University Hospitals Birmingham NHS Foundation Trust

SCREENING FOR FABRY DISEASE IN HAEMODIALYSIS POPULATION

SoFAH STUDY

PROTOCOL VERSION 1.3 (27th Sept 2022)

RESEARCH REFERENCE NUMBERS

IRAS Number:	281233
SPONSORS Number:	RRK7404
ISRCTN Number	ISRCTN44751506

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

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

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

For and on behalf of the Study Sponsor:

Signature:
.....

Date:
...../...../.....

Name (please print):
.....

Position:
.....

Chief Investigator:

Signature:
.....

Date:
...../...../.....

Name: (please print):
.....

Statistician:

Signature:
.....

Name: (please print):
.....

Position:
.....

TRIAL SUMMARY

Study Title	Screening for Fabry Disease in Haemodialysis Population	
Internal ref. no. (or short title)	SoFAH study	
Study Design	Epidemiology; Multi-centre; Cross-sectional study	
Study Participants	Patients receiving haemodialysis under the care of eight renal units in the West Midlands	
Planned Sample Size	1,800 patients	
Follow up duration	*Single visit – clinical follow up possible*	
Planned Study Period	12 months	
	Objectives	Outcome Measures
Primary	• To examine the prevalence of Fabry disease in patients receiving haemodialysis in the U.K.	• Dried blood spot (DBS) α-gal-A enzyme assay, Lyso-GB3 assay and GLA genetic testing
Secondary	• To examine the false positive rate of DBS test for Fabry disease in haemodialysis population. • To examine the phenotypical and genetic characteristics of any new cases of Fabry disease identified by the study. • To compare the characteristics of previously undiagnosed Fabry disease cases to patients without Fabry disease amongst haemodialysis population. • Cascade screening and three generation genetic testing of index patients	• Age • Gender • Ethnicity • Cardiovascular history • Previous renal diagnosis • Other known co-morbidities (from clinical notes) • Renal biopsy results where available • α-gal-A enzyme results • Lyso-GB3 assay • Genetic mutational analysis results

FUNDING AND SUPPORT

FUNDER(S)	FINANCIAL AND NON FINANCIAL SUPPORT GIVEN
Sanofi- Genzyme Address: 4620 Kingsgate, Cascade Way, Oxford Business Park South, Oxford, OX4 2SU, UK. Tel No.: 01865 405200	Financial support for the running of trial
Kidney Research UK Address: Nene Hall, Lynch Wood, Peterborough, PE2 6FZ Tel no.: 0300 303 1100	Kidney Research UK will provide communications and engagement consultancy to the project to help raise its profile, and, subject to the study findings, help to illustrate the strategic benefits of furthering this research.
ARCHIMEDLife Laboratories Address: Leberstraβe 20/2 1110 Vienna, Austria Email: info@archimedlife.com	ARCHIMEDlife laboratories will process and analyse all specimens for Fabry test as per SoFAH study protocol. ARCHIMEDlife will also set up results
University of Leeds **Health Economics, Dr David Meads** Address: Room 11.63, Level 11, Worsley Building, Clarendon Way Email: D.Meads@leeds.ac.uk	An economic evaluation will be conducted by Dr David Meads from the perspective of the health and personal social services care provider in England based on the outcome of the SoFAH screening study.

KEY TRIAL CONTACTS

Chief Investigator	Prof Indranil Dasgupta Email address: indranil.dasgupta@uhb.nhs.uk Tel number: +44 (0)121 424 2158 Fax number: +44 (0)121 424 1159
Trial Co-Ordinator	 Manjinder Sandhu Email Address: Sofahtrial@uhb.nhs.uk Telephone : +44 (0)7721 856018
Sponsor	University Hospitals Birmingham NHS Foundation Trust Mindelsohn Way, Birmingham, B15 2TH.
Funder(s)	Sanofi- Genzyme Address: 4620 Kingsgate, Cascade Way, Oxford Business Park South, Oxford, OX4 2SU, UK. Tel No.: 01865 405200
Key Protocol Contributors	Prof Indranil Dasgupta Email address: indranil.dasgupta@uhb.nhs.uk Dr Khai Ping Ng Email address: khaiping.ng@nhs.net Dr Tarek Hiwot Email address: Tarekegn.Geberhiwot@uhb.nhs.uk
Health Economist	Dr David Meads Email: D.Meads@leeds.ac.uk
Committees	Prof Indranil Dasgupta Email address: indranil.dasgupta@uhb.nhs.uk Tel number: +44 (0)121 424 2158 Dr Khai Ping Ng Email address: khaiping.ng@nhs.net Dr Tarek Hiwot Email address: Tarekegn.Geberhiwot@uhb.nhs.uk Trial manager Research nurse Data manager Full contact details including phone, email and fax numbers

ROLE OF STUDY SPONSOR AND FUNDER

The study is sponsored by University Hospital Birmingham NHS Foundation trust, which oversees the conduct of the study.

Sanofi-Genzyme provides financial support to the study.

ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

SoFAH study will be coordinated by the study coordinator at the Research and Development department, University Hospitals Birmingham NHS foundation Trust, according to the current guidelines for Good Clinical Practice. Participating renal units may be monitored by trial manager to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki.

The chief investigator (CI) takes overall responsibility for the conduct of study. A principal investigator (PI) will be nominated at each renal unit and will take responsibility for all activity conducted at site. Any delegated or devolved responsibility will be documented on the delegation log. It is the PI's responsibility to ensure that staffs are appropriately trained to perform the tasks that they are delegated to and that it is appropriately documented on the delegation log.

Protocol contributors

Prof Indranil Dasgupta designed the study. Dr Khai Ping Ng drafted the protocol.

KEY WORDS: Fabry Disease, Haemodialysis, prevalence, undiagnosed, Midlands, UK.

LIST of CONTENTS

LIST OF ABBREVIATIONS

CI	Chief Investigator
EQ5D-5L	Euro Quality of Life 5D-5L questionnaire
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
ICERs	Incremental Cost-Effectiveness Ratios
IQR	Inter-quartile range
Lyso-GB3	Globotriaosylsphingosine
PI	Principal Investigator
PIS	Patient Information Sheet
PSA	Probabilistic Sensitivity Analyses
QALY	Quality Adjusted Life Year
REC	Research Ethics Committee

TRIAL FLOW CHART

Figure 1.0: Trial flow chart

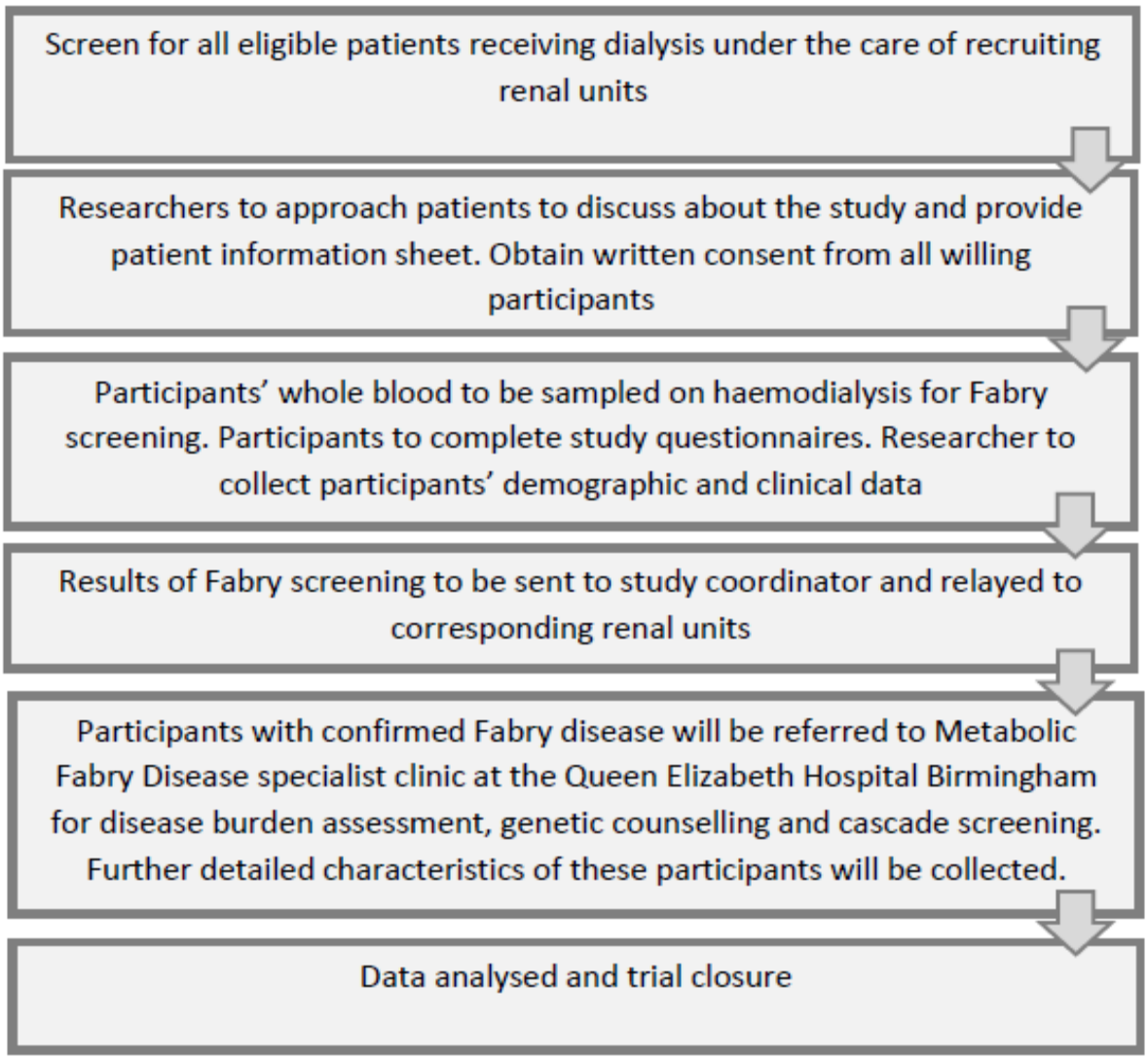
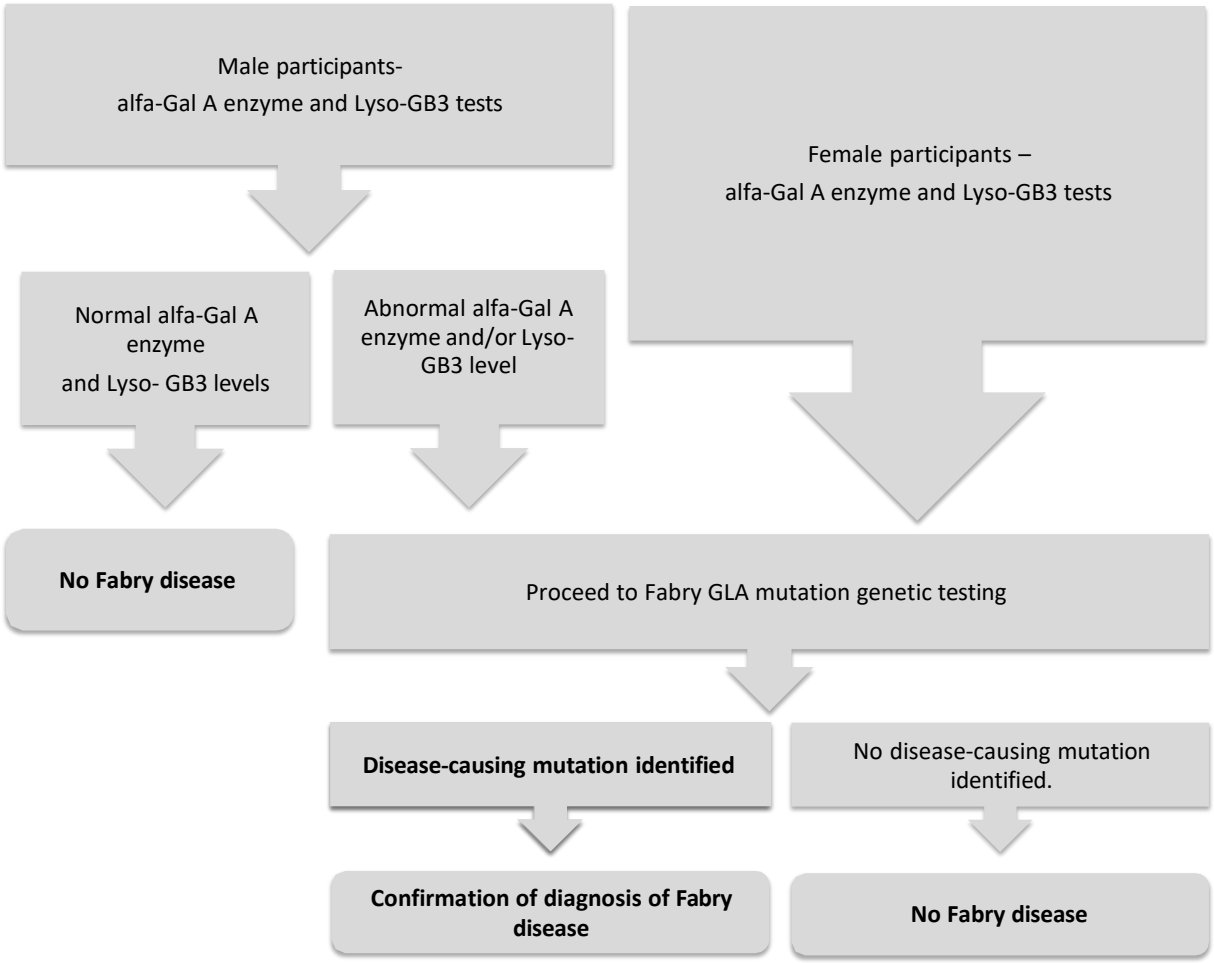


Figure 2.0: SoFAH screening algorithm



STUDY PROTOCOL

SoFAH study: Screening for Fabry Disease in Haemodialysis Population

1 BACKGROUND

Fabry disease is an X-linked inherited lysosomal storage disorder. A mutation of the GLA gene leads to the deficiency of a lysosomal hydrolase enzyme, α-galactosidase A. It results in progressive accumulation of glycophospholipids (predominantly globotriaosylceramide 3, Gb3) in cells throughout the body, resulting in multisystem disorder and premature death [1-3]. Clinical manifestations include anhidrosis or hypohidrosis, acroparaesthesia, corneal opacities, angiokeratomas, sensorineural deafness, non-specific bowel disturbance, progressive proteinuric kidney disease, fibrotic cardiac disease, progressive hypertrophic cardiomyopathy and cerebrovascular disease [2]. Though Fabry disease is an X-link disorder, both male and female can be affected. Patients may present in either classic form, which has severe clinical phenotype, or atypical variant, which presents later in the 3rd to 7th decade of life [3, 4]

The prevalence of Fabry disease was previously estimated to be 1 in 117,000 to 1 in 833,000 [5, 6]. However, recent large genetic screening programs of newborns reported incidence of 1 in 1,300 to 1 in 7,800 of Fabry disease in male [7-10], demonstrating that Fabry disease is more frequent that previously expected.

A systematic review carried out nearly a decade ago estimated prevalence of Fabry Disease amongst end-stage kidney disease population to be 0.33% in men and 0.1% in women [11]. However, two large national screening studies in Europe, using blood spot test in end stage kidney disease patients on haemodialysis suggested the prevalence is much higher at around 3.5%. [15,16]. The diagnosis of Fabry Disease is frequently delayed; according to a registry study the average delay is 14 years in males and 19 years in females [7] [11]. In the UK, screening is not routinely done but available in the forms of dried blood spots which measures the α-galactosidase A activity or detection of urine total globotriaosylceramide detection.

Female carriers may have normal to low enzyme activity due to mosaic x-inactivation, therefore plasma level of Lyso-GB3 should be measure and genetic testing may be required.

2 RATIONALE

Due to its non-specific manifestations, Fabry disease especially the later-onset variant, is often under-diagnosed. In addition, as mentioned above, there is often a significant delay in diagnosis [7]. Our experience in the University Hospitals Birmingham Fabry Disease Clinic suggests that the disease is often diagnosed late in kidney patients. Screening of high-risk groups is therefore important for case finding [12]. Though a previous UK based screening of 155 male on haemodialysis did not identify any new cases of Fabry disease, it was limited by its small sample size [13]. The prevalence has been found to be higher in other populations [15,16]. The aim of this study is to evaluate the prevalence of Fabry disease in a large UK haemodialysis population, especially in those who are not known to have a diagnosis of their primary kidney disease (chronic kidney disease of unknown aetiology). This may ultimately change the investigation protocol for patients with chronic kidney disease especially those with an unknown cause. This will also lead to cascade screening of relatives of identified patients which in turn will facilitate early institution of enzyme replacement therapy. Moreover, the identified patients in the haemodialysis population may benefit from enzyme replacement therapy in terms of their cardiac involvement, i.e. coronary artery disease, cardiac failure and death [17].

2.1 Assessment and management of risk

There is no significant additional risk associated with the screening test as blood is sampled during haemodialysis.

All male participants are screened using alfa-galactosidase enzyme and Lyso-GB3 assay. A high threshold of < 30% for alfa-galactosidase enzyme level and Lyso-GB3 level of > 2.7 nm are set to avoid false negative results. If either of the enzyme or Lyso GB3 level is abnormal, further

genetic testing for GLA mutation will be performed in order to confirm the diagnosis of Fabry disease.

Due to mosaic x-inactivation, all female participants will be screened using alfa-galactosidase enzyme level, lyso-GB3 assay as well as genetic mutation test in order to confirm diagnosis of Fabry disease.

There is a potential risk of psychological impact on patient who is diagnosed with Fabry disease through this study. This risk will be mitigated and managed by the support of University Hospitals Birmingham Fabry Disease specialist clinic as part of their routine NHS care for further assessment, counselling and support. In addition, this will also lead to cascade screening of relatives of newly diagnosed participants by the specialist Fabry disease clinic.

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.1 Primary objective

To estimate the prevalence of Fabry Disease in a large haemodialysis population in the United Kingdom.

3.2 Secondary objectives

- To examine the false positive rate of dried-blood spot alfa-galactosidase enzyme test for Fabry disease in male and female haemodialysis population.
- To examine the false positive rate of dried-blood spot lyso-GB3 test for Fabry disease in male and female haemodialysis population.
- To examine the phenotypical and genetic characteristics of any new cases of Fabry disease identified by the SoFAH study.
- To determine cost-effectiveness of screening for Fabry disease in haemodialysis population.

3.3 Outcome measures

3.3.1 Primary endpoint/outcome

- Prevalence of Fabry disease in haemodialysis population in the UK as defied by dried blood spot alfa-galactosidase A enzyme activity, Lyso-Gb3 level and genetic mutation of GLA analysis.

3.3.2 Secondary endpoints/outcomes

- Clinical characteristics of new cases of Fabry disease identified by the study, including age, gender, ethnicity, duration of onset, cardiovascular history, previous renal diagnosis and previous renal biopsy report.
- Fabry disease symptoms questionnaire results.
- EQ5D-5L questionnaire

3.3.3 Exploratory endpoints/outcomes

None

4 STUDY DESIGN

SoFAH is a cross-sectional, epidemiological screening study of Fabry disease.

5 STUDY SETTING

It is a multi-centre study, including all haemodialysis population under the care of eight renal units in the Midlands, in the UK.

Eight centres will be included in the study (data retrieved from the renal registry 2015) giving an estimated study population of 2,451.

Sites	**Number of patients on haemodialysis**
University Hospitals Birmingham NHS Trust	450
Derby Royal Hospital	220
Leicester General Hospital	400
Royal Stoke University Hospital	334
University Hospital Coventry and Warwickshire	354
Dudley Russells Hall Hospital	172
Wolverhampton New Cross Hospital	318
Royal Shrewsbury Hospital	203
Total	**2451**

6 ELIGIBILITY CRITERIA

6.1 Inclusion criteria

All patients receiving haemodialysis under the care of the eight participating renal units:

- Age 18 years and above
- Capable of giving informed consent

6.2 Exclusion criteria

- Patient with known diagnosis of Fabry Disease.
- Patient unable to give consent .

7 STUDY PROCEDURES

Refer to Figure 1.0: Study Flow Chart and Figure 2.0: SoFAH Fabry Screening algorithm.

7.1 Recruitment

7.1.1 Patient identification

All adult patients receiving haemodialysis under the care of the eight participating renal units are identified by the local PIs and recruited by the local members of the care team.

- Each renal unit provides the list of patients on haemodialysis to the SoFAH study researcher who is part of patient's usual care team.
- Researcher provides all eligible patients with Patient Information Sheet on SoFAH study.
- Researcher obtains informed, written consent from willing participants during their haemodialysis session.

- All patients will be given unique SoFAH research study numbers upon recruitment. All recruited patients will be registered via a secure, centralised and web-based REDCap database. Local PIs will keep the logs of recruited patients and their corresponding unique study numbers. All consent form and research data will only be stored under study numbers without patients' identifiable data.

Pseudonymised screening log will also be maintained at each site in order to monitor reasons for non-participation and ensure representativeness of recruited participants.

Data will be recorded on paper CRF and entered onto an electronic database which will be developed for the study. The database will be developed by the study coordinator and accessible at all sites.

7.2 Consent

All eligible patients are given SoFAH patient information sheet (PIS) and consent form by the Researcher during one of their haemodialysis sessions. All potential participants will be given sufficient time to consider their participation in the study and opportunity to ask questions regarding the study after receiving the PIS, following which the consent will be taken.

Written, informed consent is obtained by Researcher prior to the participant undergoing procedures that are specifically for the purposes of the study. In the case of participants who cannot read or write or require translators, the study will allow a witness to sign on a participant's behalf (in the case of problems with reading or writing), allow a witness to date the form on behalf of the participant and allow hospital or personal interpreter.

The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment and must be provided with a contact point where he/she may obtain further information about the trial. Where a participant is required to re-consent or new information is required to be provided to a

participant it is the responsibility of the (PI) principal investigators to ensure this is done in a timely manner.

The PI retains overall responsibility for the informed consent of participants at their sites and ensures that the Researcher who are delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki. The PI takes responsibility for ensuring that all vulnerable subjects are protected and participate voluntarily in an environment free from coercion or undue influence.

7.2.1 Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable

All patients will be consented for Fabry disease genetic testing.

7.3 Baseline data

All consented participants' demographic and clinical data will be collected, which include age, gender, ethnicity, clinical symptoms associated with Fabry disease, cardiovascular history, previous renal diagnosis, previous renal biopsy report and dialysis vintage. Data will be collected on paper CRF and entered into study database.

7.4 Screening of Fabry Disease

- Whole venous blood samples (2.5 ml) will be sampled from all participants during haemodialysis from the dialysis line and apply to the dried blood spot card (see appendix 1).
- All dried blood spot specimens will only have study number and no patient identifiable data.

- Dried blood spot specimens will be sent for analysis to the Archimed Laboratories, Austria Europe. All specimens will be labelled with unique SOFAH research study number and no personal data will be sent to Austria.
- The dried blood spot results will be returned by the laboratories to the study coordinator and chief investigator via a secured portal system set up by the Archimed Laboratories.

7.5 Long term follow-up assessments

All participants who were tested negative for Fabry disease will be notified via letter by the research team and chief investigator. No further visits or follow-up will be required unless the participant's Fabry test is positive.

The local kidney consultants (PI) involved in this study will inform the participants who are tested positive for Fabry disease of the new diagnosis. They will be referred to the specialist Fabry disease clinic at the Queen Elizabeth Hospital in Birmingham (UHB) as part of their routine NHS care for further assessment, counselling and support. In addition, this will also lead to cascade screening of relatives of newly diagnosed participants by the specialist Fabry disease clinic as part of standard of care, which in turn will facilitate early institution of enzyme replacement therapy.

7.6 Withdrawal criteria

Participants will be withdrawn from the study if they choose not to continue with the study. No further new information about the patient will be collected, although information which has already been collected may continue to be used in order to preserve the value of the study. If patient was tested positive for Fabry disease prior to withdrawal, the research team and local PI will make arrangements for future care to continue, which include referral to the specialist Fabry disease clinic at UHB.

7.7 Storage and analysis of samples

All dried blood spot cards will be transported for analysis to the Archimed Laboratories, Vienna via arranged delivery/courier service within 2 days of sampling. Until then the cards will be stored securely in dry condition, room temperature in the research office. Specimens will be disposed of 6 months after analysis by the Archimed Laboratories.

7.8 End of Study

The study ends after all participants complete their Fabry disease screening tests, and that the results for the screening tests are available and complete.

8 STATISTICS AND DATA ANALYSIS

8.1 Sample size calculation

All the adult haemodialysis patients, approximately 2,451 patients, under the care of the eight renal units are invited to the study. We estimate that the response rate for patients agreeing to participate to be 80% [14]. Hence, we aim to have a total of 1,800 participants recruited into the study. Given the prevalence of Fabry disease in dialysis population estimated to be 0.3% [11], there will be approximately 6 patients with Fabry disease in our study population. However, screening was mainly done using alfa-galactosidase enzyme test, which is not reliable in diagnosing female patients with Fabry disease. Using alfa-galactosidase enzyme, Lyso-GB3 and GLA genetic tests, SoFAH study aims to provide more accurate prevalence rate of Fabry disease amongst the haemodialysis population in the UK.

8.2 Planned recruitment rate

We aim to recruit 70 participants per week across all sites over 12 months. Seven Researchers working at 2 days per week will be involved in the recruitment. Each will aim to recruit 10 patients per week.

8.3 Statistical analysis plan *(see under 10.3.3)*

- Descriptive statistics will be used to assess prevalence of Fabry disease and secondary objectives
- Cost-effectiveness analysis of screening for Fabry disease among an unselected population of haemodialysis patients based on the outcome of the descriptive screening study.

8.3.1 Summary of baseline data and flow of patients

Demographic and clinical data of the participants, which include age, gender, ethnicity, clinical symptoms associated with Fabry disease, cardiovascular history, previous renal diagnosis, previous renal biopsy report and dialysis vintage are collected via renal database and electronic patient medical record after enrolment.

All participants are asked to complete a Fabry disease symptoms paper questionnaire and EQ5D-5L paper questionnaire during dialysis. Participants with language barrier, or unable to read or write in English can have help from Researchers or haemodialysis nurses in order to complete the forms. This will be monitored regularly by the trial coordinator.

(Please refer to Figure 1.0 for flow of patients)

8.3.2 Primary outcome analysis

The primary outcome of the prevalence of Fabry disease in haemodialysis population will be reported using descriptive statistics.

8.3.3 Secondary outcome analysis

The secondary outcomes on genetic and clinical characteristics of new cases of Fabry disease identified in the study will be reported using descriptive statistics. Comparative analysis of demographic and clinical differences between patients with Fabry disease and those without Fabry disease in the study population will be performed using SPSS.

Numerical values are expressed as mean (standard deviation) for parametric data or median (interquartile range (IQR)) for non-parametric data. Non-parametric variables will be log transformed prior to analysis to achieve normal distribution or, if this is not achieved, variables will be analysed using non-parametric test. Categorical values will be presented as percentage (number of participants). Parametric continuous data will be compared by means of student t-test, whilst non-parametric continuous data were compared using Mann-Whitney test. Categorical data will be compared by means of χ^2 test. Statistical significance is defined as two-tailed p value <0.05.

8.4 Subgroup analyses

None

8.5 Adjusted analysis

None

8.6 Interim analysis and criteria for the premature termination of the trial

None

8.7 Subject population

All consented participants will be recruited into the study.

8.8 Procedure(s) to account for missing or spurious data

In the case of missing enzyme or lyso-GB3 results, participants will be asked if they agree for repeat blood sampling on their next haemodialysis session.

Researchers at each site will ask patients via telephone or face-to-face for any missing data on their demographics or clinical symptoms. Principal investigators will be involved to facilitate the completeness of the clinical data of their recruited participants to minimise missing data.

The reasons for any missing data are to be documented in the case report form.

Missing data will be excluded from the analysis.

8.9 Other statistical considerations.

8.9.1 Economic evaluation

Given budgetary constraints, any health care investment requires evidence of value for money to inform guideline development and ensure uptake by clinicians. An economic evaluation will be conducted from the perspective of the health and personal social services care provider in England. The evaluation will follow the NICE reference case for technology appraisals and as such the primary endpoint will be cost per quality-adjusted life year (QALY) over a lifetime horizon.

We will compare the cost-effectiveness of Fabry disease screening strategies vs. usual care (no screening). We will develop a decision-analytic model, mapping out the patient pathway and estimating lifetime costs, benefits and harms of the screening strategies. The model will be

developed with patient and clinician input but is likely to include screening costs, early intervention with enzyme replacement therapy, prevention of kidney disease progression and dialysis and cardiovascular disease. We will use data from the proposed observational study to inform diagnostic yield and Fabry disease complication rates. These will be supplemented by targeted literature reviews.

We will conduct analyses to extend the evaluation beyond the incident patient to family members who could benefit from early treatment following diagnoses in cascade screening. We will estimate the budget impact of rolling out the screening strategies.

We will conduct extensive one-way and scenario sensitivity analyses to identify key drivers of cost-effectiveness and areas of uncertainty. We will conduct probabilistic sensitivity analyses (PSA) to characterise parameter uncertainty and present results in terms of incremental cost-effectiveness ratios (ICERs), cost-effectiveness acceptability curves and net-benefit distributions.

9. STUDY MANAGEMENT

SoFAH study will be coordinated by the SoFAH research team at the University Hospitals Birmingham NHS Trust according to the current guidelines for Good Clinical Practice. The conduct of the research will be overseen by the CI. The study management committee (membership detailed in the administrative information on page 6) will monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself. The study management group will meet 3 monthly to provide oversight of the developing trial with more frequent operational meeting of the CI, trial manager and trial team as required.

The responsibility for engaging with sites during termination of site activity and end of study will be overseen by the CI or their delegate.

CI is responsible for the design, conduct, data analyses and reporting of SoFAH study. Trial coordinator will be responsible for the day to day operations of the SoFAH study. The coordinator will visit recruiting sites to engage the local research team, perform site initiation and training, maintain study master file, ensure data integrity and arrange regular monitoring meetings. Local PIs will be responsible for providing eligible list of patients for recruitment, review DBS results on Archimed laboratory results portal and inform each participants of their results. Local site researchers will be responsible for recruitment of eligible haemodialysis patients, ensure the participants meet eligibility criteria, complete informed consent, complete CRFs, obtain DBS specimens, liaise with Archimed laboratories to transport specimens for analysis and enter data onto the SoFAH database.

10. DATA MANAGEMENT

Data protection/confidentiality

All information collected will be subject to the Data Protection Act and will be kept strictly confidential, in line with the UK General Data Protection Regulation (GDPR).

Patient identifiable data will be collected from a combination of sources including directly from participants and their medical records. All identifiable data will be collected following patient's consent. Patient identifiable data are stored only on their corresponding recruiting site, in a separate place to the pseudonymised data. Case report forms (CRFs) must be completed and signed/dated by the research team as soon as the required information is available. Paper records will be kept securely in locked filing cabinets within secure areas on each site. In all cases, it remains the responsibility of the investigator to ensure that they have been completed correctly and that the data are accurate. Entries should be made in ballpoint

pen preferably in black ink and must be legible. Any errors should be crossed out and with a single stroke, the correction inserted and the change initiated and dated. If it is not clear why a change has been made, an explanation should be written next to the change.

All participants will be allocated a study number and this will be used throughout the study. Pseudoanonymised data will be entered electronically onto REDCap secured, web-based database developed specifically for the use of SoFAH research team. Data entered will be consistent with the source data and the discrepancies will be explained. All missing and ambiguous data will be queried. Any data will be stored securely and only accessed by the researchers for the purpose of this study.

Any samples sent away to the Archimed laboratories will only use the study number so that patient data and identity are kept confidential. Archimed laboratories will set up a dedicated SoFAH electronic reporting portal. After sampling, each DBS specimen will be registered via the corresponding study number and DBS card number onto the SoFAH Archimed electronic portal before transporting for analysis to Archimed laboratory. The DBS will be sent via pre-arranged weekly courier service from each site directly to the Archimed laboratories. The reporting portal will only contain study number and gender for each participant. The DBS results will be reported via the SoFAH Archimed electronic result portal. The site research team is responsible for entering the DBS results onto SoFAH REDCap database. The local site PI is responsible for informing the participants of the DBS results and referring any patient with new diagnosis of Fabry disease to the specialist Fabry disease clinic at the University Hospital Birmingham NHS Trust.

Both SoFAH REDCap and Archimed databases will only be accessible to persons authorised by the CI. New requests for access to the database will have to be approved by the trial co-ordinator. All members of the research team are trained in the appropriate use of confidential information and will receive training on how to access/use the databases.

Data processing and transfer of data to Health Economist statistician

Only pseudoanonymised data will be transferred to the statistician (Dr David Meads) at the University of Leeds for health economic analysis at the end of the study. The statistician will have no access to patient identifiable data. Agreement will be made between the sponsor (University Hospitals Birmingham NHS Trust) and the University of Leeds prior to transfer of data.

Trial documentation and archiving

Upon completion of study, all sites will be responsible for archiving the trial data, including data generated from the Archimed laboratories, for 10 years. The CI will have responsibility for access to the archived material.

Preparation and submission of Annual Safety Report

As the study will be completed within 12 months, annual report will not be required.

11. AUDIT & MONITORING

To enable monitoring and audit, SoFAH research team will keep records, all original signed informed consent forms and copies of all case report forms, at the recruiting sites.

The study may be audited by University Hospitals Birmingham NHS Foundation Trust under their remit as Sponsor and other regulatory bodies to ensure adherence to Good Clinical Practice and the UK Policy Framework for Health and Social Care Research.

Monitoring of study data shall include confirmation of subject eligibility and informed consent; adherence to the study protocol, source data verification; data storage and data transfer procedures; local quality control checks and procedures especially with regard to questionnaire data from patients who need help in completion, back-up and disaster recovery of any local databases and validation of data manipulation.

The CI, or a nominated designee of the CI, shall carry out monitoring of study data as an on-going activity. SoFAH trial coordinator will be in regular contact with the recruiting sites to check on progress and answer any queries. Trial coordinator will also monitor consent forms and data entered on the SoFAH database for the 1st and 10th recruited participants from each sites in order to ensure compliance with protocol and data integrity. The first 10 questionnaire data by participants who required help with completion will also be monitored to ensure consistent data and avoid missing data. In addition, DBS results will be monitored for any missing data, which can occur due to poor sampling technique or storage condition prior to transport. Recruiting sites with 3 or more missing DBS results will trigger a DBS re-training session for the site researchers

Study conduct will be subject to systems audit of the trial master file for inclusion of essential documents; permissions to conduct the trial; study delegation log; curriculum vitae 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, timeliness of visits); accountability of study materials and equipment calibration logs.

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

12. REGULATORY APPROVALS

The study will not commence until HRA approval has been obtained from the HRA and sites have confirmed participation in the study. Approvals will be obtained from the Research Ethics Committee (REC) prior to the commencement of this study. Confirmation of Capability and Capacity will be obtained from the each site prior to any research activity being undertaken. The study will be conducted in accordance with principles of the International Conference on Harmonization (ICH) Good Clinical Practice (GCP).

12.1 Sponsorship and Indemnity

University Hospitals Birmingham NHS Foundation Trust will act as the Sponsor to this study. Delegated responsibilities will be assigned to the Chief Investigator. University Hospitals Birmingham NHS Foundation Trust holds standard NHS Hospital indemnity with NHS Litigation Authority for NHS Trusts in England, which apply to this study.

13. DISSEMINATION POLICY

Kidney Research UK is involved in this study and will provide communications and engagement consultancy to the project to help raise its profile, and, subject to the study findings, help to illustrate the strategic benefits of furthering this research. In addition, Kidney Research UK will also facilitate communications of future patient benefit potential of the study to the Charity's patient and supporter audiences during the study, support the progress of the study through advising on patient-facing materials as necessary and support the academic and lay dissemination of its results.

The findings of this study will be reported at appropriate conferences and the aim is to publish them in a relevant open access journal. A summary report will be produced adherent to the funder's guidelines at the completion of the project. Participants in the study will also be informed of lay summary of the study outcome via a letter sent by the SoFAH research team.

14. REFERENCES

1. Mahmud HM: Fabry's disease--a comprehensive review on pathogenesis, diagnosis and treatment. JPMA The Journal of the Pakistan Medical Association 2014, 64(2):189-194.
2. Schiffmann R, Hughes DA, Linthorst GE, Ortiz A, Svarstad E, Warnock DG, West ML, Wanner C: Screening, diagnosis, and management of patients with Fabry disease: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. Kidney international 2017, 91(2):284-293.
3. Mehta A, Ricci R, Widmer U, Dehout F, Garcia de Lorenzo A, Kampmann C, Linhart A, Sunder-Plassmann G, Ries M, Beck M: Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey. European journal of clinical investigation 2004, 34(3):236-242.
4. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, Yoshida A, Kuriyama M, Hayashibe H, Sakuraba H et al: An atypical variant of Fabry's disease in men with left ventricular hypertrophy. The New England journal of medicine 1995, 333(5):288-293.
5. Meikle PJ, Hopwood JJ, Clague AE, Carey WF: Prevalence of lysosomal storage disorders. Jama 1999, 281(3):249-254.
6. Pinto R, Caseiro C, Lemos M, Lopes L, Fontes A, Ribeiro H, Pinto E, Silva E, Rocha S, Marcao A et al: Prevalence of lysosomal storage diseases in Portugal. European journal of human genetics : EJHG 2004, 12(2):87-92.
7. Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, Ponzone A, Desnick RJ: High incidence of later-onset fabry disease revealed by newborn screening. American journal of human genetics 2006, 79(1):31-40.
8. Scott CR, Elliott S, Buroker N, Thomas LI, Keutzer J, Glass M, Gelb MH, Turecek F: Identification of infants at risk for developing Fabry, Pompe, or mucopolysaccharidosis-I from newborn blood spots by tandem mass spectrometry. The Journal of pediatrics 2013, 163(2):498-503.
9. Hopkins PV, Campbell C, Klug T, Rogers S, Raburn-Miller J, Kiesling J: Lysosomal storage disorder screening implementation: findings from the first six months of full population pilot testing in Missouri. The Journal of pediatrics 2015, 166(1):172-177.
10. Hwu WL, Chien YH, Lee NC, Chiang SC, Dobrovolny R, Huang AC, Yeh HY, Chao MC, Lin SJ, Kitagawa T et al: Newborn screening for Fabry disease in Taiwan reveals a high incidence of the later-onset GLA mutation c.936+919G>A (IVS4+919G>A). Human mutation 2009, 30(10):1397-1405.
11. Linthorst GE, Bouwman MG, Wijburg FA, Aerts JM, Poorthuis BJ, Hollak CE: Screening for Fabry disease in high-risk populations: a systematic review. Journal of medical genetics 2010, 47(4):217-222.
12. Terryn W, Cochat P, Froissart R, Ortiz A, Pirson Y, Poppe B, Serra A, Van Biesen W, Vanholder R, Wanner C: Fabry nephropathy: indications for screening and guidance for diagnosis and treatment by the European Renal Best Practice. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2013, 28(3):505-517.
13. Wallin EF, Clatworthy MR, Pritchard NR: Fabry disease: results of the first UK hemodialysis screening study. Clinical nephrology 2011, 75(6):506-510.
14. Merta M, Reiterova J, Ledvinova J, Poupetova H, Dobrovolny R, Rysava R, Maixnerova D, Bultas J, Motan J, Slivkova J et al: A nationwide blood spot screening study for Fabry disease in the Czech Republic haemodialysis patient population. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2007, 22(1):179-186.
15. Kotanko P, Kramar R, Devrnja D, Paschke E, Voigtländer T, Auinger M, Pagliardini S, Spada M, Demmelbauer K, Lorenz M, Hauser AC, Kofler HJ, Lhotta K, Neyer U, Pronai W, Wallner M, Wieser C, Wiesholzer M, Zodl H, Födinger M, Sunder-Plassmann G. Results of a nationwide

screening for Anderson-Fabry disease among dialysis patients. J Am Soc Nephrol. 2004 May;15(5):1323-9. Erratum in: J Am Soc Nephrol. 2004

16. Merta M, Reiterova J, Ledvinova J, Poupetová H, Dobrovolny R, Rysavá R, Maixnerová D, Bultas J, Motán J, Slivkova J, Sobotova D, Smrzova J, Tesar V. A nationwide blood spot screening study for Fabry disease in the Czech Republic haemodialysis patient population. Nephrol Dial Transplant. 2007 Jan;22(1):179-86. Epub 2006 Oct 13.

17. Antonio Pisani, Letizia Spinelli, Massimo Sabbatini et al. Enzyme Replacement Therapy in Fabry Disease Patients Undergoing Dialysis: Effects on Quality of Life and Organ Involvement. American Journal of Kidney Disease. 2005; 46(1): 120-127

15. Appendices

Appendix 1: Blood sampling procedure using Dried Blood Spot Card

Blood Sampling using a Dried Blood Spot Card

ARCHIMED Life Science GmbH

Step 1: Preparation

Each ARCHIMEDlife Sampling Kit contains a Dried Blood Spot (DBS) card, an Informed Consent Form (ICF) and a pre-addressed return envelope.

Please fill in **all patient information** on the DBS card.
Slightly fold the flap back so that the filter paper does not touch the paper back during blood collection.

Avoid touching the blood sampling circles.

Step 2: Blood sampling

Take a sample of blood from a finger stick.*

- Choose finger for sampling (2nd or 3rd index finger recommended).
- Stimulate blood flow by shaking hand briefly or massage finger from hand to fingertip a few times.
- Clean finger with isopropyl alcohol and allow to dry completely.
- Perform stick with incision device and gently wipe off first drop of blood using a sterile dry gauze or cotton ball.
- Allow formation of a large drop of blood.
- Lightly touch the blood drop to one side of the filter paper allowing blood to fill the preprinted circle completely. (Figure 1)
  Do not touch the DBS card directly to the finger stick site!
- Repeat until all circles are filled (a minimum of three circles is required).

PLEASE NOTE:
Front and back side of the blood sampling circles must be completely filled and evenly saturated. Do not layer blood. Fill in at least three circles!

Alternate sampling options:

- Blood samples from collected from venipuncture into EDTA tubes can be used (no heparin or citrate blood). Apply 75 µL using a micropipette into the middle of each sampling circle. Do not touch the pipette to the DBS card. (Figure 2)
- Blood samples from heel stick can be used (for use with newborns only). Visit **www.archimedlife.com/dbs** to download our Neonatal DBS Sample Collection instruction. (Figure 3)

Figure 1

Backside of filterpaper:

Figure 2

Figure 3

Step 3: Drying

The DBS card must remain open at room temperature for four hours for the blood spots to dry completely. Do not use radiators, hair dryers or sunlight to accelerate the process.

Once completely dried, close the DBS card and put it into the provided return envelope together with the signed ICF. The completed Sampling Kit should only be stored at room temperature until shipment.

Step 4: Sample return

Please send the completed Sampling Kit to us. Return the kit as soon as possible, ideally within **24 hours** of sample collection. It is highly recommended to send the Sampling Kit to our lab no more than three days after sample collection. Please ensure Sampling Kits are stored at room temperature during that time.

ARCHIMEDlife | One drop of blood for a better life
www.archimedlife.com

For any questions or other additional information, please contact us at info@archimedlife.com

* Sample collection is intended to be performed by trained personnel. Samples should be taken directly from the finger stick onto filter paper. Do not use blood containing anticoagulant such as heparin or citrate.

A020106 EN04