

Predicting the post-dialysis rebound of β 2-microglobulin

Short Title: β 2M post-HD rebound prediction

Protocol Reference/RD no: 2025-60

Version number: 1.0

Version date: 07.10.2025

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Study Sponsor: East & North Hertfordshire NHS Teaching Trust, Lister Hospital, Coreys Mill Lane, Stevenage, SG1 4AB

IRAS ID: 365263

This protocol has regard for HRA guidance

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[Project Full Title]

Predicting the post-dialysis rebound of β2-microglobulin

[Short Title]

β2M post-HD rebound prediction

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Protocol RD Number:	2025-60
IRAS ID:	365263
REC Number:	26/WM/0022
ISRCTN Number:	NA
Date:	07.10.2025
Amended:	

Protocol Signature Page

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Approved by Chief Investigator:

Shaza Adam

M.B.,B.S | MRCP

Date: 07/10/2025

Investigator's Agreement

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 requirements.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor, East & North Hertfordshire Teaching NHS Trust.

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.

Signature

Shaza Adam

Name of Principal Investigator

Date 07.10.2025

Study Administration

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Protocol Synopsis

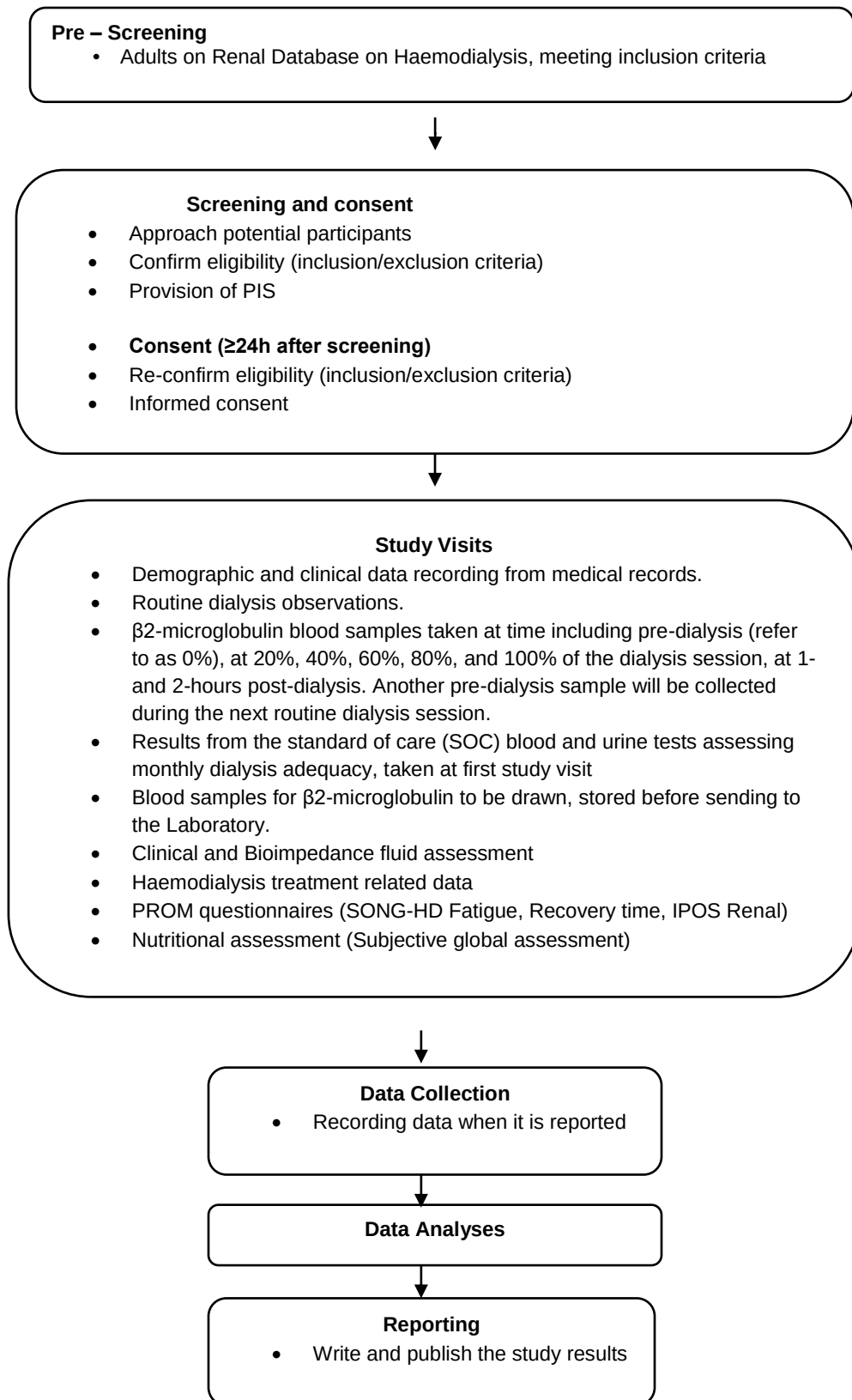
Study Title	Predicting the post-dialysis rebound of β2-microglobulin
Short Title	β2M post-HD rebound prediction
Type of Study	Cross-sectional prospective non-interventional Study
Study Period	01/02/2026 -01/08/2026
Objectives	Primary Objective: To develop a predictive model for post-dialysis rebound of β2-microglobulin, facilitating more precise assessment of solute clearance Secondary objectives: The findings from this pilot study will provide the foundation for submitting a larger grant proposal aimed at evaluating the potential of β2-microglobulin as a dialysis quality marker.
Outcome measures	Primary outcomes measures: 1. To develop a predictive model for the post-dialysis rebound of β2-microglobulin. This will be developed using a predictive model based on β2-microglobulin measurement obtained at multiple time points including pre-dialysis, intradialytic and post-dialysis. β2-microglobulin samples will be taken at time points including pre-dialysis (referred to it as 0%), at 20%, 40%, 60%, 80%, and 100% of the dialysis session. After dialysis samples will also be taken at 1- and 2-hours post-dialysis and additionally pre-dialysis at the next routine dialysis session (after approximately 48 hours). 2. Using the above estimate of post-dialysis β2-microglobulin, to derive an accurate estimate of β2-microglobulin removal ratio and renal clearance as a measure of dialysis adequacy/quality. We will apply a recently developed two-pool kinetic model for β2-microglobulin to the data to assess the performance of this kinetic model in predicting both the levels during dialysis and the post-dialysis rebound. This will include assessing the model's ability to predict reduction ratio of β2-microglobulin and assessing how the modelled total dialysis session β2-microglobulin removed compares to the measured reduction ratio in this study. Secondary outcome measures We will measure:

	1. Small panel of solute clearance markers (urea and creatinine). 2. Patient-Reported Outcome Measure (PROM) that are linked to quality dialysis, dialysis-related symptoms and measurable physiological changes that occur during dialysis (e.g. blood pressure, hypotension).
Study Design and Methodology	This will be a cross-sectional single center study recruiting 30 adult patients with End Stage Renal Failure (ESRF) on hemodialysis. Participants will have 2 study visits, during their routine hemodialysis session. During the study visits the participants will have the following data recorded: 1. Demographic and clinical data. 2. β2-microglobulin blood samples taken at time including pre-dialysis (refer to as 0%), at 20%, 40%, 60%, 80%, and 100% of the dialysis session, at 1- and 2-hours post-dialysis. Another pre-dialysis sample will be collected during the next routine dialysis session. 3. Results from the standard of care (SOC) blood and urine tests assessing monthly dialysis adequacy, taken at first study visit 4. Routine dialysis observations. 5. Clinical And Bioimpedance fluid assessment (Standard of care) 6. PROM questionnaires (SONG-HD Fatigue, Recovery time, IPOS Renal) 7. Nutritional Assessment (Subjective global assessment) 8. Dialysis Treatment Parameters. The first study visit will be coordinated with the routine dialysis adequacy urine and blood test, which includes monthly pre- and post-dialysis measures of solute clearance and 48 hours Urine collection. Using both regression-based and software modelling, we will derive an algorithm to predict the equilibrated post-dialysis β2-microglobulin concentration from the measured β2-microglobulin results during the dialysis session. This approach will enable accurate estimation of β2-microglobulin clearance as a measure of dialysis adequacy.

Indication	β2-microglobulin is of significant interest as an indicator of middle molecule solute clearance in haemodialysis and haemodiafiltration. This study aims to develop a predictive model for the post-dialysis rebound of β2-microglobulin, thereby enabling a more accurate assessment of middle molecule solute clearance achieved by dialysis. This study will permit an algorithm to predict post-dialysis β2-microglobulin and we anticipate applying for funding, likely through a NIHR RfPB grant (or similar) to support a larger use of clearance of this molecule to assess dialysis quality.
Planned Trial sites	Single Centre
No of Participants	30 participants
Eligibility Criteria	Inclusion criteria: • Age 18 years or above. • Ability to give informed consent. • End Stage Renal Disease established on maintenance haemodialysis for at least 3 months. Exclusion criteria: • Inability to give informed consent. • Acute infection in last 2 weeks.
Treatment	Not applicable
Assessment Schedule	Blood β2-microglobulin samples will be collected at defined time points during two routine and consecutive haemodialysis sessions, referred to as Session HD1 and Session HD2, to comprehensively characterize β2-microglobulin kinetics. 1. Session HD1(Initial Study Visit): A. Results of pre-dialysis sample collected at time 0% (immediately before starting dialysis), representing baseline β2-microglobulin level. This will be obtained as part of the routine 6 monthly dialysis β2-microglobulin testing. B. Five intra-dialytic samples collected at 20%, 40%, 60%, 80%, and 100% of the total dialysis duration, to monitor β2-microglobulin removal during the session. C. Two post-dialysis samples collected at 1 hour and 2 hours after completion of Session HD1, to assess post-dialysis β2-microglobulin rebound.

	D. Results from the standard of care (SOC) blood and urine tests assessing monthly dialysis adequacy. Derived from routine monthly dialysis adequacy testing. 2. Session HD2 (Subsequent routine dialysis Session and last study visit): A. Pre-dialysis sample collected at time 0% of Session HD 2 (immediately before dialysis begins), providing further information on interdialytic β2-microglobulin levels Other assessment measures will be collected during dialysis session HD1; these include demographic data, routine dialysis observation, dialysis treatment related data, PROM and nutritional measures.
Statistical Methodology and analysis	Using both regression-based and software modelling, we will derive an algorithm to predict the equilibrated post-dialysis β2-microglobulin concentration from measured β2-microglobulin levels during dialysis. This approach will enable accurate estimation of β2-microglobulin clearance as a measure of dialysis adequacy.

Figure 1. Trial Flow chart



Study Glossary

<i>B2M</i>	<i>β2-microglobulin</i>
CI	Chief Investigator
ENHT	East and North Hertfordshire Teaching NHS Trust
ESRF	End Stage Renal Failure
HD	Haemodialysis
HDF	Haemodiafiltration
kDa	Kilodalton
PI	Principal Investigator
PROM	Patient-Reported Outcome Measure
RDA	Dialysis-Related Amyloidosis
REC	Research Ethics Committee
RKF	Residual Kidney Function
SOC	Standard of Care
SOPs	Standard Operating Procedures

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1. INTRODUCTION

1.1 Background

β 2-microglobulin, is an 11.8 kDa polypeptide that forms the non-variable chain of Major Histocompatibility Complex I (MHC class I) which is present on the surface of all nucleated cells (1). Upon dissociation of MHC class I, β 2-microglobulin is continuously released from the surface of cells into circulation. The production rate of β 2-microglobulin is constant, at a level of 2–4 mg/kg/daily, and in normal subjects, the plasma concentration varies from 1 to 3 μ g/mL with a half-life of 3 hours approximately. Most of the β 2-microglobulin is eliminated by glomerular filtration and subsequent reabsorption and catabolism by the proximal tubules. Therefore, the levels of β 2-microglobulin are inversely related to the glomerular filtration rate; in end stage renal failure (ESRF) patients, serum β 2-microglobulin levels increase up to 60-fold (2).

In patients with chronic kidney disease (CKD) and ESRF undergoing dialysis, serum β 2-microglobulin levels are influenced primarily by Residual Kidney Function (RKF), inflammation, and nutritional status. Accumulation of β 2-microglobulin predisposes dialysis patients to conditions such as dialysis related amyloidosis and increase risk of deaths (3- 4).

Patients with chronic kidney disease suffer from various systemic diseases due to uremic toxins, cardiovascular disease, infections and malignancies. Among uremic toxins related syndrome (a condition that results from retention of solutes with biologic toxicity), Dialysis-Related Amyloidosis (DRA) and it is characterized by the deposition of amyloid fibrils predominantly in bone and joint tissues, leading to progressive structural damage. β 2-microglobulin has been identified as the principal component of these amyloid deposits. Incidence of this complication increases with the duration of dialytic therapy and the age of the patient (5). Therefore, β 2 microglobulin is both directly a uraemic toxin and a marker of residual renal function since its levels are related to level of RKF.

Clearance of β 2-microglobulin and other middle molecules is enhanced by the convective transport achieved in high-flux hemodialysis (HD) and, more effectively, in hemodiafiltration (HDF), where both convective and diffusive mechanisms operate. The extent of dialysis clearance depends on measurable dialyzer factors such as sieving coefficient and protein binding, as well as patient-related factors. Since β 2-microglobulin is largely distributed in the extracellular fluid, its removal during dialysis requires transcapillary transfer from the interstitial to the vascular compartment at a rate of about 100 mL/min. This relatively slow intercompartmental exchange limits total body clearance because dialysis removes it principally from the blood. This leads to a post-dialysis rebound in plasma β 2-microglobulin concentration. Our unit has previously published work on the kinetics of β 2-microglobulin showing it rebounds significantly after dialysis (6). These kinetics have previously been described by a two-compartment model which divides the body into two “pools”, and this model has been previously applied to other middle molecules such as cystatin C of similar molecular weight (7). In a previous inpatient study at Lister Hospital, post-dialysis equilibration of β 2-microglobulin was observed to occur approximately 2 hours after treatment, consistent with the two-compartment kinetic model.

To understand the amount of β 2-microglobulin removed from pre- and post-dialysis blood levels of this molecule, one needs to account for the rebound effect since the post-dialysis level will over-

estimate the removal (8). This study therefore is designed to generate a method of estimating the level of β 2-microglobulin at the end of dialysis after the rebound has occurred so we can estimate the amount removed during dialysis.

1.2 Preclinical/Clinical Data

It is well recognized that presence of Residual Kidney Function (RKF), even at a low level, is associated with a lower mortality risk in hemodialysis patients (9) the work by the Lister Hospital renal research team in the past support that pre-dialysis β 2-microglobulin blood levels can provide estimates of RKF and can have clinical utility as marker for dialysis adequacy (10).

Previous studies have investigated kinetic modeling approaches on data from the HEMO study and on a sample of patients undergoing high-flux haemodialysis to quantify the generation and removal of β 2-microglobulin: A two-pool kinetic model of β 2-microglobulin yielded values of reduction ratio and pre-dialysis serum concentration that were consistent with measured values with various hemodiafiltration and hemodialysis treatment regimens (11).

1.3 Rationale

To allow the clearance of β 2-microglobulin to be properly assessed as an indicator of dialysis quality requires the post-dialysis level to be measured, and this level rebounds in the first few hours after dialysis by around one third due to equilibration after dialysis in different body compartments. We are proposing a study that is designed to allow us to predict the equilibrated level of β 2-microglobulin post dialysis so that the true post-dialysis concentration can be assessed, and so that the clearance of β 2-microglobulin can be assessed from fall in β 2-microglobulin pre-dialysis to post dialysis (equilibrated).

β 2-microglobulin is influenced by residual renal function and could serve as a potential indicator of dialysis adequacy. So, in this study we are aiming to develop a predictive model for post-dialysis rebound of β 2-microglobulin, facilitating more precise assessment of solute clearance.

In our unpublished data from the Lister Hospital/University of Hertfordshire, colleagues have developed a kinetic model for β 2-microglobulin removal that estimates equilibrated β 2-microglobulin concentrations based on pre- and post-dialysis levels as well as the total amount removed. However, this model has not yet been validated and requires further evaluation

2. STUDY OBJECTIVES AND DESIGN

2.1 Objectives

2.1.2 Primary objective:

To develop a predictive model for post-dialysis rebound of β 2-microglobulin, facilitating more precise assessment of solute clearance.

2.1.2 Secondary objectives:

The findings from this pilot study will provide the foundation for submitting a larger grant proposal aimed at evaluating the potential of β 2-microglobulin as a dialysis quality marker.

2.2 Endpoints/outcomes

2.2.1 Primary Outcomes:

- a) Development of a predictive model for the post-dialysis rebound of β 2-microglobulin

This will be developed using a predictive model based on β 2-microglobulin measurement obtained at multiple time points including pre-dialysis, intradialytic and post-dialysis. β 2-microglobulin samples will be taken at time including pre-dialysis (refer to it as 0%), at 20%, 40%, 60%, 80%, and 100% of the dialysis session, at 1- and 2-hours post-dialysis. Additionally, another pre-dialysis sample will be collected at the next routine dialysis session, providing further information on interdialytic β 2-microglobulin levels model created.

- b) Use of the above predictive model for post-dialysis β 2-microglobulin, to derive an accurate estimate of β 2-microglobulin removal ratio and renal clearance as a measure of dialysis adequacy/quality.

We will apply also a recently developed two-pool kinetic model for β 2-microglobulin to the data to assess the performance of this kinetic model in predicting both the levels during dialysis and the post-dialysis rebound and the relationship of the reduction ratio to mass of β 2-microglobulin removed.

2.2.2 Secondary outcomes:

We will measure:

- a) Small solute clearance markers (urea and creatinine).
- b) Patient-Reported Outcome Measure (PROM) that are linked to quality dialysis, dialysis-related symptoms and measurable physiological changes that occur during dialysis (e.g. blood pressure, hypotension).

2.3 Study Design

Cross-sectional, prospective design.

2.3.1 Number of subjects

We plan to recruit 30 adult patients with End Stage Renal Failure (ESRF) who have been on maintenance haemodialysis for at least 3 months, receiving either high flux hemodialysis or hemodiafiltration. All patients will be dialysed using Fresenius 5008, 5008S or 6008 machines.

2.3.2 Study Duration

Recruitment will occur over 6 months with data analysis immediately after completion of recruitment following sample analyses. It is anticipated that the study data will be analysed within 12 months of study initiation.

2.3.3 End of Study

We will finish recruitment and sample analysis by 01/08/2026. Data analysis will be completed within 6 months of this data (01/02/2027).

3. SUBJECT SELECTION AND RECRUITMENT

3.1 Inclusion Criteria

- a) Age 18 years or above.
- b) Ability to give informed consent.
- c) ESRF established on maintenance haemodialysis for at least 3 months.

3.2 Exclusion Criteria

- a) Infection within 14 days prior to study consent.
- b) Inability to give informed consent.

3.3 Subject Recruitment and Screening

Potential participants will be identified from the East and North Hertfordshire Teaching NHS Trust renal database as dialysis patients. Patients for approach to the study must have undergone haemodialysis for at least 3 months and are able to provide written informed consent.

Eligible patients will be invited to participate in the study. Screening will be performed by the research team, who are also part of the clinical staff at the study site.

4. SUBJECT WITHDRAWAL

Subjects are free to withdraw consent for inclusion in the study at any time. At the point of withdrawal, no further data will be collected. However, data and samples collected up to the date/time of withdrawal may be used for analysis if the subject consents. Withdrawal from the study will be documented in the patient's medical notes.

The study is very short study (Over 2 days) and we would not anticipate participants to lose capacity during study but in the event of this happens we would withdraw them from the study but retain any study data collected while they had capacity.

5. STUDY PROCEDURES AND SCHEDULE OF ASSESSMENTS

5.1 Pre-screening

Patients on Haemodialysis will be identified using the East and North Hertfordshire Teaching NHS Trust renal database (Clinical Vision).

Inclusion/exclusion criteria will be checked on pre-screening.

5.2 Screening

Patients identified at pre-screening as potentially eligible will be approached for the study. The study will be explained to them, and they will be provided with a Participant Information Sheet (PIS). Patients approached will have the opportunity to think, discuss with family, friends and ask any questions before consenting.

Inclusion and exclusion criteria will be re-confirmed, particularly checking if a recent infection has occurred in the previous 14 days (an exclusion criterion). If a patient fails screening, they can be re-approached for screening after 14 days.

5.3 Informed consent

Patients approached at screening, after a minimum of 24h, will be re-approached for consent. All patients participating in the study will be required to provide written consent. Participants will give consent to a research nurse, or study investigator.

5.4 Study Procedures by Visit

Participants will be visited during two consecutive routine dialysis sessions, with no additional visits required. The first visit (Session HD1) will coincide with the day scheduled for monthly dialysis adequacy testing. During this visit, participants will be asked to remain for two hours post-dialysis for collection of β2-microglobulin samples at 1- and 2-hour post-dialysis.

During the subsequent routine dialysis session (Session HD2), a second pre-dialysis β2-microglobulin sample will be collected, and the study sample collection will then be completed.

The following data will be collected during first visit (Session HD1)

➤ **Medical History and demographic data**

Demographic data, medical history and current medication data will be obtained from electronic medical records. Charlson Co-morbidity Index will be recorded from the co-morbidities listed in the electronic medical records.

➤ **Clinical Observations**

During the study visit, participants will be assessed for standard of care pre and post-dialysis clinical observations including pre and post dialysis blood pressure (BP), pre and post dialysis heart Rate (HR), pre and post dialysis body temperature (T), Pre- and post-dialysis body weight and Episodes of intradialytic hypotension and their management will be recorded. Intradialytic hypotension is defined by a drop in systolic blood pressure ≥ 20 mmHg or decrease in MAP by 10 mmHg associated with symptoms (Dizziness, muscle cramps, fainting, nausea, vomiting, abdominal discomfort, restlessness, anxiety).

➤ **Fluid status assessment**

Fluid status assessment will be performed pre- dialysis. It will include clinical examination (Chest auscultation, Juglar venous pulse, and assessment for dependent oedema) and bioimpedance assessment (ECF Vol and OHV) using Fresenius body composition monitor (BCM) machine. Fluid assessment will be performed by the research team on all the study during the participant's routine dialysis session.

➤ **Nutritional assessment**

Nutritional assessment will be performed using modified Subjective Global Assessment (SGA) instrument to quantitatively measure malnutrition score. This study procedure will be performed by a trained member of the research team during the participant's routine dialysis session.

➤ **Patient Reported Outcome Measures (PROM)- Questionnaires**

The following questionnaires which are likely indicators of symptoms linked to dialysis quality will be completed by the participants.

- a) IPOS Renal
- b) SONG-HD Fatigue
- c) Recovery time after dialysis (categorised as <2, 2-6, 7-12, >12 hours)

Participants can take help from family, friends and member of the clinical team or the research team in filling the questionnaire. However, interpretation of the questions to form an answer will be done by the participants.

IPOS renal and SONG-HD fatigue will be completed on the study day immediately post dialysis or during dialysis session. Patients will be asked to record post dialysis recovery time and return this when they next attend for dialysis.

If a participant reports any significant negative symptoms with high score in the Questionnaires provided, such as experiencing depression most of the time or always for the past week, we will escalate this urgently to the Renal Psychologist who is part of the Renal social support team

➤ **Dialysis related data documentation**

The following routinely collected data will also be recorded as part of the study data: Time on dialysis (Td), Average blood flow rate (Qb), Average dialysate flow rate (Qd), UF volume, Dialysate sodium, Dialysate Temperature, Type of dialysis access, Type of dialyser, Dialyser surface Area, Dialyser mass transfer area co-efficient (KoA), dialyser ultrafiltration coefficient (KUF), HDF use (yes/no) and HDF volume. These dialysis parameters are readily available from the dialysis machine at the end of the HD session. Hypotensive episodes and their management will also be recorded.

➤ **β 2-microglobulin and solute of clearance measurement**

Blood β 2-microglobulin samples will be collected at defined time points during two routine and consecutive haemodialysis sessions, referred to as Session HD1 and Session HD2, to comprehensively characterize β 2-microglobulin kinetics.

1) Session HD1 (First Study Visit)

- a) Results of pre-dialysis sample collected at time 0% (immediately before starting dialysis), representing baseline β 2-microglobulin level. This will be obtained as part of the routine 6 monthly dialysis β 2-microglobulin testing.
- b) Five intra-dialytic samples collected at 20%, 40%, 60%, 80%, and 100% of the total dialysis duration, to monitor β 2-microglobulin removal during the session.
- c) Two post-dialysis samples collected at 1 hour and 2 hours after completion of Session HD1, to assess post-dialysis β 2-microglobulin rebound.
- d) Results from the standard of care (SOC) blood and urine tests assessing monthly dialysis adequacy. Derived from routine monthly dialysis adequacy testing.

2) Session HD2 (Subsequent routine dialysis Session and last study visit):

- a) Pre-dialysis sample collected at time 0% of Session HD2 (immediately before dialysis begins), providing further information on interdialytic β 2-microglobulin levels.

5.5 Blood sampling procedure

Pre-dialysis blood samples (3ml in serum separator tubes) will be obtained from the dialysis access port coming from the patient, either from the arteriovenous fistula or dialysis catheter. These will be taken from the Arterial “A” sampling port. The first sample will be obtained immediately on starting

dialysis. The intra-dialytic samples will be then taken at time intervals reflecting 20%, 40%, 60%, 80% and 100% of the dialysis time (e.g. If 4 hours dialysis then sample at start, 0.8h, 1.6h, 2.4h, 3.2h, 4h). The end of dialysis (100% dialysis time) sample will be taken with a procedure to reduce vascular access recirculation (“Slow-Flow Technique”): blood flow is reduced to 100ml/min for 15 seconds and then the sample is obtained from the “A” port.

Post-dialysis blood samples at 1 hour and 2 hours the patient will be disconnected from the machine. For these samples two options are available. Peripheral venepuncture will be used as the first option but if a patient has an existing dialysis catheter and poor veins for venepuncture then this may be used.

To assist patients the research team will ensure that patients have comfortable place to rest during this two-hour waiting period and transport back to their place of residence will be rearranged if needed. The study team will coordinate with the patient transport service to ensure patients can leave as soon as possible after the 2 hour blood test.

5.6 Storage and transportation of the samples

Blood samples will be centrifuged. (3000 rpm. 15 mins) and then aliquoted in duplicate (one for analysis, one for storage), then stored at –80 degrees centigrade (°C).

Samples will be analysed for β2-microglobulin. The analysis sample will be destroyed after data has been analysed. The stored sample will be kept for up to 3 years.

Table 2: Schedule of assessments

Study Period				
Procedures	Pre-Screening	Screening	Session HD1	Session HD2
Identifying a potentially eligible patient	X			
Inclusion/Exclusion criteria checks	X	X		
Provision of patient information sheet and informed consent form		X		
Written informed consent*		X		
Demographic data, medical history			X	
Clinical assessment			X	
Bioimpedance			X	

Nutritional assessment			X	
Dialysis related data recording			X	
Pre-dialysis β2-microglobulin sample collected at time 0			X	
Intra-dialytic β2-microglobulin samples collected at 20%, 40%, 60%, 80%, and 100% of the total dialysis duration			X	
Post-dialysis samples collected at 1 hour and 2 hours after completion of dialysis			X	
Pre-dialysis β2-microglobulin before second dialysis session				X

5.7 End of study procedures

The study will end once the final β2-microglobulin sample has been analysed. This refers to the second pre-dialysis β2-microglobulin sample collected from the last participant during their second (final) study visit. And this sample will be analysed within 2 months from date of collection.

6. STATISTICS AND DATA ANALYSIS

6.1 Sample Size determination

Assuming a moderate effect size (Cohen D 0.3), a sample size of 26 subjects is required for a multiple linear regression with two covariates (weight and dialysis duration) with 80% and alpha 0.05. Hence, a slightly larger sample size of 30 has been selected. This is identical to the numbers used in the previously published study for urea rebound which has been in general clinical use for 30 years.

6.2 Subject Population(s) for Analysis

All subjects included in this study will be included in analysis

6.3 Statistical Methods

We will develop regression equations to predict the magnitude of post-dialysis β2-microglobulin rebound. This is likely to involve linear regression if the relationships are found to be linear. The sample size will permit inclusion of up to two variables in the same model, but we will explore the optimum parameters for predicting magnitude of rebound. This will follow the principles used to

predict the rebound of urea as previously published in (Tattersall et al, "The post-hemodialysis rebound: predicting and quantifying its effect on Kt/V", Kidney Int 1996) and describes the general approach to be taken. We will develop the best equation for prediction of post-dialysis rebound.

We will apply also a recently developed two-pool kinetic model for β2-microglobulin to the data to assess the performance of this kinetic model in predicting both the levels during dialysis and the post-dialysis rebound.

7. DATA HANDLING AND RECORD KEEPING

7.1 Confidentiality

Information about study subjects will be kept confidential and managed according to Trust requirements. Participants will be informed of who will have access to this information, on the participants' information sheet (PIS). Patients participating will be assigned a unique study identification number. This identification number will be linked to the patient's name, NHS number and date of birth in the study folder recruitment log which will be stored in the site file in paper form. A password protected copy of this file with patient identifiable information will be stored electronically on NHS computer. Only the members of the research team on delegation log will have access to this information.

Data collected for the study will be recorded in a password protected spreadsheet kept on the East and North Hertfordshire Teaching NHS Trust (ENHT) server. The study data will have patient identifiers (NHS number, name, DOB) removed and will be therefore anonymous.

7.2 Source Documents, data handling and record keeping:

Data will be entered by GCP trained members of research team only who are on the study delegation log. This data will be electronically captured and stored by the research team on the NHS computer systems using standard password-controlled secure data storage.

Original paper consent forms will be stored in a site file in a secure locked room at the study site. An electronic study participant log with their personal identifiers (name, NHS number, date of birth) linking these to their anonymous study ID will be retained for up to 3 years after the study end-date. This will be retained electronically on the East and North Hertfordshire Teaching NHS Trust server, password protected. The study paper site file will be kept for 3 years after the study end-date in a secure locked room on ENHT premises.

Information about study subjects will be kept confidential and managed according to the Sponsor's policies and SOPs. Subject's names will not appear on any of the data sheets being transported off site; the link between the patient's personal details and their anonymity number will be kept separately from all other data. No personal data will be passed on to other researchers or organisations.

7.3 Records Retention

All essential study documents will be stored for 3 years, and the research data will be used solely for the purposes outlined for this study. Archiving will be authorised by the Sponsor following submission of the end of study report. Destruction of essential documents will require authorisation from the Sponsor. Archiving this study will be carried out according to the Sponsor's operating procedure.

8. STUDY MONITORING, AUDITING AND INSPECTING

8.1 Study Monitoring Plan, Auditing and Inspecting

The study will be monitored according to the study-specific monitoring plan. The Sponsor operates a risk-based monitoring programme for all East and North Hertfordshire Teaching NH Trust (ENHT) sponsored projects. Proportionate monitoring will involve completion of remote monitoring forms by the Principal/Chief Investigator or delegated individual.

If the Sponsor becomes aware of concerns regarding the project activity, including any deviations from the study protocol or GCP, further triggered monitoring may be implemented. This may involve on-site monitoring visits.

The ongoing study monitoring will be via the weekly renal research meetings of the research group at the study site (ENHT) where study progress and data collection issues will be reviewed. CI/PI/Co-investigators/research nurses will be present in this meeting.

The Chief Investigator will permit necessary audit and inspections per the Sponsor's SOPs and any appropriate inspections by relevant regulatory authorities.

9. REGULATORY OBLIGATIONS AND COMPLIANCE

This study is to be conducted according to EU and international standards of Good Clinical Practice and International Conference on Harmonisation guidelines, applicable government regulations and Ethics policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Independent Ethics Committee (IEC), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IEC concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IEC members and their affiliates to the sponsor.

9.1 Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol.

- a) Prospective, planned deviations or waivers to the protocol are not allowed and must not be used e.g. it is not acceptable to enroll a subject if they do not meet the eligibility criteria or restrictions specified in the trial protocol

- b) Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.
- c) Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

9.2 Notification of Serious Breaches to GCP and/or the protocol.

A “serious breach” is a breach which is likely to effect to a significant degree –

- (a) the safety or physical or mental integrity of the subjects of the study; or
- (b) the scientific value of the study

The sponsor will be notified immediately of any case where the above definition applies during the study conduct phase.

10. STUDY FINANCES

10.1 Funding Source

The analysis for this study has been funded by the East and North Hertfordshire Teaching NHS Trust Research Capability Funding 2025/2026.

10.2 Conflict

There are no conflicts of interest to declare.

10.3 Subject Stipends or Payments

All study procedures will be done as part of routine clinical care (routine dialysis session). There will not be any extra visits needed for any of the study activity. Participants will not be paid for participating in the study.

11. INDEMNITY

The standard NHS Indemnity procedure will apply for this study in case of harm to participants arising from the management, design or conduct of the research. All participants will be recruited from an NHS site.

12. PUBLICATION PLAN

The results of this study will be submitted for publication in relevant peer-reviewed academic medical journals and at conferences as soon as possible after study completion.

Study participants will be informed of the research findings when available. We intend to provide a plain English study summary to our local Lister Kidney Patient’s Association for their local newsletter

to provide feedback to the East and North Hertfordshire Teaching NHS Trust patients who have taken part in the study or may have heard about the study.

Once completed, we anticipate requesting funding to support a larger study of use of clearance of this molecule to assess dialysis quality.

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APPENDICES

Appendix 1 - PIS (Participant Information Sheet)

Appendix 2 - ICF (Informed Consent Form)

Appendix 3 - Questionnaires

Appendix 4- Nutritional assessment tool

