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Associations of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence

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Short title: Skin autofluorescence as a risk marker in people receiving dialysis

Acronym: AGED (**A**dvanced **G**lycation **E**nd-products in **D**ialysis Patients)

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Trial / Study Statistician: Not required, all statistical analysis will be performed by the investigating team

Trial / Study Coordinating Centre: Renal Unit of the Department of Nephrology Royal Derby Hospital, Uttoxeter Rd, Derby, DE22 3NE

SYNOPSIS

Title	Associations of advanced glycation end-product accumulation and adverse outcomes in peritoneal dialysis and haemodialysis patients and the impact of a dietetic intervention on skin autofluorescence.
Acronym	AGED
Short title	Skin autofluorescence as a risk marker in people receiving dialysis.
Chief Investigator	Professor Maarten Taal
Objectives	**Study 1:** • To investigate whether the skin autofluorescence (SAF) levels increase over time in prevalent and incident people on peritoneal dialysis (PD) and haemodialysis (HD). • To investigate one-year survival in relation to increased SAF levels. • To determine the most important factors associated with changes in SAF over time. • To investigate the association of increased SAF levels with morbidity, changes in visual function, health-related quality of life and nutritional status. **Study 2:** • To investigate whether the correction of malnutrition by an intensive nutritional intervention results in a decrease in SAF levels in people on PD and HD.
Trial Configuration	**Study 1:** longitudinal/prospective. **Study 2:** observational non-randomized proof of principle study.
Setting	Outpatient secondary care setting.
Sample size estimate	**Sample size Study 1:** The primary outcome of the study is one-year survival in relation to increased SAF levels in people on HD and PD. With a power of 80%, a two-sided alpha of 5% and an expected hazard ratio of 3.5 and 2.0 in people on PD and HD, respectively, 100 HD and 40 PD participants will be needed. **Sample size Study 2:** Since this is an observational proof of principle study, it would be reasonable to include 40 dialysis participants (either HD or PD).
Number of participants	**Study 1:** • 100 participants undergoing maintenance HD treatment (dialysis vintage greater than 3 months). • 40 PD dialysis participants with a dialysis vintage greater than 3 months. • 30 incident participants starting either HD or PD treatment (dialysis vintage $\leq$3 months). **Study 2:** 40 malnourished participants on maintenance HD and PD.
Eligibility criteria	**HD cohort:** • Age: $\geq$18 years (no upper age limit). • At least three dialysis sessions per week for 3 or 4 hours. • Dialysis with biocompatible membranes.

	• Able to give informed consent. **PD cohort:** • Age: ≥18 years (no upper age limit). • Dialysis with lactate/bicarbonate-buffered solutions with different glucose concentrations as prescribed for routine clinical care. • Able to give informed consent.
Description of interventions	**Study 1:** no intervention. **Study 2**: Dietitian supervised nutritional support aimed at improving malnutrition in addition to standard dietetic advice consistent with United Kingdom Renal Association guidelines (Wright and Jones, 2010).
Duration of study	**Study 1:** Participant recruitment will begin on June 2016 with an anticipated recruitment period of 14 months. Therefore, all baseline data and measurements will be collected and performed between June 2016 and August 2017. Participant Duration: once recruited, all participants will be followed-up for up to five years; consequently, it is expected that the study will be completed by August 2022. **Study 2:** Participant recruitment will begin in December 2017 with an anticipated recruitment period of 6 months. Therefore, all baseline data and measurements will be collected and performed between December 2017 and June 2018. Participant Duration: once recruited, all participants will be followed-up for 24 months; consequently, it is expected that the study will be completed by June 2020.
Randomization and blinding	**Studies 1 and 2:** no randomization required.
Outcome measures	**Study 1:** • Primary outcomes: 1. Changes in SAF levels over 1 year. 2. One-year survival. • Secondary outcomes: factors associated with the accumulation of AGE, morbidity, biochemistry, visual function, quality of life and nutritional status. **Study 2:** • Primary outcomes: changes in SAF levels. • Secondary outcomes: biochemistry, visual function, quality of life and nutritional status.
Statistical methods	Intragroup comparisons will be performed using Wilcoxon test or paired t-test for dimensional variables and McNemar test for categorical variables. Intergroup comparisons will be performed using Mann Whitney test or Student t test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. To determine the significance and strength of associations, Pearson's correlation coefficient will be used for continuous variables and Spearman rank for nonparametric variables. Linear

	regression analysis will be used to identify determinants of advanced glycation end-product (AGE) accumulation. Cox proportional hazards models will be used to investigate the prognostic value of the accumulation of AGE for predicting mortality. A p value ≤0.05 will be considered to have statistical significance.

ABBREVIATIONS

AE Adverse Event

AGE Advanced glycation end-products

CI Chief Investigator overall

CKD Chronic kidney disease

CLF Collagen linked fluorescence

CML Carboxymethyl lysine

CRF Case Report Form

EDTRS Early Treatment Diabetic Retinopathy Study

ESKD End stage kidney disease

GCP Good Clinical Practice

HD Haemodialysis

ICF Informed Consent Form

IL-6 Interleukin 6

NHS National Health Service

PD Peritoneal dialysis

REC Research Ethics Committee

R&D Research and Development department

SAE Serious Adverse Event

SAF Skin autofluorescence

SF-36 36-Item Short Form Health Survey

SGA Subjective global assessment

TNF-α Tumour necrosis factor-α

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TRIAL / STUDY BACKGROUND INFORMATION AND RATIONALE

Chronic kidney disease (CKD) is a global public health problem and is associated with multiple adverse outcomes including reduced survival, especially in people requiring renal replacement therapy (peritoneal dialysis (PD), haemodialysis [HD] and transplantation) (Zhang and Rothenbacher, 2008). Multiple risk factors lead to the development and/or progression of CKD, such as obesity, hyperlipidaemia, glomerulonephritis, intercurrent infections, smoking, type 2 diabetes and hypertension, the two latter being considered as leading causes of CKD worldwide (Zhang and Rothenbacher, 2008; Junaid Nazar et al., 2014).

People on dialysis develop a variety of complications/abnormalities as a result of loss of endocrine or exocrine function of the kidneys, including anaemia, metabolic acidosis, bone and mineral disorders, fluid overload, hypertension, electrolyte disturbances and dyslipidaemia (KDIGO, 2013). In recent years, inflammation, oxidative stress and endothelial dysfunction (other common abnormalities in people on dialysis) have become areas of interest because of their strong relationship with higher rates of cardiovascular morbidity and mortality in people on dialysis (Oleniuc et al., 2012).

Advanced glycation end-products (AGE) are uremic toxins markedly increased in people on dialysis. Formation of AGE starts with a non-enzymatic reaction between proteins and glucose molecules called the Maillard reaction; however, AGE are also formed more rapidly during oxidative stress with the subsequent formation of reactive carbonyl compounds like methyl glyoxal. At this point, AGE synthesis is irreversible and AGE will cross-link with tissue proteins; it seems that collagen in the skin and vascular basement membranes are especially susceptible to AGE accumulation and subsequent injury. AGE also interact with specific AGE receptors that will lead to the activation of systemic inflammation by increasing the release of cytokines and, consequently, exacerbate tissue damage (Arsov et al., 2014). Importantly, AGE are also formed in food during cooking with dry heat at high temperatures such as in frying, grilling or roasting and about 10% of ingested AGE are absorbed.

Skin autofluorescence (SAF) is a relatively new technique that measures the skin accumulation of AGE. It is a non-invasive, operator independent, quick (less than 5 minutes) and easy to perform technique that utilizes the fluorescent properties of AGE, like the extensively used collagen linked fluorescence (CLF) method, and has been validated with specific AGE measurements and CLF in skin biopsies (Oleniuc et al., 2012). It has been reported that SAF is strongly correlated with overall and cardiovascular mortality in people with diabetes and undergoing HD (Hartog et al., 2007; Graff et al, 2014). Several factors have been associated with higher SAF values in people on dialysis in cross-sectional studies, for instance, chronological age in both dialysis modalities, glucose exposure from peritoneal dialysis fluid and dialysis vintage only in people on PD and presence of diabetes in people on HD (McIntyre et al., 2010; Smit et al., 2010).

AGE accumulation is postulated as the one of the modulating factors that drives visual disorders (Kandarakis et al., 2014); the increased accumulation secondary to hyperglycaemia in diabetes is thought to cause vascular basement membrane thickening and destruction of pericytes from the retinal capillary bed in diabetic retinopathy (Singh et al., 2014). The accumulation of AGE has also been reported to be a mechanism in the deterioration in visual acuity associated with increasing age (Ishibashi et al., 1998). Separately, the accumulation of AGE in other metabolic diseases (e.g. end stage kidney disease [ESKD]) has been found to cause nerve and eye dysfunction. Ocular abnormalities and therefore visual disturbances are reported in people with ESKD but the mechanism for this has not been fully explored or understood. In animal studies, AGE accumulation is seen

in the lens, cornea and vitreous humour (Darlene et al., 2011). In humans, systemic AGE levels and visual acuity scoring have not been previously investigated.

Because of the adverse outcomes strongly associated with higher levels of SAF, several options focused on reducing the accumulation of AGE have been proposed. One of these promising interventions is the reduction of dietary AGE; it has been suggested that cooking techniques that avoid very high temperatures such as poaching, steaming, stewing and boiling can significantly reduce the AGE content of food when compared to frying, broiling, grilling and roasting (Uribarri et al., 2010); nevertheless, most of the evidence regarding dietary modifications to reduce exogenous AGE is of low quality and therefore further studies are required (Kellow and Savige, 2013).

Nevertheless, analysis of baseline data from Study 1 has identified strong associations between higher SAF and malnutrition whereas no correlations were observed between higher SAF and high dietary AGE intake. Correction of malnutrition may therefore represent a more important dietary intervention to reduce accumulation of AGE in people receiving dialysis. We further reasoned that placing people with malnutrition on a restrictive diet may worsen their malnutrition and we have therefore adapted our original research plan to include an observational study to assess the impact of correcting malnutrition on SAF by providing a dietitian supervised nutritional support intervention, which essentially involves the usual/standard dietetic care/advice provided by the NHS with some additional supervision, follow-up and approved dietary supplements (also provided by the NHS), rather than a randomised trial of dietary AGE restriction.

The results from the present project will benefit people on dialysis because they may demonstrate that the correction of malnutrition decreases the SAF levels in this population. Published observational studies suggest that reduction of SAF levels will in turn be associated with a reduction in the high morbidity and mortality rates associated with chronic dialysis and, consequently, healthcare costs. Improved survival and reduced comorbidity would also be expected to improve the quality of life of people on dialysis.

TRIAL / STUDY OBJECTIVES AND PURPOSE

PURPOSE

The purpose of the present project is to investigate the association between AGE accumulation and adverse outcomes (mainly mortality) and the impact of a dietary intervention on SAF levels in people on HD and PD.

PRIMARY OBJECTIVES

Study 1:

- To investigate whether the SAF levels increase over time in prevalent and incident HD and PD participants.
- To investigate one-year survival in relation to increased SAF levels.

Study 2:

- To investigate whether the correction of malnutrition by an intensive nutritional intervention results in a decrease in SAF levels in people on PD and HD.

SECONDARY OBJECTIVES

Study 1:

- To determine the most important factors associated with change in SAF over time.
- To investigate the association of increased SAF levels with morbidity, health-related quality of life and nutritional status.
- To investigate the association between SAF levels and changes in visual function.

Study 2:

- To investigate the effect of an intensive nutritional intervention on the improvement of health-related quality of life and nutritional markers.

DETAILS OF DEVICE(S)

No products will be used in the present project.

Description

The medical device that will be used in the present project is a validated “AGE reader Standard Unit (SU)”. The AGE reader is a medical device used in clinical research which can non-invasively measure tissue accumulation of AGE through a fluorescent technique called SAF.

The AGE reader SU illuminates a skin surface of 1 cm^2, guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ≈370 nm). Light from the skin is measured with a spectrometer (AVS-USB2000, Avantes Inc., Eerbeek, The Netherlands) in the 300-600 nm range, using a 200-µm glass fibre. SAF will be measured as the ratio between emission and excitation, calculated in arbitrary units (AU), by dividing the intensity of the fluorescent light coming from the skin (measured as area under the curve [AUC] of fluorescent wave lengths between 420 and 600 nm) by the intensity of the emitted light (measured as AUC of wave lengths between 300 and 420 nm) multiplied by 100. For each participant, three measurements will be performed on the volar surface of the lower arm at ≈10 cm below the elbow, ensuring that the area has normal skin without visible vessels, scars or other abnormalities. For HD participants, only the non-fistula arm will be used and evaluations will take place prior to dialysis sessions (Siriopol et al., 2015). This technique is non-invasive and there is no risk of harm to the participant. Measurements take only a few minutes.

The AGE reader SU will be used by Daniela Viramontes Horner (PhD student) following user instructions provided by the manufacturer.

Manufacture

The name of the manufacturer is DiagnOptics Technologies BV (Aarhusweg 4-9, Groningen, The Netherlands)

The AGE reader SU is a distributed CE marked product covered by the "CE Marking of Conformity Certificate", reference number: 2085393CE01, delivered by DEKRA Certification B.V., Arnhem, The Netherlands, Notified Body Identification Number 0344, and conforms to the required technical documentation, in accordance with Annex VII of the "EC-Directive", the Council Directive 93/42/EEC of 14 June 1993, concerning medical devices.

The AGE reader SU will be used in the present project in accordance with this CE marking.

Packaging and labelling

Storage, dispensing and return

The AGE reader SU will be stored securely, in a locked room, or locked cupboard or cabinet. Access to the medical device will be limited to the study staff and investigators.

Placebo
No placebo products will be used in the present project.

Known Side Effects
The AGE-Reader has been extensively tested in thousands of adults for several years without any adverse effects. The AGE Reader has CE approval (2085393CE01) to be used as a medical device and thus has passed the rigid safety standard. The amount of ultraviolet light exposure is actually far below the safety limits.

Reference source:
AGE reader. Cardiovascular risk assessment. http://www.diagnoptics.com/age-reader/ [Accessed on 12th May, 2016]

TRIAL / STUDY DESIGN

TRIAL / STUDY CONFIGURATION
Studies 1 and 2 will be single-centre.

Study 1: longitudinal/prospective.
Study 2: observational non-randomized proof of principle study where malnourished dialysis participants will receive a dietitian supervised intensive nutritional support.

Primary endpoints
Study 1:
1. Changes in SAF levels after 12 months of follow-up.
2. One-year survival.

Study 2: changes in SAF levels after 6 months of a dietetic intervention (i.e. intensive nutritional support).

Secondary endpoints
Study 1:
- Factors associated with the accumulation of AGE.
- Association of SAF levels with morbidity, biochemical variables (haemoglobin, haemoglobin A1C (HbA1C), urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides, and intact parathyroid hormone), visual function, circulating AGE levels (carboxymethyl lysine [CML] and pentosidine), inflammatory biomarkers (tumour necrosis factor-α [TNF-α], interleukin 6 [IL-6], C reactive protein [CRP] and others) health-related quality of life and nutritional status (energy, protein and fat intake, dietary AGE intake, anthropometry and subjective global assessment [SGA]).

Study 2:
- Association of SAF levels with morbidity, biochemical variables (haemoglobin, HbA1C, urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides, and intact parathyroid hormone), visual function, circulating AGE levels (CML and pentosidine), inflammatory biomarkers (TNF-α, IL-6, CRP and others) health-related quality of life and nutritional status (energy, protein and fat intake, dietary AGE intake, anthropometry and SGA).

Safety endpoints

- There will not be any safety endpoints for these studies.

Stopping rules and discontinuation

Participant Withdrawal

Participants will be withdrawn from the study if they withdraw their consent at any time. Participants may be withdrawn from the study at the discretion of the Investigator. Participants will be made aware that withdrawal will not affect their future care. Participants who withdraw will be asked for permission to retain data already collected for use in the final analysis.

If participants fail to attend for their study visits, a member of the research team will try to contact them by telephone within 24 hours of their appointment and by written correspondence within a week of the missed appointment. Multiple attempts at contact will be made by the research team over a period of a month before stating that a participant has been lost to follow-up.

Participants will be withdrawn from the study if they:

- Withdraw their consent at any point.
- Become pregnant.
- Decline to comply with the dietetic intervention (Study 2 only).

Criteria for terminating the study
Study 1 is observational and will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

The intervention in Study 2 is not expected to be associated with any adverse effects. Study 2 will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

RANDOMIZATION AND BLINDING

Maintenance of randomization codes and procedures for breaking code

TRIAL/STUDY MANAGEMENT

The Chief Investigator (CI; Professor Maarten Taal) has overall responsibility for the study and shall oversee all study management. The data custodian will be the CI.

The study will be coordinated from the University of Nottingham campus at the Royal Derby Hospital. Data will be collected at the Renal Unit of the Department of Nephrology at the Royal Derby Hospital (Derby Teaching Hospitals NHS Foundation Trust) and stored at the University of Nottingham.

Daniela Viramontes Horner (PhD student), Dr. Nick Selby and Professor Maarten Taal are responsible for study planning, recruitment, consenting, data collection, handling and interpretation.

DURATION OF THE TRIAL / STUDY AND PARTICIPANT INVOLVEMENT

Study Duration:

Study 1:
Participant recruitment will begin on June 2016 with an anticipated recruitment period of 14 months. Therefore, all baseline data and measurements will be collected and performed between June 2016 and August 2017.

Participant Duration: once recruited, all participants will be followed-up for up to five years; consequently, it is expected that the study will be completed by August 2022.

Study 2:
Participant recruitment will begin in December 2017 with an anticipated recruitment period of 6 months. Therefore, all baseline data and measurements will be collected and performed between December 2017 and June 2018.

Participant Duration: once recruited, all participants will be followed-up for 24 months; consequently, it is expected that the study will be completed by June 2020.

End of the Trial

The end of studies 1 and 2 will be when long term follow-up has been completed.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

Potential participants on HD and PD will be recruited from the Renal Unit of the Department of Nephrology at the Royal Derby Hospital. The initial details of the study and participant information sheet will be provided by the usual care team (which may include the researcher). Participants will then be given at least 24 hours to consider whether they wish to participate, as well as ask any questions about the study, before being re-contacted by the investigators.

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the study, the participant information sheets, and consent forms, but the consent forms and information sheets will not be available in other languages.

It will be explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

The participant's general practitioner will not be informed about the study because there are no implications for other aspects of treatment.

Eligibility criteria

Inclusion criteria
HD cohort:
- Age: ≥18 years (no upper age limit).
- At least three dialysis sessions per week for 3 or 4 hours.

- Dialysis with biocompatible membranes.
- Able to give informed consent.

PD cohort:
- Age: ≥18 years (no upper age limit).
- Dialysis with lactate/bicarbonate-buffered solutions with different glucose concentrations as prescribed for routine clinical care.
- Able to give informed consent.

Exclusion criteria
- Does not wish to participate.
- Renal transplant.
- Pregnancy or breast feeding or intending pregnancy.
- Expected survival less than one year (for Study 1).

Expected duration of participant participation
Participants will be participating in the Study 1 for five years and in the Study 2 for 24 months.

Removal of participants from therapy or assessments/Participant Withdrawal
Participants will be withdrawn from the study if they withdraw their consent at any point, receive a renal transplant or decline to comply with the dietetic intervention (Study 2). Female participants will be withdrawn from the study if they become pregnant.

Participants may be withdrawn from the study either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and, with their consent, may still be used in the final analysis.

If participants fail to attend for their study visits, a member of the research team will try to contact them by telephone within 24 hours of their appointment and by written correspondence within a week of the missed appointment. Multiple attempts at contact will be made by the research team over a period of a month before stating that a participant has been lost to follow-up.

With participant consent, data that is collected prior to withdrawal will be kept and analysed as part of the data set. The date, time and reason for participant withdrawal will be documented on the case report form (CRF).

To ensure attainment of adequate numbers of participants for the analysis to be valid, the research team will attempt to recruit other participants to replace any who withdraw from the study.

Informed consent
The process for obtaining participant informed consent will be in accordance with the Research Ethics Committee (REC) guidance, Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced.

The research team have both training and experience in assessing participant capacity and gaining informed consent in the clinical context.

Detailed information will be provided on the study to ensure that participants understand the purpose and potential risks involved. The study will not involve participants who are unable to represent their own interests or are particularly susceptible to coercion.

All participants will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the study. The Investigator will explain the details of the study and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.

The decision regarding participation in the study is entirely voluntary. The Investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care.

Informed consent will be obtained from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one will be kept by the Investigator, and a third will be retained in the participant's hospital records.

Should there be any subsequent substantial amendment to the final protocol, which might affect a participant's participation in the study, continuing consent will be obtained using an amended Consent Form which will be signed by the participant.

TRIAL / STUDY TREATMENT AND REGIMEN

Study 1 - longitudinal/prospective study

100 participants undergoing maintenance HD treatment (dialysis vintage greater than 3 months), 40 PD participants with a dialysis vintage greater than 3 months and up to 30 incident participants starting either HD or PD treatment (dialysis vintage less than 3 months) will be recruited from the Renal Unit of the Department of Nephrology at the Royal Derby Hospital and will be followed-up for five years.

Data collection and study procedures/assessments

Once participants have given their informed consent, relevant participant characteristics will be extracted from electronic data captured in medical records and from a self-report questionnaire at baseline and will include demographic characteristics, educational level, occupation status, family history, duration of renal replacement therapy, dialysis adequacy, aetiology of CKD, medical history (e.g. history of cardiovascular disease, diabetes and hypertension), current co-morbidities, alcohol history, smoking status and current medication. For the PD participants, information regarding the type of PD and the volume of the different PD solutions will also be recorded.

The accumulation of AGE will be assessed by measuring SAF using a validated Autofluorescence Reader (AGE Reader, DiagnOptics, Groningen, The Netherlands) at baseline, 3, 6, 9 and 12 months of follow-up. The method has been previously described by Meerwaldt et al. (2005). As described in the study by Siriopol et al. (2015), "the autofluorescence reader illuminates a skin surface of 1 cm^2, guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ≈370 nm). Light from the skin is measured with a spectrometer (AVS-USB2000, Avantes Inc., Eerbeek, The Netherlands) in the 300-600 nm range, using a 200-μm glass fibre". SAF will be measured as the ratio between emission and excitation, calculated in arbitrary units (AU), by

dividing the intensity of the fluorescent light coming from the skin (measured as area under the curve [AUC] of fluorescent wave lengths between 420 and 600 nm) by the intensity of the emitted light (measured as AUC of wave lengths between 300 and 420 nm) multiplied by 100. For each participant, three measurements will be performed on the volar surface of the lower arm at ≈10 cm below the elbow, ensuring that the area has normal skin without visible vessels, scars or other abnormalities. For HD participants, only the non-fistula arm will be used and readings will be undertaken within the first hour of HD treatment (Siriopol et al., 2015).

Routine blood/laboratory tests will be performed at baseline and monthly and will include haemoglobin, HbA1C, urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides and intact parathyroid hormone. Pre- and post-dialysis blood samples (10 mL) will be taken at baseline, 3, 6, 9 and 12 months of follow-up and centrifuged to obtain at least 2 mL of serum and 2 mL of plasma which will be frozen and stored at -80°C for subsequent analysis of circulating AGE levels (CML and pentosidine) and markers of inflammation (TNF-α, IL-6, CRP and others).

Nutritional assessments will be performed at baseline and at 6 and 12 months of follow-up and will include: three 24-h dietary recalls to assess energy, fat and protein intake, a validated food frequency questionnaire to evaluate dietary AGE intake; the 7-point scale SGA for the classification of the nutritional status (normal, mild-moderate malnutrition and severe malnutrition) and anthropometric measurements and indexes (weight, height, mid-arm circumference, triceps skinfold thickness, body mass index, hand-grip strength and mid-arm muscle area).

Participants will have all aspects of their visual acuity assessed at baseline and 12 months. Those on HD will be tested before and after a single mid-week dialysis session. Participants will initially be asked a screening questionnaire to assess whether or not they note a change in their visual acuity or experience visual disturbances peri dialysis. The assessments will include near, distance, contrast and colour vision assessed using the Jaeger near vision chart, standardized Early Treatment Diabetic Retinopathy Study (EDTRS) testing, Pelli-Robson Contrast Sensitivity Chart, and Ishihara Color Vision Test, respectively. Participants will be asked to bring their glasses or wear their contact lenses on the study day to assess best corrected visual acuity.

Health-related quality of life will be evaluated at baseline and at 6 and 12 months of follow-up with the 36-Item Short Form Health Survey (SF-36) and EQ5D questionnaire.

After the first year of the study, SAF readings and questionnaires will be repeated once a year for up to five years.

Study 2 - Observational non-randomised proof of principle study

Starting December 2017, 40 malnourished (i.e. SGA scores from 1 to 5) participants on maintenance HD and PD will be recruited from the Renal Unit of the Department of Nephrology at the Royal Derby Hospital and then will be followed-up for 24 months. Therefore, Study 2 will be running alongside Study 1. Depending on the results of the longitudinal/prospective study, we will decide to include incident HD and PD participants as well.

Description of intervention

Once the identified malnourished participants have given their informed consent, they will receive intensive dietitian supervised nutritional support with the aim of improving their

malnutrition. In addition, participants will receive standard dietary advice for people on dialysis based on the Nutritional Guidelines in CKD published by the Renal Association in March 2010 in the UK (Wright and Jones, 2010) and will include the following: energy (35 kcal/kg/day) and protein intake (1.2 g/kg/day), as well as potassium, phosphate and sodium restriction, according with biochemical blood parameters.

Adherence to the intervention will be evaluated by means of three 24-h dietary recalls every month (1 dialysis day, 1 non-dialysis day and 1 weekend day for the HD participants and 2 weekdays and 1 weekend day for the PD participants).

Data collection and study procedures/assessments

Relevant participant characteristics will be extracted from electronic data captured in medical records and from a self-report questionnaire at baseline and will include demographic characteristics, educational level, occupation status, family history, duration of renal replacement therapy, dialysis adequacy, aetiology of CKD, medical history (e.g. history of cardiovascular disease, diabetes and hypertension), current co-morbidities, alcohol history, smoking status and current medication. For the PD participants, information regarding the type of PD and the volume of the different PD solutions will also be recorded.

The accumulation of AGE will be assessed by measuring SAF using a validated Autofluorescence Reader (AGE Reader, DiagnOptics, Groningen, The Netherlands) at baseline, 3 and 6 months of follow-up according to the method described above.

Routine blood/laboratory tests will be performed at baseline and monthly and will include haemoglobin, HbA1C, urea, creatinine, potassium, phosphate, calcium, sodium, albumin, total proteins, cholesterol, triglycerides and intact parathyroid hormone. Pre- and post-dialysis blood samples (10 mL) will be taken at baseline, 3 and 6 months of follow-up and centrifuged to obtain at least 2 mL of serum and 2 mL of plasma which will be frozen and stored at -80°C for subsequent analysis of circulating AGE levels (CML and pentosidine) and markers of inflammation (TNF-α, IL-6, CRP and others).

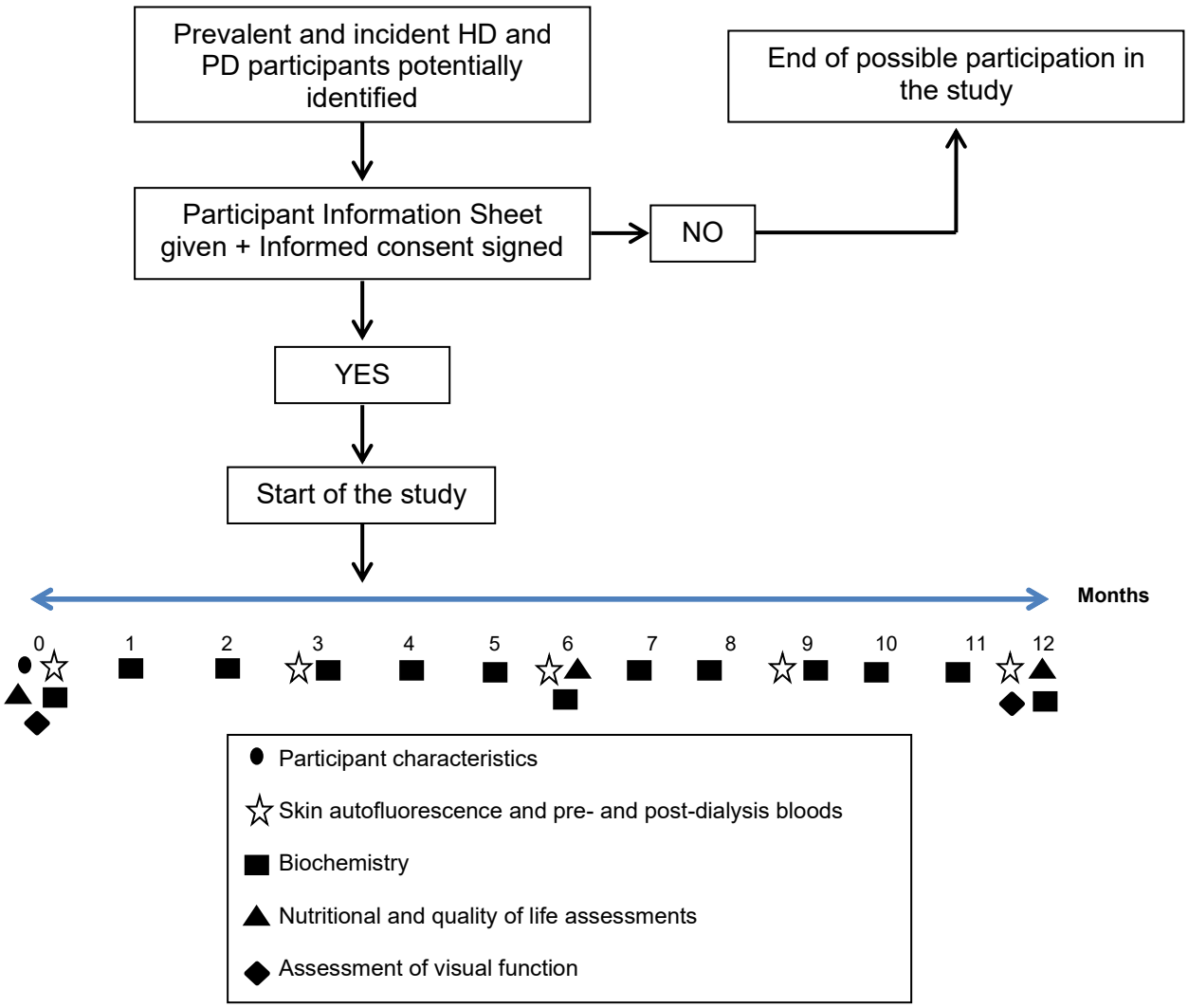
Nutritional assessments will be performed at baseline and at 6th month of treatment and will include: three 24-h dietary recalls to assess energy, fat and protein intake, a validated food frequency questionnaire to evaluate dietary AGE intake, the 7-point scale SGA for the classification of the nutritional status (normal, mild-moderate malnutrition and severe malnutrition) and anthropometric measurements and indexes (weight, height, mid-arm circumference, triceps skinfold thickness, body mass index, hand-grip strength and mid-arm muscle area).

Participants will have all aspects of their visual acuity assessed at baseline and 6 months. Those on HD will be tested before and after a single mid-week dialysis session. Participants will initially be asked a screening questionnaire to assess whether or not they note a change in their visual acuity or experience visual disturbances peri dialysis. The assessments will include near, distance, contrast and colour vision assessed using the Jaeger near vision chart, standardized EDTRS testing, Pelli-Robson Contrast Sensitivity Chart, and Ishihara Color Vision Test respectively. Participants will be asked to bring their glasses or wear their contact lenses on the study day to assess best corrected visual acuity.

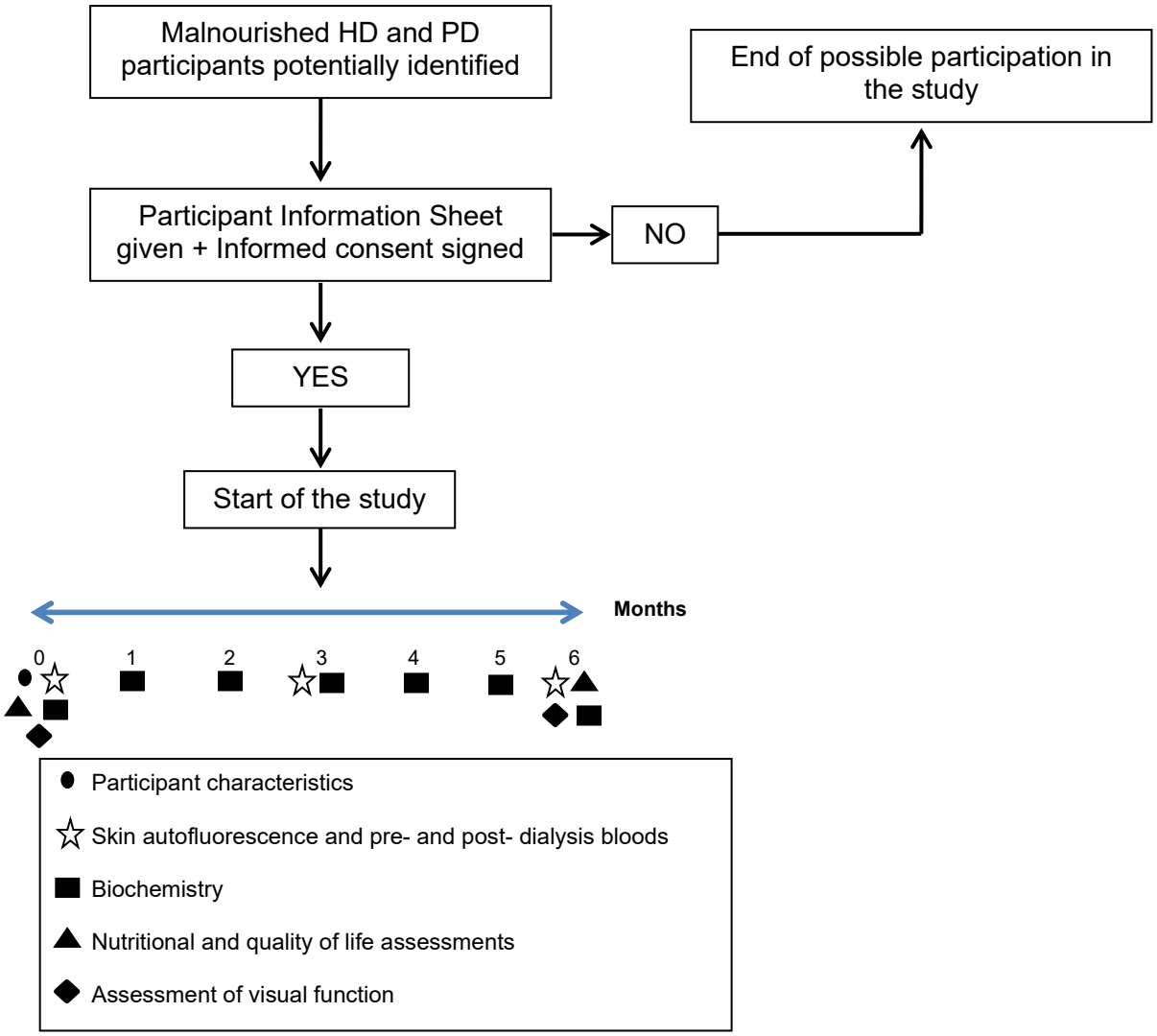
Health-related quality of life will be evaluated at baseline and at 6th month of follow-up with the SF-36 and EQ5D questionnaire.

After the first 6 months of the study, SAF readings and questionnaires will be repeated once every 6 months for up to 2 years.

Schematic diagram of study procedures and stages – Study 1 (timeline showing only the first year of study)



Schematic diagram of study procedures and stages – Study 2 (timeline showing only the first 6 months of the study)



Compliance

The Study 1 (prospective/longitudinal) involves SAF measurements and visual function, nutritional and quality of life assessments during one year of follow-up. Therefore, there is no need for an assessment of participant compliance with the study protocol. Should the participant not complete the study day, this will be classified as a withdrawal rather than non-compliance (see Section **Participant Withdrawal**).

Compliance with the intervention of Study 2 will be evaluated by means of three 24-h dietary recalls every month (1 dialysis day, 1 non-dialysis day and 1 weekend day for the HD participants and 2 weekdays and 1 weekend day for the PD participants).

Criteria for terminating trial

Study 1 is observational and will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

The intervention in Study 2 is not expected to be associated with any adverse effects. Study 2 will therefore only be terminated if it becomes clear that for unforeseen reasons the study is not feasible.

TRANSPORT AND STORAGE OF THE TISSUES

Blood samples (10 mL) will be collected at intervals as described and centrifuged. At least 2 mL of serum and 2 mL of plasma will be stored at -80°C for future analysis.

Samples and data will be stored in a linked anonymised format. Samples will be labelled using a combination of study reference, unique study identifier and cross referenced with location code numbers to permit accurate linkage to study data and the consent form.

The master database will be held by the CI in a password encrypted file.

Where participants do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004.

LABORATORY ANALYSES

Routine blood/laboratory tests will be analysed in a local NHS laboratory at the Royal Derby Hospital, which is serviced and managed according to national standards and governance frameworks. No DNA or RNA extraction will occur as part of this study. Assays for CML and inflammatory markers will be conducted in research laboratories at the University of Nottingham.

STATISTICS

Methods

Statistical analysis will be conducted by Daniela Viramontes Horner (PhD student), Professor Maarten Taal and Dr. Nick Selby. Data management and statistical analyses will be performed using statistical software SPSS version 22.0.

Data will be presented as mean ± standard deviation, median (percentiles 25%-75%), or percentages, as appropriate.

To compare baseline versus final evaluations, Wilcoxon test or paired t-test will be used in the case of dimensional variables, and McNemar test in the case of categorical variables. Intergroups comparisons will be performed using Mann Whitney test or Student t test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. To determine the significance and strength of associations, Pearson's correlation coefficient will be used for analyses of associations between continuous variables and Spearman rank for nonparametric variables. Linear regression analysis will be used to identify determinants of AGE accumulation. Cox proportional hazards models will be used to investigate the prognostic value of the accumulation of AGE for predicting mortality. A p-value less than or equal to 0.05 will be considered to have statistical significance.

Sample size calculation of Study 1 was performed by using the software nQuery Advisor v.6.0.

Sample size Study 1:

The primary outcome for sample size determination is one-year survival in relation to increased SAF levels in people on HD and PD. With a power of 80%, a two-sided alpha of 5% and an expected hazard ratio of 3.5 and 2.0 in people on PD and HD, respectively (based on similar findings of the studies of Kimura et al. [2014] and Siriopol et al. [2015]), 100 HD and 40 PD participants will be needed.

Sample size Study 2
Since this is a proof of principle study, it would be reasonable to include 40 dialysis participants (either HD or PD).

Assessment of efficacy

Primary endpoints

Study 1:
- Mean change in SAF levels (AU) from baseline to 12 months of follow-up.
- One-year survival.

Study 2:
Mean change in SAF levels (AU) after 6 months of a dietitian supervised intensive nutritional support intervention

Secondary endpoints

Study 1:
- Regression coefficients for identifying factors associated with the accumulation of AGE.
- Correlation coefficients for analysing the association of SAF levels with morbidity, biochemistry, circulating AGE levels, inflammatory biomarkers, visual function, health-related quality of life and nutritional status.

Study 2:
- Changes in biochemistry, circulating AGE levels, inflammatory biomarkers, visual function, health-related quality of life and nutritional status.

Assessment of safety

Tolerability and safety of the nutritional support intervention during the proof of principle study (Study 2) will be assessed by direct interview. If any serious adverse event (SAE) is reported during the study, it will be recorded on an appropriate SAE report form.

Procedures for missing, unused and spurious data

All SAF measurements, biochemistry, nutritional and quality of life assessments for Study 1 and 2 will be used in the statistical analysis, including data from participants who did not complete the entire study protocol.

Definition of populations analysed

Study 1:
Full Analysis set: All participants included in the protocol for whom at least one post-baseline assessment of the primary endpoint is available.

Per protocol set: All participants in the Full Analysis set who are deemed to have no major protocol violations that could interfere with the objectives of the study.

Study 2:
Full Analysis set: All randomised participants, who participated in at least one treatment and for whom at least one post-baseline assessment of the primary endpoint is available.

Per protocol set: All participants in the Full Analysis set who are deemed to have no major protocol violations that could interfere with the objectives of the study.

ADVERSE EVENTS

The occurrence of an adverse event as a result of participation within this study is not expected and as such no adverse event data will be collected.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The study will not be initiated before the protocol, informed consent forms and participant information sheets have received approval / favourable opinion from the REC, and the respective NHS Research & Development (R&D) department. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of GCP, and the Department of Health Research Governance Framework for Health and Social care, 2005.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, GCP and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Case Report Forms

Case Report Forms (CRFs) will be the primary data collection instrument. All data requested on the CRF will be recorded. Data collected includes inclusion and exclusion criteria, demographic characteristics, educational level, occupation status, family history, duration of renal replacement therapy, dialysis adequacy, aetiology of CKD, medical history (e.g. history of cardiovascular disease, diabetes and hypertension), current co-morbidities, alcohol history, smoking status, current medication, type of PD, volume of PD solutions SAF measurements, routine blood tests, circulating AGE levels, inflammatory biomarkers, visual function, nutritional and quality of life assessments.

Each participant will be assigned a study identity code number for use on CRFs, other study documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and final two digits from their year of birth.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Study Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the study, in accordance with regulatory requirements and for follow-up as required.

CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Sample Labelling

Each participant will be assigned a study identity code number for use on the samples, consent forms and other study documents and the electronic database. The identifier will include subject number, participant initials (of first and last names separated by a hyphen or a middle name initial when available) and final two digits from their year of birth.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only study staff as listed on the Delegation Log

shall have access to study documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the CI, Sponsor's designee and inspection by relevant regulatory authorities (e.g. DH, Human Tissue Authority).

DATA PROTECTION

All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The CRF will only collect the minimum required information for the purposes of the study. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the study staff and investigators and relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

Information about the study in the participant's medical records/hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for study participants and study staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Study conduct may be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the study; Trial Delegation Log; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); AE recording and reporting; accountability of study materials and equipment calibration logs.

The Study Coordinator/Academic Supervisor, or where required, a nominated designee of the Sponsor, shall carry out a site systems audit at least yearly and an audit report shall be made to the Trial Steering Committee.

TRIAL DATA

Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Study Coordinator/Academic Supervisor, or where required, a nominated designee of the Sponsor, shall carry out monitoring of study data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the study database will be checked. Where corrections are required these will carry a full audit trail and justification.

Study data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and study documents held by the CI on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all study databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this study at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files. Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

The results of this study will be submitted to peer-reviewed journals for publication as soon as data analysis is completed. The results will also be presented at national and international scientific conferences. Participants will not be identified in any publications. Participants will be contacted either by email or a letter sent to their addresses about the findings of the research (unless participants advise the research team that they do not wish to be contacted). In addition, participants will be informed of the results of the study via a departmental research newsletter, which is made available to all people on dialysis. The results of this study will form part of PhD thesis for Daniela Viramontes Horner. The study has been registered on www.clinicaltrials.gov (Trial registration number: NCT02878317)

USER AND PUBLIC INVOLVEMENT

Currently a patient focus group including people on HD is being established.

STUDY FINANCES

Funding source

This study is funded by internal funds from the University of Nottingham and Department of Renal Medicine, Royal Derby Hospital.

Participant stipends and payments

Participants will not be paid to participate in the study. Travel expenses will be offered for any hospital visits in excess of usual care. The majority of study assessments will be timed to take place at the time of routine visits for dialysis treatment or assessment.

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name)_____

Signature:_____

Date: _____

Co- investigator: (name) _____

Signature:_____

Date: _____

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