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The impact of vascular calcification on ambulatory and central aortic blood pressure in a South African dwelling dialysis population

A clinical, radiological and pathophysiological study of vascular health in a young prevalent dialysis population in a developing country

by

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**Faculty of Health Sciences
UNIVERSITY OF CAPE TOWN**

Date of submission: 19 December 2011

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DECLARATION

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Date: 19 December 2011

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PART A: Study Protocol

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The impact of vascular calcification on ambulatory and central aortic blood pressure in a South African dwelling dialysis population

A clinical, radiological and pathophysiological study of vascular health in a young prevalent dialysis population in a developing country

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1. Executive summary

Chronic kidney disease is a common and treatable condition associated with extremely high cardiovascular mortality rates(1). The reasons for this mortality are poorly understood, but it is likely to be related to the extensive vascular calcification that develops in this population. Calcification (in particular medial wall calcification, also called Monckeberg's sclerosis) and consequent stiffening of the vasculature leads to increased pulse wave velocity which has been shown to be associated with increased mortality rates (2, 3). We propose that the link between increased pulse wave velocity and mortality may well be as a result of central aortic blood pressure and would like to measure this in our dialysis population. Central aortic blood pressure has recently been shown to be an important factor in determining outcomes based on the differential effects of antihypertensive drugs (4), but this has not been studied in dialysis populations or in Africans. There is also some evidence that modification of treatment according to vascular risk can improve outcomes.

Additionally, there is recent evidence for the validity of simple measures of vascular calcification via use of plain radiographs instead of expensive cardiac CT and we would like to explore this possibility further (5).

This is a cross sectional study of the prevalence of vascular calcification, both in the abdominal aorta and the coronary circulation, and their effects on central aortic blood pressure, ambulatory blood pressure and left ventricular mass in prevalent dialysis patients being treated at the Groote Schuur Hospital Renal Unit and at the University of Cape Town Private Academic Hospital (UCT PAH). Assessment of calcification will be done by means of plane abdominal radiograph as well as cardiac CT while central aortic pressure, ambulatory blood pressure and radial augmentation index will be measured via the BPro® Radial Pulse Wave Acquisition Device and A-PULSE CASP® Software (HealthStats) system. Left ventricular hypertrophy will be assessed via electro- and echocardiography.

The results of this study will provide us with important information about our local population which will inform us as to their extent of disease. Ideally, we would like to do a longitudinal follow-up with repeat assessments done after 12 months but this will be dependent on funding. Future treatment decisions, based on this risk profile, will therefore be assessed and ultimately, this should impact positively on patient outcomes. Additionally, we hope to validate simple measures of calcification as called for by international consensus groups.

2. Relevance of this study

To our knowledge, no study has examined the influence of vascular calcification on central aortic and ambulatory blood pressure in dialysis patients. As discussed above, this may provide us with important information that may ultimately improve care for dialysis and pre-dialysis patients.

The assessment of extra-coronary calcification and vascular function in relation to coronary calcification has not been performed in Africa where a younger dialysis population exists and where genetic and environmental influences may affect mineral metabolism and hence cardiovascular risk. Many environmental differences exist that could account for differences in vascular and bone health such as exposure to sunlight and Vit D use, diet and salt consumption, dialysis quality, membrane type, aluminium toxicity, iron loading, water strontium content and limited access to more effective phosphate binders (6).

Validation of a simple method for the assessment of vascular calcification will provide clinicians in our environment with a cost-effective means of determining a vascular risk profile from which patients stand to benefit the most as compared with more costly investigations. Determining a baseline calcification score in this way will also allow researchers to design prospective trials comparing progression of disease based on differing therapies and assess mortality prediction through follow up as recommended in the recent KDIGO report (7). Therefore, this is likely to add information to traditional risk prediction models which cannot explain all of the cardiovascular risk attributable to the dialysis population.

3. Specific objectives

Primary objectives

1. To determine the prevalence of vascular calcification in prevalent dialysis patients in the Groote Schuur Hospital and UCT PAH Renal units as assessed by coronary CT and abdominal radiograph.
2. To determine if vascular calcification influences the central aortic blood pressure, augmentation index and 24 hour ambulatory BP in dialysis patients.
3. To compare coronary calcification and central aortic blood pressure with healthy age, sex and smoking matched controls.

Secondary objectives

1. To examine the relative contribution of comorbid illness (E.g. diabetes mellitus), ethnicity, age, dialysis vintage, calcium exposure, vitamin D status, calcium/phosphate product, blood pressure and smoking status towards vascular health in this cohort.
2. To examine the effects of vascular calcification on left ventricular hypertrophy (LVH) as assessed by electrocardiography (ECG) and echocardiography.
3. To examine the differences (if any) between ethnicity and dialysis modality, and vascular health.

Ideally, we would like to do a longitudinal study with reappraisal after 12 months but this is funding dependant

STUDY METHODS

1. Study design and rationale

The study will take the form of a cross-sectional survey of all prevalent haemodialysis and peritoneal dialysis patients ≥ 18 years of age at Groote Schuur Hospital and UCT PAH with a duration of dialysis ≥ 3 months (approximately 100 patients). Controls will be enrolled after advertising locally for the appropriate persons. Patients will be examined and relevant data such as blood pressure (BP), pulse pressure, body mass index (BMI), N-N distance (sternal notch to navel distance) will be noted. The presence of abdominal aortic calcification will be determined by lateral lumbar radiograph as described by Kaupilla (8). Coronary calcium score will be determined through coronary CT scanning with the use of a multidetector CT scanner. Vascular function and 24 hr ambulatory and central blood pressure will be assessed through radial pulse wave analysis as measured via the BPro® Radial Pulse Wave Acquisition Device and A-PULSE CASP® Software (HealthStats) system which is an FDA-approved device. All available laboratory parameters such as calcium, phosphate and their product will be recorded as well as alkaline phosphatase and parathyroid hormone trends. Electrocardiography and echocardiography will be performed to assess for the presence of left ventricular hypertrophy (LVH). Controls will undergo coronary CT scanning and BPro analysis. Analysis of data will then be done looking for significant differences in baseline characteristics between populations and relating these to the degree of vascular calcification and the vascular functional assessment.

The above protocol will be subject to subject to the approval of the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town.

2. Subject selection and enrolment

Subjects will be approached within the dialysis unit at Groote Schuur Hospital and at the UCT PAH. Controls will be selected after advertising appropriately. We will obtain written informed consent prior to performing any screening procedures.

Screening examinations will be used to determine the eligibility of each candidate for study inclusion.

Inclusion Criteria

- Adult patients receiving haemo- and/or peritoneal dialysis for ≥ 3 month's duration.
- Ability and willingness of subject or legal guardian/representative to provide informed consent
- Men and women age ≥ 18 years
- Controls to have a normal clinical examination, normal urine dipstick and estimated GFR ≥ 60 ml/min

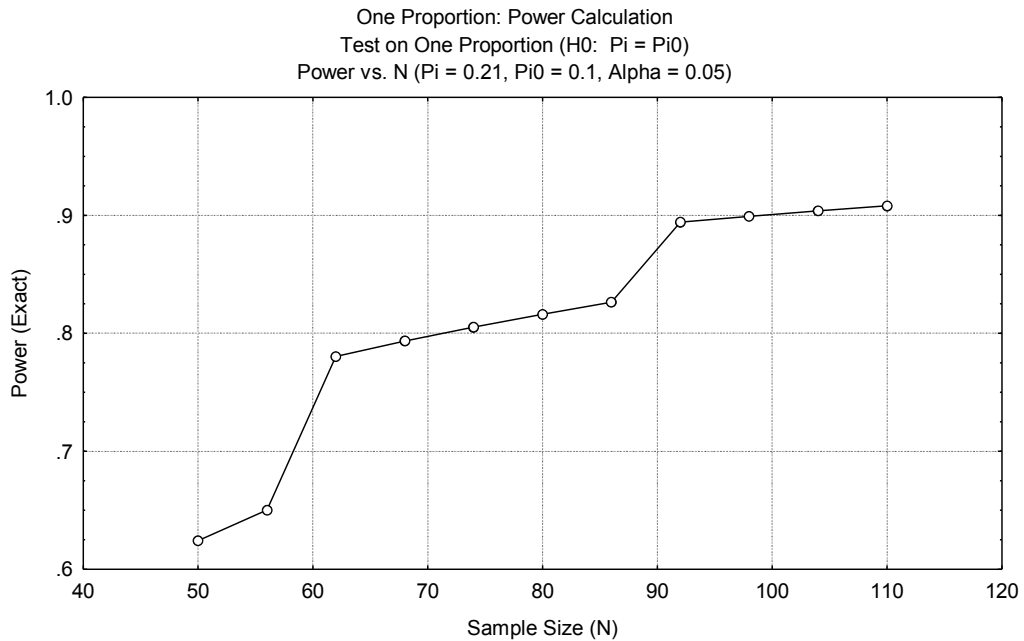
Exclusion Criteria

- Planned or current pregnancy within the next 6 months due to radiation risks (subjects to have a documented negative pregnancy test prior to inclusion)

It should be noted that some conditions will exclude individual subjects from certain of the tests, but these subjects will still be included for analysis of available data. The absence of both radial pulses or sustained arrhythmia precludes BPro analysis. Previous coronary artery bypass or stenting will preclude coronary CT.

Sample Size Calculation

In previous studies, the prevalence of coronary calcium score ≥ 1000 was 21% (9). In our population, it is likely that the prevalence of coronary calcium score ≥ 1000 will be lower due to the younger age of our cohort and lower prevalence of diabetes (selection bias due to acceptance onto the hospital dialysis programme criteria). Thus, if we assume a prevalence of this outcome to be between 10 and 21%, then a sample size of 71 patients will be required for a power of 80%. Thus, we intend to recruit 80 patients.



Power implication of different sample sizes presented in the above graph.

Calculation of sample size of control group:

There is no published data on coronary calcification scores in the South Africa population. We can, therefore, only make estimates based on previously published studies from abroad. The data published in various studies has not been reported in the same format and so in choosing the difference between cases and controls, we have chosen to use the proportion of patients with a coronary calcium score of <100.

In a study published in Brazil (10), 101 prevalent haemodialysis patients were analysed. Importantly, the mean age of the study was 48 which may compare adequately with our younger population versus most other studies where the average age has been significantly older. In this study, the proportion of patients with a coronary calcium score of <100 was 48%.

In the Multi-Ethnic Study of Atherosclerosis (MESA) (11), the distribution of coronary artery calcium score by race, gender, and age was evaluated in asymptomatic individuals who were apparently free of cardiovascular disease. These data give us some idea of percentiles for coronary calcium score in a healthy, non-selected population similar to what we could expect for our controls. Given that the 90th centile for coronary calcium score in those aged 45-54 years in whites was 8 for women and 110 for men and in blacks 9 for women and 45 for men, it should be

safe to assume that the proportion of both populations with a coronary calcium score <100 is at least 90%.

We have therefore used the above proportions to predict the sample size of controls required for our study assuming a case series of 75 patients. (This was based on the original power calculation stating a minimum of 70 patients is required for a predicted frequency of severe calcification, CAS >1000, between 10 and 21%) Therefore, for a case series of 75 patients, we can expect to need at least 13 controls. These should be matched for age, sex and smoking status but be free from Chronic Kidney Disease (normal urine dipstick and estimated GFR) and other known cardiovascular disease. Given the uncertainty surrounding our population, we have thus decided to include 80 subjects and 20 controls.

Proportion Difference Power / Sample Size Calculation
(Revised 10/30/2009 - Also display results of uncorrected ("classical") calculation.)

This screen computes the sample size required to detect a difference between two proportions.

Note: Before using this page for the first time, make sure you read the [JavaStat user interface guidelines](#) for important information about interacting with JavaStat pages.

Significance Level (alpha):	0.05	(Usually 0.05)
Power (% chance of detecting):	80	(Usually 80)
Group 1 Population Proportion:	0.48	(Between 0.0 and 1.0)
Group 2 Population Proportion:	0.9	(Between 0.0 and 1.0)
Relative Sample Sizes Required (Group 2 / Group 1):	0.177	(For equal samples, use 1.0)

Sample Size Required

	Group 1	Group 2	Total
"Classical" Calculation:	60	11	70
With Continuity Correction:	75	13	88

Note: This page incorporates a continuity correction to the usual sample-size formula based on the normal approximation to the binomial distribution. This correction increases the sample size (for each group) by an

3. Study enrolment procedures

Once a candidate for study entry has been identified, details of the study will be carefully discussed with the subject. All potential participants will be informed of the purpose, scope and details of the study with the aid of an approved patient information leaflet. The subject will be asked to read the information leaflet and sign the informed consent form. Ample time will be given to the subject to ask questions and review procedures. Patients will be given an original copy of the patient information leaflet and signed consent form.

Subjects from whom informed consent has been obtained, and who are eligible for the study, will be given a patient identification number (PID) and data will be entered onto protocol specific case report forms and then entered into the database.

4. Clinical and Laboratory Evaluations

The cohort will be investigated as described below. All subjects will be encouraged to undergo all tests and procedures. Radial pulse wave analysis, ambulatory blood pressure and echocardiography will be conducted in Groote Schuur Hospital but patients will be required to travel to the UCT private academic hospital (1 floor down) for the performance of lateral abdominal radiograph and coronary CT scan.

Screening/Entry Evaluations will be done on the same day. All imaging and data collection will be collected within a total period of one month from enrolment. It is envisaged that subjects will be required to attend between 2 and 3 additional visits for study purposes between regular dialysis days. Transport costs will be compensated accordingly. Controls will be required to have a full clinical examination, urine dipstick test and serum creatinine to exclude chronic kidney disease or other chronic illness prior to inclusion

4.1 Medical Questionnaire

An investigator will complete a detailed medical questionnaire. Details of age, sex, self reported population group, dialysis vintage, phosphate binder, smoking history and history of diabetes mellitus, hypertension, dyslipidaemia, cardiovascular history including angina, heart failure, myocardial infarction, revascularization, vascular surgery and current medications will be recorded and clinic records screened for all supportive data including drug history. Trained investigators will administer the questionnaires, in a standardised manner. Quality control will be ensured by the coordinator.

4.2 Clinical Assessment

- Physical Examination:

A limited physical examination will be performed and relevant information such as blood pressure (BP), pulse pressure, pulse rate and presence and site of AV fistula will be recorded. BP will be measured in the seated position in the arm that does not contain an arteriovenous fistula after a minimum period of 5 minutes rest with the mean of the last two readings once stable readings are achieved as per the South African Hypertension Society guidelines (12).

- Height: Recorded in cm.
- Weight: Recorded in Kg.
- N-N distance (Sternal Notch to Naval) in cm.

4.3 Radiology

These tests will be performed at the 2 Military Hospital in Wynberg.

4.3.1 Lateral Abdominal Radiograph

The standard technique of exposing the lateral lumbar spine in standing position (with 100 cm film distance, 94 KVP, and 33–200 mAs) will be used. The radiograph should include the last two thoracic vertebrae and the first two sacral vertebrae with a minimum of 4cm soft tissue being visible anterior to the lumbar spines. The estimated dose of radiation is 1mSv. The approach to be used in terms of scoring calcific deposits in the abdominal aorta will be very similar to that described by Kaupilla in 1997 (See appendix A for full description) (8). The radiographs will be read by two investigators who should reach consensus on every radiograph. Only the segments of abdominal aorta in front of the first to the fourth lumbar vertebra will be considered. Points will be assigned from 0 to 3 according to the length of each

calcified plaque (0: none; 1: $<1/3^{\text{rd}}$ of segment length; 2 $\geq 1/3^{\text{rd}}$ but $< 2/3^{\text{rds}}$ of segment length; 3: $\geq 2/3^{\text{rds}}$ of segment length) identified along the anterior and posterior profiles of the aorta in front of each of the lumbar vertebrae. Using this numerical grading, each patient will be assigned a score between 0 and 24 with higher scores indicating a greater degree of calcification of the abdominal aorta. The abdominal aortic calcification scores will be divided into approximate tertiles for statistical analysis purposes (scores of 0, 1–6 and ≥ 7).

4.3.2 Coronary CT

Spiral CT will be used to determine coronary calcium score using the volume method which has been validated and described in detail previously (13) (See Appendix B for full description). The estimated dose of radiation is 1-2.5mSv. The total yearly dose of radiation recommended for non-medical personnel is 5mSv (5). The data acquisition parameters will be: 120 kVp, 400 mAs, nominal slice width 2.5 mm (effective width 3.2 mm), gantry rotation time 0.5 s, table feed 7.5 mmus [pitch 0.375 (34 slices per rotation)]. Data will be reconstructed with a 1808 linear interpolation algorithm providing a temporal resolution of 270 ms, retrospective ECG gating during diastole, 1.3 mm longitudinal increment, 512x512 matrix, field of view 25 cm², medium body (C) filter, and no edge enhancement. Data will then be transferred to a workstation and analysed with appropriate software. The scans will be read by an experienced investigator. Coronary artery calcium scores will be divided into three levels (<100 , 100–999, and ≥ 1000) based on cut points that have been shown to be associated with increased cardiovascular disease incidence in general population studies.

4.4 Echocardiography

Understanding the limitations of its use, assessment of left ventricular mass will be done via use of M-mode echocardiography and this will be calculated using the Penn convention as described previously:

$LV\ mass(Penn) = 1.04 ([LVIDD + PWTD + IVSTD]^3 - [LVIDD]^3) - 13,6$ (14). All echocardiograms will be done by the same experienced investigator. Left ventricular hypertrophy will be defined as $>125\text{ g/m}^2$ in males and $>110\text{ g/m}^2$ in females as per European Society of Hypertension (ESH) guidelines (15).

4.5 Electrocardiography

All patients will have a resting electrocardiogram (ECG) performed. These will be analysed by two experienced investigators who should reach consensus on the interpretation of each ECG. Both the Sokolow-Lyons index ($SV_1 + RV_{5-6} > 38 \text{ mm} = \text{LVH}$) and Cornell voltage QRS duration product ($> 2440 \text{ mm} \cdot \text{ms} = \text{LVH}$) will be reported on quantitatively, and the presence or absence of left ventricular hypertrophy noted as defined by the ESH guidelines.

4.6 Ambulatory Blood Pressure and Pulse Waveform Analysis

Vascular function and 24 hr ambulatory and central blood pressure will be assessed through radial pulse wave analysis as measured via the FDA-approved BPro® Radial Pulse Wave Acquisition Device and A-PULSE CASP® Software (HealthStats) system. The BPro™ has been validated against the AAMI and ESH protocols and passed both validations (16). It carries a CE mark and is approved for clinical use by the FDA. The BPro™ records pressure wave forms calibrated to the brachial BP through use of an FDA-approved oscillometric monitor (MC300, HealthStats) and samples up to 96 X 10 second blocks of time, over 24 hours. This will provide a 24 hour profile and summary of an individual's systolic, diastolic and mean arterial pressures. It also gives information on BP dipping status. The A-PULSE CASP® software makes use of an "npoint forward moving average" (NpMA) method to calculate central aortic systolic pressure and radial augmentation index. The device has been validated against the Sphygmocor™ device using all CAFÉ study data and the correlation was $r^2=0.993$. Additionally, central aortic pressures were recorded in vivo at the aortic root using a Millar's SPC-454D tonometer (Millar's instruments, Texas U.S.A) in 20 patients undergoing routine cardiac catheterization and the pressures compared with that obtained by the BPro device. The correlation between the BPro™ readings of CASP and the direct measurement of aortic CASP was $r^2=0.9835$. (Unpublished as yet)

Practically, the device will be applied as a wrist watch on the arm that does not contain an AVF. It will then be calibrated to brachial blood pressure after the individual has rested for 5 minutes via use of the MC3000 oscillometric device. The device is then connected to the software and real time pulse wave morphology captured. Thereafter, it will be worn by the individual for a 24 hour period whereupon the device will be retrieved, interrogated and ambulatory data captured.

4.7 Blood Investigations

We will also record all available laboratory parameters such as haemoglobin, calcium, phosphate, alkaline phosphatase, 25-OH Vit D3 and parathyroid hormone for the last two years on each patient where such data is available. This information is part of standard dialysis care for all patients in the unit and therefore no venepuncture should be necessary as part of the study. However, it may be necessary to do some tests if the test has not been performed by the dialysis unit already. Controls will have a serum creatinine with estimated glomerular filtration rate done and analysed at a local private laboratory.

5. Criteria for Discontinuation

- Request by subject to terminate treatment
- Clinical reasons considered serious or life-threatening by the physician
- Subject repeatedly non-compliant with visits and procedures
- Withdrawal of consent

6. Analysis of Data and Statistical Considerations

The primary comparison will be between subjects that have significant vascular calcification and those that do not. This will be done via the cross-sectional design of the study. Variables that will be considered include:

- Age, smoking status and population group
- Dialysis vintage
- Diabetes Mellitus and other comorbid illness
- Phosphate binder and Vitamin D used and duration thereof.
- Calcium, phosphate, ALP, PTH and Hb values
- Radiographic investigation (radiographs, CT, Echo)
- Central aortic blood pressure, radial augmentation index and the presence of LVH

We will produce summary statistics (mean, median, standard deviation, and quartiles) for the above variables. T-test, Mann-Whitney, ANOVA and χ^2 tests will

be used to compare values obtained for patients with and without vascular calcification. Logistic univariate and multivariate analysis will be employed to determine differences in risk factors (if any) for vascular calcification between these groups.

7. Data Collection and Monitoring and Adverse Event Reporting

Case report forms (CRF) will be provided for each subject. Subjects will not be identified by name on data spreadsheets or on any external communication. Subjects will be identified by the patient identification number (PID)

8. Ethical Considerations

Research Ethics Committee (REC) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the REC responsible for oversight of the study. A signed consent form will be obtained from the subject (or parent, legal guardian, or person with power of attorney for subjects who cannot consent for themselves, such as those below the legal age of consent). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, parent, or legal guardian, and this fact will be documented in the subject's record. This protocol complies with the latest version of the Declaration of Helsinki (2008) and the Department of Health: Ethics in Health Research: Principles Structures and Processes (2004).

Perceived Risks and Benefits

There are very few perceived risks involved in the study the main ones being additional radiation via CT and abdominal radiograph. However, the exposure from these investigations is well below the recommended additional yearly exposure of 5 mSv as discussed above. In fact, all of the investigations we are performing would be considered the standard of care in some centres without resource constraints.

We feel that the information gained through our study will impact positively on our patient care. Any problems identified through investigation will be made known to the participant and with consent, to the doctor responsible for their care.

Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept locked. All computer entries and networking will be done with coded numbers only in order to protect confidentiality. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB or the Department of Health.

The study may be discontinued at any time by the IRB, the Department of Health, or other government agencies as part of their duties to ensure that research subjects are protected.

9. Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study.

10. Timetable

2010 January to April	Protocol development, funding applications, development of radiographic reporting techniques and questionnaire as well as local ethics committee approval
2010 May	Collection of data begins
2011 May	Submit annual progress report to IRB
2011 July to September	Analysis and reporting of data
2011 September to December	Complete papers and submit for publication/thesis

11. Budget

Study-related Costs*

Research Nurse	R 184 800.00
Data typist and statistician	R 45 000.00
Lateral Lumbar Radiograph (100 pts @ R266 each)	R 26 600.00
Echocardiography (100 patients @R670 each)	R 67 000.00
Coronary CT (100 patients @ R2827 each)	R 282 700.00
BPRO Radial pulse wave system (2 units at R60 000 each)	R 120 000.00
Travel costs (100 patients @ R50 each for 6 visits)	R 30 000.00
Consumables	R 10 000.00
Equipment	R 20 000.00
Travel and conference attendance	R 20 000.00

TOTAL

R 806 100.00

**Expenses calculated according to National Reference Price List 2009 plus 10% inflationary allowance based on CPIX*

Appendix A: (Method of assessing aortic calcification as per Kaupilla (Kauppila et al. 1997)

Kaupilla’s approach as described in their article as follows: “was to assess both the location and severity of calcific deposits in relation to segmental lumbar arteries and we assessed aortic calcifications at each vertebral segment. Calcific deposits were regarded as present if densities were visible in an area parallel to the lumbar spine and anterior to the lower part of the spine. Aortic densities at the upper part of the lumbar spine often overlapped the vertebrae, as the abdominal aorta in the L1-L2 region is often lateral to the spine, while the lower abdominal aorta in the L3 and L4 region is situated anterior to the spine. Densities overlapping the vertebrae were scored present only if they extended from or formed a clear pattern with those of the lower part of the aorta.” “Lesions were graded as follows: 0, no aortic calcific deposits; 1, small scattered calcific deposits filling less than 1:3 of the longitudinal wall of the aorta; 2, one third or more, but less than two thirds or more of the longitudinal wall of the aorta calcified; 3, two thirds or more of the longitudinal wall of the

aorta calcified. Individual level-specific severity scores were summarized to yield three different composite scores for aortic calcifications. In the affected segments score (0–4), the number of individual aortic segments which showed any calcification were calculated. In the anterior and posterior affected score (0–8), the number of individual aortic segments, both anterior and posterior, which showed any calcification were summed. In the antero–posterior severity score (0–24), the scores of individual aortic segments both for the posterior and anterior wall were summed.

Example of the scoring system used on a patient below:

Scoring System for Lumbar Aortic Calcification

	Posterior	Anterior	Sum A+P	Affected A+P	Affected A or P	
L1	0	0	0	0	0	
L2	2	1	3	2	1	
L3	3	3	6	2	1	
L4	3	3	6	2	1	
			15	6	3	Total
			24	8	4	Maximum

Appendix B (Description of Coronary Calcium Score Method as per (Moe et al. 2003)):

“CT scans were performed with the quad-slice technique on the model MX 8000 scanner (Philips Medical Systems, Cleveland, OH). The data acquisition parameters were: 120 kVp, 400 mAs, nominal slice width 2.5 mm (effective width 3.2 mm), gantry rotation time 0.5 s, table feed 7.5 mmus [pitch 0.375 (34 slices/rotation)]. Data were reconstructed with a 1808 linear interpolation algorithm providing a temporal resolution of 270 ms, retrospective ECG gating during diastole, 1.3 mm longitudinal increment, 512x512 matrix, field of view 25 cm², medium body (C) filter, and no edge enhancement. Data were transferred to a workstation and analysed with HeartBeat-CS software (MX View, Marconi Medical Systems, Cleveland, OH). On the basis of the ECG tracing, the software program automatically selected a reduced set of diastolic images from each cardiac cycle. The proximal coronary arteries were scored, beginning with the first image in which a coronary artery was seen (usually the left anterior descending) and continuing for 6 cm along the long axis of the patient [6]. All pixels with density ≥ 130 Hounsfield units (HU) were highlighted automatically in colour on the images. The observer placed an electronic region of interest (ROI) around each highlighted CAC and assigned one of four locations to each calcified plaque: left main, left anterior descending (LAD), circumflex or right coronary artery. Branches of the LAD, circumflex and right coronary arteries were considered parts of those arteries. The descending aorta was evaluated over the 6 cm in the z-axis direction. A minimum plaque area of 0.5 mm² was used to reduce errors due to noise.” Calcium scoring by volume will be used. “This method, called average-continuous, uses a weighting factor $(F) = \frac{A}{100} - 0.5$, where A is the average density of each plaque on each image. The score for

each plaque is calculated by multiplying the area of each plaque in mm² (to generate a volume determination) by the weighting factor. The score for the entire specimen equals the sum of the scores for each plaque. As above, this score is then multiplied by 1.07. This technique is called ‘calcification score by volume’.”

12. References

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University of Cape Town

PART B: Literature Review

University of Cape Town

LITERATURE REVIEW

Summary

Vascular calcification and stiffening are common in dialysis patients and are associated with adverse cardiovascular outcomes. Vascular calcification in ESRD appears to be linked to abnormal bone physiology and the risk factors for its development (such as calcium load and ethnicity) require investigation. It may be possible to assess for the presence of vascular calcification by simple and inexpensive means but this requires validation in our context. Furthermore, the prevalence of vascular calcification in African patients is not known. The reasons for increased cardiovascular mortality in CKD remain incompletely understood. Although the pathophysiology is likely multifactorial, alterations in central haemodynamics may play a significant role in this and this requires further investigation.

Cardiovascular mortality in end stage renal disease

Chronic kidney disease (CKD), is both common, harmful and treatable (1). It is estimated that approximately 10% of the world's population suffer from CKD (2). While there is no reliable estimate of the burden of CKD in South Africa, it is thought to be similar to global trends. Even more alarming is the 67% increase in premature adult deaths secondary to kidney disease in South Africa in just 7 years from 1999 to 2006 (3). Haemodialysis (HD) patients in particular, experience extremely high rates of cardiovascular mortality, 20 – 30 times higher than that of age-matched peers (4, 5). Almost certainly, this is in large part secondary to accelerated vascular disease comprising both arteriosclerosis and atherosclerosis. However, the reasons behind the rapid development of this state are incompletely understood and are not entirely explained by traditional risk factors. While traditional risk factors may account for much of the mortality in the uraemic state, many novel cardiovascular risk factors exist such as vascular calcification, raised aortic pulse wave velocity (PWV), raised central aortic systolic pressure (CASP) and loss of nocturnal blood pressure (BP) dipping that could explain the high incidence of cardiac death (6). This has been especially highlighted by the minimal impact of statin therapy to impact on cardiovascular mortality in patients with end stage renal disease

(ESRD)(7-9) . These data have called into question traditional beliefs about risk factors for cardiovascular disease in the context of CKD and there has thus been a call for more intense research in this area to better define contributing risk factors and the underlying process. One such process is the rapid development of arterial calcification in patients with end-stage kidney disease.

Vascular Calcification in renal disease

Vascular calcification is extremely common in CKD-5D and reported prevalence rates are >80% in studies published in Europe and in the United States (10, 11). While the pathogenesis is certainly complex, it involves endothelial dysfunction, abnormalities of mineral metabolism and altered levels of endogenous inhibitors of calcification (12). Vascular calcification may localise to the intima (often in association with atherosclerosis) or to the media (common in renal disease and related to disordered mineral metabolism) or both. While at lower risk of mortality than those with intimal calcification, patients with medial calcification are still at much higher risk of death than age-matched peers (13). Most data relates to haemodialysis populations. Very little is known about the relative risks of vascular calcification with different dialysis modalities however it seems that vascular calcification is also highly prevalent in peritoneal dialysis (PD) populations (14). Established risk factors for the development of VC in published studies include age, time on dialysis, the presence of diabetes, a history of vascular disease and raised calcium/phosphate product (11, 15). Unfortunately, there is no published data describing the prevalence of VC in Sub-Saharan Africa.

The cost of calcium loading

There appears to be a strong link between the presence of vascular calcification and chronic kidney disease bone mineralisation disorders (CKD-BMD) (16). In particular, the presence of low bone turnover as determined by bone biopsy appears to correlate with the extent of extra-coronary calcification (17). Additionally, calcium load (orally, or through dialysate exposure) has been shown to correlate with vascular calcification and its progression in haemodialysis patients (18, 19). Moreover, the dosage of calcium containing phosphate binders has been shown to have the greatest impact on vascular calcification and stiffness in the presence of adynamic bone disease (16). Of concern is the relative increase in the prevalence

of adynamic bone disease despite the reduction in aluminium exposure over time (20). It is thought by many investigators that this may be due to excessive use of calcium based phosphate binders and vitamin D analogues and as such, it has been proposed that judicious management of calcium homeostasis in CKD could delay the development of vascular calcification and hence lower mortality (21). Sevelamer, a non-calcium containing phosphate binder, has been shown in two randomised controlled trials to retard the progression of vascular calcification in haemodialysis (HD) patients (18, 19, 22) and in one study (23) was associated with lower mortality in incident HD populations. The mechanism for this effect may be due to improvements in bone turnover as shown in a prospective study of Lanthanum Carbonate, another non-calcium containing phosphate binder (24). The reduction in coronary calcification with sevelamar use was not found in a third study (25) but this may have been due to inclusion of patients with other well established risks for calcification apart from CKD (26).

Patients at the highest risk for death (those with prominent vascular calcification) may stand to benefit the most from these costly drugs which are not widely available in resource poor settings. However, it has been suggested that further studies are needed before firm recommendations to this effect can be made (27). Currently, the latest guidelines from the KDIGO group have suggested restricting the dose of calcium-based phosphate binders in the presence of arterial calcification and/or adynamic bone disease and/or if serum PTH levels are persistently low (28).

Endogenous inhibitors of calcification

There exist several endogenous circulating or tissue bound factors that inhibit the development of vascular calcification in vivo. These include factors such as matrix gla protein, fetuin A and osteopontin. Levels of these inhibitors have been shown to be altered in renal disease and may therefore predispose to the development of VC (29). There is no published data on these inhibitors in African patients.

The role of ethnicity and geography

There exist significant differences between the rates of coronary artery calcium score in white populations of different continents (30). Furthermore, there exist differences in the rate of coronary (31) and extra-coronary calcification (32) between

different ethnic groups. It has been suggested that some of these differences could be accounted for by differing vitamin D metabolism (33). Despite this, a recent cross sectional survey of prevalent dialysis patients showed no differences in markers of vasculopathy (PWV), Coronary calcium score and Thoracic calcification as measured by Electron Beam CT) between white and black subjects. Importantly, both centres in this survey were in the United States (34) and it remains to be determined whether this holds true in South Africans.

The effects of Vascular calcification: stiffening and the link with mortality

The presence of vascular calcification is a strong predictive marker for cardiovascular (CVS) mortality in any population and vascular calcification is particularly prevalent in the dialysis population where CVS disease accounts for much of the mortality (35-37). Coronary artery calcium presence and the coronary artery calcium score (as determined by CT) are strongly associated with cardiovascular mortality in dialysis populations (13, 38). While ischaemic heart disease is an important contributor to death (5.6%) in these populations, the leading cause of death in dialysis patients is in fact sudden cardiac arrest or arrhythmia accounting for 26.3% of deaths (39). The link between vascular calcification and subsequent mortality is unknown but haemodynamic factors are likely to play a significant role. VC is associated with increased aortic PWV (11) and this in turn, is associated with raised CASP and reduced coronary perfusion due to the summation of propagated and rapidly reflected pressure waves at the aortic root (40, 41). Whether vascular calcification is directly linked with central pressures is however, unknown since there are many determinants of aortic stiffening other than vascular calcification (42). Furthermore, a damaged and stiff aorta may well be a target for the deposition of calcium due to exposure of calcium binding sites on fragmented elastin fibres. As a result, central pressures may vary significantly from what may be expected based on brachial blood pressure alone and may result in the misclassification of many hypertensives (43). In addition to VC, loss of the normal nocturnal BP drop has also been identified as a potential contributor to the high cardiovascular burden in CKD (44). Non-dipping status is associated with greater proteinuria and more rapid loss of glomerular filtration rate (GFR) in CKD (45, 46), and in end stage renal disease has correlated better with left ventricular mass index (LVMI) than clinic measurements alone (47, 48).

Detection of Vascular Calcification: CT as the gold standard

Since the advent of 64-slice multidetector CT scanning, coronary calcium content can be reliably measured non-invasively in dialysis patients. As mentioned above, the extent of calcium presence correlates well with hard end-points and survival in CKD-5D. Coronary CT cannot distinguish between arterial intimal and medial calcification but does correlate well with the presence and amount of atherosclerotic plaque. While the presence of calcium confers a higher risk on an individual, the absence of any calcium is associated with a very low mid-term risk of coronary events. Furthermore, adding the coronary calcium score to traditional risk factor prediction models improves risk stratification (49). Of course, the major disadvantage of coronary CT is its high expensive and lack of availability in resource-poor settings.

A call for accessible screening: The role of plane radiographs

The Global Bone and Mineral Initiative Working Group of the Kidney Disease Improving Global Outcomes (KDIGO) managed by the National Kidney Foundation has recommended screening for the presence of cardiovascular calcification with simple office-based methods to make it accessible to a greater number of nephrologists (28). This is particularly relevant to our setting in Africa where studies are severely lacking. Additionally, validation of these methods in our population is required.

It has recently been shown that there exists a good correlation between coronary artery calcium score (CAS) and the presence of abdominal aortic calcification (AAC) as determined by lateral plane radiograph of the abdomen as well as pulse wave velocity (PWV) (11) in developed nations. Furthermore, it has been shown that as with the CAS, the degree of AAC is also predictive of mortality in haemodialysis patients (36, 50) and in transplant recipients (51). This provides an inexpensive means to determine the presence of vascular calcification and hence risk in resource poor settings (52) which may lead to differences in treatments and improved outcomes.

Current gaps in knowledge and reasons for our study

To our knowledge, no study has examined the influence of vascular calcification on central aortic and ambulatory blood pressure in dialysis patients. As discussed above, this will provide us with important information that may ultimately improve care for dialysis and pre-dialysis patients.

There is no published data on the prevalence of vascular calcification in African dialysis patients. In Africa, a younger dialysis population exists and genetic and environmental influences may affect mineral metabolism and hence cardiovascular risk. Many environmental differences exist that could account for differences in vascular and bone health such as exposure to sunlight and Vit D3 use, diet and salt consumption, dialysis quality, membrane type, aluminium toxicity, iron loading, water strontium content and limited access to more effective phosphate binders (53).

Validation of a simple method for the assessment of vascular calcification will provide clinicians in our environment with a cost-effective means of determining a vascular risk profile from which patients stand to benefit the most as compared with more costly therapy. Determining a baseline calcification score in this way will also allow researchers to design prospective trials comparing progression of disease based on differing therapies and assess mortality prediction through follow up as recommended in the recent KDIGO report. Therefore, this is likely to add information to traditional risk prediction models which cannot explain all of the cardiovascular risk attributable to the dialysis population.

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PART C: Study Manuscript

University of Cape Town

**VASCULAR CALCIFICATION IN SOUTH AFRICAN DIALYSIS PATIENTS:
ETHNIC VARIATION, PREVALENCE, DETECTION AND HAEMODYNAMIC
EFFECTS**

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BACKGROUND

In Sub-Saharan Africa, the prevalence of vascular calcification (VC) in CKD-5D is unknown. We undertook to determine the effect of ethnicity on VC, the risk factors for VC, the utility of abdominal X-ray (AXR) in predicting coronary calcium score (CCS) and the effect of VC on central aortic systolic pressure (CASP) and left ventricular mass index (LVMI) in South African dialysis patients.

METHODS

74 CKD-5D patients and 20 healthy controls were studied prospectively. All subjects underwent chest CT for CCS, AXR for abdominal aortic calcium score (AACS), echocardiography for LVMI. Ambulatory blood pressure monitoring (ABPM) and CASP were generated via radial artery applanation tonometry.

RESULTS

Overall, 38.6% of cases demonstrated $CCS \geq 1$. On multivariate analysis, positive predictive factors included age and prior cardiovascular disease. Blacks had a median CCS of 0 (IQR 0) while non-blacks a median CCS of 66 (IQR 383), $p < 0.001$; controls had a CCS of 0 (IQR 0). Black race remained a significant negative predictor for coronary calcification after adjustment for known precipitants, $PR = 0.14$ and 95% CI: 0.0-0.53. VC was not associated with any ABPM parameters; interdialytic office BP was highly predictive of ABPM ($r=0.9$, $p=0.9$). Using receiver operator characteristic (ROC) curves, an AACS of ≥ 1 showed an area under the curve of 0.83 to predict a $CCS > 10$.

CONCLUSIONS

Black race significantly protects from VC in South African CKD-5D patients and warrants further study. The AXR is a useful screening tool for CCS in our population. VC does not appear to influence CASP in our population.

Keywords: vascular calcification, ethnicity, dialysis, central aortic systolic pressure, applanation tonometry, ambulatory blood pressure monitoring

SUMMARY OF MAIN MESSAGE IN PAPER

Black South African CKD-5D patients appear protected against vascular calcification despite adjusting for known precipitants. While it is plausible that this could be due to inherited differences in endogenous inhibitors of calcification, this remains to be investigated. Lateral abdominal X-ray is a good screening test for coronary calcification. VC was not associated with elevated central pressures and office BP was highly predictive of ABPM.

INTRODUCTION

Haemodialysis (HD) patients experience extremely high rates of cardiovascular mortality, 20 – 30 times higher than that of age-matched peers (1, 2). Coronary artery calcium (CAC) presence, and the coronary artery calcium score (CCS) as determined by cardiac computed tomography (CT), are strongly predictive of cardiovascular mortality in dialysis populations, (3, 4) which most often takes the form of sudden cardiac death (5). Vascular calcification (VC) is evident in >80% of the dialysis population in Europe and the US (6-8). The prevalence of vascular calcification in Sub-Saharan Africa is unknown.

VC is associated with stiffening of the aorta and increased aortic pulse wave velocity (PWV). This is associated with raised central aortic systolic pressure (CASP) and reduced coronary perfusion (9, 10). Brachial pressure may thus underestimate central pressure (11). Whether VC is directly linked with central pressures is however, unknown since a damaged and stiff aorta may also be a target for the deposition of calcium (12). CASP can be calculated from applanation tonometry-derived peripheral pulse waveforms thus avoiding invasive central pressure determination (13). The major disadvantage of standard techniques (such as carotid-femoral PWV) however, is the one-dimensional static measurement obtained, with no information on ambulatory values or nocturnal dipping status. Loss of the normal nocturnal blood pressure (BP) drop has also been identified as a potential contributor to the high cardiovascular burden in CKD (14) and in end stage renal disease it has correlated better with left ventricular mass index (LVMI) than office BP measurements alone (15, 16). There have been calls for the routine use of ABPM in clinical studies of CKD given the lack of information on BP variability (14, 17).

CAC is best detected on a cardiac CT scan which is very expensive and not universally available. Studies from the USA have shown a reasonable correlation between CCS and the presence of abdominal aortic calcification (AAC) as determined by a lateral radiograph of the abdomen (AXR) (18). As for coronary calcification, the degree of AAC is also predictive of mortality in haemodialysis patients (6, 19) and in kidney transplant recipients (20). The Global Bone and Mineral Initiative Working Group of the Kidney Disease Improving Global Outcomes (KDIGO) has recommended screening for the presence of cardiovascular calcification with simple office-based methods such as echocardiogram or lateral abdominal radiograph to make it accessible to a greater number of nephrologists (21). Validation of these methods in our population is required.

We undertook to do this by performing simultaneous cardiac CT and AXR in a prospective, cross-sectional descriptive study of prevalent haemo- and peritoneal dialysis patients.

Patient demographics were recorded in an attempt to identify risk factors for vascular calcification and to assess the effect of ethnicity on VC. Furthermore, we sought to prospectively evaluate whether the presence of VC in our CKD-5D cohort had any relationship to ambulatory CASP, LVMI and nocturnal dipping status using the BPro® Radial Pulse Wave Acquisition Device. To our knowledge, no study has been published examining ambulatory CASP in dialysis patients. Finally, we sought to determine the utility of interdialytic office brachial and central BP measurement in predicting ambulatory BP parameters.

SUBJECTS AND METHODS

Patient and Control Selection

The study was approved by the Research Ethics Committee of the University of Cape Town, South Africa. Cases were selected if they were on maintenance dialysis ≥ 3 month's duration and were able to sign informed consent. 66 Prevalent HD and 8 prevalent peritoneal dialysis (PD) patients ≥ 18 years old were enrolled from Groote Schuur Hospital, Cape Town giving a total number of recruits of 75. Patients were excluded if they were pregnant or planning a pregnancy, had sustained arrhythmias or prior coronary stenting. One patient was excluded for loss to follow-up, thus the final case sample was 74 participants. Age, sex and smoking matched healthy controls from the same residential area as cases were selected if they were free from cardiovascular disease with normal clinical and laboratory examinations (including eGFR ≥ 60 ml/min and urinary albumin excretion ≤ 2.5 mg/mmol creatinine). 27 Controls were screened, 5 being excluded for hypertension and 2 for low-grade proteinuria giving a final control sample size of 20.

Laboratory Data

The following clinical and demographic parameters were recorded on entry to the study: blood pressure, cardiac rhythm, age, sex, ethnicity (patient-reported), body mass index (BMI), time on dialysis, dialysis modality, smoking habits, history of atherosclerotic vascular disease or diabetes, and medication history. All relevant routine quarterly laboratory parameters (haemoglobin, albumin, calcium, phosphate, alkaline phosphatase, 25-OH-Vitamin D, parathyroid hormone and total cholesterol) on each patient for the two years prior to enrolment was recorded and averaged. Controls underwent serum creatinine testing with estimated glomerular filtration rate as well as urinary albumin excretion quantification and electrocardiography. In a sub-study of 10 blacks and 10 non-blacks, total dietary

calcium and phosphate intake was estimated using a 24hr food recall questionnaire and food frequency tables.

Ambulatory and Office Blood Pressure Monitoring

The BPro® Radial Pulse Wave Acquisition Device and A-PULSE CASP® Software (HealthStats, Singapore) system uses an N-Point Moving Average method to non-invasively derive CASP from the radial arterial pressure waveform. It is a small, wrist watch-like cuffless blood pressure monitor which obtains radial pressure waveforms by applanation tonometry and is FDA approved plus carries the CE mark. For CASP, it has been validated against a generalized transfer function method (GTF-CASP [SphygmoCor system, AtCor, Sydney, Australia]) using CAFE study data as well as central aortic pressures recorded in vivo at the aortic root using a Millar's SPC-454D tonometer (Millar's instruments, Texas U.S.A) (22). For BP determination, the BPro™ has been validated against the Association for the Advancement of Medical Instrumentation (AAMI) and European Society of Hypertension (ESH) protocols and passed both validations (23). The BPro™ records pressure wave forms calibrated to the brachial BP and samples up to 96 X 10 second blocks of time, over 24 hours. This provides a 24 hour profile and summary of an individual's systolic, diastolic and mean arterial pressures. Additionally, with help from the manufacturer, we were able to convert the ABPM data into ambulatory CASP readings.

Practically, the device was applied as a wrist watch on the non-dominant arm or that which did not contain an AVF on the interdialytic day for HD patients or at a routine visit for PD patients. The device was then calibrated to office BP - brachial blood pressure obtained via use of the MC3000 oscillometric device (HealthStats) according to the recommended ESH protocol (24). The device was then connected to the software and real time pulse wave morphology captured. Thereafter, it was worn by the individual for a 24 hour period whereupon the device was retrieved and ambulatory data captured.

Abdominal X-Ray

A standard technique of exposing the lateral lumbar spine in standing position (with 100 cm film distance, 94 KVP, and 33–200 mAs) was used. Calcific deposits in the abdominal aorta were scored as described by Kaupilla (25), by a single experienced clinician (RF) blinded to clinical data.

Cardiac CT

Images were acquired using the Philips Brilliance 64 slice MDCT Scanner. A standard protocol was used as follows: tube voltage, 120 kV; tube current, 55 mAs; detector collimation, 40×0.625 mm; gantry rotation, 400 ms. CT data were transferred to the Philips Extended Brilliance Workstation Version 4.0.2.145 for analysis and CCS was calculated with the Agatston algorithm (26). All scans were evaluated by a single experienced radiologist (SM) and the intra-reader variability was tested and was below 10%.

Echocardiography

Assessment of the left ventricular mass was done via use of M-mode echocardiography and this was calculated using the Penn convention (27). Left ventricular hypertrophy was defined as >125 g/m² in males and >110 g/m² in females as per ESH guidelines (28). All scans were obtained and evaluated by a single experienced cardiologist, who was blinded to clinical data (AL).

Statistical analysis

Normality was determined with the Shapiro-Wilk test. Continuous variables are expressed as mean \pm SD or median and inter-quartile range (IQR) and compared with the two-tailed independent Student's t-test and Mann-Whitney test as appropriate. Dichotomous data are presented as percentages and compared with Chi-square tests. To evaluate the utility of the AXR in predicting coronary calcification, the receiver operator characteristic (ROC) curve analysis was used to calculate the area under the curve in relation to a CCS of ≥ 1 and ≥ 10 . Associations between elevated coronary calcium scores and various exposures are presented as prevalence ratios and 95% confidence intervals (CI) and were estimated using generalized linear modeling. Subjects of African ethnicity were classified as Black and the remainder of subjects were classified together as non-black for the purposes of the analysis. All analyses were conducted using Stata 12.0 Statistical Software (College Station, TX, USA).

With respect to control size calculation, there is no published data on coronary calcification scores in the South African population. Using data from a study published in Brazil with a comparable younger dialysis patients (29), the proportion of patients with a coronary calcium score of <100 was 48%. In the Multi-Ethnic Study of Atherosclerosis (MESA) (30), the distribution of coronary artery calcium score by race, gender, and age was evaluated in asymptomatic individuals free of cardiovascular disease. Given that the 90th percentile for coronary calcium score in those aged 45-54 years in whites was 8 for women and 110 for men and in blacks 9 for women and 45 for men, we considered it reasonable to assume that the proportion of both populations with a coronary calcium score <100 is at least 90%.

Using these proportions, we calculated a sample size of 13 controls and 75 patients but given the uncertainty surrounding the proportions, we chose to include 80 cases and 20 controls.

RESULTS

The study population consisted of 74 Cases and 20 Controls in the final analysis. Table 1 shows baseline characteristics for cases. Blacks had a mean age of 41.6 (SD 11.1) and were 53% female; Non-Blacks had a mean age of 41.9 (SD 10.0) and were 61.8% female, $p=0.9$ and 0.4 respectively. At enrolment, 39 (52.7%) had been exclusively on HD, 5 (6.8%) exclusively on PD and 30 (40.5%) on both modalities. Calcitriol was being used by 82.9% of the cases and this was not different between race groups ($p=0.8$). All patients were dialysed with a dialysate calcium content of 1.25mmol/L and were on the maximum dose of calcium carbonate phosphate binders. Table 2 shows the estimated dietary calcium and phosphorous intake in a sub-study of 20 patients.

Table 1: Baseline Characteristics of cases

Characteristic	N	Value	Range (SD/IQR)
Age, mean (years)	74	41.8	10.5
Women (%)	74	56.8	
Race, self-reported (%)			
Black	40	54.0	
Non-Black	34	46.0	
White	2	2.7	
Mixed	29	39.2	
Asian	2	2.7	
Hispanic	1	1.4	
Body Mass Index, median (kg/m ²)	72	23.3	5.4
Months on Dialysis, median	74	32.0	43.6
Haemodialysis	68	27.0	37.6
Peritoneal Dialysis	34	17.0	30.0
Diabetes (%)	74	13.5	
Tobacco Use (%)	74	41.9	
History of Cardiovascular Disease (%)	74	4.0	
ABPM systolic BP, mmHg	72	147.4	33.1
ABPM diastolic BP, mmHg	72	97.6	21.7
ABPM Peripheral Pulse Pressure, mmHg	72	49.8	15.4
ABPM Central Aortic Systolic Pressure, mmHg	72	139.2	31.3
ABPM Dipping Status (%)	72	5.3	5.5
LVMI, g/m ²	74	180.4	97.4
LVH			
By ECHO	74	86.4	
By ECG	74	70.3	
Ejection Fraction (%)	74	63	12.2
Plasma Total Cholesterol, mmol/L	66	4.12	0.86
Plasma Parathyroid Hormone, pmol/L	67	57.1	58.2
Plasma Alkaline Phosphatase, U/L	67	97.8	85.5
Plasma Corrected Calcium, mmol/L	63	2.0	0.2
Plasma Phosphate, mmol/L	64	1.7	0.4
Plasma PhosphateXCalcium product (mmol/l ²)	66	3.43	0.95
Plasma 25-OH-Vitamin D nmol/L	62	66.8	41.4
Plasma Albumin, g/L	63	39.7	3.7
Plasma Haemoglobin, g/dL	64	8.8	1.4
Patients on treatment with Calcitriol (%)	70	82.9	
Patients on treatment with Ergocalciferol (%)	69	17.4	
Number of antihypertensives used, mean	74	2.3	1.4

SD, Standard Deviation; IQR, Interquartile range; ABPM, Ambulatory Blood Pressure Monitoring; BP, Blood Pressure; LVMI, Left Ventricular Mass Index; LVH, Left Ventricular Hypertrophy

Table 2: Estimated dietary calcium and phosphorous intakes

Population	N	Calcium Intake, mg/day	SD	P-Value	Phosphorous Intake, mg/day	SD	P-Value
Blacks	10	401	224	0.3	915	287	0.9
Non-Blacks	10	527	268		906	399	

Note: Calcium intake is excluding the contribution from calcium-containing phosphate binders. SD, Standard Deviation

Race and vascular calcification

Table 3 shows the median coronary and abdominal aortic calcium scores by race as well as the proportion with calcification by race and quartile/tertile respectively. Overall, only 15.5% and 17.5% of black patients had coronary or abdominal calcification respectively compared with 67.7% and 57.6% of non-blacks respectively (both comparisons are $p < 0.001$). There was no association between dose of calcitriol and CCS ($p = 0.8$). Median 25-OH-Vitamin D levels were not different in blacks (68.4, IQR 70.4) and non-blacks (66.6, IQR 42.6), $p = 0.3$. Controls had no demonstrable VC ($p < 0.01$).

Table 3: Proportion of cases and controls with coronary and abdominal calcification

				Quartiles of Coronary Calcification Score							
Race	N	Median Score	IQR	0		1-9		10-99		≥ 100	
				Prop	95%CI	Prop	95%CI	Prop	95%CI	Prop	95%CI
Black cases	39	0	0	84.6	69.594.1	2.6	0.0-13.5	2.6	0.0-13.5	10.3	2.9-24.2
Non-Black cases	31	66	383	32.3	16.7-51.4	3.2	0.0-16.7	25.8	11.9-44.6	38.7	21.8-57.8
Controls	20	0	0	100	83.2-100.0	0	0.0-16.8	0	0.0-16.8	0	0.0-16.8

				Tertiles of Abdominal Aortic Calcium Score					
Race	N	Median Score	IQR	0		1-6		≥ 7	
				Prop	95%CI	Prop	95%CI	Prop	95%CI
Black cases	40	0	0	82.5	67.2-92.7	12.5	4.2-26.8	5.0	0.1-16.9
Non-Black cases	33	1	8.5	42.4	25.5-60.8	24.2	11.1-42.3	33.3	18.0-51.8
Controls	20	0	0	100	83.2-100.0	0	0.0-16.8	0	0.0-16.8

Prop, Proportion; IQR, Interquartile Range; PR, Prevalence Ratio; 95%CI, 95% Confidence Interval

Risk factors for vascular calcification and haemodynamic effects

Table 4 shows baseline characteristics for all subjects with and without coronary calcification and for black race. Those with coronary calcification (Ca^{++}) were older, had been on dialysis longer and had more diabetes than those without coronary calcification (Ca^{-}). Black race was associated with less coronary calcification. Gender, tobacco use, dialysis modality, history of cardiovascular disease, LVMI, all BP parameters and all biochemical parameters were not significantly different between groups. The effect of systolic BP (SBP)

on CASP was adjusted for by reporting a CASP:SBP ratio. There was also no difference in central BP parameters when comparing groups with and without abdominal aortic calcification.

In a pre-specified subgroup analysis, blacks who did develop coronary calcification (n=6) had a non-significantly higher blood pressure (systolic BP +18.3mmHg and CASP +27.7%) than blacks without coronary calcification (n=33), both p=0.1. However, there was a higher percentage of diabetes (+47%, p<0.001) and known cardiovascular disease (+30.3%, p<0.05). Black subjects with Ca⁺⁺ had higher plasma calcium, phosphate and cholesterol although this also did not reach significance (p=0.1). In non-black subjects, only age was significantly associated with Ca⁺⁺ (P<0.001).

Table 4: Baseline characteristics of cases stratified by presence of coronary calcification and race

Variable	All Races					Blacks				
	N	Ca -	N	Ca ⁺⁺	P-Value	N	Ca -	N	Ca ⁺⁺	P-Value
Age (median)	43	38.3	27	46.0	<0.01	33	39.8	6	48.3	0.1
Black Race (%)	33	84.6	6	15.4	<0.001	-	-	-	-	-
Gender, m/f ratio	43	1.1	27	0.5	0.1	33	1.1	6	0.5	0.4
Time on Dialysis before inclusion, months	43	27.0	26	51.0	<0.05	33	27.0	5	27.0	0.7
Tobacco Use (ever) (%)	43	37.2	27	51.9	0.2	33	33.3	6	50.0	0.4
Prior cardiovascular Events (%)	43	2.3	27	7.4	0.3	33	3.0	6	33.3	<0.05
Presence of Diabetes (%)	43	7.0	27	25.9	<0.05	33	3.0	6	50.0	<0.001
ABPM systolic BP, mmHg	43	145.8	26	150.1	0.6	33	143.5	5	161.8	0.1
ABPM diastolic BP, mmHg	43	97.7	26	97.3	0.9	33	94.5	5	104.1	0.2
ABPM Peripheral Pulse Pressure, mmHg	43	48.0	26	52.8	0.2	33	47.3	5	59.8	0.1
ABPM Central Aortic Systolic Pressure, mmHg	43	137.6	26	141.8	0.6	33	133.5	5	161.2	0.1
ABMP CASP/SBP ratio	43	0.9	26	0.9	0.5	33	0.9	5	0.9	0.2
ABPM Central Pulse Pressure, mmHg	43	39.9	26	44.5	0.2	33	47.0	5	59.8	0.1
Plasma Total Cholesterol, mmo/L	39	4.0	25	4.4	0.1	31	4.0	5	4.5	0.2
Plasma Parathyroid Hormone, pmol/L	39	66.0	25	65.0	0.4	31	57.9	5	67.8	0.3
Plasma Alkaline Phosphatase, U/L	39	99.8	25	79.2	0.1	31	111.0	5	79.0	0.4
Plasma Corrected Calcium, mmol/L	38	2.0	25	2.0	0.4	30	2.0	5	2.2	0.2
Plasma Phosphate, mmol/L	39	1.6	25	1.8	0.1	31	1.6	5	2.0	0.1
Plasma PhosphateXCalcium product	38	3.2	25	3.7	0.1	30	3.2	5	4.1	0.1
Plasma 25-OH-Vitamin D nmol/L	37	66.7	23	68.2	0.5	29	66.6	3	98.5	0.6

Ca -, Coronary Calcium Score = 0; Ca⁺⁺, Coronary Calcium Score = ≥1; m/f = Male:Female; ABPM, Ambulatory Blood Pressure Monitoring; BP, Blood Pressure; CASP/SBP, Central Aortic Systolic Pressure/Systolic Blood Pressure

Table 5 shows the prevalence ratios of coronary calcification by various categories in a model adjusted for age, sex and race as well as an adjusted model. Time on dialysis, presence of diabetes, history of vascular disease and phosphateXcalcium product were included in the adjusted model (these being established risk factors for vascular calcification in CKD-5D (6-8)) Using generalized linear modeling to investigate independent predictors of $CCS \geq 1$, the following factors were excluded: gender , time on dialysis , diabetes and phosphateXcalcium product (all non-significant). Independent predictors of coronary calcification were age and history of cardiovascular. Black race remained an independent negative predictor of coronary calcification.

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Table 5: Univariate and multivariate prevalence ratios of coronary calcium presence for cases

VARIABLE	CATEGORY	N	UNIVARIATE MODEL ADJUSTED FOR AGE, SEX AND RACE		MULTIVARIATE ADJUSTED MODEL
			% CAC+	CRUDE PR (95% CI)	ADJUSTED PR (95%CI)
AGE (Years)	<30	12	16.7	1.0 (Ref)	1.0 (Ref)
	30-39	15	13.3	2.6 (0.4-18.5)	1.03 (1.01-1.05)
	40-49	27	51.9	5.1 (0.9-27.8)	
	≥50	16	56.3	5.5 (1.0-30.5)	
RACE (Self Reported)	Non-Black	31	67.7	1.0 (Ref)	1.0 (Ref)
	Black	39	15.4	0.3 (0.2-0.5)	0.14 (0.0-0.53)
GENDER (M/F)	Female	39	46.2	1.0 (Ref)	1.0 (Ref)
	Male	31	29.0	0.8 (0.6-1.2)	0.91 (0.60-1.39)
TIME ON DIALYSIS (Years)	0-1.99	19	26.3	1.0 (Ref)	1.0 (Ref)
	2.0-2.99	17	23.5	0.9 (0.4-1.9)	0.65(0.31-1.38)
	3.0-5.99	15	53.3	1.1 (0.6-2.1)	0.88 (0.48-1.61)
	≥6.0	18	50.0	1.0 (0.5-2.0)	0.83 (0.48-1.61)
PRIOR CARDIOVASCULAR EVENT (Y/N)	No	67	37.3	1.0 (Ref)	1.0 (Ref)
	Yes	3	66.7	4.2 (1.4-12.4)	7.5 (1.5-36.7)
DIABETES (Y/N)	No	60	33.3	1.0 (Ref)	1.0 (Ref)
	Yes	10	70.0	1.17 (0.7-1.9)	0.93 (0.53-1.64)
CALCIUM/PHOSPHATE PRODUCT (mmol/l ²)	<3.0	22	22.7	1.0 (Ref)	1.0 (Ref)
	3.0-4.49	31	48.4	1.5 (0.9-2.4)	1.31 (0.75-2.30)
	≥4.5	10	50.0	1.4 (0.7-2.5)	1.35 (0.68-2.65)
PLASMA CALCIUM (mmol/L)	<2.0	32	37.5	1.0 (Ref)	
	≥2.0	31	41.9	1.3 (0.9-1.9)	
PLASMA PHOSPHATE (mmol/L)	<1.4	16	31.2	1.0 (Ref)	
	1.4-1.8	22	27.2	0.9 (0.3-2.5)	
	>1.8	26	53.9	1.7 (0.8-4.0)	
ABPM SYSTOLIC BP (mmHG)	<120	12	25.0	1.0 (Ref)	
	120-139	13	46.2	0.9 0.5-1.6	
	140-159	23	43.5	0.6 0.3-1.2	
	160-179	13	30.8	0.9 (0.5-1.7)	
	≥180	8	37.5	0.6 (.3-1.4)	
ABPM CASP/SBP RATIO	<0.910	15	33.3	1.0 (Ref)	
	0.91-0.929	20	35.0	1.1 (0.6-2.0)	
	0.930-0.939	13	30.8	1.1 (0.6-2.1)	
	≥0.940	16	62.5	1.4 (0.8-2.5)	
BP DIPPING STATUS (%)	≤0	11	54.6	1.0 (Ref)	
	0.001-4.9	19	47.4	0.8 (0.5-1.3)	
	5-9.9	21	28.6	1.1 (0.6-1.9)	
	≥10	11	36.4	1.3 (0.7-2.3)	
PLASMA CHOLESTEROL (mmol/L)	<4.0	27	22.2	1.0 (Ref)	
	4.0-4.99	29	58.6	1.0 (0.7-1.5)	
	≥5.0	8	25.0	0.8 (0.5-1.7)	
PLASMA 25-HYDROXY-VIT D (nmol/L)	<50	13	53.9	1.0 (Ref)	
	50-74	21	28.6	1.2 (0.7-2.1)	
	75-89	9	44.4	1.1 (0.7-1.7)	
	≥90	17	35.3	0.7 (0.4-1.2)	

%CAC+, Percentage with coronary calcium score ≥1; PR (95% CI), Prevalence Ratio (95% Confidence Interval); M/F, Male/Female; ABPM, Ambulatory Blood Pressure Monitoring; BP, Blood Pressure; CASP/SBP, Central Aortic Systolic Pressure/Systolic Blood Pressure

Comparing cases with controls

Table 6 shows the differences in baseline characteristics between cases and controls. There was no difference with respect to age, gender, race and smoking status. BP, LVMI and CASP were all significantly higher in cases while dipping was significantly lower. After adjustment for SBP, CASP was not different between cases and controls.

Table 6: Baseline characteristics of cases versus controls

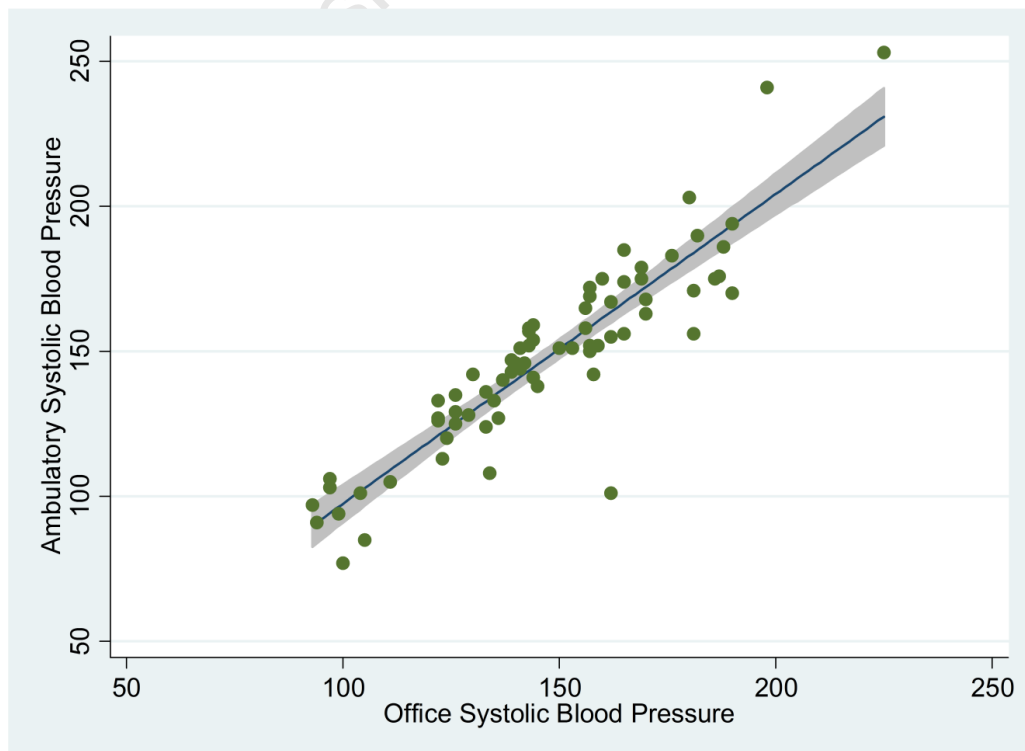
Variable	Cases		Controls		P-Value
	N	Value	N	Value	
Age (median)	74	41.8	20	39.7	0.4
Gender, m/f ratio	74	0.8	20	0.8	0.9
Tobacco Use (%)	74	41.9	20	42.1	1.0
Black Race (%)	74	54.0	20	50.0	0.8
ABPM systolic BP, mmHg	72	147.4	20	130.6	p<0.01
ABPM diastolic BP, mmHg	72	97.6	20	85.8	p<0.01
ABPM Central Aortic Systolic Pressure, mmHg	72	139.2	20	123.3	p<0.05
ABMP CASP/SBP ratio	72	0.9	20	0.9	0.9
ABPM SYS-CASP Difference , mmHg	72	8.3	20	7.3	0.1
ABPM Dipping Status (%)	72	5.3	18	9.3	p<0.01
LVMI, g/m ²	74	180.4	20	97.3	p<0.001
LVH (On echo criteria), %	74	86.4	20	5.0	p<0.001

m/f, male/female; ABPM, Ambulatory Blood Pressure Monitoring; BP, Blood Pressure; CASP/SBP, Central Aortic Systolic Pressure/Systolic Blood Pressure; SYS, Systolic Pressure; LVMI, Left Ventricular Mass Index; LVH, Left Ventricular Hypertrophy

Office versus ambulatory blood pressure

Figure 1 shows the correlation of Office with ambulatory systolic blood pressure. Office systolic BP and CASP correlated well with ambulatory blood pressure (both $r=0.9$, $p=0.9$).

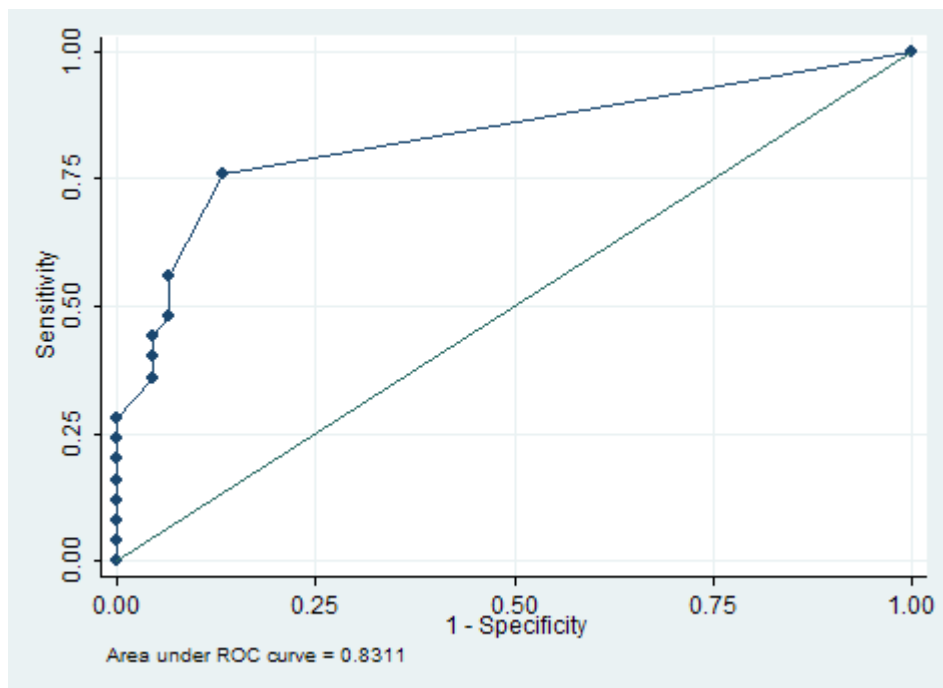
Figure 1: Correlation of office and ambulatory systolic blood pressure



Abdominal aortic x-ray as a screening test for vascular calcification

Figure 2 shows the receiver operator characteristics curve for predicting a CCS of ≥ 10 in cases using an abdominal calcification score of 1. The area under the curve (AUC) was 0.83 with a sensitivity of 76% and a specificity of 86.7%, resulting in the correct classification of 82.9% of patients. Using a cut point of abdominal score ≥ 2 reduced sensitivity to 56.0% and raised specificity to 93.3%. Using an abdominal score of 1 to rather predict a coronary calcium score of ≥ 1 yielded much the same findings as the first analysis (AUC of 0.82, sensitivity of 74.1%, specificity of 88.4% and also correctly classifying 82.9% of patients).

Figure 2: ROC Curve for predicting coronary calcium score ≥ 1 using abdominal aortic calcium score of 1



DISCUSSION

This is a prospective cross-sectional study of 74 CKD-5D patients in a South African public sector dialysis unit. To our knowledge, this is the first descriptive study of vascular calcification in CKD-5D in Sub-Saharan Africa. The young age of participants represents a population selection bias because of stringent public sector dialysis inclusion criteria. Patients are selected for state-funded dialysis based on their suitability for transplantation, amongst other things. This also explains the low BMI and low prevalence of diabetes and cardiovascular disease in the cohort (table 1). The low haemoglobin is in keeping with a funding restriction on erythropoietin prescription.

A key finding in this study was the significantly lower prevalence of coronary calcification in blacks versus non-blacks (table 3). In the USA, a recent cross sectional survey of prevalent dialysis patients showed no differences in coronary and thoracic calcification between white and black subjects (31). In the general population, however, significant differences exist between the coronary calcium content in white populations of different continents (32) and in the rate of coronary (33) and extra-coronary calcification (34) between different ethnic groups. In Africa, genetic and environmental influences may affect mineral metabolism and hence cardiovascular risk. In our cohort, the use of calcitriol was common practice but was not different in blacks. In contrast to other studies from abroad, (35) 25-Hydroxy-Vitamin D levels were not lower in blacks. Moreover, estimated dietary calcium and phosphate intakes were not different between groups. Since we are unable to account for any external differences between the groups, it may be that inherited differences in endogenous inhibitors of calcification exist between blacks and non-blacks. This requires further study.

The 6 black patients with Ca^{++} , had a non-significantly higher blood pressure, more diabetes and more cardiovascular disease than blacks without coronary calcification. Blacks with calcification also exhibited a trend to higher calcium and phosphate levels although this was also not significant. The low numbers in this sub-group precluded any meaningful multivariate analysis.

Contrary to our expectations, the presence of VC or CKD-5D was not associated with higher ambulatory or office CASP when adjusting for SBP with an ABPM CASP/SBP ratio of 0.943 for Ca^- versus 0.945 for Ca^{++} , $p=0.5$ in cases and 0.944 for controls, $p=0.9$. The reasons for this are unclear but it may be that VC is not directly responsible for aortic stiffening and that the association with PWV is not causative since there are many factors such as elastin fragmentation, endothelial dysfunction and advanced glycation that affect aortic stiffness other than calcification (12). Alternatively, since vascular micro-calcifications may be present in uraemic subjects without apparent radiologically visible calcium, (36) it is possible that vascular stiffening occurs earlier on and obscures any differences in CASP. However, this does not explain the lack of difference between cases and healthy age-matched controls and calls into question the use of the N-Point Moving Average method to non-invasively derive CASP. These results require confirmation using established methodologies such as PWV. Of further interest, is the lack of any relationship between vascular calcification and other predictors of outcome in CKD such as LVMI and. Since VC predicts outcome so well in other CKD-5D patients, it will be important to follow our cohort longitudinally to establish whether these markers also predict risk in our patients.

Interdialytic office blood pressure and CASP correlated very well with ambulatory BP measurements. This has important implications since the FDA has called for the inclusion of CASP into clinical studies of blood pressure (9). Non-dipping was particularly prevalent as in other studies of CKD (37) and was worse in the cases. ABPM has been shown to be superior to office and intradialytic measurements in predicting outcomes for CKD-5D patients (38, 39) and it remains to be determined whether this applies to our cohort.

The AXR provided a good screening test for the presence of a $CCS \geq 1$ with an AUC of 0.83. This compares favourably to a study from the United States where the AUC using AXR was 0.78 in predicting a coronary calcium score ≥ 30 (18). These findings have important implications in providing a cost-effective screening test for vascular calcification in Sub-Saharan Africa where resources are particularly limited.

There are several limitations to our study. First, the patients in our cohort are young and one cannot be certain whether these findings would be reproduced in an older cohort of patients more similar to what has been reported on from abroad. Second, whites were poorly represented in our cohort and further study in this population would help to explore the differences between race groups more fully. Third, we were not able to measure PWV in our study and this would have helped to reconcile the lack of effect of VC and CHD-5D status on CASP. Fourth, we were unable to measure calcium flux in the different race groups which could affect the prevalence of vascular calcification. Finally, while we are attempting to investigate the variation in dietary intake of calcium and phosphate between race groups, we lack a locally validated and accurate system for quantifying intake, and this needs to be confirmed in a larger study. The strengths of our study include the prospective nature, the inclusion of virtually all available patients in the unit, the very low rate of dropout and the inclusion of a control group.

In conclusion, in South African dialysis patients, black race appears to confer significant protection against the presence of coronary calcification. This requires further investigation as well as confirmation in other cohorts. Vascular calcification is not associated with changes in central aortic systolic pressure. Interdialytic office blood pressure and central aortic systolic pressure, when measured according to ESH standards, correlate very well with ambulatory measurements. Finally, the lateral abdominal x-ray is a good screening test for the presence of coronary calcium.

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Conflict of interest statement

Genzyme Corporation provided statistical advice during protocol design but was at no stage involved in the collection, analysis, interpretation and reporting of data herein.

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PART D: Supporting Documents

PATIENT INFORMATION AND INFORMED CONSENT: CASES

STUDY TITLE: “The impact of vascular calcification on ambulatory and central aortic blood pressure in a South African dwelling dialysis population”

INTRODUCTION

You are invited to participate in this study. This information will help you decide if you would like to participate. Before you agree, you should fully understand what is involved. If you have any questions, which you do not understand, please do not hesitate to discuss this with the study doctor. You should not agree unless you are completely happy about this study.

WHY IS THIS STUDY BEING DONE?

You are invited to participate in this research study. You have previously advanced chronic kidney disease and as such, require dialysis regularly in order to prolong life. It is a fact that people on dialysis suffer more from diseases of the heart and blood vessels (cardiovascular disease) than the general population. Large amounts of calcium build up in their blood vessels and it is likely that this is related to the increased chances of cardiovascular related death. Recent research has suggested that the link may be through a higher blood pressure in the great vessels connected to the heart. We would like to examine this link in our unit and therefore request your participation in this research.

WHY ARE YOU BEING ASKED TO TAKE PART?

As you are on long term dialysis, you are at risk of calcium build up and are thus a good person for us assess.

WHAT IS THE DURATION OF THE STUDY AND WHAT PROCEDURES WILL BE PERFORMED?

If you decide to participate, you will be one of 100 participants.

The study will be running for approximately 18 months, however, it will take 1 month or less to acquire the necessary information from you. Once you have agreed to participate, you be required to wear a device similar to a wrist watch for about 24 hours. This will gather information on your blood pressure and is not painful in any way. Thereafter, we will arrange for you to have a special CT scan of your heart and a plain x-ray of your abdomen that we use to determine the amount of calcium in your blood vessels. Also, we will arrange a special ultrasound of your heart to look for the effects of high blood pressure on the heart muscle. Ideally, we would like to repeat these measurements after a period of 1 year in order to assess for any change over time.

WILL THIS INCONVENIENCE ME IN ANY WAY?

Participation in the study will mean having to attend hospital between 2 and 4 extra times on the days in between your dialysis days. Every effort will be made to accommodate your needs in terms of timing and an amount of R50 will be given to compensate you for transport costs if you come on a non-dialysis day and R150 for attendance at 2 Military hospital.

WHAT WILL HAPPEN IF YOU DECIDE NOT TO TAKE PART IN THE STUDY?

Your participation is entirely voluntary and you can refuse to participate or stop at any time without stating a reason. Your withdrawal will not affect your access to future medical care. The investigator retains the right to withdraw you from the study if it is your best interest or you do not follow the guidelines and regulations of the trial.

WHAT ARE THE RISKS AND DISCOMFORTS OF THIS STUDY?

The only risk incurred in the study is the extra exposure to radiation that you will receive as a result of the CT scan and X-ray. However, the amount of radiation is small and well within that recommended to be safe by expert authorities. Blood tests are unlikely to be required but may result in a bruise at the puncture site, swelling of the vein, infection, or bleeding. In experienced hands this is highly unlikely and only minor discomfort may occur.

ARE THERE BENEFITS TO YOU FOR BEING IN THIS STUDY?

There may be benefits to you if we find your blood pressure is inadequately controlled and we can thus give this information to your doctor with your permission. The benefits of this study may impact future patient care positively but ultimately, this may not affect you.

WHAT WILL HAPPEN WHEN THE STUDY IS OVER?

With your permission, your results will be shared with the doctor responsible for your care. Any information collected about you will be kept securely and no information will be divulged to any third party without your prior consent or as required by law. No blood samples will be stored

HAS THE STUDY RECEIVED ETHICAL APPROVAL?

The study protocol has been approved by the Research Ethics Committee of the University of Cape Town, and the Medicines Control Council of South Africa.

ARE THERE ANY WARNINGS OR RESTRICTIONS FOR MY ACTIVITY?

No.

INSURANCE AND FINANCIAL ARRANGEMENTS

All the study doctors are covered by insurance. If any trial related injury occurs, UCT has a No Fault Insurance Policy for trial-related injuries.

The policy states:

“ The University of Cape Town (UCT) undertakes that in the event of you suffering any significant deterioration in health or well-being, or from any unexpected sensitivity or toxicity, that is caused by your participation in the study, it will provide immediate medical care. UCT has appropriate insurance cover to provide prompt payment of compensation for any trial-related injury according to the guidelines outlined by the Association of the British Pharmaceutical Industry, ABPI 1991. Broadly-speaking, the ABPI guidelines recommend that the insured company (UCT), without legal commitment, should compensate you without you having to prove that UCT is at fault. An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request

SOURCE OF ADDITIONAL INFORMATION

For the duration of the study you will be under the care of Dr Freercks and Professor's Rayner and Swanepoel. If at any time you have symptoms causing you problems or questions please do not hesitate to contact them at this telephone number (021) 404 3318. In addition, you may contact the UCT Ethics Committee should you have any concerns about the study or your treatment on tel. 021 4066626 or at shuretta.thomas@uct.ac.za.

CONFIDENTIALITY

All information during the course of the trial is strictly confidential. Each patient will be allocated a study number – only investigators will be aware of the link between the hospital folder number and study number. Data will be reported in scientific journals, but will not include information that identifies you.

It is important that the Medicines Control Council of South Africa and the Research Ethics Committee of the University of Cape Town be able to review records of the trial, but only in relation to their regulatory obligations.

The Protocol of this clinical trial was submitted for approval to the University of Cape Town Research Ethics Committee, a research ethics committee registered with the National Health Research Ethics Council. The study has been structured in accordance with the Guidelines on Clinical Trials and Ethics in Health Research, published by the Department of Health and the Declaration of Helsinki 2008.

INFORMED CONSENT

I hereby confirm that I have been informed by the study doctor, Dr Freercks or Prof's Rayner/Swanepoel about the nature, conduct, benefits and risks of this clinical trial. I have also received, read, and understand the written Patient Information and Consent form.

I am aware that the results of the trial will be anonymously processed into a trial report.

I may, at any stage, withdraw my consent and participation without prejudice.

I have had sufficient opportunity to ask questions declare myself prepared to participate in the trial.

Patient's Name:(print)

Patient's Signature:..... **Date:**

Study Doctor's Name: (print)

Study Doctor's Signature:**Date:**

Witness Name: (print) (Required only if interpreter used)

Witness Signature:

PATIENT INFORMATION AND INFORMED CONSENT: CONTROLS

STUDY TITLE:“The impact of vascular calcification on ambulatory and central aortic blood pressure in a South African dwelling dialysis population”

INTRODUCTION

You are invited to participate in this study. This information will help you decide if you would like to participate. Before you agree, you should fully understand what is involved. If you have any questions, which you do not understand, please do not hesitate to discuss this with the study doctor. You should not agree unless you are completely happy about this study.

WHY IS THIS STUDY BEING DONE?

You are invited to participate in this research study. The patients enrolled in this study have previously been diagnosed with advanced chronic kidney disease and as such, require dialysis regularly in order to prolong life. It is a fact that people on dialysis suffer more from diseases of the heart and blood vessels (cardiovascular disease) than the general population. Large amounts of calcium build up in their blood vessels and it is likely that this is related to the increased chances of cardiovascular related death. Recent research has suggested that the link may be through a higher blood pressure in the great vessels connected to the heart. We would like to examine this link in our unit and are therefore enrolling patients from our unit to participate.

WHY ARE YOU BEING ASKED TO TAKE PART?

It would greatly add to the conclusions drawn from this study to be able to compare our findings with healthy subjects from the same population. Although you are apparently healthy, it will be necessary to conduct a thorough physical examination and performs some simple tests to confirm this.

WHAT IS THE DURATION OF THE STUDY AND WHAT TESTSWILL BE PERFORMED?

If you decide to participate, you will be one of 100 participants of which 80 will be patients and 20 will be healthy controls such as yourself.

The study will be running for approximately 18 months, however, it will take 1 month or less to acquire the necessary information from you. Once you have agreed to participate, you will undergo a thorough physical examination to exclude any chronic diseases as well as a simple urine dipstick. You will also be required to have one blood test to ensure that your kidney function id within normal limits for your age. If all of the above is satisfactory, you be required to wear a device similar to a wrist watch for about 24 hours. This will gather information on your blood pressure and is not painful in any way. Thereafter, we will arrange for you to have a special CT scan of your heart which will allow us to detect the degree of calcium buildup present, if any. year in order to assess for any change over time.

WILL THIS INCONVENIENCE ME IN ANY WAY?

Participation in the study will mean having to attend hospital between 2 and 4 times Every effort will be made to accommodate your needs in terms of timing and an amount of R100 per visit will be given to compensate you for transport costs.

WHAT WILL HAPPEN IF YOU DECIDE NOT TO TAKE PART IN THE STUDY?

Your participation is entirely voluntary and you can refuse to participate or stop at any time without stating a reason. Your withdrawal will not affect your access to future medical care. The investigator

retains the right to withdraw you from the study if it is your best interest or you do not follow the guidelines and regulations of the trial.

WHAT ARE THE RISKS AND DISCOMFORTS OF THIS STUDY?

The only risk incurred in the study is the extra exposure to radiation that you will receive as a result of the CT scan. However, the amount of radiation is small and well within that recommended to be safe by expert authorities. Blood tests may result in a bruise at the puncture site, swelling of the vein, infection, or bleeding. In experienced hands this is highly unlikely and only minor discomfort may occur.

ARE THERE BENEFITS TO YOU FOR BEING IN THIS STUDY?

There are no direct benefits to you other than your contribution to our research. If the amount of calcium is considered to be high in your arteries, you will be referred to your health care provider with the result for further workup and care. The benefits of this study may impact future patient care positively but ultimately, this may not affect you.

WHAT WILL HAPPEN WHEN THE STUDY IS OVER?

With your permission, your results will be shared with the doctor responsible for your care. Any information collected about you will be kept securely and no information will be divulged to any third party without your prior consent or as required by law. No blood samples will be stored

HAS THE STUDY RECEIVED ETHICAL APPROVAL?

The study protocol has been approved by the Research Ethics Committee of the University of Cape Town, and the Medicines Control Council of South Africa.

ARE THERE ANY WARNINGS OR RESTRICTIONS FOR MY ACTIVITY?

No.

INSURANCE AND FINANCIAL ARRANGEMENTS

All the study doctors are covered by insurance. If any trial related injury occurs, UCT has a No Fault Insurance Policy for trial-related injuries.

The policy states:

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I have had sufficient opportunity to ask questions declare myself prepared to participate in the trial.

Patient's Name:(print)

Patient's Signature:..... **Date:**

Study Doctor's Name: (print)

Study Doctor's Signature:**Date:**

Witness Name: (print) (Required only if interpreter used)

Witness Signature:

SCANNED COPY OF DATA CAPTURE FORM

CASE REPORT FORM

The impact of vascular calcification on ambulatory and central aortic blood pressure in a South African dwelling dialysis population

HOSPITAL NUMBER _____ ID NUMBER _____

CONSENT SIGNED _____ CASE No _____

RACE (Circle): B / W / MIXED / ASIAN GENDER: M/F _____

DIALYSIS DAY/MODALITY: _____ MEETS INCLUSION CRITERIA: (Circle) Y / N
 ANY EXCLUSION CRITERIA: (Circle) Y / N
 (see last page for list)

DOB: _____

YRS ON DIALYSIS: PD HD TELEPHONE NUMBERS: 1. _____
 2. _____
 3. _____

SMOKING STATUS: (Circle as appropriate) N / Y / P / Previous / Current IF SO: Pack Years (if applicable): _____

PRIMARY DIAGNOSIS & CAUSE FOR CKD: _____

HOSPITAL STICKER

Known Diabetic: Y / N
 Known Vasc. Dx (Angina/MI/CVA/PVD): Y / N
 Known HPT: Y / N

PRIOR TRANSPLANTATION AND REASON FOR GRAFT FAILURE: Y / N _____

PRIOR PARATHYROIDECTOMY: _____

- MEDICATIONS: (And Dose)
- | | |
|------------------|-----------------------|
| 1. Titrallac | 8. Vasodilator |
| 2. I Alpha D3 | 9. ARVs |
| 3. Ergocaliferol | 10. ESA |
| 4. CCB | 11. Non Ca PO4 binder |
| 5. ACE/ARB | 12. Warfarin |
| 6. Vasopres | 13. Loop/Furosemide |
| 7. Statin | 14. Minoxidil |
| 15. Other | |

PAST CABG-STENT IN CHEST & DATE: yes/no, if yes CABG or STEN _____

HISTORIC LABORATORY RESULTS (LAST 8 RESULTS AS AVAILABLE APPROX 3 MONTHLY)

TEST	RESULT	COOR CALC	PHOSPHORUS	CRP or product	ALK PHOS	PTH (appx/1)	HS	TOTAL CHOL	LDL CHOL	LDL % DL	LDL Acid	LDL CoQ	HS Status	HS Type
Q1 (most distant)														
Q2														
Q3														
Q4														
Q5														
Q6														
Q7														
Q8 (most recent)														

STUDY INVESTIGATIONS

EXAMINATION: PULSE RATE: _____
(5 MIN REST)

BP: ____/____ MAP: _____ RHYTHM REG/IRREG: _____

Height (m): _____ Weight (kg): _____ N-N Distance (cm): _____

CURRENT VASCULAR ACCESS ARM: (Circle) Left / Right / TC / PC / Leg

RADIAL PULSE PALPABLE: (Circle) Left / Right / Both / Neither

RESTING ECG: DATE DONE: _____

PRIMARY READER (NAME): _____

RATE: _____ RHYTHM: _____

SOKOLOW-LYONS: $(SV_1 + RV_5 > 38 \text{ mm} = \text{LVH})$ _____

CORNELL PRODUCT: $([RV_5 + SV_3] \times \text{QRS duration})$ _____

SECOND READER AGREES: (Circle) Y / N NAME: _____
(IF NO, MUST REVIEW TOGETHER AND REACH CONSENSUS)

BPRO: DATE DONE: _____

Exclusions:
Sinus brad arrhythmia/AF
Absent radial pulse

A-PULSE DATA:
SYS PRESSURE: _____
DIASTOLIC: _____
PULSE PRESSURE (Sys - Dias): _____
CASP: _____
MAP: _____
PULSE RATE: _____
AUG PRESSURE: _____
AUG INDEX: _____
PRI (RELATIVE TIME SYS PRESS AND AUG): _____
SUG (SYST UPSTROKE GRADIENT): _____

BPro DATA:
Avg. 24 Hr BP: _____ / _____
Avg. 24 Hr MAP: _____ / _____
Avg. DAY BP: _____ / _____
Avg. NIGHT BP: _____ / _____
% DIPPING: _____ / _____

ECHOCARDIOGRAPHY: DATE DONE: _____

LVID: _____
PWTD: _____
IVST: _____
LV Mass: _____
LV Mass Index (Corrected for BSA): _____

$LV\ mass\ (g) / P_{ann} = (1.04 [(LV\ IDD + PWTD + IVSTD)^2 - (LV\ IDD)^2] - 13.6) / BSA$ (in square m)

PREGNANCY TEST (if female - circle): POS / NEG / Not applicable

CORONARY CT: DATE DONE: _____

Exclusions: **CORONARY CALCIUM SCORE:** _____
Prior CABG/Stent
Can't lie supine **SIGNIFICANT ARTIFACT INTERFERENCE: (Circle) Y / N**

ABDOMINAL RADIOGRAPH:

DATE DONE: _____

PRIMARY READER (NAME): _____

VERTABRA	POSTERIOR SCORE (0-3)	ANTERIOR SCORE (0-3)	TOTAL (A+P)
L1			
L2			
L3			
L4			
TOTAL			

SECOND READER AGREES: (Circle) Y / N NAME: _____
 (IF NO, MUST REVIEW TOGETHER AND REACH CONSENSUS)

Inclusion Criteria

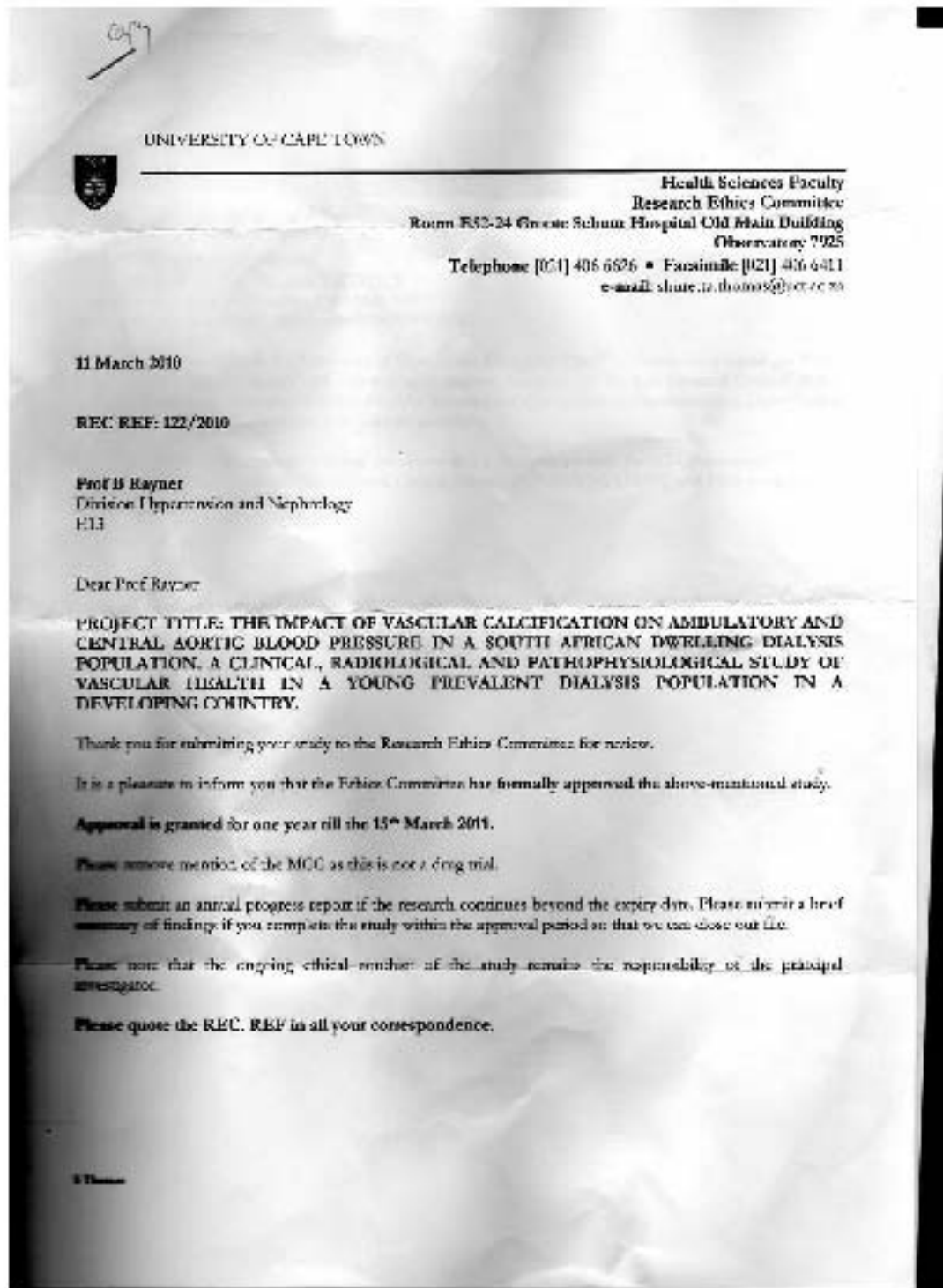
- Adult patients receiving haemo- and/or peritoneal dialysis for a 3 month's duration.
- Ability and willingness of subject or legal guardian/representative to provide informed consent
- Men and women age ≥ 18 years

Exclusion Criteria

- Planned or current pregnancy within the next 6 months due to radiation risks

It should be noted that some conditions will exclude individual subjects from certain of the tests, but these subjects will still be included for analysis of available data. The absence of both radial pulses or sustained arrhythmia precludes BPro analysis. Previous coronary artery bypass or stenting will preclude coronary CT.

UCT ETHICS APPROVAL: INITIAL



UCT ETHICS APPROVAL: ADDITION OF CONTROLS



UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

26 May 2010

REC REF: 122/2010

Prof BL Rayner
Medicine

Dear Prof Rayner

PROJECT TITLE: THE IMPACT OF VASCULAR CLACIFICATION ON AMBULATORY AND CENTRAL AORTIC BLOOD PRESSURE IN A SOUTH AFRICAN DWELLING DIALYSIS POPULATION

Thank you for your letter to the Research Ethics Committee dated 25 May 2010.

It is a pleasure to inform you that the Ethics Committee has approved the amendment (to include healthy controls) to the above study

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC, REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS