
6. Dr Saif Al-Chalabi, study coordinator

2.2 Sponsor contact information

Northern Care Alliance NHS Foundation Trust has accepted the responsibilities of Sponsorship for the study. The sponsor's representative is Professor Steve Woby.

Study Sponsor: Northern Care Alliance NHS Foundation Trust Trust
Sponsor Reference: S19REN08-S
Contact Details: Professor Steve Woby, Managing Director of R&I
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2.3 Funding

Northern Care Alliance NHS Foundation Trust
Kidneys for life: "Proteomic analysis of molecules exchanged using new medium cut-off dialysis membrane. "£10000 to be paid six monthly in arrears on production of expenditure report. All grants must be claimed by 31 December 2019 unless permission has been sought to extend this date.
Baxter: Investigator initiated research grant £10,600 upon receipt of executed Agreement and proof of registration on www.clinicaltrials.gov

2.4 Organisational Structure and Responsibilities

Principal Investigator: Dr. Dimitrios Poulikakos

- Design and conduct of study
- Preparation of protocol and revisions
- Organising study meetings
- Review of participants laboratory results
- Reviewing progress of the study and agreeing to changes in the protocol if necessary
- Oversight of patient safety by conducting regular meetings with study team
- Publication of study reports
- Study budget holder
- Responsible for data management plan

Sub-investigators:

1. Dr Smeeta Sinha: review of study progress, data analysis, validation, writing editing reports/manuscripts
2. Dr Darren Green: data analysis, validation, writing editing reports/manuscripts
3. Professor Philip Kalra: data analysis, review of drafts
4. Dr Sarah Withers: analysis of study samples, data analysis, writing editing reports.
5. Study Co-ordinator:Dr Saif Al-Chalabi

Study Management Team:

1. Dr. Smeeta Sinha
2. Dr Saif Al-Chalabi
3. Sarah Withers

3 Contact Details

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4 Background Information and Rationale

Despite technological advances in the field of renal replacement therapy, mortality in haemodialysis (HD) patients remains high, comparable with cancer patients. The leading cause of death is cardiovascular disease. It is postulated that specific factors related to end stage renal disease play a detrimental role in increasing cardiac risk. One factor that has been identified is FGF-23, a molecule that increases with progressive renal dysfunction and causes uraemic cardiomyopathy in experimental models. FGF 23 levels remain high in HD patients receiving current standard dialysis treatment that removes largely small molecules. The new Baxter medium cut-off membrane (MCO, Theranova, Baxter Healthcare) has a higher molecular weight cut-off than conventional membranes that facilitates removal of larger i.e. medium-sized molecules. Studies on the new MCO membrane have reported increased exchange of beta 2 microglobulin (11.8kDa), myoglobin (17kDa), kappa free light chains (22.5kDa) complement factor D (24kDa), alpha 1 microglobulin (33kDa), YKL-40 (40kDa) and lambda free light chains (45kDa) (http://www.hdxtheranova.com/clinicaldata.html). However, there are no data on the impact of these membranes on FGF-23 (32kDa)

This study will investigate the difference in clearance of FGF-23 between MCO membranes and conventional dialysis membranes. It will also analyse the pattern of production, intra-dialytic removal and re-accumulation of FGF-23.

5 Aims of the proposed research

Primary Objective:

1. Comparison of FGF 23 clearance between conventional and medium cut off membranes.

Secondary Objectives:

1. Assessment of intra-subject stability of FGF 23 clearance

2. Examine rate of FGF 23 re-accumulation rate

3. Examine impact of type of membrane, phosphate, calcium and urea clearance and PTH levels on FGF-23 clearance

6 Study Design

This will be a prospective randomised case-crossover design study. This design helps mitigate confounding variables as each patient acts as their own control.

Eligible participants will be stable haemodialysis patients on haemodialysis at Salford HD Unit and Bolton dialysis unit which is a satellite unit of Northern Care Alliance NHS Foundation Trust. Only patients who do not pass significant amount of urine (which indirectly indicates their own residual kidney function) and who have otherwise adequate dialysis treatment will be included (to allow comparison between different patients). Patients with active infection or cancer will not be included in the study as they are usually not in a steady state biochemical equilibrium to be able to assess impact of additional intervention.

Patients will be identified via electronic patient records (EPR) by the study coordinator and the research nurses from the Vascular Research Team (Research and Innovation Department, Northern Care Alliance NHS Foundation Trust). They will be approached by the research nurses and study coordinator during their routine vist for dialysis treatment and will be consented for entry into the study at their next routine appointment,. After informed consent, the patient will be randomly assigned to continue their dialysis treatments via either of the following two options via closed envelope.

Option 1: They will have one week of their usual thrice weekly dialysis treatment in a monitored fashion with traditional dialysis membrane. During this week, blood samples will be taken before and after dialysis via the dialysis circuits (without vene-puncture). Returned dialysate will also be collected and analyzed. Patients will then have three weeks of wash out period (when they will have their usual dialysis treatment). This will be followed by one week of monitored dialysis treatment with MCO dialysis membrane when again blood samples will be taken before and after dialysis and returned dialysate will be collected and analyzed.

Option 2: They will have one week of monitored dialysis treatment with MCO dialysis membrane. During this week, blood samples will be taken before and after dialysis via the dialysis circuits (without vene-puncture). Returned dialysate will also be collected and analyzed. This will be followed by three weeks of wash out period (when they will have their usual dialysis treatment). This will be followed by one week of monitored dialysis treatment with traditional dialysis membrane when again blood and dialysate samples will be taken.

Blood samples are routinely collected each month on dialysis without additional vene-puncture. Monitored weeks will be timed to coincide with such collection thereby reducing excessive blood sampling. Returned dialysate is usually discarded and its sampling is not expected to cause extra discomfort to the patient.

Data will be anonymized and statistical analysis applied.

7 Detailed Plan of Investigation

7.1 Recruitment procedure

We aim to recruit 20 patients for our study. Eligible study participants receiving regular HD at Salford and Bolton HD units will be identified by the study coordinator and the research nurses from the Vascular Research Team (Research and Innovation Department, Northern Care Alliance NHS Foundation Trust) from the electronic patient records. Patients will be approached by a member of the research team during their routine visit for dialysis treatment and will be provided written and verbal information about the study in a clinic room to ensure the privacy. Participants will have sufficient time (at least 24 hours) to consider their involvement in the study and consent may be taken at their next routine appointment by the research nurses or the study coordinator following .response to any relevant questions in a clinic room to ensure the privacy.

7.2 Study protocol

7.2.1 Inclusion/Exclusion Criteria

7.2.1.a Inclusion Criteria:

- Patients aged 18 years and over with end stage renal failure on regular haemo-dialysis

7.2.1.b Exclusion Criteria:

- Lack of capacity to consent to treatment
- Significant residual urine output (> 500 ml of urine per 24 hours)
- Poor dialysis adequacy (URR<65%)
- < 18 years old
- Active infection
- Active malignancy

7.3 Study duration

Recruitment is expected to take about 6 weeks. After recruitment, the study itself will continue for five weeks in total; extendable to seven weeks in cases where wash out period needs to be extended due to acute illness. Last patient visit is expected to be after approximately 11 weeks. Processing of biological samples, statistical analysis and reporting of the results will be completed in 12 months.

7.4 Sample collection and handling

This study requires the collection of blood, which can easily be obtained, without additional venepuncture, directly from the dialysis machine before and after a monitored session. One 5ml lithium heparin blood tube and one 5ml serum blood tube, one EDTA and one 3.2% sodium citrate tube will be collected before and after monitored dialysis sessions. The serum will be left at room temperature for 15 minutes to clot before centrifugation to separate serum from clot. Serum will be pipetted into 1.5ml cryovials for storage at -80degC. The lithium heparin blood tube will be centrifuged to separate plasma from red cells. *The sodium citrate tubes will undergo two centrifuge spins, one at low speed and one high speed to remove remaining debris.*Plasma will be pipetted into 1.5ml cryovials for storage at -80degC.

7.5 Sample analysis

Routine biochemistry bone profile will be performed at the Salford Hospital biochemistry laboratory. Samples will also be transferred to the Department of Biochemistry, Research Institute Maastricht (CARIM), Maastricht University to be analysed for

FGF-23 levels and for selected relevant inflammatory calcification and coagulation factors.

7.6 Consent procedure

Potential participants meeting inclusion criteria will be approached by members of the research team at the Bolton haemodialysis satellite unit which is part of Northern Care Alliance NHS Foundation Trust or at Salford Dialysis Unit. All members of research team will have GCP training. In a private setting, potential participants will be asked from the research team about their willingness to be informed about this research study. Members of the research team will assess capacity of the patient. If the potential participant is deemed to have capacity, sufficient details regarding the study will be provided in order to gain informed consent. Information will be provided in form of verbal discussion and written leaflet. If patients require more time to make a decision they can take the information home and consent may be taken at their next routine appointment.

Effort

will be made to make information as accurate, balanced, and free of misleading information as possible. It will be ensured that potential participants are aware that the study is designed to assess impact of MCO dialyses membrane on blood levels of a molecule with potential harmful effects and no immediate clinical benefit is likely to emerge as a consequence. It will be explained that likely benefit will be a more clear understanding of clearance of this protein with MCO membranes which will help guide future research. Blood sample storage for potential future testing will be discussed and it will be explained that the data will be pseudoanonymised. In order to minimize risk of pressured decision, it will be clarified that their decision to participate in research study will have no bearing on their regular dialysis treatment or future interaction with dialysis/clinical staff. Alternative option of continuing with their usual dialysis will be emphasized to allow consent without any pressure.

7.7 Outcome measurement

FGF 23 clearance based on blood and dialysate testing for FGF 23 levels.

7.8 Withdrawal criteria

Participants will be allowed to withdraw from the study at any point. We will not collect any further data from this participant however will use data available during their time in the study for the final study analysis. If it is in the participant's best interest the clinician in charge of the study may also choose to withdraw the participant.

8 Regulatory and Ethical Considerations

8.1 Study conduct

- The study will be conducted in accordance with the UK Policy Framework for Health and Social Care Research and other applicable guidance.
- The study will not commence until all regulatory approvals are in place, which will include HRA Approval, REC Approval and confirmation from local R&D that the Trust has capacity and capability to carry out the research.

8.2 Monitoring and audit

- The study will be subject to the standard procedures for monitoring and auditing of studies by the sponsor.
- Any changes to the protocol will be agreed with the sponsor prior to submission to NHS research ethics committee for review with the exception of where urgent safety measures apply.
- All staff working in the study will have completed appropriate training to undertake the duties delegated to them by the Principal investigator such an ICH-GCP.

8.3 Protocol deviations

- Any deviations to the protocol will be reported to the sponsor within 24 hours of the occurrence to allow an impact assessment to be completed.
- Consideration will be given to the nature of the deviation, its causes and the potential impact on the study.
- Where necessary, a deviation from the protocol may lead to an amendment to the protocol

8.4 Study progress reports

The PI and research team will submit progress reports to the Sponsor as requested and prior to submission to NHS REC, in accordance with the terms and condition of the study approval.

8.5 Stopping rules

It is not anticipated that the study will be stopped prior to its intended end-date. However, the study will be halted if:

- New information comes to light which means that the aims of the study are futile.
- Safety issues come to light regarding the intervention.
- Resources to conduct the study are no longer available.

9 Record keeping and data management

- All outcome measures will be recorded on standardised data collection forms. These, together with the exclusion criteria screening proformas and consent forms, will all be stored in a secure area at the Vascular Research Team, Research and Innovation Department at Northern Care Alliance NHS Foundation Trust.
- Given this is a prospective data collection, the data will be pseudo anonymised before being stored. A separate database with matched patient study code numbers will contain patient names, date of birth, hospital numbers and contact details. The data will be stored on the NHS computer with following layers of security.
    - Patient data will only be saved on NHS computers.
    - NHS Computer will be located in secure NHS facility (swipe card/code protected entrance to the room) where only health care professionals are allowed to access.
    - Computers will be protected by start-up password.
    - Files will be password protected.
    - The PI, research nurses and study coordinators will have access to the data.
- Under the General Data Protection Regulation (GDPR), data will be anonymized, encrypted where necessary and access will be strictly restricted. Use of laptops and other portable devices will be avoided. Where it is necessary for them to be used, data will be encrypted and the data uploaded onto a secure server or desktop as soon as possible and the data removed from the portable device as soon as possible and using appropriate data destruction software. Under no circumstances, patients' or research participants'' personal data will be stored on a home or other personal computer.

10 Statistical analyses and data handling

Statistical support has been provided by Calvin Heal, Biostats Research Assistant (Research Assistant at University of Manchester.
The clearance of FGF 23 will be calculated as ($FGF23_{pre}$ – $FGF\ 23_{post}$)/$FGF\ 23_{pre}$ for each dialysis session and intra-subject stability will be assessed with repeated measures ANOVA or Friedman test, depending on the distribution of the data.

The re-accumulation rate of FGF 23 will be measured as ($FGF23_{pre}$ – $FGF\ 23_{post}$ previous HD session)/$FGF\text{-}23_{post}$ previous HD session.

The average FGF 23 clearance values on conventional membranes will be compared with average values on medium cut-off membranes with paired-t test or Wilcoxon signed-rank test depending on the distribution of the data (irrespective of whether intra-subject stability is confirmed or not)

Further analysis will depend on the results of the intra-subject stability of FGF 23 clearance, that is, either it may be appropriate that the first HD session of each monitored session will be used for the analysis or each dialysis may need to be treated as separate event. In this case calcium, phosphate, URR, PTH, type of membrane and FGF -23 will be entered in a multiple linear regression analysis to determine relationships with FGF 23 clearance.

The data collected will be stored pseudo anonymised. A separate database with matched patient study code numbers will contain patient names, date of birth, hospital numbers and contact details. The data will be stored on the laptop computer and will be password protected with restricted access.

Power Calculation;
Sample size was calculated calculation was based on the recent study by Damasiewicz et al (6). If the log difference is 7.47 or greater, with 20 patients the study has a 80 % chance of detecting it at a two-sided 0.05 significance level.

11 Dissemination of Results and Publication Policy

Results will be presented in local/regional meetings and published in a journal. Results of the study will be publicised more widely via the Citizen Scientist initiative (see http://www.citizenscientist.org.uk/).

12 References

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2. Gao S, Xu J, Zhang S, Jin J. Meta-Analysis of the Association between Fibroblast Growth Factor 23 and Mortality and Cardiovascular Events in Hemodialysis Patients. Blood Purif. 2019;47 Suppl 1:24-30. doi: 10.1159/000496220. Epub 2019 Jan 30.

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4. Kirsch AH, Lyko R, Nilsson LG, Beck W, Amdahl M, Lechner P, Schneider A, Wanner C, Rosenkranz AR, Krieter DH. Performance of hemodialysis with novel medium cut-off dialyzers. Nephrol Dial Transplant. 2017 Jan 1;32(1):165-172. doi: 10.1093/ndt/gfw310.

5. Damasiewicz MJ, Lu ZX, Kerr PG, Polkinghorne KR.The stability and variability of serum and plasma fibroblast growth factor-23 levels in a haemodialysis cohort. BMC Nephrol. 2018 Nov 14;19(1):325. doi: 10.1186/s12882-018-1127-7.

13 Appendix

N/A

14 List of Abbreviations

FGF-23	Fibroblast growth factor 23
HD	Haemodialysis
PTH	Parathyroid hormone
URR	Urea reduction ratio
MCO	Medium cuff off membrane
Kda	Kilodaltons