Establishing a high risk CKD cohort: Cross-sectional analysis and early outcomes

By

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Abstract

Introduction

Patients with Chronic Kidney Disease (CKD) are at increased risk of both cardiovascular disease and progression to end-stage kidney disease; our understanding of the factors that determine these poor outcomes is incomplete. The study reported in this thesis has been designed to address some of these shortfalls.

Methods

I established a prospective, observational cohort study of patients with high risk CKD as defined by i) declining kidney function and/or ii) proteinuria and/or iii) advanced (stage 4 and 5) CKD. Participants undergo repeated detailed bio-clinical assessment over a follow up period of ten years and are tracked for clinical outcomes. The baseline data are presented in this thesis along with some data form the six-month visit.

Results

I report cross-sectional data from the first 500 participants; mean age is 65 years, 60% were male and 72% white ethnicity. Mean eGFR was 27mL/min/1.73m^2 and median urine ACR was 26.9 mg/mmol. Detailed analyses demonstrated important associations between i) quality of life and unemployment, male gender, deprivation, co-morbidity and inflammation ii) arterial stiffness, inflammation and renal and cardiovascular outcomes iii) periodontitis and arterial stiffness iv) mortality, inflammation and arterial stiffness.

Conclusions

This thesis reports important new findings from patients with CKD and establishes a resource that will provide future insights that should contribute to improving clinical outcomes.

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Abbreviations

AASK; African American Study of Kidney Disease and Hypertension

ACEi; Angiotensin Converting Enzyme Inhibitor

ACR; Albumin Creatinine Ratio

ADHS; Adult dental health survey

ADMA; Asymmetric Dimethylarginine

AER; Albumin Excretion Rate

AGEs; Advanced Glycation End Products

AIx; Augmentation Index

AKI; Acute kidney injury

ALB; Albumin

APKD; Autosomal dominant polycystic kidney disease

Apo-AIV; Apolipoprotein-AIV

ARB: Angiotensin receptor blocker

ARIC; Atherosclerosis Risk In Communities

AU; Arbitrary units

BMI; Body Mass Index

BNP/Pro-NT BNP; Brain Natiuretic Peptide/Pro-N Terminal Brain Natiuretic Peptide

BOP; Bleeding On Probing

BP; Blood Pressure

CAD; coronary artery disease

CAL; Clinical Attachment Loss

CCB; Calcium Channel Blocker

Ccr; Creatinine clearance

cFLC; polyclonal free light chains

CG; Cockcroft-Gault

CKD; Chronic Kidney Disease

COPD; Chronic obstructive pulmonary disease

CRIB; Chronic Renal Impairment In Birmingham

CRIC; Chronic Renal Impairment Cohort

CRISIS; Chronic Renal Insufficiency Standards Implementation Study

CRP; C-reactive protein

Curea; Urea clearance

CV; Cardiovascular

CVD; Cardiovascular Disease

DM; Diabetes mellitus

eGFR; estimated Glomerular Filtration Rate

ESKD; End Stage Kidney Disease

FGF-23; Fibroblast Growth Factor-23

FLC; Free Light Chains

GFR; Glomerular Filtration Rate

GWAS; Genome Wide Association Study

Hb; Haemoglobin

HbA1C; Glycated haemoglobin

Hcy; Homocysteine

HTR; Hip thigh ratio

IHD; Ischaemic heart disease

IL-6; Interleukin-6

IMD; Index of multiple deprivation

KIM-1; Kidney injury molecule-1

LCKD; Longitudinal CKD

LFABP; Liver-type acid-binding protein

MCP1/CCL2; Monocyte Chemo-attractant Protein

MDRD; Modification of diet in Renal Disease

MGUS; Monoclonal Gammopathy of Uncertain Significance

MMKD; Mild to Moderate Kidney Disease

MRI; Magnetic resonance imaging

NAG; N-acetyl-β-o-glucosaminidase

ND; Not dialysed

NGAL; Neutrophil Gelatinase-Associated Lipocalin

NKF/K-DOQI; National Kidney Foundation/Kidney-Disease Outcomes Quality Initiative

PCR; Protein Creatinine Ratio

Pcr; serum creatinine concentration

PD; Periodontal disease

PER; Protein Excretion Rate

PET; Positron emission tomography

PP; pulse pressure

PPD; Probing Pocket Depth

PTH; Parathyroid Hormone

PWA; Pulse Wave Analysis

PWV; Pulse Wave Velocity

QoL; Quality of life

QUALYs; Quality adjusted life years

RCT; Randomised controlled trial

R^2ID; Renal Risk In Derby

RIISC; Renal Impairment In Secondary Care

RRT; renal replacement therapy

SEEK; Study to Evaluate Early Kidney Disease

SES; Socio-Economic Status

SF-36; Short form-36

SNP; Single Nucleotide Polymorphism

SUN; Serum urea nitrogen

SWLS; Satisfaction with life scale

TNFα; Tumour necrosis factor alfa

UKADS; UK Asian Diabetes Study

UUN; Urine urea nitrogen

vWF; Von Willebrand factor

WHR; Waist hip ratio

1. Chapter One: Introduction

1.1.Chronic Kidney Disease: a historical perspective

Historically nephrologists have focused their attention on two groups of patients, those with an acute decline of kidney function (now classified as Acute Kidney Injury (AKI)) and those requiring long-term renal replacement therapy (RRT). Most patients with chronic renal failure (now called chronic kidney disease (CKD)) received no specialist care by nephrologists until they were close to or had reached End Stage Kidney Disease (ESKD) that may require RRT (1).

The exception to this were patients with immune or inflammatory kidney disease (usually glomerulonephritis) as identified by kidney biopsy (2), where there is an intense interest in both the pathogenesis and natural history of these disorders and patients were often treated with immune-modulating drugs. However this group of patients only represent a minority of patients with CKD; the majority of patients with CKD are elderly and have sustained kidney damage as a consequence of vascular disease or one or more of hypertension, macrovascular disease and diabetes. These patients had either not been identified as having CKD and/or remained under the care of general practitioners until they reached ESKD (3-5).

There were a number of reasons for the lack of recognition of CKD as a highly important chronic disease, both for the individual patient and in terms of the organisation of clinical services and the health economic implications of the disorder, these include: (i)

the underlying renal disease was not felt to be amenable to direct treatment; (ii) there was no agreed structure to classify the severity of their renal impairment; and (iii) there was an under-appreciation of the impact of the complications of CKD on the patient. During the past 10-years these shortfalls are being systematically addressed. As a consequence of this there is now evidence developing from the UK and other countries that outcomes for people with CKD are improving (6, 7).

An important requirement for improving the outcomes of people with CKD is a detailed understanding of the natural history of the disease. On a population basis, much valuable information can now be derived from very large cohorts, where known outcomes are linked to routinely collected clinical data (8-10). Such studies informed the classification of CKD and allowed more accurate stratification of risk. However, more detailed information than this is required to elicit the natural history of CKD in subgroups of people with CKD, to better define the relationship between traditional and non-traditional risk factors for CKD, and to identify new targets for treatment for people with CKD. This information can only be provided by carefully characterised, prospectively recruited cohorts of people with CKD.

In this thesis I describe the establishment of a prospective bio-clinical cohort, the Renal Impairment In Secondary Care (RIISC) study. The study recruits patients with CKD from secondary care in Birmingham. Participants undergo six study visits over a ten-year period, at each visit a detailed bio-clinical assessment is carried out, outcomes relating to progression of renal disease, cardiovascular events and deaths are tracked. Participants must have at least one of, albuminuria quantified by a urinary albumin

creatinine ratio (ACR) of ≥70 mg/mmol, progressive stage 3 CKD or stage 4-5 CKD (non-dialysis (ND)). In addition to exclusion of patients who require any form of RRT patients with kidney disease under treatment with immune modulatory agents are also excluded.

In this introductory chapter I provide the context for the RIISC study by reviewing the current status of CKD as it relates to the research chapters that are presented in this thesis. This includes an overview of the other prospective cohorts that have been recruited to date and the relevant data that these cohorts have produced.

1.2.The classification of CKD

One of the major developments in CKD since 2002 has been the introduction of a classification system for CKD. This system was based on the adoption of the Modification of Diet in Renal Disease (MDRD) study formula to estimate the standard measurement of excretory kidney function, the glomerular filtration rate (GFR). The MDRD equation was used by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) to frame a classification system that has become widely adopted in clinical practice (11). The guidelines arose from the observations that to improve the outcomes of patients on dialysis required a focus on the health of patients at risk of ESKD and that it may be possible to slow the rate of progression and reduce the complications of CKD (11).

The formalised reporting of estimated GFR (eGFR) led to better identification of the prevalence of kidney disease, with estimates of CKD in the developed world of up to

16% of the adult population (12, 13). In the UK a recent report that utilised epidemiological data identified the prevalence of all CKD as 14% in men and 13% in women. The prevalence of CKD where there was a demonstrable reduction in excretory kidney function as defined by an eGFR of <60ml/min/1.73m^2 (stage 3-5 CKD) was 6% (14).

More accurate identification of CKD, including the use of a classification system, allows the risk stratification of patients for the purposes of patient counselling and appropriate targeting of interventions that may improve the outcomes of those affected; it also enables comparisons to be made between similar groups of patients for the purposes of research. The need for further clinical research in CKD was recognised by K/DOQI through the framing of the following research questions that were used in the development of the CKD classification system and which they wanted to address when they published the first set of guidelines for the management of patients with CKD (11, 15).

1. How is the disease to be defined? Is it on the basis of pathological features or evidence of impaired function or structure? Is there to be an element of chronicity included in the definition and how is this defined?
2. If impaired function is an important defining characteristic how is it to be measured and how reproducible and reliable are the measures to be used and are they equally reproducible and reliable in all potential patients?
3. Should any additional evidence of disease be included in the classification and if so what?

4. Are the consequences of the disease understood and is there evidence that the natural history can be altered by available interventions?

I will now discuss these questions to describe the basis of their diagnostic and classification system.

1.2.1. How is CKD to be defined?

Patients with CKD are unusual in comparison to patients in other disease groups, they do not share a common underlying diagnosis and as a result the pathophysiology and natural history may differ within the group. However it was appreciated by the authors of the guideline that these patients did have one thing in common, a chronic disease process that resulted in decreased kidney function. They also acknowledged that in different diagnoses the markers of impaired kidney function might differ. Despite these observations once kidney disease is established the features that define CKD apply across disease states and comprise evidence of damaged renal parenchyma as demonstrated by active urinary sediment and/or structural abnormality (this must be present for stages 1 and 2) and/or evidence of decreased kidney function as demonstrated by a reduced GFR and chronicity to distinguish it from AKI (11).

Until the development of the K/DOQI guideline, kidney function was primarily assessed using direct measurement of serum or plasma creatinine, formed from creatinine phosphate metabolism in skeletal muscle, to produce an approximation of kidney function. However serum creatinine levels are variably effected by other factors including muscle mass, dietary protein load, gender and direct renal tubular excretion. As a consequence (i) identical serum creatinine levels represent different levels of

kidney function in different individuals and (ii) the relationship between creatinine levels and kidney function is non-linear.

Whilst not a direct measurement of GFR, creatinine can be utilised in combination with correction factors that are specific for the individual to produce an eGFR. The importance of eGFR in providing a readout of kidney function that is used in routine clinical practice is based on the principle that true GFR is the most accurate assessment that we have available for measuring the excretory function of the kidneys. Glomerular filtration rate is defined as the volume of fluid filtered from the glomerular capillaries in a specified period of time; any substance that is freely filtered at the glomerulus, and is neither secreted nor absorbed by the kidney can be used to measure GFR; in routine clinical practice creatinine is the molecule that is utilised for this purpose. Creatinine is freely filtered at the glomerulus, is not protein bound and is not metabolised by the kidney, however as previously noted both tubular secretion and variable production is dependent upon factors that are unrelated to kidney disease are important confounders (16). Some of these confounders can be corrected for by utilising equations into which they are incorporated to produce a calculated or eGFR.

True or measured GFR can be obtained using inulin clearance (the gold standard method). The inulin clearance method requires intravenous infusion and timed urine collections over a number of hours and is therefore complex, costly and inconvenient. Furthermore inadequate urine collections can cause inaccuracy of the obtained measurement (17). More commonly, for assessment of measured GFR, isotopes such as [^{51}Cr]EDTA and ^{125}I-iothalamate can be used. However, these are also impractical to use

in routine clinical practice, as they are time consuming for the patient, expensive, require a specialised organisational infrastructure (special licensing and regulation of handing and waste disposal of the radioisotope) and exclude certain patient groups (e.g. pregnant women) (18). To overcome these obstacles other exogenous substances have also been used to measure GFR, these include the radiocontrast agents iohexol and iothalamate (19, 20). Both iohexol and iothalamate have been shown to correlate closely with gold standard measures of kidney function (inulin clearance) with excellent reproducibility and minimal renal toxicity (21-23). However there is evidence that measured GFR does not perform better than eGFR when clinically relevant outcomes were studied; in a sub-group of 1214 participants from an observational study a cross-sectional analysis was performed, GFR was estimated using both creatinine and cystatin based equations and measured using iohexol, the authors concluded that measured GFR was not superior to estimated GFR in explaining the complications of CKD (24).

The GFR obtained from tests that provide a measure of GFR produces normal values for men of around 130 ml/min/1.73m2 and for women of around 120 ml/min/1.73m2; with increasing age these 'normal' values decline (25-28). The use of measured GFR is currently restricted to situations where a precise measure of kidney function is required (for example in individuals wishing to be considered as living kidney donors) and eGFR is the current clinical standard in most other clinical scenarios. The current formula used to calculate eGFR in routine clinical practice in the UK is the four variable modification of diet in renal disease (MDRD) equation.

The MDRD equation has replaced the Cockcroft-Gault (CG) (29) formula, which was developed against creatinine clearance, introduced in 1976 and was subsequently used by many clinical services in routine practice. Indeed, the formula continues in use as it was used for the purposes of defining dose adjustments for many drugs which have significant renal clearance. However it is a complex formula, requiring anthropometric data that may not be routinely or readily available and it may provide an overestimation of GFR in some groups (30). Furthermore the CG formula is less accurate than MDRD and can vary from measured GFR by>30% (31, 32).

Figure 1-1: The Cockcroft-Gault formula for the estimation of GFR (29)

$$\left\{ \frac{[(140\text{-Age}) \times \text{weight (in kg)}]}{[72 \times \text{Serum creatinine (in mg/dL)}]} \right\} *$$

*Multiply by 0.85 if female

The MDRD formula was published in 1999 when Levey et al developed formulae to estimate GFR using serum creatinine and other readily available data as a component of a randomised controlled trial designed to assess the effect of protein restriction and blood pressure control upon the progression of CKD (33). 1628 patients with kidney disease were recruited and the study protocol included measurement of GFR with ^{125}I-iothalamate, a 24-hour urine collection and a single measurement of serum creatinine (34, 35). The recruited population were young, predominantly male and 88% were of white ethnicity, the prevalence of diabetes was low and there were no individuals with

normal kidney function (30). A stepwise regression model was utilised to predict GFR utilising training and validation samples. Seven equations were assessed and these are listed in figure 2

Figure 1-2: The equations compared in the MDRD study for the estimation of GFR (30)

Equation 1: GFR = 0.69 x [100/P_{Cr}]

Equation 2: GFR = 0.81 x [Cockcroft-Gault formula]

Equation 3: GFR = 0.81 x [C_{Cr}]

Equation 4: GFR = 1.11 x [(C_{Cr} + C_{urea})/2]

Equation 5: GFR = 1.04 x $[C_{Cr}]^{+0.751}$ x $[C_{urea}]^{+0.226}$ x [1.109 if patient black]

Equation 6: GFR = 198 x $[P_{Cr}]^{-0.858}$ x $[age]^{-0.167}$ x [0.822 if patient is female] x [1.178 if patient is black] x $[SUN]^{-0.293}$ x $[UUN]^{+0.249}$

Equation 7: GFR = 170 x $[P_{Cr}]^{-0.999}$ x $[age]^{-0.176}$ x [0.762 if patient is female] x [1.180 if patient is black] x $[SUN]^{-0.170}$ x $[Alb]^{+0.318}$

Alb, serum albumin; C_{Cr}, creatinine clearance (mL/min/1.73m^2); $C_{urea,}$ urea clearance (mL/min/1.73m^2); P_{Cr}, serum creatinine concentration (mg/dL); SUN, serum urea nitrogen concentration (mg/dL); UUN, urine urea nitrogen concentration (g/d)

The equation that resulted in the maximum R^2 value (91.2%) included urine biochemistry variables (equation 6) but is not useful for clinical practice as it requires 24-hour urine collection. Therefore equation 7 was used to interpret the study as the precision of the equation was close to that of equation 6 (R^2 90.3%) and it included routinely collected clinical data (30). The inclusion of variables associated with creatinine production (age, ethnicity and gender) contributed to the accuracy of the

equation, although the equation was not validated in individuals with normal renal function or the elderly (patients over 70 years of age were not included in the MDRD study), these omissions may limit the utility of the equation as an estimation tool across a CKD population.

The K/DOQI working group abbreviated MDRD equation 7 by removing blood urea nitrogen and serum albumin from the calculation. The resultant abbreviated, "4-variable MDRD" formula although not validated by the MDRD group, performed well compared to ^{125}I- iothalamate in an analysis of 1775 patients recruited to the African American Study of Kidney Disease and Hypertension (AASK) (36). This equation formed the basis for the 2002 classification system (11, 37). The stages of CKD that were created were based upon the eGFR; although patients with stage 1 and stage 2 CKD required additional evidence of kidney damage for classification.

Figure 1-3: The four variable MDRD equation for estimating GFR from serum creatinine (36)

$$eGFR = 32788 \times sCr\ (mmol/L)^{-1.154} \times age^{-0.203} \times [1.212 \text{ if black}] \times [0.742 \text{ if female}]$$

sCR, serum creatinine

In 2009, in an attempt to improve the accuracy of eGFR, the CKD-Epi formula was developed from a pool of ten studies which produced a total of 8254 patients (2/3 of whom were randomly selected for formula development and 1/3 for validation; a further 3896 patients from 16 studies were then used for external validation purposes) (38). The new formula performed better than the MDRD formula, especially at higher

GFRs, and there is also some evidence that it provides better cardiovascular disease (CVD) risk stratification than the MDRD formula (39). In 2013 KDIGO published an updated set of CKD guidelines and while they fall short of suggesting that the CKD-Epi formula should replace the MDRD formula its use is described as "reasonable" (40).

Work is continuing in this area and includes other markers of kidney function. A recent large community based cohort study including individuals aged >65 years (in contrast to the MDRD study), calculated the prevalence of CKD using the MDRD and CKD-Epi equations, with CKD-Epi GFR estimated both by creatinine and by cystatin C. The data obtained suggested a variance in the prevalence of CKD dependant upon the equation used, although the cystatin C based CKD-Epi estimate appeared to be the most specific (41). While this was a large study it was limited by being a cross-sectional analysis and by the absence of a gold standard measure of GFR against which the estimating equations were compared.

Figure 1-4: The CKD-Epi equation for estimating GFR from serum creatinine (38)

$$eGFR = 141 \times \min(sCr/k,1)^{a} \times \max(sCr/k,1)^{-1.209} \times 0.993^{age} \times (1.018 \text{ if female}) \times (1.159 \text{ if black})$$

sCr, serum creatinine; k ,0.7 for women and 0.9 for men; a, -0.329 for women and -0.411 for men

1.2.2. What additional evidence for renal damage is required?

The working group acknowledged that there are markers of kidney damage other than markers of impaired kidney function (reduced GFR), these include structural anomalies of the renal tract (for example polycystic kidneys or posterior urethral valves) and

active urinary sediment (haematuria or proteinuria) (15). Individuals with structural kidney disease are at risk of progression and even in the absence of evidence of reduced GFR should be considered to have CKD. The presence of haematuria or proteinuria may indicate glomerular pathology and the importance of proteinuria as a risk factor for CKD progression and CVD has become increasing recognised with time and will be described later in this chapter. The final definition of CKD by the workgroup relied on (11):

(i) The presence of kidney damage for ≥ 3 months, with kidney damage defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies and/or

(ii) GFR <60 ml/min/1.73m^2 for≥ 3 months with or without kidney damage.

1.2.3. Are the consequences of the disease understood and is there evidence that the natural history can be altered by available interventions?

At the time of development of the guideline there was considerable concern that the numbers of patients with ESKD was increasing, thus placing a significant burden on the provision of health care. Patients with CKD were known to be at risk of progression to ESKD and patients with ESKD were known to be at significant cardiovascular risk (42). In the same year that the guideline was published, the Hoorn study reported that individuals with mild kidney impairment (estimated using serum creatinine, the CG formula and the MDRD formula) were at risk of increased cardiovascular mortality (43).

The following year, in data derived from a community based observational study, Atherosclerosis Risk In Communities (ARIC), where the majority of participants had preserved kidney function; the rate of cardiovascular events over the follow up period

of around six years was higher in individuals with a reduced eGFR than those with a normal eGFR (44). The risk was incrementally higher for people with a lower eGFR and was more pronounced for individuals of African American ethnicity than White ethnicity and was independent of other CVD risk factors (44).

In a large cohort of community dwelling Americans Go et al reinforced this observation and demonstrated an independent, graded increase in risk of CVD, risk of death and hospitalisation as eGFR fell in a study with a follow up period of just under three years (45). This increased risk started with an eGFR below 60mL/min/1.73m^2 and increased with the severity of CKD. While this study did not contain information about other potential CVD risk factors it indicated that individuals with even minor degrees of renal impairment were at significant CVD risk and that a component of this risk was likely to be independent of other comorbidities that are associated with CKD (45).

The reasons for the association between CKD and CVD are only partly understood; although many risk factors for CVD are also causes or complications of CKD (diabetes, hypertension and vascular calcification), not all of this enhanced risk is explained by these traditional risk factors and it has been hypothesised that non-traditional risk factors are implicated in CVD development (46-48). The risk factors for initiation and progression of CVD in patients with CKD are further discussed further in section 2.

Progression of CKD to ESKD occurs in a minority of patients, and there is geographical variation in the incidence of progression (13). Where progression does occur there is an increased risk of CVD in addition to the risk associated with baseline and stable

impairment of kidney function (49). An analysis of the CVD health study showed that patients whose eGFR declined by more than 3mL/min/1.73m^2/year were at increased risk of all cause and cardiovascular mortality (50). In another analysis of the same cohort the association between cardiovascular events and rate of decline of kidney function was examined; the incidence of all types of cardiovascular events was higher in those patients with a more rapid decline of kidney function, this was independent of demographic factors, CVD risk factors and baseline kidney function (49). These observations suggest that the presence and progression of CKD are both important CVD risk factors, the possible mechanisms for this will be discussed in section 2.

The importance of CKD as a CVD risk factor, and the hypothesis that the enhanced CVD risk could be mitigated by early identification of CKD with treatment of known risk factors such as hypertension, dyslipidaemia and diabetes, as well as an increased understanding of the complications of CKD, was a major drive for the development of the K/DOQI CKD classification system and guidelines. At the heart of the classification system was the division of CKD into stages based upon eGFR using the modified MDRD formula. The stages of CKD defined by the group are shown in table 1.

Table 1-1: The stages of CKD as defined by the 2002 KDOQI guideline(11)

Stage	eGFR (mL/min/1.73m^2)	Description
1	≥ 90	Normal or increased GFR, with other evidence of kidney damage
2	60-89	Slight decrease in GFR, with other evidence of kidney damage
3	30-59	Moderate decrease in GFR, with or without other evidence of kidney damage
4	15-29	Severe decrease in GFR, with or without other evidence of kidney damage
5	<15	Established renal failure

GFR, glomerular filtration rate.

1.3.The limitations of the staging system

The CKD staging system represented a significant step forward in the management of patients with CKD; however in the decade since the introduction of the staging system concerns have been raised that it over classifies some individuals. There are two main issues that may contribute to over-classification: (i) those who do not have significant CKD but fall into the classification system as a consequence of a GFR decline as a consequence of the normal aging process; (ii) those with a single eGFR reading below 60mL/min/1.73m^2 due to inaccuracies of eGFR when the serum creatinine is at or close to the normal range. Over-classification may lead to over-inflated estimates of CKD prevalence and has distracted attention from those individuals at highest risk (51, 52).

The use eGFR was not validated in the normal population, and this represents a significant weakness in the CKD classification system; the description of CKD as a single pathological entity where all individuals can be expected to progress from one stage to another at a uniform and predictable rate is another. Whilst the natural history of progressive CKD remains poorly understood, the lack of linearity in progression is well described as is the long-term stability of CKD in many patients who are classified as having the disorder; these differences can in part be explained by the fact that CKD is not a homogenous disease process but a collection of disparate pathological entities that have simply resulted in loss of kidney function over a variable period of time (53, 54). The 2002 CKD staging system overlooked this and invited the clinician to categorise a patient without necessarily considering whether the patient had genuine CKD, was in a static or progressive state, and/or had risk factors that placed them at enhanced

cardiovascular or renal risk (55); in the more recent 2012 KDIGO guideline an emphasis is placed upon progression of CKD and proteinuria (40).

1.4.The risks associated with progressive CKD

It has long been understood that hypertension is both a cause and a consequence of CKD and that as blood pressure (BP) rises the relative risk of development of ESKD increases correspondingly. There is a strong evidence base to show that management of BP to defined targets improves long-term survival and protects against the progression of CKD in people with renal disease (56-58). As the treatment of hypertension has improved, the relative risks of CVD and progressive CKD have fallen, further confirming a patho-physiological relationship (56, 58). The presence of proteinuria in hypertensive patients is also of prognostic significance; these patients are at highest risk of progressive decline in kidney function as well as CVD (59). For this reason the guidelines included recommendations on the treatment of hypertension for patients with CKD and patients with proteinuria (usually measured as albuminuria) have more aggressive BP targets than those without proteinuria (60, 61). The principle that albuminuria is a known risk factor for both progressive CKD and CVD was first explored in patients with diabetes (62-65).

The term proteinuria is usually used to refer to the presence of albuminuria; while not all proteinuria is albuminuria it is important to note that semi-qualitative methods to identify proteinuria such as dip stick testing are most specific for the identification of albuminuria and other proteins may not be detected by this method (66). While much of the risk associated with proteinuria refers to albuminuria, it is important to note that

the urinary excretion of other proteins could be implicated in progressive CKD and CVD risk.

Albumin is the most common protein present in the urine in health and in disease; this is a function of albumin as the most prominent plasma protein, and of the molecular weight of the molecule (which ensures that around 1% of albumin is filtered by the glomerulus in health) (67). Glomerular filtered albumin is almost completely resorbed in health by proximal tubular epithelium. However when there is excess filtration of albumin by the glomerulus and/or a decrease in the re-absorptive capacity of proximal tubular epithelium, albuminuria (pathological levels of albumin in the urine) can develop (68). In addition to being a marker of risk, albuminuria may be directly injurious to intrinsic renal cells and so contribute to the progression of CKD (69), the relationship between albuminuria, GFR and CVD has been recently reviewed by a number of authors (8, 9, 70).

Traditionally albuminuria was quantified using 24 hour urine collections, as the amount of albumin excreted in the urine varied during the day; a major disadvantage of this method was that it was inconvenient for patients and collection was frequently incomplete resulting in inaccuracy. It is now acceptable to quantify albuminuria using spot urine tests, either for ACR or protein creatinine ratio (PCR), the sample does not have to be a first void sample although if one is available this is preferable as it correlates most closely with 24-hour measurements and can exclude orthostatic proteinuria (71). The K/DOQI reference ranges for albuminuria using ACR and the equivalent in both PCR and 24-hour urine results are shown in table 2.

Table 1-2: Interpretation of various methods of measuring proteinuria

	Normal	High	Very high
ACR (mg/mmol)	<3	3-30	>30
PCR (mg/mmol)	<15	15-50	>50
AER (mg/day)	<10	10-300	>300
PER (mg/day)	<50	50-500	>500
Urine diptest	-ve to trace	Trace to 1+	>1+

ACR, Albumin creatinine ratio; PCR, protein creatinine ratio; AER, albumin excretion rate; PER, protein excretion rate

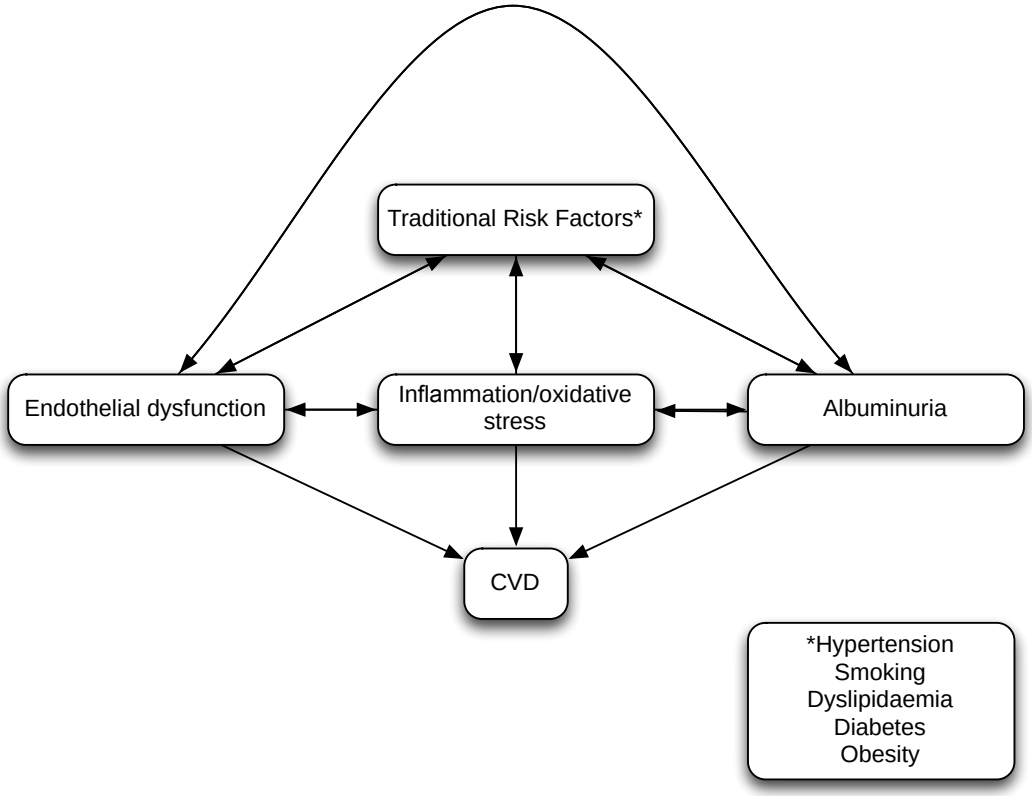
Albuminuria has traditionally been divided into microalbuminuria and overt albuminuria; the term microalbuminuria being first coined in the early 1980s by Viberti and Svendson and was defined as albuminuria that was below the level detectable on urine dip-stick testing but at a level that was predictive of the development of overt nephropathy (72, 73). Subsequently there has been a great deal of research confirming that microalbuminuria (defined as 30-300mg/24 hours) is both a renal and cardiovascular risk factor, however in recent years there has been an understanding that even albuminuria below the microalbuminuria cut off and into the normal range is a risk factor for cardiovascular events and progression to overt nephropathy (74-76). This more sophisticated appreciation of albuminuria as a continuous risk factor has led some to call for an end to the distinction between microalbuminuria and overt albuminuria (77). This has led to the description of albuminuria as normal, high or very high as shown in table 2 (40).

Albuminuria can result from a primary renal pathology (such as intrinsic glomerular disease), from any pathology that affects the glomeruli (such as diabetic glomerulosclerosis) or as a result of hyperfiltration where discrete nephron loss leads to compensatory hyperfiltration of surrounding nephrons, these hyperfiltrating nephrons contribute to an increase in urinary albumin secretion. Albuminuria can be considered not only as contributing to the risk of progressive CKD and as an early marker of kidney damage but also as a CVD risk factor (78, 79).

1.5.Albuminuria and cardiovascular disease

The pathophysiology of CVD in patients with CKD is complex as so many of the risk factors are inter-related, albuminuria is an excellent example of this, figure 5 illustrates some of these inter-relationships associated with albuminuria.

Figure 1-5: The interaction between albuminuria and cardiovascular risk



1.5.1. Endothelial dysfunction and albuminuria

The central pathological link between albuminuria and CVD is proposed to be endothelial dysfunction. The endothelium is a single layer of cells that line the vessel wall, and becomes activated in response to shear stress related injury, resulting in reduced vessel dilation and increased adhesion of leucocytes and platelets (80). A consequence of repeated activation of the endothelium is endothelial dysfunction resulting in the disruption of the homeostatic function of the endothelium (81). Subsequently, markers of inflammation are found in tandem with lipid accumulation in vessel walls (82). The location of the endothelium has traditionally made measurement of its function complex and invasive, however a number of recent advances have resulted in greater ease and accuracy of such measures, the most frequently used methods are summarised in table 3.

Table 1-3: Methods of measuring endothelial function

Technique	Description	Shortcomings
Direct coronary measurement (83)	Coronary angiography with pharmacological stimuli to assess vasodilation	Invasive
MRI/PET coronary imaging (84, 85)	Allows quantification of myocardial function and microvascular function, PET is the method of choice for myocardial blood flow assessment	Expensive
Venous occlusion plethysmography (86)	Measurement of muscular blood flow by assessment of tissue volume change induced by the inflation of a cuff proximally	Invasive
Flow mediated dilation (87)	Vessels are imaged after induced hyperaemia and diameter measured before and after removal of hyperaemia	Significant inter and intra operator variability
Pulse wave analysis (88)	Non-invasive arterial waveform imaging to measure the augmentation index (the difference between the 1st and 2nd systolic peak)	Little data available yet on relationship with treatment and clinical outcomes
Peripheral artery tonometry (89)	After induced hyperaemia the digital pulse wave amplitude is measured	Yet to be validated in large cohorts
Plasma concentration of Von-Willebrand factor (vWF) (90, 91)	vWF has been shown in in-vitro and in-vivo studies to be released from the endothelium in response to damage	Limited information that vWF is causative in the process of endothelial dysfunction, also known to be an acute phase reactant

MRI, magnetic resonance imaging; PET, positron emission tomography

Albuminuria was first proposed as a marker of endothelial dysfunction by Stehouwer et al in 1992 in a group of patients with non-insulin dependent diabetes, at baseline 1/3 of the patients had microalbuminuria; after a follow up period of between 9 and 53 months. Both the baseline level and the change of endothelial function (measured using vWF) predicted development of albuminuria (92). Albuminuria predicted cardiovascular events only in patients where vWF levels were above the median at baseline, these data suggesting for the first time that albuminuria and endothelial dysfunction may be linked in the pathogenesis of CVD (92).

There have been a number of subsequent studies investigating the relationship between proteinuria and endothelial dysfunction, in a cohort of 328 patients with type 2 diabetics followed up for a mean of 9 years a series of serum markers of inflammation and endothelial function and a 24-hour urine collection for protein excretion measurement were carried out (93). Individuals with higher urinary albumin excretion, increased markers of inflammation and increased markers of endothelial dysfunction were at increased risk of death; these associations were independent of traditional risk factors and also of each other (93). The authors also reported that the presence of traditional risk factors was correlated with markers of inflammation and endothelial dysfunction; these findings suggested that individuals with type 2 diabetes are at enhanced cardiovascular risk in part due to endothelial dysfunction, inflammation and albuminuria and that these risk factors are independent of one another (93).

In a population based cohort of 645 patients with and without diabetes the relationship between microalbuminuria (based on a single ACR) and endothelial function (using flow

mediated dilatation) were explored (94). The authors reported that individuals with microalbuminuria had decreased flow mediated dilatation regardless of whether they had diabetes or not, they hypothesised that impaired nitric oxide synthesis might be responsible for this finding (94). The cardiovascular outcomes of these individuals were not reported.

The relationship between kidney function, albuminuria and mortality was explored in the HUNT II study, a community based health study with prolonged follow up (95). Participants were asked to provide three urine samples in which the ACR was measured; they also underwent estimation of GFR (by MDRD) and collection of data pertaining to medical history, anthropomorphics and blood pressure (95). Cardiovascular events and deaths were tracked and found to be strongly correlated with both impaired kidney function and increased urinary albumin excretion, the risk was additive suggesting that when both albuminuria and impaired kidney function are used for risk stratification more accurate estimates are achieved (95).

In another community based study Astor et al reported data from the NHANES III cohort, urine samples were obtained for ACR measurement and GFR was estimated using the MDRD formula, data pertaining to cardiovascular risk factors were also collected (96). Lower eGFR was associated with increased cardiovascular events and all cause mortality, when eGFR and albuminuria were considered together the association with all cause mortality and cardiovascular events was significant (96).

1.5.2. Albuminuria and progressive kidney disease

In 1976 Kussman et al observed that the number of patients with diabetes who were reaching ESKD was rising and the natural history of kidney disease in these patients was not clear; to investigate this a retrospective analysis of juvenile patients with diabetes reaching ESKD was performed (97). They reported that at the time of identification of proteinuria there were few complications of diabetes present but as time progressed the amount of proteinuria increased and kidney function fell in tandem with the development of multiple diabetic complications, they concluded that proteinuria is an early marker of diabetic nephropathy but was unlikely to be treatable by any means other than renal replacement therapy when the need arose (97).

The relationship between proteinuria and progressive CKD was described in 1984 by Mogensen et al in a cohort of patients with type 2 diabetes, urinary albumin excretion was measured and a comparison was made between the outcomes of normal controls, patients with diabetes and albumin excretion of 30 -140μg/mL and patients with diabetes and "heavy proteinuria" (>140μg/mL urinary albumin excretion) after 9 years of follow-up (98). The group with 30-140μg/mL albuminuria were more likely to have progressed to clinically detectable proteinuria than the normal controls and were also at increased risk of death, the group with baseline overt proteinuria had the poorest outcomes (98).

When Verhave et al reported data in 2004 on the association between microalbuminuria and CKD risk in non-diabetic subjects they were the first to do so (99). The PREVEND study was drawn from the general population of Groningen in the

Netherlands, 6022 individuals underwent estimation of GFR (using both the Cockcroft-Gault and the MDRD formulae) and measurement of urinary albumin excretion (using the mean of two 24 hour urine collections) at baseline and after four years (99). At the follow up visit just over 4% of the cohort had a new finding of an eGFR<60mL/min/1.73m^2 and higher baseline urinary albumin excretion was independently predictive of the development of impaired eGFR (99).

In another large community study, carried out in Alberta Canada, nearly 1 million adults underwent estimation of GFR (using the MDRD equation) and urinary albumin excretion (using a single spot sample for ACR or urine dip stick testing with mild proteinuria being defined as trace or 1+ and heavy proteinuria being defined as 2+), all baseline measures were repeated over a six month run in period (100). Adjusted all cause mortality was higher in those with a lower eGFR or proteinuria, as was progression to ESKD or doubling of serum creatinine (100).

As albuminuria was shown to be an independent predictor of both cardiovascular events and progression of CKD in patients with and without diabetes, the K/DOQI classification system was amended in 2004 to include the suffix “p” to denote the presence of proteinuria (101). Despite this, there remained calls for further improvement of the classification system, the reasons for this were summarised in an editorial by Poggio et al and essentially focus on both the inaccuracy of estimation of GFR (especially in certain demographic groups) and the lack of focus on albuminuria as a risk factor for adverse renal and cardiovascular outcomes (102).

In response to this, in 2009, the CKD classification system was further updated to place more of an emphasis on risk of progression and the significance of proteinuria as a renal cardiovascular risk factor; the updated classification is shown in table 4.

The natural conclusion of the K/DOQI classification system and the increased understanding of the cardiovascular implications of CKD was the development of best practice guidelines for the management of patients with CKD. These included recommendations on blood pressure targets and which anti-hypertensive agents should be used, and a focus on the monitoring and management of secondary complications of CKD (61, 103, 104).

Table 1-4: The updated CKD classification system (105)

Composite Ranking for Relative Risks by GFR and Albuminuria (KDIGO 2009)				Albuminuria stages, description, and range (mg/g)				
				A1		A2	A3	
				Optimal to high-normal		High	Very high to nephrotic	
				< 10	10-29	30-299	300-2000	> 2000
GFR stages, description, and range (mL/min/1.73m²)	G1	Optimal	> 105					
			90-104					
	G2	Low-normal	75-89					
			60-74					
	G3a	Mild-moderate	45-59					
	G3b	Moderate-severe	30-44					
	G4	Severe	15-29					
	G5	Kidney failure	< 15					

The heat map in table 4 (reproduced with permission) illustrates how patients can be risk stratified, with those in the red boxes being at highest risk based on eGFR and

albuminuria. In 2011 the CKD consortium published data showing associations between eGFR, albuminuria and all-cause and cardiovascular mortality (9) and eGFR, albuminuria and adverse renal outcomes (8). As a consequence this new classification is an integral part of the 2012 KDIGO guidelines.

However albuminuria is not the only non-traditional risk factor for CKD progression and cardiovascular disease, other proposed biomarkers of CKD progression will be discussed in section 9. In understanding the clinical relevance of any CVD risk factor an understanding of the clinical and surrogate end-points that are used in CKD studies is required.

1.6.The end-points utilised in CKD studies

1.6.1. Clinical end-points

A clinical end point is a definitive physical event that a patient has reached; this may be a cardiovascular event, progression to ESKD and requiring RRT or death. While a clinical end point provides certainty that the pathological process in question has occurred and the use of categorical outcomes makes analysis less complex, patients may take many years to reach an end point and this can make associations difficult to assess and studies of intervention very expensive. It can also be argued that by the time an end point has occurred any opportunity to reverse the pathological process has been missed. Another disadvantage of a clinical end point is that patients who do not meet them (either because their disease has progressed slowly or they have died before they were able to reach them) are not considered to have “progressed”. However this might

not be a genuine reflection of their risk; the use of composite end points that include death goes some way to overcome this.

1.6.2. Surrogate end-points

For the reasons listed above surrogate end points are often used; a surrogate end point is one where a stage that is intermediate to the end point is identified and measured, this might include a change in kidney function (106) or the development of albuminuria. Biomarkers such as creatinine (used in estimating equations) and albuminuria are often used as surrogate end points, with a biomarker described as a substance measured in a biological sample that is related to a pathological process, although other physiological markers can also be described as biomarkers; for example, fever is a biomarker of infection. Finding a marker that is genuinely an intermediate step in the process, that is unaffected by any other process and that is reliably and reproducibly measureable is a challenge and may explain why surrogate end points are not as robust as clinical end points. Despite this the use of biomarkers can enable relatively rapid assessment of efficacy of a certain diagnostic technique or intervention.

In studies of renal interventions or of novel biomarkers both clinical and surrogate end points have been utilised, these can be broadly divided into end points related to markers or measures of renal function or markers related to proteinuria.

Kidney function based clinical and surrogate end points

There are both clinical and surrogate end points that are based on changes in kidney function, the most commonly used clinical end point being progression to ESKD and commencement of RRT. Surrogate end points include rate of change of kidney function

through slope based analyses; the utility of this approach is limited by the fact that kidney function rarely declines in a linear fashion (106).

Another surrogate end point is the absolute change in kidney function between two time points, a doubling of serum creatinine or halving of eGFR is often utilised. Whilst the pattern of decline of kidney function is not a limitation, the variability of the most commonly used marker of kidney function (a creatinine based eGFR) means that a difference in eGFR on two occasions may not represent a genuine change in GFR between those time points (35). The variability in creatinine based estimates of GFR may not simply be related to factors associated with creatinine production but may be a feature of the intra-test variability of creatinine; in the MDRD study this was quoted as 9.4% (35). In effect a surrogate marker of kidney function is being used to define a surrogate end point.

Albuminuria based surrogate end points

While albuminuria is known to be of pathophysiological relevance to the progression of CKD it is not a proven intermediate step in the path to ESKD, as not all patients with significant albuminuria will progress to ESKD (107). Using albuminuria as a surrogate end point has been popular in interventional studies of patients with diabetes where the disease process is known to involve the development of albuminuria. While there is a strong and significant association between albuminuria and progressive CKD it cannot be definitively stated that the effect of a certain intervention on albuminuria is the same as its effect on renal progression (106). Another potential limitation is the intra-test variability of measures of albuminuria (using the ACR) which can be as high as 60% (108).

Table 5 summarises some major randomised controlled trials of interventions to reduce the rate of renal progression and the various renal end points used.

Table 1-5: RCTs of renal progression and end points used

Study	Hypothesis/aim	Renal end point(s) used
HOPE (109)	Ramipril reduces cardiovascular and microvascular complications of diabetes	Development of "overt nephropathy" – total urinary albumin >300mg in a 24 hour urine sample or ACR >36mg/mmol
BENEDICT (110)	ACEi and CCB alone and in-combination can reduce microalbuminuria	Time to transition from urinary albumin < 20μg/mL to > 20μg/mL in two of three consecutive overnight urine samples
MARVAL (111)	Valsartan reduces microalbuminuria independent of BP lowering	Percentage change in urinary ACR
Irbesartan Diabetic nephropathy trial (112)	Irbesartan slows progression of diabetic nephropathy compared to a CCB alone	Composite of doubling of serum creatinine, the development of ESRD or all cause mortality
DCCT (113)	That intensive control of diabetes reduces the development and progression of long-term complications	The development of microalbuminuria, albuminuria
MDRD (114)	That dietary protein restriction reduces the rate of renal progression	Rate of GFR decline (iothalamate clearance) by analysis of slope of GFR
AASK (115)	To compare BP targets on the effect of renal progression	Rate of change of eGFR and a composite of 50% reduction of eGFR, ESRD or death
Breyer (116)	That Captopril would reduce rate of renal progression	Primary endpoint: doubling serum creatinine, secondary end points: ESKD, death
REIN (117)	That Ramipril is renoprotective in patients without proteinuria	Rate of change of GFR, time to ESKD

Abbreviations; ACEi, angiotensin inhibitor; CCB, calcium channel blocker

The purpose of the studies in table 5 has been to identify interventions that could reduce the rate of progression of CKD, however the natural history of CKD remains poorly understood and the determinants of progression are not completely understood. To address this a number of cohort studies have been developed to better describe the determinants of progression of kidney disease and cardiovascular risk in patients with CKD.

1.7.CKD cohort studies

The existing observational CKD cohorts can be broadly divided into those where the aim is to better describe the natural history of CKD in certain specific groups (for example low risk primary care or high secondary care settings) and those where the role of specific biomarkers in the progression of CKD is studied. There is often overlap between these groups. A number of the natural history cohorts were established at the time of the K/DOQI classification with the purpose of directing future clinical guidance or classification systems. I have selected prospective observational studies (with at least a two year follow up period and at least 250 participants, CKD cohorts that fulfilled these criteria are summarised in table 6.

Table 1-6: Observational CKD cohorts

Cohort	Population	Aims	Year recruitment commenced	Number recruited
Chronic Renal Impairment in Birmingham (CRIB) (118)	CKD with Creatinine >1.47mg/dL (130mmol/L) pre-dialysis	To explore the relationship between kidney fucntion and CVD	1997	369
Mild to Moderate Kidney Disease study(MMKD) (119)	Patients who had attended secondary care nephrology clinics at least twice	To investigate risk factors and risk markers for progressive CKD	1997	277
Longitudinal Chronic Kidney Disease Study(LCKD) (120)	Secondary care, GFR<50mL/min/1.73m^2 on two occaisions	To understand the course of CKD and the determinants of patient outcomes	2000	820
Chronic Renal Insufficiency Standards Implementation Study (CRISIS) (121)	Secondary care stage 3-5 CKD (pre-dialysis)	To investigate the determinants of patient coutcomes	2002	1325
Chronic Renal Insufficiency Cohort (CRIC) (122, 123)	Secondary care, all CKD stages	To identify risk factors for the progression of CKD and the development of CVD	2003	3612
Study for the evaluation of early kidney disease (SEEK) (124)	Predominantly primary care (29% from secondary care), inclusion based upon single eGFR ≤60mL/min/1.73m^2	To identify risk factors for CVD in CKD	2004	1814
Chronic Kidney Disease Japan Cohort (CKD JAC) (125)	Japanese (or Asian patients living in Japan) adults with eGFR 10-59mL/min/1.73m^2	Identify risk factors for progression of CKD in Japanese subjects, identify the risks for CVD in CKD, to assess	2007	2977

		the impact of CKD on quality of life		
Renal Risk In Derby (R^2ID) (126)	Primary care, eGFR 30-59mL/min/1.73m^2 on more than two occaisions three months apart	To determine renal and cardiovascular risk factors in stage 3 CKD	2008	1800
German CKD study (GCKD) (127)	Secondary care, eGFR 30-60mL/min/1.73m^2 or significant proteinuria (UAE >300mg/g) with a eGFR>60mL/min/1.73m^2	To identify and validate risk factors for progression of CKD, and developement of CVD, to determine gender based differences in risk, to assess impact of CKD on quality of life	2010	4914

Despite the existence of these cohort studies, many of which have recruited large numbers of patients, our knowledge of the natural history of progressive CKD remains incomplete, to explore how established cohorts are contributing to the evidence base in this area each of the cohorts outlined in table 6 will now be described in detail.

1.7.1. Chronic Renal impairment In Birmingham (CRIB)

The CRIB study was one of the earliest cohort studies and pre-dates the K/DOQI staging system, its primary aim was to establish why patients with chronic renal impairment (the term CKD had yet to be coined) were at enhanced cardiovascular risk. To achieve this, patients were recruited from a teaching hospital nephrology department in Birmingham. The cohort comprised patients with a serum creatinine >130μmol/L and two age and sex matched control groups (one comprised of healthy individuals and one of patients with angiographically proven coronary artery disease). At recruitment participants underwent detailed questioning about medical history and medications, their height, weight and blood pressure was recorded and a 12 lead ECG was obtained (118). Blood was collected for measurement of cholesterol, lipoproteins, troponin, β natiuretic peptide and homocysteine (128). The follow up period was a mean of 6 years but no further clinical assessment of the participants took place during that period, the outcomes reported were ESKD and all cause mortality (128). The strengths of this cohort were the wide inclusion criteria and prolonged follow up period; the cohort was limited by the content of the patient assessment and the fact that it was not repeated during the follow up period, and the relatively small number of patients recruited.

1.7.2. Mild to Moderate Kidney Disease Study (MMKD)

In the MMKD study patients with a variety of causes of kidney disease were recruited from renal clinics in Austria and Germany with the purpose of identifying risk factors associated with the progression of CKD. Participants required stable but impaired kidney function for

three months prior to recruitment and individuals with a serum creatinine > 6mg/dL (528μmol/L) were excluded (119). At recruitment participants had GFR measured using iohexol, blood and urine were collected for biomarker analysis and data were collected on past medical history. Participants were then followed up for a median of 53 months without any further clinical data collection (129, 130). The outcomes studied were doubling of serum creatinine and/or progression to ESKD (131). The strengths of this cohort were that data were largely collected by a single operator, the follow up period was long and measured GFR was available through the inclusion of iohexol GFR measurement, however the clinical assessment was limited and carried out at baseline only and the inclusion of patients deemed to be "stable" at recruitment might have influenced the number who later progressed.

1.7.3. Longitudinal Chronic kidney Disease Study (LCKD)

The LCKD study was set up in the United States with the aim of prospectively following up a group of patients with CKD to gain an understanding of both the natural history of CKD and the determinants of patient outcomes (120). Patients with a GFR <50mL/min/1.73m^2 on two separate occasions (using the MDRD formula) and who were not on dialysis were eligible to participate, the baseline assessment included data on past medical history and medication use, quality of life assessment and blood and urine collection for biomarker analysis; a sub group of participants also underwent cardiovascular assessment which included echocardiography, flow mediated vasodilation, pulse wave velocity, 24hr heart rate monitoring and spiral CT (these investigations were done on two occaisions one year apart) (120).

The follow up period was planned to be at least four years (final data are yet to be published so the actual follow up period is unknown) and the outcomes studied were rate of change of GFR (defined as the absolute change in GFR from baseline to end, the change in GFR per

month and the slope of GFR change), progression of CVD, deaths and arrival at ESKD (120). The strengths of this cohort include the large numbers, the detailed baseline assessment (especially in the sub-group who underwent detailed cardiac assessment) and the repeated data collection during the follow up period, the inclusion criteria are potential limiting factors as the range of CKD included was extremely broad and the rate of progression could be expected to be low for the group as a whole.

1.7.4. Chronic Renal Insufficiency Implementation Study (CRISIS)

The CRISIS study commenced recruitment in Manchester in 2002; its aims were to identify the factors associated with vascular stiffness in patients with stage 3 -5 CKD (defined using the MDRD formula and K/DOQI classification) and to prospectively track patient outcomes (121). At recruitment participants underwent medical history questioning including medication history, and blood and urine collection for biomarker analysis, they also undergo measures of vascular stiffness (pulse wave velovicity and augmentation index) (121). The strengths of this cohort are that it includes a well defined cohort of patients who undergo a detailed assessment, including vascular measures, it is not yet clear what the follow up period will be for the cohort.

1.7.5. Chronic Renal Insufficiency Cohort Study (CRIC)

The CRIC study commenced recruitment in 2003 with the aim of improving the understanding of the role of both traditional and non-traditional risk markers for CKD progression and CVD and also understanding how the complications of CKD influence health service provision (123). The criteria for recruitment comprised patients were under the care of renal services with MDRD derived eGFRs that were age stratified: 20mL/min/1.73m^2 lower limit for all ages; 70mL/min/1.73m^2 the upper limit for those<44 years old, 60mL/min/1.73m^2 upper limit for 45-64 year olds; 50mL/min/1.73m^2 upper limit for those aged 65-74, half of the

participants were diabetics (123). Participants underwent a detailed assessment which included medical history, medication history, anthropomorphic measures, blood pressure, assessment of peripheral vascular health (ankle-brachial pressure index), 12 lead ECG and echocardiography (in one-third measurement of coronary artery calcification using spiral CT) and quality of life (QoL) assessment; one third of the cohort also had GFR measurement using ^{125}I-iothalamate (123). Participants were followed up six monthly and blood and urine samples were collected annually, the cardiac assessments were performed at year one and four; outcome data were collected regarding progression of CKD (absolute change in GFR, arrival at ESKD and halving of GFR), subclinical cardiac disease, overt cardiac disease and death (123).

CRIC was a well-designed cohort study with detailed assessment (including the sub groups with detailed cardiovascular assessment and measured GFR); repeated assessments, prolonged follow up and very large numbers are also strengths. The low levels of proteinuria observed in participants may reduce the applicability of results obtained and the risk of CVD in the cohort (122).

1.7.6. Study to Evaluate Early Kidney Disease (SEEK)

The SEEK study commenced recruitment in 2004 with the aim of identifying the prevalence of renal bone mineral disorders in patients with CKD, participants had to have an eGFR (based on the MDRD formula) <60mL/min/1.73m^2 (124). Baseline data were collected regarding medical history, medication history and blood and urine samples were collected (124). The main strength of this cohort is the large number of recruited patients, however it is limited by the content of the clinical assessment, the broad inclusion criteria and the lack of repeated clinical assessment.

1.7.7. Chronic Kidney Disease Japan cohort (CKD-JAC)

The CKD-JAC study commenced recruitment in 2007 and aimed to recruit 3000 individuals with an eGFR (estimated using a modified MDRD equation validated in Japanese subjects) of 10-59mL/min/1.73m^2; the aims of the study are to identify the risk factors for both progression of CKD and development of CVD in a Japanese population (125). It is not clear whether the population is drawn from primary or secondary care or a combination, at recruitment participants undergo an assessment which included demographics, past medical history, family history, medication history, anthropomorphic assessment, measurement of BP, heart rate and ankle-brachial pressure index and assessment of quality of life (QoL) using the kidney disease quality of life (KDQoL) questionnaire (125).

Participants are followed up until death or withdrawal and the same data are collected at 2 year intervals and an annual ECG and echo are performed; the outcomes of interest include progression of CKD (defined as "reduction of eGFR"), CVD events, all-cause mortality and impairment of QoL (125). A strength of this cohort is the prolonged follow up, repeated assessment and large size, a weakness the problem of reproducibility in a western population.

1.7.8. Renal Risk in Derby study (R^{2}ID)

The R^{2}ID study commenced recruitment in 2008; it is a primary care cohort of patients with CKD stage 3 which aims to examine the renal and cardiovascular risks associated with CKD stage 3 (4, 132). At recruitment data were collected on medical history, medication history, diet, anthropomorphic measurements, blood pressure (BP), pulse wave velocity (PWV), measurement of skin advanced glycation end products (AGEs) and blood and urine collection (132). Follow up is on going and the outcomes studied include rate of progression of CKD and cardiovascular events and deaths. The strength of the cohort lies in its targeted focus on

patients with perceived lower risk CKD in primary care (such patients forming the majority of patients with CKD); the detailed and repeated assessment and the prolonged follow up.

1.7.9. The German Chronic Kidney Disease (GCKD) study

The GCKD study was designed to identify risk factors for progression of CKD and identification of CVD risk in CKD in the German CKD population, there is also a focus on QoL which is assessed using the KDQoL; eligible participants have stage 3 CKD (or stage 1 or 2 in the presence of significant proteinuria), are under secondary care follow up and are Caucasian (127). At baseline participants under an assessment which includes included demographics, past medical history, family history, medication history, anthropomorphic assessment, measurement of BP, heart rate, a single lead ECG and assessment of QoL; the assessment is repeated at two year intervals until death, withdrawal or study completion (ten years) (127). The strength of this cohort may lie in the large numbers of patients recruited and repeated assessment, a weakness in the low risk population recruited and limited bio-clinical assessment. To date no data have been published from this cohort.

1.8.Biomarkers in CKD

While most of these cohort studies have involved the collection of biological samples for the biomarker analysis there have been a number of other studies where the aim was to investigate the role of biomarkers in CKD progression. In addition to the known traditional risk factors found in patients with CKD it is now appreciated that much of the risk that patients with CKD are exposed to (both renal and cardiovascular) cannot be accounted for by these (48). The complex relationship between traditional and non-traditional risk factors and renal and cardiovascular risk is illustrated in figure 5. There are numerous biomarkers that are proposed as of potential clinical relevance in CKD, some as markers of the presence of CKD, some as risk markers of progression of CKD and some as indicators of cardiovascular

risk in patients with CKD. Table 7 summarises the most frequently investigated biomarkers. In order to merit inclusion the biomarker in questions had to have been investigated in at least one good quality study (usually observational) with at least 100 participants. Where I have indicated that there is evidence this was demonstrated in more than one such study, equivocal indicates that there were contradictory findings from studies and no indicates that there was no evidence that the biomarker was related to the outcome of interest.

The characteristics of a good biomarker are summarised in figure 6, not all of the biomarkers described meet these criteria.

Figure 1-6: The characteristics of a clinically useful biomarker (133, 134)

- Reliability –acceptable levels of sensitivity and specificity
- Reproducibility – acceptable levels of inter and intra patient variability
- Physiologically plausible
- Availability
- Not prohibitively expensive
- Practicality – collected from a biological specimen that is easy to obtain and acceptable to the patient resulting in a sample that is easy to handle

Table 1-7: Putative CKD biomarkers

Pathophysiological process	Biomarker	Evidence for presence of CKD	Evidence for progression of CKD	Evidence for CVD risk
Glomerular filtration	Albumin (135)	Yes	Yes	Yes
	Creatinine	Yes	No	Yes
	Cystatin C (136, 137)	Yes	No	Yes
	B trace protein (138, 139)	Yes	No	Yes
	Podocin (140)	Yes	Equivocal	No
	Nephrin (140)	Yes	Yes	No
	FLCs (141)	Yes	Equivocal	Equivocal
Tubulo-interstitial damage	NGAL (142)	Yes	Yes	Yes
	KIM-1 (143)	Yes	Yes	No
	NAG (144, 145)	No	No	Yes
	L-FABP (140, 146)	Yes	Yes	Yes
Endothelial dysfunction	ADMA (147, 148)	Yes	Yes	Yes
	Oxidised LDL	No	No	Yes
	AGEs (126, 149)	Yes	Yes	Yes
	Uric acid (150)	Yes	No	Yes
Inflammation	CRP/hsCRP(151, 152)	Equivocal	Equivocal	Yes
	Pentraxin 3(153)	Yes	No	Yes
	IL-6(154)	Yes	No	No
	TNFα (154)	Yes	Yes	No
	Hcy (155)	Yes	Yes	No
	MCP1/CCL2 (156)	Equivocal	Equivocal	Equivocal
Cardiac biomarkers	BNP (157)	No	Yes	Yes
	NT proBNP (157)	Yes	Yes	Yes
	Troponin (158)	Yes	Yes	Yes
Metabolic dysfunction	Adiponectin (159, 160)	Yes	Yes	Yes
	FGF23 (161)	Yes	Yes	Yes
	Phosphate (162)	Yes	Yes	Yes
	ApoA-IV(163)	Yes	Yes	Yes
	Bicarbonate (164, 165)	Yes	Equivocal	No
Markers of vascular dysfunction	PWV (166, 167)	Yes	Yes	Yes

FLC; free light chains, NGAL; neutrophil gelatinase-associated lipocalin, KIM-1; Kidney injury molecule 1, NAG; N-acetyl-β-O-glucosaminidase, L-FABP; liver-type fatty acid binding protein, ADMA; asymmetric dimethylarginine, AGEs; advanced gylcation end products, CRP; C-reactive protein, IL 18; interleukin 18, Hcy; homocysteine, CCP1/CCL2; monocyte chemoattractant protein/chemokine ligand 2, ANP; atrial natiuretic peptide, BNP; brain natiuretic peptide, NT-proBNP; N-terminal brain natiuretic peptide, FGF 23; fibroblast growth factor 23, Apo A-IV; Apolipoprotein A-IV

1.9.The components of the RIISC bio-clinical assessment that are presented in this thesis

1.9.1. Biomarkers

Albumin

The evidence base for the role of albuminuria in progressive CKD has already been described in section 1.5.2 (8-10).

Cystatin-C

Cystatin C is a low molecular weight protein which is freely filtered at the glomerulus (168), it is considered a useful marker of glomerular filtration because it can detect small deteriorations in kidney function that are not detectable by creatinine (169). Cystatin C was used by the MMKD study group in comparison with measured GFR and creatinine based estimates of GFR and was found to provide reliable risk prediction for the progression of CKD (137). In the REGARDS cohort of 26 643 adults cystatin C and creatinine based estimates of GFR were used to categorise patients into groups, after a median follow up of 4.6 years the highest risk of progression to ESKD was in those classified as having CKD by all markers, the second highest group was those not identified by creatinine based estimates but identified by both ACR and cystatin C (137).

In an analysis of the MDRD cohort measured GFR was compared to creatinine and cystatin C as risk factors for cardiovascular mortality and progression to ESKD, after a median follow up of 10 years cystatin C was more strongly associated with both all cause and cardiovascular mortality (136). In a review of the role of cystatin C in the assessment of cardiovascular risk the authors concluded that an elevated cystatin C was a useful marker of increased

cardiovascular risk as an indicator of "pre-clinical" CKD associated with adverse outcomes (possible via a mechanism of involvement in the atherosclerotic process) (170).

Free Light Chains (FLCs)

Immunoglobulin FLCs are bi-products of immunoglobulin synthesis, they undergo clearance by the kidneys (though there is some reticulo-endothelial clearance) and consequently elevated polyclonal FLCs have been found in patients with renal impairment (171, 172). Because of the molecular weight of FLCs (22.5kDa in monomeric form and 45kDa when in dimeric form as frequently occurs) it was hypothesised by Hutchison et al that FLCs might be an early marker of diabetic nephropathy, preceding microalbuminuria (141). In the prospective UK Asian Diabetes Study (UKADS) three groups were compared; a control non-diabetic group, a South Asian diabetic group and a Caucasian diabetic group, the majority of the diabetic patients did not have microalbuminuria (69%) (141). The diabetic patients had significantly elevated levels of both kappa and lambda light chains compared to the control patients, even when matched for renal function, the South Asian diabetic patients had higher FLCs than the Caucasian diabetics (141). Analysis of follow up data showed that patients with abnormal serum FLCs were more likely to go on to develop microalbuminuria than those with normal serum FLCs at baseline (141).

Haynes et al examined the role of both polyclonal and monoclonal FLC in progression to ESKD and death in data collected from the CRIB cohort described previously. They hypothesised that as markers of the adaptive immune system, FLC are potential markers of sub-clinical inflammation and that they may also have an immunomodulatory effect that had not been previously studied on clinical outcomes in CKD (173, 174). In a cohort of 364 patients, serum

FLCs were measured and serum immunofixation and electrophoresis was also undertaken; the outcomes of interest were arrival at ESKD and all cause mortality (173).

Just under 10% of the cohort had evidence of monoclonal gammopathy of undetermined significance (MGUS), this was higher that previous prevalence studies (though this was the first time that the prevalence of MGUS in a CKD cohort had been published) (173, 175). While the presence of MGUS was associated with arrival at ESKD this effect disappeared when adjustment was made for renal function, and there was no association between MGUS and risk of death (173).

Neither polyclonal excess of kappa or lambda FLCs were associated with an increased risk of progression to ESKD after correction for eGFR, however a relationship between lambda FLC and mortality was found (173). As a result of these findings it is unclear if FLCs have a role in the risk stratification of CKD beyond being a marker of impaired renal clearance and further studies are in process.

C-reactive protein (CRP)

C-reactive protein is a systemic marker of inflammation, there is some evidence that systemic inflammation may be a non-traditional risk factor for both CVD and CKD progression; in the MDRD study the acute phase reactant ferritin was associated with eGFR decline and inflammation has been shown to propagate glomerulosclerosis in animal models (176, 177).

In an analysis from the MDRD study Sarnak et al hypothesised that markers of inflammation and nutritional status were associated with CKD progression; to address this they measured

CRP and leptin (which is known to be elevated in obesity and patients who are obese have been shown to be at risk of glomerulosclerosis) in 804 patients (151, 178). The MDRD cohort was divided into study A and study B, study A was comprised of patients with a baseline GFR of 25-55mL/min/1.73m^2, study B of patients with a GFR of 13-24mL/min (in all patients GFR was measured using ^{125} I-iothalamate), at baseline the mean CRP in study A was 0.48 and in study B was 0.46 (151). The groups were followed up for a mean of 2.2 years and the end point of rate of change of GFR was used, in neither study groups was baseline CRP associated with rate of GFR decline (151).

These results are interesting because they were unexpected, as CRP had been previously shown to be associated with both CVD and CKD and there is a plausible patho-physiological process by which this might be the case. However it is important to note that CRP is a surrogate marker of inflammation and the MDRD cohort while large and well characterised (with measures of GFR rather than estimates) is not a wholly representative CKD population (176).

CRP was found to be a predictor of CVD in a dialysis population. In a cohort of 163 Japanese haemodialysis patients (who were divided into two groups dependent upon their baseline CRP level, where CRP was analysed as a categorical variable with patients whose CRP was <10mg/L were described as having low levels and those with levels >10mg/L as high levels) the five year survival was significantly worse in the group with higher CRPs than lower CRPs (44% versus 82%) (152). The dialysis population differs from the CKD population and the reasons for the presence of inflammation may also differ, however these results suggest that in this context inflammation is related to cardiovascular outcomes (152). In a cohort of

patients on peritoneal dialysis similar findings have been reported, Noh et al described a cohort of 106 stable peritoneal dialysis patients with two years of follow up, those with higher CRP levels at baseline had significantly poorer survival (179).

Pro-inflammatory cytokines

There are a number of pro-inflammatory cytokines that have been associated with endothelial dysfunction, the most frequently studied are tumour necrosis factor α (TNFα) and interleukin-6 (IL-6) (180). In a small, cross-sectional analysis of patients with stage 3-5 CKD compared to healthy subjects all markers of inflammation were significantly higher in the patients with CKD, IL-6 was significantly higher in those with pre-existing CKD (181).

In a cross-sectional analysis of patients with CKD (some on dialysis, some pre-dialysis), patients with normal kidney function but CVD and healthy controls, pro-inflammatory cytokines were measured along with markers of lipoprotein oxidation; in patients with CKD IL-6, TNF α and CRP were all significantly higher than in the CVD and healthy controls (154).

In a post hoc analysis of an RCT of a statin, baseline CRP and TNF receptor ii (TNFrii) levels were measured and the rate of decline of kidney function used as the outcome measure; after a median duration of follow up of 58 months both CRP and TNFrii levels were independently associated with faster rate of decline of kidney function (182).

Uric acid

Uric acid has been shown to be associated with CVD, in a rat model hyperuricaemia caused hypertension and renal vascular changes in the absence of crystal deposition (183-185). It has been hypothesised that this effect is mediated via inflammation, endothelial dysfunction and

oxidative stress (186, 187). In an epidemiological study Cain et al demonstrated that hyperuricaemia was associated with prevalent CKD and concluded that the role of uric acid in CVD may be via CKD, they were unable to draw conclusions about whether uric acid had a role in the initiation of progression of CKD (150). However in the atherosclerosis risk in communities (ARIC) study the relationship between uric acid and mortality was shown to be significant only in individuals without CKD and not in those with CKD (188).

In an interventional study of allopurinol versus control with outcomes of hospitalisation, cardiovascular events, ESKD and mortality a cohort of 113 patients with an eGFR <60mL/min were recruited (189). The baseline uric acid levels were higher in the group allocated to allopurinol treatment than the control group, and after the follow up period were significantly lower than the control group. In the allopurinol group the rate of decline of renal function was largely unchanged but the control group had evidence of progression; there was no difference in BP or albuminuria between the groups (189). As the groups were small the rate of cardiovascular events was low as was progression to ESKD, however there was a reduction in both hospitalisations and cardiovascular events in the allopurinol group, these data suggest that hyperuricaemia may be implicated in progressive CKD and that treatment with allopurinol is well tolerated and can mitigate that risk (189).

Phosphate

Disturbance of calcium and phosphate metabolism is related to cardiovascular disease in patients with CKD, whether or not there is also a relationship with progression of CKD is as yet unknown (190).

In a rat model hyperphosphataemia was found to be associated with progression of renal failure and the development of fibrosis; to address whether this observation would be replicated in humans Voormolen at al conducted an observational study of 448 patients with CKD (191, 192). The PREPARE study included incident CKD stage 4 and 5 patients (pre-dialysis) and followed them up for a mean of just 337 days, the aim of the study was to examine the relationship between plasma phosphate and progression of CKD (defined as rate of change of MDRD eGFR) (192). They found that as plasma phosphate increased the rate of renal progression also increase (for each mg/dl higher plasma phosphate the decline in eGFR increased by 0.154mL/min/1.73m^2/month, this was independent of other traditional risk factors (192).

In a slightly smaller study of 225 patients with less advanced CKD (stages II-V pre-dialysis), Chue et al measured a variety of bone biomarkers and measures of arterial stiffness, they followed up the patients for a mean of 924 days and the main outcome was rate of change of GFR (MDRD based estimates) using a linear regression method (162). As with the PREPARE cohort serum phosphate independently predicted rate of decline of GFR, a 1mmol/L increase in phosphate being associated with a 0.34mL/min/1.73m^2/month faster rate of decline (162).

Bicarbonate

Metabolic acidosis is a common complication of CKD and is especially prevalent when the eGFR falls below 30mL/min; the effects of metabolic acidosis include increased protein catabolism and amino acid oxidation and potentially progressive CKD (193-195). However the experimental evidence for treatment of metabolic acidosis with bicarbonate is mixed with some authors demonstrating a positive effect (196, 197) and other showing no benefit (198).

In an animal based study the influence of dietary bicarbonate supplementation upon decrease in eGFR was studied; rats fed on soy protein with dietary acid supplementation developed metabolic acidosis and decline in eGFR compared to rats fed on a diet supplement with sodium bicarbonate (though this effect was only seen after the sodium bicarbonate induced increase in blood pressure was treated) (199).

Shah et al carried out an epidemiological study of over 5000 adults from primary care, renal function and acidosis (measured using estimated GFR and serum bicarbonate respectively) were considered in relation to progressive renal impairment which was defined as a greater than 50% fall in eGFR or progression to an eGFR<15mL/min/1.73m^2 (164). Participants with lower bicarbonate levels were more likely to progress than those with higher bicarbonate levels, when the bicarbonate levels were divided into groups the hazard ratio for progression with a bicarbonate level <22mEq/L was 1.54 compared to a hazard ratio of 1.14 if the bicarbonate level was >27mEq/L (164).

To address the hypothesis that treatment of acidosis would reduce the rate of progressive CKD Brito-Ashurst et al carried out a randomised controlled trial; 134 adults with CKD (where the creatinine clearance, as measured using 24 hour urine samples, was between 15-30mL/min/1.73m^2) and a serum bicarbonate level of 16-20mmol/L were randomised to either receive supplementation with sodium bicarbonate or standard therapy (165). The primary end point was rate of change of GFR and the proportion of patients who sustained a rapid decline (3mL/min/1.73m^2/year) and progression to ESKD; the rate of decline was significantly slower in patients receiving bicarbonate supplementation than the control group

(1.73 versus 5.93mL/min/1.73m^2/year), fewer patients receiving bicarbonate supplementation experienced an accelerated decline and fewer progressed to ESKD (165).

These data suggest that bicarbonate may be a biomarker of progressive CKD and that treatment with bicarbonate supplementation may reduce the risk of progression but this has yet to be studied in large prospective CKD cohorts.

1.9.2. Markers of arterial stiffness and microvascular disease

A characteristic feature of arterial disease in CKD is thickening and calcification of the medial arterial layer, known as arteriosclerosis (166). In its purest form, media calcification is concentric and does not extend into the arterial lumen. Increased collagen content, calcification, hyperplasia and hypertrophy of the vascular smooth muscle cells results in wall thickening and increased arterial stiffness. Although associations have also been established between the degree of arterial stiffness and atheromatous plaque burden (200), recent studies have failed to demonstrate a significant influence of traditional atherosclerotic risk factors on the development of arteriosclerosis (201), suggesting that alternative 'non-atherogenic' factors drive this process. There is certainly some overlap, however, as endothelial dysfunction and reduced Nitric Oxide bioavailability have been shown to contribute to arterial stiffening (202). There is a strong association between arterial stiffening and mortality in CKD (166).

The pathophysiological effects of arteriosclerosis and arterial stiffening are best understood by an appreciation of the normal physiology of the aorta and large arteries. Their major functions are not only to deliver blood around the body but also to buffer the oscillatory changes in BP that result from intermittent ventricular ejection. The highly distensible arterial

system ensures that most tissues receive near steady flow with no exposure to peak systolic pressures; this mechanism is so efficient that there is almost no drop in peripheral mean arterial pressure compared to the ascending aorta (203). Loss of arterial distensibility results in a more rigid aorta that is less able to accommodate the volume of blood ejected by the left ventricle, resulting in greater pressure augmentation in systole and higher pulse pressures (204). As arterial stiffness increases the loss of arterial distensibility exposes the myocardium, brain and kidneys to higher systolic pressures and greater pressure fluctuations arising from increased pulse pressures, resulting in myocardial and cerebral microvascular damage and an increased risk of heart failure, arrhythmia and stroke (205). While the high systolic pressure increases left ventricular afterload, lower diastolic pressure reduces diastolic coronary perfusion promoting ischaemia and placing greater reliance on systolic coronary perfusion (206, 207).

Pulse wave velocity (PWV)

Arterial stiffness is increased in patients with early stage CKD (166). Aortic PWV is currently considered to be the "gold-standard" measurement of arterial stiffness (208). Measures derived from central pulse wave analysis (PWA) (central systolic pressure, pulse pressure and augmentation index [AIx]) are considered indirect, surrogate markers of arterial stiffness and provide additional information on arterial wave reflections (208). Increasingly, these markers are recognised as powerful predictors of cardiovascular mortality and morbidity in patients with CKD (166, 208).

Theoretically, arterial stiffness should also lead to renal vascular damage and progressive renal impairment by similar mechanisms to those described above (166). Three small studies have found an association; Taal *et al* in 2007 used radial-dorsalis pedis PWV as a measure of

arterial stiffness in 35 patients with advanced stage IV and V CKD and found PWV and AIx, predicted progression to ESKD (209). In a Japanese study of 41 subjects with non-diabetic CKD AIx predicted a greater decline in renal function (210). Interestingly, a subsequent study by this group in 42 patients failed to replicate this finding and did not demonstrate any relationship between PWV or AIx and progression of renal dysfunction (211). A third study of 133 patients with stage III-IV CKD showed PWV to be a predictor of decline in renal function (212). However, a larger study of 235 patients with CKD and longer follow-up failed to show any association between PWV and progression of CKD (213). This latter study is in keeping with a prospective longitudinal analysis of the Framingham Offspring Study which did not find an association between baseline aortic PWV and incident CKD or microalbuminuria (214). The differences between all of these studies serve to highlight that very little is actually known about the natural history of arterial stiffness and kidney disease and in particular the complex interactions between age, uraemia, blood pressure and medication in CKD patients (166). Clearly larger longitudinal studies are needed to resolve this and interventional studies targeting arterial stiffness as a means of lowering cardiovascular events may then be warranted.

Advanced glycation end products (AGEs)

Advanced glycation end products are the result of the non-enzymatic glycation and oxidation of proteins, lipids and amino acids in a process known as the Maillard reaction; increased formation of AGEs are found in hyperglycaemic states, chronic inflammatory states, as part of the aging process and in renal impairment (215-217). Upon binding to the cell surface receptor they are able to modulate a number of intracellular processes and it is via this mechanism that they are thought to enhance cardiovascular risk (218, 219). The accumulation of AGEs has been shown to correlate well with renal function and death in patients with CKD, dialysis patients and renal transplant recipients (220-223).

In a prospective cohort of 1700 patients with CKD stage 3, skin AGE accumulation (measured by skin autofluorescence) were independently associated with a number of traditional and non-traditional risk factors for CVD (126). In a cohort of 386 haemodialysis patients, Nishizawa et al measured plasma pentosidine and found that patients in the upper tertile of pentosidine levels had more cardiovascular events during the follow up period (149).

In a sub-group analysis of an RCT designed to explored the efficacy of the ACEi captopril at preventing renal progression in patients with diabetes, 67 patients who sustained a doubling of creatinine levels during follow up were matched with 67 patients who had progressed but had not doubled their creatinine (they were matched for intervention, baseline creatinine and proteinuria) (224, 225). Patients who had doubled their creatinine had higher levels of both pentosidine and neopterin at baseline (there were no differences in the levels of the inflammatory markers IL-6 and CRP), from this observation it is possible that AGEs could be implicated in the progression of diabetic CKD (225).

1.9.3. Genetics and CKD

The influence of genetic factors on CKD progression has yet to be elucidated, in one study no relationship was found between several single nucleotide polymorphisms (SNPs) and progressive CKD (226) . When a genome wide association study (GWAS) was performed a gene related to uromodulin was shown to be associated with renal function, although its relationship to renal progression has yet to be studied (227). In a study of dialysis patients, patients with “mild” CKD and a group of healthy controls, polymorphisms of genes that influence endothelial function were explored; the authors reported that some genotypes were

found more frequently in some diagnostic groups than others; this is an interesting area for future work (228).

In an analysis of nine cohort studies, containing over 23 000 participants, a GWAS was performed; serum creatinine, eGFR and cystatin C were used as measures of renal function (229). There were 109 SNPs associated with serum creatinine, these were distributed over five loci, only one of these had previously been described as having an association with kidney function; when potential associations between the loci and eGFR or cystatin C were investigated two of the four loci were associated with eGFR but not cystatin or CKD, none of the four novel loci were associated with weight, hypertension or diabetes (229).

In another large GWAS study over 130 000 individuals were included; the aim of the study was to stratify participants by four key risk factors, hypertension, age, gender and diabetes to identify novel loci (230). Six new loci were identified that were associated with eGFR, there was variability with some loci being more pronounced in younger patients and some being more frequent in certain ethnic groups (230).

1.9.4. The anthropomorphic phenotype and CKD

Globally increasing cardiovascular mortality (231) together with the recognition of kidney disease as a cardiovascular risk factor (45, 70) has led to greater interest in the relationship between obesity and kidney disease. A growing number of studies have concluded that adulthood obesity increases the risk for development of kidney disease (232).

Obesity is associated with a number of conditions known to increase the risk of CKD including hypertension, diabetes mellitus and heart failure (233). Several studies have shown an association between adult obesity and CKD with approximately 25% of CKD in Western populations being attributable to obesity (232). A recent study has also confirmed that a large proportion of the association between low socio-economic status (SES) and CKD can also be explained by obesity (234). Studies looking at the relationship between fat distribution and CKD have produced conflicting results (232). Furthermore, very few studies have examined longitudinally the relationship between obesity and progression of CKD (232). Intriguingly, small studies in patients after bariatric surgery show improvements in blood pressure control, proteinuria and inflammatory markers as well as in GFR although this last parameter needs to be interpreted with caution and confirmed in larger studies with harder end-points (235).

The current understanding of the biological mechanisms for the effects of obesity on CKD remains limited. Obesity may promote kidney damage directly through haemodynamic and hormonal effects or indirectly by favouring the development of diabetes and hypertension, disorders with strong kidney involvement (232).

It has been postulated by Heitmann et al that thigh circumference may be a cardiovascular risk factor in a prospective community based study of 2987 individuals. Decreased thigh circumference was related to increased risk of cardiovascular death and morbidity, this difference was independent of body mass index (BMI), percentage of body fat and waist circumference (236). To date there is no published evidence that thigh circumference influences the progression of CKD or the CVD risk experienced by individuals with CKD.

1.9.5. The periodontal phenotype and CKD

There is emerging interest in the potential association between chronic periodontal inflammation and endothelial dysfunction (237), this is based upon the hypothesis that atherosclerosis is an inflammatory disease and that chronic periodontitis contributes to the systemic inflammatory burden and thus potentiates atherosclerosis (238-240).

1.9.6. The socio-economic phenotype and quality of life measures in CKD

Socioeconomic factors are known to influence both the prevalence and severity of chronic disease (241, 242). Population studies conducted in both the United States and Europe have demonstrated an increased risk of CKD in individuals of lower socioeconomic status (SES) (243-250).

The influence of race and SES has been explored in North American studies where African-American subjects were more likely to be of lower SES and have a corresponding increased risk of prevalence and severity of CKD (243-249).

In a retrospective cross-sectional analysis of 1657 patients with CKD who were referred to a secondary care renal service in Sheffield, Bello et al studied the association between area-level SES and severity of established CKD at presentation and whether any association was independent of other established risk factors (251). They included all referred patients with a known eGFR <60mL/min/1.73m^2 for at least six-months who were not on any form of RRT (i.e. non dialysis stage 3-5 CKD (ND CKD)); the socio-demographic parameters analysed were age, gender, ethnicity (Caucasian versus non-Caucasian) and full postal code (251). They described an association between poor SES and severity of CKD at presentation that was independent of a number of socio-demographic, lifestyle and clinical variables (251).

However the relationship between SES and CKD is complicated by the influence of other established risk factors for CKD, which are known to be related to both CKD and reduced SES (252-255).

1.10. The development of renal risk scores

The disparity in clinical outcomes of individuals with CKD makes accurate risk stratification a holy grail of CKD management; being able to accurately predict which patients will remain stable, which will experience a slow linear or stepwise decline and which are at risk of an inexorable, accelerated decline towards ESKD would allow clinicians to focus treatment on those at highest risk.

It is clear that there are many factors that influence risk of progression (a likely combination of nephron loss, inflammation and endothelial dysfunction caused by both traditional and non-traditional risk factors); the observation that a single insult (e.g. unilateral nephrectomy) is usually associated with good renal outcomes supports this (256, 257). Any risk scoring system would necessarily include clinical and laboratory variables in combination with sophisticated mathematical modelling similar to that used to construct the Framingham cardiovascular risk scoring system (258).

In an analysis of data from the RENAAL study (reduction of renal end points in patients with diabetic nephropathy using the angiotensin blocker Losartan), a proposed risk stratification system for arrival at ESKD included urinary ACR, serum albumin, creatinine and haemoglobin.

The formulae for risk of progression to ESKD and death are shown in figure 7 (259). The authors concluded that the use of the formulae improved the prediction of arrival at ESKD from 50% when only albuminuria was included, to >80% when all four clinical variables were included (259). It is important to note that this formula was developed from a diabetic cohort and may not be applicable to the non-diabetic population.

Figure 1-7: Equations for the prediction of progression to ESKD and death from the RENAAL study (259)

ESKD = (1.96 x log[ACR])-(0.78 x sAlb[g/dL])+(1.28 x sCr[mg/dL])-(0.11 x Hb[g/dL])

Death = (1.14 x log[ACR])-(.061 x sAlb[g/dL])+(0.97 x sCr[mg/dL])-(0.07 x Hb[g/dL])+(0.08 x HbA1C[%])

ESKD, End stage kidney disease; ACR, Albumin creatinine ratio; Alb, Albumin; sCr, Serum creatinine; Hb, Haemoglobin; HbA1C, glycated haemoglobin

In another attempt to devise a renal risk stratification tool Tangri et al used a variety of routinely collected demographic, clinical and laboratory data in two independent cohort studies where there was a variety of distribution of renal impairment and causes of renal impairment (260). The total population was large (n=8391) and the end point used was progression to ESKD (identified from national registry data); the development cohort consisted of 3449 individuals and the validation cohort of 4942 individuals, the two cohorts did not materially differ from one another at baseline (260). They used seven different equations (the composition of which are shown in figure 8) and compared them to determine which provided the most accurate prediction of risk of progression.

Figure 1-8: The components of the risk equations devised by Tangri et al (260)

Model 1: Age and Gender

Model 2: Baseline eGFR, age and gender

Model 3: Baseline eGFR, age, gender and log urine ACR

Model 4: Baseline eGFR, age, gender, log urine ACR, diabetes and hypertension diagnoses

Model 5: Baseline eGFR, age, gender, log urine ACR, systolic BP (per 10mmgHg), diastolic BP (per 10mmgHg) and body weight (per 10kg)

Model 6: Baseline eGFR, age, gender, log urine ACR, serum albumin (per 0.5g/dL), serum phosphate (per 1.0mg/dl), serum bicarbonate (per 1.0mEq/L) and serum calcium (per mg/dL)

Model 7: Baseline eGFR, age, gender, log urine ACR, systolic BP (per 10mmgHg), diastolic BP (per 10mmgHg) and body weight (per 10kg), serum albumin (per 0.5g/dL), serum phosphate (per 1.0mg/dl), serum bicarbonate (per 1.0mEq/L) and serum calcium (per mg/dL)

eGFR, estimated GFR; ACR, Albumin creatinine ratio; BP, Blood pressure

The C statistic was higher for model 6 compared with models 2 and 3 and no further in sensitivity or specificity was observed by the additional of extra clinical variables (model 7), the C statistics for model 7 and model 6 were 0.835 (95% CI 0.819-0.851) and 0.851 (95% CI 0.825-0.857) respectively (260). An equation based on model 6 is consequently the one used in a smart phone risk stratification tool developed by the authors (261). While this is a very promising risk stratification tool it has yet to be validated in other cohorts and may be limited by its lack of ethnic diversity and inclusion of referred patients only.

It is clear that there are many unknowns in the field of CKD; what is true CKD and how much of what we currently classify as CKD is simply part of the normal aging process, what are the risks posed to individuals as a result of CKD (both renal and cardiovascular) and by what

mechanisms these act, what are the determinants of progression and CVD risk and what interventions can mitigate these risks and how can patients with CKD be accurately risk stratified to allow attention to be focused on those at highest risk?

1.11. Introductory Conclusions

In this thesis I present data derived from an index cohort that I established to address shortfalls in evidence in CKD. This is a long-term project that aims to address the hypothesis that there are non-traditional determinants of clinical outcome in progressive CKD and that these may be amenable to therapeutic approaches; the early data derived from this project provides the basis of the work that I present in this thesis.

The research questions will be addressed in the results chapters that follow are;

1. What is the baseline demographic, vascular, inflammatory and periodontal phenotype of patients with high risk CKD managed in secondary care?
2. What is the socio-economic phenotype of the cohort and what are the determinants of quality of life, functional status and symptom burden in a high risk CKD cohort?
3. What are the associations between the established cardiovascular and renal phenotype and the vascular and periodontal phenotype?
4. What are the early determinants of progression to ESKD and death in the cohort?

Chapter one contains a detailed description of the methodology used in the RIISC study, and its evidence base. Chapter two contains the descriptive baseline demographic characteristics of the RIISC cohort and chapter three contains the descriptive socio-economic and quality of

life characteristics of the cohort. Chapter four describes the vascular characteristics of the cohort, chapter 5 describes the periodontal characteristics of the cohort and chapter 6 describes the early outcomes data. The results presented are then discussed in the final chapter.

2. Chapter two: Methods

The study protocol was approved by the South Birmingham Local Research Ethics committee (reference 10/H1207/6) and University Hospitals Birmingham Research and Development department (reference RRK3917). The inclusion and exclusion criteria are shown in table 1.

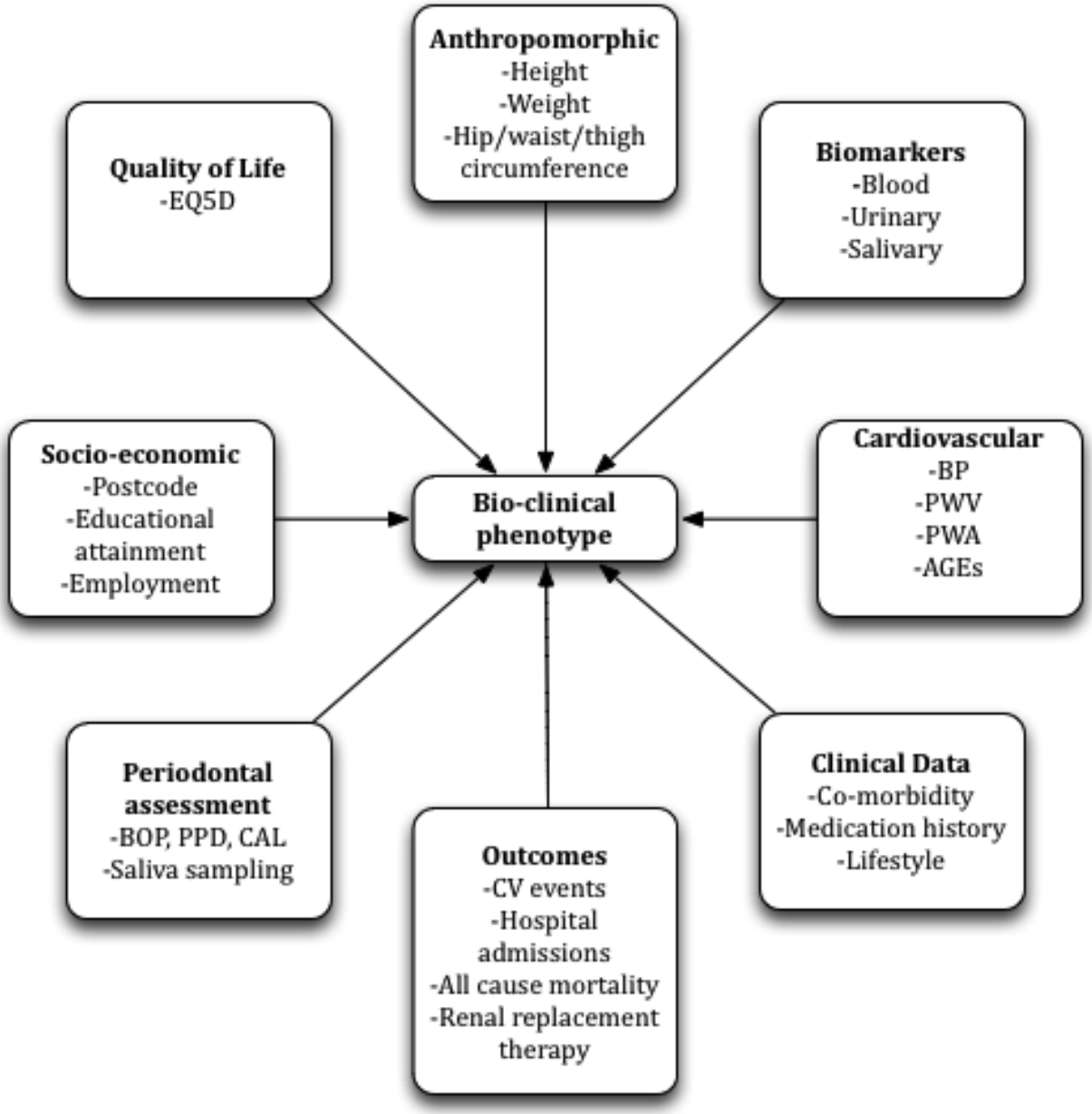
Table 2-1: The inclusion and exclusion criteria of the RIISC study

Inclusion criteria (at least one required)	Exclusion criteria
Stage 3 CKD with either/or; i) decline of eGFR of ≥5mls/min/year or ≥10mls/min/5years	Renal Replacement therapy
ii) ACR ≥70 mg/mmol on three occasions	Immunosuppression for renal disease
CKD 4 or 5 (pre-dialysis) (these patients may also have progression or proteinuria inclusion criteria)	

Patients with progressive CKD are identified from secondary care renal clinics, where they have been under follow-up for at least one year, by an automated IT system that reports ACR data and generates an automated assessment of the rate of decline of kidney function (see below). Written information is sent to patients in advance of their attendance at the study clinic; for those patients who do not speak English, translated information is sent in audio format (as it is known that patients who do not speak English may not be able to read in their own language) (262).

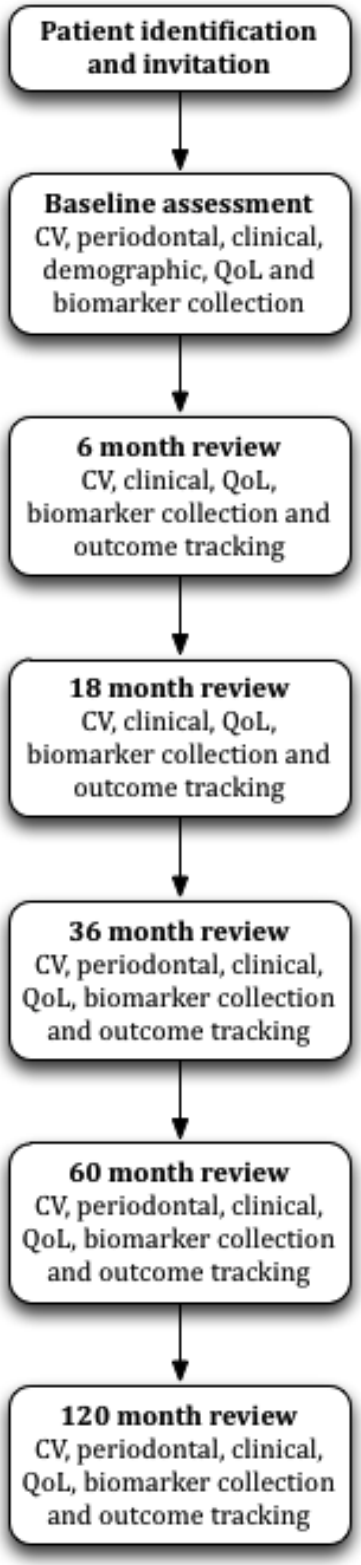
Participants undergo a detailed bio-clinical assessment an overview of which is shown in figure 1. The study reviews are integrated into the routine clinical follow up process and participants are followed up for 10 years or until they reach a defined clinical end-point (ESKD or death). The timepoints of the study visits and the data collected at each timepoint is shown in figure 2.

Figure 2-1: The composition of the bio-clinical assessment



BP; Blood pressure, PWV; Pulse wave velocity, PWA; pulse wave analysis, AGEs; Advanced glycation end products, CV; Cardiovascular, BOP; bleeding on probing, PPD; probing pocket depth, CAL; clinical attachment loss.

Figure 2-2: Timeline of study visits and assessments performed



Each study visit is arranged around routine clinic visits, so patients are not required to attend the clinic more frequently than their clinical condition and current guidelines dictate (61). Outcomes and endpoints embedded in RIISC are shown in table 2. Patients who reach ESKD are withdrawn from further follow-up, although ethical permission to continue to collect cardiovascular events and mortality data on these patients has been obtained.

Table 2-2: Clinical outcomes and endpoints

Clinical outcomes	Clinical Endpoints
Cardiovascular events	Death
Hospitalisations (and days hospitalised)	ESKD (as defined a requirement for renal replacement therapy)
Progression of CKD as measured by decline in eGFR calculated by linear regression	

The study specific assessments and the evidence base for these are described below.

2.1.Assessment of rate of renal decline

An assessment of the rate of decline of renal function is important for both the identification of potential participants and to measure the outcomes of those participants. However measuring rate of change of kidney function is complex (because renal decline is rarely a linear phenomenon) (263) and there is no gold standard methodology. A linear regression method is used to measure renal decline, utilising eGFR as calculated by the 4-variable MDRD formula with serum creatinine that is IDMS traceable. For screening rate of decline, each potential participant must have at least 6 eGFR results (obtained between a 12 and 60 month period), to allow an accurate assessment of the rate of change of eGFR with time (162).

In the MDRD study the intra-test variability of the creatinine based eGFR was 9.4%, the variability being greatest at the extremes of GFR (30). In a study examining the accuracy of creatinine based eGFR equations in clinical studies in comparison to iothalamate based GFR measurements, Levey at al in 1993 recommended that to reduce the intra-test variability at least four measures should be used (35). This approach has now been validated by a number of studies and there is consensus that using between four and six eGFRs collected over a period of at least one year is a more accurate way of assessing decline than percentage change in creatinine based on two results (54, 162, 248).

The presence of significant albuminuria is also part of the inclusion criteria, early work on urinary albumin creatinine ratio measurement found that there was significant intra-test variability associated with this method (around 60%) (108). To reduce the impact of this variability potential participants are required to have three ACR measurements greater than 70mg/mmol (the cut off limit defined by NICE as "higher level proteinuria" (61).

2.2. Assessment of socioeconomic status, quality of life (QoL) and demographics

2.2.1. Assessment of socioeconomic status (SES)

Participants are asked a series of questions that related to SES, these include full postal code, educational attainment, current employment and current or last job type (the questions asked of participants are included in appendix 8). In previous analyses household income was used to assess SES (254) however to avoid alienating

participants (and because the evidence to suggest that this was an essential component of the assessment of SES was lacking (264)) this was not included in the RIISC protocol.

The UK Index of Multiple Deprivation (IMD) score is a measure of SES; using a number of indicators (covering economic, social and housing metrics) to produce a deprivation score for each electoral ward in England (265). In this thesis the rank rather than the score obtained is described.

2.2.2. Assessment of functional status

The most frequently used measure of functional status is the Karnofski performance index, which is comprised of 11 statements ranging from 100 (representing normal functioning) to 0 (representing death) (266). In a study evaluating the prevalence of depression in individuals on dialysis compared to individuals being managed conservatively, depressed individuals had poorer functional status by the Karnofsky index; there was no significant difference in the prevalence of depression between the dialysis patients and the conservatively managed patients (267).

The Karnofsky index has not been included in the RIISC protocol as functional status is not a stated outcome measure, limited information on functional status can be achieved with other tools.

2.2.3. Assessment of quality of life

There are numerous instruments for the measurement of QoL (268), all have their strengths and limitations, as QoL is a highly subjective concept. The instruments may be symptom based, satisfaction based or organ system specific.

2.2.4. The satisfaction with life scale

The satisfaction with life scale (SWLS) (269) is a measure of general well being and consists of a five-item scale with a 1-7 satisfaction rating for each item, poor scores on this scale have been described in individuals with CKD (270). A commonly used generic measure of health related QoL is the RAND 36-item questionnaire (also known as the short form-36 or SF-36) (271). It consists of 36 questions covering well-being, functional status and perceptions of health status; higher scores representing better perception of health. In a study of patients with a variety of stages of CKD, impaired QoL defined by SF-36 scores was observed even in the early stages of CKD (272).

The short form-36/KDQOL

The SF-36 consists of 36 questions covering well-being, functional status and perceptions of health status and it has been adapted for use in patients with renal disease (primarily aimed at those on maintenance dialysis) as the Kidney Disease Questionnaire (KDQOL) (273). The Kidney Disease Questionnaire (KDQOL) is a derivation of the SF-36 tailored to patients with kidney disease (primarily aimed at those on dialysis), it is comprised of five domains covering physical symptoms, fatigue, depression, relationships with others and frustration (273).

In a study of 205 patients with pre-dialysis CKD (stages 4 and 5) the KDQOL was administered; the mean scores obtained suggested that there was considerably impaired functional status compared to population norms (274).

However, while the SF-36 and KDQOL are detailed assessment tools they are time-consuming to administer, to overcome this abbreviated tools have been devised. An example of this is the EQ5D(275).

The EQ5D

The most credible assessment tool which combines accuracy with brevity is the EQ5D (275) which has been evaluated in a number of chronic disease groups and was found to perform well (276). A recent report commissioned by the department of Health to review patient reported outcomes for adults with CKD recommended the EQ5D among the preference-based measures with more supporting evidence, with the caveat that there was a lack of evidence of responsiveness and use in quality and outcome (277). This level of recommendation, together with the brevity of the tool and it's utility in assessing quality adjusted life years (QALYs), led to the choice of the three level EQ5D for assessment of QoL in RIISC participants (278, 279).

The EQ5D is comprised of two sections; a structured five-domain section which focuses on functional status and symptom burden where participants are asked whether they have no, moderate or severe problems or symptoms and a visual analogue scale (VAS) where participants are asked to rate how good or bad they perceive their health to be on that day on a scale of 1-100 (where 1 represents the poorest possible health and 100 the best possible). A research nurse administers the EQ5D at the time of consent (and re-affirmation of consent at follow-up visits) in a private room.

Participants are asked to describe their ethnicity according to the categories used in the United Kingdom census of 2011; in the analyses described in this thesis the categories were grouped into White, Asian, Black or Other.

2.3.Clinical assessment

Participants undergo a detailed clinical assessment at each time-point; data are collected regarding renal diagnosis, all co-morbidity and lifestyle factors. Co-morbidity data were obtained by clarifying that already held medical records are correct and any additional co-morbidities were identified by asking "have you ever been told by a doctor that you have...?" and "have you ever taken any treatment for….?". The questions asked are included in appendix 10.

Cardiovascular diseases were strictly defined as follows; ischaemic heart disease was defined as previous angiographically demonstrated atherosclerosis, coronary artery bypass grafting, echocardiogram evidence of regional wall dyskinesia or a diagnosis of angina based upon exercise testing; cerebrovascular disease was defined as radiologically proven ischaemic or haemorrhagic stroke, known carotid occlusive disease or clinical evidence of transient ischaemic attacks; peripheral vascular disease was defined as vascular insufficiency requiring limb amputation or re-vascularisation, abdominal aortic aneurysm or clinical evidence of vascular insufficiency.

Co-morbidity was scored using the Charlson co-morbidity index, this was devised in 1987 as a tool for the prediction of 10 year mortality in individuals with a range of chronic conditions; the case definitions quoted in the original paper were used and are

shown in table 3 (280). This has been updated by Hall et al to include an age-adjusted score using an excel based calculator; this tool was used to classify co-morbidity in RIISC participants (281). All data required for co-morbidity scoring were collected though only cardiovascular co-morbidity data are presented in this thesis.

Table 2-3: The Charlson co-morbidity score and case definitions (280)

Condition	Definition
Myocardial infarction	One or more probable or definite myocardial infarctions in patients who have been hospitalised or had enzyme or electrocardiographic changes
Biventricular heart failure	Exertional or paroxysmal nocturnal dyspnoea and who have responded symptomatically (or on physical examination) to digitalis, diuretics or afterload reducing agents
Peripheral vascular disease	Intermittent claudication or intervention for arterial insufficiency, gangrene or acute arterial insufficiency or a thoracic or abdominal aortic aneurysm of >6cm
Cerebrovascular disease	History of cerebrovascular accident with minor or no residual deficit or transient ischaemic attacks
Dementia	Chronic cognitive deficit
Chronic pulmonary disease	Dyspnoea on slight activity with or without treatment and those who are dyspnoeic on moderate activity despite treatment. Severe pulmonary disease includes those who are dyspnoeic at rest, who require constant supplemental oxygen or who have CO_2 retention
Peptic ulcer disease	Requirement for treatment of a peptic ulcer including those who have bled from ulcers
Mild liver disease	Cirrhosis without portal hypertension or chronic hepatitis
Diabetes with no end organ damage	As described
Hemiplegia from any cause	As described
Moderate/severe renal disease	Moderate renal disease; serum creatinine >3mg/dL (>265.2µmol/L)
	Severe renal disease; patients on dialysis, those who have had a transplant or those with uraemia
Diabetes with end organ damage	As described
Any tumour	Solid tumours without documented metastases but treated within the last five years, examples include breast, colon, ling and a variety of other tumours
Leukaemia	Acute and chronic myeloid leukaemia, acute and chronic lymphocytic leukaemia and polycythaemia vera
Lymphoma	Hodgkin's, lymphosarcoma, Waldenstrom's macroglobulinaemia, myeloma and other lymphomas
Moderate or severe liver disease	Moderate liver disease; cirrhosis with portal hypertension without bleeding
	Severe liver disease; cirrhosis, portal hypertension and variceal

	bleeding
Metastatic solid tumour	As described
AIDS	As described

2.4.Cardiovascular assessment

All cardiovascular measurements are conducted in a room maintained at a constant temperature (22-24°C), using standardised operating procedures by trained personnel, at the same time of day (for each patient and at each time-point) and prior to phlebotomy and periodontal probing.

2.4.1. Peripheral blood pressure measurement

Participants have their BP measured using the BpTRU method after a five-minute rest. This is an oscillometric method that takes six consecutive readings and averages the last five measurements. This method has been shown to produce readings that are comparable to the daytime averages obtained by 24 hour ambulatory BP monitoring (282-286). Routine clinic blood pressure readings may be inaccurate because of the absence of a prior rest, the single reading obtained or the environment in which the readings are taken. In a cohort of patients with CKD, BpTRU readings were significantly lower than routinely obtained clinic readings and correlated closely with 24-hour ambulatory BP daytime average readings (and 24-hour readings per se) (287). The SOP for the measurement of BP using the BpTRU™ device is included in appendix 1.

2.4.2. Measurement of Arterial stiffness and central blood pressure

There are many commercially available systems for measuring PWV (288, 289). In this study the Vicorder™ system was chosen. The Vicorder™ device has been developed to

measure aortic PWV with little operator training and in a non-intrusive manner. It has been found to have very good intra- and inter-observer repeatabilities in a number of conditions and produces comparable results to those obtained using the widely used method of applanation tonometry (290-292).

Participants are asked to lie flat on a couch; a cuff is placed around the neck with the inflating balloon over the carotid artery (the patient is reassured that when inflated this is no tighter than a fitted shirt collar) and another cuff around the top of the leg. The distance between the two is measured and data are recorded using the Vicorder™ system. There is no requirement for a period of relaxation prior to the recording of data. Three PWV readings are taken (if the reading is more than 10% from the expected normal reading of 7m/s then another three readings are taken until two readings within 10% of one another are obtained.

The Vicorder™ system also generates the AIx and central BP of participants. The SOP for measurement of PWV and central BP is included in appendix 2.

2.4.3. Advanced glycation end products

Advanced glycation end product levels are to be measured by two complementary methods in RIISC. Firstly, AGE accumulation in the skin will be measured by skin autofluorescence (AGEreader™ (293)), secondly, serum concentrations of the AGE marker pentosidine will also be measured, however only data pertaining to skin autofluorescence are presented in this thesis. In 2004 Meerwaldt et al described a close

correlation between skin autofluorescence an AGEs measured in skin biopsy samples in studies with both prevalent dialysis patients and those with persevered renal function (diabetic and non-diabetic subjects) (294), in 2005 they described a close correlation between AGEs and measures of inflammation (CRP) in a study of haemodialysis patients (221). However, as several studies conducted in patients following kidney transplantation and in dialysis patients have not shown close correlations between AGEs measured in the skin (by skin biopsy rather than autofluorescence) and serum markers, in due course I will explore the relationship between AGEs measured using both skin autofluorescence and the serum marker pentosidine (295-297). The detailed SOP for AGE measurement in included in appendix 3.

2.5.Anthropomorphic assessment

RIISC participants have their height, weight, hip, waist and thigh circumference measurements taken using a standardised method (following the standard operating procedures included in appendices 4 and 5); BMI, hip/thigh ratio (HTR) and waist/hip ratio (WHR) are then calculated.

2.6.Periodontal assessment

RIISC participants undergo a full mouth periodontal assessment which comprises measurement of pocket probing depth (PPD), a measure of current disease status; recording of bleeding on probing (BOP), a measure of periodontal inflammation; clinical attachment loss (CAL), a measure of lifetime disease experience (carried out by a trained dental hygienist supported by a trained dental surgeon). Saliva samples are collected for non-presumptive proteomic analysis and plaque samples are being collected for molecular microbiome analysis to address the hypothesis that the nature

of the subgingival biofilm may correlate with renal status, however these data are not presented in this thesis (298, 299). The SOP for the periodontal assessment is included in appendix 7.

2.7.Biomarkers

There are a number of biomarkers that have been associated with CKD. These include: (i) markers of renal impairment; (ii) risk factors for CVD; and (iii) risk factors for progressive CKD. To date some studies of renal biomarkers have been limited by methodological shortfalls (the methods used for measuring renal progression, the large number of biomarkers studied and the exclusion of certain groups of patients). Table 3 describes the index biomarkers selected in the RIISC study and the current evidence of their possible role in the progression of CKD.

The biomarkers listed in table 3 have all been identified as being associated with progressive CKD in human studies of at least 50 patients, there are a number of other putative biomarkers (such as pro-inflammatory cytokines and vitamin D isotypes) where such evidence does not currently exist but where early experimental work suggests a plausible link with renal progression, RIISC aims to clarify the role that these biomarkers have (alone or in combination with each other) in the progression of CKD.

The appropriate collection and sample handling method of samples for biomarker analysis is important as many putative biomarkers are unstable and degrade rapidly from biological samples, it is accepted that this may limit their wider clinical application and to address this concern a sub-study investigating the reproducibility and stability of

certain biomarkers is to be carried out. As part of the RIISC protocol all samples are handled as described in appendix 6 (300-302).

Table 2-4: Biomarkers measured as part of the RIISC protocol and presented in this thesis

Biomarker	Patho-physiological basis	Number of patients	Definition progression	Evidence to date
Cystatin C	Marker of kidney function (136)	117	Doubling serum creatinine or ESKD	Cystatin C predicted renal decline (doubling of Creatinine or arrival at ESKD) in the MMKD study (303)
C-reactive protein (CRP)	Marker of inflammation	804	Rate of change of eGFR	Neither serum CRP or leptin predicted renal progression (151)
Free light chains (FLCs)	Marker of renal function and possible inflammation (141)	282 healthy controls, 772 South Asian diabetics, 91 Caucasian diabetics	Development of microalbuminuria	Elevated serum FLCs were a risk factor for the development of microalbuminuria (141)
IL-6	Pro-inflammatory cytokine			Small studies have suggested that polymorphisms in genes that encode inflammatory markers may influence progression of atherosclerosis and progressive CKD (304)

2.8.Genetic analysis

Samples are collected for genetic analysis using the PAXgene™ system; this is described in detail in the standard operating procedure included in appendix 6.

2.9.Clinical outcome data

The outcome measures reported in this thesis relate to arrival at ESKD and death, data pertaining to ESKD (defined as requirement for dialysis for more than 90 days or a pre-emptive renal transplant) were collected from renal registry data sources and data pertaining to death were collected from both trust IT and health authority data sources.

2.10. Data collection and analysis

The aim of RIISC is to recruit a minimum of 1000 participants; this thesis reports the cross-sectional analysis of the baseline data obtained from the first 500 recruits. This will allow robust interpretation of the relationship between the variables that will be under study in the cohort and their relationship to clinical outcomes. Data collected is stored in a specially designed database that allows detailed recording of the demographic and phenotypic characteristics of the cohort across multiple sites; data can be rapidly retrieved and analysed.

2.10.1. Statistical analysis

With an assumed event rate (death or progression to end stage kidney disease) of 10% per year (based upon epidemiological data) a cohort of 1000 patients followed up for a ten year period would give a minimal detectable hazard ratio of 1.238.

The data were analysed using SPSS v19, where data were parametrically distributed mean and standard deviation are shown, where non-parametrically distributed median and interquartile range are shown.

The statistical test used and the justification for it is described prior to each analysis in the results chapters. Binary logistic regression is used where categorical dependent variables are present, goodness of fit being assessed by the R^2 value.

3. Results 1: Descriptive characteristics of the RIISC cohort

The methodologies described in chapter 2 were carefully designed to provide accurate information on the cohort at inception and during follow-up. The cohort studies that have previously been set up and report bio-clinical data have been reviewed in chapter 1. As anticipated there are major similarities between the data collected in RIISC with other prospective cohorts, however there are also fundamental differences; these relate to both the characteristics of the participants recruited and the data set acquired.

In this chapter I have: (i) described the baseline demographic and clinical characteristics of the cohort; (ii) compared the characteristics of those patients who were recruited into the study with those who were eligible for recruitment and approached for inclusion in the study but did not wish to participate; (iii) explored the difference in key clinical variables between the baseline and six-month visits of the recruited cohort and iv) compared the demographics of RIISC participants to those of other CKD cohort studies. Haemodynamic data including BP are reported in the cardiovascular phenotype chapter

All clinical and demographic data presented relates to the first 500 participants recruited to the RIISC study between October 2010 and February 2013, of these 305 (61%) had reached the six-month time-point at the time of analysis and data from that visit is also shown.

3.1. The baseline demographic, clinical and anthropomorphic characteristics of the cohort

The eGFR was derived from the IDMS traceable serum creatinine level incorporated into the MDRD formula; Black ethnicity was corrected for. The urinary ACR was also measured at the baseline visit, for 57 individuals these data were incomplete.

Participants were recruited based on the eligibility criteria stated in the methodology chapter. Participants could have one or more of the eligibility criteria, so for example some individuals with stage 3 could have been recruited as consequence of both progression of CKD and proteinuria. Some individuals with stage 4 or stage 5 (pre-dialysis) CKD may have fulfilled three criteria for recruitment.

At the time of screening potential participants required an eGFR <60mL/min/1.73m^2 in addition to one or more additional criteria for recruitment; however in 10 patients the eGFR at the time of the baseline visit had increased to ≥60mL/min/1.73m^2. As these individuals had the necessary stipulated prior decline in eGFR at the time of screening and/or significant proteinuria they entered the study.

Participants were divided by stage of CKD for the purposes of the analysis shown in tables 1-5; the cohort was comprised predominantly of patients with stage 3B CKD and stage 4 CKD (n=122 and n=293 respectively), the remainder had stage 1

and stage 2 CKD (n=11), stage 3A CKD (n=28) and stage 5 CKD (n= 40). It was not possible to determine the CKD stage for 6 participants because there were insufficient creatinine data available.

Table 1 contains the baseline demographic and clinical characteristics of the recruited cohort and Table 2 the baseline laboratory data of the cohort.

These data show that age increase with severity of CKD, there is a higher prevalence of glomerulonephritis in individuals with less severe stages of CKD (a finding almost certainly explicable by the inclusion criteria; those individuals without stage 4 or 5 CKD were required to have significant proteinuria or progressive CKD). The ACR appears to demonstrate a bi-modal distribution with individuals with stage 1 and 2 and stage 5 CKD having the greatest ACRs. Individuals with stage 1 and 2 CKD have a significantly higher cholesterol than those with other stages (a likely reflection of the high prevalence of the glomerulonephritides in this stage) and individuals with stage 5 CKD have higher levels of uric acid.

Table 3-1: The baseline demographic characteristics of the RIISC cohort stratified by CKD stage

	All n=500	1&2 n=11	3A n=28	3B n=122	4 n=293	5 n=40	p-value
Males (%)	60	73	84	60	59	55	0.423
Age* (years)	65 (16)	41 (12)	53 (17)	61 (15)	66 (16)	65 (16)	<0.001
Ethnicity (%):							
White	72	55	64	74	74	66	0.441
Asian	16	27	21	13	14	26	0.291
Black	10	9	11	12	10	8	0.986
Other	2	9	4	1	2	0	0.291
Renal diagnosis (%):							
Hypertensive/ischaemic	30	37	21	28	30	32	0.370
Diabetic nephropathy	12	27	21	15	10	10	0.915
APKD	7	0	11	12	5	8	0.086
Glomerulonephritis	18	9	32	19	17	15	0.003
Reflux/pyelonephritis/obstruction	7	0	4	2	10	5	0.071
Unknown	16	0	4	17	19	15	0.087
Other	10	27	7	7	9	15	0.990
Current smokers (%)	14	18	7	14	14	10	0.794
Previous smokers (%)	42	45	50	34	42	58	0.091

* Values expressed as mean (SD), ANOVA performed; APKD, autosomal dominant polycystic kidney disease, for categorical variables Chi squared test performed

Table 3-2: The baseline laboratory characteristics of the cohort

	All	1&2	3A	3B	4	5	p-value
Creatinine (µmol/L)	221 (89)	87 (21)	128 (37)	159 (25)	236 (49)	405 (134)	<0.001
eGFR (mL/min/1.73m²)	27 (12)	70 (25)	49 (4)	35 (4)	23 (4)	12 (2)	<0.001
Cystatin C (mg/L)	2.6 (0.8)	1.2 (0.3)	1.6 (0.3)	2.2 (0.5)	2.8 (0.6)	3.7 (0.7)	<0.001
ACR* (mg/mmol)	26.9 (5.4-107.8)	117.9 (64.5-345.5)	93.6 (8.9-150.7)	17.9 (3.9-109)	22.9 (4.4-86.4)	70.7 (11.7-200.4)	<0.001
Haemoglobin (g/dL)	12.4 (4.5)	14.0 (1.5)	13.2 (3.1)	12.6 (2.4)	12.3 (5.5)	11.5 (2.4)	0.290
Corrected Calcium (mmol/L)	2.24 (0.14)	2.25 (0.09)	2.31 (1.28)	2.24 (0.12)	2.23 (0.14)	2.26 (0.21)	0.082
Phosphate (mmol/L)	1.38 (5.49)	1.03 (0.16)	1.05 (0.18)	1.07 (0.25)	1.57 (7.13)	1.39 (0.32)	0.932
Serum albumin (g/L)	44 (19)	41 (1)	43 (6)	44 (3)	43 (4)	53 (6)	0.048
Bicarbonate (mmol/L)	25.7 (16.5)	25.6 (2.5)	25.7 (3.3)	26.1 (2.9)	25.9 (21.2)	22.7 (3.3)	0.838
HbA1C (mmol/mol)	43.2 (38.8-55.2)	48 (26)	47 (21)	47 (14)	50 (16)	50 (17)	0.529
Cholesterol (mmol/L)	4.7 (1.3)	5.5 (0.8)	5.6 (1.4)	4.8 (1.4)	4.6 (1.2)	4.5 (1.1)	<0.001
Uric acid	462 (134)	364 (165)	386 (124)	437 (129)	484 (122)	486 (137)	<0.001

Values shown as mean (SD), ANOVA performed in all except for ACR* data shown as median and interquartile ranges (Kruskall-Wallis test performed)

In line with recent changes to the classification of CKD, albumin creatinine ratios were grouped into "no proteinuria" (ACR<2.9mg/mmol), "high proteinuria" (ACR 3-29mg/mmol) and "very high proteinuria" (ACR >30mg/mmol). In table 3 the distribution of ACR groups by CKD stage is shown as percentages.

Table 3-3: Proteinuria and CKD stage

	All	1&2	3A	3B	4	5	p-value
No proteinuria (A1)	18	0	8	20	19	11	0.214
High proteinuria (A2)	34	9	32	37	35	28	0.370
Very high proteinuria (A3)	48	91	60	43	46	61	0.017

Chi squared tests performed

These data confirm the findings presented in table 2, that there are two peaks in very high proteinuria.

3.2.The baseline co-morbidity of the RIISC population

For the purpose of this analysis co-morbidities were divided into the presence or absence of diabetes, cardiovascular diseases, chronic obstructive pulmonary disease and malignancy (past or current). The Charlson Co-morbidity Index was calculated for each participant, the age-adjusted score is shown in this analysis, higher scores represent greater co-morbidity. Co-morbidity data are shown in table 4.

Just under 40% of the cohort had diabetes, however the prevalence of diabetic nephropathy was 12% (29% of diabetic patients in the cohort having diabetic nephropathy). This is consistent with previous work indicating that fewer than 40% of diabetics have diabetic nephropathy (305). In this cohort the diagnosis

of diabetic nephropathy was only made in the presence of proliferative retinopathy requiring laser treatment, or based upon renal histology.

There were no significant differences in established CVD burden by CKD stage, however those with the most severe CKD had the highest co-morbidity.

Table 3-4: The baseline co-morbidity of RIISC participants by CKD stage

	All patients	Stage 1&2	Stage 3A	Stage 3B	Stage 4	Stage 5	p-value
Diabetes (%)	39	27	21	35	44	40	0.104
Ischaemic Heart Disease (%)	24	18	18	23	23	35	0.464
Cerebrovascular Disease (%)	12	9	14	14	13	13	0.507
Peripheral Vascular Disease (%)	12	9	4	13	13	8	0.526
Any cardiovascular disease (%)	34	18	32	39	33	38	0.526
Chronic Obstructive Pulmonary Disease (%)	13	27	7	13	13	8	0.415
Malignancy (%)	16	9	7	15	17	18	0.626
Median age adjusted Charlson Co-morbidity Index*	5 (2-7)	1 (0-2)	2 (2-4)	4 (1-6.75)	5 (3-8)	7 (4.25-8.75)	<0.01

*Data shown as median and inter-quartile range, Kruskall-Wallis test performed, for categorical variables Chi squared test performed.

The use of agents to block the renin-angiotensin system was common with 66% of the cohort prescribed an ACEi or angiotensin receptor blocker (ARB) at inception, of these 69% were diabetic (there was no significant difference between the percentage of diabetics and non-diabetics prescribed an ACEi or an ARB, p=0.300). At recruitment 5% were prescribed both an ACEi and an ARB, of these 39% were diabetic, the small percentage of participants prescribed dual blockade may represent the increasing understanding of the potential harms of such regimes (306). For those patients prescribed dual blockade the majority (87%) had very high albuminuria (ACR≥30mg/mmol).

3.3.The anthropomorphic phenotype of the cohort

Participants underwent detailed anthropomorphic assessment as described in chapter 2 section 1.5. All the anthropomorphic data were parametrically distributed. Anthropomorphic data are shown in table 5. The data show that there were no significant anthropomorphic differences between the stages of CKD.

Table 3-5: Anthropomorphic phenotype of RIISC participants

	All	Stage 1&2	Stage 3A	Stage 3B	Stage 4	Stage 5	p-value
Body Mass Index (kg/m^2)	29.7 (6.7)	32.3 (4.9)	27.8 (5.8)	29.9 (6.4)	29.8 (6.8)	29.6 (7.4)	0.383
Waist circumference (cm)	102.8 (17.2)	104.7 (16.9)	100.1 (15.3)	103.5 (16.2)	102.9 (17.5)	102.6 (19.7)	0.911
Hip circumference (cm)	106.6 (14.6)	108.2 (11.0)	102.9 (10.7)	107.2 (14.2)	107.1 (15.2)	106.0 (15.2)	0.682
Thigh circumference (cm)	53.8 (9.5)	57.3 (9.9)	51.6 (7.3)	54.5 (7.5)	53.8 (10.0)	53.4 (12.2)	0.455
Hip Waist Ratio	1.13 (3.72)	0.96 (0.11)	0.98 (0.09)	0.96 (0.12)	1.24 (4.85)	0.96 (0.12)	0.960
Hip Thigh Ratio	2.74 (11.7)	1.94 (0.37)	2.01 (0.23)	1.97 (0.26)	3.26 (15.23)	2.03 (0.15)	0.845

All variables parametrically distributed so ANOVA performed

Diabetes has been shown to be associated with negative anthropomorphic characteristics (elevated BMI (307) and increased waist circumference (308)) while other anthropomorphic characteristics (increased hip and thigh circumference) may be protective (308). An association between diabetes, obesity and chronic kidney disease was described in the NHANES survey (12).

To ascertain the association between diabetes and the anthropomorphic characteristics of the cohort, the group was divided into diabetics and non-diabetics and anthropormophic data compared between these two groups, the results are shown in figures 1-3. I then assessed whether diabetes was independently associated with adverse anthropomorphic features by utilizing a multivariable analysis incorporating all variables with a significant univariate association with anthropomorphic characteristics.

Figure 3-1: BMI in diabetics and non-diabetics

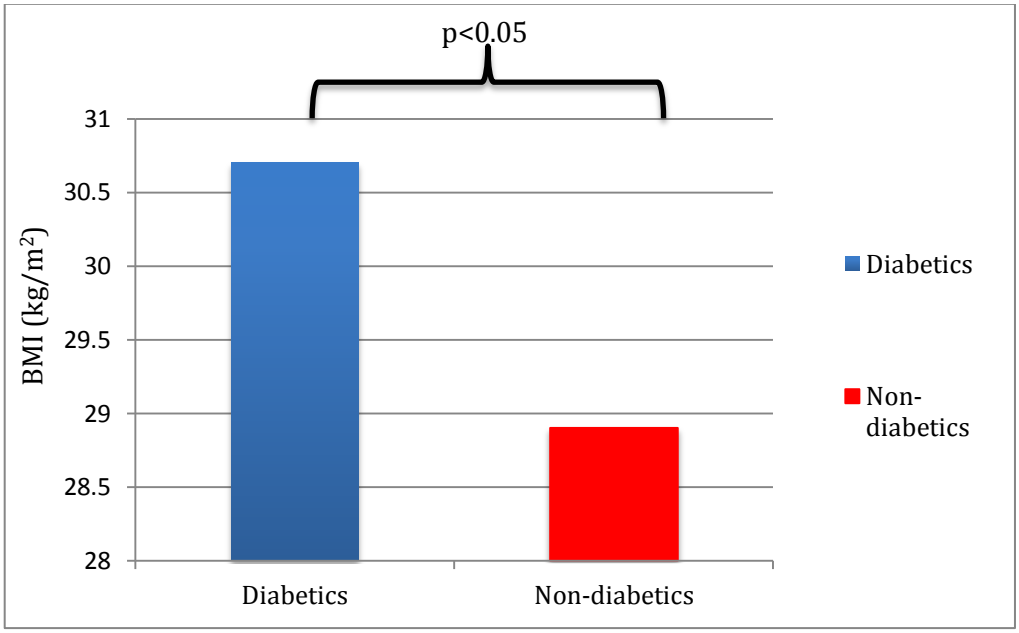


Figure 3-2: Waist and hip circumference in diabetics and non-diabetics

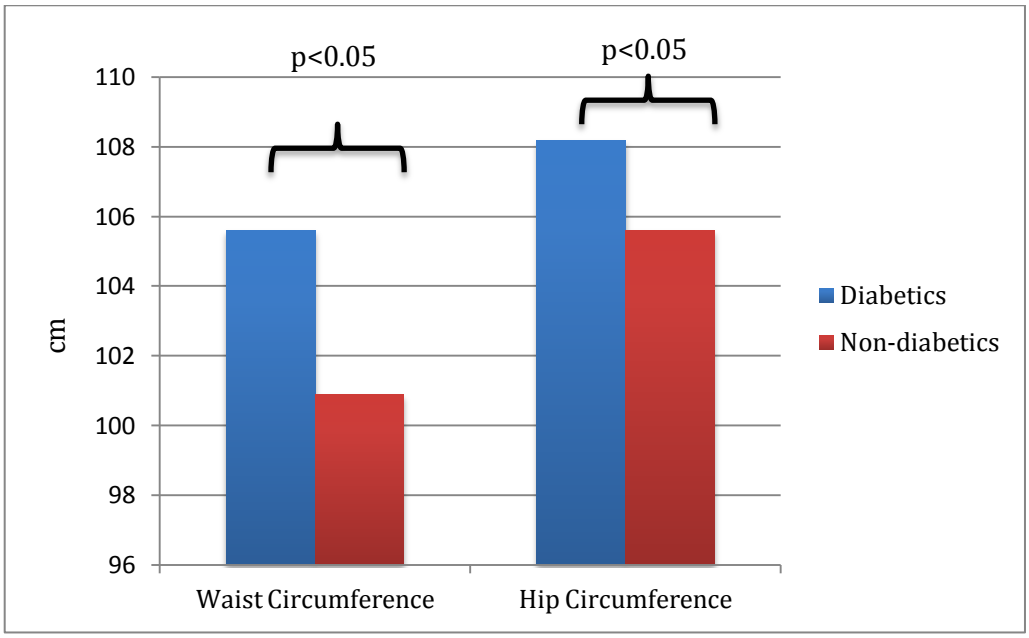
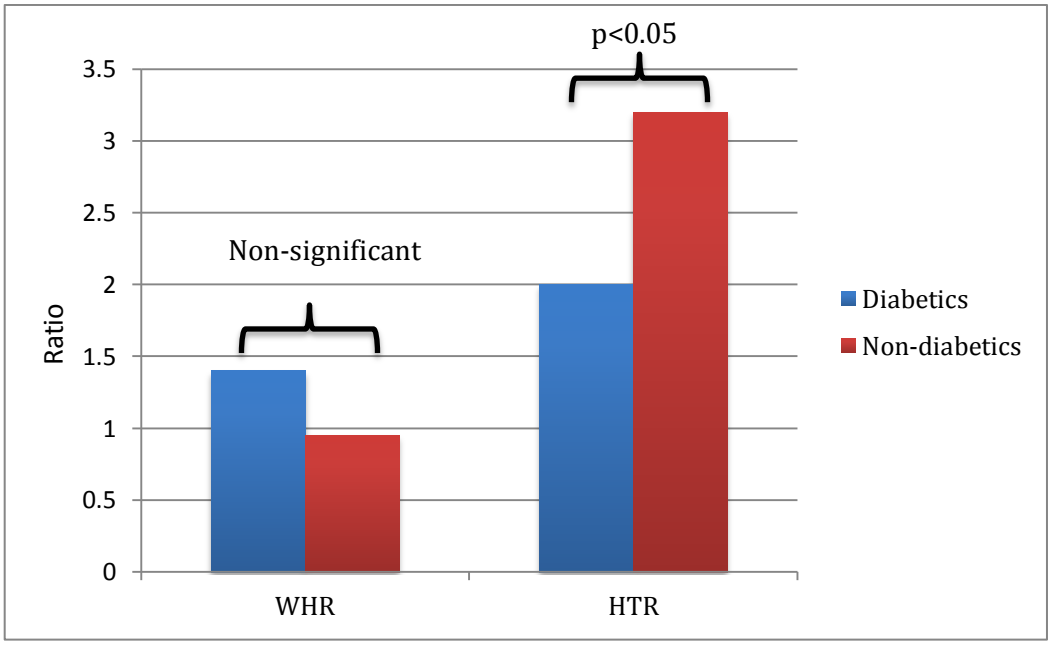


Figure 3-3: Waist hip ratio and hip thigh ratio in diabetics and non-diabetics



These data show that diabetic participants had significantly higher BMI, significantly larger waist and hip circumference and significantly lower HTR. There was no significant difference in WHR.

To identify variables associated with increased body mass index a univariate analysis was performed (the dependent variable being the presence of a body mass index in the upper quartile).

Figure 4 shows the variables included into in the analysis; those with a significance <0.1 (indicated by *) were then included in a multi-variate analysis, the results of which are shown in table 6.

Figure 3-4: Variables a priori considered to be a priori associated with elevated BMI

Demographic:	Age
	Gender*
	Ethnicity*
Clinical:	Diabetes*
	Cardiovascular disease
	Charlson co-morbidity Index
Socio-economic:	Current smoking
	Previous smoking
	Deprivation score*
Laboratory:	eGFR
	ACR
	CRP*
	Polyclonal free light chains
	HbA1C*

*reached significance in the univariate analysis

Table 3-6: Binary logistic regression of variables associated with increased BMI

	p-value	Odds ratio	95% confidence intervals
HbA1C	0.000	1.034	1.018, 1.050
Male gender	0.145	NA	NA
Black ethnicity	0.180	NA	NA
Presence of diabetes	0.863	NA	NA
hsCRP	0.086	NA	NA
Deprivation score	0.505	NA	NA

These results show that increased glycated haemoglobin (the odd ratios quoted refer to each 1% increase in HbA1C) was the only variable independently associated with elevated BMI in this cohort; the reasons for this require further study.

3.4.The representativeness of the cohort of the population from which it was recruited

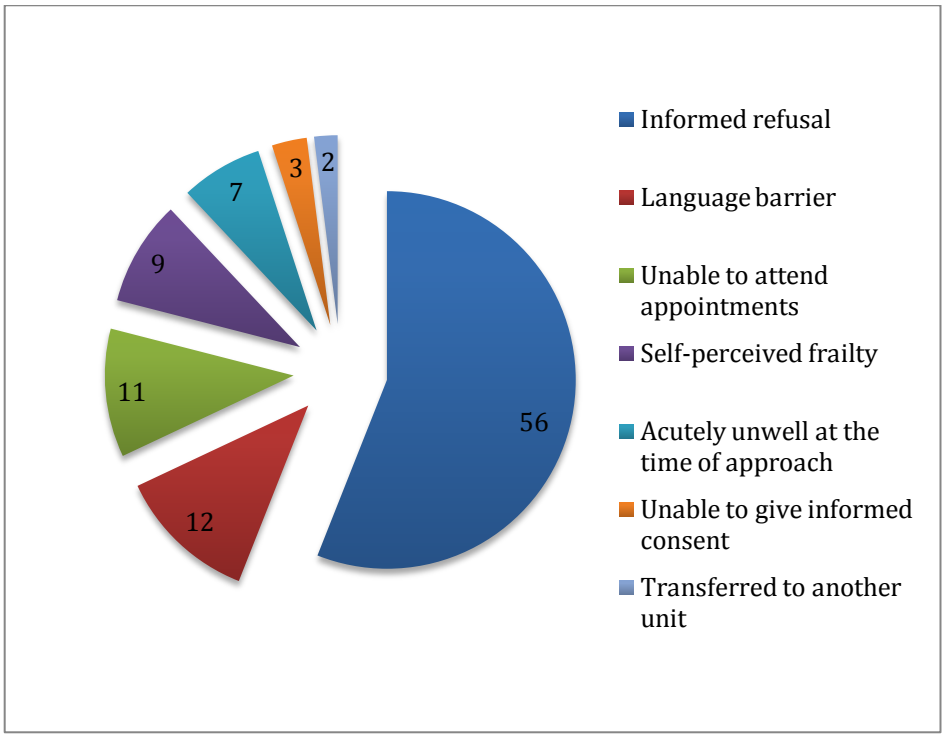
Interpreting the results of a cohort study requires an understanding of whether those that consent to participate are representative of the population from which they are recruited. To assess the representativeness of the cohort a cross-sectional analysis of the first 533 eligible patients who were approached about inclusion in the study was performed. 309 patients from this group participated in the study and 224 patients who met criteria for recruitment did not consent to participate. Advice was sought from the R&D department who felt that as this

included routinely collected, anonymised data no separate ethics application was required.

Potential participants indicated that they did not wish to participate at one of two time-points in the study recruitment period: (i) when they were sent the patient information sheet in advance of the study clinic (18% declined at this stage): (ii) on attendance at the clinic (77% declined at this stage). In addition 5% failed to attend for an initial study clinic visit on more than three occasions, and this was taken as evidence of declining to participate.

The reasons for non-consent provided by those who attended the clinic are shown in figure 5. The commonest reason given (in 56%) for not participating was resistance to participation in medical research. Other reasons included self-perceived frailty and logistical issues.

Figure 3-5: Reasons for non-recruitment (% shown)



Language barrier refers to the consenter's inability to communicate with a potential participant with sufficient clarity to obtain informed consent despite translation.

To determine the representativeness of the recruited cohort, routinely collected clinical data from both the participants and the eligible but non-consenting patients were compared, this is shown in table 7. Advice was sought from the trust R&D department who felt that a separate ethics amendment was not required for this work. The comparison includes, ethnicity, gender, eGFR and deprivation rank (using the IMD), co-morbidity could not be compared, as those who did not consent did not undergo the same detailed clinical questioning as those who did. The majority of the data were parametrically distributed. Data that were non-parametrically distributed were analysed using non-parametric tests.

Table 3-7: Characteristics of eligible patients, recruited and not recruited

	Recruited	Not recruited	p-value
Age	62 (17)	71 (13)	0.000
% Males	42%	53%	0.011
Ethnicity (%)			
White	71	63	0.039
Asian	16	18	0.531
Black	11	17	0.036
Other	2	2	0.886
ACR (mg/mmol)*	33.0 (5.5-132.5)	21.6 (3.4-94.8)	0.150
eGFR (mL/min)	26 (11)	25 (9)	0.064
IMD score*	7855 (3209-17747)	7683 (3556-16381)	0.860

* data shown as median and interquartile range, Mann-Whitney tests performed, for parametric variables unpaired T-tests performed, for categorical variables Chi squared tests performed

ACR; albumin creatinine ratio, eGFR; estimated glomerular filtration rate, IMD; index of multiple deprivation score

These data illustrate that the patients who declined to participate were significantly older, were more likely to be male and of non-white ethnicity. There was no significant difference in the eGFR, ACR or in the IMD rank.

A multivariate analysis into the variables associated with recruitment was performed, increasing age ($p<0.001$), non-white ethnicity ($p<0.001$) and male gender ($p=0.009$) were all independently associated with non-recruitment into the study.

3.5.Laboratory variables at baseline and six-month visits

The laboratory parameters included in this analysis were haemoglobin, calcium and phosphate, bicarbonate, glycated haemoglobin and uric acid (185, 309); these were selected as they have all been shown to be independently associated with patient outcomes in CKD (162, 165, 192, 310-312).

However the only variable independently associated with outcomes where there is an evidence base that intervention improves outcomes is glycaemic control (313), for the other parameters (with the exception of bicarbonate) there are treatment recommendations, although this is not based on level 1 evidence (40). There are two potential sources of improvement in laboratory variables between baseline, regression to the mean and the recruitment effect.

Recruitment into clinical studies has previously been shown to influence clinical outcomes (314), the only way to determine whether this was a factor in the

RIISC study a control group would need to be studied as there was no control group available it is not possible to exclude this as a source of variability.

To determine the effect of regression to mean I explored changes in variables of interest between visit 1 and visit 2 (6-month), the data are shown in table 8.

Table 3-8: Change in key laboratory parameters between baseline and six-months

	Baseline	Six-months	p-value
Haemoglobin (g/dL)	12.6 (2.2)	12.0 (2.5)	0.091
Corrected calcium (mmol/L)	2.19 (0.31)	2.16 (0.47)	0.192
Phosphate (mmol/L)	1.13 (0.28)	1.15 (0.29)	0.496
Bicarbonate (mmol/L)	24.7 (4.9)	25.5 (14.8)	0.337
Cholesterol (mmol/L)	4.6 (1.2)	4.6 (1.3)	0.689
HbA1C (mol/mmol)	49.3 (19.9)	48.9 (16.6)	0.614
eGFR (mL/min)	27 (10)	26 (10)	0.012
ACR (mg/mmol)*	27.9 (4.8-120.0)	30.3 (5.1-129.0)	0.296

*median (IQR), Mann-Whitney test performed, for parametric variables unpaired T-tests performed

This analysis showed no significant inter-individual changes in any of the variables shown except for eGFR. At a population level unpaired T-tests (as all variables were parametrically distributed) were performed and again there were no significant differences between the time-points.

The difference between the eGFR at baseline and six-months was examined, though it must be appreciated that true progression cannot be accurately identified using just two estimates of GFR or over such a short period of time. The mean difference in eGFR was -0.69(4.7) mL/min. Paired T-tests revealed that the eGFR at 6 months was significantly lower than baseline eGFRs, p=0.012.

3.6.The demographics of the RIISC cohort compared to other established CKD cohorts

As described in chapter 1 and chapter 2 there are a number of other established CKD cohorts, these cohorts have been drawn from different populations with different aims and objectives. Where data are available the baseline demographic characteristics of these other cohorts have been compared to the RIISC cohort in table 9. To convert ACR data from mg/g to mg/mmol multiply by 0.113.

Table 3-9: Comparison of baseline characteristics of other prospective observational CKD cohorts

	Number	Mean age	% males	%white	Mean eGFR	Urinary protein excretion	Co-morbidity
CRIB (128)	382	61.5 (10.7)	64.9	88	21.8 (10.7)mL/min/1.73m^2	460 (88.1-257)mg/g	44.8% CVD 17.3% DM
MMKD (119)	227	45.7 (12.6)	68	100	70 (42)mL/min/1.73m^2	0.9 (0.9)g/24hr	NA
LCKD (120)	622	60.4	56	75	23.2mL/min/1.73m^2	NA	27% CVD 38% DM
CRISIS (121, 315)	1325	65.1	63.7	NA	30.9mL/min/1.73m^2	0.8g/24hr	47% CAD 32% DM
CRIC (122)	3612	58.2 (11)	54	45	43.3 (13.5)mL/min/1.73m^2	0.17 (0.07-0.81)g/24hr	22% CAD 47% DM
SEEK (124)	1814	71.1	47.7	87.9	47 (17.7)mL/min/1.73m^2	NA	33% CAD 48% DM
R^{2}ID (132)	1741	72.9 (9)	40	97.5	52.5 (10.4)mL/min/1.73m^2	0.33 (0-0.15)mg/mmol	22% CVD 17% DM
CKD-JAC (316)	2977	60.8 (11.6)	62.1	0	28.6 (11.8)mL/min/1.73m^2	976 (1340) mg/g	15% IHD 38% DM
RIISC	500	65 (16)	60	72	27 (12) mL/min	26.9 (5.4-107.8) mg/mmol	24% IHD 39% DM

Abbreviations: CRIB, Chronic Renal Impairment In Birmingham; MMKD, Mild to Moderate Kidney Disease; LCKD, Longitudinal Chronic Kidney Disease; CRISIS, Chronic Renal Insufficiency In Salford; CRIC, Chronic Renal Insufficiency Cohort; SEEK, Study to Evaluate Early Kidney Disease; RRID, Renal Risk In Derby; CVD, Cardiovascular disease; DM, Diabetes Mellitus; CAD, Coronary Artery Disease; IHD, Ischaemic Heart Disease.

3.7.Discussion and conclusions

This is a cohort of individuals who met criteria for secondary care follow up as directed by national guidance (61), the cohort is drawn from the Birmingham area where the local population is ethnically diverse (in the 2011 census 5.5% of the population were of South Asian origin (317)) . Consistent with this RIISC cohort was comprised of a substantial proportion of non-white participants, mainly of South Asian ethnicity (who are known to be at enhanced risk of both CKD and CVD, largely as a consequence of increased prevalence of diabetes within this population (318)).

The analysis of the eligible versus recruited population illustrated that South Asian patients were appropriately represented in the recruited population, however Black individuals (who have been shown to be over-represented in renal replacement programs in the United States (319)) are under-represented in the cohort. It is not clear what the barriers to research participation were in this group, detailed data on reasons for non-consent by ethnic group were not collected.

Population based work from the UK has shown that the prevalence of CKD increases with increasing age; in a large, primary care based dataset more than 70% of patients with an eGFR <30mL/min/1.73m^2 were older than 70 years of age (320). Indeed, much of the CKD found in older individuals is at the less severe end of the spectrum and may be managed solely in primary care (a

finding supported by the older age of the participants in the R²ID cohort (132)). The finding that the mean age of the cohort is 65 years of age is consistent with a secondary care, high-risk cohort.

Recent published data from the CKD prognosis consortium has confirmed that the risk of ESKD and death is higher in individuals with lower eGFRs and higher levels of proteinuria (70); for RIISC the level of proteinuria is higher than all other cohorts reported to date except for CRIB; proteinuria levels are maintained across all age ranges and stages of CKD by eGFR, confirming that this is a high risk cohort. In a separate analysis from the same cohort males were found to have an increased all-cause and cardiovascular mortality at all levels of eGFR and proteinuria compared to women but that decreased eGFR and increased albuminuria placed both genders at increased risk of progression to ESKD (135).

There is a well described association between cardiovascular disease and CKD (45), the presence of established cardiovascular disease at recruitment in a significant proportion of the cohort is in keeping with the observations of other researchers (118, 124, 315). The finding that overall co-morbidity increases with increasing severity of CKD in this cohort can be explained by increasing age with severity of CKD, however the Charlson index used provides an age adjusted score to account for this and this is not an observation that has been previously published. An explanation for this observation might be that CKD progression shares risk factors with other co-morbidities (in addition to the well described

overlap in risk factors for CKD and CVD) such as malignancy and chronic respiratory disease.

The co-existence of diabetes and CKD is well known; the prevalence of diabetes in this cohort is consistent with other secondary care CKD cohorts and with the findings of the CKD prognosis consortium's recent meta-analysis (135). In this cohort diabetic individuals have significantly higher body mass indexes and waist and hip circumference, although there was no significant increase in waist to hip ratio, in contrast with previous work that has suggested that central obesity is prevalent in individuals with diabetes and CKD (321).

The baseline demographic and descriptive characteristics of the cohort indicate that the recruited population is broadly representative of the eligible population and that there are consistencies between this and other prospective CKD cohorts; the high prevalence of established vascular disease, diabetes, proteinuria and reduced eGFR at inception suggest that this is a cohort of individuals at substantial cardiovascular and renal risk. In the next chapter I describe the baseline socioeconomic status and quality of life characteristics of RIISC.

4. Results 2: What are the lifestyle and socio-economic characteristics of RIISC participants and what impact does CKD have on self-reported quality of life?

The data presented here relates to the first 500 participants recruited to the RIISC study, and the 305 who had undergone a six-month follow up visit at the time of the analysis. Data are presented as means with standard deviation or medians with interquartile ranges. The data has been used to analyse how QoL relates to other variables of importance collected as part of the study.

4.1.The socio-economic phenotype of the cohort

Participants were asked questions relating to socio-economic status including educational attainment and employment (both whether they were employed or not and what their current or last job was); the questions that were asked are included in appendix 8. Data relating to the baseline socioeconomic status are shown in table 1.

The IMD rank was used to stratify levels of deprivation, this utilises the full postal code. A rank of 1 represents the most deprived area and a score of 32482 the least deprived area it is also possible to use the IMD score (which is used to compare areas, a limitation of using the rank rather than score data is that differences in deprivation may be more difficult to detect). The median IMD rank was 7576 (2874-15140). There was no significant difference in IMD rank when analysed by CKD stage (p=0.768).

Table 4-1: The socio-economic status of the RIISC cohort by CKD stage

	All n=494	1&2 n=11	3A n=28	3B n=121	4 n=294	5 n=40	p-value
Highest qualification:							
None	49	27	14	43	56	45	<0.002
GCSE/O' level	21	37	21	24	19	25	0.428
NVQ	8	0	7	5	10	5	0.300
A'level	8	9	22	9	5	10	0.037
Undergraduate degree	10	9	25	16	7	5	0.010
Higher degree	4	18	11	3	3	10	0.010
Current employment status:							
Unemployed	19	9	18	18	21	23	<0.002
Employed	28	73	54	36	20	55	<0.002
Retired	53	18	28	46	59	22	<0.001
Current or last job type:							
Unskilled manual	23	20	8	16	27	22	0.086
Skilled manual	38	20	27	42	37	42	0.464
Clerical	14	10	15	15	15	11	0.969
Managerial	8	20	15	9	7	3	0.237
Professional	17	30	35	18	14	22	0.048

Results shown as %, Chi squared tests performed

These data show a significant difference in both formal qualifications and being of working age but unemployed between stages of CKD.

Data were also collected regarding smoking habits (past and current) and alcohol consumption. Thirteen per-cent of the cohort were current smokers (n=67); 41% had previously been smokers (n=207). Of those who were either current or past smokers the smoking exposure was calculated using pack years (number of cigarettes smoked a day multiplied by the number of years of smoking divided by 20); the mean number of pack years was 25 (22).

With regard to alcohol consumption, just under 50% regularly drank alcohol (n=229) and 5% (n=23) reported regularly consuming more than the recommended limits of alcohol (more than 14 units a week for women and more than 21 units a week for men).

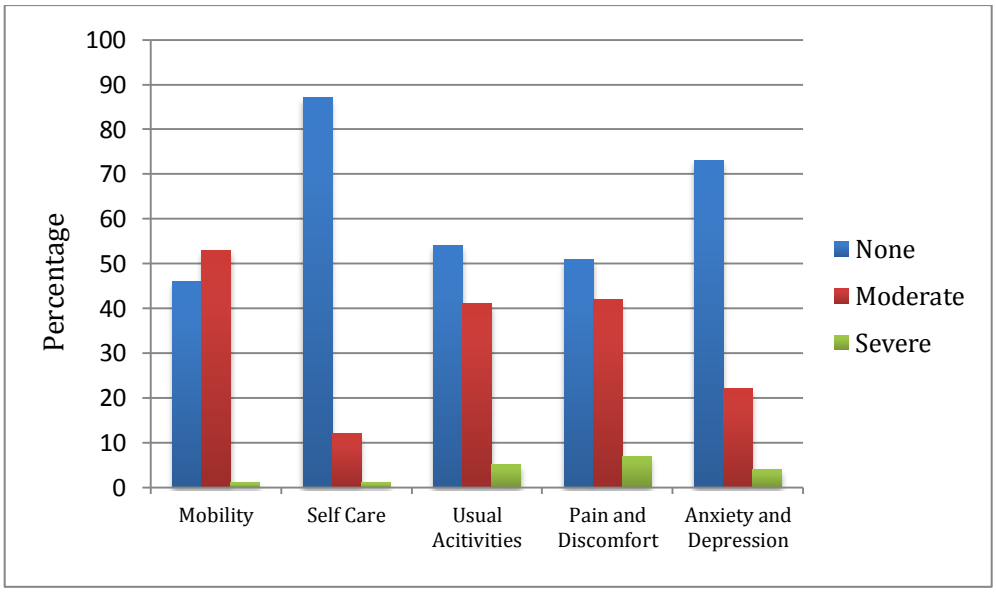
4.2. Functional status and symptom burden in RIISC participants: the baseline phenotype and the determinants of impaired functional status and symptom burden

The EQ5D instrument is comprised of two sections; a five domain structured questionnaire where participants are given three options for each domain and a VAS where participants are asked to rate their health from 0-100 (where a score of 0 represents the worst perceived health and a score of 100 the best perceived health). The instrument was administered by a research nurse at the time of initial consent and at each study visit (the EQ5D is included in appendix 9). Here

I report the results of the structured section, which deals with physical functioning and symptom burden.

The five domain questionnaire contains the following sections; mobility, self-care, usual activities (work, study, housework or seeing family or friends), pain/discomfort and anxiety/depression. In each section participants could indicate that they either had no problems, some problems or severe problems (each domain is worded for clarity, for example in the mobility domain severe problems are described as 'being immobile'). The scores in each domain are presented in figure 1.

Figure 4-1: The baseline five domain EQ5D scores



These data show that the prevalence of reported "severe" problems in each domain was very low.

Responses in this section where then dichotomised to whether the individual had indicated that they had either no problems/symptoms in each domain or moderate or severe problems in each domain. Using this method 54% reported moderate or severe impairment of mobility, 13% reported some or severe difficulties performing self-care, 46% reported some or severe difficulties performing usual activities, 49% reported moderate or severe pain or discomfort and 27% reported moderate or severe anxiety or depression.

To identify variables that might be associated with these outcomes, univariate and then multi-variate analyses were performed of a series of established and dynamic phenotypic characteristics (these are shown in figure 2) the dynamic phenotype refers to those variables that can be considered to be modifiable and the established phenotype refer to those that are fixed; variables that reached a significance of <0.1, as indicated by symbols, were included in the multi-variate analyses shown in tables 2-6.

Figure 4-2: Variables from the established and dynamic phenotype that may influence functional status and symptom burden

The established phenotype	The dynamic phenotype
Age*❖§✦	Current smoking
Gender§¥	Current alcohol intake❖
Ethnicity	BMI❖§✦
Diabetes*§✦	WHR❖¥
CVD*§	Hb
COPD	eGFR*§❖¥
Malignancy*§✦	HbA1C*❖§✦¥
Co-morbidity index*❖§✦	hsCRP*❖§✦
Previous smoking*	Unemployment*❖§✦¥
IMD rank*§✦❖¥	

* moderate or severe impairment: mobility

❖ moderate or severe impairment: self-care

§ moderate or severe impairment: usual activities

✦ moderate or severe impairment: pain/discomfort

¥moderate or severe impairment: anxiety/depression

Abbreviations: CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease; IMD, index of deprivation; BMI, body mass index; WHR, waist hip ratio; Hb, haemoglobin; eGFR, estimated glomerular filtration rate; HbA1C, glycated haemoglobin; hsCRP, highly sensitive CRP.

Table 4-2: Multivariate analysis of variables associated with impaired mobility

	p-value	Odds ratio	95% confidence intervals
Age	0.001	1.055	1.022, 1.089
Unemployment	<0.002	6.437	2.771, 14.954
Diabetes	0.791		
Cardiovascular disease	0.714		
Malignancy	0.373		
Co-morbidity	0.838		
Previous smoking	0.713		
Rank of deprivation	0.966		
eGFR	0.828		
HbA1C	0.986		
hsCRP	0.883		

Table 4-3: Multivariate analysis of variables associated with impaired self-care

	p-value	Odds ratio	95% confidence intervals
Unemployment	0.007	8.882	1.836, 42.961
BMI	0.021	1.089	1.013, 1.172
Age	0.489		
Co-morbidity	0.507		
HbA1C	0.689		
hsCRP	0.197		

Table 4-4: Multivariate analysis of variables associated with impaired usual activities

	p-value	Odds ratio	95% confidence intervals
HbA1C	0.011	1.032	1.007, 1.057
Unemployment	0.000	6.732	2.836, 13.878
Age	0.195		
Male gender	0.089		
Diabetes	0.913		
Cardiovascular disease	0.366		
Malignancy	0.900		
Co-morbidity	0.974		
Rank of deprivation	0.998		
BMI	0.850		
eGFR	0.432		
hsCRP	0.273		

Table 4-5: Multivariate analysis of variables associated with increased pain/discomfort

	p-value	Odds ratio	95% confidence intervals
Unemployment	<0.002	5.896	2.796, 12.554
Age	0.100		
White ethnicity	0.984		
Black ethnicity	0.256		
Diabetes	0.545		
Malignancy	0.616		
Co-morbidity	0.202		
Index of deprivation	0.683		
Regular alcohol intake	0.761		
BMI	0.499		
HbA1C	0.776		
hsCRP	0.554		

Table 4-6: Multivariate analysis of variables associated with increased anxiety/depression

	p-value	Odds ratio	95% confidence intervals
Male gender	0.028	2.118	1.083, 4.143
Rank of deprivation	0.025	1.000	1.000, 1.000
Unemployment	0.022	2.179	1.117, 4.249
eGFR	0.877		
WHR	0.718		
HbA1C	0.667		

These data show that unemployment was independently associated with moderate or severe problems in each domain.

4.3.Self reported health perception in RIISC participants: the baseline phenotype and the determinants of poor perception of health

The scores obtained from the VAS were parametrically distributed with a mean score of 62 (21). To identity correlations between the VAS and other continuous variables a series of linear regressions was performed. The results are shown in table 7.

Table 4-7: Linear regression between VAS and other continuous variables

		Age	Charlson co-morbidity	BMI	eGFR	Hb	ACR*	HbA1C*	hsCRP*	IMD rank*
VAS	Pearson's	-0.157	-0.204	-0.172	0.119	0.044	0.021	-0.178	-0.218	0.132
	significance	<0.002	<0.002	<0.002	0.008	0.334	0.641	<0.002	<0.002	0.003

VAS, visual analogue scale; BMI, body mass index; eGFR, estimated glomerular filtration rate; ACR, albumin creatinine ratio; HbA1C, glycated haemoglobin; hsCRP, highly sensitive C-reactive protein; IMD, index of deprivation. *data log transformed to achieve normal distribution

These data show that there are significant correlations between increasing age, increasing co-morbidity, increasing BMI, decreasing eGFR, increasing HbA1C, increasing hsCRP and lower VAS scores.

To further explore the potential determinants of impaired self perceived health status the VAS score were dichotomised into whether or not an individual had a VAS score in the bottom quartile of scores or not. A univariate analysis of variables associated with bottom quartile VAS scores was performed; the variables included are shown in figure 3. Those variables that reached significance (<0.1) in the univariate analysis are indicated with an asterix and were included in the multivariate analysis; the results of which are shown in table 8.

Figure 4-3: Variables from the established, dynamic and functional phenotypes included in the univariate analysis

The established phenotype	The dynamic phenotype	The functional phenotype
Age	Current smoking	Impaired mobility*
Gender	Current alcohol intake	Impaired self-care*
Ethnicity*	BMI*	Impaired usual activities*
Diabetes	WHR	Pain/discomfort*
CVD	Hb	Anxiety/depression*
COPD	eGFR	
Malignancy	HbA1C*	
Co-morbidity index*	hsCRP*	
Previous smoking	Unemployment*	
	IMD rank	

Abbreviations: CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease; IMD, index of deprivation; BMI, body mass index; WHR, waist hip ratio; Hb, haemoglobin; eGFR, estimated glomerular filtration rate; HbA1C, glycated haemoglobin; hsCRP, highly sensitive CRP

Table 4-8: Multivariate analysis of variables associated with poor perception of health state

	p-value	Odds ratio	95% confidence intervals
HbA1C	0.045	1.025	1.001, 1.050
hsCRP	0.028	1.041	1.004, 1.080
The presence of moderate or severe anxiety or depression	0.001	5.024	1.967, 12.828
White ethnicity	0.581		
Black ethnicity	0.265		
Co-morbidity	0.227		
BMI	0.268		
Unemployment	0.463		
Presence of moderate or severely impaired mobility	0.501		
Presence of moderate or severely impaired self care	0.195		
Presence of moderate or severely impaired usual activities	0.205		
Presence of moderate or severe pain or discomfort	0.215		

These data show that poor glycaemic control, increased systemic inflammation and moderate or severe depression were all independently associated with low VAS scores.

4.4.Variability in self-reported functional status and symptom burden between baseline and six-months

The results obtained form the five-domain section of the EQ5D were compared for those individuals who had undergone both baseline and six month visits, the data were again dichotomised to those who indicated that they had moderate or severe problems in each category compared to those who indicated they had no problems in each category. The results are shown graphically in figure 4(a-e).

Figure 4-4: The five domain section of the EQ5D at baseline and six-months

Figure 4a: The percentage of patients with moderate or severely impaired mobility at baseline and six-months

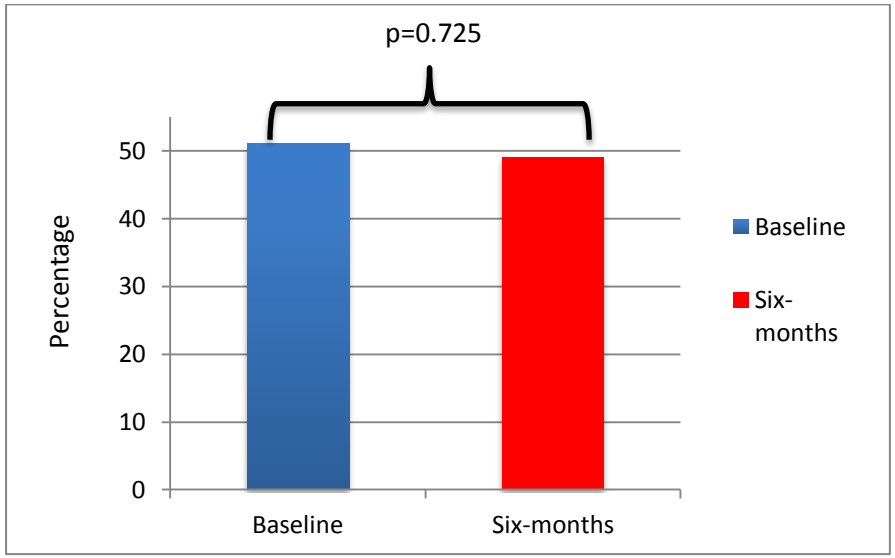


Figure 4b: The percentage of patients with moderate or severely impaired self-care at baseline and six-months

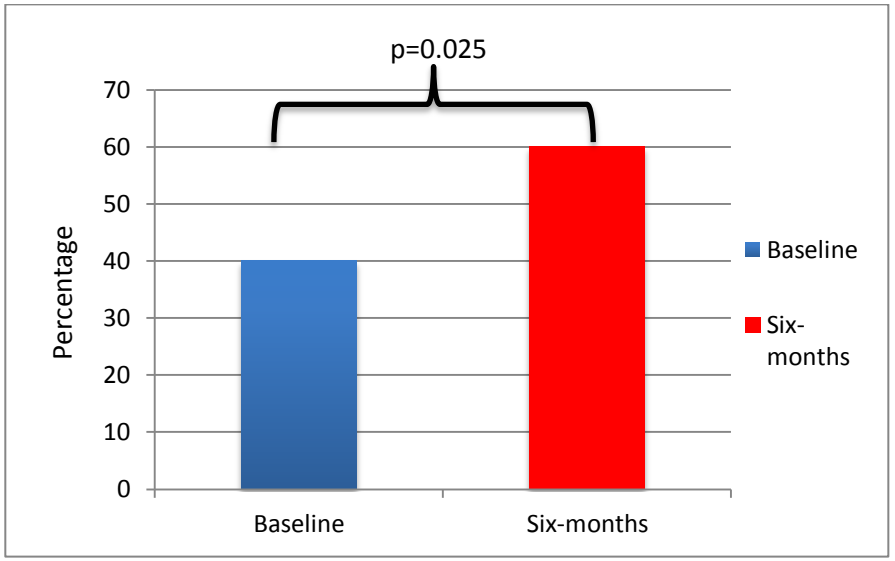


Figure 4c: The percentage of patients with moderate or severely impaired usual activities at baseline and six-months

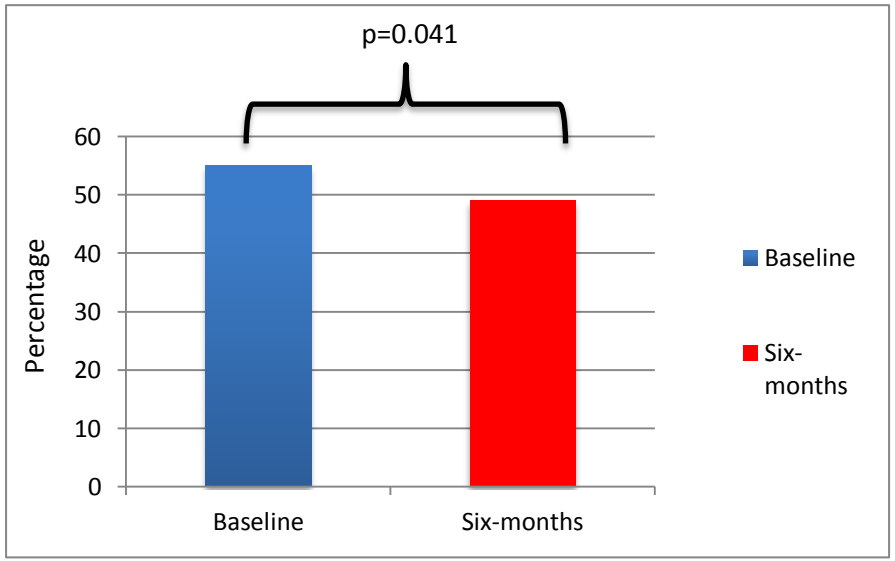


Figure 4d: The percentage of patients with moderate or severe pain or discomfort at baseline and six-months

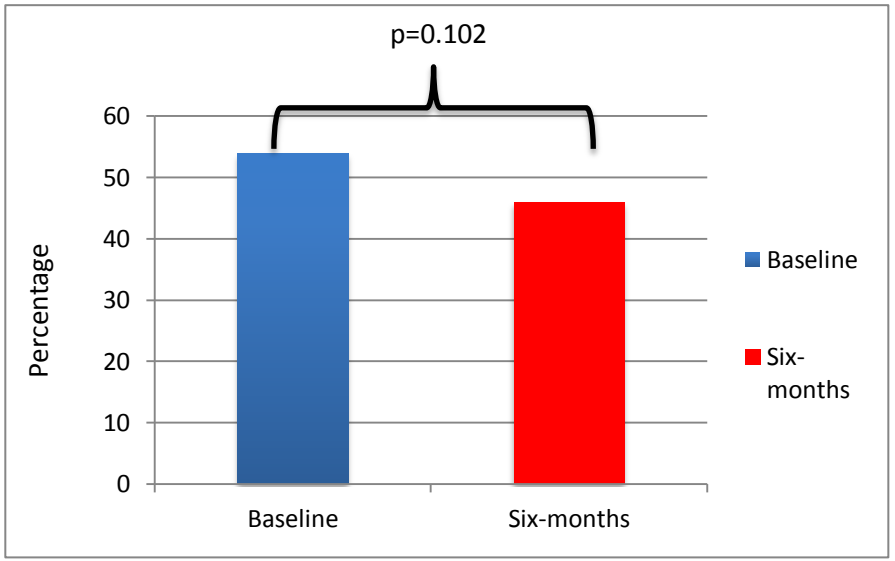
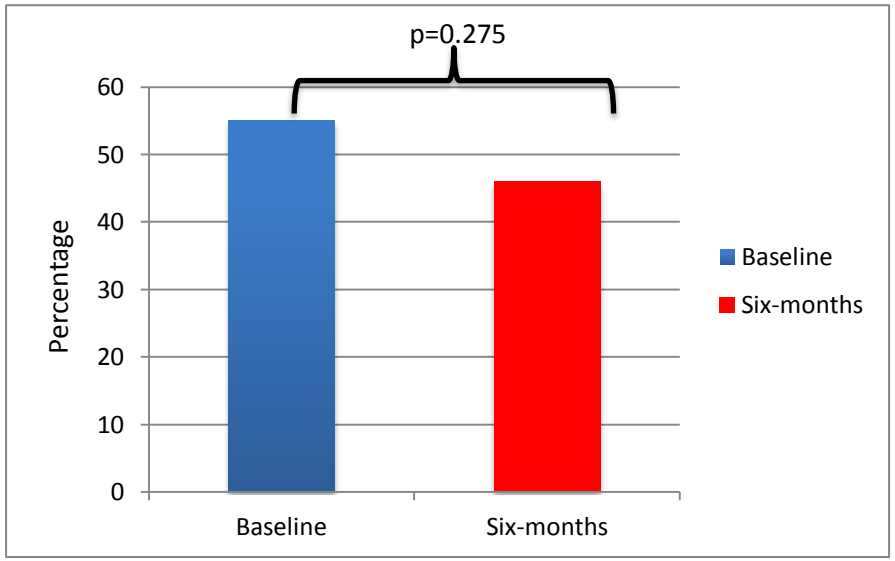


Figure 4e: The percentage of patients with moderate or severe anxiety or depression at baseline and six-months

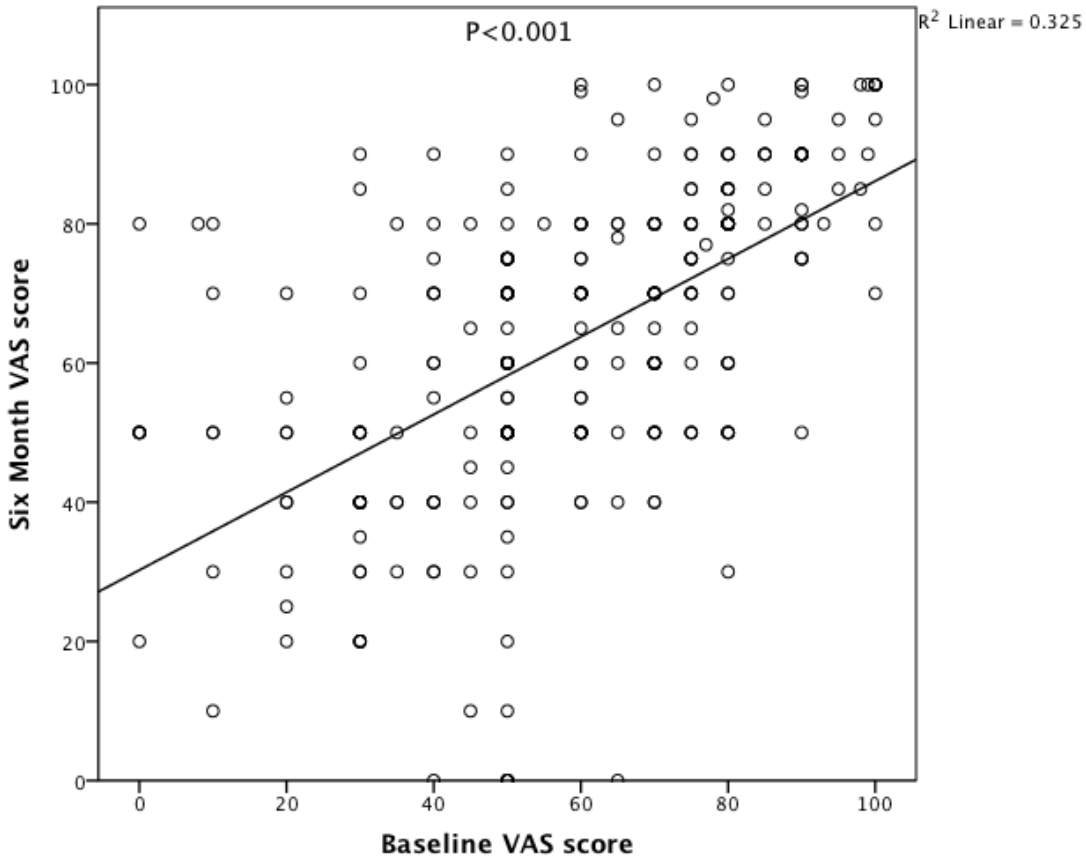


These data show that there is no significant difference in the prevalence of moderate or severe mobility problems, moderate or severe pain or discomfort or anxiety or depression between baseline and six-months; however more participants reported moderate or severe impairment of self-care at six-months than baseline and fewer reported moderate or severely impaired performance of usual activities at six-months than baseline.

4.5.Variability in health perception between baseline and six-months

To examine the correlation between the VAS scores (on a population level) at each time-point for those participants who had attended both visits a linear regression was performed. The results are shown graphically in figure 5.

Figure 4-5: The correlation between baseline and six-month VAS scores



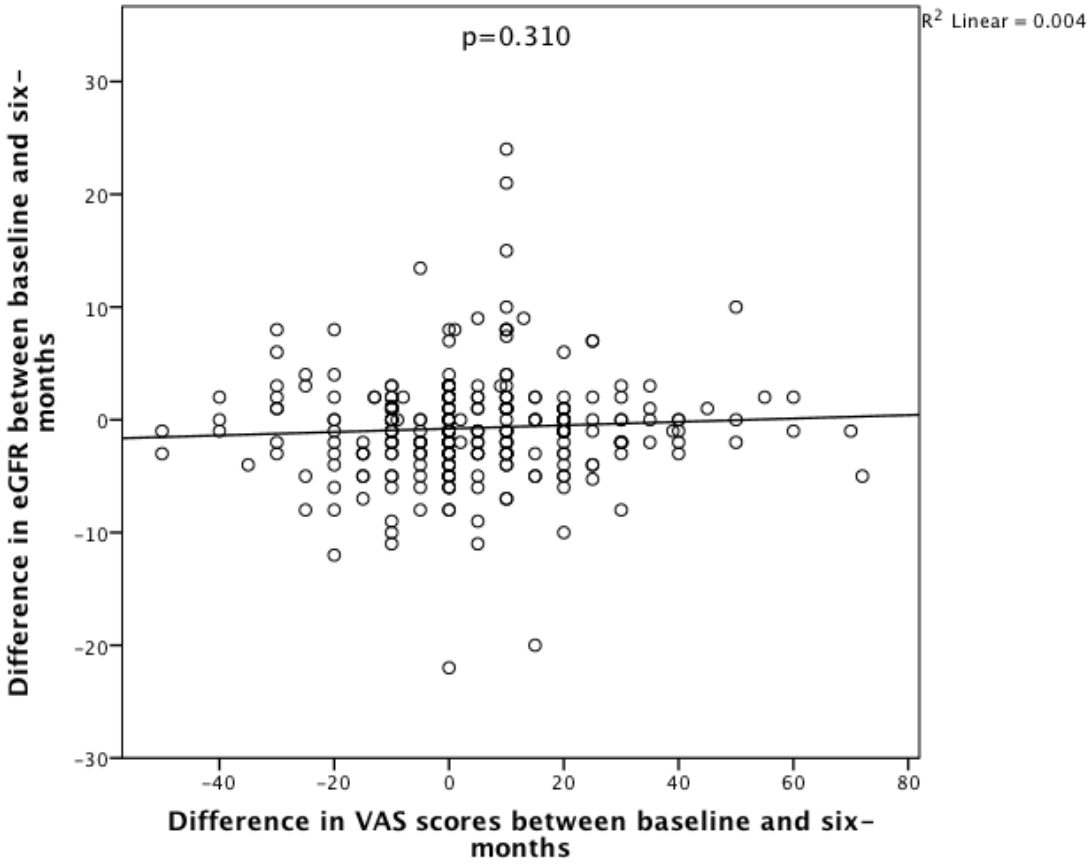
This illustrates a positive correlation between baseline and six-month VAS scores.

To examine the intra-individual variation between the time-points a paired T-test was performed, this showed that the six-month scores were significantly higher than the baseline scores ($p<0.002$). It is not clear why this was, an explanation may be that by the six-month visit participants knew what to expect from the questionnaire and might have given more considered responses at this stage.

4.6.Influence of key clinical variables on self reported health state

In the first results chapter I demonstrated that there was a significant drop in eGFR between baseline and six-month visits. I hypothesised that this might influence self-perception of health state. Correlations between changes in eGFR and changes in VAS scores are shown in figure 6

Figure 4-6: Correlation between changes in eGFR and changes in perception of health state



It can be seen that there is no correlation between declining kidney function and changes in VAS scores.

4.7.Conclusions

This analysis focused on the first 500 patients recruited to the RIISC study. The large majority of the data that I have presented in this chapter are from the baseline visit and describe cross-sectional relationships. As the study matures a dynamic picture of the relationship between QoL and key bio-clinical characteristics will develop and this will produce a strong resource for assessing which variables are the major determinants of changing QoL with time.

There is an increasing focus on QoL and patient reported outcomes in chronic disease. This is of great relevance for patients with CKD, where there is a lack of high quality RCTs of intervention, particularly for patients with stages 4 and 5 CKD. As shown in the data presented in this chapter, a large proportion of patients with CKD have significant impairment of QoL. Focusing on interventions that can improve QoL could lead to real improvements for patients with CKD.

Where other CKD cohorts have reported the socio-economic status of the participants some of the findings are consistent with this study. For example, 33% of the CRIC cohort had less than a high school education and 48% were unemployed, findings which are consistent with RIISC (322). Factors associated with poverty and socio-economic status have been shown to influence rate of progression to ESKD and future work will identify if this association is also present in the RIISC cohort (323).

The analysis that I have performed identified associations between both potentially modifiable (or dynamic) and non-modifiable (established) risk factors. The rationale for taking this approach to the analysis was that better identification of the impact of modifiable risk factors on QoL would direct future studies of intervention, although this distinction requires care as some potentially modifiable risk factors may be difficult to change.

For policy makers, one important observation from RIISC is the association between unemployment and functional status and symptom burden. In one other study that has utilized EQ5D to measure health related QoL in patients with CKD progressive CKD, anaemia, malnutrition, diabetes and cardiovascular disease associated with worse QoL; unemployment was not considered (324).

A further interesting observation in this analysis is the independent association between both glycaemic control and inflammation and QoL. This requires further assessment, as both glycaemic control and inflammatory status may be important areas for intervention.

The primary limitation of the data described in this chapter is that the study is not designed principally a study of the impact of CKD upon QoL and functional status and therefore comprehensive assessments of functional status and QoL were not used. The EQ5D is a validated measure but provides only limited information, other instruments that provide more detailed information were not used because while they provide more detailed (and often disease specific) information they are often time-consuming and difficult to administer. A further limitation may be the method used to assess socio-economic status, in a detailed review of this area Braveman et al described the many methods available for assessing socio-economic status and recognized the limitations of any given system (264).

5. Results 3: The cardiovascular phenotype of the RIISC cohort

The major association between CKD and poor clinical outcomes is with increased morbidity and mortality from CVD. Therefore ascertaining the cardiovascular phenotype of a high risk CKD population is of great importance both in measuring the pre-existing burden of CVD and assessing those factors that are likely determinants of long-term clinical outcomes. I have divided the cardiovascular phenotype into established (variables that are irreversible) and dynamic (those where there is potential for reversal with a putative impact on outcomes).

In this chapter I have analysed data from the first 500 participants recruited to the RIISC study and focused on defining the cardiovascular phenotype of the cohort and the relationship of this to other bio-clinical features of interest. In addition to confirming associations that have been demonstrated in other cohort studies, the analysis shows novel associations that may be important as non-traditional risk factors for CVD in people with CKD.

The overall demographics of the cohort were presented in chapter 3. In this chapter baseline data are presented on both established CVD and dynamic markers of cardiovascular risk such as BP (utilizing the BpTru™ system), vascular phenotype (by the Vicorder™ system) and AGE (by skin autofluorescence). The methods used to measure these data are described in chapter 2 and detailed SOPs are included in the appendices 2 and 3.

The mean age of patients with any established CVD was 68 (14), significantly older than those without established CVD ($p<0.001$), 78% of participants with CVD were of White ethnicity (37% of White participants had CVD compared to 29% of Asian participants and 21% of Black participants), 62% of participants with CVD were male (35% of male participants and 33% of female participants had CVD).

Table 1 shows the established CVD burden of the cohort by CKD stage and table 2 the dynamic variables contributing to CVD risk by CKD stage; all continuous variables shown in the table were parametrically distributed so mean and standard deviation data are shown. For 13% of the cohort skin autofluorescence could not be performed because skin pigmentation precluded successful measurement.

Table 5-1: The baseline established cardiovascular phenotype of RIISC participants

	All	1&2	3A	3B	4	5	p-value
Ischaemic Heart Disease (%)	24	18	18	23	23	35	0.464
Cerebrovascular Disease (%)	12	9	14	14	13	13	0.507
Peripheral Vascular Disease (%)	12	9	4	13	13	8	0.526
Any Cardiovascular Disease (%)	34	18	32	39	33	38	0.526

Chi-squared tests performed

Table 5-2: The baseline dynamic cardiovascular phenotype of RIISC participants

	All	1&2	3A	3B	4	5	p-value
Systolic BP (mmHg)	129 (23)	125 (17)	123 (16)	129 (22)	129 (24)	133 (22)	0.423
Diastolic BP (mmHg)	75 (14)	81 (9)	77 (11)	77 (12)	75 (14)	74 (17)	0.440
Peripheral MAP (mmHg)	94 (16)	95 (11)	92 (11)	95 (13)	93 (15)	94 (15)	0.906
Peripheral PP (mmHg)	53 (20)	45 (13)	46 (16)	51 (20)	54 (19)	59 (22)	0.027
Central MAP (mmHg)	105 (16)	100 (10)	103 (10)	105 (17)	105 (15)	106 (15)	0.775
Central PP (mmHg)	65 (19)	55 (9)	57 (20)	65 (18)	66 (20)	69 (18)	0.039
AIx (%)	21 (10)	18 (6)	20 (8)	22 (10)	20 (10)	24 (9)	0.192
PWV (m/s)	10.2 (2.3)	9.2 (2.6)	9.3 (1.8)	10.0 (2.6)	10.3 (2.3)	10.3 (1.9)	0.100
AGEs (AU)	3.0 (0.8)	2.2 (0.7)	2.7 (0.7)	2.9 (0.8)	3.1 (0.8)	3.4 (0.8)	<0.01

Data shown as mean (standard deviation (SD)), ANOVA performed; BP, blood pressure; MAP, mean arterial pressure; PP, pulse pressure; AIx, augmentation index; PWV, pulse wave velocity; AGEs, advanced glycation end products; AU, arbitrary units

These data show that there is no significant difference in the established CV phenotype by stage of CKD. There was a significant increase in AGEs and peripheral pulse pressure (PP) and central PP between the stages of CKD. The presence of established CVD was significantly associated with AGE accumulation and higher peripheral PP ($p<0.02$ and $p=0.051$ respectively) but not other measures of peripheral or central BP or PWV.

The cohort was comprised of 39% diabetic participants and as diabetes is associated with CVD risk I hypothesised that both the presence of established CVD and the dynamic cardiovascular risk burden would be significantly higher in diabetics compared to non-diabetics. To address this hypothesis I performed a comparative analysis between diabetic patients and non-diabetic patients, the results of this are shown in tables 3 and 4.

Table 5-3: The established cardiovascular phenotype based on the presence or absence of diabetes

	Non-Diabetics	Diabetics	p-value
Ischaemic Heart Disease (%)	16	37	<0.01
Cerebrovascular Disease (%)	10	16	0.026
Peripheral Vascular Disease (%)	7	20	<0.01
Any Prior Cardiovascular Disease (%)	23	51	<0.001

Chi-squared tests performed

Table 5-4: The dynamic cardiovascular phenotype by the presence or absence of diabetes

	Non-Diabetics	Diabetics	p-value
Systolic BP (mmHg)	127 (23)	132 (22)	0.005
Diastolic BP (mmHg)	76 (14)	75 (12)	0.428
Peripheral MAP (mmHg)	93 (15)	94 (13)	0.337
Peripheral PP (mmHg)	51 (19)	56 (20)	0.002
Central MAP (mmHg)	105 (16)	106 (17)	0.266
Central PP (mmHg)	63 (18)	68 (20)	0.001
AIx (%)	21 (10)	20 (10)	0.545
PWV (m/s)	9.9 (2.4)	10.6 (2.3)	0.002
AGEs (AU)	2.9 (0.8)	3.1 (0.8)	0.004

Data shown as mean (standard deviation (SD)), unpaired T-tests performed; BP, blood pressure; MAP, mean arterial pressure; PP, pulse pressure; AIx, augmentation index; PWV, pulse wave velocity; AGEs, advanced glycation end products; AU, arbitrary units

Patients with diabetes had a greater burden of pre-existing CVD, higher systolic BP, peripheral and central PP, PWV, and AGEs; this indicated more established macrovascular and microvascular disease well as more dynamic risk in patients with diabetes than patients without diabetes.

A possible explanation for this observation is that patients with diabetes have worse kidney function and higher levels of proteinuria than those without diabetes and that the difference in kidney function and proteinuria might be responsible for the difference in vascular measures. To assess this an analysis was performed to look for differences in kidney function and proteinuria between diabetic and non-diabetic patients; an important limitation of this analysis is that as this is a referred cohort it may not be representative of patients managed in primary care.

The percentage of patients with diabetes in stage 5 CKD was 42% compared to 30% in stage 1 and stage 2 CKD, therefore to assess if there was an association between kidney function and arterial stiffness in patients with and without diabetes, I compared kidney function between the groups. This analysis demonstrated no significant difference in eGFR between patients with diabetes and those without (26 (10) and 28 (13) mL/min/1.73m^2 respectively, p=0.109). There was also no significant difference in ACR between patients with and without diabetes (22.7 (5.1-92.5) and 33.0 (6.9-128.3) mg/mmol respectively, p=0.117 (Mann-Whitney test)).

A further analysis of macro and microvascular status in participants with and without diabetes will be described in section 4.

5.1. Socio-economic status and the established and dynamic cardiovascular phenotype

The impact of socio-economic factors such as educational attainment (325), employment type (326) (though the role of unemployment is controversial and while increased CVD risk has been described in unemployed individuals this may be due to the co-existence of other risk factors) (327, 328) deprivation are known to influence cardiovascular health (329). To explore the baseline associations between the established and dynamic cardiovascular phenotype in RIISC participants by variables associated with SES a series of analyses were undertaken, continuous data were parametrically distributed and so T-tests were performed, the results are shown in tables 5-8.

Table 5-5: The established cardiovascular phenotype and SES

	No CVD	Prior CVD	p-value
No formal qualifications (%)	46	54	0.129
Unemployment (%)	39	48	0.239
Unskilled employment (%)	23	22	0.880
IMD rank*	8049 (2778-16081)	6916 (2894-13870)	0.388
Current smoking (%)	14	12	0.622
Previous smoking (%)	39	46	0.098

For categorical variables Chi-squared tests performed, *non-parametric Mann-Whitney test performed

Table 5-6: The dynamic cardiovascular phenotype by the presence of unemployment

	Working age and unemployed	Working age and employed	p-value
Peripheral MAP (mmHg)	97 (14)	96 (13)	0.608
Peripheral PP (mmHg)	51 (20)	44 (14)	0.001
Central MAP (mmHg)	107 (19)	105 (14)	0.422
Central PP (mmHg)	63 (18)	55 (13)	<0.001
AIx (%)	20 (13)	18 (10)	0.314
PWV (m/s)	9.4 (2.0)	8.8 (1.8)	0.031
AGEs (AU)	2.9 (0.9)	2.5 (0.6)	0.002

Data shown as mean (standard deviation (SD)) unpaired T tests performed; BP, blood pressure; MAP, mean arterial pressure; PP, pulse pressure; AIx, augmentation index; PWV, pulse wave velocity; AGEs, advanced glycation end products; AU, arbitrary units

Table 5-7: The dynamic cardiovascular phenotype by educational attainment

	No formal qualifications	Formal qualifications	p-value
Peripheral MAP (mmHg)	93 (16)	94 (13)	0.493
Peripheral PP (mmHg)	59 (20)	47 (17)	<0.001
Central MAP (mmHg)	106 (17)	105 (15)	0.367
Central PP (mmHg)	70 (10)	60 (17)	<0.001
AIx (%)	22 (9)	20 (11)	0.117
PWV (m/s)	10.8 (2.3)	9.5 (2.1)	<0.001
AGEs (AU)	3.2 (0.8)	2.8 (0.8)	<0.001

Data shown as mean (standard deviation (SD)) unpaired T tests performed; BP, blood pressure; MAP, mean arterial pressure; PP, pulse pressure; AIx, augmentation index; PWV, pulse wave velocity; AGEs, advanced glycation end products; AU, arbitrary units

Table 5-8: The dynamic cardiovascular phenotype by employment type

	Unskilled manual labour	Other employment type	p-value
Peripheral MAP (mmHg)	95 (14)	93 (15)	0.337
Peripheral PP (mmHg)	58 (21)	51 (19)	0.004
Central MAP (mmHg)	106 (22)	104 (14)	0.470
Central PP (mmHg)	67 (22)	64 (18)	0.100
AIx (%)	20 (12)	21 (10)	0.496
PWV (m/s)	10.4 (2.4)	10.1 (2.3)	0.167
AGEs (AU)	2.9 (0.9)	2.9 (0.8)	0.983

Data shown as mean (standard deviation (SD)) unpaired T tests performed; BP, blood pressure; MAP, mean arterial pressure; PP, pulse pressure; AIx, augmentation index; PWV, pulse wave velocity; AGEs, advanced glycation end products; AU = arbitrary units

These data suggest that in this cohort SES has little impact upon the established cardiovascular phenotype but the dynamic phenotype appears to be influenced by working age unemployment and educational attainment but less by employment type. The lack of relationship with employment type may reflect the subjective nature of the way that employment data were collected (in contrast

there was little subjectivity in the interpretation of whether an individual had certain qualifications or not and whether they were currently employed or not). It is not clear from these analyses whether the relationship between SES and the dynamic cardiovascular phenotype is confounded by the presence of other risk factors.

5.2.Kidney function and dynamic macrovascular health

To assess the relationship between markers of kidney function, Pearson's correlations were performed; the results are shown in table 9. The variables hypothesised to be markers of dynamic macrovascular health included peripheral and central systolic BP and diastolic BP, peripheral and central PP, peripheral and central MAP, PWV and AIx. There is evidence that measures of BP that include PP and MAP are associated with increased cardiovascular risk (330, 331). The results of the analysis of these relationships are shown in table 10.

Table 5-9: Correlations between measures of kidney function

	eGFR	Log ACR§	Cystatin C
Creatinine	-0.717**	-0.062	0.660**
eGFR		0.003	-0.675**
Log ACR§			-0.004

§Non-parametric data so log transformed to achieve parametric distribution, the remaining data were parametric data so Pearson's correlations shown, **significant at 0.01 level, *significant at 0.05 level; eGFR, estimated glomerular filtration rate; ACR, albumin creatinine ratio

Table 5-10: Correlations between measures of dynamic macrovascular status

	Peripheral DBP	Peripheral PP	PWV	Central PP	AIx
Peripheral SBP	0.548**	0.802**	0.346**	0.613**	0.215**
Peripheral DBP		-0.060	-0.068	-0.087	-0.040
Peripheral PP			0.402**	0.747**	0.295**
PWV				0.440**	0.056
Central PP					0.229**

Parametric data so Pearson's correlations shown, **significant at 0.01 level, *significant at 0.05 level; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; PWV, pulse wave velocity; AGEs, advanced glycation end products; AIx, augmentation index

The variables associated with dynamic vascular health were peripheral and central PP and MAP, PWV, AIx and AGEs. Linear regression analysis was performed with eGFR as a marker of kidney function.

Figure 1(a-f) shows the scatter plots of the relationships between the variables; the correlation coefficients between the variables (including other markers of kidney function, not shown in the scatter plots, cystatin C, creatinine and ACR) is shown in table 11.

Figure 5-1: Correlation between markers of kidney function and arterial stiffness

Figure 1a: eGFR and peripheral PP

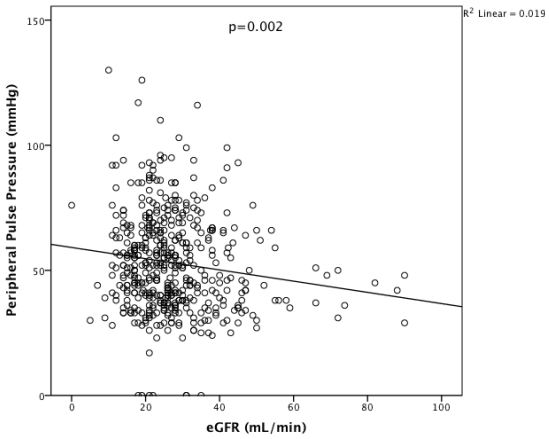


Figure 1b: eGFR and central PP

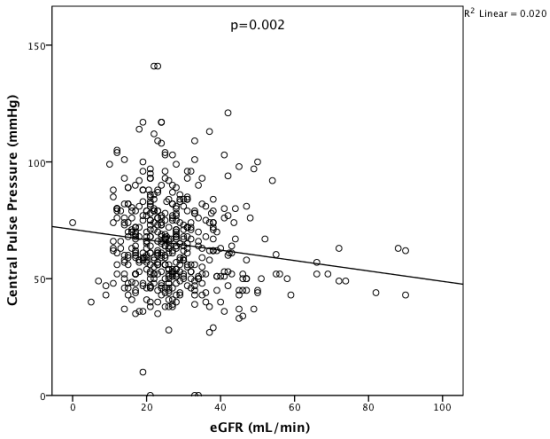


Figure 1c: eGFR and peripheral MAP

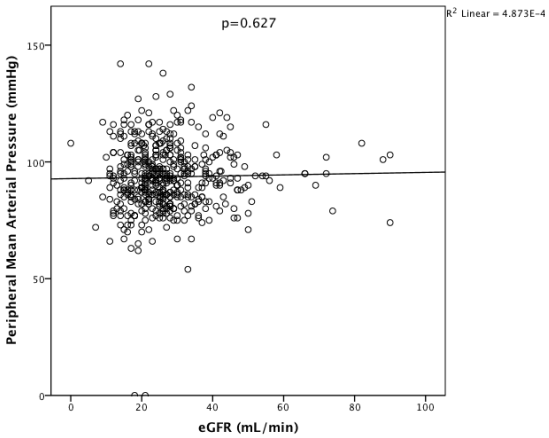


Figure 1d: eGFR and central MAP

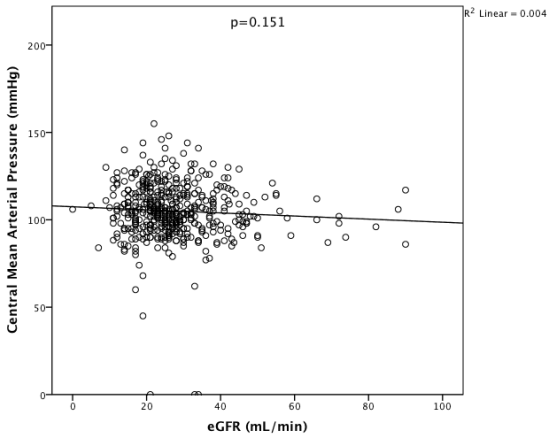


Figure 1e: eGFR and PWV

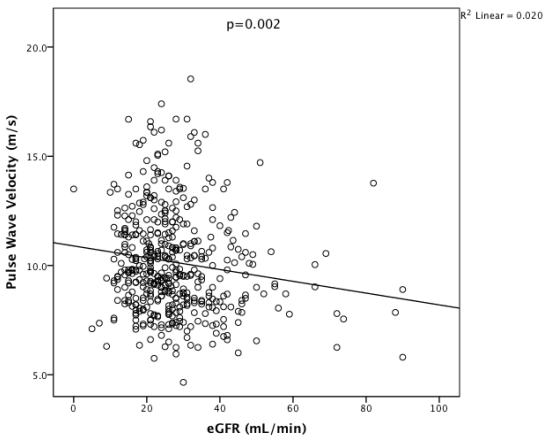


Figure 1f: eGFR and AIx

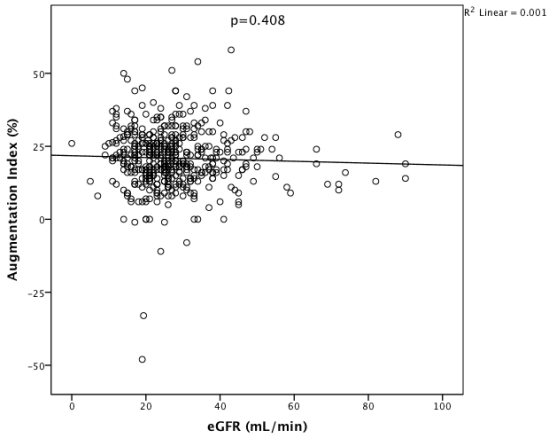


Table 5-11: Correlation between markers of kidney function and markers of dynamic vascular status

		Measures of macrovascular health					
		Peripheral PP	Central PP	Peripheral MAP	Central MAP	PWV	AIx
eGFR	Pearson's	-0.137	-0.141	0.022	-0.066	-0.140	-0.038
	Significance	**0.002**	**0.002**	0.627	0.151	**0.002**	0.408
Cystatin C	Pearson's	0.091	0.075	-0.104	-0.078	0.022	0.065
	Significance	0.090	0.173	**0.053**	0.159	0.691	0.230
Creatinine	Pearson's	0.004	-0.004	-0.007	0.031	0.009	-0.066
	Significance	0.930	0.924	0.876	0.495	0.847	0.150
ACR	Pearson's	0.003	-0.017	-0.011	-0.061	-0.004	0.032
	Significance	0.223	0.718	0.803	0.183	0.362	0.478

Non-parametric data log transformed to achieve parametric distribution; PP, Pulse pressure; MAP, Mean arterial pressure; PWV, pulse wave velocity; AIx, Augmentation index; AGEs, advanced glycation end products. Statistically significant results are shown in bold type.

These data show that as eGFR falls, peripheral and central PP and PWV rise. However there were no correlations between ACR and markers of arterial stiffness, an observation in contrast to that of Smith et al who conducted a cross-sectional analysis of patients with type 2 diabetes and found that both reduced eGFR and raised ACR were associated with higher PWV (332). The discrepancy between their findings and those presented here may be explained by the presence of two distinct sub-groups of patients; those with less advanced CKD and heavy proteinuria and those with advanced pre-dialysis CKD and minimal proteinuria by virtue of decreased glomerular filtration.

5.3. Macro-vascular status (pulse wave velocity and blood pressure) and measures of AGEs

Pulse wave velocity and BP based measures are indicators of vascular compliance and as such are indicators of macrovascular health; the accumulation of tissue AGEs have been proposed as a marker of cumulative metabolic stress with a potential role in the development of micro and macrovascular complications of diabetes (221, 333). In table 10 the measures of macrovascular health correlate closely, this finding is not unexpected.

As some of the variables used are derived from the same base variables (for example both PP and MAP are dependent upon systolic and diastolic blood pressure) tests for co-linearity were performed on the macrovascular data; these confirmed that the data were not co-linear.

In a cohort of healthy Chinese adults there was a correlation between AGEs and PWV (334); however there is no published data on the relationship between AGEs and arterial stiffness in patients with CKD. To explore associations between the macrovascular phenotype and AGE accumulation (measured as skin AF), linear regression was performed. Scatter plots are shown in figure 2 (a-f)

Figure 5-1: Correlation between measures of AGE accumulation and macrovascular status

Figure 2a: AGEs and peripheral PP

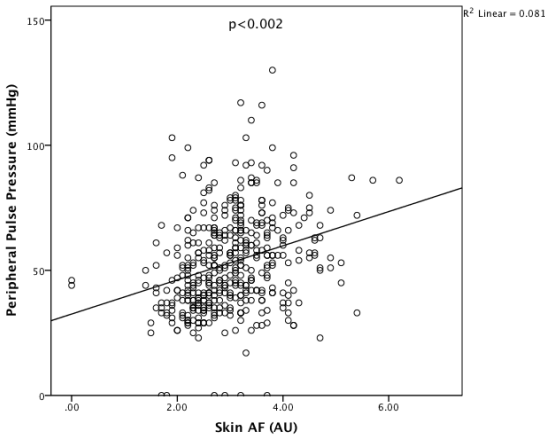


Figure 2b: AGEs and central PP

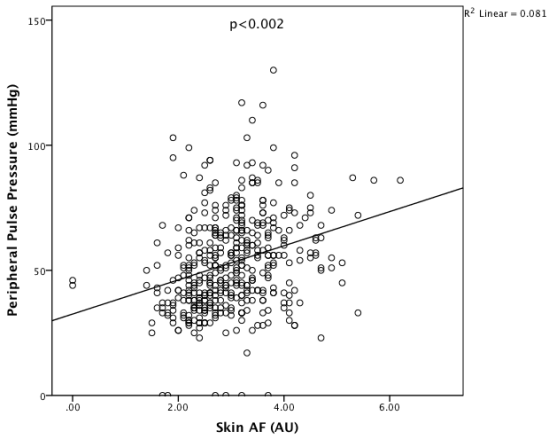


Figure 2c: AGEs and peripheral MAP

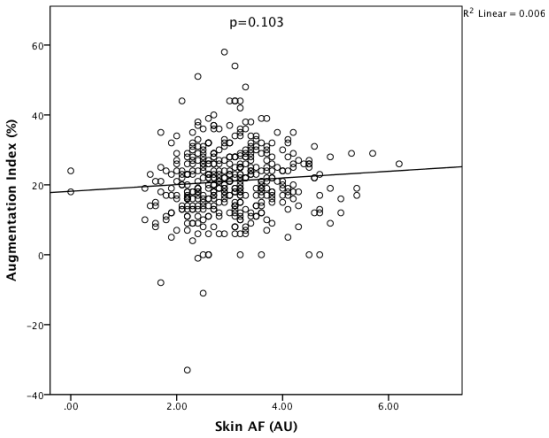


Figure 2d: AGEs and central MAP

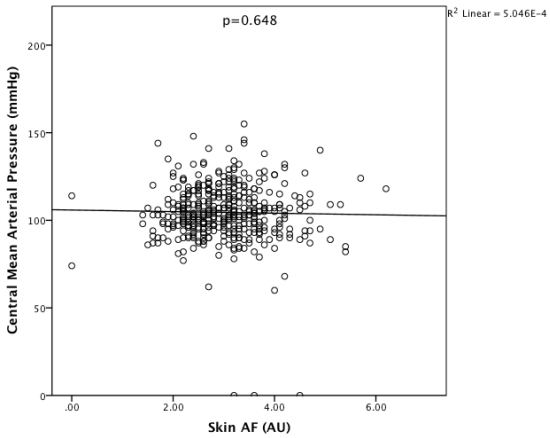


Figure 2e: AGEs and PWV

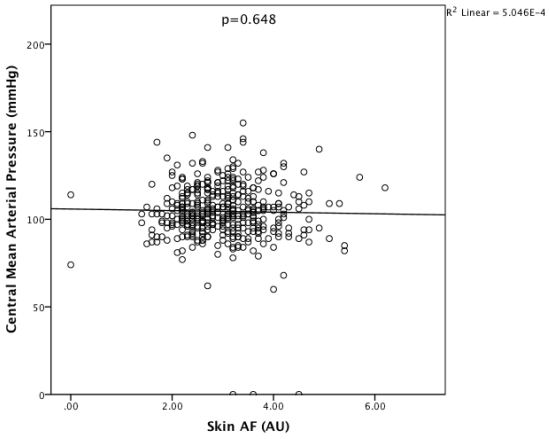
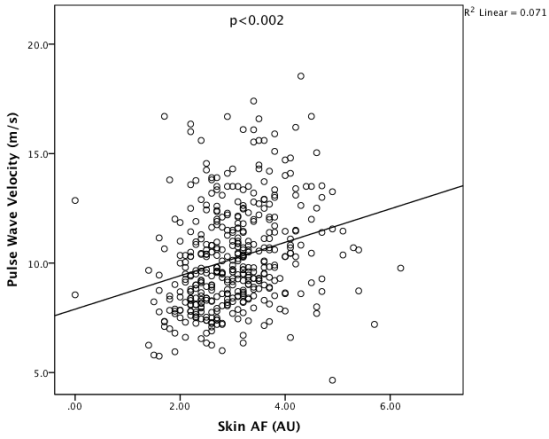


Figure 2f: AGEs and AIx



These data suggest that peripheral and central PP are associated with AGE accumulation in this cohort (both peripheral and central PP also correlated strongly with PWV); this is in keeping with the findings of Schram et al who described a correlation between plasma AGEs and increased pulse pressure in type 1 diabetic subjects (335). There was a weak (but statistically significant) positive correlation between AGEs and PWV. The negative correlation between peripheral MAP and AGEs is unexpected and has not been previously described; it remains to be seen whether this is a genuine finding or an artefact, a possible explanation may be the effect of medications (for example β-blockers, spironolactone or allopurinol).

5.4. Macrovascular status and microvascular status in diabetic and non-diabetic participants

Exposure to prolonged hyperglycaemia has a number of metabolic effects, one of which is the irreversible accumulation of advanced glycation end products which have been postulated as playing a key role in the development and progression of diabetic complications (336). Table 12 shows the correlations between measures of macrovascular health and microvascular health in patients with and without diabetes.

Table 5-1: Correlations between measures of micro- and macro vascular status in all participants, diabetics and non-diabetics

		Measures of macrovascular status					
		Peripheral PP	**Central PP**	**Peripheral MAP**	**Central MAP**	**PWV**	**AIx**
AGEs: All patients	Pearson's	0.284	0.253	-0.145	-0.022	0.284	0.080
	Significance	**<0.002**	**<0.002**	**0.003**	0.645	**<0.002**	0.103
AGEs: Non-diabetics	Pearson's	0.265	0.280	-0.180	-0.034	0.286	0.146
	Significance	**<0.002**	**<0.002**	**0.003**	0.589	**<0.002**	**0.018**
AGEs: Diabetics	Pearson's	0.278	0.174	-0.108	-0.029	0.187	-0.041
	Significance	**<0.002**	**0.030**	0.175	0.717	**0.020**	0.605

PP, Pulse pressure; MAP, Mean arterial pressure; PWV, pulse wave velocity; AIx, Augmentation index; AGEs, advanced glycation end products, significant results are shown in bold type

There were significant correlations between accepted markers of macrovascular disease and the presence of markers of microvascular disease in both diabetic and non-diabetic patients.

To explore the relationship between glycaemic control on both macrovascular and microvascular health, correlations between glycated haemoglobin (HbA1C) and measures of vascular status in were performed, as HbA1C data were non-parametrically distributed log transformation was performed to achieve parametric distribution. Scatter plots are shown in figure 3 (a-g).

Figure 5-2: Correlations between glycated haemoglobin and measures of micro-and macrovascular status

Figure 3a: HbA1C and peripheral PP

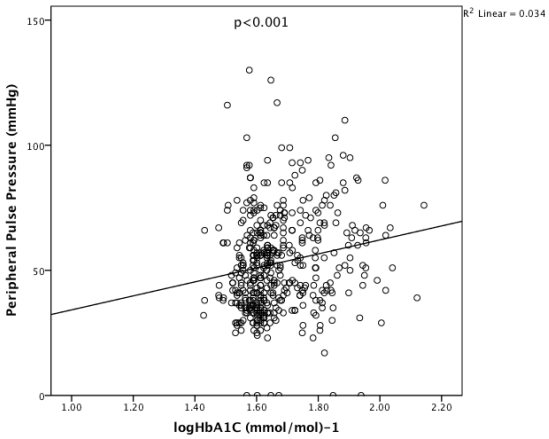


Figure 3b: HbA1C and central PP

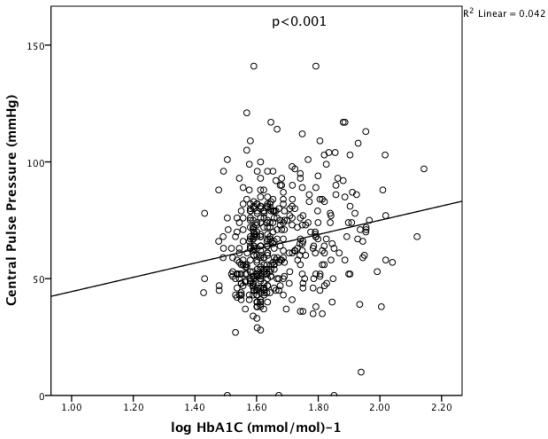


Figure 3c: HbA1C and peripheral MAP

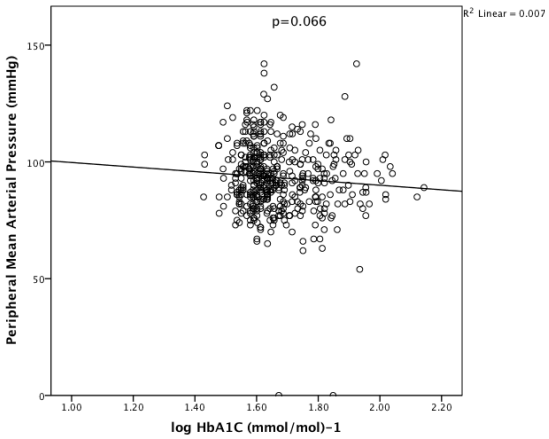


Figure 3d: HbA1C and central MAP

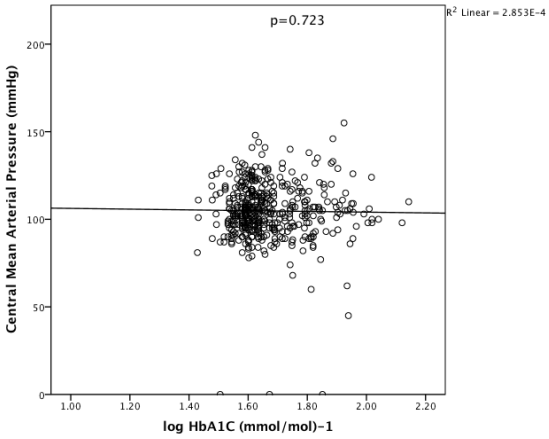


Figure 3e: HbA1C and PWV

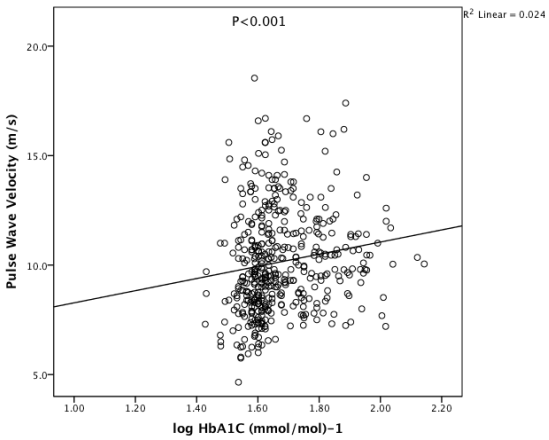


Figure 3f: HbA1C and AIx

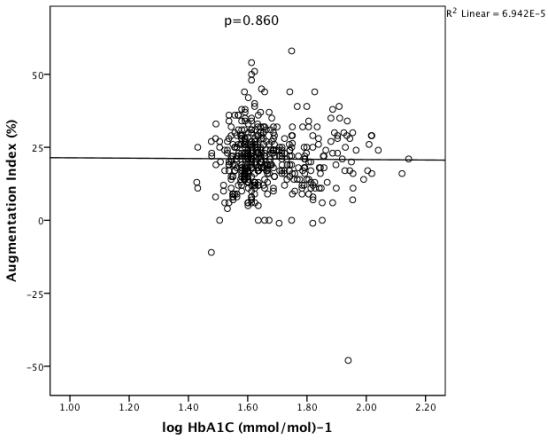
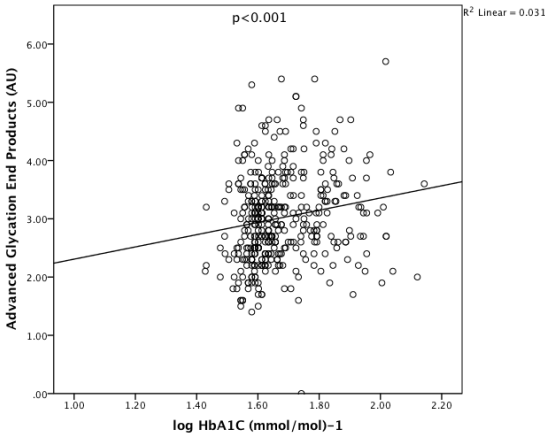


Figure 3g: HbA1C and AGEs



These data show a positive correlation between measures of arterial stiffness and microvascular status and increasing HbA1C.

5.5.The relationship between the anthropomorphic phenotype and the cardiovascular phenotype

The anthropomorphic phenotype occupies an interesting place in the nexus between established and dynamic cardiovascular risk factors; it has been shown that significant weight loss (achieved either by low calorie diet, bariatric surgery or both) was associated with reduced markers of arterial stiffness (337, 338). Furthermore in a study of otherwise healthy overweight Japanese adults, weight loss was associated with reduction in tissue AGEs (339); this suggests that AGE accumulation may be part of the dynamic rather than established cardiovascular phenotype.

In RIISC there was no significant difference in either BMI or WHR in those patients with established CVD compared to those without CVD. To explore the associations between the anthropomorphic phenotype and the dynamic cardiovascular phenotype linear regression was performed; the anthropomorphic measure used in this analysis was BMI as this correlated strongly with all other anthropomorphic measures in this cohort, scatter plots are shown in figure 4 (a-g).

Figure 5-4: Correlations between anthropomorphics and arterial stiffness

Figure 4a: BMI and peripheral PP

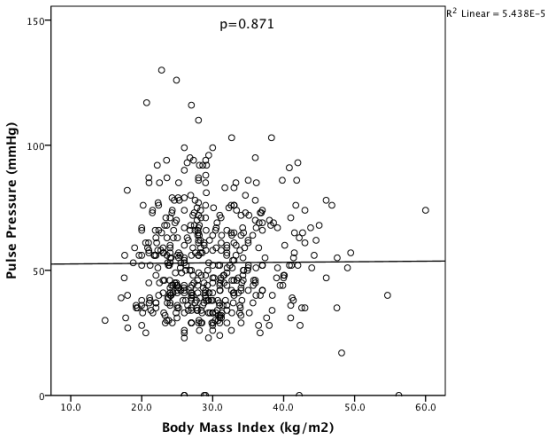


Figure 4b: BMI and central PP

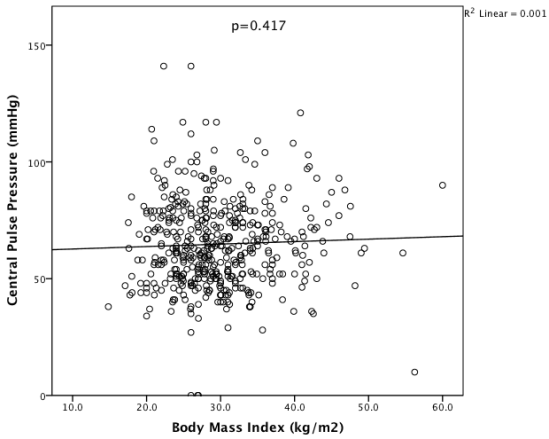


Figure 4c: BMI and peripheral MAP

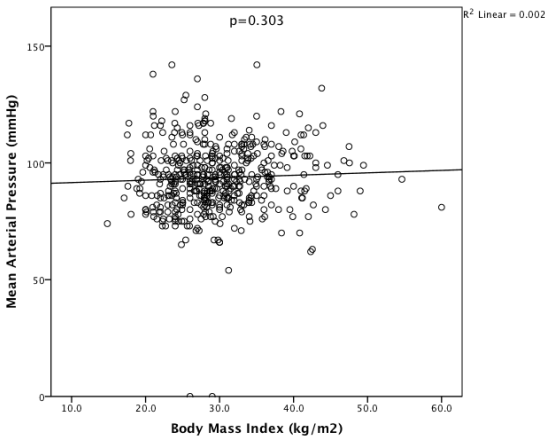


Figure 4d: BMI and central MAP

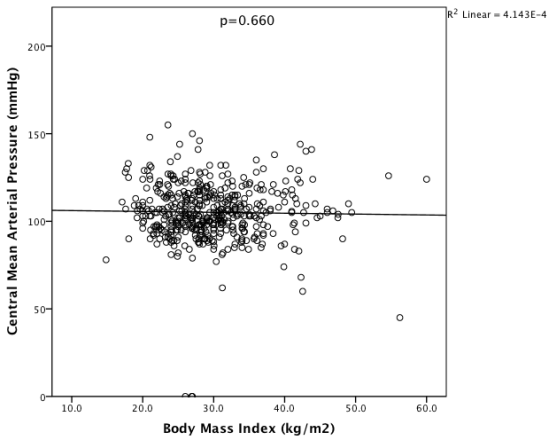


Figure 4e: BMI and PWV

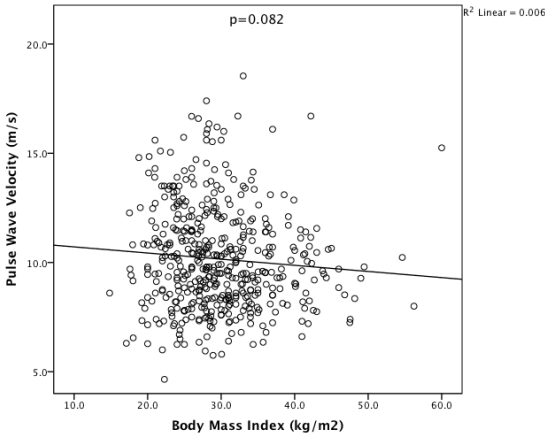


Figure 4f: BMI and AIx

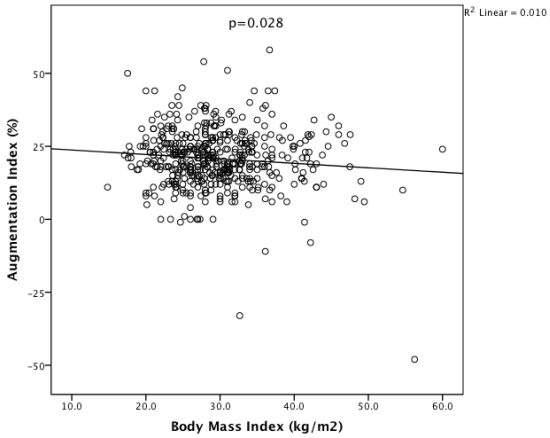
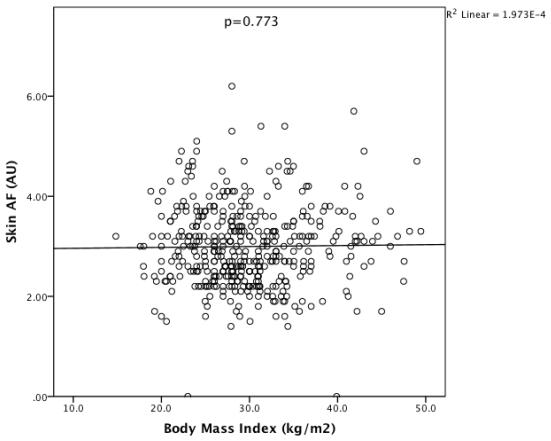


Figure 4g: BMI and AGEs



These data suggest that in this cohort the anthropomorphic phenotype is not closely related to the cardiovascular phenotype; while there were no significant associations there appears to be a paradoxical relationship between BMI and PWV, in the paediatric literature the relationship between obesity and decreased PWV has been described and has been attributed to vasodilation (340), McIntyre et al described similar findings in the R^2ID cohort (341).

The absence of any significant associations between established CVD and the anthropomorphic phenotype is an interesting observation that may be explained by the cross-sectional nature of the data; it is possible that participants who have had CV events have subsequently achieved weight loss. The lack of any correlation between the dynamic CV phenotype and the anthropomorphic phenotype is not entirely unexpected as the data relating to arterial stiffness and obesity has been contradictory with some authors reporting associations between obesity and arterial stiffness (200, 342, 343) and others finding no association (344-346).

5.6.Systemic inflammation and arterial stiffness

A correlation between systemic inflammation and arterial stiffness in 100 healthy individuals was demonstrated by Vlachopoulos et al in 2005 (347); recently Chue et al hypothesised that systemic inflammation may be an important factor in arterial stiffness in patients with CKD (166). In the CRIC cohort a cross-sectional analysis of pulse wave velocity was performed, the authors concluded that reduced renal function may contribute to arterial stiffness and that arterial stiffness may contribute to the increased cardiovascular risk experienced by individuals with CKD, however in their analysis the role of inflammation was not considered (348). To address this shortfall I utilised the RIISC cohort to assess the relationship between inflammation and cardiovascular parameters in patients with CKD.

I utilized three markers of inflammation in this analysis.

1. Highly sensitive C-reactive protein (hsCRP), a widely used biomarker of inflammation and measured in 330 participants in the cohort to date.

2. IL-6 is a pro-inflammatory cytokine and marker of systemic inflammation that has been associated with malnutrition and CVD in patients with ESKD (349). In a cohort of individuals without pre-existing CVD or CKD, elevated inflammatory markers (including IL-6 and hsCRP) were associated with decreasing kidney

function (350). IL-6 was measured using a commercially available Luminex kit and data were available for 320 of the 500 participants recruited.

3. Elevated combined polyclonal free lights chains (cFLC) have been associated with increased mortality in the Olmsted county cohort (351). In addition there is a increasing evidence that there is an independent association between mortality in chronic disease cohorts, including CKD. Polyclonal FLCs are biomarkers of chronic inflammation, kidney function and reticulo-endothelial health (352, 353). Serum FLC measurements were available for 350 of the 500 participants recruited to the RIISC study.

To assess correlation between these non-parametrically distributed markers Spearman's Correlations were performed, the results are shown in table 13.

Table 5-2: Correlation between markers of systemic inflammation

	cFLC	**IL-6**
hsCRP	0.219**	0.247**
cFLC		0.081

Non-parametric data so Spearman's correlations shown, **significant at 0.01 level; hsCRP, highly sensitive CRP; FLC, free lights chains; IL, interleukin

These data show that there are correlations between hsCRP and polyclonal FLCs and IL-6.

To explore the prevalence of inflammation in individuals with and without established cardiovascular disease non-parametric tests were performed, the results are shown in table 14.

Table 5-3: Established CVD and systemic inflammation

	No CVD	Prior CVD	p-value
hsCRP	2.7 (1.1-8.9)	3.2 (1.2-9.6)	0.570
Polyclonal FLC	73.8 (50.4-102.9)	71.4 (48.9-108.4)	0.767
IL-6	7.0 (5.0-9.0)	7.0 (6.0-10.0)	0.671

hsCRP, highly sensitive CRP; FLC, free lights chains; IL, interleukin, non-parametric unpaired T tests performed

These data show that there is no difference in markers of inflammation between participants with CVD and those without CVD.

Highly sensitive CRP, polyclonal FLCs and IL-6 were analysed as continuous variables; all were non-parametrically distributed and were therefore log-transformed before analysis to achieve parametric distribution. Linear regression analysis was performed; scatter plots are shown in figures 5-7 (a-g) and Pearson's correlations are shown in table 15.

Figure 5-5: Correlations between hsCRP and measures of arterial stiffness

Figure 5a: hsCRP and peripheral PP

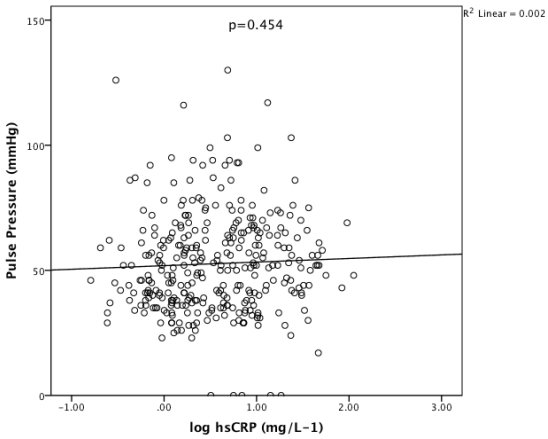


Figure 5b: hsCRP and central PP

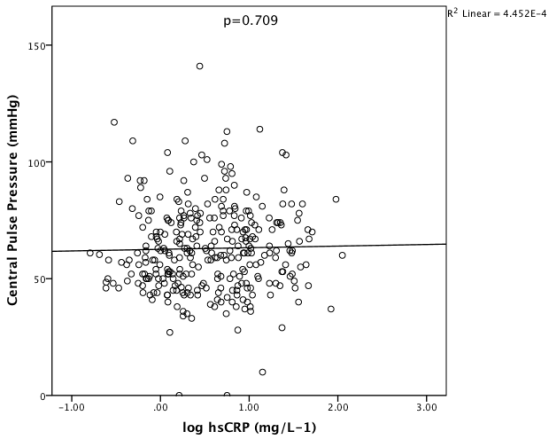


Figure 5c: hsCRP and peripheral MAP

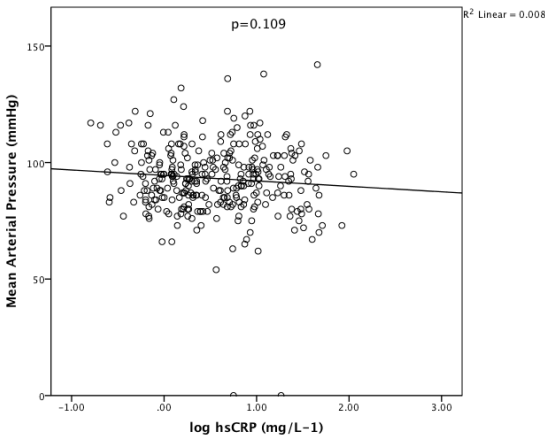


Figure 5d: hsCRP and central MAP

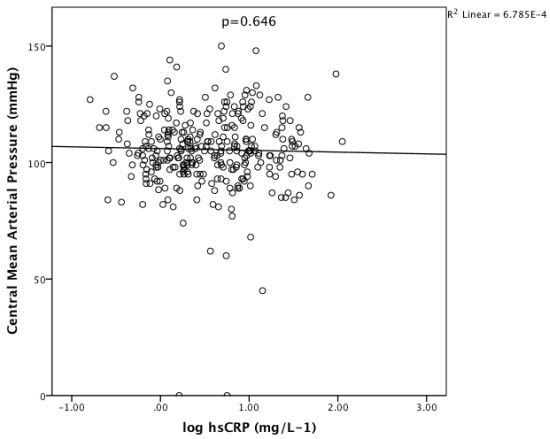


Figure 5e: hsCRP and PWV

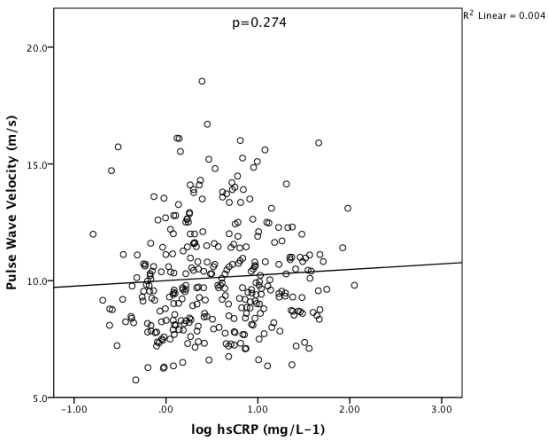


Figure 5f: hsCRP and AIx

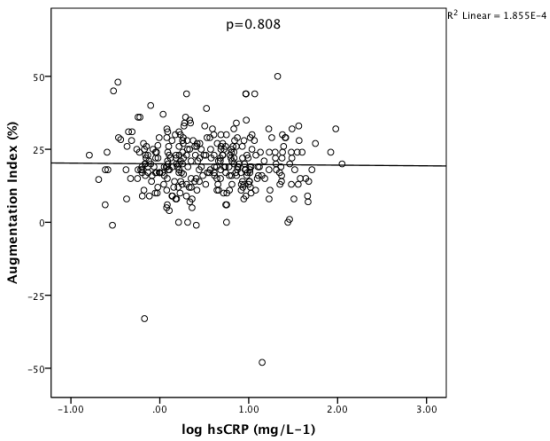


Figure 5g: hsCRP and AGEs

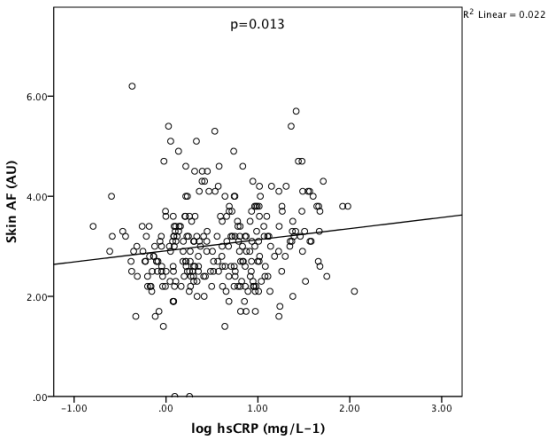


Figure 5-6: Correlations between polyclonal FLC and measures of arterial stiffness

Figure 6a: cFLC and peripheral PP

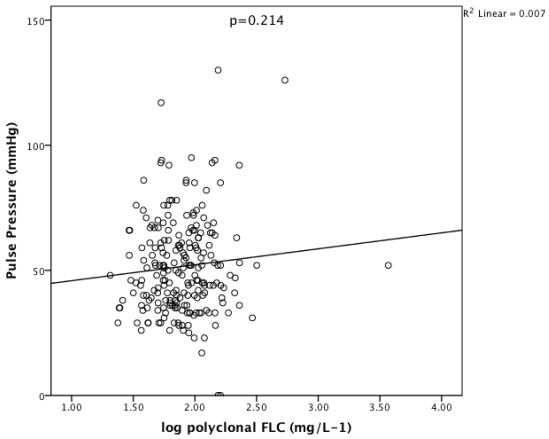


Figure 6b: polyclonal FLC and central PP

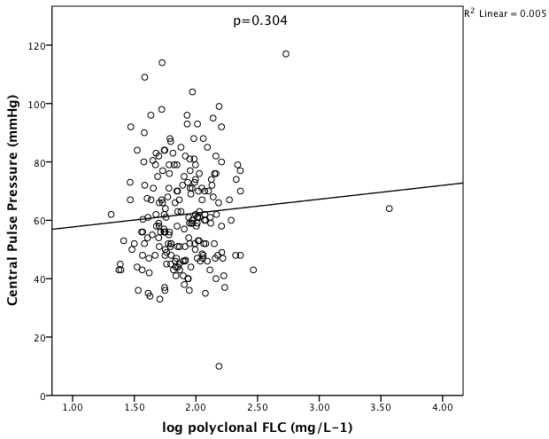


Figure 6c: cFLC and Peripheral MAP

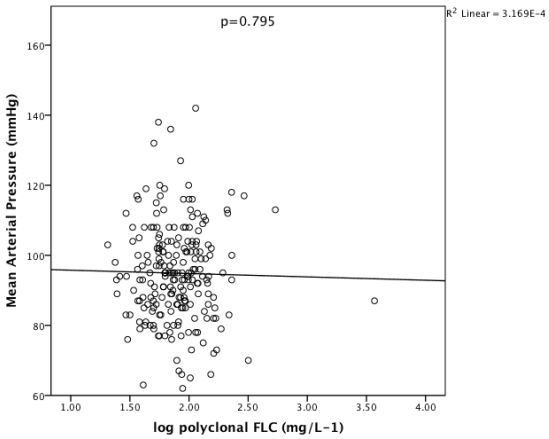


Figure 6d: cFLC and central MAP

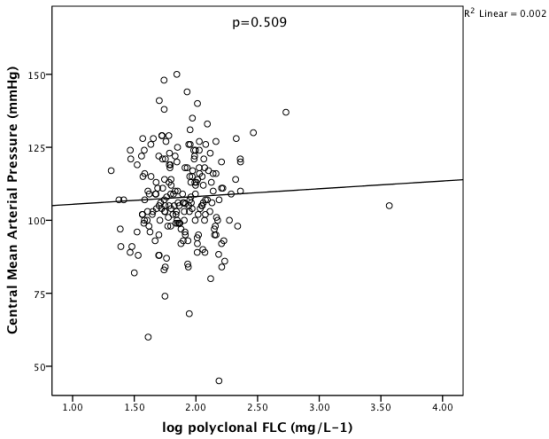


Figure 6e: cFLC and PWV

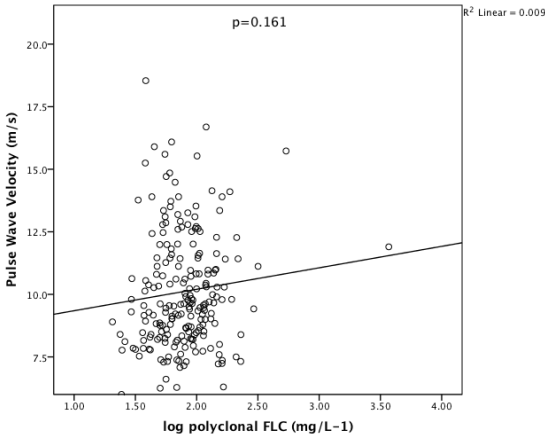


Figure 6f: cFLC and AIx

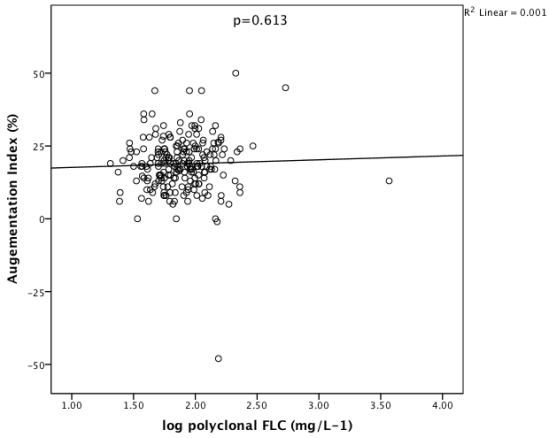


Figure 6g: cFLC and AGEs

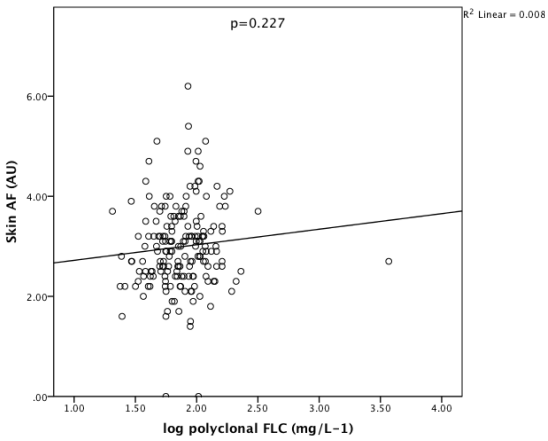


Figure 5-7: Correlations between IL-6 and measures of arterial stiffness

Figure 7a: IL-6 and peripheral PP

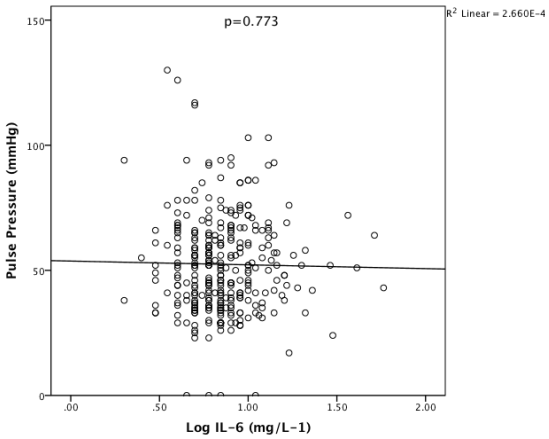


Figure 7b: IL-6 and central PP

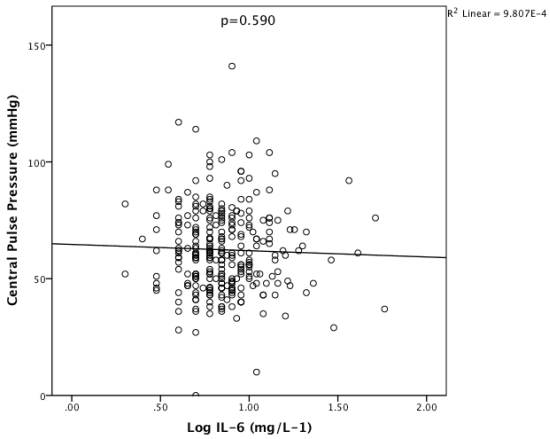


Figure 7c: IL-6 and Peripheral MAP

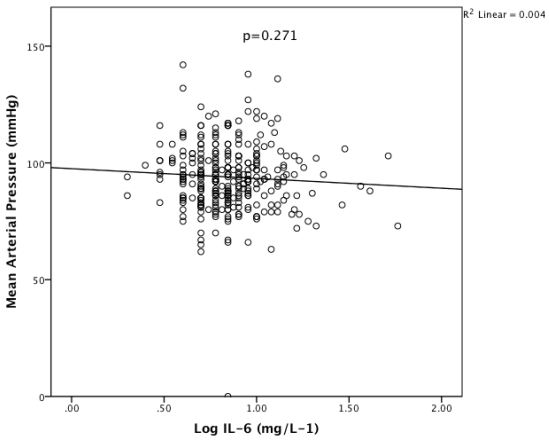


Figure 7d: IL-6 and central MAP

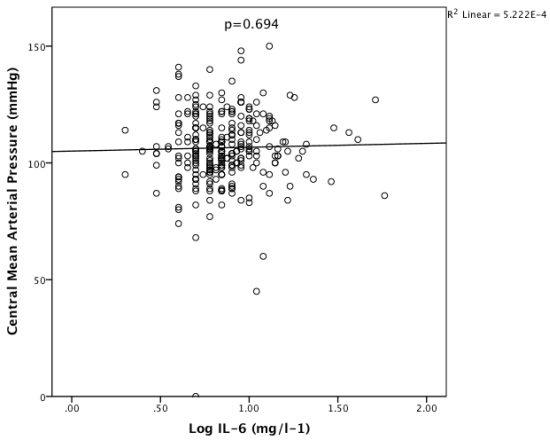


Figure 7e: IL-6 and PWV

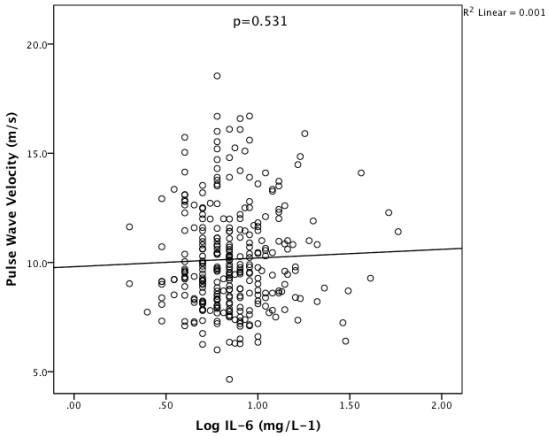


Figure 7f: IL-6 and AIx

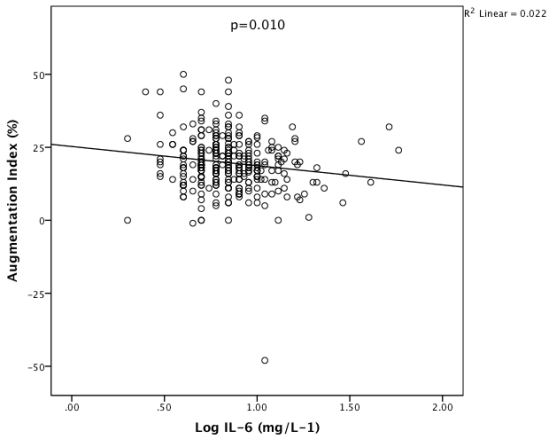


Figure 7g: IL-6 and AGEs

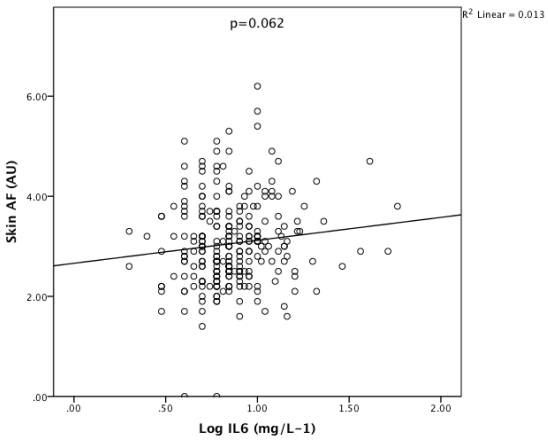


Table 5-4: Correlations between systemic inflammation and measures of vascular status

		Measures of vascular health						
		Peripheral PP	Central PP	Peripheral MAP	Central MAP	PWV	AIx	AGEs
hsCRP	Pearson's	0.041	0.021	-0.089	-0.026	0.061	-0.014	0.149
	Significance	0.454	0.709	0.109	0.646	0.274	0.808	**0.013**
Polyclonal FLCs	Pearson's	0.084	0.072	-0.018	0.046	0.096	0.035	0.090
	Significance	0.214	0.304	0.795	0.506	0.161	0.613	0.227
IL-6	Pearson's	-0.016	-0.031	-0.063	0.023	0.036	-0.147	0.115
	Significance	0.773	0.590	0.271	0.694	0.531	**0.010**	0.062

PP, Pulse pressure; MAP, Mean arterial pressure; PWV, pulse wave velocity; AIx, Augmentation index; AGEs, advanced glycation end products; hsCRP, highly sensitive CRP; FLCs, free light chains; IL-6, interleukin 6; significant correlations are shown in bold type

The data suggest a significant correlation between IL-6 and hsCRP, this is expected as IL-6 is produced in response to inflammation or infection from macrophages and adipocytes, stimulating the production of CRP from the liver (which occurs within six hours of the original insult) (354). IL-6 has been implicated in the transition from acute to chronic inflammation via a mechanism of monocyte recruitment (355).

There was a correlation between hsCRP and AGEs, the correlation between IL-6 and AGEs, approached but did not achieve significance; in a review of the literature Schwedler et al, observing a correlation between IL-6/CRP and AGEs hypothesised that CRP might stimulate AGE production in uraemia (356).

To explore the data further the IL-6 and hsCRP data were divided into quartiles and ANOVA performed; for the IL-6 data there were no significant differences between quartiles of IL-6 and peripheral and central PP and MAP, PWV and AGEs, however there was a significant difference in AIx between the quartiles. For hsCRP the only significant difference was in PWV. The plots of the significant variables are shown in figure 8 (a-b).

Figure 5-8: Quartiles of hsCRP and IL-6 and measures of arterial stiffness

Figure 8a: Quartiles of hsCRP and PWV

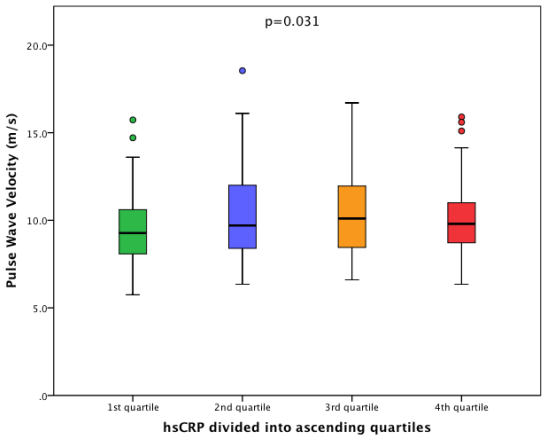
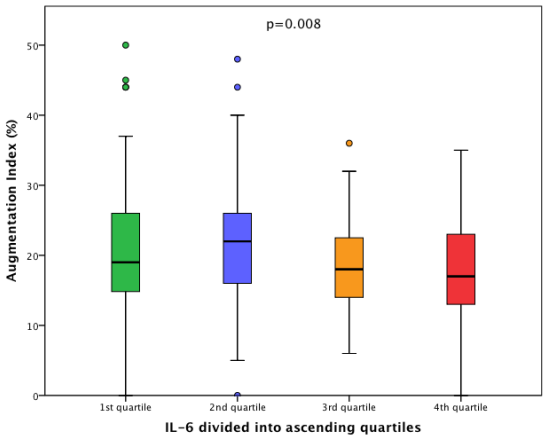


Figure 8b: Quartiles of IL-6 and AIx



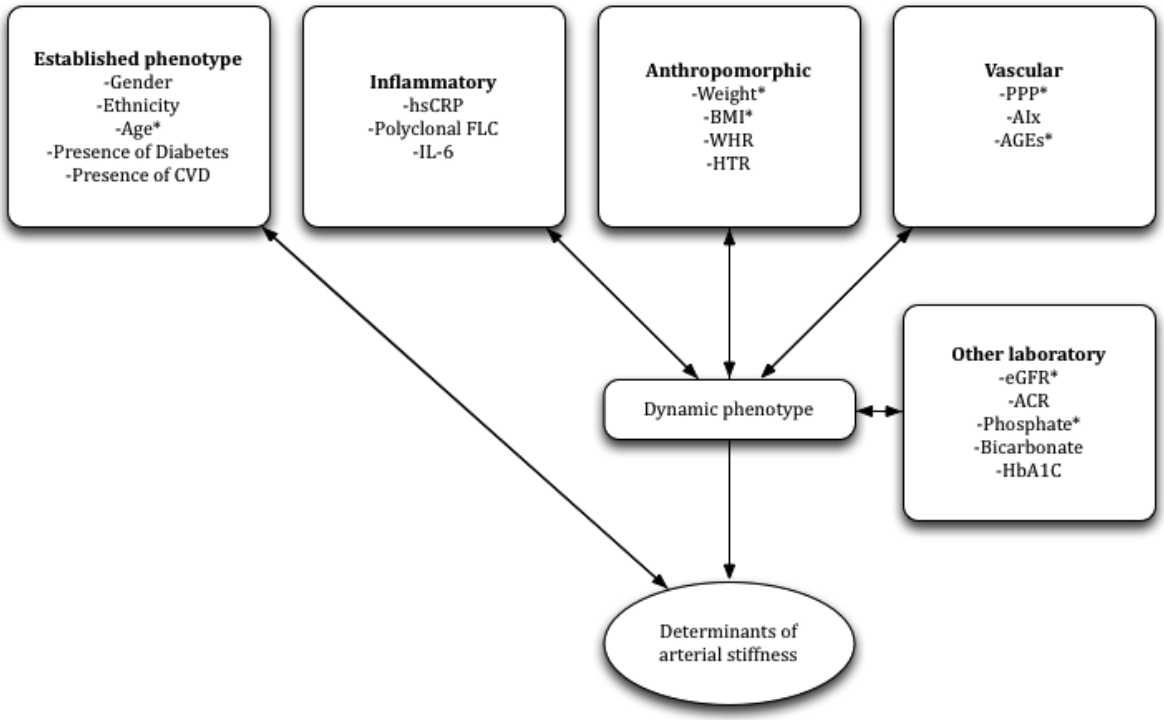
The presence of previously described correlations between IL-6/CRP and AGEs suggests that these findings are genuine rather than artifactual; it is an interesting observation that the relationship between AIx and IL-6 appears to be inverse, there have been no previously published data on the relationship between these variables. There is equivocal data on the relationship between hsCRP and arterial stiffness with some investigators reporting a positive association between CRP and PWV (357-359) and others reporting no relationship (360).

The absence of clear correlations between inflammatory markers and macrovascular status may be explained by the cross-sectional nature of the data collected and the limited number of individuals for whom inflammatory markers were available.

5.7.The determinants of vascular stiffness in the RIISC cohort

For the purposes of this analysis PWV was used as the measure of vascular stiffness, to transform it from a continuous to a categorical variable patients were described as having a PWV in the highest quartile or not. To reduce the influence of co-linearity, the measure of blood pressure that correlated most closely with eGFR (peripheral PP) was included. The variables included in the univariate analysis are shown in figure 9.

Figure 5-9: Variables included in the univariate analysis of variables potentially associated with arterial stiffness



CVD, cardiovascular disease; hsCRP, highly sensitive CRP; FLC, free light chains; IL-6, interleukin 6; BMI, body mass index; WHR, waist hip ratio; HTR, hip thigh ratio; PPP, peripheral pulse pressure; CPP, central pulse pressure; AIx, augmentation index; AGEs, advanced glycation end products

The significant variables (those that achieved a significance of <0.1 in the univariate analysis) are indicated with an asterix and were then included in the multivariate analysis (a binary logistic regression method was used). The results of the multivariate analysis are shown in table 16.

Table 5-5: Multivariate analysis of factors significantly associated with arterial stiffness

	Significance	Odds ratio	95% confidence intervals
Age	0.000	1.081	1.056, 1.107
Peripheral PP	0.002	1.023	1.008, 1.037
Weight	0.253		
BMI	0.068		
eGFR	0.457		
Phosphate	0.074		
AGEs	0.167		

All the relationships were associated with a higher level of the variable concerned apart from eGFR and BMI, which were associated with a lower level

The Pearson's correlation between age and PWV was 0.546 and the Pearson's correlation between peripheral pulse pressure and PWV was 0.402.

5.8.Conclusions

In these cross-sectional analyses, where there are significant associations causality cannot be assumed, nor is it possible to establish temporality of any of the associations described. Despite these limitations this analysis assesses complex interaction between both established injury, where there may not be a major reversible component and potentially reversible risk factors. I will now discuss the findings of this chapter by asking a number of key questions.

5.8.1. How should cardiovascular phenotype in CKD be defined?

The cardiovascular phenotype is influenced by both dynamic and established risk factors and can be described in terms of macrovascular and microvascular disease. In the Framingham cohort pulse pressure was shown to correlate more strongly with cardiovascular events than either systolic pressure or diastolic pressure alone (361),

suggesting that pulse pressure is a marker of arterial stiffness. However the relationship between arterial stiffness (defined by PWV) did not correlate well with traditional cardiovascular risk factors (apart from age and blood pressure) in a recent systematic review (362). While PP may be a close marker of arterial stiffness the observation that central PP can vary independently of PWV (363) suggests a more complex relationship between the two that is not fully understood; the gold standard marker of arterial stiffness is PWV(364).

In this cohort, measures of peripheral and central BP, PWV and AIx are used as markers of large vessel arterial stiffness and tissue AGE accumulation is used as a measure of microvascular disease.

5.8.2. How does the established cardio-vascular co-morbidity burden influence the cardiovascular phenotype?

There is a substantial burden of established CVD in this cohort and as expected diabetic patients have a higher prevalence of established CVD, which was correlated to markers of both macrovascular disease and microvascular disease. Interestingly the presence of CVD in the whole cohort is less strongly associated with markers of macrovascular and microvascular disease. An explanation for this might be the time lag between the cardiovascular event and the index study date on which the cardiovascular measures were taken.

5.8.3. What is the influence of the dynamic, anthropomorphic phenotype?

The anthropomorphic phenotype could be considered as both an established and a dynamic risk factor. In this cohort there was no association between the presence of established cardiovascular disease and elevated body mass index or other adverse anthropomorphic features, while the presence of adverse anthropomorphic features have been shown to be risk factors for the development of CKD (365), the role of anthropomorphic features in the progression of CKD is controversial (366-368).

There were no significant associations between BMI and measures of either macrovascular or microvascular disease, this is consistent with results of the Framingham observational study of healthy adults (369) but not with the findings of the R^2ID cohort where a negative correlation between BMI and pulse wave velocity was described (370). The findings of the univariate analysis of determinants of PWV are in keeping with those of the R^2ID cohort; they identified many of the same variables as being associated with increased PWV, however in the multivariate analysis the only variable of significance in common with that cohort was age (370).

5.8.4. What is the influence of the dynamic, inflammatory phenotype?

There was no clear correlation between the dynamic inflammatory phenotype and the established cardiovascular phenotype in this cross-sectional analysis, in previous longitudinal studies in both healthy individuals (371) and individuals with CKD (181) systemic inflammation was associated with subsequent cardiovascular events and

incident cardiovascular events. This may also be explained in part by the time lag between the cardiovascular event and the measurement of inflammation. As the cohort matures further analyses will better assess the relationship between systemic inflammation and cardiovascular disease.

5.8.5. What are the limitations of this analysis?

While this is a well-described cohort of high-risk CKD patients there are limitations to the analysis presented here. The cross-sectional nature of the analysis presented in this chapter means that no conclusions can be drawn regarding the temporal nature of any associations described; markers of inflammation and vascular stiffness represent the current cardiovascular and inflammatory phenotype while the presence of established cardiovascular disease represents past damage. Anthropomorphic characteristics may occupy a space between the established and the dynamic phenotype but this cannot be confirmed on the basis of the data presented here.

There are no measures of oxidative stress and limited measures of inflammation described in this analysis. More comprehensive analysis of markers of inflammation and oxidative stress in the cohort with time may influence the findings of the analyses, particularly as the cohort matures and the data can analysed in the context of hard clinical end-points.

6. Results 4: The periodontal phenotype of the RIISC cohort; the vascular and inflammatory implications of periodontitis.

There is a potential association between chronic periodontal inflammation (periodontitis) and accelerated CVD (237); the proposed mechanism is through local oral inflammation producing systemic inflammation that acts to potentiate endothelial dysfunction and atherosclerosis (238-240). There is also a potential role for oxidative stress, an association has been described between periodontitis and oxidative stress (372), although there is less evidence for this to date. Individuals with CKD (non-dialysis) and those receiving both chronic haemodialysis and peritoneal dialysis have been shown to have a higher prevalence of severe periodontitis than healthy individuals (373-375). Therefore I hypothesised that in patients recruited into RIISC the prevalence of periodontitis would be high and there would be an association between periodontitis, inflammation and vascular dysfunction.

The data presented in this chapter relate to the 469 RIISC participants (94% of the cohort) who underwent a detailed periodontal assessment. Not all RIISC participants underwent a periodontal assessment either because they did not give consent for this component of the assessment or for logistical reasons.

The periodontal assessment was conducted by a research hygienist and a dentist and comprised of measures of PPD, CAL, loss of teeth and a clinical assessment of periodontal health (which included documentation of BOP and number of loose teeth) with measurements taken on all teeth present. A description of the periodontal

assessment can be found in chapter two. There was no significant difference in the proportion of patients within each stage of CKD who underwent assessment.

Participants were classified according to a recognized classification system by whether they were dentate or edentulous, had a healthy mouth, had gingivitis (which is not a pre-cursor state to periodontitis), or had mild, moderate or severe periodontitis (376). While periodontal disease is known to be a significant cause of edentulousness (377) it is not the only cause of edentulousness so these participants were treated as a separate group in the analysis.

The assessed RIISC cohort was comprised of 83% dentate participants; table 1 represents the breakdown of those with healthy mouths, the presence of gingivitis and the presence of periodontitis in the cohort.

Table 6-1: The periodontal characteristics of the RIISC participants

		Percentage
Healthy		5
Gingivitis		13
	Mild periodontitis	42
Periodontitis	Moderate periodontitis	18
	Severe periodontitis	6
Edentulous		17

The baseline demographic characteristics of patients according to their periodontal status are shown in table 2.

Table 6-2: The baseline demographic characteristics of patients according to periodontal status

	Healthy	Gingivitis	Periodontitis	Edentulous	p-value
Males (%)	46	53	65	57	0.084
Age* (years)	65 (16)	58 (18)	61 (16)	77 (9)	<0.001
Ethnicity (%):					
White	77	74	67	81	0.227
Asian	5	10	20	13	0.005
Black	14	13	13	3	0.856
Other	4	3	3	3	0.782

For categorical variables Chi-squared tests performed, continuous variables ANOVA performed

These data show that edentulous participants are significantly older than dentate participants (when edentulous participants are removed from the analysis there is no significant difference in age between participants with healthy mouths, gingivitis or periodontitis, p=0.245). There is a significantly higher prevalence of periodontitis in Asian participants.

Edentulous participants have been excluded from all further analyses presented in this chapter for the reasons outlined previously.

6.1. Periodontitis and socio-economic status

In a community based cohort study measures of social deprivation were associated with poor dental health (378), I therefore explored the impact of socio-economic status upon periodontal health in RIISC; the results of this analysis are shown in table 2. The IMD rank is a measure of social deprivation based upon postal code, the lower the rank the greater the deprivation in that area.

Cigarette smoking has been shown to be associated with both socioeconomic status and the presence of periodontal disease (379), the prevalence of current and former smoking is also shown in table 3.

The data show that patients with periodontitis had a higher prevalence of current smoking and less social deprivation.

Table 6-3: Markers of socio-economic status and periodontal health

	All patients	Healthy	Gingivitis	Periodontitis	p-value
No formal qualifications (%)	49	41	35	44	0.091
Unemployment (%)	20	14	21	21	0.058
Unskilled employment (%)	71	32	2	19	0.229
Current smoking (%)	14	9	16	15	0.038
Previous smoking (%)	42	45	29	39	0.401
IMD rank*	7576 (2838-15140)	6264 (2744-18038)	8596 (2937-15563)	8786 (2962-15930)	0.011

* data shown as median and interquartile range, Kruskall-Wallis test performed, for categorical variables Chi-squared tests performed

6.2.The periodontal phenotype and kidney function

The periodontal phenotype by CKD stage is shown in table 4. The difference between markers of kidney function in dentate participants with and without, periodontitis is shown in table 5. The data presented illustrate that there is no significant difference in the prevalence of periodontitis by CKD stage, though individuals with periodontitis had a significantly higher serum creatinine than those with healthy mouths or gingivitis

Table 6-4: The distribution of periodontal status by CKD stage (%)

	All patients	CKD 1&2	CKD 3A	CKD 3B	CKD 4	CKD 5	p-value
Healthy	5	0	0	5	5	5	0.754
Gingivitis	13	30	20	19	10	10	0.045
Periodontitis	65	70	76	72	81	82	0.298
Moderate or severe periodontitis	36	29	38	32	36	39	0.955

Chi-squared tests performed

Table 6-5: Measures of kidney function in dentate participants with and without periodontitis

	Healthy/Gingivitis	Periodontitis	p-value
eGFR	29 (12)	27 (13)	0.193
Cystatin C	2.6 (0.7)	2.6 (0.9)	0.562
Creatinine	202 (72)	232 (99)	0.009
ACR*	30.4 (7.4-92.2)	34.6 (8.9-131.8)	0.270

*non-parametric data, median and IQR shown, Mann-Whitney test performed, parametric data unpaired

T tests performed; Abbreviations; eGFR, estimated GFR; ACR, albumin creatinine ratio

The relationship between markers of kidney function and severity of periodontitis in dentate patients with periodontitis is shown in figure 1 (a-d).

There was no difference in any of the markers of kidney function when patients with moderate or severe periodontitis were compared to all other dentate patients who were assessed.

Figure 6-1: Measures of kidney function and severity of periodontitis

Figure 1a: eGFR and periodontitis

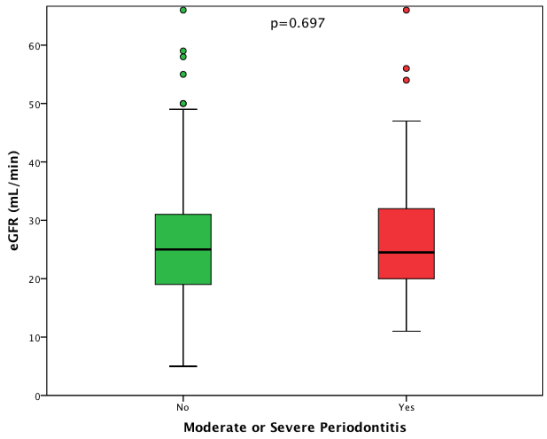


Figure 1b: Creatinine and periodontitis

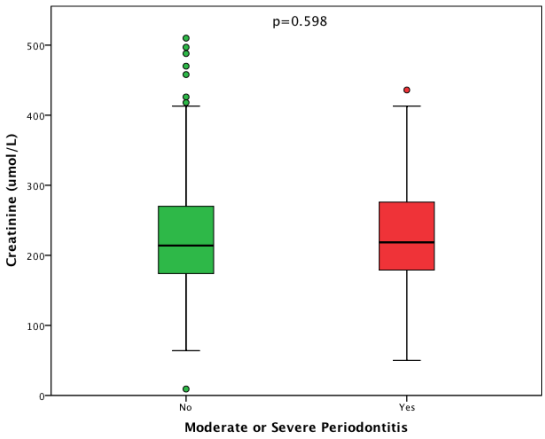


Figure 1c: Cystatin C and periodontitis

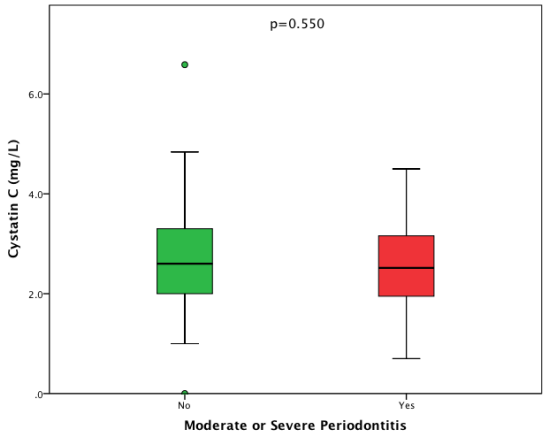
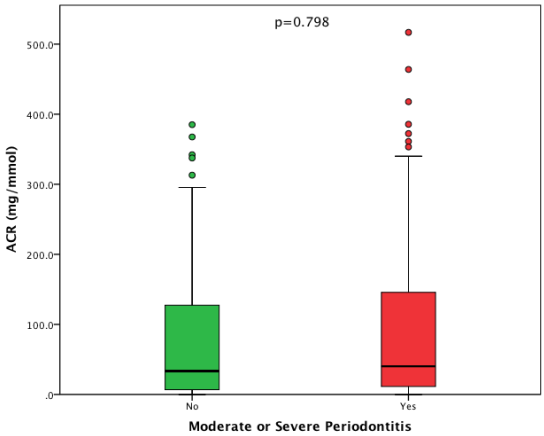


Figure 1d: ACR and periodontitis*



*Mann-Whitney test

6.3.Periodontitis and the established cardiovascular phenotype

The presence of periodontitis has been associated with a number of co-morbidities in cross-sectional studies, these include CVD and diabetes (380). A recent systematic review has confirmed the presence of a relationship between periodontal disease and CKD, and also the potential impact of treatment of periodontal disease on kidney function (381).

The cardiovascular phenotype (and other significant co-morbidities) and periodontal health in the RIISC cohort is shown in table 6.

The data presented show no significant differences in the prevalence of any of the co-morbidities studied, when patients were divided into those with moderate and severe periodontitis and those with mild periodontitis there was still no significant difference in prevalence of cardiovascular disease or any of the other co-morbidities studied.

Table 6-6: Co-morbidity and periodontal disease in the dentate participants of the RIISC cohort

	All patients	Healthy	Gingivitis	Periodontitis	p-value
Diabetes (%)	39	27	53	40	0.923
Ischaemic heart disease (%)	23	27	23	20	0.312
Cerebrovascular Disease (%)	12	5	10	12	0.174
Peripheral Vascular Disease (%)	12	18	16	9	0.493
Cardiovascular disease (%)	33	36	34	30	0.653
COPD (%)	13	23	11	14	0.212
Malignancy (%)	16	9	11	14	0.500
Charlson co-morbidity index	5 (2-7)	5.5 (1-8)	4 (0.75-7)	4 (2-6)	0.646

Chi-squared tests performed

6.4.Periodontitis and arterial stiffness

Periodontitis has been proposed as a cause of arterial stiffness via increased systemic inflammation (237); in a case control study of individuals with periodontitis who either had hypertension or no cardiovascular risk factors, periodontitis was associated with endothelial dysfunction in both groups (382). In a study of renal transplant recipients the presence of advanced periodontal disease was associated with the presence of left ventricular hypertrophy (383); however the relationship between the periodontal phenotype and the vascular phenotype in a CKD cohort has not yet been described.

The difference in markers of arterial stiffness between those dentate individuals with and without any degree of periodontitis is shown in table 7.

Table 6-7: Markers of arterial stiffness in dentate participants with and without periodontitis

	Healthy/Gingivitis	Periodontitis	p-value
Peripheral pulse pressure (mmHg)	53 (19)	51 (19)	0.551
Central pulse pressure (mmHg)	64 (20)	63 (18)	0.817
Peripheral mean arterial pressure (mmHg)	94 (17)	94 (15)	0.892
Central mean arterial pressure (mmHg)	103 (21)	106 (16)	0.242
Pulse wave velocity (m/s)	9.7 (10.1)	9.9 (2.3)	0.377
AIx (%)	21.1 (10.1)	19.8 (10.5)	0.318
Advanced glycation end products (AU)	2.9 (0.9)	2.9 (0.7)	0.547

Parametric data so mean (SD) shown, unpaired T tests performed

These data show no significant difference in measures of arterial stiffness when patients without periodontitis were compared with those with all severities of periodontitis.

In the next analysis dentate individuals with periodontitis were considered with regard to arterial stiffness and severity of periodontitis, unpaired T tests were performed and the results are shown in the box and whisker plots in figure 2 (a-g).

Figure 6-2: Measures of arterial stiffness and severity of periodontitis

Figure 2a: Peripheral PP and severity of periodontitis

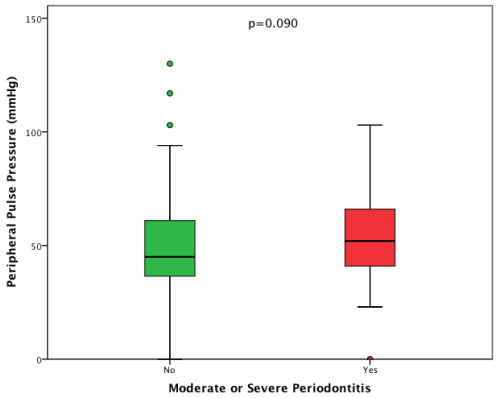


Figure 2b: Central PP and severity of periodontitis

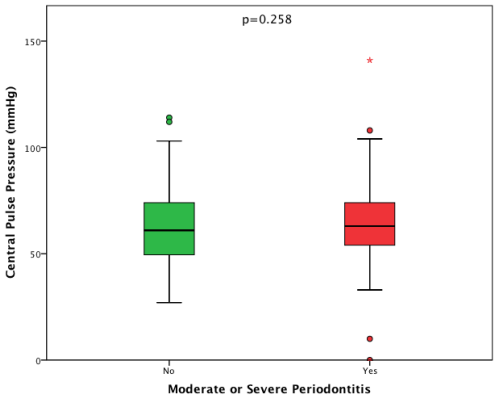


Figure 2c: Peripheral MAP and severity of periodontitis

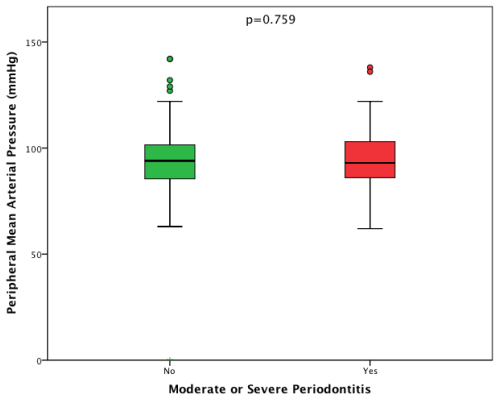


Figure 2d: Central MAP and severity of periodontitis

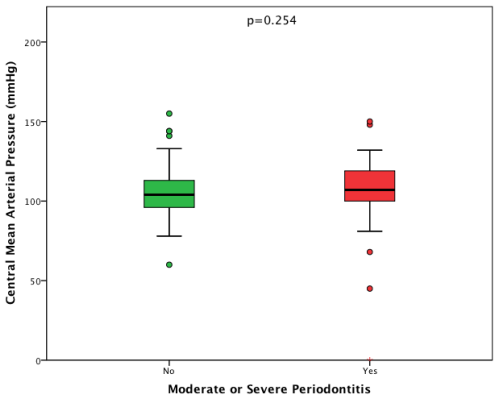


Figure 2e: PWV and periodontitis

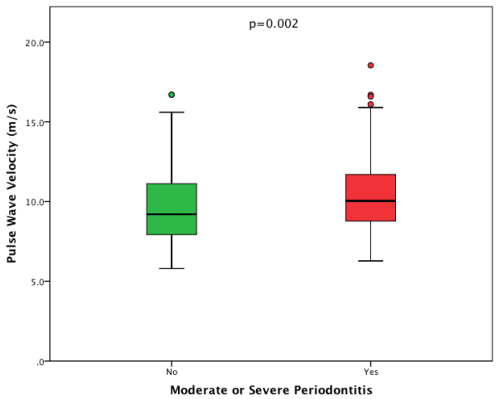


Figure 2f: AIx and periodontitis

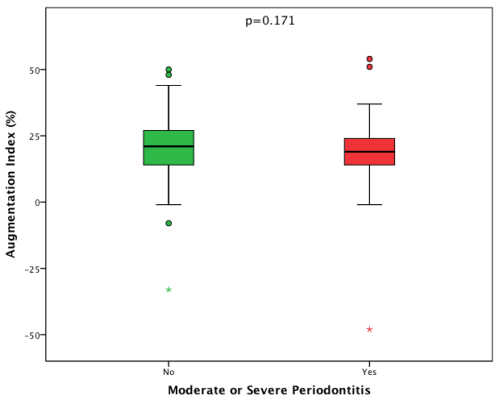
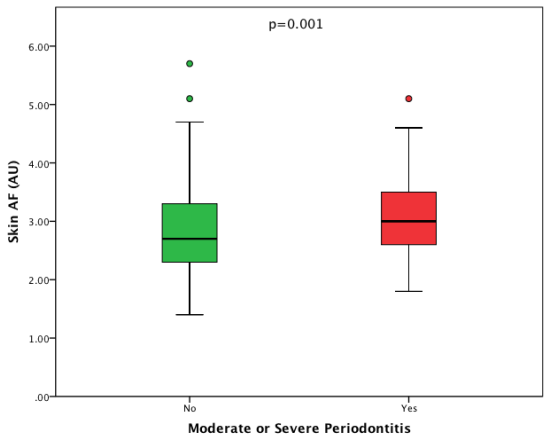


Figure 2g: AGEs and periodontitis



These data show that when participants with periodontitis are divided in moderate or severe versus mild, those with the most severe periodontitis have significantly higher PWV and AGEs.

6.5.The relationship between the anthropomorphic phenotype and the periodontal phenotype

In a prospective observational study of healthy adults the relationship between baseline weight and subsequent progression of periodontitis was explored; higher baseline weight and weight gain were risk factors for the progression of periodontitis (384). However, Shultis et al described a contrasting finding in a prospective observational study of American Indian subjects with type 2 diabetes, reporting that body mass index was negatively associated with the severity of periodontitis (385).

The difference between anthropomorphic markers in dentate participants with, and without, periodontitis is shown in table 8 and indicates that there is no

relationship between anthropomorphic status and periodontal health in patients with CKD.

Table 6-8: Anthropomorphic characteristics of dentate participants, with and without, periodontitis

	Healthy/Gingivitis	Periodontitis	p-value
BMI	30.6 (6.2)	29.5 (6.9)	0.181
Waist/Hip ratio	0.96 (0.09)	0.96 (0.13)	0.771
Hip/Thigh ratio	2.03 (0.26)	3.19 (14.9)	0.480

Parametric data so mean (SD) shown and unpaired T tests performed

These data show no significant difference in anthropomorphic measures by presence of periodontitis.

The relationship between anthropomorphic status and severity of periodontitis in dentate patients with periodontitis was explored using unpaired T tests, the results are shown in figure 3 (a-c).

Figure 6-3: Anthropomorphics and severity of periodontitis

Figure 3a: BMI and severity of periodontitis

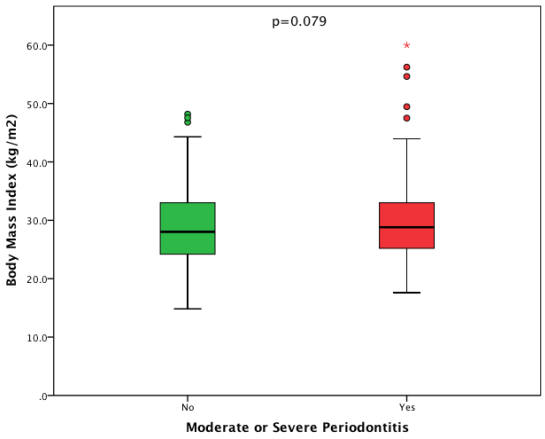


Figure 3b: HTR and severity of periodontitis

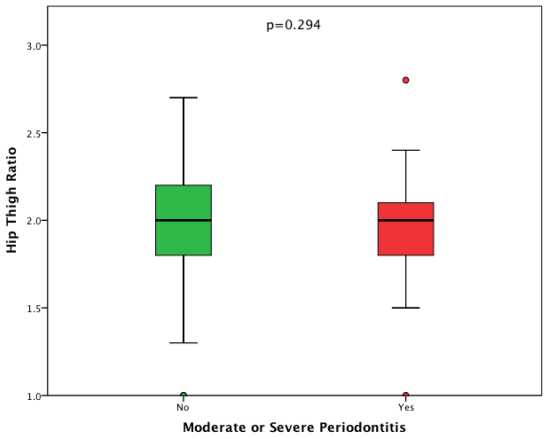
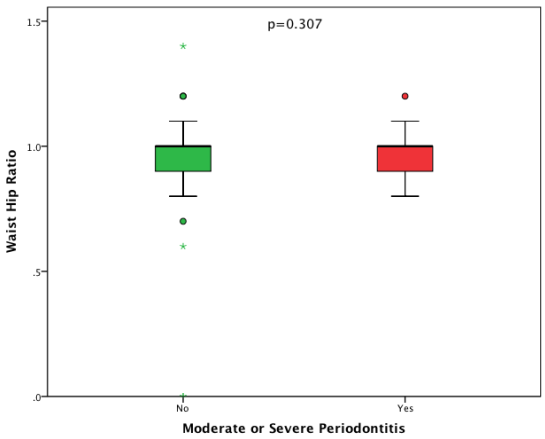


Figure 3c: WHR and severity of periodontitis



These data show no difference in anthropomorphic measures by severity of periodontitis.

6.6.Periodontitis and systemic inflammation

Periodontitis is the most prevalent inflammatory disease in humans (386) and has been associated with systemic inflammation and as a non-traditional risk factor for CVD (237, 387). I assessed the association between periodontitis and the following markers of inflammation, highly sensitive C-reactive protein, polyclonal FLC and IL-6.

The difference in inflammatory markers between dentate individuals with and without any degree of periodontitis is shown in table 9, as the inflammatory markers were all non-parametrically distributed, median and interquartile ranges are shown and Mann-Whitney tests were performed.

Table 6-9: Inflammatory markers in dentate participants with and without periodontitis

	Healthy/Gingivitis	Periodontitis	p-value
hsCRP (mg/L)	2.1 (1.0-8.4)	3.0 (1.2-9.3)	0.524
Polyclonal FLC (mg/L)	71.4 (47.7-101.9)	72.5 (51.6-114.7)	0.360
IL-6 (mg/L)	6.0 (5.0-8.5)	7.0 (5.0-10.0)	0.120

Non-parametric data so median (IQR) shown, Mann Whitney tests performed

These data show no significant difference in inflammatory markers in participants with and without periodontitis.

In the next analysis dentate individuals with periodontitis were assessed for the relationship between systemic inflammation and severity of periodontitis; the results are shown in the box and whisker plots in figure 4 (a-d), the Mann Whitney test was used to determine significance as the data were non-parametric.

Figure 6-4: Inflammatory markers and severity of periodontitis

Figure 4a: hsCRP and severity of periodontitis

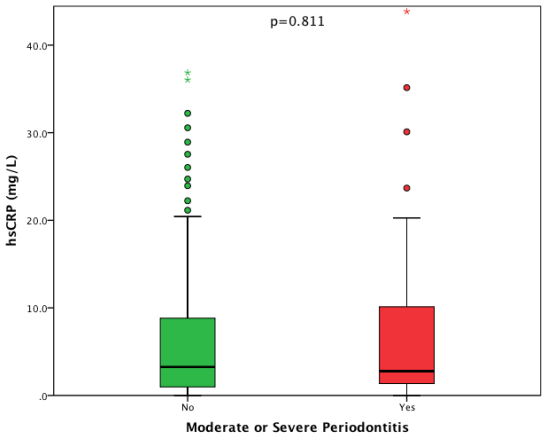


Figure 4b: polyclonal FLC and severity of periodontitis

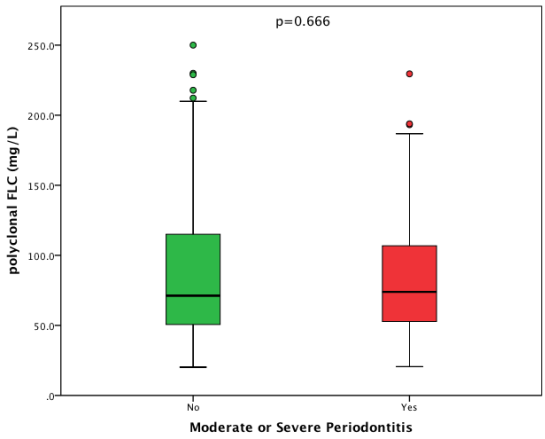
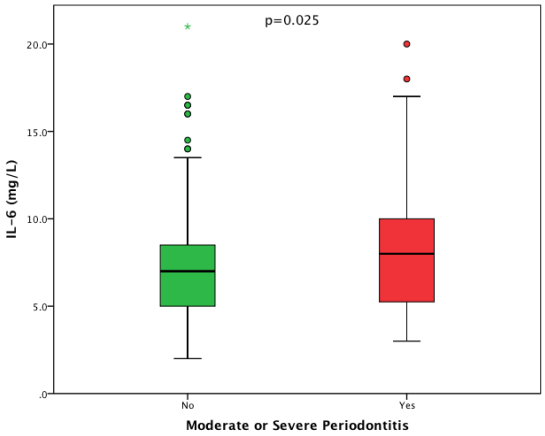


Figure 4c: IL-6 and severity of periodontitis



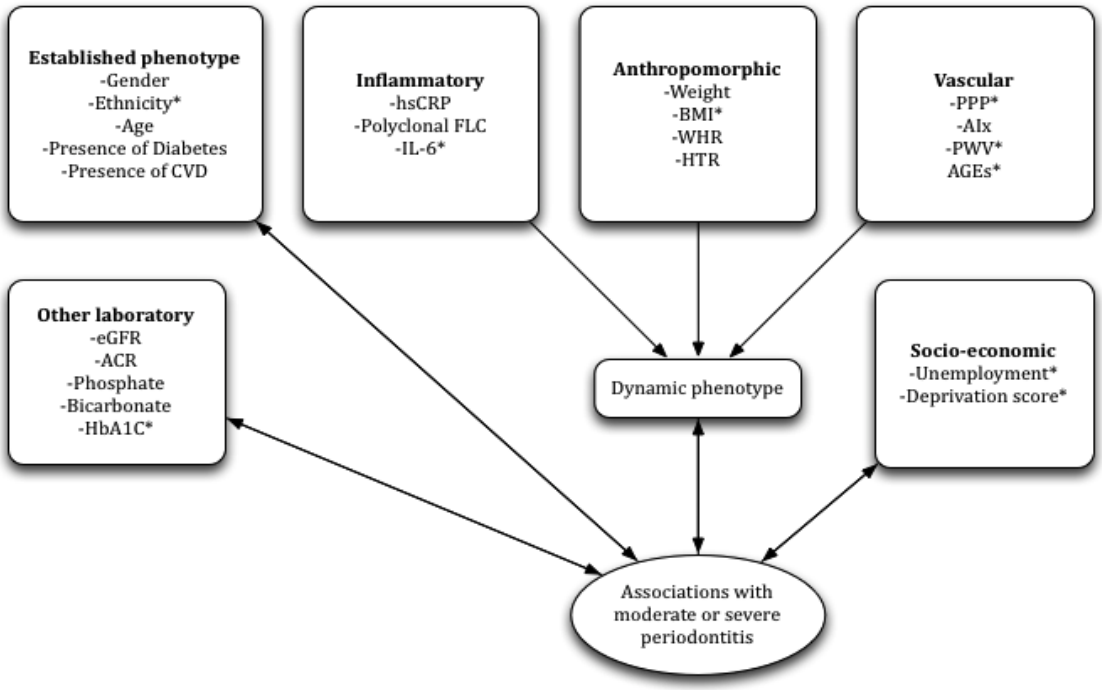
These data show that IL-6 increases as severity of periodontitis increases. This is an interesting observation and one that is not consistent with previous findings that both pro-inflammatory cytokines and CRP are elevated in patients with periodontitis compared to healthy controls (388), in a small study of haemodialysis patients the presence of periodontitis was associated with both increased CRP concentrations and measures of atherosclerosis (389).

It is not clear what the mechanism between the increased IL-6 concentrations observed in this cohort and the severity of periodontitis might be, a limitation of the analysis is that the data are cross-sectional (this is a significant weakness if the hypothesis that periodontitis causes systemic inflammation by repeated exposure of the circulation to bursts of inflammation caused by tooth brushing, eating or dental probing is correct (390)) another weakness is the limited number of inflammatory markers measures and the absence of any markers of oxidative stress; these will be overcome in more detailed analyses of the cohort in the future.

6.7.The determinants of periodontal status in the RIISC cohort

For the purposes of this analysis dentate participants were dichotomised into whether they had either moderate or severe periodontitis or not. A univariate analysis was performed, a multivariate analysis (binary logistic regression) including those variables that reached a significance of <0.1 (indicated by an *) was then performed, the results are shown in table 10. The variables included in the univariate analysis are shown in figure 5.

Figure 6-5: The variables included in the univariate analysis of factors potentially associated with moderate or severe periodontitis



CVD, cardiovascular disease; hsCRP, highly sensitive CRP; FLC, free light chains; IL-6, interleukin 6; BMI, body mass index; WHR, waist hip ratio; HTR, hip thigh ratio; PPP, peripheral pulse pressure; CPP, central pulse pressure; PMAP, peripheral mean arterial pressure; CMAP, central mean arterial pressure; AIx, augmentation index; AGEs, advanced glycation end products; eGFR, estimated glomerular filtration rate; ACR, albumin creatinine ratio

Table 6-10: Multivariate analysis of variables associated with moderate or severe periodontitis

	Significance	Odds ratio	95% confidence intervals
Pulse wave velocity	0.005	1.568	1.147, 2.144
White ethnicity	0.548		
IL-6	0.548		
Body mass index	0.438		
Pulse pressure	0.440		
Advanced glycation end products	0.118		
Unemployment	0.081		
Deprivation	0.852		
HbA1C	0.634		

For all the continuous variables any associations were positive

These data show that only increased pulse wave velocity was independently associated with the presence of moderate or severe periodontitis.

6.8.Discussion

6.8.1. The prevalence of periodontitis

Previous work has focused on the potential utility of a diagnosis of periodontitis as a risk factor for either incident CKD or future CKD risk (391-393). In one of the largest population based studies (comprising more than 12 000 North American adults selected to be representative of the general population) Fisher et al reported a periodontitis prevalence of 6%; in the same cohort the prevalence of CKD (defined as an estimated GFR 15-59mL/min using the four variable MDRD equation) was 3.6% (391).

In a Polish study investigators examined the periodontal health of 106 individuals with kidney disease (35 haemodialysis patients, 33 peritoneal

dialysis patients and 38 pre-dialysis CKD patients) and compared them to healthy volunteers with and without CKD (374). They reported that the prevalence of periodontitis was greater in the cohort with kidney disease (though they do not quote an exact periodontitis prevalence) and that within this group the haemodialysis patients had the most severe disease (374). In a small cohort of haemodialysis patients (n=51) Buhlin et al reported a prevalence of severe periodontitis of 35% (394).

In a subgroup analysis of the NHANES III cohort only patients with CKD were included, the prevalence of periodontitis was just under 15%, though the prevalence reached 39% in non-Hispanic black patients (395), in a further analysis the authors described that there was a significant dose response with individuals with the most advanced CKD having the most severe periodontitis in a Mexican sub-group (396).

We report a prevalence of periodontitis of 65%, with 36% of these individuals having moderate or severe periodontitis (a total of 23% of the cohort who underwent a periodontal examination), with 17% of the cohort being edentulous. This suggests that periodontitis is more prevalent in the RIISC population than in previously published populations, as these other cohorts were comprised of primarily American or Scandinavian participants it is possible that they were not comparable to the RIISC population. A further point of comparison is with the Adult Dental Health Survey (ADHS), an epidemiological study of adults from the West Midlands area (the same geographical area from

which the RIISC cohort is drawn). In the ADHS the prevalence of periodontitis was 55% with 45% being healthy and 10% being edentulous (397). The two cohorts do have demographic differences, the ADHS population is significantly younger with a greater percentage of females and is composed of predominantly white individuals (397). This observation suggests that the prevalence of periodontitis might be higher in the British population compared to other countries, though the prevalence of periodontitis and edentulousness in the RIISC population appears slightly higher than in the general population of the same geographical area.

A potential explanation for the disparity in prevalence of periodontitis between different populations is that the method of defining periodontitis was not comparable; in RIISC validated case definitions were used and each participant underwent a detailed calibrated periodontal assessment (299). It is possible that in other populations less rigorous methods were used and this led to an under-diagnosis of periodontitis.

The increased prevalence of periodontitis in the RIISC cohort is in keeping with previous observations that CKD and periodontitis are associated, however as this is a cross-sectional analysis no temporal relationship can be established.

6.8.2. The relationship between periodontal disease and arterial stiffness

Previous authors have explored the relationship between CVD and periodontitis, (398-401). In a review of atherosclerosis and periodontitis Friedewald et al concluded that there was evidence to support an association between periodontitis and atherosclerotic CVD but that it was unclear if this was an independent relationship or not and what the pathophysiological basis for the relationship might be (402).

Increased pulse wave velocity PWV has been found to be associated with progressive CKD and cardiovascular events (167, 403, 404). To my knowledge, to date, there have been no studies which have reported detailed cardiovascular and periodontal assessments and their inter-relationship in patients with CKD from a prospective observational study, therefore it was not possible to compare the results of this study with those of any other cohort.

The finding that both PWV and AGEs increase with severity of periodontitis suggests that periodontitis is associated with an increased risk for the initiation or accelerated development of macrovascular and microvascular disease in CKD. However there was only an association between two of the seven markers of vascular health used and periodontitis.

The finding that increased arterial stiffness (as defined by increasing pulse wave velocity) was independently associated with the severity of periodontitis also supports this hypothesis. However none of the components of either the established or dynamic cardiovascular phenotype were associated with the presence of periodontitis (or the severity of periodontitis).

The strengths of this work are that it explores a novel area, includes a large group of patients who are very well characterized from a vascular, clinical and periodontal perspective and will provide follow up data. The weakness of this analysis is that the relationship between dynamic risk factors and established phenotypes is difficult to explore in a cross-sectional analysis.

6.9. Conclusions

This is the first time that the relationship between periodontitis and CVD in patients with CKD has been explored. This cross-sectional analysis suggests that periodontitis is associated with arterial stiffness in patients with CKD, however maturation of the cohort is required to define the clinical implications of this relationship.

7. Results 5: Early outcomes of RIISC study participants

At the time of writing 500 participants have been recruited to the RIISC study, of these 305 have reached the six-month visit and 104 have reached the 18-month visit. In this chapter I present the early outcomes of participants; including withdrawals from the study, commencement of RRT and deaths.

7.1.Withdrawal from the study

There have been 27 withdrawals from the study. The median time to withdrawal from consent was 185 (149-548) days. Participants were under no obligation to identify the reason for withdrawal and 37% did not do so; where reasons were provided these are shown in Table 1.

Table 7-1: Reasons for withdrawal

	Percentage
No reason given	37
Unable to attend morning clinics	22
Increased frailty/co-morbidity	19
Perception of increased number of appointments	13
Unable to tolerate study assessments	6
Psychiatric problems	3

The baseline characteristics of those who withdraw from the study compared to those who did not are shown in table 2. There was no significant difference between the two groups

Table 7-2: The demographic characteristics of those who withdrew from the study compared to those who did not

	Remained in study (n=473)	Withdrawn from study (n=27)	p-value
Males (%)	61	48	0.181
Age	63 (16)	66 (19)	0.396
Ethnicity (%):			
White	71	74	0.764
Asian	16	4	0.218
Black	11	11	0.932
Other	2	7	0.039
eGFR	28 (12)	23 (7)	0.218
ACR*	26.9 (5.5-107.8)	43.5 (2.5-124.5)	0.989
Age adjusted Charlson Co-morbidity Index*	5 (2-7)	6 (0-8)	0.723
IMD rank*	7484 (2794-15139)	8667 (3199-16607)	0.796

*data shown as median (IQR), Mann-Whitney U tests performed, for categorical variables Chi-squared tests performed

7.2.Renal replacement therapy

7.2.1. The baseline characteristics of participants who required RRT

Twenty seven participants progressed to RRT (as defined by requirement for haemodialysis or peritoneal dialysis for more than 90 days or receiving a pre-emptive renal transplant); 70% (n=19) commenced haemodialysis, 19% peritoneal (n=5) dialysis and 11% (n=3) received a pre-emptive transplant. Renal replacement therapy was commenced at a median of 333 (146-575) days from recruitment. The baseline characteristics of participants who required RRT compared to those who remained independent of dialysis are shown in table 3. The renal diagnoses and baseline cardiovascular co-morbidity of the two groups are shown in table 4.

The data show that patients who progressed to ESKD were significantly younger, were more likely to be of Asian ethnicity, had lower baseline eGFRs and higher baseline ACRs.

There was no significant difference between the renal diagnostic groups in progression to ESKD, the only significant difference in co-morbidity was a significantly lower prevalence of PVD in those who progressed to ESKD but with a low event rate and a small sample this may be a spurious finding.

Table 7-3: The demographic characteristics of participants remaining RRT independent compared to those who commenced RRT

	RRT independent (n=473)	Commenced RRT (n=27)	p-value
Males (%)	61	44	0.597
Age	64 (16)	54 (18)	0.001
Ethnicity (%):			
White	73	48	0.006
Asian	15	37	0.002
Black	10	15	0.467
Other	2	0	0.445
eGFR	28 (12)	17 (6)	0.001
ACR*	23.0 (4.8-93.4)	239.2 (128.3-342.1)	<0.001
Age adjusted Charlson Co-morbidity Index*	5 (2-7)	3 (2-7)	0.162
IMD Rank*	7668 (2874-15219)	7070 (1815-11296)	0.302

*data shown as median (IQR), Mann-Whitney U tests performed, for categorical variables Chi-squared tests performed

Table 7-4: The baseline established cardiovascular diagnoses of participants who required RRT compared to those who did not

	RRT independent	Commenced RRT	p-value
Diabetes (%)	40	44	0.581
Ischaemic heart disease (%)	25	15	0.251
Cerebrovascular disease (%)	12	15	0.670
Peripheral vascular disease	13	0	0.049
Chronic Obstructive Pulmonary Disease (%)	12	15	0.670
Malignancy (%)	16	15	0.863

Chi-squared tests performed

7.2.2. The dynamic cardiovascular phenotype and progression to RRT

The previous table illustrated that in this cohort there was no increased prevalence of established cardiovascular disease in participants who progressed to RRT compared to those who did not; Taal et al demonstrated that PWV and AIx were independent predictors of progression to ESKD in patients with stage 4 and 5 CKD (167). To explore the early associations between the dynamic cardiovascular phenotype and progression to RRT a series of analyses was performed using unpaired T tests, the results are shown in figure 1 (a-g).

The data show that the peripheral MAP is significantly higher in participants who progress to ESKD and the AIx is significantly lower in those who progress to ESKD.

Figure 7-1: The dynamic cardiovascular phenotype and progression to RRT

Figure 1a: Peripheral PP

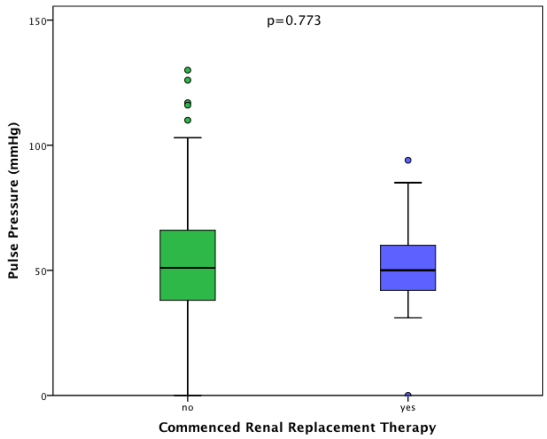


Figure 1b: Central PP

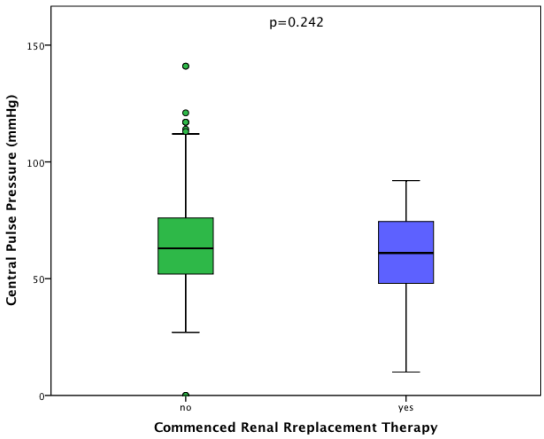


Figure 1c: Peripheral MAP

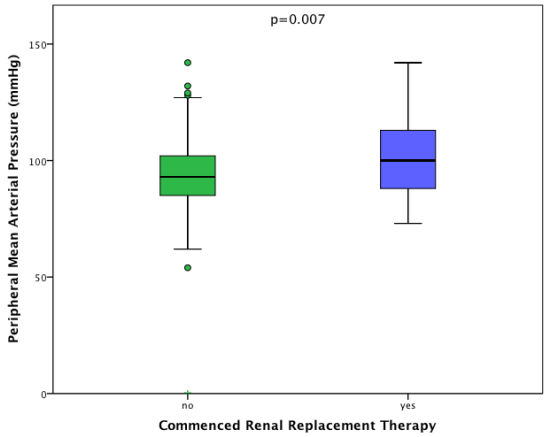


Figure 1d: Central MAP

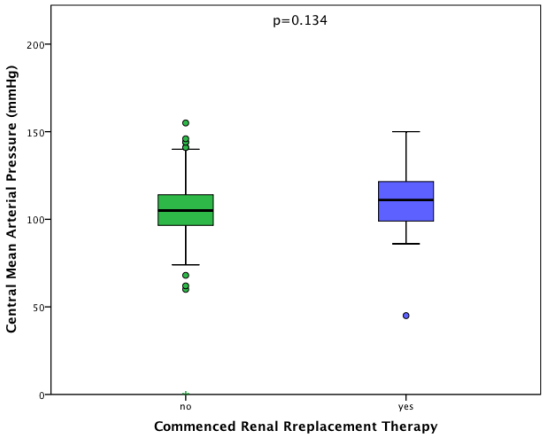


Figure 1e: PWV

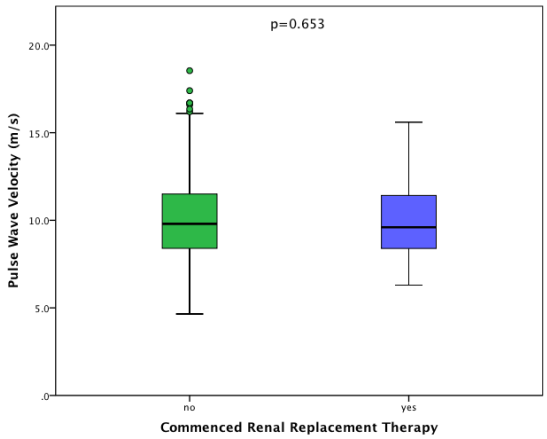


Figure 1f: Augmentation Index

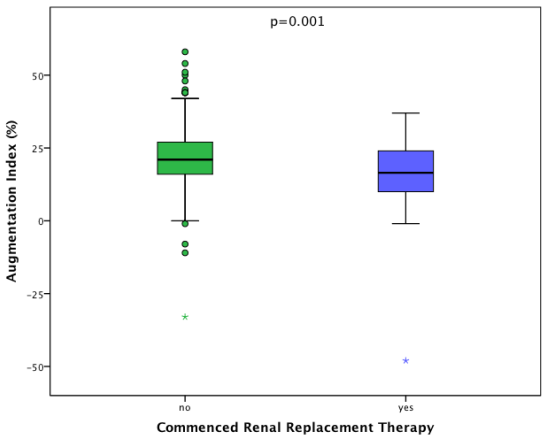
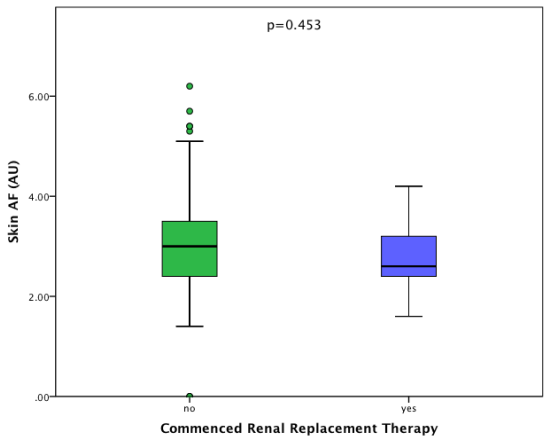


Figure 1g: AGEs



7.2.3. The dynamic inflammatory and anthropomorphic phenotype and progression to RRT

The association between systemic inflammation and the progression of CKD has been previously demonstrated; Tonelli et al demonstrated that higher levels of CRP and TNFα were independently associated with progression of CKD (182). To explore the early potential associations between inflammation and progression to RRT in this cohort a series of analyses were carried out using Mann-Whitney tests, the results are shown in figure 2 (a-c).

The association between BMI and other anthropomorphic measures in the progression of CKD is controversial with a number of studies suggesting no association between BMI and progression of CKD (366, 367, 405); however other authors describe an association between central obesity and risk factors for CKD progression (341) and Othman et al described BMI as an independent risk factor for progression of CKD in a cohort of non-diabetic subjects (406). To explore the

early associations between anthropomorphic measures and progression to RRT a series of analyses were carried out using unpaired T tests, the results are shown in figure 2 (d-f).

These data show that participants who progressed to RRT had significantly higher baseline polyclonal FLCs, this is likely to be a representation of kidney function.

Figure 7-2: Inflammation and anthropomorphics and progression to RRT

Figure 2a: hsCRP

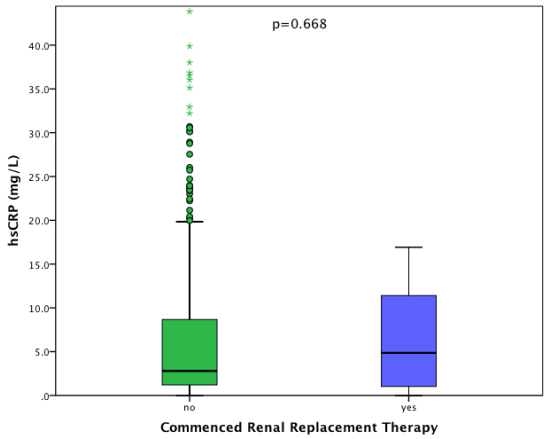


Figure 2b: IL-6

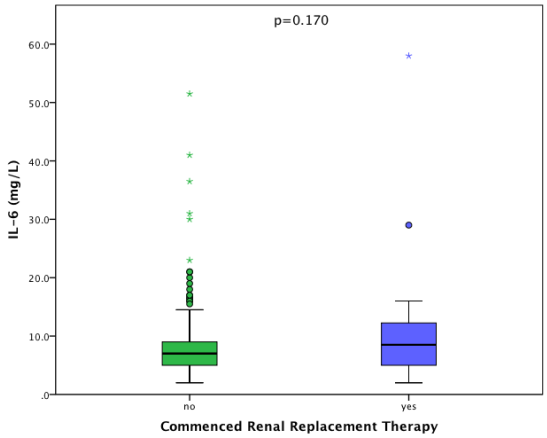


Figure 2c: cFLC

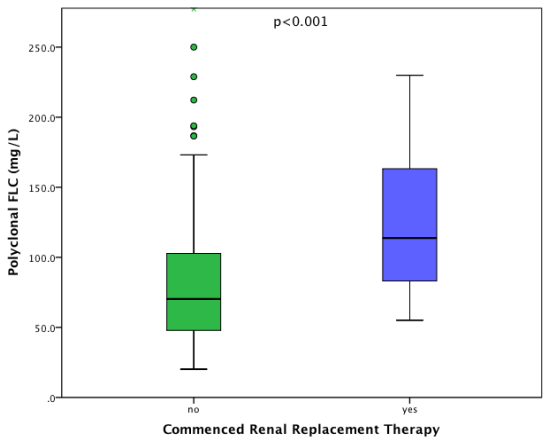


Figure 2d: BMI

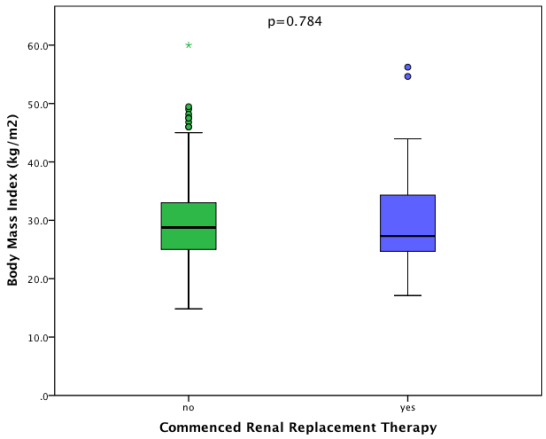


Figure 2e: WHR

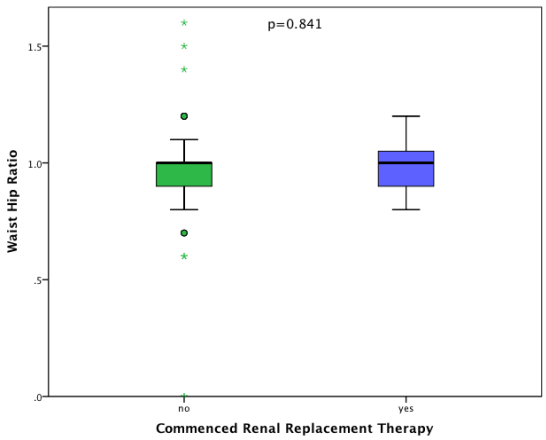
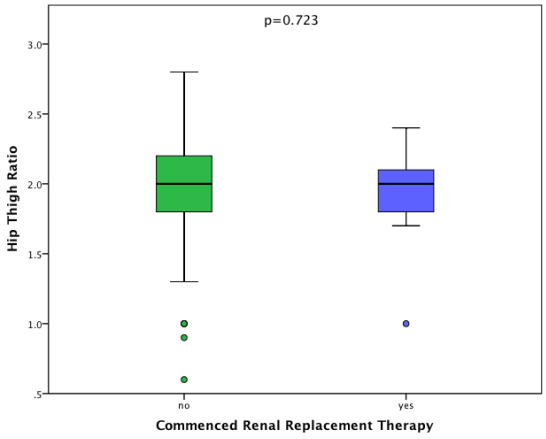


Figure 2f: HTR



7.2.4. The determinants of progression to RRT

To identify the associations of progression to RRT a univariate analysis was performed. The variables included comprise aspects of the established and dynamic phenotype and are shown in figure 3; those variables that reached a significance of <0.1 (indicated with an asterix) were included in a multivariate analysis (a binary logistic regression), the results of which are shown in table 5.

Figure 7-3: Variables included in the univariate analysis of determinants of progression to RRT

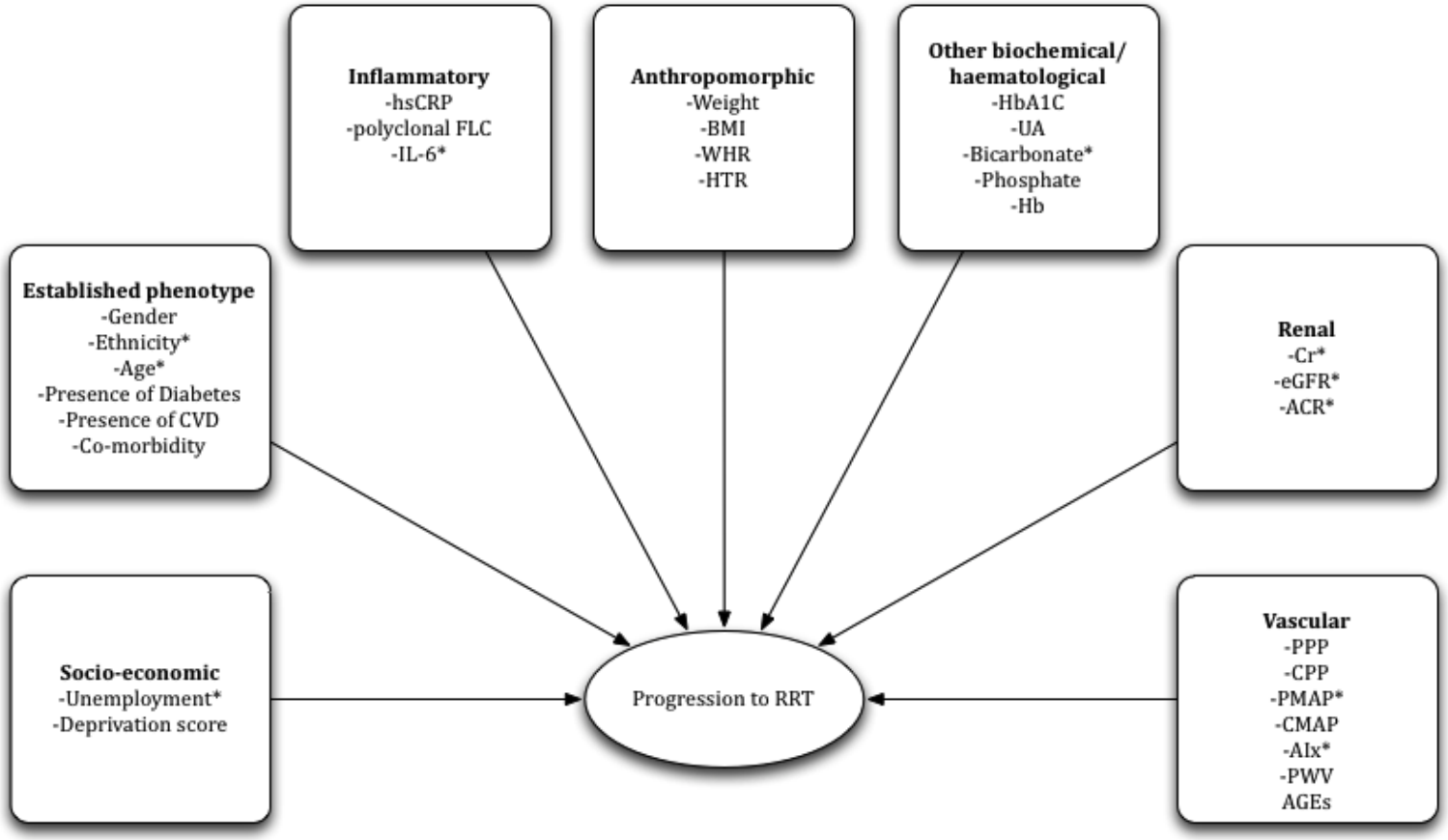


Table 7-5: Multivariate analysis of variables associated with progression to RRT

	Significance	Odds ratio	95% confidence intervals
eGFR	<0.001	0.826	0.742, 0.920
ACR>30mg/mmol	0.009	58.797	2.881, 1229.868
AIx	0.032	0.893	0.805, 0.990
White ethnicity	0.862		
Asian ethnicity	0.654		
Decreasing age	0.139		
Creatinine	0.409		
IL-6	0.933		
Bicarbonate	0.863		
MAP	0.170		
Unemployment	0.121		

For creatinine, IL-6 and MAP a higher level of the variable was associated with the outcome, for eGFR, AIx, age and bicarbonate a low level of the variable was associated with the outcome

7.3.Survival in the RIISC cohort

There were 21 deaths in the cohort, deaths occurred at a median of 237 (160-395) days following recruitment, only one death occurred in a patient who had progressed to RRT (the death occurred 67 days after the commencement of dialysis).

The characteristics of participants who died compared to those who survived are shown in table 6; the data show that participants who died were significantly older and more co-morbid than those who survived.

Table 7-6: The Demographic characteristics of participants who died compared to those who survived

	Survived (n=479)	Died (n=21)	p-value
Males (%)	60	66	0.549
Age	63 (16)	78 (9)	<0.001
Ethnicity (%):			
White	71	76	0.630
Asian	16	10	0.418
Black	10	14	0.578
Other	2	0	0.503
eGFR	28 (12)	22 (9)	0.067
ACR*	27.5 (5.4-110.0)	18.7 (6.7-82.5)	0.526
Age adjusted Charlson Co-morbidity Index*	5 (2-7)	8 (6.5-9)	<0.001
IMD rank*	8009 (2808-15180)	6093 (3767-10490)	0.614

*data shown as median (IQR), Mann-Whitney U tests performed, for categorical variables Chi-squared tests performed

7.3.1. The established cardiovascular phenotype and survival in the RIISC cohort

To examine the prevalence of established cardiovascular disease in RIISC participants who both died and survived a comparative analysis was performed, the results are shown in table 7.

Table 7-7: The established cardiovascular and co-morbid phenotype in participants who died and those who survived

	Survived	Died	p-value
Diabetes (%)	40	24	0.135
Ischaemic heart disease (%)	24	35	0.306
Cerebrovascular disease (%)	13	5	0.287
Peripheral vascular disease	13	0	0.084
Chronic Obstructive Pulmonary Disease (%)	13	5	0.278
Malignancy (%)	24	35	0.306

Chi-squared tests performed

These data show that there are no significant differences between the groups.

7.3.2. The dynamic cardiovascular phenotype and survival in the RIISC cohort

In a recent meta-analysis Vlachopoulos et al found that aortic stiffness (defined as aortic pulse wave velocity) was a strong predictor of future cardiovascular events and all cause mortality; the predictive ability being higher in those individuals with higher baseline cardiovascular risk (407). As this is a cohort at substantial cardiovascular risk (by virtue of renal impairment and the high prevalence of established cardiovascular disease and diabetes) I hypothesised that participants who died would have a greater burden of micro and macro-

vascular disease than those who survived. To test this hypothesis a number of analyses were performed, the results are shown in figure 4 (a-g).

Figure 7-4: The dynamic vascular phenotype and survival

Figure 4a: Peripheral PP

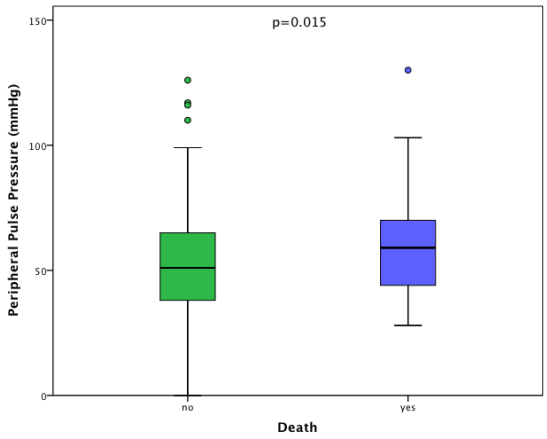


Figure 4b: Central PP

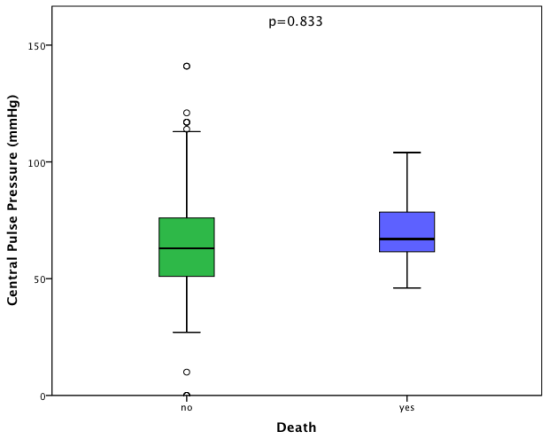


Figure 4c: Peripheral MAP

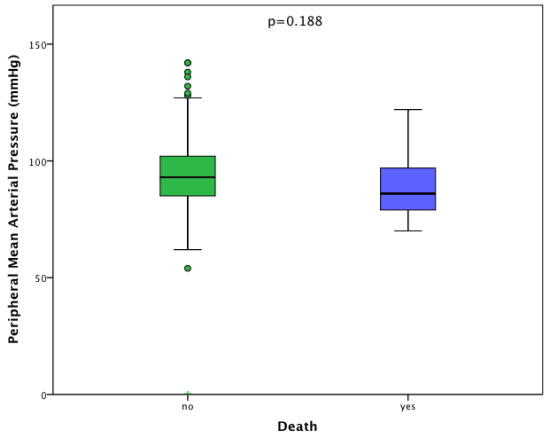


Figure 4d: Central MAP

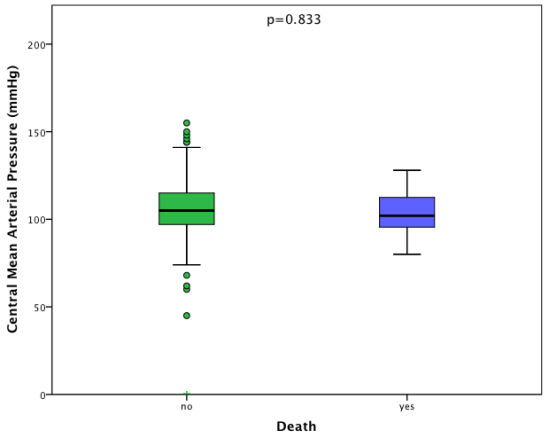


Figure 4e: PWV

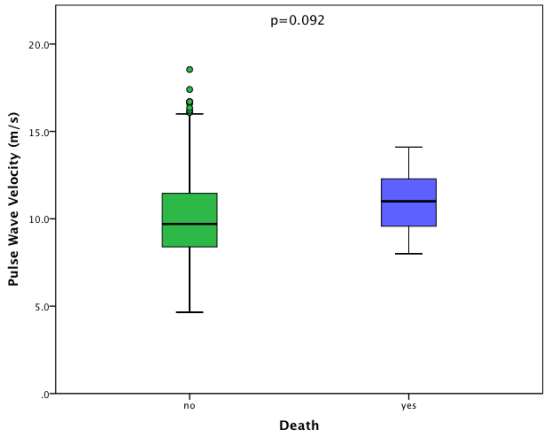


Figure 4f: Augmentation Index

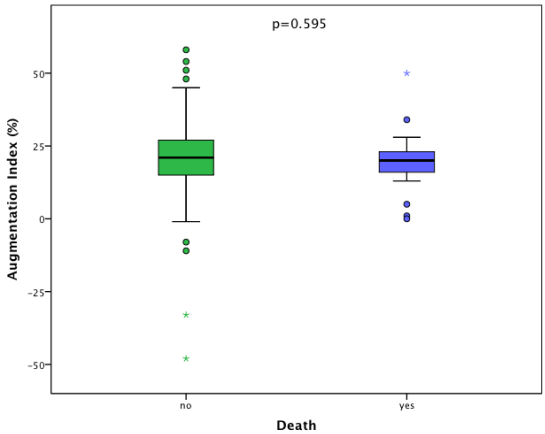
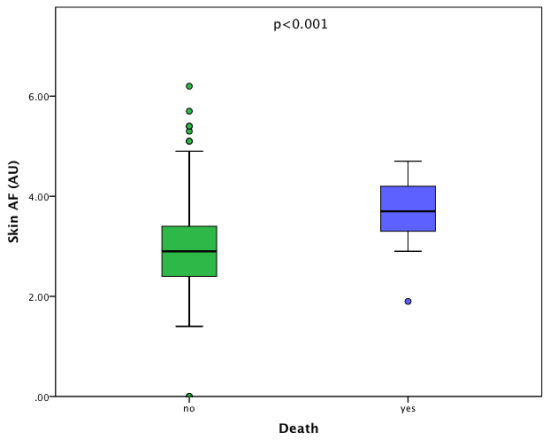


Figure 4g: AGEs



These data show that patients who died had a significantly higher peripheral PP and AGEs than those who survived.

7.3.3. The dynamic inflammatory and anthropomorphic phenotype and survival in the RIISC cohort

Malnutrition and inflammation have been proposed as possible causes of poor survival in patients with CKD (408). To explore the associations in this cohort a number of analyses were performed using Mann-Whitney tests for the non-parametrically distributed inflammatory markers and unpaired T tests for the anthropomorphic data, the results are shown in figure 5 (a-f).

The data show that both hsCRP and polyclonal FLC concentrations are increased in patients who did not survive; there are no anthropomorphic differences between survivors and non-survivors.

Figure 7-5: Inflammation and anthropomorphics and survival

Figure 5a: hsCRP

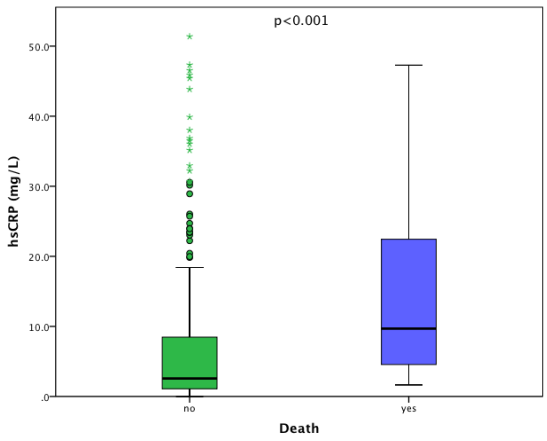


Figure 5d: BMI

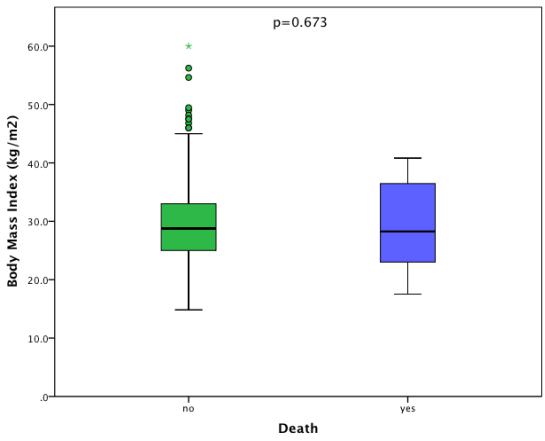


Figure 5b: IL-6

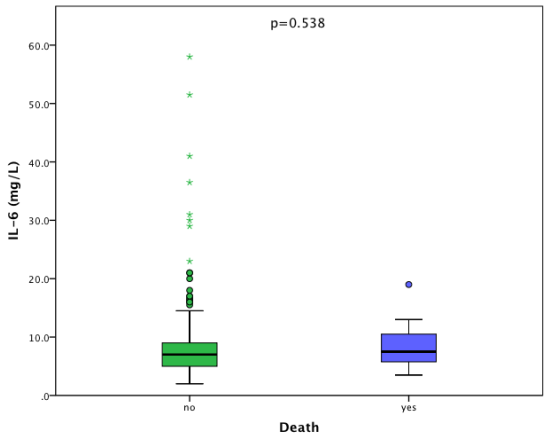


Figure 5e: WHR

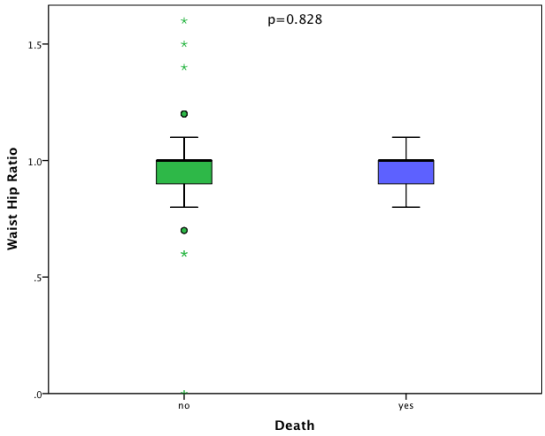


Figure 5c: cFLC

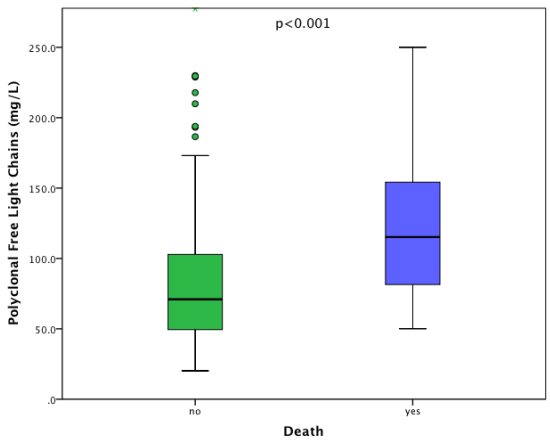
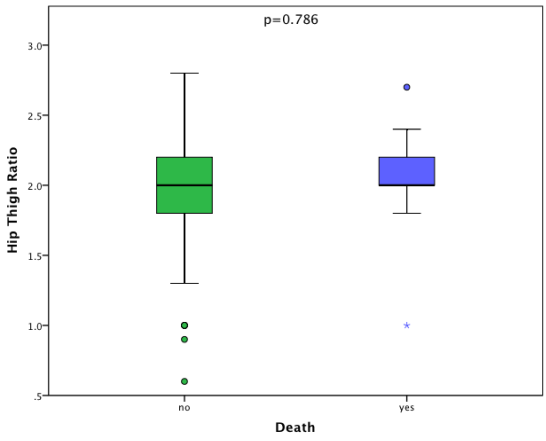


Figure 5f: HTR



7.3.4. The determinants of all-cause mortality in the RIISC cohort

To identify the determinants of survival a univariate analysis was performed; the variables included were components of both the established and dynamic phenotype. The results are shown in figure 6; those variables that reached a significance of <0.1 (indicted in figure 6 with an asterix) were included in a multivariate analysis (a binary logistic regression), the results of which are shown in table 8.

Figure 7-6: The variables included in the univariate analysis of determinants of survival

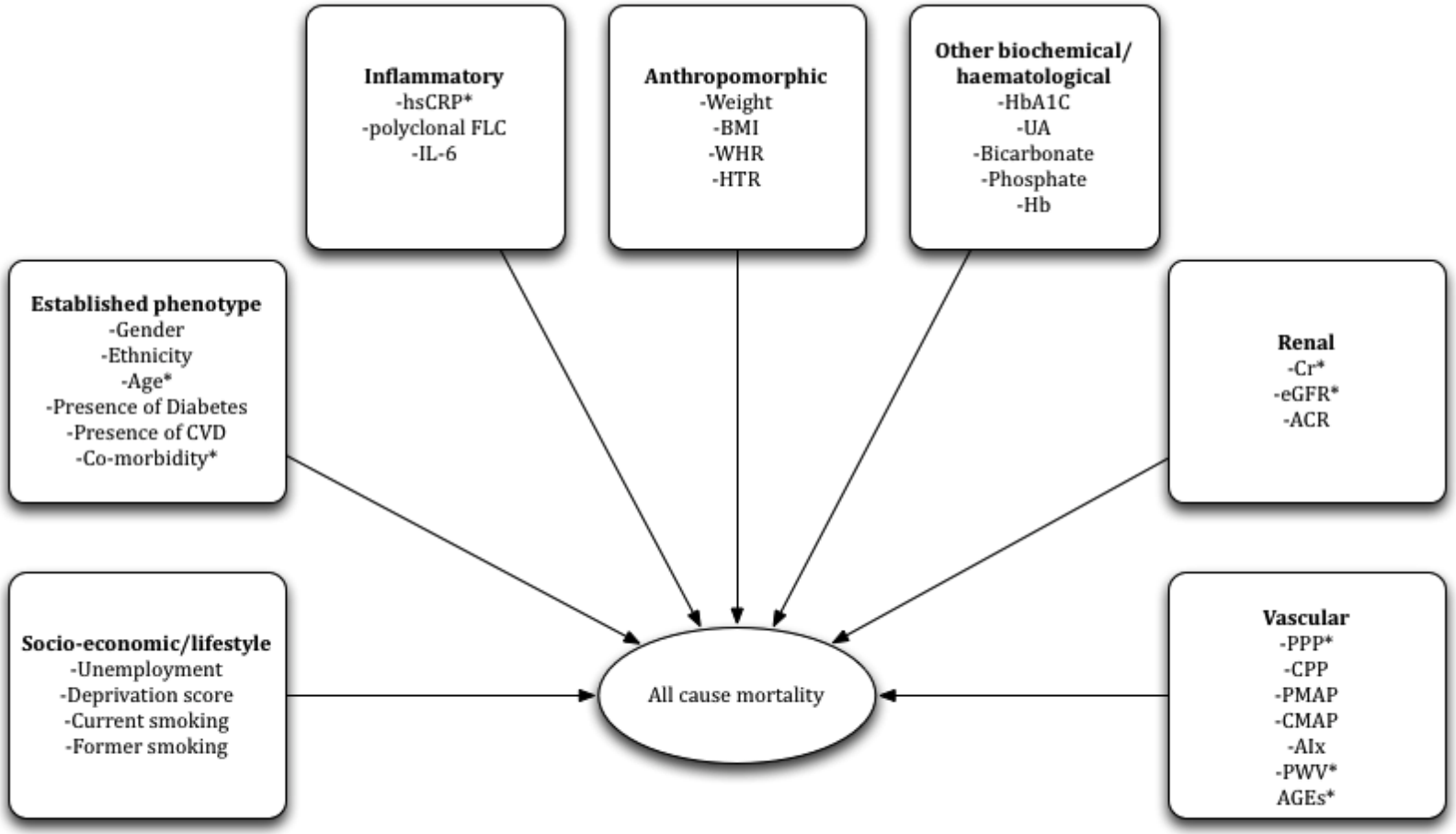


Table 7-8: Multivariate analysis of variables associated with all-cause mortality

	Significance	Odds ratio	95% confidence intervals
Co-morbidity	0.001	1.476	1.180, 1.845
AGEs	0.043	1.941	1.021, 3.691
Age	0.278		
Creatinine	0.143		
eGFR	0.369		
hsCRP	0.239		
Pulse pressure	0.161		
Pulse wave velocity	0.811		

For the continuous variables an increase in the variable was associated with the outcome of interest, except for eGFR where a decrease in level was associated with the outcome of interest

These data show that increased co-morbidity and AGE accumulation are independently associated with all cause mortality.

7.4.Composite of death and renal replacement therapy

47 participants reached the composite end point of death or renal replacement therapy (one participant died following commencement of renal replacement therapy, this was counted as a single event). The baseline characteristics of participants who reached the composite end-point compared to those who did not are shown in table 9. The data show that participants who reached the composite end point had significantly lower baseline eGFRs and significantly higher baseline ACRs than those who did not.

Table 7-9: The baseline characteristics in relationship to composite end-point

	No composite outcome (n=453)	**Reached composite outcome (n=47)**	**p-value**
Males (%)	60	60	0.903
Age	64 (16)	64 (18)	0.923
Ethnicity (%):			
White	73	60	0.056
Asian	15	25	0.056
Black	10	15	0.318
Other	2	0	0.303
eGFR	28 (12)	19 (8)	<0.001
ACR*	23.3 (4.7-95.3)	128.3 (17.0-295.4)	<0.001
Age adjusted Charlson Co-morbidity Index*	5 (2-7)	6 (3-8)	0.079
Index of deprivation*	8199 (2848-15397)	6394 (2260-11058)	0.259

*data shown as median (IQR), Mann-Whitney tests performed; for categorical variables Chi-squared tests performed

7.4.1. The established cardiovascular and co-morbid phenotype and the composite end-point

The baseline cardiovascular and co-morbid phenotype is shown in table 10.

Table 7-10: The baseline cardiovascular and co-morbid phenotype and the composite end-point

	Composite not reached	Composite reached	p-value
Diabetes (%)	40	36	0.634
Ischaemic heart disease (%)	24	23	0.920
Cerebrovascular disease (%)	12	11	0.731
Peripheral vascular disease (%)	13	0	0.008
Chronic Obstructive Pulmonary Disease (%)	13	6	0.189
Malignancy (%)	16	17	0.847

Chi-squared tests performed

These data show that there was a significantly lower prevalence of PVD in patients who reached the composite end-point.

7.4.2. The dynamic cardiovascular phenotype and the composite end point

When renal replacement therapy and survival were considered separately aspects of the dynamic cardiovascular phenotype were associated with adverse outcomes, to explore the relationship a series of analyses were undertaken using paired T tests, the results are shown in figure 8 (a-g). These data show that the baseline AIx was significantly lower in patients who reached the composite end-point than those who did not.

Figure 7-7: The dynamic vascular phenotype and the composite end-point

Figure 7a: Peripheral PP

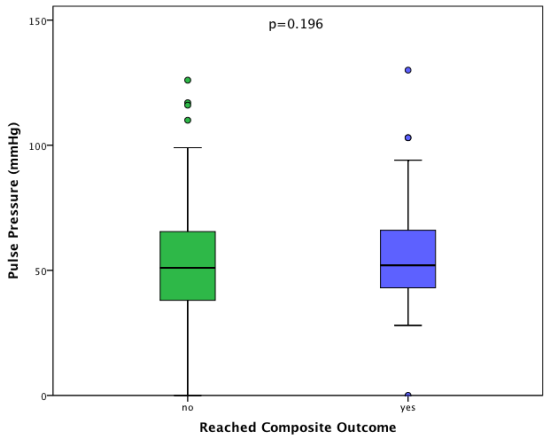


Figure 7b: Central PP

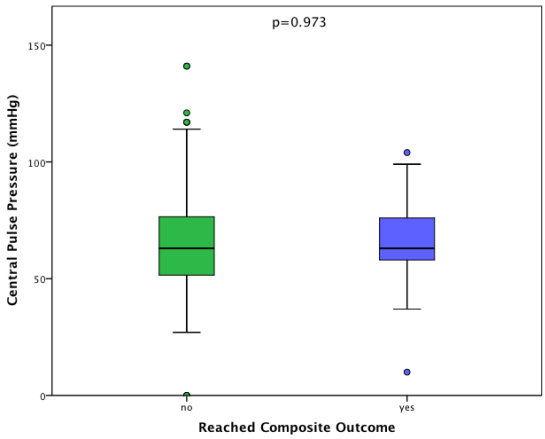


Figure 7c: Peripheral MAP

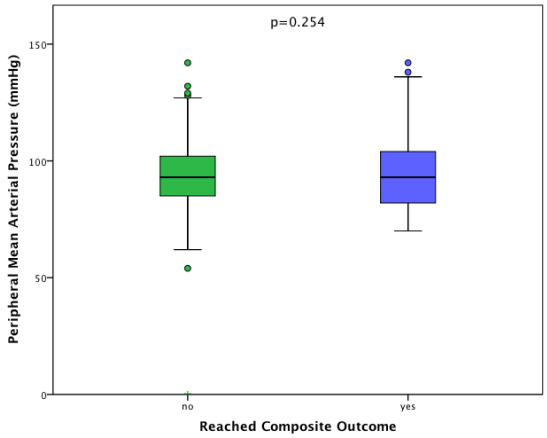


Figure 7d: Central MAP

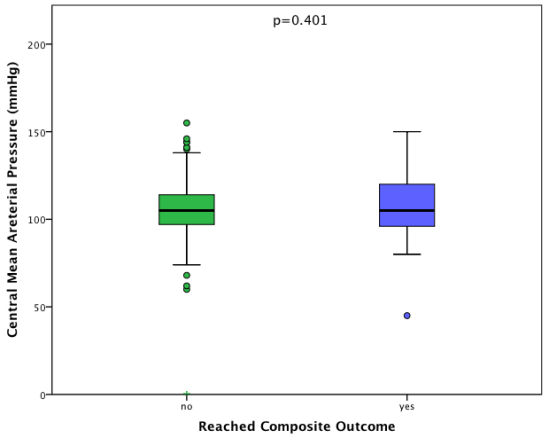


Figure 7e: PWV

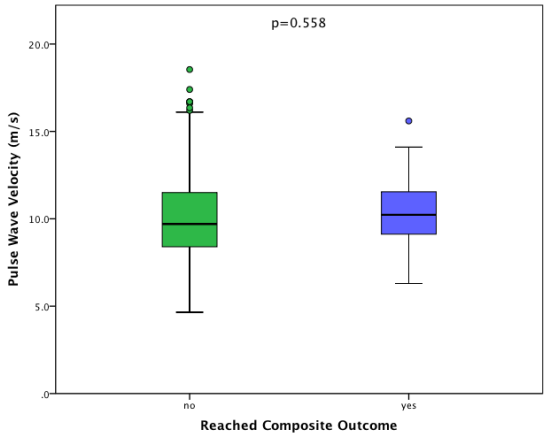


Figure 7f: AIx

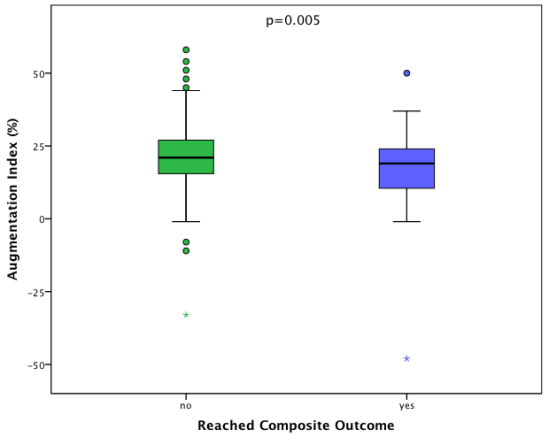
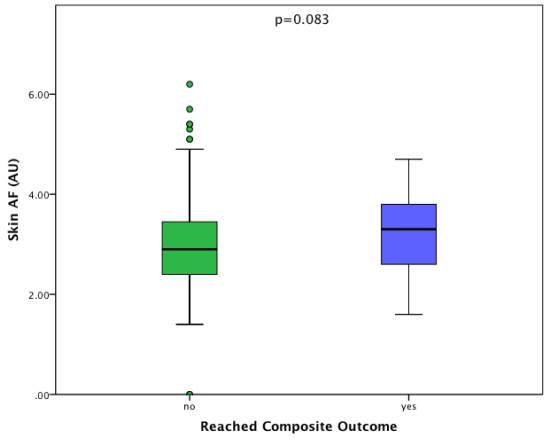


Figure 7g: AGEs



7.4.3. The dynamic inflammatory and anthropomorphic phenotype of participants who reached the composite outcome

Inflammatory markers were elevated in patients who reached RRT and also in patients who died, I hypothesised that inflammation would be associated with the composite outcomes. No anthropomorphic measures were associated with either arrival at RRT or survival; I hypothesised that there would be no difference in anthropomorphic measures in those participants who reached the composite end-point compared to those who did not.

Figure 9 (a-f) illustrates the results of the analyses performed (Mann-Whitney U tests for non-parametric inflammatory data and unpaired T-tests for anthropomorphic data). The data show that both hsCRP and polyclonal FLCs concentrations were higher in those who reached the composite end-point than

those who did not, as hypothesised there were no differences in anthropomorphic features between the groups.

Figure 7-8: Inflammation and anthropomorphics and the composite end-point

Figure 8a: hsCRP

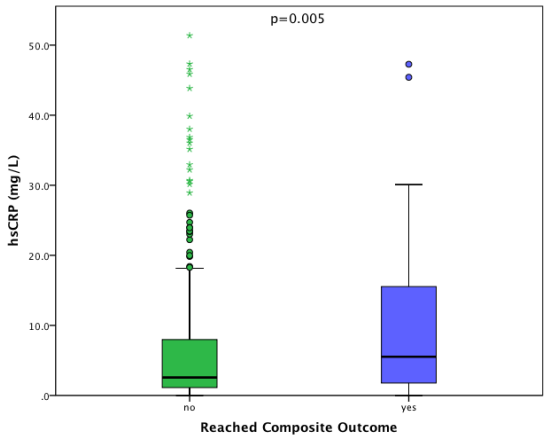


Figure 8b: IL-6

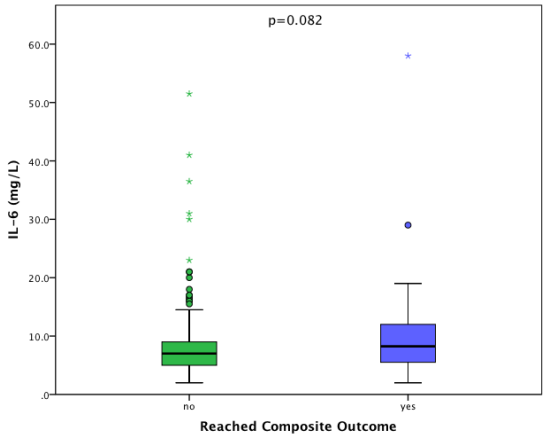


Figure 8c: cFLC

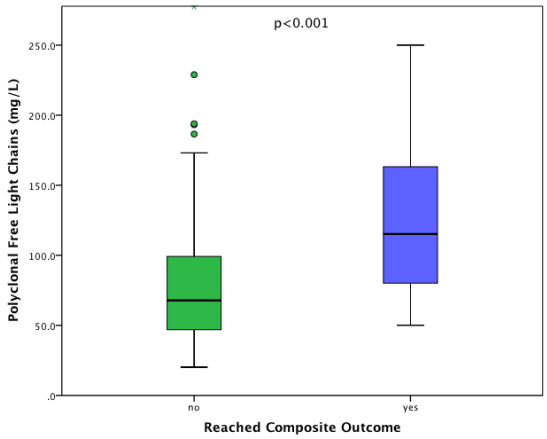


Figure 8d: BMI

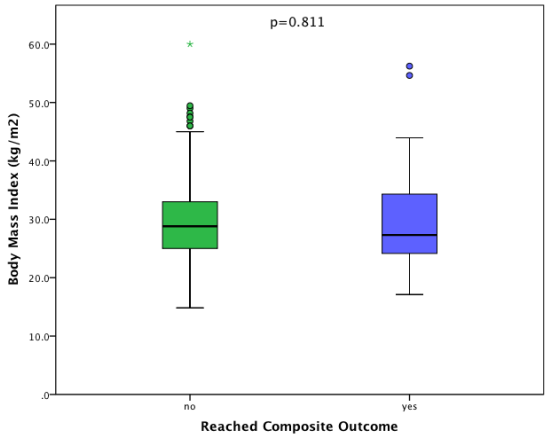


Figure 8e: WHR

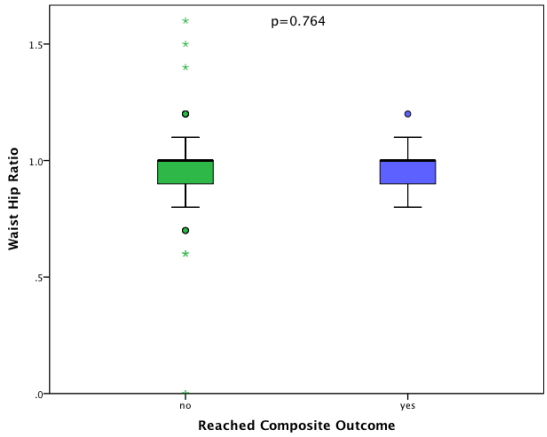
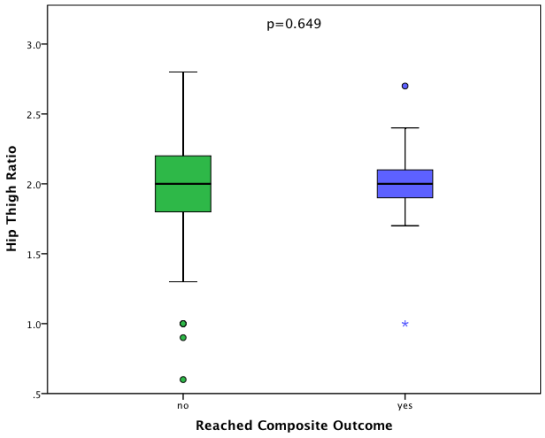


Figure 8f: HTR


p=0.649
Hip Thigh Ratio
3.0
2.5
2.0
1.5
1.0
.5
no
yes
Reached Composite Outcome

7.4.4. The determinants of reaching the composite outcomes

The same univariate analysis was performed for the composite outcome as for each individual outcome, those variables that reached a significance of <0.1 (indicated in figure 10 with an asterix) were then included in a multivariate analysis, the results of which are shown in table 11.

Figure 7-9: Variables included in the univariate analysis of determinants of the composite end-point

Table 7-11: Multivariate analysis of variables associated with reaching the composite end-point

	Significance	Odds ratio	95% confidence intervals
Current smoking	0.026	8.491	1.294, 55.705
Creatinine	0.006	1.010	1.003, 1.016
ACR	0.001	1.007	1.003, 1.011
White ethnicity	0.946		
Asian ethnicity	0.710		
Unemployment	0.237		
eGFR	0.241		
hsCRP	0.490		
IL-6	0.556		
Bicarbonate	0.420		
Haemoglobin	0.745		
AIx	0.708		
AGEs	0.876		

For creatinine, ACR, hsCRP, IL-6 and AGEs a higher level of the variable was associated with the outcome, for eGFR, bicarbonate, haemoglobin and augmentation index a lower level of the variable was associated with the outcomes

These data show that smoking, increased serum creatinine (but not eGFR) and increased ACR were independently associated with arrival at the composite end-point.

7.4.5. Kaplan-Meier Survival curves for categorical outcomes

The only categorical variables that were independently associated with either renal replacement therapy, survival or the composite outcome were the presence of an ACR>30mg/mmol (RRT outcome) and current smoking (composite outcome).

Kaplan-Meier survival plots for these categorical variables are shown in figures 11 and 12.

Figure 7-10: Kaplan Meier survival curve of proteinuria and RRT

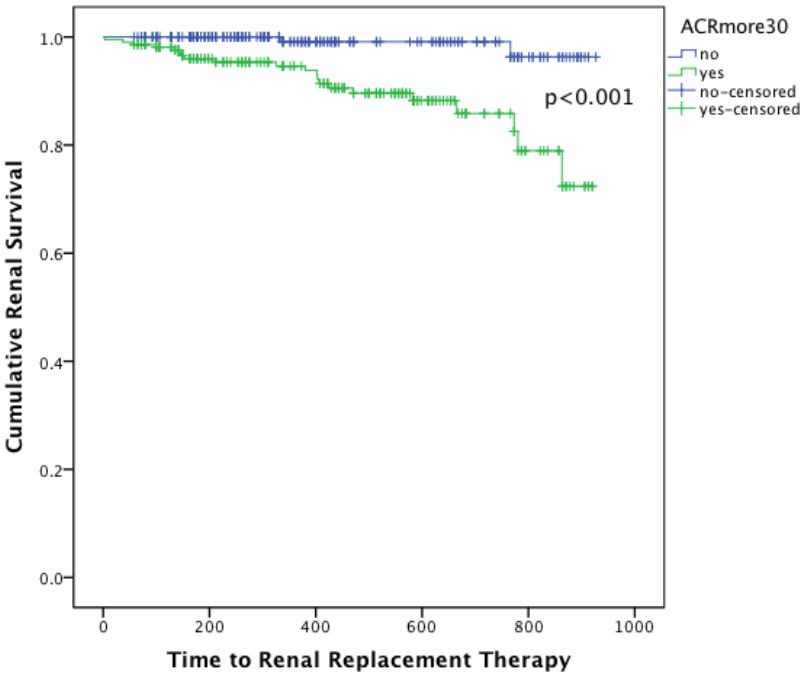
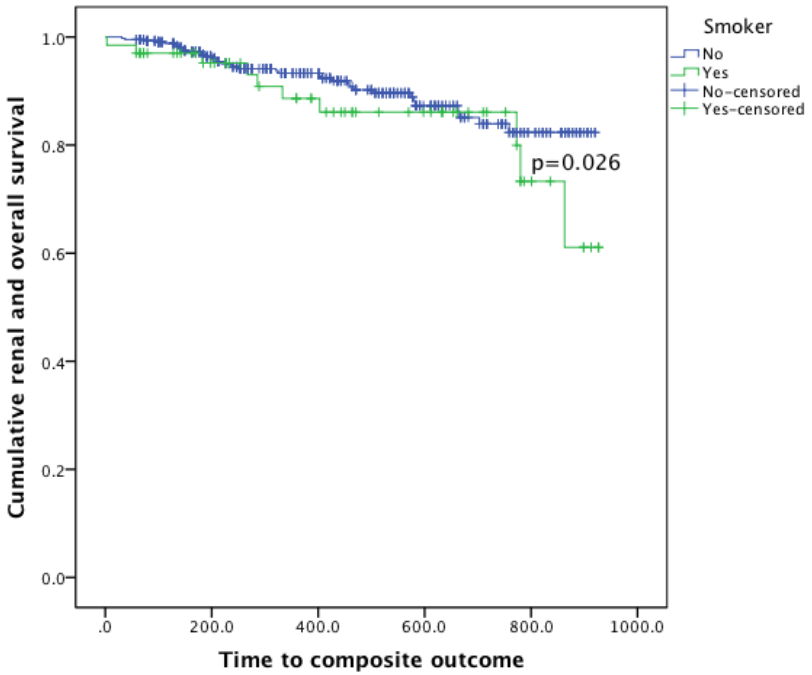


Figure 7-11: Kaplan-Meier survival curve of current smoking and composite end-point



7.5.Discussion and conclusions

7.5.1. The influence of established cardiovascular co-morbidity upon outcomes in the RIISC cohort

In this cohort, with the low event rate, there was no influence of established cardiovascular co-morbidity on subsequent arrival at RRT or all cause mortality. An explanation for this might be that the time scale studied was too short and that with time the influence of cardiovascular co-morbidity will become clearer. The low event rate might also explain this finding. I have not examined the influence of prior cardiovascular co-morbidity upon subsequent cardiovascular events for reasons that will be discussed when I consider the limitations of the data and outcomes analysis.

7.5.2. The influence of the dynamic cardiovascular phenotype upon outcomes in the RIISC cohort

In this cohort there were associations between both RRT and all-cause mortality and two measures of the dynamic vascular phenotype; decreased augmentation index was independently associated with arrival at RRT and increased AGEs were independently associated with all cause mortality. The association between reduced AIx and progression to RRT is an interesting one; increased AIx has previously been described as associated with increased cardiovascular risk in a healthy population (409) and coronary obstruction in a CKD population (410).

In the MMKD study there was a modest association between lower AIx and CKD, the authors hypothesised that this might be related to reduced arterial

compliance in central compared to peripheral arteries (411); this is the first cohort where an association between reduced augmentation index and progression of CKD has been shown, as the cohort matures and the event rate rises it will become clear if this is a genuine finding or an artefact.

The accumulation of AGEs has been shown to be independently associated with mortality in patients on haemodialysis (221), AGE accumulation has also been associated with both cardiovascular and renal risk factors in patients with CKD (126). The association between increased AGE accumulation and all cause mortality in this cohort is plausible.

7.5.3. The dynamic inflammatory and anthropomorphic phenotype and outcomes in the RIISC cohort

In this cohort there were associations between markers of inflammation and both all-cause mortality and the composite outcome; this is not an unexpected observation and supports the hypothesis that inflammation plays a pathogenic role in cardiovascular disease propagation, systemic inflammation can also lead to all-cause mortality through other, non-cardiovascular, pathways such as infection and malignancy. Interestingly none of the inflammatory markers were independently associated with the outcomes studied. As the cohort matures the role of inflammation in progression of CKD, cardiovascular risk and all-cause mortality may become clearer.

In this cohort there were no associations between any of the anthropomorphic measures studied (a strength of the analysis was that markers of central obesity as well as body mass index were included) and any of the outcomes of interest. This supports previous observations that obesity may not influence progression of CKD (366, 367).

7.5.4. The limitations of this analysis

There are a number of limitations and I will discuss them in turn;

The event rate

This is an early analysis that took place after a relatively short period of follow up, the median duration of follow up at the time of the analysis was 366 (191-612) days. Because the outcomes studied might be expected to take place over a prolonged time-scale the rate of events was low, this suggests that the significant associations are likely to be observed in further analysis of the cohort after more prolonged follow up.

The renal outcome measure

The use of progression to RRT or ESKD is a blunt instrument to ascertain renal progression, as significant declines in kidney function will not included. However there was insufficient follow-up renal data to accurately assess a decline in eGFR using a linear regression method. As the cohort matures it will be possible to assess renal progression using this method.

8. Discussion and conclusions

As the results are discussed in some detail at the end of each chapter here I will focus upon the following areas; the strengths and limitations of both the study and the data presented and how the cohort can be utilised in further research. I finish by providing an executive summary of the results obtained to date.

8.1.Strengths and limitations

8.1.1. The strengths of the RIISC protocol

While there have been a number of other CKD cohort studies, some which are on-going, the RIISC study is unusual for the following reasons;

i. It is comprised of a high-risk secondary care cohort recruited from a patient group that are consistent with the NICE CKD guideline for secondary care referral and follow-up. Therefore participants in RIISC are at both enhanced cardiovascular and renal risk. As a result of this the event rate will be high and the data obtained could provide essential clinical information to identify those individuals at greatest risk of poor outcome. It is recognised by kidney specialists that there is marked heterogeneity in the natural history of CKD.

ii. The cohort undergo a detailed and highly reproducible bio-clinical assessment based upon rigorously applied SOPs, this provides confidence that the data obtained are robust and variability in any measurements will be limited to the biological variability of the individuals under study and the variability of the measurement approach used for any individual bio-clinical variable measured under standard operating conditions.

iii. The cohort undergo repeated bio-clinical assessment in addition to outcomes tracking, this will provide an opportunity to identify the impact of the relationship between changing bio-clinical parameters and clinical outcomes. Of studies that are currently in their active phase only the R^2ID and CRIC cohort participants undergo repeated clinical assessment during the follow up period (122, 132), though in the case of the R^2ID cohort the participants are drawn from a different CKD population.

iv. It is now a multi-centre study with patients being currently recruited form both University Hospital Birmingham and Heartlands Hospital Birmingham (although the data presented here are from a single centre); this allows recruitment of a diverse population to ensure that the cohort is representative of a high-risk CKD population.

v. There is an emphasis on broadening participation to ensure that the recruited cohort is representative; an example of this is the provision of study information in multiple languages and audio format and the use of translators to increase participation from ethnic minority patients. The high ethnic minority participation in RIISC is a particularly strong aspect of the study.

8.1.2. Weaknesses and areas of controversy in the RIISC protocol

When designing an observational study it is important to prioritise certain aspects of the bio-clinical assessment, while the RIISC assessment is very detailed there were a number of omissions from the protocol which could be considered as weaknesses and which could limit the clinical utility of the data

obtained. These omissions, and the reason for the omission, are summarised in table 1.

Table 8-1: RIISC protocol; areas of controversy

Omission	Rationale
No gold standard measure of kidney function used for either screening or renal progression	While inulin and iohexol clearance are the gold standard measures of kidney function, radioisotope methods are accepted as they are easier and less expensive (21). However these are still invasive and costly and would increase the burden on potential and actual participants. The MDRD equation with IDMS traceable creatinine results was chosen because it is part of routine clinical practice (thus making our cohort representative of the CKD population). The application of other creatinine-based equations (e.g. CKD EPI) will also be explored.
No dietary restrictions placed upon patients prior to clinic attendance	Serum creatinine is affected by diet and meat consumption prior to testing can influence the result obtained (35). In some studies participants are asked to refrain from eating meat in the 24 hours preceding testing (126), however we decided that this placed an additional burden on patients and would make results obtained not generalisable to routine clinical practice.
No cardiac imaging (CT or echocardiography)	While coronary calcification has been described in CKD and detailed cardiac imaging has been conducted as baseline in some cohort studies; this is invasive and adds complexity to the protocol. The non-invasive measures of arterial stiffness have been shown to correlate well with more invasive methods (120, 123, 364).
No use of Dexa scanning to measure bone health	Patients with CKD are known to be at risk of bone loss and fractures, renal bone disease is also a risk factor progression and cardiovascular events (412). Dexa scanning is the gold standard measurement of bone density but novel biomarkers of bone turnover, such as FGF 23, have been shown to be associated with progressive CKD and cardiovascular risk, without radiation exposure and at lower cost and inconvenience to the participant (413).
The use of a short quality of life questionnaire that is non-renal specific	There are a number of renal specific quality of life measures available, they vary in detail but tend to focus on symptom burden specific to the renal population. The SF 36 is a generic questionnaire that has been validated in CKD, though there is no evidence that using it in combination with the KDQOL questionnaire is additive (414-416). There is evidence that the EQ5D in combination with the KDQOL provide complementary information on patient perception of disease; however even the abbreviated the KDQOL contains 36 questions (some being very detailed) and would be difficult to complete for patients who do not speak English as a 1st language (as many of the eligible population may not) (414, 416).
No data on income collected	While many authors report that income is an important measure of SES (254), such questions can alienate participants and it has been described as non-essential for the assessment of SES (264)
The recruitment of patients from secondary care only	The majority of CKD is managed in the community (primary care) (417) the data obtained from this, higher risk, cohort may not be applicable to primary care patients. However the focus on RIISC is specifically on those patients at highest risk of progression to ESKD and under secondary care follow-up; that is those patients who have the highest disease burden.

A potential area of concern for recruitment of the cohort is that as clinical practice changes it is feasible that referral patterns from primary care will change (with fewer patients being referred) and larger numbers of patients will be discharged from secondary to primary care for on-going management; this could reduce the pool of eligible patients for recruitment.

8.1.3. The strengths of the data presented

As already described the data were collected in a robust and reproducible way, the following are specific examples of this;

i. Care was taken to assess the representativeness of the cohort, to this end data were presented on those who declined to consent and those who consented and then withdrew. By understanding the barriers to participation it may be possible to increase access to research by addressing these.

ii. The baseline demographics were compared to the other CKD cohorts and this confirmed RIISC as a high risk cohort compared to other cohorts (as defined by kidney function and proteinuria) except for the CRIB cohort which was similar but smaller than RIISC and without the detailed bio-clinical assessment or repeated assessment and the CRISIS cohort where the baseline eGFR of participants was similar to that of RIISC participants.

iii. Blood and urine samples were collected in a structured manner and were immediately processed as outlined by standard operating procedures. Routinely measured variables were all measured in a single hospital laboratory; those measured specifically for the study were stored and batch analysed at a single laboratory to minimise inter-assay variability

iv. The impact of clinical variables on QoL was considered

v. Data (clinical and QoL related) were compared between the baseline and six-month visits to examine the variability of the measures.

vi. The periodontal assessment was carried out by a calibrated research dentist and hygienist to minimise inter-operator variability

8.1.4. The weaknesses of the data presented

Despite the strengths described above there are a number of flaws in the data presented which are described below;

i. There was no use of GP records for the compiling of co-morbidity data so it is possible that the data could be incomplete. This will be addressed over the next 12 months of follow-up.

ii. All eGFR data presented were creatinine based MDRD estimations, as this is the current clinical standard for estimating GFR and is the basis of the CKD classification system. However there is the potential to analyse the data in respect of other CKD equations including equations that incorporate cystatin C as a variable.

iii. Very few renal diagnoses were based upon renal histology; the large majority were clinical diagnoses.

iv. It was not possible to compare the baseline co-morbidity of those who consented and those who did not as detailed co-morbidity data was not collected for those who did not consent. This could mean that the non-consenting group could have significantly more co-morbidity than the recruited group which in turn would make the cohort less representative of the eligible population.

v. Even though attempts were made to ensure that the recruited population was representative of the eligible population, analysis showed that the recruited population were younger than the non-recruited population and were more likely to be of white ethnicity. This discrepancy may reduce the clinical applicability of the data obtained

vi. The question on job type in the SES assessment was subjective and as a result the data obtained may be inaccurate, the demographics section of the assessment was carried out by trained research personnel but no example of job types were provided to calibrate the data.

vii. The EQ5D was occasionally translated verbally into other languages, event though print versions of the instrument are available in a number of languages. The EQ5D was administered by a member of the research team rather than allowing the patient to complete it alone.

viii. Inflammation data were not available for the whole cohort, when the data are obtained for the whole cohort the results may differ, a very limited number of inflammatory markers were analysed for the purpose of this analysis, it is the intention that many more inflammatory markers will be measured in the complete cohort when recruitment has completed

8.2. The RIISC cohort and future areas of research

In addition to completing recruitment, follow up and re-exploring the associations presented in this thesis there are a number of other potential areas of clinical research that RIISC could be utilised for: I will discuss four of these.

8.2.1. The use of RIISC as a validation cohort for biomarker studies

While there have been many biomarker studies in CKD, they have been limited by the often small numbers of patients recruited and the variety of end-points studied. Such studies may involve a limited phenotyping exercise of participants, which may be limited to the degree of renal impairment or a specific diagnostic group such as diabetes. Furthermore most studies involve measurement of a specific biomarker and then a variable duration of follow up, after which the outcome of interest (often a surrogate outcome such as doubling of creatinine) is recorded (141, 142, 147, 148, 155, 157, 161, 192, 418).

This methodology fails to take into account the potential confounding factors of the differing phenotype of individuals with CKD and the effect of changing management of CKD over time upon outcomes. The use and limitations of surrogate outcomes was described in the introduction, the presence of a robust and repeated bio-clinical assessment with gold standard sample handling and the ability to assess progression using a linear regression method makes the RIISC cohort a strong resource for validation of biomarkers.

Another potential weakness of previous biomarker studies that limits their wider clinical utility is that many of the markers studied may be unstable, requiring sample handling that may not be possible in routine practice (419, 420). The impact of different sample handling methods on biomarker measurement is another area where the RIISC cohort could be utilised with a sub-study designed to address this important area.

8.2.2. The use of RIISC in the development of studies of intervention

An area of particular interest for intervention is periodontal disease; it has been hypothesised that by treating periodontal disease endothelial function could be improved via reduction of systemic inflammation (237). As periodontitis has been associated with CKD in cross-sectional analyses (392, 421) there is interest in the potential impact of periodontal treatment on kidney function. In a recent systematic review Chambrone et al concluded that while there is sufficient evidence to link periodontitis to CKD, there are insufficient trials of intervention to determine the role of periodontal treatment upon progression of CKD (422).

It is not known what the long-term implications of periodontal treatment are upon CKD, whether any treatment effects persist and what the mechanism of any effect might be. To address these shortfalls in knowledge the RIISC methodology could be reproduced and the RIISC cohort utilised as a matched control group for such an intervention.

8.2.3. Validation of renal risk scores

In chapter one I described a number of renal risk scoring systems, despite the potential for significant clinical benefit from such risk scores none are currently in general use. The scoring system of interest is the one devised by Tangri et al (260) which is available as a smartphone application; however despite the large development and validation cohorts used in the creation of the risk equation it is not clear how it will apply to a high risk UK based population with a different ethnic composition.

As all the data required for this risk equation is routinely collected as part of the RIISC study (and indeed in usual clinical practice) and the equation is freely available it would be possible to combine the data obtained from RIISC with other contemporary UK based cohorts to validate the equation. Other risk scoring systems could be validated in the same way.

8.2.4. The use of RIISC to understand the dynamic vascular phenotype

Throughout this thesis I have referred to the dynamic and established vascular phenotype to differentiate potentially modifiable risk factors from those that are fixed; there is limited understanding of the natural history of the dynamic vascular phenotype in patients with CKD. There are a number of unanswered questions with regard to the vascular phenotype;

i. How does the contemporary management of CKD influence the dynamic vascular phenotype?
ii. How reproducible are measures of vascular health such as PWV, peripheral and central BP based measures and measures of AGEs?
iii. How well does the AGEreader™ perform compared to other measures of tissue AGE accumulation in patients with CKD?
iv. Do assessments like pulse wave velocity (which require specialist equipment) add to risk prediction over and above routinely collected measures of arterial stiffness like pulse pressure?

The RIISC methodology will provide answers to these and as a result should provide data that guide the assessment and management of high risk CKD in the future.

8.3. Executive conclusions

I will divide this into two groups; the first relates to the QoL and SES status section of the results chapters and the second to the vascular, periodontal and outcomes sections of the results chapters

8.3.1. Quality of life and socioeconomic status in RIISC participants

The data presented suggest that this is a cohort of variable SES; the prevalence of working age unemployment was high, interestingly the index of deprivation was not associated with severity of CKD as has been described in other studies (251). No participants refused to answer questions pertaining to SES suggesting that the methodology was acceptable, however further information might have been obtained from household income data.

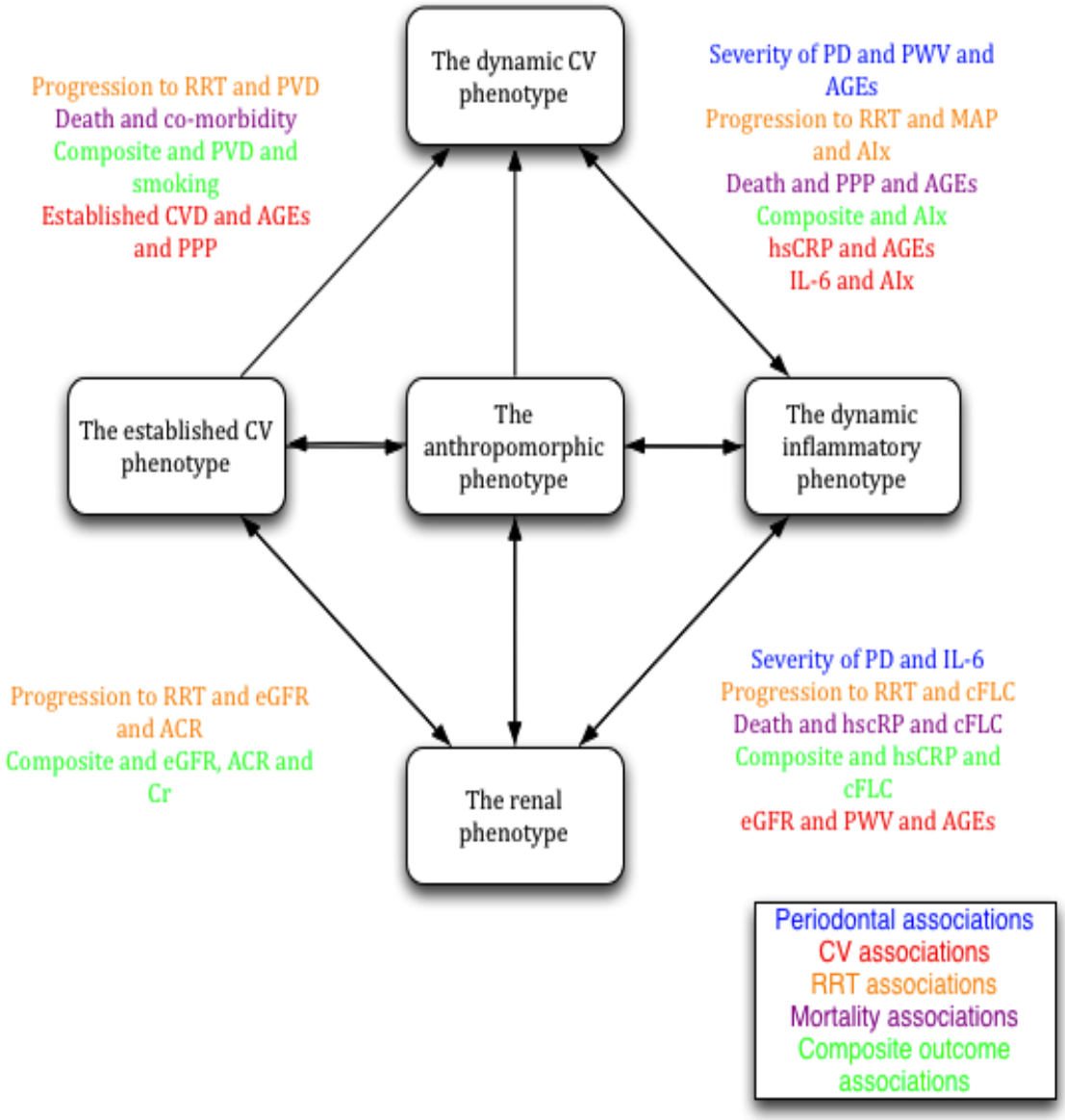
Quality of life was impaired in the cohort; in the five-domain section of EQ5D less than half of participants reported no mobility problems, just under half reported some impairment of usual activities and just under half reported regular pain or discomfort. Interestingly the prevalence of impairment of self-care was low. As a cohort of older adults with at least one chronic disease it is not unexpected that functional status is impaired in this group.

The importance of unemployment as a determinant of functional status is interesting and has not been previously reported; however unemployment was not independently associated with poor health perception, an interesting finding given the apparent influence of unemployment in the five-domain section of the EQ5D. The influence of potentially modifiable factors, such as employment, inflammation and glycaemic control, offers an interesting insight into potential future studies of intervention.

8.3.2. The vascular, inflammatory and periodontal phenotype and participant outcomes

There are many potentially inter-related variables described in these results chapters, to illustrate what the data presented tells us about this I have combined the findings in figure 1.

Figure 8-1: Correlations between the dynamic and established phenotype



Abbreviations; CV, cardiovascular; PD, periodontal disease; PWV, pulse wave velocity; AGEs, advanced glycation end products; RRT, renal replacement therapy; MAP, mean arterial pressure; AIx, augmentation index; PPP, peripheral pulse pressure; hsCRP, highly sensitive CRP, IL-6, interleukin-6; eGFR, estimated glomerular filtration rate; cFLC, polyclonal free light chains; ACR, albumin creatinine ratio; Cr, creatinine; CVD, cardiovascular disease; PVD, peripheral vascular disease

An interesting observation from the data presented is that the established CV phenotype was not associated with either CKD stage, systemic inflammation or the presence or severity of periodontitis. There was also no association with measures of SES and the presence of established CVD appeared to have no influence on either functional status or perception of health state. In contrast aspects of the dynamic CV phenotype were associated with kidney function, systemic inflammation and severity of periodontitis. There were also associations between measures of SES and the dynamic CV phenotype

As this is cross-sectional data, and the date of previous CV events was not recorded it is possible that the discrepancy described above is related to the time lag between the index CV event and the measurement of dynamic characteristics. It is not understood at what stage dynamic CV risk factors become established CV events, the detailed and repeated bio-clinical assessment of RIISC participants, the prolonged follow up period and the high expected event rate should help to illuminate this most important of unknowns.

8.4. Conclusions

The RIISC study is a prospective, observational cohort study, embedded in a secondary care setting with the aim of recruiting patients with CKD who are at enhanced CV and renal risk. Participants undergo repeated, detailed (and evidence based) bio-clinical assessment and are followed up for ten years, though the data presented in this thesis relates to baseline and six-month follow data only. The study was designed to recruit a high risk but real life cohort of

secondary care CKD patients, providing data that are clinically relevant and widely applicable.

The overall purpose of the study is to answer the following research questions;

i. What is the baseline demographic, vascular, inflammatory and periodontal phenotype of patients with high risk CKD managed in secondary care?
ii. What are the implications of SES on high risk CKD and how is QoL affected by it?
iii. How does the phenotype change with time and how is this related to clinical management driven by contemporary guidelines?
iv. What are the determinants of CKD progression, adverse cardiovascular outcomes and death and what links these risk factors?

In this thesis I have presented index, cross-sectional data relating to the 1st half of the cohort to be recruited. The data I have presented attempts to address points i and ii; as the cohort reaches its target recruitment of 1000 patients and follow up progresses it should be possible to address points iii and iv.

It is my view that research can be said to be useful if a clinician can answer the following questions in the affirmative; is the population studied representative of my patients? Is any intervention or assessment carried out reproducible in clinical practice or is it prohibitively complex, expensive or invasive? Are the outcomes used of relevance to my patients?

I have previously described the emphasis placed on ensuring representativeness of the cohort and I believe that the data presented illustrate that this has largely been achieved. While the RIISC assessment is detailed, time consuming, involves a large multi-professional team and specialist and expensive equipment it is not envisaged that this assessment will become part of the routine management of patients with CKD, more that the assessment will provide data that allow a better understanding of the natural history of CKD. The outcomes used include both surrogate and hard end-points, all of which are of clinical significance at both an individual and health economic level.

I submit that the RIISC methodology is such that the data obtained could guide the management and influence the outcomes of many thousands of patients with progressive or high risk CKD managed in the secondary care setting.

9. Appendix 1: Measurement of blood pressure using the BpTRU™ device (SOP)

Purpose

To obtain blood pressure readings on patients in the RIISC study which are consistent with the study protocol.

All participants will have their blood pressure recorded at all time-points

Preparation and Method

Patients will have rested in a quiet room for 5 minutes prior to taking a measurement.
Patients will have the monitor sited at the same level as their heart with their back and arm supported in a relaxed position. Both feet should be flat on the floor.
They will be asked not to talk while the recording is taking place.
Align the artery indicator on the cuff with the patient's brachial artery. Wrap the cuff around the arm and check that the white index marking on the edge of the cuff falls within the white range markings on the inside surface of the cuff.
If the index does not fall within the range markers, replace the cuff with a smaller or larger size.
Ensure the cuff is tight but allow two fingers to be inserted between cuff and arm.

Taking a BP measurement.

Turn on machine or press the Clear button to clear memory between patients.
Attach cuff to upper arm of patient
Use the cycle button to select an automatic series of measurements (indicated by a character from 1-5 in the Cycle display.)
Press the BP start button to begin the measurement. (Wait 5 seconds after turning on the BpTRU™ before pressing the start button.)
Press the Stop button at any time to stop the measurement and deflate the cuff or to pause between measurements.

Results

A tone will sound at the completion of six measurements.
After 5 seconds the reading display will show "A" and the average readings of the last 5 measurements is displayed.

10. Appendix 2: Measurement of arterial stiffness using the Vicorder™ device (423)

Purpose

This SOP describes procedures to ensure the correct use of the Vicorder™ Equipment for the RIISC study to obtain measurements that are consistent with the study protocol.

All participants will have their pulse wave velocity and pulse wave analysis measured at all time-points

Method

Vicorder™ readings will be recorded at all study time points: baseline, 6 months, 18 months, 3 years, 5 years and 10 years.

Take 3 readings; if there is a more than 10% deviance from expected normal of 7m/s; continue to take readings until there are two within 10% of one another. If the first three readings are above 12m/s then take another three readings.

Note which leg and arm used for readings and enter data. Use same arm and leg throughout study at all time points. If at any time point this is different, record reason for change.

Ensure room temperature kept between 22 and 24 degree Celsius: use temperature log sheet to record.

Ensure that all data collected is stored in spreadsheet.

11. Appendix 3: Measurement of advanced glycation end products using the AGEreader™ device (293)

Purpose

The purpose of this SOP is to ensure the correct use of the AGEreader™ Equipment for the RIISC study. The AGEreader™ is a proprietary device that can non-invasively assess the tissue accumulation of Advanced Glycation End products (AGEs) and obtain measurements that are consistent with the study protocol.

All participants will have their AGEs measured at all time-points

Intended Use

Measurements should be done on the dominant arm on healthy undamaged skin
without birthmarks or excessive hair growth, tattoos or scars. Self tanning agents must not be used for at least 2 days. If patient has used self tanning agents document and inform the patient not to use next time 2 days before the appointment. Sun-blockers and other skin care products should be removed before measurement.

Pigmented skin

The device and its software have been validated in patients with Fitzpatrick class 1-4 skin colour. For measurements on patients with Fitzpatrick class 5-6 (dark brown or black), users should check with the manufacturer or distributor for the correct software version in order to avoid unreliable results. If a measurement is performed on a skin type that is too dark to give a reliable result, the AGEreader™ will give a warning.

UV-Radiation

Using the guidelines of the ICNIRP it is concluded that during AGEreader™ measurements, as intended, even when repeated up to a 100 times on the same skin site within an 8-hour period, the local radiation exposure on the skin of the patients, and to the eyes of patients and operators remain considerably below the maximum allowed values for that period. Radiation exposure to the eyes normally does not occur. Exposure of the eyes longer than 60 seconds per 8-hour period should be avoided (ie do not look directly into the UV light)

Procedure and method

Follow the instructions as set out in AGEreader™ operator manual 2010 to be found with equipment (293).

12. Appendix 4: Measurement of height and weight (424)

12.1. Measurement of height

Purpose

The purpose of this SOP is to ensure that all height readings obtained for the purposes of the RIISC study are accurate, reproducible and consistent with protocol requirements.

The height of all participants is recorded at all time-points

Preparation and method

Participant to remove shoes and to stand with feet together, flat on the base plate and with heels against the back of the plate, and to stand as tall as possible. Arms should be held loosely at the side. Tilt the head to the Frankfort plane position, so that an imaginary line passing through the external ear canal and across the top of the lower bone of the eye socket immediately under the eye would be parallel to the floor (i.e. horizontal). Check the position by holding the Frankfort plane card beside the participant's face. Ask the participant to take a deep breath in, re-check the Frankfort plane position and bring the headpiece down on the centre of the participant's head and check the level using the spirit level. Take the reading to the nearest 1 cm and record

12.2. Measurement of weight

Purpose

The purpose of this SOP is to ensure that all weights recorded are accurate, reproducible and consistent with protocol requirements.

All participants will be weighed at all time points

Preparation and Method

Place a clean paper towel on footplate of scales.
Measure weight with the participant wearing skirt or trousers and shirt, but no jacket or jersey and no shoes.
Place the scales on a hard floor. If there is no hard surface available, place the scales onto the wooden board, on the floor. Reset the zero button, be sure the scales measure in kilograms. When the zero shows ask the participant to step on, without hesitation, and then read off the flashing answer, and record value.

Sitting weight will be recorded on patients who cannot stand but on mechanical scales and only if safe to transfer patient with assistance.

13. Appendix 5: Measurement of waist, hip and thigh circumference (424, 425)

Purpose

To obtain measurement of waist, hip and thigh circumference measurements for the purpose of the RISC study that are accurate, reproducible and consistent with protocol requirements.

All participants will have their waist, hip & thigh circumference recorded at all time-points

Preparation and method

Ask the participant to face you and to stand straight with feet together and looking straight ahead. Stand to the right of the participant. Hold the tape in your right hand with the side of the tape where the scale begins facing you. Pass the other end of the tape round the back flank with your left hand and ask the participant to hold it whilst you retrieve the end of the tape from his/her left hand.
This should leave you standing slightly to the participant's left when you draw the tape taut.

Waist circumference

Make two marks with a waterproof pen at the costal margin (lower rib) and the iliac crest. Apply tape at a point midway between these two points, in line with the mid axilla. Measure on the skin if possible.
Ensure that the tape is horizontal.

Ask the participant to breathe out gently and to look straight ahead (to prevent them from contracting their muscles or holding their breath). Pull tape taut and measure to the nearest cm at the end of a normal expiration and record value. If participant is tense, repeat the measurement and take the new reading if it is higher.

Hip circumference

Locate the greater trochanter (this will be at the widest part of the hips, at the level of the buttock line). To check the levels you have to position the tape on the right flank and peer round the participant's back from their left flank to check that it is level.
While measuring ask participant to breathe out gently, to let arms hang loosely by their sides and to look straight ahead (to prevent them from contracting their muscles or holding their breath). Pull tape taut and measure to the nearest cm and record value on the questionnaire. Try to take the measurement (to the nearest cm) in mid-expiration when the abdominal muscles are maximally relaxed. If participant is tense, repeat the measurement and take the new reading if it is higher.
Waist and hip circumference should all be measured on the skin if participant consents and it should be recorded if this does not happen.

Thigh Circumference

Pass a measure immediately below the gluteal fold of the right thigh. Measure to the nearest cm. Ensure that the same leg is used for all measurements in the study. Note left or right on the database.

14. Appendix 6: Plasma, serum and urine sample handling/processing (301, 302)

Purpose

The purpose of this SOP is to ensure standardised operating procedures, when collecting blood and urine samples for the purpose of this study.

Blood, urine and saliva samples will be collected from all participants at all time-points

Introduction/Method

1. Collect blood samples using the vacutainer™ system (order of draw: 2 x red, 1 x EDTA, 1 x Paxgene™)
2. Tubes should be completely filled by the vacuum in order to obtain the correct ratio of blood to additive. Over and under filing alters the ration and changes results.
3. Thoroughly mix by inverting the tube 8-10 times
4. Leave serum (2 x red top) to clot for 1 hour at room temperature
5. Spin at 2500rpm for 10 minutes at 4°C
6. Spin the EDTA samples immediately at 2500rpm for 10 minutes at 4°C
7. Urine collected as midstream clean catch. Where possible ask the patient to provide a fresh sample. Urine samples collected more than 2 hours ago should be discarded.
8. Spin at 3000rpm for 15 minutes at 4°C

After spinning of all samples aliquot and transfer to a -80°C freezer

14.1. Processing and storing samples for genetic analysis

Sample collection

Ensure that the PAXgene™ Blood DNA Tube is at room temperature (18-25°C) prior to use. The PAXgene™ Blood DNA Tube should be the last tube drawn and fill to the line, backflow from the tube during draw should be avoided.
Transfer by a syringe is not recommended. Following draw, gently invert the PAXgene™ Blood DNA Tube 8-10 times.

Sample storage

Store the PAXgene™ Blood DNA Tube upright at room temperature until freezing at -20°C. To freeze PAXgene™ Blood DNA Tubes, stand them upright in a wire rack. If wire rack is not available, freeze horizontally in a plastic bag. Do not freeze tubes upright in a Styrofoam tray as this may cause the tubes to crack. Blood samples collected using PAXgene™ Blood DNA Tubes can be stored at 15-25°C for up to 14 days, at 2-8°C for up to 28 days, or at -20°C for up to 3 months. For long-term storage, freezing the samples at -70°C is recommended. If tubes are to be stored for no longer than 10 weeks, freeze the tubes in the wire rack at -20°C. For longer storage periods, freeze the tubes first at -20°C for 24hrs, and then transfer them to -70°C or -80°C.
Thaw PAXgene™ Blood DNA Tubes in a wire rack at ambient temperature (18-25°C) for approximately 2 hours or at 37°C in a water bath for approximately 15 minutes. After thawing, carefully invert the tube 10 times. Store the tubes on ice until you are ready to begin the PAXgene™ DNA purification procedure.

15. Appendix 7: Periodontal assessment (299)

Purpose

The purpose of this SOP is to ensure reproducible and accurate diagnosis of the severity and extent (both current and historic) of periodontal disease.

Participants will undergo a periodontal assessment at baseline, 36, 60 and 120 months.

Method

1. Explain the procedure to the patient.
2. Enquire about any medical conditions (such as a history of infective endocarditis) and medications (such as warfarin) that may render a detailed periodontal examination unsafe.
3. A trained dental professional conducts a general oral examination and notes any missing teeth.
4. Periodontal measurements are carried out on all teeth present using *the UB-WHO-CF15* constant-force periodontal probe (Implantium.co.uk).
5. For each tooth, record both probing depth and recession on the mesial and distal aspects of the buccal and palatal/lingual surfaces. So for each tooth, record 4 sets of periodontal measurements (proximal sites).
6. The Probing Depth is measured to the nearest millimetre from the base of the periodontal pocket to the gingival margin.
7. The Recession is measured to the nearest millimetre from the cement-enamel junction (CEJ) to the gingival level. If the gingival level is at the CEJ, the recession is recorded as 0mm, if the gingival level is apical to the CEJ, the recession is recorded as a positive integer and if the gingival level is coronal to the CEJ, the recession is recorded as a negative integer.
8. The total Clinical Attachment Loss (CAL) is recorded as the sum of the probing depths and recession (either 0 or positive or negative)
Clinical Attachment Loss = Probing Depth + Recession
9. On completion of these measurements for a dental quadrant, a dichotomous record of Bleeding on Probing (BoP) is recorded (either present or absent) for each site probed. This represents bleeding from the base of the pocket.

16. Appendix 8*:* Demographic data questionnaire

DOB	
Study Number	
Country of Birth	
Ethnicity	
Post Code	
Year at Address	
Highest qualification:	None
	GCSE/ O' level
	NVQ
	A' level
	Undergraduate
	Post graduate
Currently Employed	Yes
	No
	Retired/early retirement/incapacity
Job	None
	Unskilled/manual
	Skilled/manual
	Clerical
	Managerial
	Professional

18. Appendix 10: The RIISC clinical assessment

DOB	
Study number	
Medical history	IHD PVD CVD DM (type 1 / type 2) COPD Malignancy
Other medical history (including renal history)	
Current medications	
Family History	IHD PVD CVD DM (type 1 / type 2) COPD Malignancy Renal disease
Current smoker	Yes No
If yes amount	
Former smoker	Yes No
If yes amount	
Year stopped	
Current alcohol consumption	Yes no
If yes amount (units/week)	

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