Defining normal bone turnover by mortality risk: a study of bone alkaline phosphatase in haemodialysis patients

ALPHA Study

www.alphastudy.org.uk

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Sponsor:	**East Kent Hospitals University NHS Foundation Trust**
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East Kent Hospitals University NHS
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List of Abbreviations

ACR	Albumin to creatinine ratio
AE	Adverse Event
ALP	Alkaline phosphatase
AR	Adverse Reaction
CI	Chief Investigator
CKD	Chronic kidney disease
CKD-MBD	Chronic kidney disease-mineral bone disorder
CRF	Case Report Form
eGFR	Estimated glomerular filtration rate
EKHUFT	East Kent Hospitals University NHS Foundation Trust
GFR	Glomerular filtration rate
GCP	Good Clinical Practice
KKRG	Kent Kidney Research Group
MDRD	Modification of Diet in Renal Disease
NIHR	National Institute for Health Service Research
PI	Principal Investigator
PIS	Participant Information Sheet
PTH	Parathyroid hormone
MREC	Main Research Ethics Committee
SAE	Serious Adverse Event
SSA	Site Specific Assessment
SMG	Study Management Group

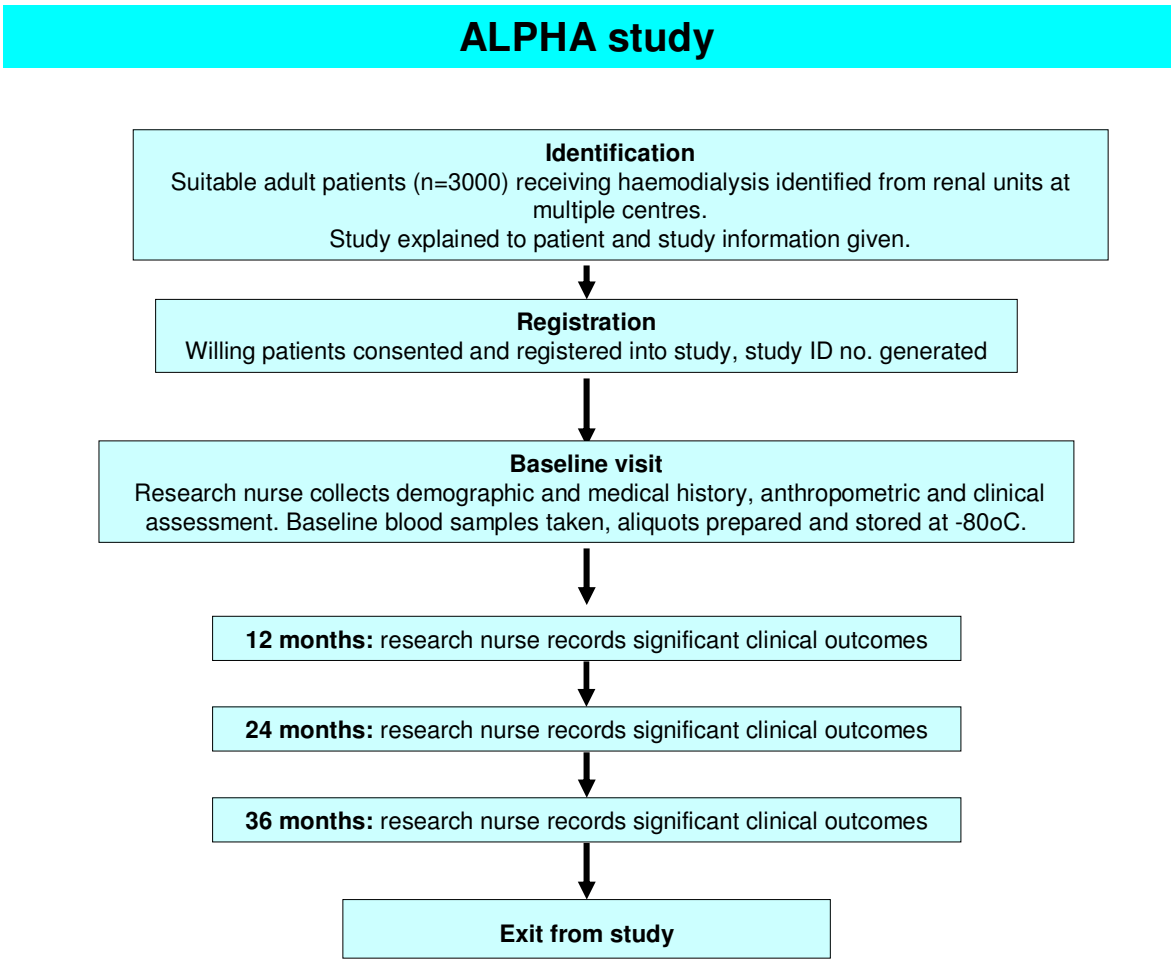
1 Summary & Study Schema

EXECUTIVE SUMMARY	
Title	Defining normal bone turnover by mortality risk: a study of bone alkaline phosphatase in haemodialysis patients
Acronym	Alpha
Study design and methods	Multi-centre UK prospective longitudinal cohort study – 3000 haemodialysis patients will have baseline (month 0) blood tests for bone alkaline phosphatase and other potential markers of bone and mineral metabolism. They will be followed for up to 5 years and all cause and cardiovascular mortality will be recorded.
Total number of participants planned	3000
Study duration per participant	36 months
Accrual period	12 months
Estimated total study duration	72 months
Primary study objective	To define an optimal range of bone ALP concentration in terms of morbidity and mortality.
Main inclusion criteria	Prevalent haemodialysis patients in the participating renal units who have been receiving dialysis for at least three months. Written informed consent
Main exclusion criteria	Age <21 years (N.B. bone may still be actively growing in males up to 20 y) Known current malignancy, liver disease Patients receiving cinacalcet, bisphosphonates, denosumab, strontium. Patients having had a parathyroidectomy Bone fracture within 3 months of recruitment

LAY SUMMARY

People with kidney disease develop abnormalities of the day to day routine maintenance of their bones and the metals, minerals and chemical messengers associated with bone health. This may cause bone disease but may also lead to blood vessels becoming 'furred-up' or hardened with calcium. These hardened vessels can ultimately cause death or disability due to cardiovascular disease (e.g. strokes, heart attacks, amputations). In clinical practice these bone and mineral abnormalities are managed using a range of treatments (e.g. vitamin D and phosphate binders). The initiation and dose of drugs given are largely guided by laboratory tests, including measurement of a chemical messenger called parathyroid hormone. However, parathyroid hormone is not a good marker for bone disease in people who also have kidney failure. There is some concern that treatments guided by parathyroid hormone may actually make the underlying condition worse. At present most members of the renal multidisciplinary team along with the patients spend considerable time adjusting treatment that has a poor scientific basis using our current bone-mineral markers. Alternative markers are needed. One such marker is called bone-specific alkaline phosphatase; we think this marker may more accurately reflect the disorders of bone and minerals in this situation. Little is known about whether bone alkaline phosphatase predicts outcomes in people with kidney failure. We plan to measure this marker in people receiving haemodialysis to establish whether it can predict outcomes (overall death rate, cardiovascular death rate and cardiovascular disease (e.g. heart attacks). Patients will have a simple blood test taken whilst on dialysis. We will then monitor their condition over the next three years and see whether there is any relationship between the marker levels and outcomes such as heart disease and strokes. We hope to identify an optimal 'healthy' level of the marker which could then become a treatment target in future studies.

Study Schema



2 Introduction

2.1 Abstract of research

Chronic kidney disease-mineral bone disorder (CKD-MBD) comprises mineral, bone and calcific cardiovascular abnormalities that develop as a complication of chronic kidney disease (CKD). Clinical management is based on surrogate laboratory markers, in particular parathyroid hormone (PTH). PTH is a flawed marker of CKD-MBD and there is a need for better markers. At present most members of the renal multidisciplinary team along with the patients spend considerable time adjusting treatment that has a poor scientific basis using our current bone-mineral markers. There is interest in the use of bone-specific alkaline phosphatase (ALP) to monitor CKD-MBD and there is some evidence linking total ALP and bone ALP levels to outcomes. We are carrying out a prospective study in which we will assess the value of bone ALP as a marker of mortality and cardiovascular morbidity in a large (n=3000) cohort of haemodialysis patients recruited across UK renal units. The strength of this association will be compared to that of PTH. Informed consent will be obtained. Baseline demographic, laboratory and pharmacologic details will be collected on each patient at entry into the study. Blood will be taken predialysis from participating patients at study outset and total ALP, bone ALP and PTH measured. Patients will be followed for at least two years for relevant outcomes: all cause mortality (death), treatment failure (death or transfer to peritoneal dialysis) and cardiovascular morbidity. Data analysis will be aimed at evaluating bone ALP activity as a predictor of all-cause mortality, with a complementary analysis in which the outcome is cardiovascular mortality (fatal myocardial infarction and stroke) and cardiovascular morbidity (non-fatal myocardial infarction and stroke). The purpose of the study will be to define an optimal range of bone ALP concentration in terms of morbidity and mortality. Future studies could use the mortality-defined optimal bone ALP concentration in haemodialysis patients as a treatment target, for example as a rationale for trials of treatments that lower ALP without increasing phosphate.

2.2 Study purpose

Chronic kidney disease-mineral bone disorder (CKD-MBD) comprises mineral, bone, and calcific cardiovascular abnormalities that develop as a complication of chronic kidney disease (CKD).[1, 2] In addition to skeletal manifestations, there is concern regarding vascular calcification that occurs in CKD-MBD as a result of the

underlying disease and its management. Clinical management is based on surrogate laboratory markers, with guidelines[1-3] recommending measurement of calcium, phosphate, alkaline phosphatase (ALP) and parathyroid hormone (PTH). PTH is commonly considered the most sensitive marker of underlying disordered metabolism.[2, 4] However, PTH is a flawed marker of CKD-MBD and there is a need for better markers.[1] There is interest in the use of bone ALP to monitor CKD-MBD.[5] Total ALP is known to be related to mortality in haemodialysis patients[6] but there are currently only limited data describing the relationship between mortality and bone-specific ALP in dialysis patients.[7] Given the difficulties associated with bone biopsy (painful, invasive), new evidence from large cohorts is unlikely to be gleaned from studies comparing marker concentrations with histomorphometric analysis. We are carrying out a prospective study in which we will assess the value of bone ALP as a marker of mortality and cardiovascular morbidity in a large cohort of haemodialysis patients recruited across several renal units. The strength of this association will be compared to that of PTH. The purpose of the study will be to define an optimal range of bone ALP concentration in terms of morbidity and mortality and in this way add to the currently deficient evidence basis for management of CKD-MBD using existing and novel biomarkers.

2.3 Rationale for the study

PTH is measured as a marker of CKD-MBD but reflects parathyroid activity rather than bone remodelling:[8] whilst it has high sensitivity for detecting hyperparathyroid renal bone disease its specificity is poor.[9] Other pitfalls of PTH in this situation include (1) standardisation-related assay variation;[10] (2) non-specificity of assays;[11] variation in the PTH-histology relationship due to (3) skeletal resistance;[12] (4) race;[13] and (5) influence of body mass index;[14] (6) poor histological correlation to target ranges;[8, 15] (7) profound effects of venous sampling site on concentration;[16] (8) high biological variation;[17] and (9) weak [18] or absent[19] relationship with mortality. These issues raise significant concerns over the validity of national guidelines recommending specific target concentrations of PTH, as currently used in UK renal units. The current target ranges are wide and somewhat arbitrary. Having originally been defined as 2 to 4 times the upper limit of normal based on histomorphometry,[20] recent recommendations suggest widening this to 2 to 9 times.[1] It ís increasingly clear that we do not really know what the optimal target range of PTH should be in dialysis patients and how it is affected by the above factors.

Serum total ALP was historically used to predict bone turnover but its use was limited by its non-specificity for bone disease. ALP is more sensitive to the effects of vitamin D replacement than PTH[21] and has a strong independent linear positive association with mortality in both patients with kidney disease[6] and in the non-CKD population,[22] possibly linked through vascular calcification.[23]

Bone-specific ALP concentration correlates with PTH concentration in haemodialysis patients,[4] being increased in high-turnover bone disease and being more specific for this condition than PTH or total ALP.[5, 9] Unlike PTH, its concentration directly reflects osteoblastic activity, it does not accumulate in blood with declining GFR,[24] it is strongly and independently related to bone mineral density[25, 26] and is also a good predictor, with DEXA-derived bone mineral density, of fracture risk in dialysis patients[27] Biological variation of bone ALP is approximately half that of PTH.[28] Mechanistically, there is evidence implicating bone ALP as a proponent of vascular calcification in CKD-MBD.[29, 30] Bone ALP is an excellent marker of bone turnover in haemodialysis patients[5, 13, 31] but its use has not become widespread, probably reflecting perceived difficulties of measurement and the primary role assigned to PTH in dialysis management.

Two recent studies have assessed the prognostic value of bone ALP in CKD patients. Amongst 135 CKD patients not on dialysis bone ALP was an independent predictor of cardiovascular events, those with bone ALP in the upper tertile having an adjusted hazard ratio for cardiovascular events of 3.88 (95% CI 1.03 to 14.59).[24] Recently, Drechsler et al have shown bone ALP to be a significant adjusted predictor of short-term (6-month) all cause and cardiovascular mortality amongst a mixture of 800 haemodialysis and peritoneal dialysis patients sampled 12 months after commencing dialysis.[7] After 4 years bone ALP, but not total ALP, remained predictive of cardiovascular mortality.[7]

2.4 Assessment and management of risk

The main risk to participants in this study is from venepuncture.

3 Study Design

We aim to prospectively recruit 3000 haemodialysis patients across UK renal units.

4 Study Objectives

The aims of the study are:

1. To test whether bone ALP is an independent predictor of mortality in patients receiving haemodialysis.
2. To test whether bone ALP is an independent predictor of cardiovascular mortality/morbidity in patients receiving haemodialysis.
3. To define an optimal range of bone ALP concentration in terms of morbidity and mortality.

5 Selection of Participants

Participants who potentially fulfil the inclusion criteria for this study must have their eligibility confirmed by medically qualified personnel with access to and a full understanding of the potential participant's medical history. If eligibility has been assessed and documented by medically qualified personnel, then the process of receiving informed consent may be delegated as appropriate.

5.1 Inclusion criteria

- Prevalent haemodialysis patients in the participating renal units who have been receiving dialysis for at least three months.
- Written informed consent

5.2 Exclusion criteria

- Age <21 years (N.B. bone may still be actively growing in males up to 20 y)
- Known current malignancy or liver disease
- Patients receiving cinacalcet, bisphosphonates, denosumab, strontium.
- Patients having had a parathyroidectomy
- Bone fracture within 3 months of recruitment

6 Recruitment

The patients will have the study explained to them by a consultant or research nurse and will be given a patient information sheet. These will be available in English and other languages as reflects the ethnic mix of the recruiting units. Patients will have as long as they require to consider whether or not they wish to take part in the study. Written informed consent will be obtained.

7 Study Procedures and Schedule of Assessments

7.1 Screening procedures

Eligibility will be assessed against the inclusion and exclusion criteria as described above. In some cases the research nurse or participant's clinician may introduce the study to the participant before providing them with the invitation letter and patient information sheet. Potential participants will have as long as they require to read the study information and consider whether to take part in the study. Assuming they are willing to participate they will be asked to provide signed informed consent (see below) and will be entered into the study.

7.2 Informed consent procedure

Eligibility should be assessed and documented by a clinician or research nurse and then the process of obtaining written informed consent may be delegated as appropriate (to a suitably trained member of the local research team).

Potential participants will initially be provided with a written Participant Information Sheet (PIS) and a covering letter explaining the study to them and inviting them to participate in the study. They will have time to consider the study and decide whether or not they wish to take part, and to discuss the study with their family and friends if they would like to. If the potential participant has any questions or queries about the study during this time they will have the opportunity to discuss the study with the research nurse. The research nurse will explain that there is no obligation for a patient to enter the study, that study entry is entirely voluntary, and that it is up to the patient to decide whether or not they would like to join. It will also be explained that the patient can withdraw at any time during the study, without having to give a reason and that their decision will not affect the standard of care they receive. Translated material and translators may be used for non-English speakers. The potential participant will then either be contacted by, or will contact, a member of the research team at their local centre. If they remain willing to participate in the study they will be asked to provide signed informed consent.

Once consent is obtained the research nurse will register the patient into the study (see section 7.3). Informed consent will be obtained before any study-related

procedures are undertaken. A copy of the signed informed consent form will be given to the participant. The original signed form will be retained at the study site in the Investigator Site File and a copy placed in the medical notes. A copy will also be sent to the ALPHA Study Office.

7.2.1 Withdrawal

Participants may withdraw at any time during the study if they choose not to continue or if their clinical team feel that continued participation in the study is inappropriate.

7.3 Registration procedures

After all eligibility criteria have been confirmed and informed consent has been received, the participants can be registered into the study.

Telephone and online registration
Participants can be entered into the study via a secure 24 hour internet based registration service at https://www.alphastudy.org. Each researcher will be provided with a unique log-in username and password in order to access the online system. Online registration is available 24 hours a day, 7 days a week, apart from short periods of scheduled maintenance and occasional network problems. Upon registration a unique study specific patient number will be generated.

Baseline demographic, anthropometric and clinical data should be entered directly into the ALPHA study website at https://www.alphastudy.org. The website will also ask the investigator to confirm that the patient does not meet any of the exclusion criteria. Most of the required data is expected to be available in the individual renal unit databases (e.g. RenalPlus, Proton, VitalData, Emed, etc): in centres where such a system is not available a data collection form can be provided. Advice notes regarding data entry may be found in Appendix 1. Once data has been entered a unique study number will be generated for each patient which should act as the primary study identifier.

7.4 Baseline details

The following data will be collected on each patient at entry into the study once consent has been obtained:

- Baseline data will include name, date of birth, gender, ethnicity, height (measured to the nearest 1 cm with a rigid stadiometer), postdialysis weight (measured in light indoor clothing to the nearest 0.1 kg) and hospital/NHS number as required by the dedicated study website (https://www.alphastudy.org)

- Comorbidity data will be collected as required by the dedicated study website (https://www.alphastudy.org)
- Record details of the patients dialysis treatment and predialysis blood pressure as required by the dedicated study website (https://www.alphastudy.org)
- Record relevant medication details as required by the dedicated study website (https://www.alphastudy.org)

7.5 Baseline blood samples

After study entry as described above, at the patient's next haemodialysis session:

- Blood (30 mL total: 20 mL clotted blood sample (plain or gel separator tubes) and 10 mL K_2EDTA blood sample) should be taken for serum alkaline phosphatase and PTH measurement and sample storage (see below Section 8.3). **Blood should only be taken predialysis. Record whether blood was from arteriovenous fistulas/grafts or from a central venous catheter (line).**
- Record serum/plasma creatinine, urea, potassium, bicarbonate, albumin, calcium (uncorrected/unadjusted), phosphate and blood haemoglobin results from the local laboratory on the date corresponding to the baseline sample
- Record the most recent local laboratory serum total cholesterol, C-reactive protein (CRP), URR, and Kt/V results and the date of those results

7.6 Follow-up Assessments

Annually, at 12, 24 and 36 months, record significant events as required by the dedicated study website (**https://www.alphastudy.org.uk**). Follow-up assessments should take place within ± one month of actual due date.

7.7 Blinding of Test Results

Results of tests undertaken specifically for the purposes of the study will not be made available to treating clinicians and participants and therefore will not influence patient management. There is no possibility of releasing results to clinicians because they are being tested in batches throughout the study. Study samples will be labelled with study ID, and tested blinded to clinical information and results of all previous test results. Clinicians will have access to standard laboratory tests as per usual practice.

7.8 Assessment schedule

Table 1: Schedule of visits (V) and assessments by month (M)

	Screening	V1 M1 (consent, registration and baseline assessments)	V2 M1	V3 M12	V4 M24	V5 M36
Identify suitable participants, review inc./exc. criteria. Explain study. Participant information sheet and cover letter to be given to potential participants.	x					
Confirm willingness, eligibility, consent, register in study		x				
Demographic and medical history, anthropometric and clinical assessment		x				
Baseline blood tests and aliquot storage			x			
Record significant outcomes				x	x	x

7.9 Study Duration

The recruitment period will end once 3000 participants have been entered into the study, and the last participant has completed the baseline assessment. The recruitment period is expected to last 12 months. The follow-up phase of the study will cease when the last participant has completed 36 month follow-up.

8 Study Procedures

8.1 Management of Participants

Throughout the study, the participants will be managed according to their usual care as determined by their local clinical staff. None of the study test results will be

available to participants or treating clinicians, and thus they will not alter any management decisions.

8.2 Blood collection

Blood samples will be collected predialysis from arteriovenous fistulas/grafts/dialysis catheters using standard procedures (the site of blood collection must be recorded). Blood will be collected in appropriate plain or gel separating or anticoagulated tubes following the manufacturer's recommended order of draw.

8.3 Preparation of serum/plasma aliquots and sample storage

Samples should be transported to the local laboratory, where plasma/serum should be separated from cells by centrifugation within 4 h of venepuncture. Centrifugation should be approximately 2000 g for 10 minutes at room temperature. If in doubt follow your normal local laboratory procedures for sample centrifugation for ALP measurement.

Prepare approximately 10 x 1 mL aliquots of serum from the clotted blood samples and approximately 5 x 1 mL aliquots of plasma from the EDTA blood samples. Aliquots should be placed into 1.5 mL Sarstedt microtubes with screw cap lids (supplied by EKHUFT) and frozen in the upright position. Tubes should be labelled with type of specimen (P=plasma, S=serum), patient's initials and unique study number and date of birth.

Aliquots of serum/plasma should then be stored at -80^{o}c pending transportation in batches to the central laboratory at Kent and Canterbury Hospital.

Study measurements (PTH, bone ALP and ALP) and potential future markers will be undertaken in the Clinical Biochemistry laboratory at Kent and Canterbury Hospital, Ethelbert Road, Canterbury, Kent CT1 3NG.

N.B. Potential future markers may include, but not be limited to, asymmetric dimethylarginine, beta trace protein, B-type natriuretic peptide, clusterin, C-reactive protein, cystatin C, growth differentiation factor 15, hepatocyte growth factor, 1,25-dihydroxyvitamin D, fibroblast growth factor 23, fibulin-1, 25-hydroxyvitamin D, interleukin-18, insulin-like growth factor-binding protein 7, kidney injury molecule-1, matrix gla protein, neutrophil gelatinase associated lipocalin, tissue inhibitor of metalloproteinases-2, symmetric dimethylarginine, trefoil factor-3, troponin I and T.

8.4 Laboratory procedures

All analyses will be undertaken in accredited laboratories by staff registered with the Health Care Professions Council following standard operating procedures under the

quality management system of East Kent Hospitals University NHS Foundation Trust.

Total and bone-specific ALP and PTH will be measured at baseline. Total ALP will be measured using the method recommended by the International Federation of Clinical Chemistry. Bone ALP will be measured using an enzyme immunoassay specific for the bone isoform of ALP (Ostase® BAP EIA, IDS Ltd.). This assay was originally marketed as the Hybritech Tandem-MP Ostase immunoenzymetric assay and has been widely used in clinical and research studies. Bone ALP is known to be stable in unseparated whole blood for up to 7 days at room temperature and for longer periods when separated and refrigerated. PTH will be measured by a standard intact PTH assay that also cross-reacts with the PTH_{7-84} fragment. Plasma concentrations of intact PTH will be measured using direct chemiluminescent two-site sandwich immunoassays on an Abbott Architect analyser (Abbott Diagnostics Ltd., Berkshire, UK). PTH has been shown to be stable at room temperature for up to 24 h in EDTA whole blood[32] and for longer periods when separated and refrigerated.

9 Recording and Reporting of Adverse Events

The study is observational in nature and therefore adverse event procedures are not required.

10 Data Management and Quality Assurance

Data from this trial will be handled by the Kent Kidney Research Group (KKRG). The KKRG recognises the responsibilities of a data management centre with respect to the ethical practice of research and the adequate protection of human subjects.

10.1 Confidentiality

The study will collect personal data about participants, medical records will be reviewed for all patients and routine physical examinations will be performed.

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998. Patients will be identified using only their initials and unique study number and date of birth on the samples and any correspondence between the Study Office and the

participating site. Should paper data collection forms be required in some centres, the same will apply.

Should data collection forms be requited at some centres, investigators will keep their own study file logs which link patients with anonymised data collection forms. The investigator must maintain documents not for submission to the Study Office (e.g. Patient Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete study records, provided that patient confidentiality is protected.

The Study Office will maintain the confidentiality of all patient data and will not disclose information by which patients may be identified to any third party other than those directly involved in the treatment of the patient and organisations for which the patient has given explicit consent for data transfer. Representatives of the ALPHA study team may be required to have access to patient's notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

The patient consent form, which will be sent to the ALPHA Study Office will, out of necessity, contain identifiable personal data. These will be stored separately from the study record. The consent form will be sent to study office, only with the patient's consent, to monitor that the consent process has been completed correctly.

Participants study data will be stored on a secure computer database, and all personal information obtained for the study will be held securely and treated as strictly confidential. Any data processed outside of the EKHUFT will be anonymised.

10.2 Long-Term Storage of Data

In line with the Medicines for Human Use (Clinical Trials) Regulations, once data collection is complete on all participants, all data will be stored for at least 5 years (but ideally not less than 15 years). Any queries or concerns about the data, conduct or conclusions of the trial can also be resolved in this time. Limited data on the participants and records of any adverse events may be kept for longer if recommended by an independent advisory board.

Trial data will be stored within EKHUFT under controlled conditions for at least 3 years after closure. Long-term offsite data archiving facilities will be considered for storage after this time.

10.3 Data collection

It is anticipated that most data will be entered directly into the study website using information available in individual renal unit databases. Authorised staff at sites will

require an individual secure login username and password to access this online data entry system. When necessary, paper data collection forms must be completed, signed/dated and returned to the Study Office by the Investigator or an authorised member of the site research team.

Entries on paper data collection forms should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change. Data reported on each form should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the form. The completed originals should be sent to the Study Office and a copy filed in the Investigator Site File.

All local laboratory test results will be entered onto the online system by the investigator at the individual renal units. All study test results (PTH, ALP and bone ALP generated in the central testing laboratories will be entered onto the system by staff at the central laboratory. Authorised staff at the laboratories will require an individual secure login username and password to access this online data entry system. Note that this data will only be viewable on the website by the central laboratory researchers. Relevant source data in the laboratories will be stored for a minimum of 10 years.

10.4 Data handling and analysis

Data analysis will be undertaken in East Kent Hospitals University NHS Foundation Trust.

Personal data and sensitive information required for the ALPHA study will be collected directly from renal unit databases, study participants and their hospital notes. Samples will be coded with the participant's unique study number and date of birth in the dd/mm/yyyy format. The consent form will also be posted to the ALPHA study office. All personal information received in paper format for the study will be held securely and treated as strictly confidential according to EKHUFT policies. All staff involved in the ALPHA study share the same duty of care to prevent unauthorised disclosure of personal information. No data that could be used to identify an individual will be published. Data will be stored on a secure server under the provisions of the Data Protection Act and/or applicable laws and regulations.

11 Archiving

Archiving will be authorised by the EKHUFT following submission of the end of study report.

Principal Investigators are responsible for the secure archiving of essential study documents (for their site) as per their NHS Trust policy. All essential documents will be archived for a minimum of 5 years after completion of study.

12 Statistical Considerations

12.1 Sample size

In the study of Regidor et al[6] three year mortality in the lower two tertiles of ALP activity was 27%; we have assumed that 2-year mortality was therefore approximately 18% and survival was 82%. We have made an assumption that a 25% increase in mortality (77.5% survival at two years) in the higher tertile of ALP activity would be a clinically useful difference in terms of prognostication. With a significance level of 0.05 and with 80% power, the study requires a total sample size of 2943, which we have approximated to 3000 (www.stattools.net/SSizSurvival_Pgm.php).

12.2 Statistical analysis

The analysis will be aimed at evaluating bone ALP activity as a predictor of all-cause mortality, with a complementary analysis in which the outcome is cardiovascular mortality (fatal myocardial infarction and stroke) and cardiovascular morbidity (non-fatal myocardial infarction and stroke). Baseline bone ALP data will be divided into tertiles. Exposure will be calculated from date of blood draw until date of death or censorship. Survival in the tertiles will be compared using Kaplan-Meier survival analysis with log rank testing. Separate analyses of initial bone ALP activity in central venous catheter and fistula access patients, in patients referred > or <90 days prior to commencing dialysis, and in new and established haemodialysis patients will also be undertaken.

Logistic regression will be used to assess the relationship between bone ALP activity and all-cause mortality.

To determine whether the prognostic value of bone ALP is independent of other risk factors, a Cox's proportional hazards regression will be performed. Unadjusted and adjusted hazard ratios for death will be calculated using the Cox proportional hazards ratio method, with time-dependent models as necessary for repeated measures of covariates. Unadjusted hazard ratios will be calculated for age, gender, body mass index, mean arterial blood pressure, diabetes mellitus, smoking status, comorbidity, haemoglobin, CRP, albumin, cholesterol, phosphate and PTH concentrations. Variables that have a significant ($p<0.05$) hazard ratio will be included in a logistic regression model, and manual backward elimination will be used to remove any variables that become non-significant. Baseline bone ALP activity will then be entered into the model separately as a categorical variable as defined above to provide a hazard ratio for bone ALP adjusted for other clinical variables. Receiver-operator curve (ROC) analysis for serum bone ALP as a predictor of death will be undertaken to determine the cut-off value for predicting death. A further analysis of the prognostic power of bone ALP will be undertaken using a net reclassification index approach.

12.3 End of study

The end of study will be 6 months after the last data capture. The last data capture will be 36 months following recruitment of the last participant.

13 Direct Access to Source Data

The investigator(s)/institution(s) will permit study-related monitoring, audits and REC review, providing direct access to source data/documents. Study participants are informed of this during the informed consent discussion and will consent to provide access to their medical notes.

14 Ethics Requirements

The Sponsor will ensure that the study protocol, PIS, consent form and submitted supporting documents have been approved by the MREC, prior to any participant recruitment. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethical approval prior to implementation.

Before a site can enrol participants into the study, the Principal Investigator or designee must apply for NHS permission from their Trust Research & Development (R&D) and be granted written permission. It is the responsibility of the Principal Investigator or designee at each site to ensure that all subsequent amendments gain the necessary approval. This does not affect

the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

Within 90 days after the end of the study, the Chief Investigator/Sponsor will ensure that the MREC is notified that the study has finished. If the study is terminated prematurely, those reports will be made within 15 days after the end of the study.

The Chief Investigator will supply the Sponsor with a summary report of the clinical study, which will then be submitted to the MREC within one year after the end of the study.

15 Monitoring Requirement for the Study

Monitoring of this study will be to ensure compliance with GCP.

There will be no independent study steering committee or independent data monitoring committee.

16 Finance

The British Renal Society and Kidney Patient's Association are funding this study with support from the Alan Squirrell Artificial Kidney Unit Trust.

17 Indemnity

This is a clinician-initiated study, ABPI guidelines for patient compensation by the pharmaceutical industry will not apply.

The Sponsor holds Public Liability (negligent harm) and Clinical Trial (negligent harm) insurance policies, which apply to this study. Participants may be able to claim compensation, if they can prove that either Co-Sponsor has been negligent. However, as this study is being carried out in a hospital setting, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical study. Compensation is only available via NHS indemnity in the event of clinical negligence being proven. The Sponsor does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees.

Participants *may* also be able to claim compensation for injury caused by participation in this study without the need to prove negligence on the part of the Sponsor or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the relevant Insurers.

There are no specific arrangements for compensation made in respect of any serious adverse events occurring though participation in the study, whether from the side effects listed, or others yet unforeseen.

Hospitals selected to participate in this study shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary should be provided to Sponsor upon request.

18 Dissemination and Publication

The Chief Investigator will coordinate dissemination of data from this study. All publications and presentations, including abstracts, relating to the study will be authorised by all co-investigators. The results of the analysis will be published in a peer reviewed journal.

All contributors to the study will be listed, with their contribution identified. Study participants will be sent a summary of the final results of the study, which will contain a reference to the full paper.

To safeguard the scientific integrity of the study, data will not be presented in public before the main results are published without the prior consent of the Chief Investigator.

19 Statement of Compliance

The study will be conducted in compliance with the approved protocol, EU GCP and the Research Governance Framework.

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Appendix 1: Notes regarding entering data on ALPHA study website

DEMOGRAPHICS

Name/DOB/Sex: Self explanatory.

Ethnicity:

Choose one of 5 options as per drop-down menu (as self-reported by patient).

Height:

Height in centimetres (whole numbers)

Weight:

Weight in kilograms – post dialysis dry weight/target weight as far as possible (whole numbers)

Post code:

Postcode as listed in the IT system of the hospital that provides primary care for the patient.

Renal unit:

Name of renal unit to which patient belongs (drop-down menu)

Hospital number:

Unique id number (with or without letters) in the hospital with primary care for patient (hospital that undertakes the patient's dialysis treatment)

NHS number (for patients in England, Wales and Northern Ireland only):

Invalid NHS numbers will not be accepted / saved (internal modulus 11 algorithm check)

CHI number (for patients in Scotland only)

Invalid CHI number will not be accepted / saved

Exclusions

Yes/no –self-explanatory (see information icons)

Date of data entry:

Date when you started filling in the demographics page for this patient for the first time (may or may not be the same date as obtaining consent from the patient for study participation)

COMORBIDITY

(N.B. cannot save data on this page without entering details on demographic page first)

For the following data items, please read case notes (admission clerking notes, inter-specialty referral letters, discharge summaries are particularly useful), clinic letters, local renal IT systems or based on reports from patient/patient's named consultant. Record data items as per information gathered from above sources. If in doubt for any item check with the patient's named consultant nephrologist.

Primary Renal diagnosis

Drop-down list. This data item indicates cause of kidney failure. Usually specified in clinic letters, local renal IT systems, patient reported cause etc.

Diabetes

This data item indicates whether the patient has diabetes or not (irrespective of whether the diabetes caused kidney failure or not)

If 'yes' is checked pop up box of type I or II

Ischaemic heart disease

This data item indicates whether the patient suffers from / has suffered from Ischaemic/coronary heart disease. If 'yes' is checked pop up box of:

Angina – diagnosis of angina as recorded in case notes or reported by patient. Usually implies typical sounding cardiac chest pain, often on exertion, relieved by GTN/rest etc.

MI – myocardial infarction/heart attack

CABG or coronary angioplasty – patient has had an intervention for presumed ischaemic heart disease (with or without previous history of angina/MI).

Heart failure

This data item indicates whether the patient suffers from heart failure. Indicate 'yes' if any of the following items appear to have been diagnosed according to the case notes/clinic letters

Congestive cardiac failure or CCF

Left ventricular failure or LVF

Right ventricular failure of RVF

LV or RV dysfunction on ECHO

Ejection fraction or EF <30% on ECHO

Atrial Fibrillation (AF)

This data item indicates whether the patient is in AF currently. Do not choose 'yes' if patient had previous episodes of AF but is not in AF currently.

Cerebrovascular disease

This data item indicates whether the patient has had symptomatic cerebrovascular disease or cerebrovascular intervention. If 'yes' is ticked pop up box of type of event:

TIA – Indicate if TIA (transient ischaemic accident) /mini-stroke/transient stroke appears in case notes/letters

Stroke (includes CVA [cerebro-vascular accident], stroke, hemiplegia, cerebral haemorrhage, subarachnoid haemorrhage, subdural haemorrhage)

Peripheral vascular disease

This data item indicates whether the patient suffers from peripheral (usually lower limb) vascular disease. If 'yes' is ticked pop up box of type of event:

Ischaemic/neuropathic ulcers - claudication – indicate if claudication (lower limb pain on walking) appears in case notes

Angioplasty/vascular graft/aneurysm/stent – iliac or femoral or popliteal or profunda or anterior tibial or posterior tibial artery intervention (angioplasty, endartrectomy, bypass etc) appears in case notes

Amputation for peripheral vascular disease (PVD) – indicated if any amputation of any part of any limb (except traumatic amputation or penile amputation) appears in case notes

Malignancy

This data item indicates whether the patient has been diagnosed with one or more malignancies in the past. If any malignancy has been recorded in the case notes, tick 'yes' and then specify which type of malignancy from the drop down menu. Please note – tick 'yes' only for a malignancy. Benign tumours (such as breast adenoma, colon polyp, skin warts/actinic keratosis, etc. do not count as malignancy).

Blood Borne Viruses (BBV)

This data item indicates whether the patient suffers/has suffered from BBV infection. If Hepatitis C/B/HIV infection (past or present) or Hep C/B PCR or antibody positive or HIV PCR/antibody positive is recorded in case notes tick 'yes' and then indicate which/how many viral infections is/are relevant to the patient.

Smoking

This data item captures whether the patient's smoking history is available in the case notes and therefore fill in the data item purely based on information available whether from the notes or the patient.

Transplant status

Listed or intended for transplant, yes/no

Previous transplant yes/no; give date

LABORATORY RESULTS

Enter the blood results closest to the date of recruitment

Enter local routine results from today's pre-dialysis sample

Enter most recent results and sample date for Cholesterol, CRP, URR, Kt/V

Enter results in following format:

creatinine (umol/L) whole numbers

urea (mmol/L) one decimal place

potassium (mmol/L) one decimal place

bicarbonate (mmol/L) whole numbers

albumin (g/L) whole numbers

uncorrected calcium (mmol/L) one or two decimal places acceptable

phosphate (mmol/L) two decimal places

Hb (g/L) whole numbers

cholesterol (mmol/L) one decimal place

CRP (mg/L) one decimal place

URR (%, urea reduction ratio) whole numbers

Kt/V two decimal places

URR

This is used to test the adequacy of the dialysis, it compares the amount of urea in the blood before and after dialysis. Expressed as a %

Kt/V

This test compares the amount of fluid that is cleared of urea during a dialysis session to the total amount of fluid in the body. This test is usually calculated by the nephrologist or unit sister and may be found on the renal IT system.

Study samples for sending to Canterbury Lab

Indicate that additional ALPHA study bloods have been taken and aliquots of serum and plasma saved at -80^{o}C. (See section 7.5 for bloods to be taken and section 8.3 for details of aliquot preparation and storage).

DIALYSIS

Treatment of modality

This should be the dialysis modality that the patient started on when dialysis first commenced. The type of dialysis modality is self explanatory and if not clear please check with the HD unit sister to confirm between HD-v-HDF.

Start date of renal replacement therapy (RRT)

Indicated when the patient commenced long term / permanent haemodialysis treatment. If the patient started dialysis as an 'acute patient' recovered renal function for a little while and then re-started dialysis, indicate date when dialysis was re-started. If in doubt check with local renal IT system or ask consultant nephrologist / dialysis unit sister.

Date started haemodialysis

Vascular access

The types of access are self explanatory. Non-tunnelled lines are also often referred to as 'Vascath' and tunnelled lines are often referred to as 'Permcath' or 'Tesio'.

If patients were using one needle in AVF/AVG and one needle in tunnelled line/non-tunnelled line – tick tunnelled line/non-tunnelled line as access used.

MEDICATION

Statins – used to lower cholesterol, drug name will end in statin, but if using a trade name check with the unit sister/BNF if unsure.

EPO/ESA – Erythropoietin stimulating drugs for anaemia, if unsure check with unit sister.

Vitamin D analogues – drop down list to choose from.

Anti-hypertensives – information lists what may be used, indicate how many the patient is prescribed.

Beta-blockers- commonly used ones include acebutolol, atenolol, bisoprolol, metoprolol, nadolol, nebivolol, propranolol. If unsure ask unit sister.

Phosphate binders- have information lists, and alucaps/aluminium hydroxide

EVENTS

This page is to be completed/updated at each annual follow-up (12, 24 and 36 months after study entry). Data to be entered within ± one month of actual follow-up due date. The data entered is cumulative during the period of the study (i.e. events for all years will be shown, not just the current year).

Enter:

Reason for exiting study (if relevant)

New cardiovascular events (MI, stroke/TIA, PVD)

Death (date and cause)

If no events, then please enter 'None' in remarks box.