STUDIES OF NOVEL RISK FACTORS ASSOCIATED WITH CHRONIC KIDNEY DISEASE IN ETHNICALLY DIVERSE, HIGH RISK POPULATIONS

By

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ABSTRACT

Chronic Kidney Disease (CKD) is common and often has a major impact on the health of those affected. In this thesis I have focused on areas of uncertainty that may have major implications for patient care.

Firstly, I assessed the determinants of increased mortality in a multi-ethnic primary care population. Secondly, I investigated the differential progression of CKD between ethnicities. Thirdly, I assessed if tryptase, as a marker of mast cell activation, could be used to stratify risk in CKD. Finally, I investigated the impact of CKD on health related quality of life (HRQL) and the association between HRQL and clinical end-points.

I found that: (i) comorbidity has a profound impact at a population level on survival in CKD; (ii) albuminuria is the principle modifiable risk factor for progression to end-stage renal disease (ESRD) in people of South Asian ethnicity; (iii) serum tryptase is an independent prognostic factor for ESRD in patients with CKD receiving treatment with an ACEi or ARB; and (iv) Low HRQL is common in CKD and reduced HRQL is associated with a higher risk for death.

The findings from this thesis contribute to the understanding of CKD in ethnically diverse, high-risk populations and form the basis for further studies.

DEDICATION

To Ellie and Madeleine,

for your belief, support and love.

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TABLE OF CONTENTS

FIGURES

TABLES

ABBREVIATIONS

ACEi; Angiotensin converting enzyme inhibitor

ACR; Albumin creatinine ratio

ADPKD; Autosomal dominant polycystic kidney disease

AF; Atrial fibrillation

AGE; Advanced glycation end products

AIx; Augmentation index

APOL1; Apolipoprotein L1

ARB; Angiotensin II receptor blocker

AT; Angiotensin

BMI; Body mass index

BP; Blood pressure

CanPREDDICT; Canadian Study of Prediction of Death, Dialysis and Interim Cardiovascular Events

CCI; Charlson comorbidity index

cFLC: Combined serum free light chains

CI; Confidence interval

CKD JAC; Chronic kidney disease in Japan cohort

CKD-EPI; Chronic kidney disease Epidemiology Collaboration

CKD-REIN; Chronic kidney disease – Renal Epidemiology and Information Network

CKD; Chronic kidney disease

CRF; Case report form

CRIB; Chronic Renal Impairment in Birmingham

CRISIS; Chronic Renal Insufficiency Standards Implementation Study

CRP; C reactive protein

CV; Cardiovascular

DBP; Diastolic blood pressure

DKD; Diabetic kidney disease

DNA; Deoxyribonucleic acid

eGFR; estimated glomerular filtration rate

Eq5D; EuroQol five dimension questionnaire

ESRD; End-stage renal disease

FBC; Full blood count

GCKD; German CKD cohort

GP; General practice

HbA1c; Glycated haemoglobin

HDL; High density lipoprotein

HEFT; Heart of England National Health Service Foundation Trust

HES; Hospital episodes statistics

HF; Heart Failure

HoB; Heart of Birmingham

HR; Hazard ratio

HRQL; Health related quality of life

IDMS; Isotope dilution mass spectrometry

IHD; Ischaemic heart disease

IMD; Index of multiple deprivation

INET-CKD: International Network of Chronic Kidney Disease Cohort Studies

IQR; Interquartile range

IT; Information technology

KDIGO; Kidney Disease: Improving Global Outcomes

KM; Kaplan Meier

LSOA; Lower layer super output area

MAP; Mean arterial pressure

MDRD; Modification of diet in renal disease

NHS; National Health Service

NICE; National Institute for Health and Care Excellence

NKF KDOQI; National Kidney Foundation – Kidney Disease Initiative

ONS; Office for National Statistics

OR; Odds Ratio

PAR 2; Protease activated receptor 2

PCR; Protein creatinine ratio

PCT; Primary care trust

PIS; Patient information sheet

PP; Pulse pressure

PROMS; Patient reported outcome measures

PVD; Peripheral vascular disease

PWV; Pulse wave velocity

QoF; Quality and outcomes framework

R^2ID; Renal Risk in Derby

RAAS; Renin angiotensin aldosterone system

RIISC; Renal Impairment in Secondary Care

RRT; Renal replacement therapy

SBP; Systolic blood pressure

SD; Standard Deviation

SES; Socioeconomic status

SHR; Subdistribution hazard ratio

SOPs; Standard operating procedures

TGF β; Transforming growth factor beta

THIN; The health improvement network

TP-CKD; Transforming participation in chronic kidney disease

U&E; Urea and electrolytes

UHB; University Hospitals Birmingham National Health Service Foundation Trust

UK; United Kingdom

VAS; Visual analogue scale

LIST OF PUBLICATIONS BY THE CANDIDATE

Directly contributing to this thesis

Contributing to Chapter 3

Jesky M, Lambert A, Burden AC, Cockwell P. The impact of chronic kidney disease and cardiovascular comorbidity on mortality in a multiethnic population: a retrospective cohort study. BMJ Open. 2013;3(12):e003458. Epub 2013/12/05

Contributing to Chapter 5

Jesky MD, Stringer SJ, Fenton A, Ng KP, Yadav P, Ndumbo M, McCann K,

Plant T, Dasgupta I, Harding SJ, Drayson MT, Redegeld F, Ferro CJ, Cockwell P. Serum tryptase concentration and progression to end-stage renal disease. European journal of clinical investigation. 2016;46(5):460-74. Epub 2016/03/22.

Contributing to Chapter 6

Jesky MD, Dutton M, Dasgupta I, Yadav P, Ng KP, Fenton A, Kyte D, Ferro CJ, Calvert M, Cockwell P, Stringer SJ. Health-Related Quality of Life Impacts Mortality but Not Progression to End-Stage Renal Disease in Pre-Dialysis Chronic Kidney Disease: A Prospective Observational Study. PLoS One. 2016;11(11):e0165675. Epub 2016/11/11

Other publications associated with advanced chronic kidney disease cohort

Stringer S, Sharma P, Dutton M, Jesky M, Ng K, Kaur O, Chapple I, Dietrich T,

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1 INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem. It affects up to one in seven adults (1-5), and the prevalence of CKD varies with age, gender and ethnic mix of the population studied (6-13). It is increasingly seen as a global health problem due to its high prevalence and association with adverse outcomes irrespective of the country reporting CKD prevalence and outcomes (4, 14-16).

The accepted definition for CKD was initially proposed by National Kidney Foundation – Kidney Disease Initiative (NKF-KDOQI) in 2002 (17) and is shown in Figure 1-1.

Figure 1-1. Definition of chronic kidney disease (17)

Kidney damage for ≥ 3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased glomerular filtration rate (GFR), manifest by either

- Pathophysiological abnormalities
- Markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests.
- GFR <60 mL/min/1.73m^2 for ≥ 3 months, with or without kidney damage.

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate

A diagnosis of CKD requires knowledge of kidney function as measured by glomerular filtration rate (GFR) or estimated (e)GFR and urinalysis for quantification of albuminuria or proteinuria. Individuals with a normal eGFR and no proteinuria may still have CKD as diagnosed by other urinary abnormalities or structural problems of kidneys, demonstrated on imaging.

In this introduction I discuss aspects of CKD of particular relevance to the hypotheses addressed and results reported in this thesis.

1.1 Assessments of renal function

1.1.1 Creatinine

Serum creatinine is an end-product of muscle catabolism. It is not protein bound and is freely filtered across the glomerulus. Due to its ease of measurement and widespread availability of the assay, it is the most commonly used marker of glomerular filtration (18). The generation of creatinine is proportional to muscle mass, and therefore may vary by age, gender, ethnicity and body size. Whilst its utility as an index of renal function has been superseded by more accurate estimates of renal function, it remains a key component of the majority of eGFR equations discussed in Section 1.1.4.

Given the importance of creatinine in nephrology practice and beyond, the ability to accurately assess creatinine is vital. Previously, different assays showed significant variation, some by as much as 30% (19). In order to reduce intra-laboratory variation,

manufacturers of creatinine assays calibrate their assay to an isotope dilution mass spectrometry (IDMS) traceable value. Whist this has improved the situation, a recent study has demonstrated some variability remains (20).

1.1.2 Cystatin C

Cystatin C is a protein produced at an apparently constant rate by all nucleated human cells. Its low molecular mass allows it to be freely filtered by the glomerulus before being resorbed and catabolised by tubular epithielial cells; consequentially, only a small amount is excreted in the urine (18). Cystatin C can be used as an alternative to creatinine to estimate GFR (21). Its utility has been increased with more affordable assays, a standardisation of measurement and an increased recognition that it may be a better marker of adverse prognosis in CKD patients than creatinine (22, 23). As discussed in Section 1.1.4.2, it has been incorporated into the latest Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations (24).

1.1.3 Measured GFR

Historically, the *gold-standard* technique to measure GFR has been inulin clearance. However, this test is invasive and time-consuming, ideally requiring a continuous infusion of inulin to achieve a steady state and multiple timed urine collections (18). More recently, radiolabelled plasma clearance methods, which are closely related to inulin clearance, have been used, including in the development a number of the eGFR equations discussed below in Section 1.1.4. GFR is calculated from plasma clearance after a bolus intravenous injection of an exogenous filtration marker (25). Several of these substances involve the use of radioactive agents (e.g. ^{99m}Tc-DTPA and ^{52}Cr-

EDTA). Iohexol is a nonradioactive contrast agent which performs well against inulin clearance (25). However, even these *direct* measurements of GFR exhibit variability (26).

1.1.4 Estimated Glomerular Filtration Rate Equations

Different equations can be used to calculate the eGFR. Historically, the Cockcroft-Gault formula (27), developed against creatinine clearance and introduced in 1976, was used. The formula, illustrated in Figure 1-2, requires an anthropometric measurement (i.e. weight), a value not routinely available from a laboratory perspective, thereby limiting its clinical utility. Whilst the Cockroft-Gault formula has been superseded by other equations, it had a key role in defining dose adjustments for medications with significant renal clearance.

Figure 1-2. Cockcroft-Gault formula (27)

$$\left[\frac{(140-\text{Age}) \times \text{Weight (kg)}}{72 \times 88.4 \times \text{Serum Creatinine } (\mu\text{mol/L})}\right] \times 0.85 \text{ if Female}$$

1.1.4.1 MDRD Equation

The Modification of Diet in Renal Disease (MDRD) group developed a formula to predict GFR from serum creatinine concentration and other factors (28). They evaluated numerous equations (Figure 1-3), which included serum and urine variables.

Figure 1-3. Equations evaluated in the MDRD study (28)

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 $[UUN]^{+0.249}$

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

The equation with routinely available clinical data (Figure 1-3, equation 5) was subsequently refined further with removal of serum urea to form the 4-variable MDRD formula (Figure 1-4); this equation had the greatest utility for clinical practice with eGFR derived from serum creatinine, age, gender and ethnicity. This equation performed well in comparison with plasma clearance methods (29) and was subsequently incorporated into national and international guidelines. It is the equation provided by most UK laboratory reports and has been validated for IDMS traceable creatinine (30).

Figure 1-4. 4-variable MDRD Equation (29)

GFR (ml/min/1.73m^2) =186 x [P_{Cr} x 88.4]$^{-1.154}$ x [age]$^{-0.203}$ x [0.742 if patient is female] x [1.210 if patient is black]

Abbreviations: GFR, glomerular filtration rate; P_{Cr}, plasma creatinine concentration (μmol/L)

The MDRD equation was developed and validated in people with CKD. It has been shown to lose precision and underestimate measured GFR at higher levels of GFR; this is most notable for GFRs greater than 60/ml/min/1.73m^2 (31, 32). UK guidelines recommend that eGFR values above 60/ml/min/1.73m^2 be interpreted with caution, *'bearing in mind that estimates of GFR become less accurate as true GFR increases'* (33).

1.1.4.2 CKD-EPI Equations

To improve the utility of kidney function estimation in clinical practice, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) developed new estimating equations for GFR, which involved a two slope-linear spline (essentially different equations depending on level of renal function). One equation, which has been introduced into clinical practice, uses serum creatinine (28) whilst others utilise cystatin C with or without serum creatinine (21). The CKD-EPI creatinine equation is currently being rolled out in UK clinical chemistry laboratories and is recommended in the current version of the NICE CKD guideline (33). The equations are shown in Table 1-1.

Table 1-1. Final equations evaluated in the CKD-EPI study. Adapted from (21) to incorporate UK creatinine units

Equation and Gender	Serum Creatinine µmol/L	Serum Cystatin C mg/l	Equation for Estimating GFR
CKD-EPI creatinine			
Female	≤61.9		$144 \times ((\text{Scr} \times 88.4)/0.7)^{-0.329} \times 0.993^{\text{Age}}$ [× 1.159 if black]
Female	>61.9		$144 \times ((\text{Scr} \times 88.4)/0.7)^{-1.209} \times 0.993^{\text{Age}}$ [× 1.159 if black]
Male	≤79.6		$141 \times ((\text{Scr} \times 88.4)/0.9)^{-0.411} \times 0.993^{\text{Age}}$ [× 1.159 if black]
Male	>79.6		$141 \times ((\text{Scr} \times 88.4)/0.9)^{-1.209} \times 0.993^{\text{Age}}$ [× 1.159 if black]
CKD-EPI cystatin C equation			
Female or Male		≤0.8	$133 \times (\text{Scys}/0.8)^{-0.499} \times 0.996^{\text{Age}}$ [× 0.932 if female]
Female or Male		>0.8	$133 \times (\text{Scys}/0.8)^{-1.328} \times 0.996^{\text{Age}}$ [× 0.932 if female]
CKD-EPI creatinine-cystatin C equation			
Female	≤61.9	≤0.8	$130 \times ((\text{Scr} \times 88.4)/0.7)^{-0.248} \times (\text{Scys}/0.8)^{-0.375} \times 0.995^{\text{Age}}$ [× 1.08 if black]
		>0.8	$130 \times ((\text{Scr} \times 88.4)/0.7)^{-0.248} \times (\text{Scys}/0.8)^{-0.711} \times 0.995^{\text{Age}}$ [× 1.08 if black]
Female	>61.9	≤0.8	$130 \times ((\text{Scr} \times 88.4)/0.7)^{-0.601} \times (\text{Scys}/0.8)^{-0.375} \times 0.995^{\text{Age}}$ [× 1.08 if black]
		>0.8	$130 \times ((\text{Scr} \times 88.4)/0.7)^{-0.601} \times (\text{Scys}/0.8)^{-0.711} \times 0.995^{\text{Age}}$ [× 1.08 if black]
Male	≤79.6	≤0.8	$135 \times ((\text{Scr} \times 88.4)/0.9)^{-0.207} \times (\text{Scys}/0.8)^{-0.375} \times 0.995^{\text{Age}}$ [× 1.08 if black]
		>0.8	$135 \times ((\text{Scr} \times 88.4)/0.9)^{-0.207} \times (\text{Scys}/0.8)^{-0.711} \times 0.995^{\text{Age}}$ [× 1.08 if black]
Male	>79.6	≤0.8	$135 \times ((\text{Scr} \times 88.4)/0.9)^{-0.601} \times (\text{Scys}/0.8)^{-0.375} \times 0.995^{\text{Age}}$ [× 1.08 if black]
		>0.8	$135 \times ((\text{Scr} \times 88.4)/0.9)^{-0.601} \times (\text{Scys}/0.8)^{-0.711} \times 0.995^{\text{Age}}$ [× 1.08 if black]

Abbreviations: Scr, serum creatinine (µmol/L); Scys, serum cystatin (mg/l)

1.1.4.3 Comparative performance of eGFR equations

When evaluating the accuracy of eGFR equations, a frequently used technique is to assess the percentage of eGFR that differs by more than 30% from the actual GFR (P_{30}). In development and validation cohorts the CKD-EPI creatinine equation has a lower P_{30} for overall eGFR, eGFR below 60ml/min/1.73m^2 and eGFR equal to or above 60ml/min/1.73m^2 compared to the MDRD eGFR (32). Additionally the CKD-EPI creatinine-cystatin C equation performed better than the CKD-EPI equations with creatinine or cystatin C alone (21). Whilst this is likely to influence assessments of prevalence of CKD on a population level, it is unclear if the use of a different equation has any impact on the clinical care of an individual patient (34).

1.1.5 Ethnicity

The application of a correction factor for ethnicity is required in both the MDRD and CKD-EPI equations. Many large databases [Section 1.9.2.1] either provide no or very limited ethnicity data, making the true prevalence of CKD difficult to estimate as their eGFR records cannot be corrected for ethnicity (1).

Previous studies have validated the eGFR equations in the North American African-Caribbean population (29) and some Asian populations (35-37). However, there are limited data amongst certain Asian populations, including people from Bangladesh, India and Pakistan (38); indeed, these groups form an integral part of the West Midlands population and, as discussed later in Section 1.5.1, appear to have a faster rate of progression to end stage renal disease (ESRD) (2, 6, 39).

1.1.6 Assessment of proteinuria

The significance of proteinuria, and in particular albuminuria, in the natural history of diabetic kidney disease (DKD) has been recognised for many years (40, 41). The cumulative impact of the eGFR and albuminuria in CKD secondary to other aetiologies was discussed at the Kidney Disease: Improving Global Outcomes (KDIGO) controversies conference (42) before being comprehensively studied by the Chronic Kidney Disease Prognosis Consortium (43-46). These landmark studies were a key component of the drive to incorporate the quantification of albuminuria into a CKD staging system (Section 1.2.2).

Guidelines suggest the use of urine albumin creatinine ratio (ACR) as the standard of care for quantification of albuminuria in routine clinic practice, although some units continue to measure protein creatinine ratio (PCR). Table 1-2 indicates the conversion between urine protein concentration, ACR and PCR.

Table 1-2. Comparisons between different tests to quantify proteinuria

Albumin Creatinine Ratio (mg/mmol)	Protein Creatinine Ratio (mg/mmol)	Urine Protein concentration (g/L)
30	50	0.5
70	100	1

1.2 Diagnosing CKD

Estimated glomerular filtration rate (eGFR) is more sensitive for diagnosing CKD than serum creatinine alone (47) because, as discussed in Section 1.1.1, the generation of creatinine varies by age, gender, ethnicity and body size. Therefore the GFR for an

80 year old white lady with a creatinine of 150 μmol/L with be different to the GFR for a 30 year old black man with an identical serum creatinine.

1.2.1 2002 Staging system

The first internationally accepted staging system was proposed in February 2002 by the Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF). This divided CKD into 5 stages based on eGFR (48, 49). A higher stage (from 1-5) indicates worse kidney function and patients are categorised into groups which are broadly associated with an increased risk of progression to end-stage renal failure and mortality the higher the stage of CKD (50). This staging system is illustrated below (Table 1-3).

Table 1-3. CKD stages as defined by K/DOQI guidelines (49)

Stage	GFR ($ml/min/1.73m^2$)	Description
1	≥90	Normal or increased GFR, with other evidence of kidney disease
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 evidence of kidney damage
5	<15	Established renal failure

Abbreviations: GFR, glomerular filtration rate

1.2.2 2012 Update to the Staging System

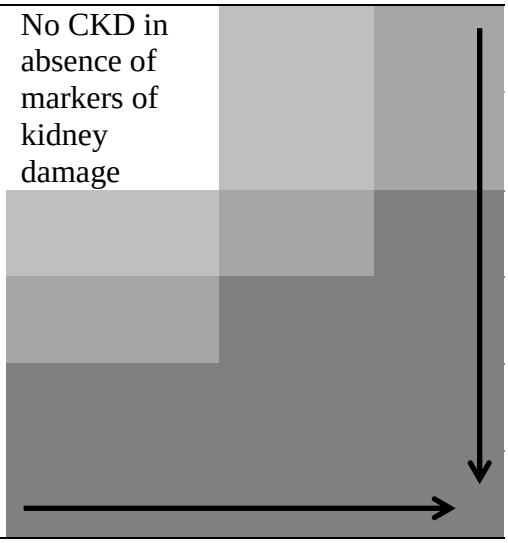
As discussed previously in Section 1.1.6, the risk conveyed by CKD is associated with both the level of secretory renal function (i.e. creatinine or eGFR) and albuminuria, usually quantified in clinical practice as an urinary ACR (42, 44-46, 51, 52). Large epidemiological studies suggest that an elevated ACR, regardless of eGFR (53), is associated with a high risk of progression. Recent studies show that levels previously seen as normal convey a higher risk of death (42).

In recognition of the importance of albuminuria, KDIGO introduced an amendment to the CKD classification (42) and subsequently developed and published clinical practice guidelines for CKD in 2012 (54) (Table 1-4). This updated staging system has been adopted internationally including by the UK, where it forms part of the 2014 update of the National Institute of Health and Care Excellence (NICE) CKD guidelines (33).

Table 1-4. Updated CKD staging system from 2012 KDIGO clinical practice guidelines (54). Figure adapted from UK guidelines (33)

Darker shades and arrows indicate increased risks of adverse outcomes

GFR and ACR categories and risks of adverse outcomes			ACR Categories (mg/mmol) and range		
			<3	3-30	>30
			A1	A2	A3
GFR categories (ml/min/1.73m^2) and range	≥90	G1	No CKD in absence of markers of kidney damage		
	60-89	G2			
	45-59	G3a			
	30-44	G3b			
	15-29	G4			
	<15	G5			



Abbreviations: ACR, albumin creatinine ratio; GFR, glomerular filtration rate

1.2.3 Number of eGFR readings required for diagnosis

The diagnosis of CKD requires the abnormal eGFR or ACR to persist for at least three months. Guidelines indicate that, to confirm a diagnosis of CKD, at least two eGFR readings, separated by not less than a period of 90 days are required (33). Similarly, an initial ACR between 3mg/mmol and 70mg/mmol should be confirmed with a subsequent sample (33).

Many previous studies investigating the prevalence of CKD have relied on a single eGFR, which is likely to overestimate the prevalence. In the UK, studies which have

relied on single measurements have estimated a higher prevalence than those relying on multiple readings (1, 55, 56).

1.3 Adverse outcomes associated with CKD

Chronic kidney disease is often asymptomatic until advanced stages. Consequently, it is often under-recognised, despite frequently coexisting with conditions such as cardiovascular disease or diabetes (57). It is estimated that 30% of individuals with advanced CKD are referred late to nephrology services from primary and secondary care (58). This has implications for risk factor assessment and modification and timely provision of information and resources, such as preparation for ESRD; early identification facilitates more time for discussion regarding dialysis modality and access or plans for kidney transplantation which are linked to an improvement in quality of life and increased survival (59, 60).

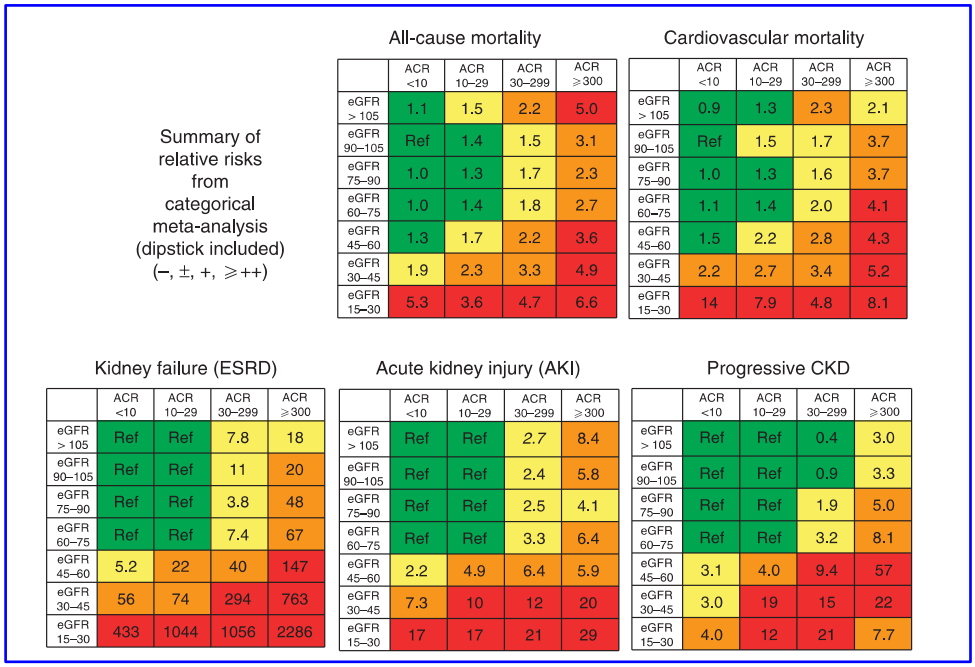
Advanced CKD does not just result in kidney specific complications. It is associated with a profound increase in morbidity and mortality; risk of death, cardiovascular events and hospitalisations all rise with CKD stage (50). Mortality is associated with both cardiovascular (50, 61) and non-cardiovascular causes (62). Even less advanced CKD represents a significant risk factor for cardiovascular disease and increased morbidity and mortality.

The Chronic Kidney Disease Prognosis Consortium have performed numerous meta-analyses assessing the impact of eGFR and albuminuria on outcomes in general and high risk population cohorts (63). They have confirmed lower eGFR and higher

albuminuria as risk factors for ESRD, acute kidney injury, progressive CKD and mortality (43, 44, 46). This has enabled the production of *heat maps* to provide a visual representation of relative risks (Figure 1-5) (42).

In the UK, there have been major initiatives to improve the early identification of people with CKD. These initiatives include the incentivised testing of kidney function in people with comorbidities associated with CKD, and the inclusion of patients with known CKD on a register held by the general practice. Most of these initiatives have been introduced through the UK quality and outcomes framework (QoF) (64) and have been complemented by the NICE CKD guideline which defines thresholds for referral of patients to secondary care nephrology services (33).

Figure 1-5. Visual Representations of relative risks for adverse events in general population cohorts with albumin creatinine ratio available. Adapted from (42)



Abbreviations: ACR, albumin creatinine ratio (mg/g*); eGFR, estimated glomerular filtration rate (ml/min/1.73m^2). * Approximate conversion of ACR to UK units: mg/mmol = [mg/g] /10

1.3.1 Endpoints associated with CKD

Endpoints can relate to clinical or surrogate events (65). Definitions of these endpoints can be found in Figure 1-6. As surrogate endpoints are typically a biological marker (biomarker), the definition for this is also provided.

Figure 1-6. Definitions of clinical and surrogate endpoints (65)

Clinical endpoint – A characteristic or variable that reflects how a patient feels, functions or survives.

Surrogate endpoint – A biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack or benefit or harm) based on epidemiological, therapeutic, pathophysiologic, or other scientific evidence.

Biological Marker (biomarker) – A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

The endpoints used in this thesis are clinical; the two key outcomes of interest are death or progression to ESRD (defined as the initiation of renal replacement therapy (RRT) i.e. chronic dialysis or renal transplantation).

Whilst clinical endpoints may be suitable for high risk populations, there is often a significant time period from the point of first detection in CKD to a clinical endpoint.

Guidelines suggest that early intervention (Section 1.7) in patients with CKD will have a greater effect on slowing the progression of CKD and delaying the time to ESRD. However, in order to prove such strategies to delay progression work, appropriate endpoints need to be selected; this is where surrogate endpoints may be helpful as they should allow a distal endpoint to be replaced with a more proximal one (66). Previous studies have used an increase in albuminuria (67, 68) or the doubling of creatinine (69) as a surrogate for progression of renal disease.

A doubling of serum creatinine corresponds to a 57% reduction in eGFR which may still require long follow up periods (70). Attention has therefore focused on whether declines in eGFR smaller than the equivalent of a doubling of serum creatinine could be used whilst still being associated with the risk of ESRD. This has been investigated by the CKD Prognosis Consortium investigators, and they proposed a 30% reduction in eGFR over two years as a surrogate marker (70). A scientific workshop sponsored by the NKF and the United States Food and Drug Administration proposed a more conservative 40% decline over two-three years given the potential for acute (i.e. treatment of concomitant illness) effects on eGFR (71).

A further endpoint of interest in studies of CKD include the incidence of cardiovascular events. Tracking these events accurately in routine clinical care is challenging and ideally requires an informatics solution to access national data regarding hospital admissions and diagnosis, such as Hospital Episodes Statistics (HES) data. This dataset has been utilised for recent studies investigation the outcomes of patients with CKD (72). The use of HES data requires a specific clause in the ethical permission; this was not initially obtained during the design of the Renal

Impairment in Secondary Care (RIISC) study that forms a significant part of this thesis (73).

1.3.2 Health-Related Quality of life

Early symptoms of CKD tend to be non-specific, such as fatigue, before symptoms more easily attributable to kidney disease develop (74). This burden of symptoms may impact negatively on health-related quality of life (HRQL).

There is increasing evidence of an association between pre-dialysis CKD and impaired HRQL as assessed by a variety of patient reported outcome measures (PROMs) (75-78). However, information on the relationship between HRQL, CKD and other adverse outcomes is limited; this is an area I explore further in Chapter 6. Additionally, information on the measures of HRQL utilised in this thesis can be found in Chapter 2 (Section 2.4.2).

1.3.3 Burden on Society

Kerr and colleagues performed economic modelling to estimate the annual cost of CKD (stage G3-G5) to the NHS in England (79). The direct cost to the English National Health Service (NHS) in 2009-10 was estimated as £1.44-1.45 billion or approximately 1.3% of all NHS spending during that year. More than half of this was spent on RRT with significant additional costs due to the excess strokes and myocardial infarctions sustained in patients with CKD.

In the developing world, for example in South Asian countries, CKD is a major public contributor to the overall burden of non-communicable disease (80). Many countries in the developing world do not have comprehensive health care systems with universal access, and RRT will be unaffordable to many (81).

1.4 Risk Prediction for Patients with CKD

Section 1.3 described major outcomes associated with CKD. Previous work focusing on modelling risk of adverse events in CKD are discussed below.

1.4.1 ESRD

1.4.1.1 Risk equations

Providing patients with clinically relevant information regarding the probability of specific clinical events and outcomes (endpoints), requires an ability to utilise large quantities of data in a way that can practically inform the decision making progress. The incorporation of risk scores into nephrology practice, something used widely by other specialties (82), is facilitating this (83).

Whilst earlier scoring systems had been developed (84), it was the publication of a validated scoring system by Tangri and colleagues in 2011 (85) which resulted in the increased use of risk scoring systems in CKD. This has subsequently undergone extensive validation in international cohorts, including the addition of both four-variable (age, gender, eGFR, ACR) and eight-variable (age, gender, eGFR, ACR, calcium, phosphate, bicarbonate, albumin) variants and the addition of an adjustment factor for populations outside North America (86). The variables used in the Tangri

equations and original Hazard Ratios [HRs] and 95% confidence intervals are shown in Table 1-5.

Table 1-5. Hazard Ratios (and 95% confidence intervals) in the original 4- and 8-variable equations. Adapted from (86)

	4-variable HR (95% CI)	**8-variable HR (95% CI)**
Age (per 10 year increase)	0.80 (0.75-0.86)	0.82 (0.77-0.88)
Male Gender	1.28 (1.04-1.58)	1.17 (0.95-1.46)
eGFR (per 5 ml/min/1.73m^2 increase)	0.57 (0.54-0.61)	0.61 (0.58-0.65)
ACR (mg/g, per log increase)	1.57 (1.44-1.71)	1.40 (1.28-1.53)
Calcium (per 1mg/dl)		0.80 (0.68-0.95)
Phosphate (per 1mg/dl)		1.30 (1.18-1.43)
Bicarbonate (per 1 mEq/l)		0.93 (0.90-0.96)
Albumin (per 1 g/dl)		0.71 (0.56-0.90)

Abbreviations: ACR, albumin creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio

An alternative risk equation in an American cohort has recently been proposed incorporating age, gender, eGFR, proteinuria/ albuminuria, haemoglobin concentration, systolic blood pressure, antihypertensive medication use, and a modified Diabetes Complications Severity Index (87). Interestingly, given the recognition of albuminuria as such a prominent continuous risk factor for progression, it only incorporates ACR as a dichotomous variable (with 30mg/g [3.39mg/mmol] as the cut point).

Reviewing the conception and validation cohorts for the prediction models, the absence of large South Asian populations in these studies is noticeable (63). This is an important omission, and raises the question of validity of such equations in ethnically diverse populations.

In the manuscripts describing the prediction models by both Tangri and Schroeder, it is the use of four variables (age, gender, eGFR and ACR) that convey the strongest risk; whilst additional variables do improve the discrimination and calibration of models, their impact is modest. This may suggest that attempting to add additional biochemical or standard demographic factors to population based models is likely to have limited effect, and perhaps emphasis should be placed on a more personalised approach focusing on sub-groups of patients (stratified medicine; Section 1.8).

1.4.1.2 Aetiology of renal disease as a risk factor for renal progression

Whilst there is significant variation in eGFR decline between individuals with the same aetiology of renal disease, patients affected by some specific kidney diseases progress more rapidly, these include autosomal dominant polycystic kidney disease (ADPKD) and diabetic kidney disease (DKD) (88-90).

1.4.2 Death

An ability to predict death, especially death prior to the progression for ESRD, is valuable as it influences the need to provide information and interventions (for example arteriovenous fistula formation) (91). However, this can be challenging as multiple non-renal and renal confounders, including both lower eGFR and higher albuminuria, have been shown to be associated with death (43). Non-renal determinants associated with an increasing mortality risk include increasing age, male gender, comorbidity, and lifestyle factors (e.g. smoking) (92, 93).

Bansal and colleagues developed a prediction equation to estimate the five-year risk of mortality among individuals aged 65 years or older with CKD in the Cardiovascular Health Study (94). They identified nine clinical variables (age, gender, ethnicity [white/ black], eGFR, ACR, diabetes, smoking, prevalent heart failure, prevalent stroke) influencing risk of death; these and their associated weighting scores are shown in Table 1-6. Estimate of risk for five year mortality depended on the total points and ranged from 3.87% for zero points to 93.7% for ten or more points.

Table 1-6. Points associated with each risk factor associated with mortality in Cardiovascular Health Study. Adapted from (94)

Risk Factor Categories		Points	Risk Factor Categories		Points
Age (years)			**ACR >3.39 mg/mmol**		
	70-74	0		No	0
	75-79	1		Yes	1
	80-84	2	**Diabetes**		
	≥85	4		No	0
Gender				Yes	1
	Female	0	**Smoking**		
	Male	1		Never	0
Ethnicity				Former	1
	Black	0		Current	2
	White	1	**Prevalent Heart Failure**		
eGFR (ml/min/1.73m^2)				No	0
	50-59	0		Yes	2
			Prevalent		
	40-49	1	**Stroke**		
	30-39	2		No	0
	<30	4		Yes	1

Abbreviations: ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate

Consistent with the lack of information on risk factors for progression to ESRD, there are also limited data for the determinants of mortality risk in South Asian patients with CKD, despite previous studies indicating survival differences between ethnic groups (11, 95-99).

1.5 Additional Demographic Variables

The ability to predict the risk of adverse events for patients with CKD is an aspect of clinical research which impacts directly on nephrology practice. Whilst prediction equations make use of demographic factors such as age and gender, two components that are notably absent in the majority of equations are ethnicity and socio-economic status (SES). These are both important considerations in an area as diverse as the West Midlands (see Section 2.1). Whilst these variables are increasingly the subject of discussion, data from UK populations are relatively sparse. The next sections in this introduction provide a concise review of the association between ethnicity and SES on outcomes.

1.5.1 Influence of Ethnicity on Adverse Outcomes

Section 1.1.5 described how knowledge of ethnicity is needed for correction factors for the most commonly used eGFR equations, and that certain ethnicities appear to have a faster rate of progression to ESRD. This section provides a concise review of the relevant literature assessing the influence of ethnicity on mortality, CKD and cardiovascular disease. Due to the population mix in the West Midlands, it mainly focuses on three main ethnicities; white (Caucasian), South Asian (including individuals of Bangladeshi, Indian and Pakistani descent) and black (individuals from or who have ancestors from Africa or the Caribbean).

An important consideration in the relationships between ethnicity and the adverse outcomes discussed below is whether ethnicity is a risk factor *per se*, or whether any association may be due to other differences between ethnicities which are themselves

associated with adverse outcomes; for example, the prevalence of diabetes is higher in certain ethnic groups and other differences may exist in SES (see Section 1.5.2), the proportion of individuals who undertake high risk behaviours (such as smoking) or the age distribution of the population studied (100-102).

1.5.1.1 Ethnicity and Chronic Kidney Disease

The majority of work investigating global differences in CKD has focused on people with end stage renal disease. Jha and colleagues investigated CKD from a global perspective and acknowledged precise calculations of the prevalence of early stage CKD are difficult, with individual studies confounded by heterogeneity of the population screened and that, within countries, some subgroups (including different ethnic groups) may be at increased risk of developing CKD, CKD disease progression, or both (4). It is therefore important to investigate observed differences within ethnicities in the population to be studied; interestingly, systematic reviews estimating ethnic differences have frequently not included data from the United Kingdom (12).

A study investigating CKD prevalence in a nationally representative sample of Health Survey for England data did not identify any statistically significant difference in CKD prevalence by ethnicity, although the authors acknowledged there were very few cases from the key minority ethnic groups; therefore it is questionable if this study produced robust data on ethnic differences (2). Another UK study, this time investigating the prevalence of CKD in people with diabetes, found lower rates of all CKD (stage G3 and above), but an increased odds ratio for more severe CKD (G4/5

or proteinuria) in people of South Asian and black ethnicities (6). This higher proportion of more severe CKD is consistent with other UK data suggesting an increased update of renal replacement therapy by South Asian and black populations (39), and a lower median age of starting renal replacement therapy (103). Despite this increased requirement for RRT, people of asian and black ethnicities have a reduced likelihood of receiving a living donor kidney transplantation (104).

1.5.1.2 Ethnicity and Mortality

Ethnicity information is not captured on death certificates in England and Wales. Consequently, there are limited published data on any population differences in mortality between ethnicities in the UK. Studies have modelled the relationship between mortality, age, deprivation and ethnic proportion and suggest broadly similar life expectancies for white and Asian populations but lower life expectancy for black men and women (105).

Information investigating mortality and ethnicity in CKD populations not on dialysis is also limited, but a study investigating mortality in a Canadian CKD cohort of three different ethnicities (Caucasian, Oriental Asian and South Asian) found all cause mortality rates were higher in Caucasian than the other two ethnicities (11). The Kidney Early Evaluation Programme, based in the United States of America, also identified mortality differences by ethnicity; with white ethnicity as the reference population, Hispanics and Asians (predominantly Oriental Asians) had a lower risk of death, African Americans had a similar risk, and American-Indians and Alaska Natives had a higher risk of death (106).

It is not possible to conclude from these data whether differences in mortality identified in studies investigating populations with CKD were due the presence of kidney disease or linked to differences in the population studied. This again highlights the need to analyse populations of direct relevance to clinical practice within the UK, and will be a focus of this thesis.

1.5.1.3 Ethnicity and Cardiovascular Disease

United Kingdom standardised mortality ratios (SMR) for ischaemic heart disease vary by ethnicity (107). When compared to the white population, South Asians have a higher SMR and the black population have a lower SMR. There is also evidence that the decline in cardiovascular disease observed in the white UK population has not been mirrored in these ethnic minority populations (107).

Several studies have investigated why this observed disparity in cardiovascular disease exists. Rana and colleagues conducted a systematic review and meta-analysis investigating any difference between cardiovascular risk factors and disease management practices in white and South Asian residents of Canada (108), a country with universal health coverage. Including 50 articles with over 5.8 million individuals, they did not demonstrate any differences in access to cardiovascular investigations or outcomes but did show a higher prevalence and incidence of cardiovascular disease and increased risk factors including diabetes, hypertension, higher body fat and a more sedentary lifestyle. This emphasises the significance of the clinical and

demographic differences between ethnicities, and the need to ensure these potential are recognised in any analyses conducted.

Studies have also investigated whether there are difference in outcomes following myocardial infarctions or interventional cardiology procedures. Jones and colleagues utilised the British Cardiovascular Intervention Society national database to perform a retrospective analysis of 279,256 patients undergoing primary coronary intervention between 2004 and 2011 (100). In similarity to the meta-analysis described above, they identified differences in demographics and risk factors at presentation; South Asians were younger and had more extensive disease and a greater prevalence of risk factors including diabetes. However, after correcting for these differences, in-hospital and medium-term mortality of South Asians was no worse than that of Caucasians. Other studies yield conflicting results, with Gijsberts and colleagues finding Indian and Malay patients had a higher risk for all-cause mortality following ST-elevation myocardial infarctions than Caucasians, albeit in a study that looked at different ethnic groups in different health care settings (Netherlands and Singapore) (109).

1.5.2 Influence of Socioeconomic Status on Adverse Outcomes

Socio-economic inequalities in health have been described in many North American and European studies, with associations found between SES and outcomes including all-cause mortality, cardiovascular disease and prevalence and progression of CKD. Many of these studies describe populations and access to healthcare that is markedly different to the population of the United Kingdom (UK), thereby making it unclear how applicable these findings are to our local population. Section 2.1.4 provides

specific SES information for the West Midlands, and I have summarised the recent and most pertinent published literature below.

1.5.2.1 Socio-economic Status and Chronic Kidney Disease

Whilst there are limited published data on the association between SES and CKD in the United Kingdom, the majority suggest an inverse relationship between SES and CKD.

Bello and colleagues investigated the association between deprivation and CKD stage at presentation referred to the nephrology unit in Sheffield, UK (110). Deprivation was divided into quintiles using the Index of Multiple Deprivation score (see Section 2.2.7) and the study found that people who lived in areas with low SES were at greatest risk for more advanced CKD at presentation. However, in a multivariable analysis, the observed difference was attenuated in all but the most deprived quintile; the significant factors that remained were the most deprived SES quintile, hypertension and diabetes. A higher prevalence of CKD G3-G5 and higher albuminuria, both risk factors for progression of CKD (Section 1.2.2), were found in individuals with a lower SES in an combined analysis of data from the Health Survey for England 2009 and 2010 (111). Taken together, these studies suggest that those from the most deprived areas potentially have more severe CKD at presentation *and* increased risk factors for progression to ESRD. UK studies also identify that people from more deprived areas have higher incident and prevalent rates of renal replacement therapy (112) but less access to living donor transplantation (104, 113), a

treatment associated with better life expectancy and quality of life when compared to other modalities (114, 115).

1.5.2.2 Socio-economic Status and Mortality

Stringini and colleagues recently published a multicohort study and meta-analysis, synthesising data from 48 independent prospective cohort studies from high income countries, including the UK (116). Socioeconomic status was indexed by occupation, coded into the European Socio-Economic Classification, and the study demonstrated that participants with lower SES had a higher mortality risk and reduced life expectancy both in models adjusted for age, marital status and ethnicity and for a more comprehensive multivariable model. Their analyses suggests low SES was associated with 2.1 years of life lost between 40 and 85 years of age, compared to 0.7 years lost for obesity, 3.9 years lost for diabetes an 4.8 years lost for current smoking. In their discussion, and in similarity to ethnicity, the authors describe how SES is often *intertwined* with other risk factors for health or disease (such as smoking status or levels of health education). Additionally, they recognise other measures of SES which assess more than a single component may be helpful when measuring on a population basis. Indeed, the measure of SES utilised elsewhere in this thesis, the Index of Multiple Deprivation (see Section 2.2.7) utilises seven domains to assess SES. Although there were low levels of heterogeneity (i.e. variation) across the cohorts, one potential criticism of the meta-analysis is the lack of inclusion of any analysis investigating the impact of universal health care provision on outcomes.

1.5.2.3 Socio-economic Status and Cardiovascular Disease

The Scottish Intercollegiate Group Network published their guideline entitled 'Risk Estimation and the Prevention of Cardiovascular Disease' in 2017 (117) and included the statement that *'for given levels of other factors, populations which are more deprived have higher cardiovascular disease risk'*. A UK based study validating the QRISK2 cardiovascular disease algorithm in patients from different ethnicities and SES was conducted by Hippisley-Cox and colleagues (118). Socioeconomic status was assessed by the Townsend deprivation score (119) and divided into quintiles. Cardiovascular disease incidence rates increased with each deprivation quintile, and remained after adjustment for other risk factors. In similarity to mortality and SES, the reasons for the association between cardiovascular risk factors and SES are considered multifactorial; studies have shown that individuals who have most to gain from assessment and risk factor modification are the least likely to attend screening programmes (120) and strategies, including within Birmingham (121), aim to reduce this disparity.

1.6 Novel Biomarkers in Chronic Kidney Disease

Previous sections have described the variables associated with progression to ESRD (Section 1.4.1) and death (Section 1.4.2) in individuals with CKD, including descriptions of the most commonly used risk prediction equations and a recognition of the potential significance of ethnicity and socioeconomic status (Section 1.5). In addition to the widely used and readily available assays, there has been significant research into novel biomarkers which may improve risk stratification in the CKD population (122, 123). Many such biomarkers have been studied in basic science and clinical settings.

Whilst several biomarkers have been identified as being associated with renal progression and/ or death, their use on a population level only leads to a modest improvement, at best, when combined with established clinical characteristics and laboratory variables.

This thesis does not aim to comprehensively describe or assess the utility of a range of biomarkers in the populations studied. However, certain biomarkers were assessed as a component of the study carried out in the high-risk CKD population ((73); see Section 2.3 for details of study). Here I discuss these biomarkers which have been comprehensively investigated (high sensitivity c-reactive protein, serum immunoglobulin free light chains) or, in the case of serum tryptase (Chapter 5), studied for the first time in a clinical context.

1.6.1 High Sensitivity C-Reactive protein

C-reactive protein (CRP) is a protein produced by hepatocytes and is widely used in the monitoring of acute inflammatory conditions. The development of high sensitivity CRP (hsCRP) assays have allowed accurate measurement of CRP concentrations into the normal range. High sensitivity CRP has been demonstrated to provide information regarding future risk of cardiovascular events and mortality (124, 125), including in the dialysis population (126).

Studies investigating the relationship between elevated CRP concentrations and progression to ESRD have yielded conflicting results. Sarnak and colleagues found no independent association between CRP and GFR decline in the MDRD study (127)

whereas an association of CRP with progression to ESRD, and the composite of death and ESRD, was found in a post hoc analysis of the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) study (128).

1.6.2 Polyclonal Serum Free Light Chains

Polyclonal serum free light chains (sFLCs) are produced during immunoglobulin synthesis and their circulating concentrations are a balance between production (potentially representing activity of the adaptive immune system (129)) and clearance. Whilst there is some reticulo-endothelial clearance, the majority of sFLCs are cleared by the kidney; consequently concentrations of sFLCs are elevated in individuals with renal impairment (130). Two types of sFLCs are produced, kappa and lambda, which can be analysed together (combined FLC; cFLC), as separate isotypes, or as a ratio (kappa-lambda ratio).

Hutchison and colleagues investigated FLC concentrations in patients with type II diabetes before the onset of overt renal disease and demonstrated evidence of elevated serum FLC before other evidence of renal dysfunction (131). Analysis of the relationship between sFLCs and adverse outcomes in CKD have been studied by several research groups (including our Birmingham group), with conflicting results. Of five prospective studies to date in CKD, four studied UK populations (132-135) and one investigated sFLC in a French cohort (136). Three of these studies (Assi; n=1695, Hutchison; n=848 and Ritchie; n=872) reported an independent relationship between sFLC concentrations and death. Haynes and colleagues (n=364) found the association between sFLCs and ESRD was explained by baseline eGFR. Desjardins

and colleagues (n=133) analysed Kappa and Lambda FLC separately and found Kappa FLC was associated with mortality in univariable but not multivariable analyses; no association was demonstrated with Lambda FLC in either univariable or multivariable analyses.

Two studies analysed the association between sFLC and progression to ESRD (132, 134). Haynes and colleagues reported an increased risk of ESRD, but significance was lost when eGFR was included in the multivariable analyses. However, Ritchie and colleagues, analysing sFLC concentrations within the chronic renal insufficiency standards implementation study (CRISIS; see Section 1.10.3.2) identified an increased risk of ESRD in the highest two quartiles of sFLC.

The studies discussed above suggest, but are certainly not conclusive of, an independent relationship between sFLCs and death, and possibly progression to ESRD. These data, and our group's interests and expertise in FLCs, justify their inclusion and further analyses.

1.6.3 Mast Cell activation and serum tryptase

Agents which block the renin angiotensin aldosterone system (RAAS) pathway, such as angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) have an ability to reduce proteinuria above their blood pressure lowering effects (discussed in Section 1.7). However, not all activation of angiotensin (AT) relies on the pathway blocked by ACEi/ARBs; indeed the concept of RAAS

breakthough refers to the ability of AT to be activated by pathways which bypass the protective effects of ACEi/ARB (137).

Mast cells are predominantly known for their roles in allergic disease and host defence against parasites (138). However these cells have a diverse functional capability beyond these roles; mast cell degranulation releases preformed mediators and compounds, including growth factors, proteases, leukotrienes, cytokines and chemokines; these are responsible for a range of physiological effects (139). Whilst mast cells are found infrequently in normal kidney tissue, an increased number of mast cells has been demonstrated to be associated with the severity of interstitial fibrosis in patients with progressive CKD due to a variety of aetiologies (140-149).

The association between mast cells and renal dysfunction may be explained in part by the biological effect of mast cell proteases. Tryptase is an inflammatory and profibrotic protease that is released from mast cells (150, 151) and serum tryptase concentrations have been shown to increase with worsening renal impairment (152). Chymase, the other major protease released from mast cells, can convert ATI to ATII and activate transforming growth factor beta (TGFβ) (153, 154). There are no previously published studies investigating the relationship between protease concentration and clinical outcomes in individuals with CKD, and I have investigated this as a component of this thesis. I focused on serum tryptase due to the previous published literature on association with renal impairment and the commercial availability of a sandwich immunoassay against tryptase (Phadia AB, Uppsala, Sweden; Section 2.4.5.3).

1.7 Current Treatment Strategies

Unless caused by a specific immune medicated process (e.g. a primary or secondary glomerulonephritis that may be responsive to treatment with immunosuppression) individuals with CKD have limited effective treatment options.

Accurate management of hypertension, particularly in proteinuric kidney disease is the cornerstone of treatment for the large majority of patients. Angiotensin converting enzyme inhibitors (ACEi)/angiotensin II blockers (ARB) further reduce CKD progression in patients with proteinuric CKD compared to other anti-hypertensive agents (155). Cholesterol lowering therapy (statins and ezetimibe) reduce atherosclerotic events (156) but do not slow the rate of progression of CKD.

Certain therapies have been demonstrated to be beneficial in specific aetiologies. Tolvaptan has been demonstrated to slow the increase in total kidney volume and the decline in kidney function in patients with ADPKD (157) and has been licensed for use, in select circumstances, by NICE (158). Several potential agents may reduce renal progression in diabetes; empagliflozin and canagliflozin, examples of sodium-glucose cotransporter 2 inhibitors, have recently been shown to decrease albuminuria and CKD progression in a study of type 2 diabetics with an eGFR >55ml/min/1.73m^2 (159, 160) and there have been encouraging results in recent phase 2 studies suggesting selective inhibition of C-C chemokine receptor type 2 may slow renal progression in DKD (161). However, caution is needed given phase 3 studies of intervention in CKD have usually not confirmed positive results from phase 2 studies (162-164).

Given this limited arsenal of proven therapies, identification of people at greatest risk remains paramount, both to provide an opportunity to counsel and modify risk factors as appropriate, but also to identify those who may benefit most from targeted intervention in a clinical or clinical trial setting.

1.8 Stratified medicine

Risk prediction equations, such as the one proposed by Tangri and colleagues (Section 1.4) (85), provide tools to stratify risk based on common biochemical and demographic markers. Attempts to improve population based models through the addition of standard demographic factors or standard or novel biomarkers to date has only led to modest improvement in the performance of these models.

Alternative approaches are therefore needed. These include: an urgent need to focus on patients who are at high risk of progression, including as a consequence of ethnicity, a better understanding of impact of HRQL on patients with CKD, and a focus on identifying which patients respond to (and would therefore benefit from) specific treatments and then to identify why this is.

This movement towards a more personalised approach to healthcare delivery, whereby detailed risk stratification may enable care to be directed to those at greatest risk is referred to as stratified medicine. This was defined by the PROGRESS group as '*the targeting of treatments (including pharmacological and non-pharmacological*

interventions) according to the biological or risk characteristics shared by subgroups of patients' (165).

1.9 Research Questions Addressed in this Thesis

Given the need to stratify risk as reliably as possible on a population and individual basis, and given the relative paucity of data regarding outcomes for patients of South Asian ethnicity, this thesis describes my research investigating the following

- Do certain ethnic groups have a higher risk of adverse outcomes, including progression to ESRD or death?
- Could novel biological markers help predict who may, or may not, respond to certain therapies? I assessed the association between serum tryptase, a product of mast cell activation, and progression to ESRD or death in patients with advanced CKD. Patients were stratified by the use of ACEi/ARBs.
- What is the relationship between HRQL and CKD stage, and the impact of HRQL on progression to ESRD or risk of death?

1.10 Data Sources

To achieve answers to these research questions, appropriate data sources need to be available in order to be interrogated. Whilst the specifics of these are examined in the methods section, the key features indicating high quality data are discussed below.

1.10.1 High Quality Data

There does not seem to be a specific definition or set criteria to what constitutes high quality data. However, key features can be found in Figure 1-7.

Figure 1-7. Features of high quality data

Accuracy and reliability – Have the variables been entered correctly?
Validity – Are they representative of the population being studied?
Timeliness – Are the data recent?
Relevance – Does it contain the information required for the appropriate analyses to be performed?

As I wished to perform analyses relevant to the UK, and in particular the ethnically and socio-economically diverse population in the West Midlands, the source data needed to be relevant and transferable to the local population. Potential sources of these data are discussed below.

1.10.2 Primary Care Data

Medical records held by primary care practices in the UK are extensive, including details regarding diagnoses and prescribed drugs, and whether an individual is on a disease-specific risk register. Additionally these records are often linked to laboratory and blood pressure data. Each practice has an information governance policy and may store the data in different ways, depending on the information technology (IT) system used. As there is likely to be variation between practices, both from a clinical and data collection point of view, an ideal dataset would cover a number of practices thereby being as representative as possible to the population studied. These datasets may be national or local and, for it to be useful in examining the relationship between CKD and outcomes, would require a measurement of renal function.

1.10.2.1 National Primary Care Data

The Health Improvement Network (THIN) dataset is a large, UK database of patients registered in over 400 primary care practices covering more than 3.7 million active patients. It has been utilised for multiple epidemiological research studies (166). It provides information on comorbidities including CKD, and contains eGFR and ACR data; indeed it has been used locally to investigate the accuracy of primary care coding of CKD (1). This work by Jain and colleagues identified inaccuracies in the coding of patients onto CKD risk registers. A further limitation of this dataset is the lack of patient level ethnicity data; eGFR may therefore not have been adjusted for ethnicity and comparisons between ethnicities would not be possible, making it unsuitable for use in this thesis.

1.10.2.2 Local Primary Care Data

In recognition that people living in inner-city Birmingham have a lower life expectancy than the national average, with unmet healthcare needs, resources and technology were allocated with an aim to improve the quality of healthcare. This included a targeted data collection across the majority of primary care practices in Heart of Birmingham (HoB) primary care trust (PCT), initially aimed at increasing the diagnosis of diabetes, cardiovascular and kidney disease among its deprived and ethnically diverse population (the ‘deadly trio’ programme) (167). This database is local, contains appropriate information on renal function comorbidities and has detailed demographic information, including self-reported ethnicity.

1.10.3 Secondary Care data

There are numerous secondary care datasets investigating CKD. Internationally, the largest of the studies have been included as part of the CKD prognosis consortium (63); these studies typically have at least 1,000 participants and information at baseline on eGFR and albuminuria. The studies included in the CKD prognosis consortium can be broadly divided into three types; general population, high risk cohorts selecting people at high cardiovascular risk and cohorts specifically selecting subjects with CKD. Additionally, whilst the populations covered are diverse, the consortium recognise that some ethnicities are under-represented; black populations are mainly from within the USA and asian populations are predominantly Eastern Asian (63). Even if it were possible to obtain permission for the use of these data, the applicability of these data to the local West Midlands' population is limited.

A further global network of CKD cohorts, the international network of CKD disease cohort studies (iNET-CKD), includes twelve prospective cohort studies and two registries covering 21 countries (168). The goals of iNET-CKD are the ability to provide mutual assistance and shared expertise through sharing research tools and technologies, to provide opportunities for collaborative research and to enhance training of both young investigators and seasoned researchers.

In addition to the studies identified in the CKD prognosis consortium or iNET-CKD, several international studies have recruited, or continue to recruit, adult patients with CKD across wide geographical areas in order to explore the determinants associated with CKD progression. These studies, and their objectives, are listed in Table 1-7.

Table 1-7. Key international studies investigating progression in adult CKD

Cohort	Population	Aims	Year recruitment commenced	Number recruited
Canadian study of prediction of death, dialysis and interim cardiovascular events (CanPREDDICT) (169)	eGFR 15-45 ml/min/1.73m^2 from outpatient nephrology clinics across Canada	1. To examine the role of both traditional risk factors and a select panel of newer, non-traditional serum and urine biomarkers, in the progression of kidney disease and CVD in patients with CKD, alone and separately. 2. To develop robust predictive models to discriminate between high and low risk patients.	2008	2546
Chronic Kidney Disease Japan Cohort (CKD JAC) (170)	Japanese (or Asian patients living in Japan) adults with eGFR 10-59m/min/1.73m^2	1. To identify risk factors for progression of CKD in Japanese subjects. 2. To identify the impact of CKD on HRQL. 3. To assess the frequency of hospitalisation and economic impact of CKD	2007	2977
Chronic Renal Insufficiency Cohort (CRIC) (171)	Secondary care, all CKD stages	To identify risk factors for the progression of CKD and the development of CVD. 2. To develop predictive models to identify high-risk subgroups, informing future treatment trials and increasing application of available therapies.	2003	3612

Table 1-7 continued…

Cohort	Population	Aims	Year recruitment commenced	Number recruited
French CKD-renal epidemiology and information network cohort (CKD-REIN) (172)	eGFR 15-59 ml/min/1.73m^2 from nephrology clinics	1. To assess the interaction between psychosocial, environmental, biological and genetic factors with outcomes. 2. To assess new biomarkers to predict outcomes. 3. To evaluate provider practices and their relation with outcomes including PROMs. 4. To identify and quantify costs, and cost effectiveness, of different treatment practices.	2014	3600*
German CKD study (GCKD) (173)	Secondary care, eGFR 30-60ml/min/1.73m^2 or significant proteinuria (albuminuria >300mg/day or proteinuria > 500 mg/day) with a eGFR>60ml/min/1.73m^2	1. To identify and validate risk factors for progression of CKD, the development of CVD, and the relationship between CKD and CVD. 2. To determine gender based differences in risk. 3. To assess impact of CKD on HRQL.	2010	4914

Abbreviations: CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR: estimated glomerular filtration rate; HRQL, health related quality of life; PROMs, patient reported outcome measures. * Signifies recruitment in progress.

Given the limitations, and potential lack of applicability, of the datasets described above, the use of UK based CKD studies may be better suited to answering my research questions. Currently there is no national secondary care CKD cohort. A summary of selected, prospective, observational UK studies of CKD, selected as they had at least a two year follow up period and greater than 250 participants, are discussed below.

1.10.3.1 Chronic Renal Impairment in Birmingham (CRIB) (174, 175)

This study was designed before the K/DOQI CKD staging system was introduced in 2002. CRIB recruited 369 individuals with CKD (serum creatinine greater than 130 μmol/L) from a single centre in Birmingham alongside control groups to investigate the relationship between kidney function and cardiovascular risk factors patients with CKD not requiring renal replacement therapy. Patient follow up was a mean of six years; no further clinical assessment took place during that period, the outcomes reported were ESRD and all cause mortality.

1.10.3.2 Chronic Renal Insufficiency Implementation Study (CRISIS) (176-178)

A single centre study from Manchester, CRISIS aimed to recruit patients 18 years and older who were referred for management of CKD and had an eGFR below 60 ml/min/1.73m^2 but had no immediate requirement to start dialysis. Recruitment started in 2002 and, by 2015, over 3000 patients were enrolled. Patients were managed in accordance with standard clinical practice guidelines and followed until death or initiation of RRT (dialysis or transplantation).

1.10.3.3 Renal Risk in Derby (R^2ID) (179)

R^2ID study commenced recruitment in 2008 and is a collaboration between primary and secondary care researchers. It is a primary care cohort of patients with stage G3 CKD (30-59 ml/min/1.73m^2) who were recruited directly from 32 participating primary care practices. It was created to examine the renal and cardiovascular risks associated with less advanced CKD, and included measurements of vascular health including PWV (Section 2.4.4.2) and advanced glycation end products (Section 2.4.4.3).

1.10.3.4 Renal Impairment in Secondary Care Study (RIISC) (73, 180)

The UK based secondary care studies discussed above all had relatively broad inclusion criteria, whether the study was based in primary or secondary care. In order to focus on the individuals at the greatest risk of progression of renal disease, our group designed the RIISC study. This focuses on advanced and/or progressive CKD and includes detailed bioclinical phenotyping collected using standard operating procedures (SOPs) within an ethnically diverse population. The RIISC study is discussed in detail in the methods section.

1.11 Introductory Conclusions

In this introduction I have described CKD and the burden it places on individuals and society. The previously identified risk factors for ESRD and death have been discussed, including the potential challenges of adding additional demographic factors or biological markers to the established risk factors on a population level. Of the factors utilized in widely accepted risk scores, it is age, gender, eGFR and ACR that

are most strongly associated with adverse outcomes. Additionally I have highlighted the paucity of data regarding in the impact of ethnicity, in particular South Asian ethnicity, and socioeconomic status, on adverse outcomes in CKD.

Through a review of the literature and, in recognition of the ethnically diverse population we cover, I have identified research questions and discussed the potential data sources that could be used to address these questions.

In this thesis I present data on a series of studies that focus on patients with CKD. I have utilised both primary care and secondary care cohorts. One cohort is representative of patients who are undergoing routine clinical care on a population basis (primary care; the 'deadly trio programme') and the other a specific group recruited through secondary care clinics (The RIISC study).

Chapter 2 describes the cohorts and statistical techniques utilised in the thesis.

Chapter 3 reports the impact of ethnicity, comorbidity and renal function on death in an ethnically diverse, primary care population.

Chapter 4 focuses on the impact of ethnicity on adverse outcomes, most notably progression to ESRD, within the secondary care nephrology setting.

Chapter 5 investigates the relationship of serum tryptase, a product of mast cell activation which may be involved in non-classical activation of the RAAS pathway,

to progression of ESRD within the secondary care RIISC study. I explore whether this relationship is influenced by participants use of angiotensin converting enzyme inhibitors or angiotensin II blockers (i.e. medication which influence the RAAS pathway).

Chapter 6 examines HRQL in the secondary care CKD population. I investigate the impact of CKD on HRQL and whether there is an association between HRQL on clinical end-points.

The cohorts and statistical techniques used will now be described in Chapter 2.

2 METHODS

In order to address the research questions summarised at the end of Chapter 1, I collected, reviewed and analysed data from both primary care and secondary care cohorts. The methodologies utilised were adjusted for the research questions and the cohorts within which a given research question was addressed.

2.1 Description of the population in the West Midlands

In order to provide context to the cohorts utilised in this thesis, a brief narrative is provided below describing the population of the West Midlands, with a particular emphasis on inner city Birmingham, from which the primary care cohort is derived.

The West Midlands has been described by the Office for National Statistics as a region of contrast and diversity (181). There is a mixture of large urban areas (Birmingham is the largest urban area outside London) and countryside, and areas with high deprivation but also areas of prosperity.

The West Midlands is home to the largest non-white population outside London, with 14% of the population classed as non-White (181). Data from the 2011 census indicate the 'Asian or Asian British' ethnic group are the biggest non-White ethnic group, followed by 'Black or Black British' (182). Table 2-1 compares the ethnicity data for England, the West Midlands and Birmingham.

Table 2-1. Ethnicity Data from 2011 Census; Birmingham compared to National and Regional Data. Adapted from (182). Data shown as percentages.

	White	Asian			Black	Mixed/ Other		
	Total	Total	South Asian	Other Asian	Total	Total	Other	Mixed
England & Wales	**86.0**	**7.5**	5.3	2.2	**3.3**	**3.2**	1.0	2.2
England	**85.4**	**7.8**	5.6	2.3	**3.5**	**3.3**	1.0	2.3
West Midlands Region	**82.7**	**10.8**	8.9	1.9	**3.3**	**3.2**	0.9	2.4
West Midlands County	**70.1**	**18.8**	15.9	2.9	**6.0**	**5.1**	1.5	3.5
Birmingham	**53.3**	**26.6**	22.5	4.1	**9.0**	**6.1**	2.0	4.4

2.1.1 Age Distribution and Educational Attainment

The West Midlands has a higher proportion of young (under 16 years) and old (over 65 years) inhabitants compared to the average for English regions (181). It therefore has a lower proportion of working age individuals (between 16 and 64 years). Additionally, 2009 data indicate the West Midlands has the highest proportion of working age people with no qualifications in England.

2.1.2 The Economy and the Labour Market

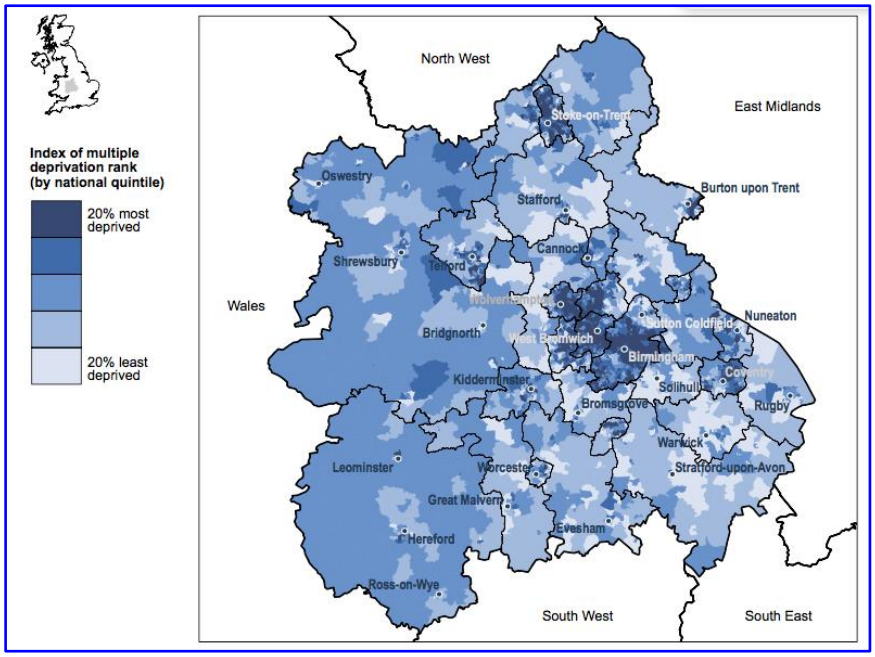
The West Midlands was the industrial and manufacturing heartland of England for many years, with the presence of core industries of coal, steel and metal working, and a concentration of motor vehicle manufacturers. This has changed dramatically in the past three decades, with major economic restructuring and a movement from manufacturing to service sector industries. The proportion of workforce employed in manufacturing industries has reduced from 22.4% in 1996 to 11.2% in 2010 and the

largest employment sectors are now ‘wholesale and retail trade; repair of vehicles’ (15.%) and ‘human health and social work activities’ (13.4%) (181).

2.1.3 Socioeconomic Status

The West Midlands is socioeconomically diverse, with some of the most and least deprived areas in England; the majority of the most deprived areas are concentrated in the regions towns and cities, although some rural areas also have high levels of deprivation. The Index of Multiple Deprivation (described in more detail in Section 2.2.7) provides a summary measure of relative deprivation based on small areas (called Lower Layer Super Output Areas; LSOAs). Figure 2-1 provides a graphical representation of the levels of deprivation within the West Midlands. Notably, the primary care population described in Section 2.2 is primarily based in the Birmingham authority area in which 56% of LSOAs are in the most deprived quintile (181).

Figure 2-1. Graphical representation of socioeconomic status of the West Midlands by Index of Multiple Deprivation quintiles (181)



The diversity described above provides an ideal population from which to explore the research questions posed in Section 1.9.

The primary and secondary care cohorts interrogated in this thesis will now be described.

2.2 Primary Care

This cohort was interrogated for the analyses described in Chapter 3.

2.2.1 Cohort Information

The cohort was derived from Heart of Birmingham Primary Care Trust (HoB PCT) which had a registered population of 312,070 on September 2008. The majority of the population (62%) were non-white (183). Sixty nine percent of the population were below 40 years of age.

Participating primary care practices gave permission for enhanced data to be collected centrally, utilising software able to identify comorbidities through their classification on chronic disease registers [Enhanced Healthcare Services, Essex, UK].

2.2.2 Ethics

Data were fully anonymised and available as a component of an on-going clinical development programme. The responsible NHS Research and Development

Consortium stated that this study did not require ethical submission to an NHS research ethics committee as it represented an evaluation of part of an on-going primary care trust (PCT) programme. For PCT data extraction the PCT professional executive committee and GP locality leads provided approval for the programme, including evaluation and publication.

2.2.3 Inclusion and Exclusion Criteria

The inclusion criteria comprised

- Registration in a practice participating in the enhanced data collection initiative.
- Individuals aged 40 years and over.

Exclusion criteria

- No kidney function checked within the last 12 months.
- Isotope Dilution Mass Spectrometry (IDMS) conversion for creatinine not available.
- Individuals who left a participating practice during the study period.
- An estimated glomerular filtration rate (eGFR) less than 15ml/min/1.73m^2.

2.2.4 Assessment of estimate Glomerular Filtration Rate

Estimated GFR reporting was not universally recorded on primary care systems in 2008. Investigations from practices participating in the enhanced data collection were sent to one of four laboratories. Whilst a standardised IDMS (Section 1.1.1) MDRD eGFR (28) was routinely reported by one laboratory, if this was not available the

eGFR was calculated utilising laboratory provided correction factors for the creatinine to generate IDMS traceable MDRD eGFR.

One general practice in the catchment area was excluded as IDMS traceable creatinine was not available from a fourth laboratory that provided blood tests specifically for that catchment area.

2.2.5 Study Duration

The follow up period was 23 months from May 2008 until February 2011. Individuals who had left the included practices during the follow up were excluded from the analyses (11.1%).

2.2.6 Data Collection

Data for the following variables were electronically collected from primary care records

- Age
- Gender
- Ethnicity. This was self-reported, considered the 'gold standard' for classification (184).
- Current smoking status
- Socio-economic status (SES) (Section 2.2.7)
- eGFR and/or creatinine
- Urinary albumin creatinine ratio (ACR)

- Vascular comorbidity (atrial fibrillation, chronic kidney disease, diabetes mellitus, heart failure, hypertension, ischaemic heart disease and stroke) as defined by a relevant clinical (Read) code specified by the UK pay for performance (QOF) business rules (185).

2.2.7 Assessment of Socioeconomic Status

Socio-economic status (SES) was assessed using the Index of Multiple Deprivation (IMD 2007) (186); this utilises the postcode from an individual's address to identify the Lower Layer Super Output Area (LSOA) where the individual resides. Each of the 32,482 LSOAs in England are assigned a score and rank for the IMD 2007, with lower ranks corresponding to the most deprived areas. The Index of Multiple Deprivation has been validated as superior to traditional deprivation indexes such as the Townsend score (119), due to its use of multiple domains reflective of socioeconomic deprivation (187). The IMD 2007 score incorporates seven areas of deprivation: income deprivation; employment deprivation; health deprivation and disability; education; skills and training deprivation; barriers to housing and services; living environment deprivation; and crime. For the analyses presented, deprivation was divided into quintiles, with the most deprived quintile as the reference population (i.e. how mortality in less deprived quintiles compared to the most deprived quintile).

2.2.8 Mortality data

Mortality data was obtained from the Primary Care Mortality Database (188), a resource developed by The NHS Information Centre in partnership with the Office for National Statistics (ONS). Data obtained from ONS records are linked to the general

practice where the individual was registered. This allowed data to be extracted for specific general practices (i.e. those within HoB PCT).

2.3 Secondary Care; The Renal Impairment in Secondary Care Study

The Renal Impairment in Secondary Care (RIISC) study was evaluated for the analyses in Chapters 4, 5 and 6.

2.3.1 Cohort Information

The RIISC study is a multicentre, longitudinal observational study of advanced and/or progressive CKD which commenced recruitment in October 2010. A detailed protocol has previously been published (73). A summary of the study can be found below, focusing on the key components utilised in this thesis.

All patients provided written consent and the study was conducted in accordance with the Declaration of Helsinki.

2.3.2 Ethics

The study protocol was approved by the South Birmingham Research Ethics Committee (number 10/H1207/6) and Research and Development department of University Hospitals Birmingham NHS Foundation Trust who were the study sponsor (RRK3917). The Trial is registered on clinicaltrials.gov, a registry and results database of publicly and privately supported clinical studies of human participants

conducted around the world (URL: https://clinicaltrials.gov/ct2/show/NCT01722383. Study identifier NCT01722383).

2.3.3 Inclusion and Exclusion Criteria

The following criteria were used to assess eligibility for study entry.

Inclusion Criteria

- An eGFR (using the four variable MDRD equation (28)) below 30 ml/min/1.73m^2.
- An ACR above 70mg/mmol on at least three occasions. Where the recruiting site routinely assessed urinary Protein Creatinine Ratio (PCR), a cut off point greater than 100mg/mmol was used.

And/or

- A decline of 5ml/min/1.73m^2/year or 10ml/min/1.73m^2 over five years (Section 2.2.4).

And

- At least one year of secondary care renal follow up.
- Six or more documented eGFR results over at least one year.

These inclusion criteria were aligned to the 2008 National Institute for Health and Care Excellence (NICE) CKD guidelines for referral to secondary care nephrology services (58).

Exclusion Criteria

- Current or previous immunosuppression for immune-mediated renal disease.
- Established on renal replacement therapy (dialysis or kidney transplantation).

Immune mediated renal disease may influence inflammatory and cardiovascular risk, either directly via immune processes or via the immunomodulatory medications used in their management**.** Similarly people established on RRT will have competing risks for adverse events. Individuals from these groups were therefore excluded from participation.

2.3.4 Assessing progression in the screening process for the RIISC cohort

A linear regression technique was used in the screening process to estimate CKD progression (73). This technique had been validated in previous research studies (189) and could be calculated relatively quickly from minimal data, something essential in a screening scenario.

An individual's eGFR values were used to estimate decline using a linear regression line calculated from the available data points. The slope of this line produced a value equivalent to eGFR change as ml/minute/1.73m^2/day which was subsequently multiplied to give estimated ml/min/1.73m^2/year and ml/min/1.73m^2/5 year values.

These analyses for renal progression were initially performed manually by the investigators before an informatics solution was sought, which calculated the slope of the line and the standard error.

2.3.5 Study Sites

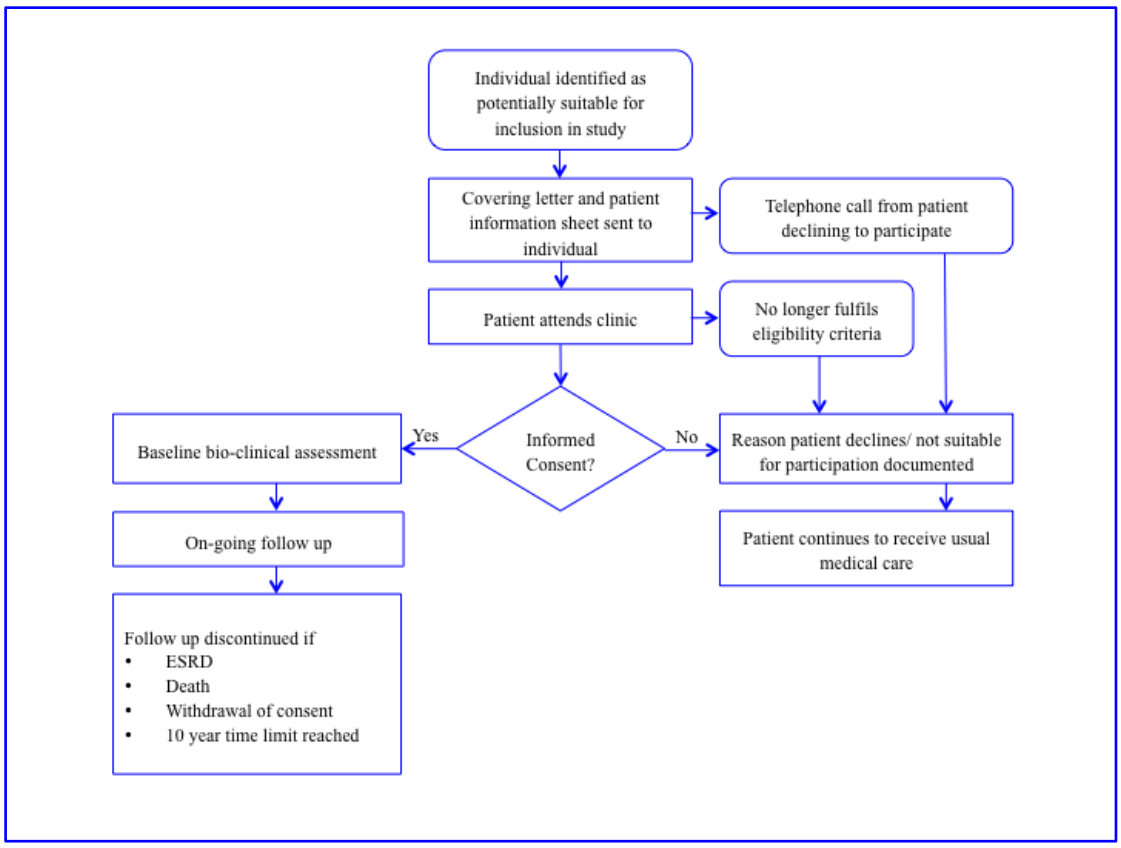
The RIISC study commenced recruitment in October 2010, initially recruiting from nephrology clinics associated with University Hospitals Birmingham NHS Foundation Trust (UHB).

Recruitment was expanded to Heart of England NHS Foundation Trust (HEFT) in October 2012 following appropriate ethical approval and site initiation visits.

2.3.6 Screening Process

Figure 2-2 illustrates the pathway from initial screening to attendance at the baseline visit. Queries regarding an individual's suitability for inclusion were discussed with the Chief Investigator or research fellow.

Figure 2-2. Screening process for eligibility for the RIISC study



Abbreviation: ESRD, end stage renal disease

If suitable, individuals were invited to participate in RIISC clinics. An appointment to a research clinic was generated and a Patient Information Sheet (PIS) sent to the patient [Appendix 1 and 2]. In order to reduce inconvenience and hospital visits, and encourage attendance, the study visit replaced a routine clinic attendance.

2.3.7 Consent

When an individual attended the research clinic, informed consent was taken by an appropriately trained and Good Clinical Practice certified investigator.

If they elected to enter the study, a consent form [Appendix 3] was signed and witnessed and the study investigations commenced.

If an individual decided not to participate in the study, the reasons for this were obtained and categorised as follows

- Informed refusal – this may have been over the telephone before a clinic attendance or during the research clinic
- Unable to give informed consent
- No longer meeting eligibility criteria including progression to ESRD prior to attendance at clinic

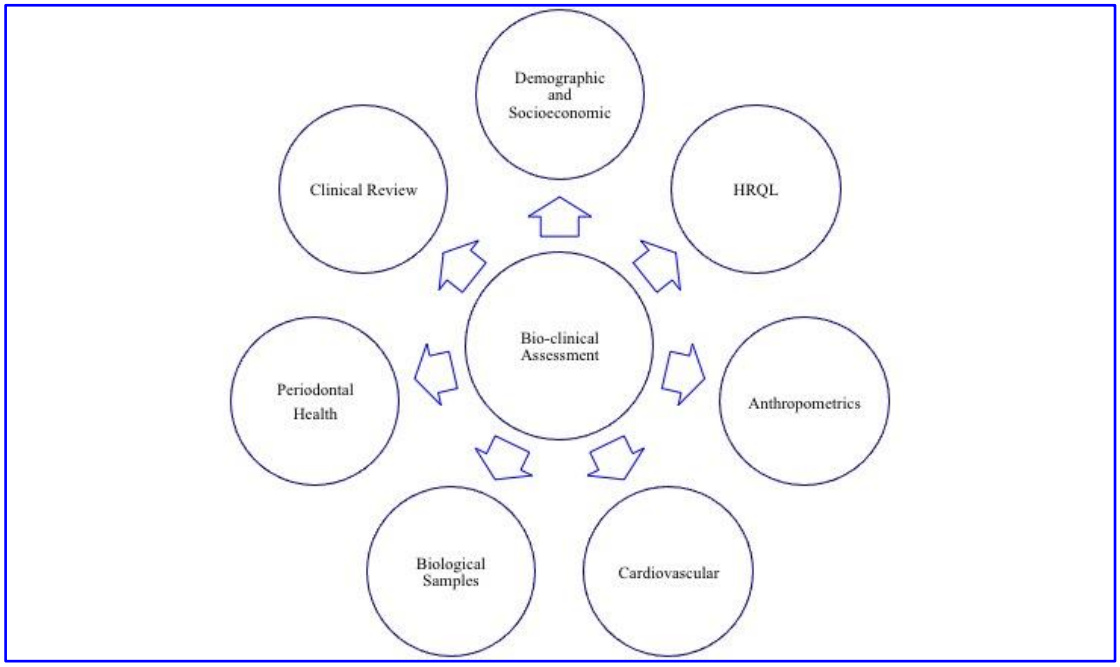
This prospective evaluation of reasons for non-consent allowed PRISMA-style flow diagrams (190) to be accurately constructed and areas where adjustments to recruiting techniques identified. An example of this was an ethics amendment submitted to the South Birmingham Research Ethics Committee, asking permission to include a summary PIS [Appendix 4] to be included with the full length PIS to provide an succinct summary of what participating in the study would involve.

If a patient elected not to enrol in the RIISC study, their normal clinic routine was followed including blood pressure check, phlebotomy, urine dipstick and protein quantification and clinical review. A routine medical review was carried out by a clinic doctor. The patients then returned to their regular clinic for follow up.

2.4 The Baseline RIISC Study Visit

Individuals giving informed consent to take part in the trial underwent a multi-faceted bio-clinical assessment (see Figure 2-3).

Figure 2-3. Overview of the RIISC Bio-clinical Assessment



Abbreviations: HRQL, health related quality of life.

The components of the assessment are described below. I have placed particular emphasis on those components used for the analyses in this thesis, but have included a brief description of other key components (e.g. different assessments of cardiovascular health) to enable an appreciation of the comprehensive bio-clinical assessment undertaken in RIISC.

2.4.1 Demographic and Socio-economic assessment

Demographics including age, gender, ethnicity and country of birth were collected. As with the primary care cohort (Section 2.2), ethnicity was self-reported, considered the gold standard for classification (184), taking into account an individual's cultural and self-identity. Ethnicity was categorised into five groups: white, black, South Asian, mixed ethnicity and other.

Socio-economic status (SES), including ethnicity, educational background, occupational status and an assessment of deprivation were documented. The catchment areas of both recruiting centres cover a wide socioeconomic spectrum and have a high proportion of non-white ethnicity.

In similarity to the technique used in the primary care cohort (Section 2.2.7), SES was assessed using the Index of Multiple Deprivation which utilises the postcode from an individual's address to assign a score and rank; a lower rank corresponds to the most deprived areas. The RIISC study used data from IMD 2010, rather than IMD 2007 used in the primary care work (191).

2.4.2 Assessment of Health Related Quality of Life

Health related quality of life (HRQL) can be assessed using disease specific or generic instruments. A systematic review of patient reported outcome measures (PROMs) used in CKD supported the use of preference-based utility measures, favouring the EuroQol, EQ-5D due to ease of use for patients and for the ability to derive utility values for health economic evaluation (192).

Data were collected from participants using the EQ-5D-3L (abbreviated to EQ-5D throughout this thesis). This is a validated, generic preference-based measure of health status that comprises a 5-question multi-attribute questionnaire and a visual analogue self-rating scale (VAS) (193) [Appendix 5]. Respondents were asked to rate severity of their current problems (level 1=no problems, level 2=some/moderate problems, level 3=severe/extreme problems) for five dimensions of health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). Health states were converted into an $EQ\text{-}5D_{index}$ score ranging from −0.594 to 1.0 (where 1 is full health and lower values indicate worse HRQL) using a set of weighted preferences produced from the UK population (194). The EQ VAS asks respondents to rate their own health state relative to full health (score=100) or worst imaginable health state (score=0).

2.4.3 Anthropometrics

Traditionally Body Mass Index (BMI; weight in kg / [height in metres]^2) has been used for an assessment of obesity and therefore a marker of cardiovascular risk. It is recognised that BMI has limitations as it does not take into account body shape and may not be as useful in the assessment of individuals of non-white ethnicity (195). One reflection of this is that waist circumference, as a measure of abdominal obesity, rather than BMI is incorporated into the consensus criteria for diagnosis of the metabolic syndrome, a cluster of risk factors for cardiovascular disease and type 2 diabetes (196).

We therefore collected the following measurements

- Height
- Weight
- Waist Circumference
- Hip Circumference
- Thigh Circumference

Readings were taken using appropriately calibrated equipment and research staff followed standard operating procedures (SOPs) to ensure consistency of results.

2.4.4 Cardiovascular Profile

2.4.4.1 Blood Pressure

Brachial blood pressure (BP) was measured using the BpTRU fully automated sphygmomanometer (BpTRU Medical Devices, Coquitlam, BC, Canada), which obtained a series of six BP readings at one minute intervals following a five minute rest period. Mean BP was derived from the average of the second to sixth BP reading. This average reading have been reported to be comparable to mean daytime blood pressure from 24 hour ambulatory BP monitoring (197) and reduces the white coat response compared to manual office BP measurements (198).

2.4.4.2 Central Blood Pressure and Arterial Stiffness

Vascular calcification is common in CKD and is associated with increased mortality, especially due to cardiovascular disease events (199). Vascular calcification in CKD results from two distinct vascular pathologies, atherosclerosis and arteriosclerosis

(200). Atherosclerosis is an intimal disease characterised by fibroatheromatous plaques whereas arteriosclerosis is a disease of the arterial medial layer due to increased collagen content, calcification and proliferation of vascular smooth muscle cells (201). It is arteriosclerosis that dominates in more severe CKD. This results in increased arterial stiffness and pulse pressure and abnormal cardiac structure and function (202) with a subsequent increased risk of cardiovascular disease events and death. Studies in a variety of populations have demonstrated the independent association of cardiovascular outcomes and PWV (203) and the relationship between CKD, PWV and death (204).

A variety of surrogate markers, using both invasive and non-invasive techniques, for arterial stiffness exist. The non-invasive Vicorder instrument [Smart Medical, Gloucestershire, UK] was utilised in the RIISC study. It measures peripheral and central blood pressure, and arterial stiffness by carotid-femoral pulse wave velocity (PWV) and calculation of augmentation index from pulse wave analysis (PWA). The Vicorder system has been shown to be operator independent and highly reproducible (205).

The following data were collected

- Peripheral systolic BP (SBP), diastolic BP (DBP), pulse pressure (PP), mean arterial pressure (MAP)
- Central SBP, DBP, PP, MAP
- Heart rate
- Carotid-femoral pulse wave velocity

Central pressure waveforms were derived and analysed using pulse wave analysis to calculate central Augmentation index (AIx) (augmentation pressure divided by pulse pressure). Augmentation index varies according to heart rate (206). To enable comparisons, AIx was corrected to a HR of 75 beats per minute (AIx_{75}).

2.4.4.3 Advanced Glycation End products

Advanced glycation end products (AGEs) are produced by the Malliard reaction, which results in irreversible covalent crosslinking of collagen and elastin with carbohydrates or carbonyl groups (207). AGEs have been implicated as a contributing factor to arteriosclerosis and cardiovascular disease (208). AGEs may accumulate in CKD due to an increased oxidative stress burden with advanced chronic kidney disease (208, 209). It has been proposed than AGEs serve as a measure of cumulative metabolic stress (210). Their levels have been shown to be independently associated with pulse wave velocity (211), mortality (212) and CKD progression (210).

Advanced glycation end product accumulation in the skin was measured by skin auto-fluorescence using a validated AGE Reader^{TM} (DiagnOptics BV, Groningen, The Netherlands).

2.4.5 Biological Samples

Routine clinical blood tests (full blood count, ferritin, urea and electrolytes, bone profile, bicarbonate, glucose, HbA1c, lipid profile) and analysis of urine (urine

dipstick and protein quantification for ACR) were performed in accordance with the current standard of care using local laboratories.

Additional blood and urine samples were processed and stored according to established trial SOPs [Appendix 6]. The trial team planned to collect the following for all patients at all time-points

- **Serum –** 5x500µl, 6x250µl and 1x100µl aliquots
- **Plasma –** 4x500µl and 1x100µl aliquots. Note; plasma was not collected for the first 150 participants at the initial (UHB) recruitment site.
- **Urine –** 5x1ml, 1x300µl and 1x100µl aliquots
- **Saliva –** 2x500µl aliquots

Samples were labelled with the site, study number, type of biological sample type and time-point and stored at minus 80°C in appropriately monitored freezers.

The PAXgene tube system (Quiagen, Venlo, Netherlands) was used to collect DNA samples at a single time-point. This typically results in DNA yields of 150-500µg. DNA is extracted at a later date according to the manufacturers instructions. In accordance to manufacturer's instructions, these samples were stored at minus 20°C for 1-7 days before being transferred to minus 80°C storage.

With particular relevance to this thesis, the following additional assays were performed:

2.4.5.1 C-Reactive Protein

C-reactive protein (CRP) was measured using the Full Range C-Reactive Protein Kit on a SPA™ automated PLUS turbidimeter (The Binding Site Group Ltd, Birmingham, UK). The normal range for CRP is between 0.1 and 9 mg/L, with 90 percent below 3 mg/L (213).

2.4.5.2 Serum Free Light Chains

Serum Kappa (κ) and Kambda (λ) free light chain (FLC) concentrations were measured by nephelometry on a Dade-Behring BNTMII Analyser (Siemens AG, Erlangen, Germany) using particle enhanced high-specificity homogenous immunoassays (Freelite™; The Binding Site Group Ltd, Birmingham, UK). The normal reference ranges for serum FLC concentrations have been previously described as κ: 3.3–19.4 mg/L and λ: 5.7–26.3 mg/L, with the assay sensitivity being demonstrated as <1 mg/L. κ and λ FLC concentrations were combined to calculate the combined FLC (cFLC) concentrations. For the purpose of analysis, data are presented as cFLC and κ-λ FLC ratio.

2.4.5.3 Serum Tryptase

Serum tryptase concentration was measured by the ImmunoCAP Tryptase sandwich immunoassay (Phadia AB, Uppsala, Sweden). Baseline tryptase levels in healthy individuals are reported by the manufacturer of the assay as 1-15 μg/L (214). This assay is utilised in Chapter 5.

2.4.6 Periodontal Assessment

The periodontal component of the RIISC study, which included saliva sampling (215) and periodontal assessment, was designed in conjunction with clinicians from the Periodontal Research Group within the School of Dentistry at the University of Birmingham. The key aim of this component was to evaluate the association between chronic periodontitis and CKD progression. A description of the rationale, methodology and baseline results has previously published (180).

2.4.7 Clinical review

The final component of the RIISC bio-clinical assessment is a clinical review by a consultant nephrologist or clinical fellow (specialty trainee or equivalent), analogous to the routine review where best practice guidelines were used to assess and manage CKD and its complications and minimise cardiovascular risk.

Data were recorded on the case report form (CRF) including:

2.4.7.1 Renal diagnosis

The known or presumed renal diagnosis was initially stated in free text and then classified as follows

- Diabetes/ Diabetic kidney disease
- Interstitial nephropathy
- Ischaemic/ hypertensive
- Adult polycystic kidney disease
- Obstructive uropathy

- Reflux nephropathy
- Primary glomerulonephritis
- Hereditary
- Other cystic renal disease
- Secondary glomerulonephritis
- Not known and other
- Not stated

The date of any renal biopsy was recorded.

2.4.7.2 Comorbidities

Medical history was listed. This enabled subjects with specific comorbidities (e.g. diabetes) to be analysed separately and for comorbidity scores to be calculated.

The Charlson Comorbidity Index (CCI) is probably the most extensively studied index (216). The CCI and case definitions for each part is presented in Table 2-2.

One constituent part of the CCI is moderate/ severe renal disease, defined as serum creatinine >3mg/dL (>265.2μmol/L). Therefore certain analyses involved including CCI *without* the renal disease points.

Table 2-2. Criteria and Scoring System for Charlson Comorbidity Index. Adapted from (216).

Domain	Definition	Points
Myocardial infarction	One or more definite or probable myocardial infarcts. Patients should have been hospitalized for chest pain or an equivalent clinical event and have had electrocardiographic and/or enzyme changes.	**1**
Congestive Heart Failure	Exertional or paroxysmal nocturnal dyspnoea and who have responded symptomatically (or on physical exertion) to digitalis, diuretics or afterload reducing agents. It does not include patients who are on one of those medications but who have had no response and no evidence of improvement of physical signs with treatment.	**1**
Peripheral Vascular Disease	Intermittent claudication or those who have had a bypass for arterial insufficiency, those with gangrene or acute arterial insufficiency, and those with treated or untreated thoracic or abdominal aortic aneurysm (6cm or more)	**1**
Cerebrovascular Disease	Patients with history of cerebrovascular accident with minor or no residua.	**1**
Dementia	Patients with moderate to severe chronic cognitive deficit resulting in impaired function from any cause.	**1**
Pulmonary disease	Patients with asthma, chronic bronchitis, emphysema, and other chronic lung disease who have ongoing symptoms such as dyspneoa or cough, with mild or moderate activity	**1**
Connective Tissue Disease (Rheumatologic)	Includes patients with systemic lupus erythematous, polymyositis, mixed connective tissue disease, rheumatoid arthritis, polymyositis, polymyalgia rheumatica, vasculitis, sarcoidosis, Sjogrens syndrome or any other systemic vasculitis.	**1**
Peptic ulcer disease	Patients who have previously required treatment for ulcer disease, including those who have bled from ulcers.	**1**

Table 2-2 continued…

Domain	Definition	Points
Liver disease	**Mild:** Chronic Hepatitis or cirrhosis without portal hypertension	**1**
	Moderate: Cirrhosis with portal hypertension, but without bleeding	**3**
	Severe: Patients with ascites, chronic jaundice, portal hypertension or a history of variceal bleeding or those who have had a liver transplant	**3**
Diabetes	**Moderate:** Patients with diabetes and previous hospitalisations for ketoacidosis, hyperosmolar coma or control and those with juvenile onset or brittle diabetes	**1**
	Severe: Patients with retinopathy, neuropathy or nephropathy attributable to diabetes	**2**
Hemiplegia (Paralysis)	Includes patients with hemiplegia or paraplegia, whether it occurred as a result of a cerebrovascular accident or other condition	**2**
Renal Disease	**Moderate:** Includes patients with a serum creatinine >3 mg/dl (>265 μmol/L)	**2**
	Severe: Includes patients on dialysis, those who have had a transplant, and those with uraemia	**2**
Tumour	Patients with solid tumours without documented metastases but initially treated in the last 5 years, including breast, colon, lung and a variety of other tumours	**2**
Lymphoma	Includes patients with Hodgkins, lymphosarcoma, Waldenstrom's macroglubulinaemia, myeloma and other lymphomas	**2**
Leukaemia	Includes patients with acute and chronic myelogenous leukaemia, acute and chronic lymphocytic leukaemia, and polycythemia vera	**2**
Metastatic Cancer	Patients with metastatic solid tumours including breast, lung, colon and other tumours	**6**
AIDS	Patients with diagnosed or probable Acquired Immunodeficiency Syndrome (AIDS), and those who are Human Immunodeficiency virus (HIV) positive and asymptomatic	**6**

2.4.7.3 Medication

Medication including doses were listed. These were then grouped according to their listing in the British National Formulary (217).

2.4.7.4 Lifestyle factors

Present and past use of tobacco and alcohol were recorded.

2.4.8 Study Duration

Patient recruitment commenced in October 2010. Patients consented for follow-up for ten years from recruitment.

The censor date for each analyses presented is stated in the relevant results section.

2.4.9 Follow-up RIISC Study Visit Schedule

Given the typically long time course of CKD until clinical endpoints, the RIISC study was designed to follow people longitudinally. Visits were scheduled for

- 6 months
- 18 months
- 36 months (3 years)
- 60 months (5 years)
- 120 months (10 years)

These visits had the same investigations performed as the baseline visit, with the exception of the dental assessment which did not occur at 6 and 18 month time-points. Additionally clinical information was sought regarding clinical events, new diagnoses and medication changes.

Participants were reviewed between these visits in their routine renal clinic at time-points appropriate to their clinical situation.

2.4.10 Study End-points

Study end-points included progression to ESRD or death. Progression to ESRD was defined as the initiation of RRT (chronic dialysis or renal transplantation). Patient mortality was captured through linkage between electronic patient records and the ONS, which collects information on all registered deaths in the UK.

2.4.11 Electronic Data Collection

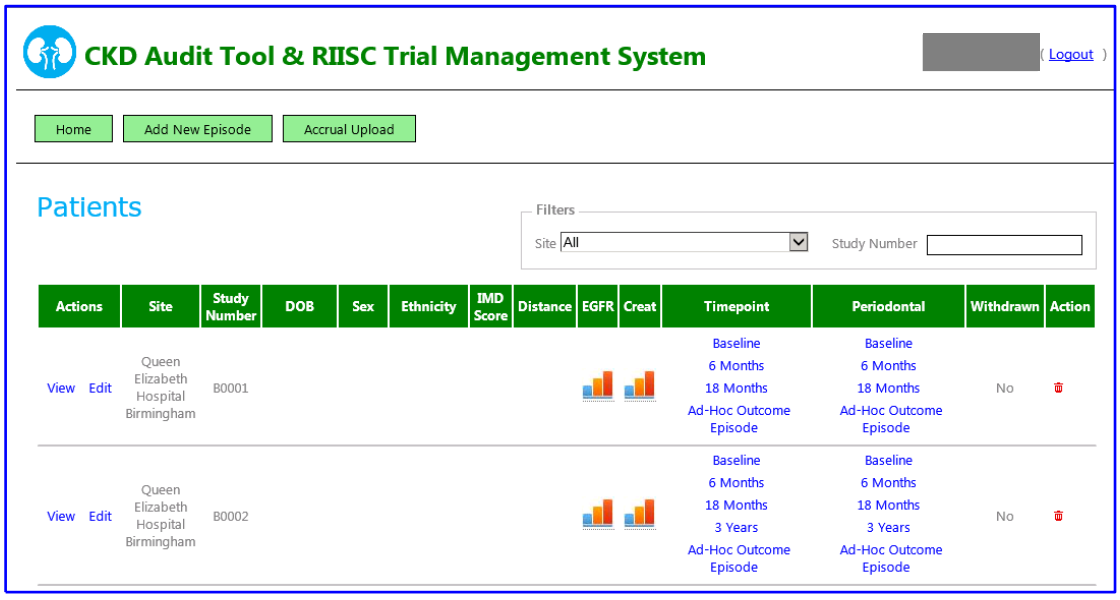
A screening spreadsheet was created by the study team to record participants, including their unique study number and dates of visits. Individuals who elected not to participate in the study were recorded, as described in Section 2.2.7, in order to document reasons for non-consent and to ensure these individuals were not re-invited to the RIISC study clinic.

Initially, data were transcribed manually from paper copies of CRFs, including measurements taken from study visits and blood results, into a database (Microsoft Access, Washington, USA). Recognising this was time-consuming and had the

potential to give rise to transcription errors (218), the informatics team at University Hospitals Birmingham were commissioned to work with us to create a bespoke informatics solution where data could be entered during the study visit (Figure 2-3). This resource was able to link to other IT systems within the hospital, including laboratory systems for blood results. Results from Heart of England NHS Foundation Trust were linked via comma separated value (CSV) files provided by the renal IT team.

An illustration of the electronic database is shown in Figure 2-4.

Figure 2-4. Screen image of bespoke RIISC electronic database.



2.5 Statistical Analyses

This section describes the general statistic techniques used in this thesis. Specific techniques or explanations pertinent to a particular results section are presented within that section.

Analyses for Chapter 3 were performed using PASW statistics 18 for Windows [IBM, Chicago, Il, USA]. Subsequent analyses (Chapters 4-6) were performed using Stata 13.1 [Statacorp, College Station, Texas, USA]. The latter enabled competing risk analyses (Section 2.5.3.3 below) to be performed.

2.5.1 Descriptive Statistics

Data are presented as mean with standard deviation (SD) or median with interquartile range (IQR) depending on distribution. Continuous variables were compared using ANOVA (parametric distribution) or Kruskal-Wallis (non-parametric distribution). Categorical variables were compared using chi-squared tests. Statistical significance was defined as a two-tailed *P*-value <0.05.

Correlation analyses were performed using Spearman's Rank Correlation. Values (R) were defined as very weak (R=0-0.19), weak (R=0.2-0.39), moderate (R=0.4-0.59), strong (R=0.6-0.79) and very strong (R>0.8) (219).

2.5.2 Regression Analyses

Both logistic and linear regression analyses are utilised in Chapter 6. These techniques are described within that section.

2.5.3 Survival Analyses

The focus of this thesis was to investigate the time from recruitment until an event of interest, for example death or progression to ESRD.

At the end-of the follow-up period, some individuals will not have experienced the event of interest, and their true time to the event is unknown. This may arise in one of the following ways (220)

- A patient has not (yet) experienced the relevant outcome.
- A patient is lost to follow-up during the study period.
- A patient experiences a different event that results in the individual no longer being followed up. A statistical approach to potentially deal with this is described in Section 2.5.3.3.

Time-to-event (survival) analyses therefore need to include those who have experienced an event and whose true time to event is unknown. This can be achieved through the use of one, or several, survival analysis techniques.

2.5.3.1 Kaplan-Meier plots

The survival probability can be estimated non-parametrically from observed survival times using the Kaplan-Meier (KM) method (220). The KM survival plot is used to provide a graphical summary of the data and can graphically demonstrate whether there is a variation in the outcome over time. The log rank test is a non-parametric technique which can compare survival in two or more groups of patients.

2.5.3.2 Cox Regression Analyses

If an adjustment for covariates or potential confounders is required, a different statistical approach is needed. The Cox proportional hazards model is the most commonly used approach for analysing multivariable survival time data in medical research (221). It provides probability (hazard ratio; HR) of an event at a given time (complete with 95% confidence intervals and *P*-value) with a specific pattern of covariates. Both univariable and multivariable regression analyses are presented.

An assumption of the Cox model is that the probability of the outcome of interest is relatively constant throughout the follow up time. This proportionality hazard assumption can be assessed by examining KM plots and log-log plots.

2.5.3.3 Competing Risk Analyses

As described above, survival analyses, by their nature, use time-to-event data (222). Survival analyses including Cox proportional hazard analyses treat all censored events as 'uninformative'; that is to say a patient being censored due to reaching the end of their follow up or due to another end-point (death in the case of ESRD or vice-versa) are treated equally. These alternative events are of clinical significance and of statistical importance; someone who has died will never reach ESRD (11). Therefore, in order to incorporate this into specific analyses, I performed competing risk analyses according to the method described by Fine and Gray (223) (Stata command stcrreg).

2.5.4 Missing data

All analyses utilise a complete case approach. However, in similarity with many clinical studies, there are some missing data (224). Data completeness is described within each section. If a component has a significant amount of missing data, for example ACR results within Chapter 3, data were analysed for all individuals identified and then repeated for individuals who had an ACR recorded.

3 RESULTS 1. The impact of ethnicity, chronic kidney disease and cardiovascular comorbidity on mortality in a multiethnic primary care population.

3.1 Preface

In Chapter 1 I discussed the paucity of data regarding the impact of ethnicity on adverse outcomes in individuals with chronic kidney disease (CKD). In this chapter I report the results of an analysis of the impact of ethnicity, comorbidity and renal function (both eGFR and ACR) on death in an ethnically diverse primary care population. These analyses utilised the enhanced primary care data collected within Heart of Birmingham Primary Care Trust (HoB PCT) (Section 2.2).

3.2 Abstract

Background. Whilst studies have reported potential survival differences between ethnic groups, there has been limited reporting on the relative impact of comorbidities including kidney function on a population basis. This study assessed the impact of CKD and cardiovascular comorbidity on mortality in a multi-ethnic, primary care population.

Methods. 31,254 Individuals aged 40 years and older, of South Asian, black or white ethnicity, registered with a general practice participating in an enhanced data collection project, and with their kidney function checked within the last 12 months, were included. The outcome measure assessed was all-cause mortality.

Results: Reduced estimated Glomerular Filtration Rate, higher albuminuria, older age, white ethnicity (versus South-Asian or black ethnicity) and increasing cardiovascular comorbidities were independent determinants of a higher mortality risk. In the multivariable model including comorbidities and kidney function, the hazard ratio for mortality for South Asians was 0.70 (95% confidence interval (CI) 0.56 – 0.87, P=0.001) and for blacks was 0.53 (95% CI 0.40 – 0.70, P<0.001), compared to whites.

Conclusions: The hazard ratio for death was lower for South Asian and black individuals compared to white individuals. This was, in part, independent of age, gender, socio-economic status (SES), kidney function and comorbidities. Risk of death was higher in individuals with CKD and in those with a higher cumulative cardiovascular comorbidity.

3.3 Introduction

Chronic kidney disease (CKD) prevalence and the risk imparted by CKD may vary by ethnicity. As discussed earlier in Section 1.5.1, some studies indicate that CKD is more common in people of white ethnicity (5, 225) but non-white ethnic groups have a faster progression to end-stage kidney disease (11, 226). Paradoxically, when treated with chronic dialysis treatment, people of non-white ethnicity have a lower mortality risk than people of white ethnicity (9, 227). An increased risk of death is also associated with other comorbidities, including hypertension, diabetes and cardiovascular disease (CVD) (228-233).

Whilst previous studies have indicated survival differences between ethnic groups (11, 95-99), there has been limited reporting in these studies on the relative impact of comorbidities including kidney function on a population basis. This paucity of data reflects a shortfall in the availability of population based primary care databases linked to estimated Glomerular Filtration Rate (eGFR) and albuminuria reporting and traceable to mortality. Furthermore there are minimal comparative data on people of South Asian ethnicity; comparative studies usually report data on Chinese-Asians (5).

In the United Kingdom, there has been a systematic improvement in chronic disease recognition through a primary care pay for performance system, the Quality and Outcomes Framework (QOF) (234, 235). This system utilises chronic disease registers for the identification, monitoring and management of patients with known comorbidities; a component of this monitoring involves measuring and documenting renal function. These disease registers can be combined with laboratory results and

linked with demographic and mortality data to better identify determinants of outcomes.

I therefore utilised a primary care cohort incorporating chronic disease registers to perform a retrospective cohort study of the relationship between CKD, cardiovascular (CV) comorbidity and mortality within a deprived, inner-city multi-ethnic population. The study hypotheses were

1. There are differences in mortality between different ethnic groups.
2. These differences in mortality are explained by known risk factors including comorbidities, renal function, demographic and socioeconomic factors.

This study incorporated all stages of kidney function, except stage G5 CKD (eGFR <15ml/min/1.73m^2), in patients with known CV comorbidities and focused on three ethnic groups: South Asian (including individuals of Bangladeshi, Indian and Pakistani descent), black (individuals from or who have ancestors from Africa or the Caribbean) and white.

3.4 Methods

A description of the cohort, ethics permission, inclusion and exclusion criteria, study design, and statistical techniques are presented in the methods section of this thesis (Sections 2.2 and 2.5).

3.4.1 Specific Statistical Analyses Related to this Chapter

All analyses were performed using PASW statistics 18 for Windows **[IBM, Chicago, Il, USA]**.

Measurements for kidney function were divided into categories; eGFR into six categories (15-29, 30-44, 45-59, 60-89, 90-119 and ≥120 ml/min/1.73m^2) with the eGFR range between 90 and 119 ml/min/1.73m^2 as the reference population. Individuals with an eGFR <15 ml/min were excluded from the analysis. ACR was divided into five categories (<1.1 mg/mmol 'optimal', 1.1-2.99 'high normal', 3-29.99 'high', 30-199.99 'very high' and ≥200 'nephrotic') in line with the KDIGO consensus conference (42).

Age was divided into six categories (50 years and under, 51-60, 61-70, 71-80, 81-90, greater than 90 years) with the youngest group serving as comparator.

The association between comorbidity, ethnicity and mortality was assessed by univariable analyses for all risk factors and then presented as three models. Choice of model variables were determined by the availability in the dataset of demographic and clinical risk factors consistent with those utilised by other investigators in previous work in similar populations (236, 237), where the variable was available in this target population. Model 1 incorporates the number of identified vascular comorbidities (zero to seven), ethnicity, age, gender, smoking status and SES. Model 2 includes eGFR level with removal of CKD from the comorbidity score (possible scores therefore zero to six) in order to avoid the association between declining renal function and the likelihood of being on the CKD register. Model 3 added ACR to the variables in Model 2.

A complete case model was used in the analyses. All data were complete with the exception of ACR. Therefore data were analysed for all individuals identified (unadjusted, Model 1 and Model 2) and then repeated for individuals who had an ACR recorded (unadjusted and Models 1-3). An 'enter' technique was used for the regression analysis.

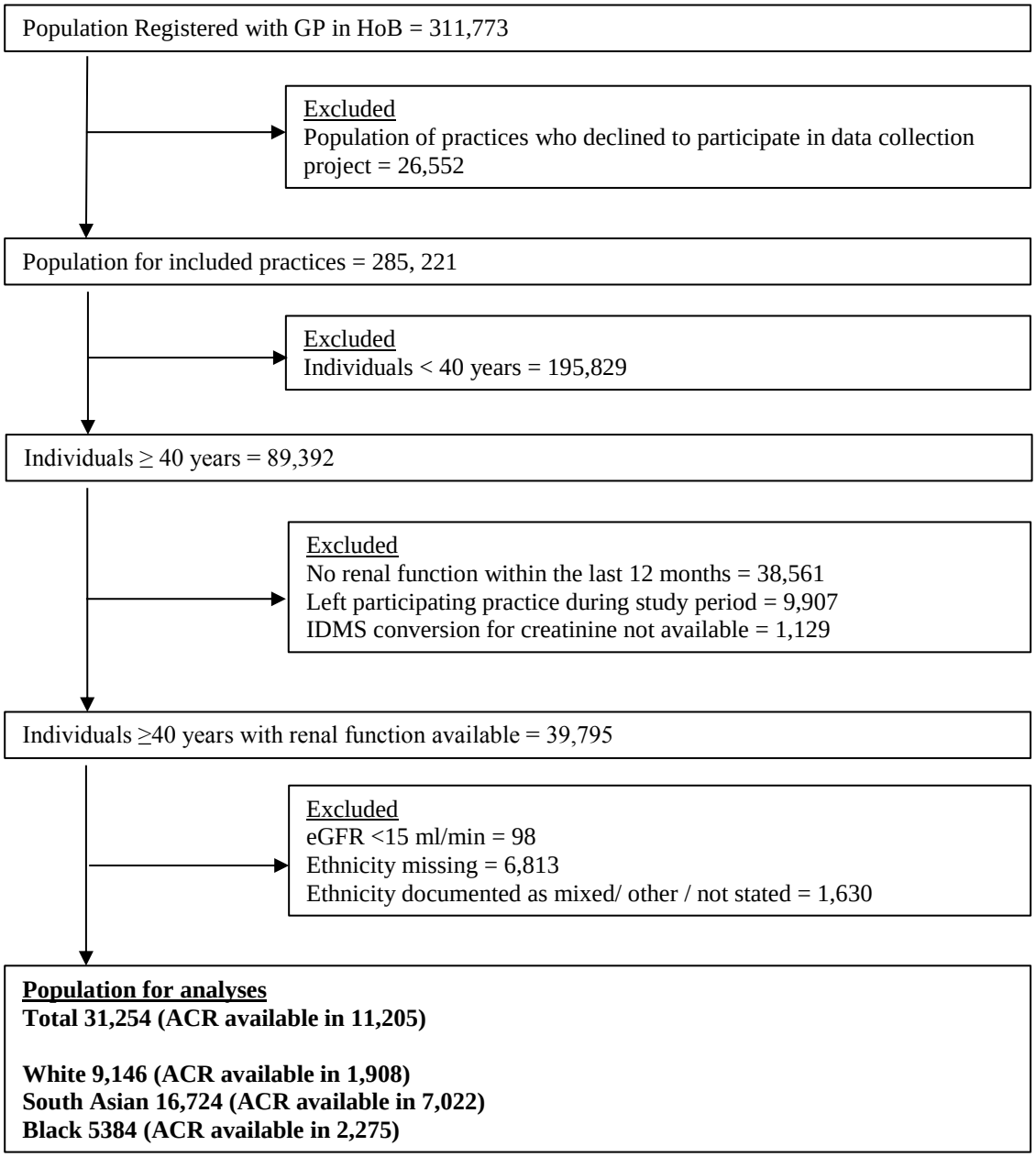
3.5 Results

3.5.1 Complete Cohort

Figure 3-1 illustrates the section process for inclusion in the study.

At inception (May 2008) 31,254 individuals fulfilled inclusion criteria for analysis. People of South Asian ethnicity formed the largest ethnic group (16,724, 53.4%), followed by people of white ethnicity (9146, 29.3%) and black ethnicity (5384, 17.2%). Baseline characteristics of the study population are shown in Table 3-1. The age distribution differed between groups with South Asians significantly younger than the other two ethnic groups. There was no significant difference in gender mix between the three ethnic groups. Smoking was least common in the South Asian group. The majority of all three ethnic groups resided in the most deprived quintile, with a higher proportion of people of South Asian and black ethnicity in this quintile than people of white ethnicity.

Figure 3-1. Flow Diagram indicating selection process for inclusion in the analyses



Abbreviations: ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; HoB, Heart of Birmingham Trust; IDMS, isotope dilution mass spectrometry.

Table 3-1. Baseline characteristics by ethnicity. Complete Cohort.

	All	White	South Asian	Black	*P*-value
Number (%)	31254 (100)	9146 (29.3)	16724 (53.4)	5384 (17.2)	
Age					
median (lower, upper quartile)	59.0 (50.0,71.0)	65.0 (55.0, 75.0)	56.0 (49.0, 68.0)	61.0 (48.0, 73.0)	<0.001
50 and under (%)	8421 (26.9)	1515 (16.6)	5124 (30.6)	1782 (33.1)	<0.001
51-60 (%)	8017 (25.7)	1948 (21.3)	5170 (30.9)	899 (16.7)	
61-70 (%)	6650 (21.3)	2459 (26.9)	3206 (19.2)	985 (18.3)	
71-80 (%)	6006 (19.2)	2109 (23.1)	2568 (15.4)	1329 (24.7)	
81-90 (%)	1974 (6.3)	1008 (11.0)	604 (3.6)	362 (6.7)	
>90 (%)	186 (0.6)	107 (1.2)	52 (0.3)	27 (0.5)	
Gender					
female (%)	15248 (48.8)	4384 (47.9)	8184 (48.9)	2680 (49.8)	0.085
Smoking					
n (%)	5150 (16.5)	2285 (25.0)	1812 (10.8)	1053 (19.6)	<0.001
IMD Rank					
Quintile 1 (least deprived) (%)	152 (0.5)	59 (0.6)	92 (0.6)	1 (0.0)	<0.001
Quintile 2 (%)	316 (1.0)	132 (1.4)	173 (1.0)	11 (0.2)	
Quintile 3(%)	3348 (10.7)	1860 (20.3)	1255 (7.5)	233 (4.3)	
Quintile 4 (%)	5144 (16.5)	2243 (24.5)	2238 (13.4)	663 (12.3)	
Quintile 5 (most deprived) (%)	22294 (71.3)	4852 (53.1)	12966 (77.5)	4476 (83.1)	
AF					
n (%)	807 (2.6)	515 (5.6)	212 (1.3)	80 (1.5)	<0.001
CKD					
n (%)	3648 (11.7)	1318 (14.4)	1691 (10.1)	639 (11.9)	<0.001
Diabetes					
n (%)	9931 (31.8)	1771 (19.4)	6415 (38.4)	1745 (32.4)	<0.001
Heart Failure					
n (%)	822 (2.6)	308 (3.4)	385 (2.3)	129 (2.4)	<0.001
Hypertension					
n (%)	16505 (52.8)	5181 (56.6)	8063 (48.2)	3261 (60.6)	<0.001
IHD					
n (%)	4226 (13.5)	1417 (15.5)	2386 (14.3)	423 (7.9)	<0.001
Stroke					
n (%)	1476 (4.7)	570 (6.2)	673 (4.0)	233 (4.4)	<0.001

Table 3-1 continued…

	All	White	South Asian	Black	*P*-value
Comorbidities					
median (lower, upper quartile)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	0.075
0 (%)	9879 (31.6)	2829 (30.9)	5459 (32.6)	1591 (29.6)	<0.001
1 (%)	10707 (34.3)	3253 (35.6)	5524 (33)	1930 (35.8)	
2 (%)	6845 (21.9)	1898 (20.8)	3694 (22.1)	1253 (23.3)	
3 (%)	2667 (8.5)	785 (8.6)	1451 (8.7)	431 (8)	
4 (%)	828 (2.6)	254 (2.8)	447 (2.7)	127 (2.4)	
5 (%)	268 (0.9)	103 (1.1)	124 (0.7)	41 (0.8)	
6 (%)	55 (0.2)	23 (0.3)	23 (0.1)	9 (0.2)	
7 (%)	5 (<0.1)	1 (<0.1)	2 (<0.1)	2 (<0.1)	
Creatinine (µmol/L)					
mean (SD)	87.0 (25.8)	88.2 (24.7)	84.6 (25.4)	92.3 (28)	<0.001
eGFR (ml/min/1.73m^2)					
median (lower, upper quartile)	80.2 (66.7, 94.3)	74.9 (62.3, 88.8)	81.3 (68.1, 95.3)	85.5 (72.3, 100.1)	<0.001
>120 (%)	1473 (4.7)	264 (2.9)	802 (4.8)	407 (7.6)	<0.001
90-120 (%)	8523 (27.3)	1842 (20.1)	4841 (28.9)	1840 (34.2)	
60-89 (%)	16373 (52.4)	5077 (55.5)	8776 (52.5)	2520 (46.8)	
45-59 (%)	3447 (11.0)	1389 (15.2)	1627 (9.7)	431 (8.0)	
30-44 (%)	1134 (3.6)	466 (5.1)	517 (3.1)	151 (2.8)	
15-29 (%)	304 (1.0)	108 (1.2)	161 (1.0)	35 (0.7)	
Died					
n (%)	1435 (4.6)	681 (7.4)	541 (3.2)	213 (4.0)	<0.001

Abbreviations: AF, atrial fibrillation; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IHD, ischaemic heart disease; IMD, index of multiple deprivation.

The number of vascular comorbidities was similar between groups, with 11-13% of each ethnic group having three or more comorbidities. Prevalence of different vascular comorbidities varied between groups: the white group had a lower reported prevalence of diabetes but a higher prevalence of CKD, atrial fibrillation, heart failure and stroke.

Median eGFR (corrected for ethnicity as appropriate) was 80.2 ml/min/1.73m^2 and was lowest in the white group (74.9 ml/min/1.73m^2 compared to 81.3 ml/min/1.73m^2 for South Asian individuals and 85.5 ml/min/1.73m^2 for those of black ethnicity; P<0.001). 21.5% of White, 13.8% of South Asian and 11.5% of Black individuals had an eGFR between 15 and 59 ml/min consistent with stage G3-G4 CKD.

At the end of the study period a higher proportion of white individuals had died (7.4%) compared to the two other ethnic groups (South Asian 3.2%, Black 4.0%; P<0.001).

3.5.2 Albumin Creatinine Ratio Cohort

An ACR had been tested in 7022 (42.0%), 2275 (24.9%) and 1908 (20.9%) of South Asian, black and white individuals respectively. Table 3-2 lists the baseline characteristics for this subgroup. The median ACR was 1.1 mg/mmol and was highest in the South Asian group (1.2 mg/mmol compared to 1.0 mg/mmol for both white and black individuals; P<0.001). Age distribution, eGFR, smoking status, and deprivation demonstrated a similar pattern to the complete cohort described above.

Those with an ACR tested were more likely to have a greater vascular comorbid burden (18-20% having three or more comorbidities). A higher proportion of individuals of South Asian descent, male gender and with diabetes had their ACR tested.

In concordance to the whole group analyses, deaths in the ACR cohort were highest amongst white individuals (7.8%) compared to the South Asian (3.6%) and black individuals (3.7%) ($P<0.001$).

Table 3-2. Baseline characteristics by ethnicity. ACR tested cohort.

	All	White	South Asian	Black	*P*-value
Number (%)	11205 (100)	1908 (17)	7022 (62.7)	2275 (20.3)	
Age (years)					
median (lower, upper quartile)	59.0 (50.0, 71.0)	65.0 (55.0, 75.0)	57.0 (50.0, 68.0)	65.0 (49.0, 74.0)	<0.001
50 and under (%)	1900 (25.9)	304 (15.9)	1961 (27.9)	635 (27.9)	<0.001
51-60 (%)	3024 (27.0)	413 (21.6)	2239 (31.9)	372 (16.4)	
61-70 (%)	2370 (21.2)	496 (26.0)	1423 (20.3)	451 (19.8)	
71-80 (%)	2251 (20.1)	456 (23.9)	1152 (16.2)	643 (28.3)	
81-90 (%)	611 (5.5)	222 (11.6)	226 (3.2)	163 (7.2)	
>90 (%)	49 (0.4)	17 (0.9)	21 (0.3)	11 (0.5)	
Gender					
female (%)	4348 (38.8)	682 (35.7)	2754 (39.2)	912 (40.1)	0.008
Smoking					
n (%)	1869 (16.7)	518 (27.1)	872 (12.4)	479 (21.1)	<0.001
IMD Rank					
Quintile 1 (least deprived) (%)	30 (0.3)	4 (0.2)	25 (0.4)	1 (0.0)	<0.001
Quintile 2 (%)	84 (0.7)	19 (1.0)	60 (0.9)	5 (0.2)	
Quintile 3(%)	712 (6.4)	233 (12.2)	540 (5.7)	78 (3.4)	
Quintile 4 (%)	1458 (13.0)	339 (17.8)	876 (12.5)	243 (10.7)	
Quintile 5 (most deprived) (%)	8921 (79.6)	1313 (68.8)	5660 (80.6)	1948 (85.6)	
AF					
n (%)	233 (2.1)	113 (5.9)	91 (1.3)	29 (1.3)	<0.001
CKD					
n (%)	1637 (14.6)	356 (18.7)	921 (13.1)	360 (15.8)	<0.001
Diabetes					
n (%)	6828 (60.9)	990 (51.9)	4505 (62.4)	1333 (58.6)	<0.001
Heart Failure					
n (%)	310 (2.8)	74 (3.9)	175 (2.5)	61 (2.7)	0.005
Hypertension					
n (%)	6189 (55.2)	1092 (57.2)	3679 (52.4)	1418 (62.3)	<0.001
IHD					
n (%)	1556 (13.9)	281 (14.7)	1071 (15.3)	201 (8.8)	<0.001
Stroke					
n (%)	480 (4.3)	97 (5.1)	283 (4.0)	100 (4.4)	0.126

Table 3-2 continued…

	All	White	South Asian	Black	*P*-value
Comorbidities					
median (lower, upper quartile)	1.0 (1.0, 2.0)	2.0 (1.0,2.0)	1.0 (1.0, 2.0)	2.0 (1.0,2.0)	0.818
0 (%)	2510 (22.4)	472 (24.7)	1514 (21.6)	524 (23.0)	<0.001
1 (%)	3139 (28.0)	466 (24.4)	2103 (29.9)	870 (25.1)	
2 (%)	3438 (30.7)	574 (30.1)	2093 (29.8)	771 (33.9)	
3 (%)	1481 (13.2)	261 (13.7)	928 (13.2)	292 (12.8)	
4 (%)	448 (4.0)	79 (4.1)	284 (4.0)	85 (3.7)	
5 (%)	154 (1.4)	46 (2.4)	83 (1.2)	25 (1.1)	
6 (%)	32 (0.3)	10 (0.5)	15 (0.2)	7 (0.3)	
7 (%)	3 (<0.1)	0 (<0.1)	2 (<0.1)	1 (<0.1)	
Creatinine (µmol/L)					
mean (SD)	89.1 (27.6)	91.8 (26.2)	86.2 (26.8)	95.8 (29.6)	<0.001
eGFR (ml/min/1.73m^2)					
median (lower, upper quartile)	81.1 (66.3, 95.9)	74.3 (59.7, 89.8)	82 (67.4, 89.8)	84.2 (70.0, 98.9)	<0.001
>120 (%)	611 (5.5)	67 (3.5)	380 (5.4)	164 (7.2)	<0.001
90-120 (%)	3234 (28.9)	404 (21.2)	2091 (29.8)	739 (32.5)	
60-89 (%)	5451 (48.6)	953 (49.9)	3453 (49.2)	1045 (45.9)	
45-59 (%)	1300 (11.6)	323 (16.9)	750 (10.7)	227 (10.0)	
30-44 (%)	487 (4.3)	131 (6.9)	274 (3.9)	82 (3.6)	
15-29 (%)	122 (1.1)	30 (1.6)	74 (1.1)	18 (0.8)	
ACR (mg/mmol)					
median (lower, upper quartile)	1.1 (0.4, 3.4)	1.0 (1.4, 2.8)	1.2 (0.5, 3.8)	1.0 (0.3, 2.9)	<0.001
Optimal (<1.1) (%)	5641 (50.3)	1026 (53.8)	3400 (48.4)	1214 (53.4)	<0.001
High Normal (1.1-2.99) (%)	2485 (22.2)	426 (22.3)	1560 (22.2)	499 (21.9)	
High (3.0-29.99) (%)	2594 (23.2)	402 (21.1)	1717 (24.4)	475 (20.9)	
Very High (30 - 200) (%)	413 (3.7)	49 (2.6)	287 (4.1)	77 (3.4)	
Nephrotic (>200) (%)	73 (0.7)	5 (0.3)	58 (0.8)	10 (0.4)	
Died					
n (%)	484 (4.3)	149 (7.8)	250 (3.6)	85 (3.7)	<0.001

Abbreviations: ACR, albumin creatinine ratio; AF, atrial fibrillation; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IHD, ischaemic heart disease; IMD, index of multiple deprivation.

3.5.3 Univariable Analysis

The univariable analysis for the complete cohort (Table 3-3) demonstrated unadjusted HRs for death of 0.42 (95% CI 0.38 – 0.47, *P*<0.001) for people of South Asian ethnicity and 0.52 (95% CI 0.45 – 0.61, *P*<0.001) for people of black ethnicity, compared to people of white ethnicity. The mortality rate increased exponentially with age and a higher HR was observed for male gender, current smokers and total number of comorbidities. No difference in mortality was found between deprivation quintiles. Using an eGFR of 90-119 ml/min/1.73m^2 as reference, a J-shaped relationship was observed with a higher risk of death seen for both higher and lower eGFR values. The HR for death increased progressively by stage of CKD with an eGFR <90 ml/min/1.73m^2.

The univariable analysis was repeated for those individuals who had their ACR reported (Table 3-3) with similar trends identified to the whole population analysis with the exception of no observed difference between individuals with an eGFR of ≥120 ml/min/1.73m^2 compared to 90-119 ml/min/1.73m^2. A progressive increase in HR for death was seen with each increasing category for ACR.

Table 3-3. Cox Proportional Hazard Regression Analysis. Univariable analyses.

	Complete Cohort HR (95% CI)	*P*-value	ACR Tested Cohort HR (95% CI)	*P*-value
Ethnicity				
White	1	(<0.001*)	1	(<0.001*)
South Asian	0.42 (0.38 - 0.47)	<0.001	0.44 (0.36 - 0.55)	<0.001
Black	0.52 (0.45 - 0.61)	<0.001	0.47 (0.36 - 0.61)	<0.001
Age (years)				
50 and under	1	(<0.001*)	1	(<0.001*)
51-60	2.13 (1.55 - 2.91)	<0.001	1.76 (1.06 - 2.92)	0.03
61-70	5.43 (4.08 - 7.23)	<0.001	4.65 (2.93 - 7.35)	<0.001
71-80	12.97 (9.89 - 17.02)	<0.001	11.36 (7.38 - 17.51)	<0.001
81-90	32.86 (29.95 - 43.26)	<0.001	24.73 (15.77 - 38.77)	<0.001
>90	90.90 (65.10 - 126.94)	<0.001	82.73 (46.68 - 146.61)	<0.001
Gender				
Female as reference	1.38 (1.24 - 1.53)	<0.001	1.40 (1.16 - 1.70)	0.001
Smoker				
Non-smoker as reference	1.15 (1.01 - 1.32)	0.036	1.26 (1.01 - 1.57)	0.044
IMD Rank				
Quintile 1 (least deprived)	0.86 (0.39 - 1.92)	0.713	<0.001 (<0.001 - >10^5)	0.939
Quintile 2	0.82 (0.47 - 1.45)	0.501	<0.001 (<0.001 - >10^5)	0.897
Quintile 3	1.00 (0.85 - 1.19)	0.983	1.15 (0.82 - 1.62)	0.419
Quintile 4	0.93 (0.80 - 1.07)	0.297	0.77 (0.58 - 1.04)	0.088
Quintile 5 (most deprived)	1	(0.802*)	1	(0.42*)

Table 3-3 continued…

	Complete Cohort HR (95% CI)	*P*-value	ACR Tested Cohort HR (95% CI)	*P*-value
AF	5.59 (4.76 - 6.57)	<0.001	6.12 (4.57 - 8.21)	<0.001
CKD	3.44 (3.07 - 3.85)	<0.001	3.50 (2.90 - 4.21)	<0.001
Diabetes	1.35 (1.21 - 1.50)	<0.001	1.94 (1.58 - 2.39)	<0.001
Heart Failure	7.62 (6.60 - 8.80)	<0.001	7.28 (5.68 - 9.33)	<0.001
Hypertension	2.08 (1.86 - 2.33)	<0.001	2.05 (1.68 - 2.50)	<0.001
IHD	2.80 (2.50 - 3.13)	<0.001	3.14 (2.59 - 3.80)	<0.001
Stroke	3.65 (3.15 - 4.23)	<0.001	3.71 (2.86 - 4.82)	<0.001
Comorbidities				
0	1	(<0.001*)	1	(<0.001*)
1	1.78 (1.49 - 2.12)	<0.001	1.63 (1.09 - 2.43)	0.016
2	2.93 (2.46 - 3.49)	<0.001	2.92 (2.02 - 4.21)	<0.001
3	5.49 (4.55 - 6.62)	<0.001	5.58 (3.84 - 8.11)	<0.001
4	9.58 (7.69 - 11.94)	<0.001	9.86 (6.51 - 14.92)	<0.001
5	17.59 (13.49 - 22.94)	<0.001	21.09 (13.48 - 33.00)	<0.001
6	28.39 (18.41 - 43.78)	<0.001	33.67 (17.52 - 64.72)	<0.001
7	11.87 (1.66 - 84.73)	0.014	29.40 (4.03 - 214.46)	0.001

Table 3-3 continued…

	Complete Cohort HR (95% CI)	*P*-value	ACR Tested Cohort HR (95% CI)	*P*-value
eGFR (ml/min/1.73m^2)				
>120	1.49 (1.11 - 2.01)	0.008	1.07 (0.60 - 1.90)	0.813
90-120	1	(<0.001*)	1	(<0.001*)
60-89	1.36 (1.16 - 1.59)	<0.001	1.50 (1.14 - 1.99)	0.04
45-59	3.85 (3.24 - 4.57)	<0.001	4.26 (3.16 - 5.74)	<0.001
30-44	6.59 (5.40 - 8.04)	<0.001	7.72 (5.56 - 10.70)	<0.001
15-29	14.47 (11.34 - 18.45)	<0.001	15.05 (9.94 - 22.80)	<0.001
ACR (mg/mmol)				
Optimal (<1.1)			1	(<0.001*)
High Normal (1.1-2.99)			1.36 (1.04 - 1.79)	0.026
High (3.0-29.99)			2.97 (2.38 - 3.70)	<0.001
Very High (30 - 200)			6.25 (4.49 - 14.01)	<0.001
Nephrotic (>200)			7.93 (4.49 - 14.01)	<0.001

Abbreviations: ACR, albumin creatinine ratio; AF, atrial fibrillation; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IHD, ischaemic heart disease; IMD, index of multiple deprivation.

* *P*-value for overall effect

3.5.4 Multivariable Analysis

Following adjustment for covariates the differences in ethnicity remained; people of South Asian and black ethnicities had a lower HR for death in all analyses.

Model 1 (complete cohort, incorporating the number of identified comorbidities) analysed the complete cohort (Table 3-4) and showed an adjusted HR for death of 0.67 (95% CI 0.60 – 0.76, $P<0.001$) for people of South Asian ethnicity and 0.59 (95% CI 0.50 – 0.70, $P<0.001$) for people of black ethnicity compared to people of white ethnicity. When the analysis was restricted to the cohort with ACR tests available (Table 3-4) the HR for death was 0.76 (95% CI 0.61 – 0.94, $P=0.011$) for people of South Asian ethnicity and 0.53 for people of black ethnicity (95% CI 0.4 – 0.69, $P<0.001$) compared to people of white ethnicity. For the complete cohort, mortality risk was lower in IMD quintiles 3 and 4 (compared to the most deprived quintile 5). No significant difference between IMD quintiles was identified in the ACR cohort. Increasing age (51 years and over in complete cohort, 61 years and over in ACR cohort), smoking status and male gender was significant in analyses for both cohorts. An increased HR for death was observed for two or more comorbidities, with the HR increasing as the number of comorbidities increased.

Table 3-4. Cox Proportional Hazard Regression Analysis. Multivariable Analyses. Model 1. (incorporating the number of identified comorbidities but not including any measurements of renal function).

	Complete Cohort HR (95% CI)	*P*-value	ACR Tested Cohort HR (95% CI)	*P*-value
Ethnicity				
White	1	(<0.001*)	1	(<0.001*)
South Asian	0.67 (0.56 - 0.76)	<0.001	0.76 (0.61 - 0.94)	0.011
Black	0.59 (0.50 - 0.70)	<0.001	0.53 (0.4 - 0.69)	<0.001
Age (years)				
50 and under	1	(<0.001*)	1	(<0.001*)
51-60	1.96 (1.43 - 2.69)	<0.001	1.54 (0.92 - 2.57)	0.101
61-70	4.51 (3.36 - 6.06)	<0.001	3.63 (2.25 - 5.87)	<0.001
71-80	10.08 (7.57 - 13.41)	<0.001	8.12 (5.12 - 12.89)	<0.001
81-90	23.97 (17.84 - 32.22)	<0.001	17.02 (10.44 - 27.73)	<0.001
>90	68.62 (48.17 - 97.76)	<0.001	61.22 (33.37 - 112.31)	<0.001
Gender				
Female as reference	1.45 (1.30 - 1.62)	<0.001	1.81 (1.48 - 2.20)	<0.001
Smoker				
Non-smoker as reference	1.72 (1.50 - 1.98)	<0.001	1.99 (1.57 - 2.52)	<0.001
IMD Rank				
Quintile 1 (least deprived)	1.08 (0.48 - 2.42)	0.849	<0.001 (<0.001 - >10^5)	0.951
Quintile 2	0.91 (0.51 - 1.60)	0.734	<0.001 (<0.001 - >10^5)	0.916
Quintile 3	0.82 (0.69 - 0.98)	0.028	0.98 (0.69 - 1.39)	0.907
Quintile 4	0.73 (0.63 - 0.85)	<0.001	0.75 (0.56 - 1.02)	0.062
Quintile 5 (most deprived)	1	(0.001*)	1	(0.478*)

Table 3-4 continued…

	Complete Cohort HR (95% CI)	*P*-value	ACR Tested Cohort HR (95% CI)	*P*-value
Comorbidities				
0	1	(<0.001*)	1	(<0.001*)
1	1.05 (0.87 - 1.25)	0.64	1.39 (0.93 - 2.10)	0.112
2	1.26 (1.05 - 1.52)	0.014	1.83 (1.24 - 2.70)	0.002
3	1.82 (1.50 - 2.23)	<0.001	2.55 (1.72 - 3.81)	<0.001
4	2.72 (2.16 - 3.44)	<0.001	3.87 (2.48 - 6.03)	<0.001
5	3.89 (2.95 - 5.14)	<0.001	6.25 (3.88 - 10.06)	<0.001
6	6.54 (4.20 - 10.16)	<0.001	10.83 (5.53 - 21.22)	<0.001
7	3.09 (0.43 - 22.08)	0.262	8.97 (1.22 - 66.15)	0.031

Abbreviations: CI, confidence interval; HR, hazard ratio; IMD, index of multiple deprivation.

* *P*-value for overall effect

Kidney function (eGFR) was incorporated into Model 2 (with the removal of CKD from the comorbidity score) and in the complete cohort (Table 3-5) the HR for people of South-Asian ethnicity was 0.68 (95% CI 0.6 – 0.77 *P*<0.001) and for people of black ethnicity was 0.79 (95% CI 0.64 – 0.98, *P*=0.032) compared to people of white ethnicity. Similarly, when the analysis was restricted to the cohort of patients with ACR tests available (Table 3-5), people of South Asian and Black ethnicity had a lower proportion of deaths compared to people of white ethnicity with HRs of 0.61 (95% CI 0.52 – 0.72, *P*<0.001) and 0.58 (95% CI 0.44 – 0.76, *P*<0.001) respectively. In the complete cohort mortality risk was lower in the IMD quintile 4. More than two comorbidities were associated with an increasing HR and an increased HR of death compared to the reference eGFR range (90-119 ml/min/1.73m^2) was seen with an eGFR ≥120 ml/min/1.73m^2 and ≥45 ml/min/1.73m^2. An eGFR of 60-89 ml/min/1.73m^2 was associated with a lower HR. In the analysis of those with ACR tested, an eGFR <60 ml/min was associated with progressively higher HR by CKD stage.

In Model 3 (all vascular comorbidities except CKD and the addition of eGFR and ACR, Table 3-6) the HR for death for people of South Asian ethnicity was 0.70 (95% CI 0.56 – 0.87, *P*=0.001) and for people of black ethnicity was 0.53 (95% CI 0.40 – 0.70, *P*<0.001) compared to people of white ethnicity (Figure 3-2). Older age, male gender, being a current smoker and increasing comorbidity (two or more) were associated with an increased HR of death (Figure 3-3). An ACR of 'high' or greater (i.e. ≥3.0 mg/mmol) and an eGFR <45 ml/min/1.73m^2 was also associated with an increased HR for death. No significant differences in HRs were observed between deprivation quintiles.

Table 3-5. Cox Proportional Hazard Regression Analysis. Multivariable Analyses. Model 2. (incorporating the number of identified comorbidities excluding CKD, and eGFR).

	Complete Cohort HR (95% CI)	*P*-value	ACR Tested Cohort HR (95% CI)	*P*-value
Ethnicity				
White	1	(<0.001*)	1	(<0.001*)
South Asian	0.68 (0.6 - 0.77)	<0.001	0.79 (0.64 - 0.98)	0.032
Black	0.61 (0.52 - 0.72)	<0.001	0.58 (0.44 - 0.76)	<0.001
Age (years)				
50 and under	1	(<0.001*)	1	(<0.001*)
51-60	2.09 (1.52 - 2.87)	<0.001	1.57 (0.94 - 2.63)	0.086
61-70	4.92 (3.65 - 6.63)	<0.001	3.70 (2.28 - 6.00)	<0.001
71-80	10.90 (8.15 - 14.58)	<0.001	7.95 (4.96 - 12.74)	<0.001
81-90	25.20 (18.63 - 34.10)	<0.001	16.16 (9.79 - 26.66)	<0.001
>90	68.19 (47.55 - 97.78)	<0.001	52.70 (28.40 - 97.77)	<0.001
Gender				
Female as reference	1.45 (1.30 - 1.61)	<0.001	1.82 (1.49 - 2.22)	<0.001
Smoker				
Non-smoker as reference	1.69 (1.47 - 1.95)	<0.001	1.96 (1.55 - 2.48)	<0.001
IMD Rank				
Quintile 1 (least deprived)	1.12 (0.50 - 2.49)	0.79	<0.001 (<0.001 - >10^5)	0.951
Quintile 2	0.90 (0.51 - 1.59)	0.707	<0.001 (<0.001 - >10^5)	0.917
Quintile 3	0.84 (0.71 - 1.00)	0.054	0.98 (0.69 - 1.40)	0.929
Quintile 4	0.74 (0.63 - 0.86)	<0.001	0.78 (0.58 - 1.05)	0.096
Quintile 5 (most deprived)	1	(0.002*)	1	(0.592*)

Table 3-5 continued…

	Complete Cohort HR (95% CI)	*P*-value	ACR Tested Cohort HR (95% CI)	*P*-value
Comorbidities				
0	1	(<0.001*)	1	(<0.001*)
1	1.02 (0.86 - 1.22)	0.788	1.46 (0.99 - 2.15)	0.055
2	1.21 (1.10 - 1.45)	0.039	1.70 (1.17 - 2.47)	0.006
3	2.12 (1.74 - 2.58)	<0.001	2.72 (1.82 - 4.06)	<0.001
4	2.64 (2.06 - 3.40)	<0.001	3.71 (2.36 - 5.84)	<0.001
5	3.64 (2.52 - 5.27)	<0.001	6.20 (3.46 - 11.12)	<0.001
6	5.07 (1.62 - 15.91)	0.005	10.02 (2.40 - 41.90)	0.002
eGFR (ml/min1.73m^2)				
>120	2.02 (1.5 - 2.72)	<0.001	1.47 (0.82 - 2.62)	0.195
90-120	1	(<0.001*)	1	(<0.001*)
60-89	0.82 (0.70 - 0.96)	0.015	0.94 (0.70 - 1.25)	0.649
45-59	1.10 (0.92 - 1.32)	0.301	1.40 (1.01 - 1.92)	0.041
30-44	1.34 (1.08 - 1.66)	0.007	1.95 (1.37 - 2.78)	<0.001
15-29	2.93 (2.27 - 3.78)	<0.001	3.26 (2.10 - 5.06)	<0.001

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IMD, index of multiple deprivation.

* *P*-value for overall effect

Table 3-6. Cox Proportional Hazard Regression Analysis. Multivariable analyses. Model 3. (incorporating the number of identified comorbidities excluding CKD, and eGFR and ACR)

	Complete Cohort HR (95% CI)	*P*-value
Ethnicity		
White	1	(<0.001*)
South Asian	0.70 (0.56 - 0.87)	0.001
Black	0.53 (0.40 - 0.70)	<0.001
Age (years)		
50 and under	1	(<0.001*)
51-60	1.52 (0.91 - 2.55)	0.112
61-70	3.52 (2.17 - 5.71)	<0.001
71-80	7.38 (4.61 - 11.82)	<0.001
81-90	15.72 (9.53 - 25.92)	<0.001
>90	51.64 (27.89 - 95.62)	<0.001
Gender		
Female as reference	1.78 (1.46 - 2.18)	<0.001
Smoker		
Non-smoker as reference	1.89 (1.49 - 2.39)	<0.001
IMD Rank		
Quintile 1 (least deprived)	<0.001 (<0.001 - >10^5)	0.952
Quintile 2	<0.001 (<0.001 - >10^5)	0.913
Quintile 3	0.98 (0.68 - 1.39)	0.902
Quintile 4	0.79 (0.59 - 1.06)	0.118
Quintile 5 (most deprived)	1	(0.65*)

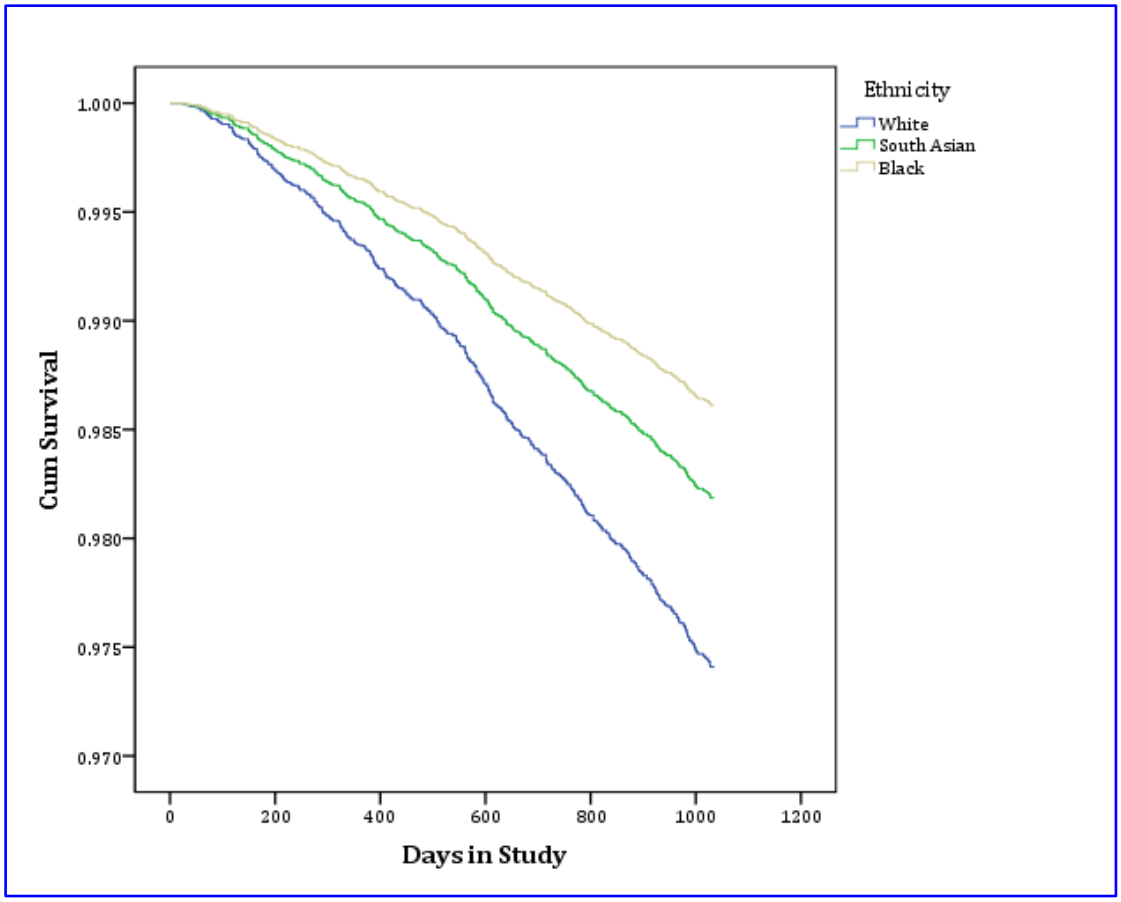
Table 3-6 continued…

	Complete Cohort HR (95% CI)	*P*-value
Comorbidities		
0	1	(<0.001*)
1	1.37 (0.93 - 2.02)	0.109
2	1.49 (1.02 - 2.17)	0.039
3	2.29 (1.53 - 3.43)	<0.001
4	3.15 (2.00 - 4.96)	<0.001
5	5.14 (2.87 - 9.21)	<0.001
6	10.54 (2.52 - 44.08)	0.001
eGFR (ml/min/1.73m^2)		
>120	1.40 (0.78 - 2.49)	0.26
90-120	1	(<0.001*)
60-89	0.91 (0.98 - 1.21)	0.505
45-59	1.28 (0.93 - 1.76)	0.126
30-44	1.57 (1.10 - 2.24)	0.014
15-29	2.07 (1.32 - 3.27)	0.002
ACR (mg/mmol)		
Optimal (<1.1)	1	(<0.001*)
High Normal (1.1-2.99)	1.03 (0.78 - 1.36)	0.821
High (3.0-29.99)	1.84 (1.46 - 2.31)	<0.001
Very High (30 - 200)	2.96 (2.13 - 4.10)	<0.001
Nephrotic (>200)	3.84 (2.11 - 6.99)	<0.001

Abbreviations: ACR, albumin creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IMD, index of multiple deprivation. * *P*-value for overall effect

Figure 3-2. Cox Regression Survival Plot indicating cumulative survival between ethnicities in Model 3 (comorbidities, eGFR and ACR).

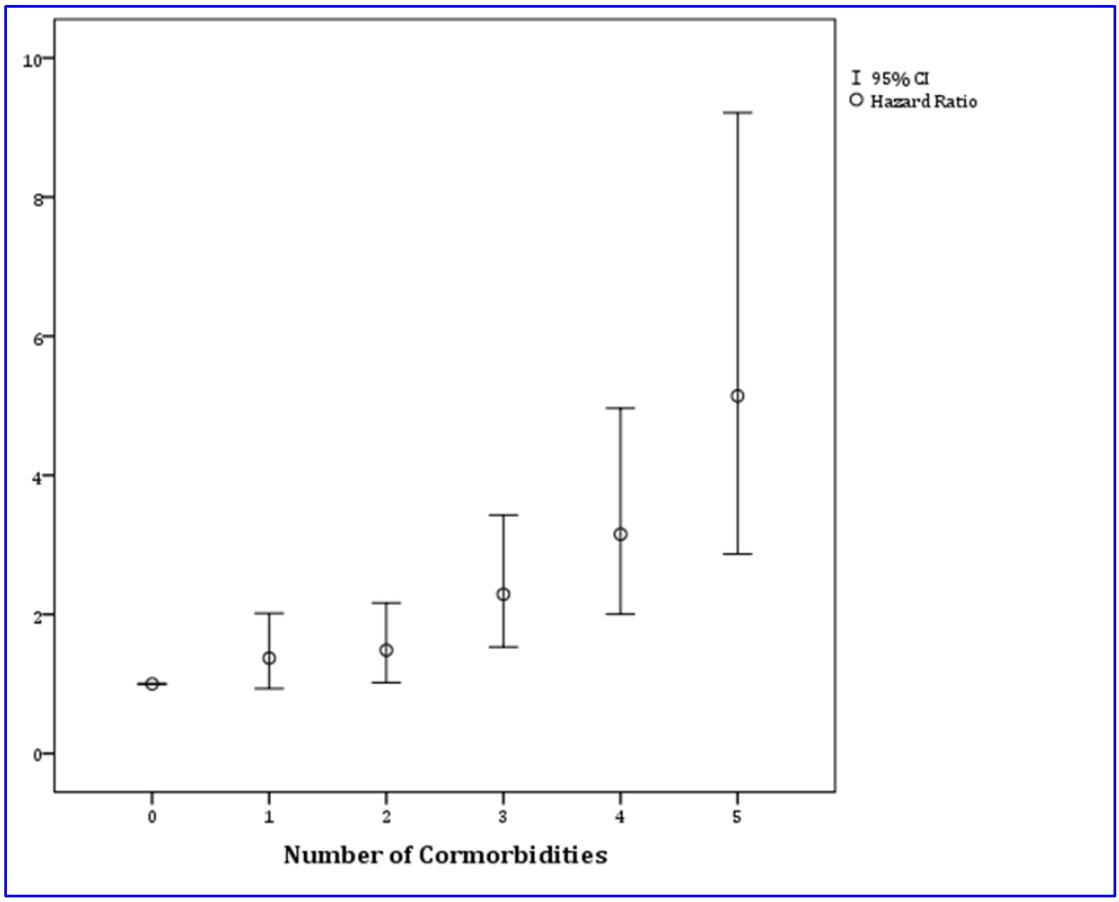
Table below survival plot demonstrates number of individuals who remained in follow up at each time-point.



	Subjects remaining in follow up Time from Recruitment (days)						
	0	**200**	**400**	**600**	**800**	**1000**	**end**
White	1908	1896	1864	1835	1798	1764	1760
South Asian	7022	6981	6938	6891	6840	6783	6775
Black	2275	2266	2251	2228	2208	2192	2191
All	11205	11143	11053	10954	10846	10739	10726

Abbreviations: ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate

Figure 3-3. Hazard ratio (HR) for death by number of comorbidities. Multivariate analysis: Model 3.



HR not illustrated for 6 comorbidities; HR 10.54 (95% CI 2.52 - 44.084)

Abbreviations: CI, confidence interval; HR, hazard ratio

3.6 Discussion

The results presented in this chapter utilised routinely available clinical and laboratory data, including kidney function assessed by eGFR and ACR, from a large primary care population. Detailed SES was included in the analyses and, importantly, three ethnic groups, South-Asian, black and white, were studied. Prior to this research, there has been uncertainty about the impact of ethnicity and SES on clinical outcomes in people with significant comorbidities including CKD. The comprehensive nature of the dataset coupled with the ability to utilise the Primary Care Mortality Database has allowed me to assess the relative impact of these factors on survival.

The previously identified associations between lower eGFR, higher ACR and increased mortality applied to this population. Furthermore, these associations remained significant when adjusted for ethnicity, age, gender, cardiovascular risk factors and SES. These results add weight to the risk stratification benefit of measuring ACR has in high risk groups.

A strong cumulative impact of comorbidity on CKD and ethnicity was shown. Whereas traditional comorbidity scores such as the Charlson Comorbidity Index (216) are difficult to calculate accurately in a large primary care setting, this study demonstrates that a simple cumulative score provides prognostic information. Individual comorbidities were present in varying frequencies within different ethnic groups, a finding consistent with that found in other ethnically diverse populations (238). Whilst individual comorbidities were associated with different mortality risks, it was the cumulative effect of comorbidities conveyed the greatest prognostic implication. A similar approach, but also including non-cardiovascular risk factors has

recently been described (239). Our study suggests that routinely collected clinical data concerning cumulative comorbidity may be utilised to quantify risk, however further work would be required to validate this as a tool for use in clinical care.

Socio-economic status was measured by the IMD 2007 score; a cumulative deprivation index score incorporating seven areas of deprivation which has been validated as superior to other deprivation scores (187). One notable finding is that there was no demonstrable association between SES and mortality when corrected for all other factors including comorbidity and ethnicity. This is in contrast to the evidence presented in Section 1.5.2, which demonstrates that there is frequently an independent relationship between SES and mortality across disease states and ethnic groups within the UK (240-243). This relationship varies by population group studied (244) and there have been limited studies investigating health disparities in similar, inner-city populations. Whilst we studied a health care system that is free at the point of care, limiting possible health access issues, the majority of individuals were from the most deprived national quintile and this study may therefore underestimate the influence of the complete spectrum of SES on mortality. To attempt to correct for this, analyses were re-run, dividing the cohort into equal quintiles. All analyses continued to indicate the effect of ethnicity and the importance of cardiovascular comorbidity and renal function. The univariable analysis and the most comprehensive multivariable analysis (data not shown) did not show any differences between most and least deprived quintiles.

One of the seven areas included in the IMD is health deprivation, raising the possibility of an inbuilt relationship between deprivation and health even before

analyses are undertaken. The possible implication of this was investigated by Adams and White (245) who analysed data having removed the health domain from IMD 2004 and found that its removal had little, practical, effect. This suggests the presence of the health domain is unlikely to influence the results.

Risk of death was lower for people of South Asian and black ethnicity compared to people of white ethnicity, and this remained in all analyses (univariable and multivariable) performed. Previous studies comparing the outcomes of different ethnic groups have been limited in their generalisability. They have either looked at disease specific mortality (11, 96, 98, 99) or have been based in populations that do not have access to free comprehensive healthcare. The finding that differences in mortality risk between ethnic groups is independent of age, gender, SES, kidney function and comorbidities require further work. Variables, such as health promotion targeted at specific groups, differences in medication usage or factors related to genetic diversity may offer potential explanations for this variation (246, 247).

3.6.1 Potential Utility of Alternative Statistical Techniques

Tables 3-1 and 3-2 demonstrate the baseline characteristics of the cohort split by ethnicity. As highlighted earlier in this chapter, differences were demonstrated between ethnicities; for example, median age was youngest in the South Asian cohort, and there were differences in SES and frequency of individual comorbidities. These variables were incorporated into the multivariable Cox regression analyses and, in recognition that certain variables (e.g. age) may not have a linear relationship with age, some were categorised. Whilst Cox modelling should correct for these

differences, one concern may be that comparing outcomes in disparate populations may lead to statistically unsafe results. In order to corroborate the results described in this chapter, an alternative statistical technique, such as propensity score matching (PSM), could be applied.

Propensity score matching is a statistical technique, most frequently using logistic regression, which provides information on the probability of receiving a treatment, or experiencing an outcome, conditional on the patient's observed characteristics (248, 249). The goal is to balance these characteristics (e.g. age, renal function, comorbidities) in the individual groups to assess whether any observed difference is due to the exposure (ethnicity in this case) or other factors. The benefits of PSM over other multiple regression techniques is not universally acknowledged, but is likely to be of most benefit when the outcome is rare and the number of observed characteristics is large (250). Propensity score matching would not account for any unmeasured characteristics.

Unfortunately, owing to an inability to access the data once a query about alternative statistical modelling was raised, I have been unable to perform PSM on these data, but acknowledge this would provide statistical weight to the results presented in this chapter.

3.6.2 Strengths and Weaknesses

A major strength in this study is the sample size, which included sixty-two practices of varying list size and number of practitioners. Ethnicity was documented in over

80% of the population studied; this is much higher than normally found in primary care records (251). Self-reporting is considered the 'gold standard' method of assessing ethnicity (184), taking into account an individual's culture and self-identity. Renal function was described in terms of eGFR and ACR.

These analyses have used data from primary care coding and recording systems, which formed part of the electronic downloads. These downloads indicate who is on a specific cardiovascular risk register and therefore may not classify people correctly. There is a relative paucity of published literature regarding the correct identification of people onto the correct cardiovascular risk registers (167, 235, 252, 253), although analysis of The Health Improvement Network database has demonstrated discrepancies between biochemically defined CKD and appearances on practice registers (1). Other surrogate measures of accuracy of the data include previous studies looking at gaming for QOF points (falsely classifying people with conditions they do not have thereby increasing revenue) or exception reporting (excluding individuals who have not had the appropriate monitoring completed) suggest that both these are rare (235, 254, 255).

When comparing the breakdown of the population studied in these analyses to the source population, it is important to highlight two key differences. Firstly, there is a relative underrepresentation of individuals of white ethnicity, consistent with previous research (256). This is most marked in those who had their ACR measured; a higher number of males and individuals with diabetes or of South Asian ethnicity had an ACR measured. Comparing the whole cohort to those who had their ACR reported showed similar trends for mortality in respect of age, eGFR, smoking status and SES,

suggests a generalisability of results. Secondly one criterion for inclusion was the recording of renal function within the previous twelve months. This is likely to have resulted in an overrepresentation of comorbidity as people with CV conditions would be more likely to have their renal function checked. A further consideration is that the accuracy and applicability of creatinine based eGFR equations, such as the formula used in this analysis, in non-white ethnic groups is a subject of ongoing research (257-259). Cystatin C based equations may be more accurate (260), but are not routinely measured in clinical practice.

It was not possible to assess the number of people who progressed to ESRD in the cohort during the study period, as the anonymous nature of the data did not allow linkage to secondary care renal data.

3.7 Conclusion to Chapter 3

These analyses show the determinants of mortality were multifactorial in a high risk population and that ethnicity should be considered as a non-traditional risk factor for mortality; the HR for death was lower for South Asian and black individuals compared to white individuals which was, in part, independent of age, gender, SES, renal function and comorbidities. Furthermore, a simple cumulative comorbidity system may have prognostic utility. Renal function (eGFR and ACR) provided additional information and gender, age and smoking status remained significant risk factors for mortality.

Further work will now be performed investigating the impact of ethnicity within a secondary care nephrology setting.

4 RESULTS 2. The impact of ethnicity on progression to end-stage renal disease in pre-dialysis chronic kidney disease.

4.1 Preface

The previous chapter investigated the impact of ethnicity and chronic kidney disease (CKD) on mortality in a the diverse central Birmingham primary care population, and demonstrated that hazard ratios for death were lower for South Asian and black individuals compared to white individuals in univariable and multivariable analyses.

Whilst the analyses intentionally did not include individuals with an estimated glomerular filtration rate (eGFR) below 15ml/min/1.73m^2, and were unable to investigate progression to end stage renal disease (ESRD) directly, the findings may impact on the secondary care nephrology population as white individuals may have an increased competing risk (i.e. death) which means they will not reach ESRD or, potentially, the need for secondary care nephrology input.

This work, coupled with evidence of higher rates of progression to ESRD in non-white ethnicities, led to an evaluation of ethnicity as a potential risk factor associated with accelerated progression of CKD in the Renal Impairment in Secondary Care (RIISC) CKD cohort. Whilst this chapter investigates the impact of both South Asian and black ethnicities compared to white ethnicity, the predominant focus is on South Asian ethnicity as there is a little published literature on the factors that influence progression in this group. To include the impact of any difference in mortality between ethnicities, competing risk regression (Section 2.4.3.3) was performed.

4.2 Abstract

Background. People of South Asian ethnicity have a higher rate of progression to ESRD compared to white individuals. A United Kingdom (UK) based, CKD cohort was utilised to assess for modifiable risk factors for accelerated progression of CKD in people of South Asian ethnicity.

Methods. 727 (white 507, South Asian 150, black 70) individuals in the Renal Impairment in Secondary Care study were included. Primary end-points included progression to ESRD or death. Survival analyses, including Cox proportional hazards and competing risk regression were performed.

Results. Median eGFR was 25.7ml/min/1.73m^2 (IQR 19.5-33.5) and median urine albumin creatinine ratio (ACR) was 32.8mg/mmol (IQR 6.7-127.6). During the follow-up period, 151 (20.8%) individuals reached ESRD and 76 (10.5%) died.

There was no difference in eGFR between ethnic groups. However there were major differences in albuminuria as measured by ACR; this was highest in South Asian participants (median 84.1mg/mmol, interquartile range (IQR) 25.9-170.2), compared to black (53.2mg/mmol, IQR 9.9-137.3) and white ethnicities (20.0mg/mmol, IQR 4.5-101.4) (*P*=0.0001).

By Cox survival analysis, individuals of South Asian (Hazard Ratio (HR) 1.62, 95% Confidence Interval (CI) 1.11-2.35, *P*=0.012) and black ethnicities (HR 1.79, 95% CI 01.10-2.90, *P*=0.019) had an increased risk of ESRD compared to individuals of white

ethnicity. Multivariable analyses attenuated this difference and ACR was the major modifiable risk factor. Other variables independently associated with worse outcome were lower age, male gender, lower eGFR, higher diastolic blood pressure, lower serum albumin, lower haemoglobin and lower triglycerides.

Conclusions. Albuminuria is the principal modifiable risk factor for progression to ESRD in people of South Asian ethnicity. Treatments to reduce albuminuria should be optimised and research is needed into why albuminuria is typically higher in this ethnic group.

4.3 Introduction

People of South Asian ethnicity are a large ethnic group in the UK. Despite a similar or lower overall prevalence of CKD compared with individuals of white ethnicity, there is both a higher rate of progression to ESRD (2, 6, 39) and a lower median age of starting renal replacement therapy (RRT) compared to people of white ethnicity (57.0 years for non-White patients compared to 66.0 years for White patients) (103).

In patients with CKD there is intense interest on risk stratification through the identification of factors that are associated with adverse outcomes. Whilst there have been many studies looking at the impact of black ethnicity on ESRD, including identification of potential genetic explanations of any differences (261), there has been no analysis specific for those of South Asian ethnicity. Consequently little is known about how risk factors for progression to ESRD in this ethnic group differ from other ethnic groups.

If differences in ESRD by ethnicity exist, potential explanations include: differences in the intrinsic characteristics of specific populations; a variation in the rate of established risk factors (12); or a competing event which prevents an individual reaching ESRD. For example, previous studies, including the analyses described in Chapter 3, have identified a lower rate of death in non-white ethnic groups (11, 262) and, if there is a different mortality rate by ethnicity, this competing risk may influence the total numbers reaching ESRD.

In order to clarify the relationships between ethnicity and established risk factors on progression to ESRD in people of South Asian ethnicity this chapter utilises the socioeconomically and ethnically diverse RIISC prospective cohort study of pre-dialysis CKD in a country with universal health coverage.

The analyses presented explore demographic, clinical and laboratory determinants on progression to ESRD for people with South Asian ethnicity, compared to people of white and black ethnicity. They focus on routinely collected clinical and laboratory data and selected additional novel risk factors to assess the relative impact of these factors on the risk of progression to ESRD. In addition to traditional survival analyses, we performed competing risk analyses to mitigate any impact of different mortality rates on ESRD.

4.4 Methods

A comprehensive description of the RIISC study and its methodology is presented in Chapter 2 (Section 2.3) and have previously been published (73, 180).

4.4.1 Ethnicity

Ethnicity and country of birth were self-reported and documented at time of recruitment. For the analyses that follow, individuals are grouped into white, South Asian (individuals of Bangladeshi, Indian and Pakistani descent) or black (individuals from or who have ancestors from Africa or the Caribbean) ethnicity; ethnicities classified as *other* are not included in the analyses.

4.4.2 Specific Statistical Analyses Related to this Chapter

Analyses were performed using Stata 13.1 (Statacorp, College Station, Texas, USA).

Techniques for the analysis of descriptive statistics and survival analyses are described in Section 2.4. Whilst competing risk analyses is also described in the methods section, I have included a short summary below to aid interpretation of the data.

4.4.2.1 Competing Risk Analyses

Survival analyses including Cox proportional hazard analyses (Section 2.4.3.2) treat all censored events as 'uninformative'. A patient may be censored at the end of their follow up or when they reach another end-point (death); both situations are treated equally but are of different clinical (and statistical) importance. To adjust for this, a competing risk analyses according to the method described by Fine and Gray (Stata command stcrreg) was performed (223). Data are presented using subdistribution hazard ratios (SHR) with 95% confidence interval (CI) and *P*-values.

4.4.2.2 Univariable and Multivariable Analyses

Ethnicity was initially analysed in univariable analyses. Multivariable analyses were then performed to include *a priori* variables (known confounding factors of age, gender, comorbidity assessed by CCI, eGFR and ACR); Model 1. Further candidate variables included any baseline characteristics demonstrating differences by ethnicity ($P<0.1$); Model 2. A backwards selection model was used to produce the final multivariable model; Model 3.

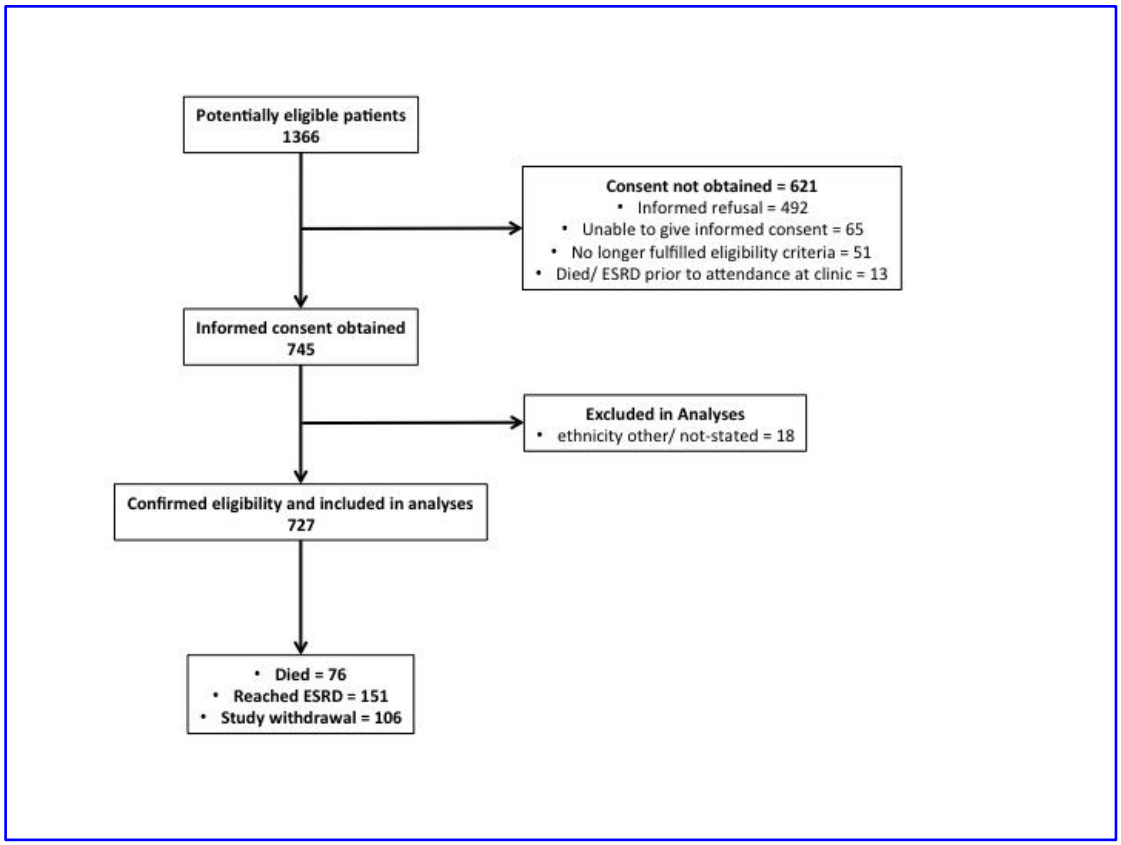
The *a priori* variables were chosen as international prediction equations identify age, gender, eGFR and ACR and being associated with the greatest risk of ESRD in (see Section 1.4 and (85-87)). Additionally Chapter 3 demonstrated the impact of comorbidity on mortality and I therefore included the Charlson Comorbidity Index as a validated cumulative score (216).

The censor date used in the analysis of these data is 24th February 2016.

4.5 Results

All participants recruited to end March 2014 (n=745) were evaluated for inclusion in this study. Eighteen patients were excluded from the analyses as they were not categorised within White (507, 68.1%), South Asian (150, 20.1%) or Black (70, 9.4%) ethnic groups. Figure 4-1 indicates the number of individuals at each stage of evaluation.

Figure 4-1. Flow diagram of study participants and outcomes.



Abbreviations: ESRD, end-stage renal disease.

Median age at recruitment was 64 years (IQR 50-77 years) and 61.1% were male. The most common primary renal diagnoses were ischaemic/hypertensive nephropathy in 25.9%, glomerulonephritis in 13.5%, diabetic kidney disease in 11.3%, polycystic kidney disease in 5.0% and not known/ other in 23.1% (Table 4-1).

Median eGFR was 25.7 ml/min/1.73m^2 (IQR 19.5-33.5) and median ACR was 32.8 mg/mmol (IQR 6.7-127.6). Table 4-2 illustrates the study population by KDIGO classification (42).

Table 4-1. Aetiology of renal disease, split by ethnicity.

	Complete Cohort n (%)	**White n (%)**	**Black n (%)**	**South Asian n (%)**	***P*-value**
Ischaemic/ hypertensive nephropathy	188 (25.9)	129 (25.4)	19 (27.1)	40 (26.7)	0.1
Primary glomeulonephritides	92 (12.7)	65 (25.8)	11 (15.7)	16 (10.7)	
Diabetic kidney disease	82 (11.3)	47 (9.3)	11 (15.7)	24 (16.0)	
Polycystic kidney disease	36 (5.0)	27 (5.3)	4 (5.7)	5 (3.3)	
Interstitial nephropathy	34 (4.7)	27 (5.3)	1 (1.4)	6 (4.0)	
Obstructive uropathy	24 (3.3)	20 (3.9)	1 (1.4)	3 (2.0)	
Reflux nephropathy	19 (2.6)	10 (2.0)	0 (0)	9 (6.0)	
Hereditary nephropathies	7 (1.0)	6 (1.2)	1 (1.4)	0 (0)	
Other cystic renal diseases	6 (0.8)	6 (1.2)	0 (0)	0 (0)	
Secondary glomerulonephritides	6 (0.8)	6 (1.2)	0 (0)	0 (0)	
Not known and other	168 (23.1)	114 (22.5)	18 (25.7)	36 (24.0)	
Not stated	65 (8.9)	50 (9.9)	4 (5.7)	11 (7.3)	
Total	**727 (100)**	**507 (100)**	**70 (100)**	**150 (100)**	

Table 4-2. Renal function of study population stratified by Kidney Disease Improving Global Outcomes (KDIGO) classification.

			ACR (mg/mmol)			
			<3	**3-30**	**>30**	**ACR not stated**
			A1	**A2**	**A3**	
			N (%)	**N (%)**	**N (%)**	**N (%)**
eGFR (ml/min/1.73m^2)	**≥60**	**G1/G2**	1 (0.1)	1 (0.1)	22 (3.0)	2 (0.3)
	45-59	**G3a**	8 (1.1)	13 (1.8)	23 (3.1)	0 (0)
	30-44	**G3b**	26 (3.6)	59 (8.1)	76 (10.5)	8 (1.1)
	15-29	**G4**	64 (8.8)	136 (18.7)	188 (25.9)	26 (3.6)
	<15	**G5**	5 (6.9)	19 (2.6)	47 (6.5)	3 (0.4)

Abbreviations: ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate

During the follow-up period 151 (20.8%) individuals reached ESRD and 76 (10.5%) died. One-hundred and six (14.6%) withdrew from follow up.

4.5.1 Baseline demographic, clinical and biochemical factors

Baseline demographic, clinical and laboratory data, split by ethnicity, are shown in Table 4-3. White participants were older, less socio-economically deprived and more likely to smoke tobacco and drink alcohol compared to participants of South Asian and black ethnicities. No difference was demonstrated between gender split or in perceived HRQL. No difference was seen in aetiology of renal disease (P=0.1).

Table 4.3. Baseline characteristics of cohort, split by ethnicity.

	Complete Cohort	White	South Asian	Black	*P*-value	Completeness of data (%)
N (%)	**727 (100)**	**507 (69.7)**	**150 (20.6)**	**70 (9.6)**		
Demographics						
Age (years) *	64 (50-77)	68 (55-78)	57 (44-69.3)	53.5 (46.5-75)	0.0001	100
Gender - Female n(%)	283 (38.9)	193 (38.1)	63 (42.0)	27 (38.5)	0.685	100
IMD Score *	31.9 (17.6-46.7)	26.0 (15.4-42.9)	38.2 (22.5-50.8)	51.1 (36.3-59.6)	0.0001	99.2
Most deprived quintile nationally n(%)	345 (47.8)	203 (40.1)	87 (58.8)	55 (79.7)	<0.001	99.4
BMI (kg/m^2) *	28.6 (24.9-33.0)	28.6 (24.7-33.3)	28 (24.9-32)	30.3 (26.2-35.3)	0.0435	98.5
Renal Function						
Creatinine (µmol/L) *	213 (167-272)	210 (167.5-263)	221 (163-289)	250.5 (173.3-332.5)	0.0305	98.5
eGFR (ml/min/1.73m^2) *	25.7 (19.5-33.5)	25.5 (10.2-33.1)	25.3 (18.3-34.8)	26.8 (19.5-38.7)	0.6011	98.5
ACR (mg/mmol) *	32.8 (6.7-127.6)	20 (4.5-101.4)	84.1 (25.9-170.2)	53.2 (9.9-137.3)	0.0001	94.9
Comorbidities						
Malignancy n(%)	110 (15.1)	96 (18.9)	6 (4.0)	8 (11.4)	<0.0001	100
Diabetes n(%)	265 (36.5)	173 (34.1)	62 (41.3)	30 (42.8)	0.137	100
COPD n(%)	78 (10.7)	65 (12.8)	9 (6.0)	4 (5.7)	0.022	100
Cerebrovascular n(%)	89 (12.2)	64 (25.4)	19 (12.7)	6 (8.6)	0.615	100
Cardiovascular n(%)	165 (22.7)	125 (24.5)	33 (22.0)	7 (10.0)	0.023	100
PVD n(%)	73 (10.0)	55 (10.8)	10 (6.7)	8 (11.4)	0.3	100
CCI *	3 (1-5)	3 (1-5)	3 (1-4)	3 (1-5)	0.0213	99.9

Table 4-3 continued…

	Complete Cohort	White	South Asian	Black	*P*-value	Completeness of data (%)
Health Related Quality of Life						
Eq5D score *	0.743 (0.656-0.883)	0.753 (0.656-0.883)	0.727 (0.636-0.858)	0.727 (0.630-1)	0.9039	98.3
Smoking Status						
Never n(%)	348 (48.2)	192 (37.9)	114 (76.0)	42(60.0)	<0.0001	99.3
Current n(%)	91 (12.6)	72 (14.2)	12 (8.0)	7 (10.0)		
Previous n(%)	283 (39.2)	238 (46.9)	24 (16.0)	21 (30.0)		
Alcohol (weekly)						
None n(%)	418 (57.5)	246 (48.5)	129 (86.0)	43 (61.4)	<0.0001	100
1-10 units n(%)	216 (29.7)	179 (35.3)	16 (10.7)	21 (30.0)		
11-20 units n(%)	63 (8.7)	55 (10.8)	5 (3.3)	3 (4.3)		
more than 20 units n(%)	30 (4.1)	27 (5.3)	0 (0)	3 (4.3)		
Blood Pressure						
Systolic BP (mmHg) **	130.4 (20.5)	130.0 (21.1)	131.6 (18.9)	131.4 (19.7)	0.6045	98.6
Diastolic BP (mmHg) **	78.8 (12.3)	75.2 (12.4)	78.8 (23.3)	79.8 (14.1)	0.0007	98.6
Mean Arterial Pressure (mmHg) **	106.1 (13.8)	104.9 (13.3)	108.2 (13.2)	110.5 (16.7)	0.0011	92.4
Medication						
ACEi/ARB n(%)	426 (58.7)	282 (55.7)	94 (62.7)	50 (71.4)	0.024	99.9
Allopurinol n(%)	131 (18.1)	102 (20.2)	22 (14.7)	7 (10.0)	0.056	99.9
Bicarbonate n(%)	107 (14.8)	70 (13.8)	25 (16.7)	12 (17.1)	0.578	99.9
Lipid Lowering medication n(%)	453 (62.4)	317 (62.6)	100 (66.7)	36 (51.4)	0.092	99.9
No. antihypertensives *	2 (1-3)	2 (1-3)	2 (1-3)	3 (2-4)	0.598	99.9

Table 4-3 continued…

	Complete Cohort	White	South Asian	Black	*P*-value	Completeness of data (%)
Biological markers						
Albumin (g/L) *	43 (40-46)	43 (41-46)	42 (38-45)	42.5 (39-44.75)	0.0006	97.9
Albumin - under 30 g/L n(%)	7 (1.0)	5 (1.0)	2 (1.4)	0 (0)	0.64	97.9
Bicarbonate (mmol/L) **	24.0 (3.7)	24.1 (3.6)	23.0 (3.8)	24.8 (4.0)	0.0006	97.2
Bicarbonate - under 20 mmol/L n(%)	104 (14.7)	67 (13.5)	30 (20.4)	7 (10.8)	0.076	97.2
Calcium (mmol/L) **	2.3 (0.1)	2.3 (0.1)	2.3 (0.2)	2.3 (0.2)	0.1653	97.5
Uncorrected calcium (mmol/L) **	2.3 (0.2)	2.3 (0.1)	2.3 (0.1)	2.3 (0.2)	0.4059	97.5
Cholesterol (mmol/L) *	4.5 (3.8-5.5)	4.5 (3.8-5.4)	4.7 (3.9-5.7)	4.4 (4-5.4)	0.4584	97.4
CRP (mg/L) *	3.0 (1.4-7.3)	3.5 (1.5-7.8)	2.7 (1.4-5.6)	2.2 (0.8-7.2)	0.0277	93.8
Ferritin (ng/mL) *	118 (58-216.5)	116 (58-208)	114 (55-199)	146 (67-247)	0.1847	94.8
Glucose (mmol/L) *	5.3 (4.7-6.8)	5.2 (4.7-6.5)	5.3 (4.6-8.2)	5.3 (4.7-7.1)	0.4961	95.9
HbA1c (IFCC; mmol/mol) *	43 (39-53)	43 (39-52)	44 (40-58)	42.5 (38.25-50)	0.1075	94.8
Haemoglobin (g/L) **	124.4 (17.2)	125.8 (16.8)	123.1 (17.1)	117.3 (17.8)	0.0003	96.1
Haemoglobin - under 100 g/L n (%)	47 (6.7)	32 (6.6)	7 (4.9)	8 (11.4)	0.197	96.1
HDL (mmol/L) *	1.2 (1.0-1.5)	1.23 (0.98-1.5)	1.12 (0.94-1.42)	1.29 (0.94-1.7)	0.0289	97.9
Phosphate (mmol/L) **	1.1 (0.2)	1.1 (0.2)	1.2 (0.3)	1.2 (0.3)	0.4897	97.1
Potassium (mmol/L) **	4.7 (0.6)	4.7 (0.6)	4.7 (0.6)	4.7 (0.5)	0.409	97.1
Triglyceride (mmol/L) *	1.6 (1.1-2.4)	1.6 (1.14-2.39)	1.8 (1.2-2.6)	1.2 (0.9-1.7)	0.0001	96.8
Uric Acid (µmol/L) *	471.2 (119.7)	469.4 (116.4)	462.6 (135.2)	503.3 (102.9)	0.0604	96.8

* median (interquartile range) ** mean (standard deviation)

Table 4-3 continued…

Abbreviations: ACEi, Angiotensin converting enzyme inhibitor; ACR, Albumin creatinine ratio; ARB, Angiotensin II Receptor Blocker; BMI, Body mass index; BP, Blood pressure; CCI, Charlson Comorbidity Index; COPD, Chronic obstructive airways disease; CRP, C-Reactive Protein; eGFR, estimated glomerular filtration rate; EQ-5D score, Health dimensions from EQ-5D health related quality of life questionnaire were converted into an EQ-$5D_{index}$ score ranging from −0.594 to 1.0 (where 1 is full health and lower values indicate worse HRQL) using a set of weighted preferences produced from the UK population (Section 2.3.2); HbA1c, Glycated haemoglobin; HDL, High density lipoprotein; PVD, Peripheral vascular disease.

;

Comorbidity was more common in participants of white ethnicity, with higher known rates of malignancy, chronic obstructive pulmonary disease and cardiovascular disease. This equated to a higher CCI in white participants. Whilst there was no difference between ethnic groups in the median number of anti-hypertensive agents used, angiotensin converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB) were more frequently prescribed in black (71.4%) and South Asian (62.7%) compared to white (55.7%) ethnic groups (P=0.024). No difference was observed in the use of lipid lowering medication or bicarbonate supplementation.

Diastolic Blood Pressure (DBP) was highest in those of non-white ethnicity (mean DBP black 79.8 mmHg, South Asian 78.8 mmHg, white 75.2 mmHg, *P*=0.0007). Similar differences were found in the mean arterial pressure (MAP).

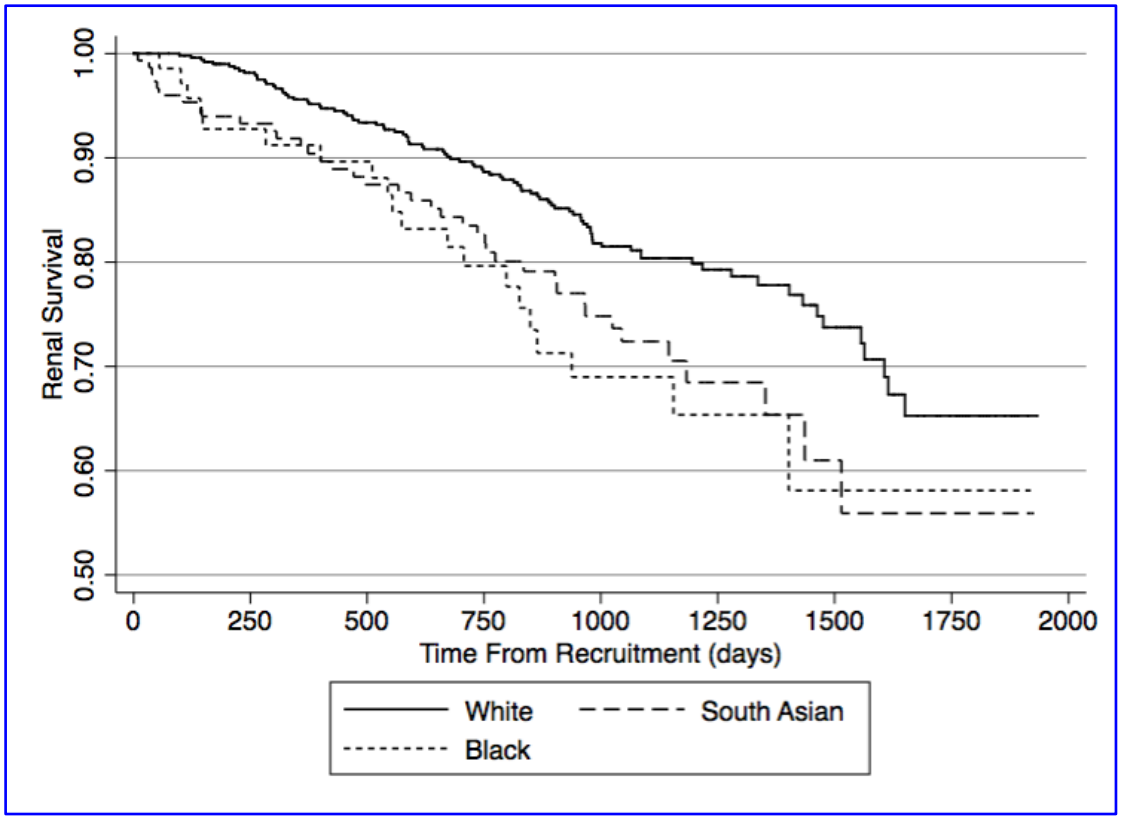
No differences were demonstrated in eGFR between ethnicities (*P*=0.6011). Proteinuria, as assessed by urine ACR, was highest in South Asian participants (median 84.1 mg/mmol), followed by participants of black ethnicity (53.2 mg/mmol) and lowest in white participants (20.0 mg/mmol) (*P*=0.0001).

Differences in biochemical parameters were noted in triglyceride, high density lipoprotein (HDL) and C reactive protein (CRP) concentrations. Albumin, bicarbonate and haemoglobin concentrations also varied, but no difference was identified when threshold levels were evaluated (albumin less than 30 g/L, bicarbonate less than 20 mmol/L, haemoglobin less than 100 g/L).

4.5.2 Progression to ESRD by ethnicity

The Kaplan-Meier survival plot illustrating progression to ESRD by ethnicity (log-rank test *P*=0.0073) is shown in Figure 4-2. By Cox survival analysis, individuals of South Asian ethnicity (HR 1.62, 95% CI 1.11-2.35, *P*=0.012) and black ethnicity (HR 1.79, 95% CI 01.10-2.90, *P*=0.019) had a statistically increased risk of ESRD compared to individuals of white ethnicity.

Figure 4-2. Kaplan-Meier survival plot illustrating progression to end-stage renal disease split by ethnicity. Log-rank test comparing ethnicities *P*=0.0073. Life table below survival plot indicates the number of individuals who remained in follow-up at each time point.



	Subjects remaining in follow up Time from Recruitment (days)							
	0	**250**	**500**	**750**	**1000**	**1250**	**1500**	**1750**
White	507	462	415	358	259	128	66	26
South Asian	150	132	117	97	65	27	12	5
Black	70	60	57	42	27	13	7	4
Total	**727**	**654**	**589**	**497**	**351**	**168**	**85**	**35**

4.5.3 Competing risk analysis

No significant difference was found for risk of death between ethnicities using Kaplan Meier (Log-rank test *P*=0.2745) or Cox proportional hazards regression (South Asian compared to white HR 0.56, 95% CI 0.28-1.14, *P*=0.113; black compared to white HR 0.96, 95% CI 0.44-2.10, *P*=0.915).

To adjust the survival analyses for risk of ESRD for the competing risk of death, competing risk analyses were performed. In these analyses, individuals of South Asian (SHR 1.65, 95% CI 1.13-2.40, *P*=0.009) and black ethnicity (SHR 1.77, 95% CI 1.08-2.91, *P*=0.023) had a higher risk of ESRD.

4.5.4 The relationship between established risk factors and progression to end-stage renal disease

On multivariable Cox regression analysis, after adjusting for age, gender, eGFR, ACR and CCI, the impact of ethnicity on ESRD risk was not significant for South Asian ethnicity (HR 0.90, 95% CI 0.58-1.38, *P*=0.625) and of borderline significance for black ethnicity (HR 1.67, 95% CI 1.00-2.79, *P*=0.05) (Table 4-4, Model 1). Lower age, lower eGFR and higher ACR were associated with ESRD risk.

Table 4-4. Multivariable survival analyses for progression to end-stage renal disease. Renal function (eGFR and ACR) analysed as continuous variables. Model 1 and 2.

	Model 1		Model 2	
	Hazard Ratio (95% CI)	***P*-value**	**Hazard Ratio (95% CI)**	***P*-value**
Ethnicity				
White	1		1	
South Asian	0.90 (0.58-1.38)	0.625	1.07 (0.61-1.89)	0.795
Black	1.67 (1.00-2.79)	0.05	1.27 (0.65-2.48)	0.478
Age (per 1 year increase)	0.97 (0.96-0.99)	<0.001	0.97 (0.95-0.99)	<0.001
Gender (male as reference)	1.33 (0.93-1.90)	0.12	2.24 (1.31-3.82)	0.003
eGFR (per 5ml/min/1.73m2 increase)	0.57 (0.51-0.64)	<0.001	0.52 (0.44-0.60)	<0.001
ACR (per 10mg/mmol increase)	1.03 (1.02-1.04)	<0.001	1.03 (1.02-1.04)	<0.001
Charlson Comorbidity Index	1.04 (0.95-1.13)	0.397	1.03 (1.02-1.16)	0.629
Socioeconomic Status				
IMD score			0.99 (0.98-1.00)	0.214
Smoking Status				
None			1	
Current			1.28 (0.65-2.51)	0.474
Previous			1.44 (0.88-2.36)	0.145
Alcohol (weekly)				
None			1	
Under 10 units			0.88 (0.53-1.44)	0.602
11-20 units			0.57 (0.25-1.28)	0.171
over 20 units			1.61 (0.61-4.25)	0.338
Blood Pressure				
AvgDBP (mmHg)			1.02 (1.00-1.04)	0.121
AvgMAP (mmHg)			1.01 (0.99-1.03)	0.419
Medication				
ACEi/ARB			1.43 (0.91-2.25)	0.119
Additional Biological markers				
Albumin (g/L)			0.93 (0.89-0.98)	0.007
Bicarbonate (mmol/L)			1.05 (0.99-1.12)	0.094
CRP (mg/L)			0.99 (0.97-1.00)	0.079
Haemoglobin (g/L)			0.97 (0.96-0.99)	<0.001
HDL (mmol/L)			0.96 (0.56-1.67)	0.898
Triglycerides (mmol/L)			0.83 (0.67-1.02)	0.078

Abbreviations: ACEi, Angiotensin converting enzyme inhibitor; ACR, Albumin creatinine ratio; ARB, Angiotensin II Receptor Blocker; CCI, Charlson comorbidity index; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; MAP; mean arterial pressure.

To quantify the significance of proteinuria and aid interpretation, the analyses were rerun with eGFR and ACR values categorised by KDIGO classification (Table 4-5, Model 1). This illustrated the significance of reduced eGFR (<30ml/min/1.73m^2 with 30-44ml/min/1.73m^2 as reference) and proteinuria (>30mg/mmol). In these analyses, South Asian ethnicity was not associated with progression to ESRD (HR 1.10, 95% CI 0.55-1.26, *P*=0.384). Black ethnicity remained significantly associated with progression (HR 1.78, 95% CI 1.09-2.93, *P*=0.022).

A further multivariable analysis was performed including *a priori* variables and factors which differed between ethnicities at baseline (Table 4-4, Model 2). A backwards stepwise technique was then performed to produce a final model with *a priori* variables and other factors which remained significant with *P*<0.05 (Table 4-6, Model 3 (Cox). In this model, both South Asian and black ethnicity were not independently significant variables. Significant factors were lower age, male gender, lower eGFR, higher ACR, higher diastolic blood pressure, lower albumin, lower haemoglobin, and lower triglycerides.

Findings from a competing risk analysis (with death as the competing risk), using the same variables, demonstrated findings consistent with the cox regression (Table 4-6, Model 3 (competing risk)).

Table 4-5. Multivariable survival analyses for progression to end-stage renal disease. Renal function (eGFR and ACR) analysed as categorical variables, classified by KDIGO CKD classification. Models 1 and 3.

	Model 1 (categorical)		Model 3 (categorical)	
	Hazard Ratio (95% CI)	***P*-value**	**Hazard Ratio (95% CI)**	***P*-value**
Ethnicity				
White	1		1	
South Asian	0.83 (0.55-1.26)	0.384	0.86 (0.56-1.32)	0.496
Black	1.78 (1.09-2.93)	0.022	1.19 (0.71-2.02)	0.511
Age (per 1 year increase)	0.98 (0.96-0.99)	<0.001	0.98 (0.96-0.99)	0.001
Gender (male as reference)	1.32 (0.92-1.87)	0.127	2.02 (1.36-2.99)	<0.001
eGFR - categorical				
G1/2 (≥60ml/min/1.73m^2)	no ESRD	1	no ESRD	1
G3a (45-59 ml/min/1.73m^2)	0.23 (0.03-1.73)	0.152	0.21 (0.03-1.64)	0.137
G3b (30-44 ml/min/1.73m^2)	1		1	
G4 (15-29 ml/min/1.73m^2)	3.50 (1.97-6.23)	<0.001	2.93 (1.63-5.24)	<0.001
G5 (<15ml/min1.73m^2)	17.53 (9.05-33.99)	<0.001	12.64 (6.32-25.26)	<0.001
ACR - categorical				
A1 (<3 mg/mmol)	1		1	
A2 (3-30 mg/mmol)	1.70 (0.78-3.72)	0.183	1.37 (0.61-3.08)	0.443
A3 (>30 mg/mmol)	4.67 (2.23-9.79)	<0.001	3.69 (1.68-8.13)	0.001
Charlson Comorbidity Index	1.07 (0.99-1.17)	0.094	1.02 (0.92-1.12)	0.754
AvgDBP (mmHg)			1.02 (1.00-1.03)	0.045
Albumin (g/L)			0.95 (0.91-0.99)	0.007
Haemoglobin (g/L)			0.97 (0.96-0.99)	<0.001
Triglycerides (mmol/L)			0.87 (0.74-1.03)	0.106

Abbreviations: ACR, albumin creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate.

Table 4-6. Multivariable survival analyses for progression to end-stage renal disease. Renal function (eGFR and ACR) analysed as continuous variables. Model 3.

	Model 3 (Cox) Hazard Ratio (95% CI)	*P*-value	Model 3 (competing risk) Subdistribution Hazard Ratio (95% CI)	*P*-value
Ethnicity				
White	1		1	
South Asian	0.97 (0.63-1.48)	0.89	0.92 (0.57-1.48)	0.722
Black	1.08 (0.63-1.86)	0.779	1.03 (0.58-1.85)	0.913
Age (per 1 year increase)	0.97 (0.96-0.99)	<0.001	0.97 (0.96-0.98)	<0.001
Gender (male as reference)	2.26 (1.51-3.37)	<0.001	2.47 (1.60-3.82)	<0.001
eGFR (per 5ml/min/1.73m^2 increase)	0.56 (0.49-0.63)	<0.001	0.56 (0.47-0.66)	<0.001
ACR (per 10mg/mmol increase)	1.04 (1.03-1.05)	<0.001	1.03 (1.02-1.04)	<0.001
Charlson Comorbidity Index	0.98 (0.89-1.08)	0.664	0.97 (0.88-1.06)	0.463
AvgDBP (mmHg)	1.02 (1.00-1.03)	0.032	1.02 (1.00-1.04)	0.022
Albumin (g/L)	0.96 (0.92-1.00)	0.031	0.95 (0.91-0.99)	0.02
Haemoglobin (g/L)	0.98 (0.97-0.99)	0.002	0.98 (0.97-0.99)	0.002
Triglycerides (mmol/L)	0.80 (0.66-0.95)	0.016	0.82 (0.68-1.00)	0.046

Abbreviations: ACR, albumin creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate.

Cox regression analysis incorporating variables from the final model (ethnicity, *a priori* variables, diastolic BP, albumin, haemoglobin, triglycerides) but with eGFR and ACR categorised was performed (Table 4-5, Model 3). This demonstrated the same variables remained significant (lower age, male gender, lower eGFR, higher ACR, higher diastolic blood pressure, lower albumin and lower haemoglobin) with the exception of triglycerides. Compared to an eGFR 30-44ml/min/1.73m^2 (G3b; the reference category), an eGFR 15-29ml/min/1.73m^2 (G4; HR 2.93, 95% CI 1.63-5.24, *P*<0.001) and eGFR <15ml/min/1.73m^2 (G5; HR 12.64, 95% CI 6.32-25.26, *P*<0.001) were associated with a higher HR for ESRD, as was an ACR >30 mg/mmol (A3; HR

3.69, 95% CI 1.68-8.13, *P*=0.001). Consistent with the analyses above, no significance was identified for differences by ethnicity.

4.6 Discussion

Whilst significant emphasis has been placed on risk stratification and identification of factors associated with ESRD, the impact of South Asian ethnicity has not been explored in detail, even though observational data shows an increased rate of ESRD in non-white ethnic groups (39). Previous studies investigating the impact of ethnicity on progression of CKD have not included large South Asian populations or have investigated the relationship in health care systems where universal coverage is not provided and access to healthcare may vary by SES.

Utilising the UK based, prospective observational RIISC study (73), I analysed progression to ESRD within the socioeconomically and ethnically diverse RIISC study, where over 30% of participants were of self-reported South Asian or black ethnicity. Whilst the median eGFR was similar between ethnicities, individuals of South Asian or black ethnicity were likely to be younger, more socio-economically deprived and have less comorbidity. Furthermore, people of South Asian or black ethnicity had higher albuminuria, assessed as urine ACR. No difference was seen in reported health-related quality of life between ethnicities.

There was an increased risk of ESRD for South Asian ethnicity compared to their white counterparts. This difference persisted in competing risk analyses (with death as the competing risk). Subsequent multivariable analyses incorporating established risk

factors (age, gender, eGFR, ACR, comorbidity) and candidate variables that differed by ethnicity (diastolic blood pressure, serum albumin, haemoglobin and triglycerides after backwards step-wise technique) attenuated the difference between ethnicities. Similar findings were identified for those of black ethnicity. Socio-economic status was not significant in the multivariable model, thereby not suggesting evidence of health inequality associated with socio-economic deprivation.

One of the most striking differences in the baseline characteristics between ethnicities was the variation in albuminuria. Given the prognostic significance of ACR in our analyses, possible explanations for this difference include:

1. The difference in ACR could be a disease specific effect. I did not identify any significant difference in overall proportion of renal aetiologies between ethnicities (*P*=0.1). However the proportion of people with diabetic kidney disease (DKD) was highest in South Asian (16.0%) and black (15.6%) compared to white ethnicities (9.3%). Whilst median ACR for South Asians with DKD was higher (155.3 mg/mmol, IQR 65.1-427) than white (64.7 mg/mmol, IQR 23-339.6) and black (58.1 mg/mmol, IQR 3.95-240.5) ethnicities, no statistical difference was found (*P*=0.187).

2. The difference in ACR may be explained by BP differences or a differential effect of ACEi/ARB. Whilst the median number of antihypertensives used were similar for all ethnicities, ACEi/ARBs were more frequently prescribed in the South Asian and black ethnic groups, and the baseline diastolic BP and MAP were both slightly, but significantly, higher in South Asian (and black) groups. Current

guidelines for hypertension suggest the selection of antihypertensive agent by ethnicity, in recognition that the pathophysiology of (non-renal) hypertension may be different. Previous research has often focused on those of black ethnicity compared to white. Data on South Asian ethnicity and response to antihypertensive treatments are limited and have not focused on patients with CKD. Studies have commented that '*South Asians appear to respond to antihypertensive drug treatment in a similar manner to whites'* but acknowledge that *'there is insufficient information on this point and South Asians are under-represented in studies'*. (263). However, a sub-analysis of the ASCOT trial suggested that South Asians may have a greater response to antihypertensives (perindopril and amlodipine) than whites (264). Using a stratified medicine approach (see Section 1.8 for definition) it may be possible to target treatments according to specific biological or risk characteristics (165). This concept will be explored further in Chapter 5.

3. There may be a genetic basis for the development of albuminuria within the South Asian population. Whilst research is again limited, proteinuria appears to be more common in non-white ethnicities within the paediatric and adolescent population, suggesting a potential genetic or environmental basis (265, 266). Additionally, non-white ethnic groups were more likely to have proteinuric DKD compared to non-proteinuria DKD (267). There may also be differences within broad categories of ethnicity; Jafar and colleagues studied determinants of proteinuria among South Asian subgroups in Pakistan, and concluded that unmeasured environmental or genetic factors account for difference in proteinuria (268). Studies have looked at variants of the gene encoding apolipoprotein L1 (APOL1),

with high risk groups having a higher risk of adverse renal outcome (261). However there was no evidence of an interaction between APOL1 and baseline proteinuria.

4.6.1 Strengths and Weaknesses

The major strength of this study is the use of a prospectively recruited, socio-economically and ethnically diverse cohort of patients with advanced and/or progressive pre-dialysis dependent CKD. Detailed demographic and clinical data were collected at initial recruitment and the participants were tracked longitudinally to record outcomes, including death and ESRD. The study focused on individuals at highest risk of ESRD and, in comparison to other studies within CKD populations in the UK (269, 270), a higher number of people reached ESRD than died.

Survival analyses were performed using both Cox proportional hazard analyses and competing risk analyses. The latter is important, as it enabled the competing risk of death to be taken into account when assessing progression to ESRD. Whilst I did not demonstrate a significant difference in the study population, the HR for death for South Asian and black ethnicities were lower. This would be consistent with the primary care data presented in Chapter 3.

A weakness, as with all observational studies, is that I have assessed association rather than causation. However, the detailed demographic and bioclinical characteristics have enabled multivariable analyses of both *a priori* and candidate variables. Whilst information regarding medication use was collected, the study team

did not record whether people had been given, or were intolerant to, medications such as ACEi/ARB.

It would have been useful if these analyses could be repeated in a replication cohort but I did not identify any national or international cohort with a significant proportion of South Asian participants.

4.6.2 Global Significance of These Findings

CKD is a major public health issue and contributes to the overall burden of non-communicable disease; this is likely to grow with the increasing prevalence of diseases including diabetes mellitus and obesity (80). Individuals of South Asian ethnicity account for approximately 25% of the world's population and international studies have identified a high prevalence of CKD within South Asian countries, including a high rate of proteinuria (4, 14, 15). Treatment of ESRD is unaffordable to many (81). If these results suggesting a higher rate of progression to ESRD, albeit explained by known risk factors, are transferable to the wider South Asian population, screening and intervention strategies for these high risk groups should be considered as part of public health strategies.

4.7 Conclusion to Chapter 4

In summary, these analyses demonstrate that those of South Asian ethnicity are at a higher risk of ESRD than their white counterparts. This difference can be explained by known risk factors for renal progression and albuminuria is the principal modifiable risk factor.

Traditional strategies to reduce albuminuria (blood pressure control, use of ACEi/ARB) need to be optimised and ongoing research into why albuminuria is typically higher in South Asian and black ethnicities is needed.

5 RESULTS 3. Serum tryptase concentration and progression to end-stage renal disease

5.1 Preface

In the previous chapter, I discussed that studies have proposed that different ethnic group may have a differential response to antihypertensive treatment (Section 4.6). This is an example of how stratified medicine (Section 1.8) may be beneficial, as it may be possible to target treatments to those most likely to respond.

I used a stratified medicine approach in the study reported in this chapter, focusing on biological determinants of response to angiotensin converting enzyme inhibitor (ACEi) and angiotensin II receptor blocker (ARB) therapy in patients with chronic kidney disease (CKD). I investigated the relationship of serum tryptase, a product of mast cell activation which may be involved in non-classical activation of the Renin-Angiotensin-Aldosterone System, to progression to end stage renal disease (ESRD) and whether this relationship is influenced by participants use of ACEi/ARB.

These analyses utilised the Renal Impairment in Secondary Care (RIISC) cohort and the detailed bioclinical information, including medication use, collected and the routine collection of additional biological samples (Section 2.3).

5.2 Abstract

Background. Mast cell activation can lead to non-classical activation of the Renin-Angiotensin-Aldosterone System (RAAS). However the relevance of this to human chronic kidney disease is unknown. I assessed the association between serum tryptase, a product of mast cell activation, and progression to ESRD or mortality in patients with advanced CKD. Patients were stratified by use of ACEi/ARB.

Methods. This was a prospective cohort study of 446 participants recruited into the RIISC study. Serum tryptase was measured at recruitment by sandwich immunoassay. Cox regression analysis was undertaken to determine variables associated with progression to ESRD or death.

Results. Serum tryptase concentration was independently associated with progression to ESRD but not with death. In patients treated with ACEi or ARB, there was a strong independent association between higher tryptase concentrations and progression to end-stage renal disease; when compared to the lowest tertile, tryptase concentrations in the middle and highest tertiles had Hazard Ratios (HR) of 5.78 (95% Confidence interval (CI) 1.19-28.03, *P*=0.029) and 6.19 (95% CI 1.49-25.69, *P*=0.012) respectively. The other independent risk factors for progression to end-stage renal disease were lower age, male gender, lower estimated glomerular filtration rate, and higher urinary albumin creatinine ratio.

Conclusion. Elevated serum tryptase concentration is an independent prognostic factor for progression to end-stage renal disease in patients with chronic kidney disease who are receiving treatment with an ACEi or ARB.

5.3 Introduction

Identifying patients with CKD at high risk of progression to ESRD is a clinical priority. Angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor II blockers (ARB) slow progression of CKD, but some patients treated with these drugs remain at high risk of progressing to ESRD (85, 88).

Mast cells may contribute to progressive renal disease through a number of pathways (139). Tryptase is an inflammatory and profibrotic protease that is released from mast cells (150, 151); it activates protease-activated receptor 2 (PAR2) which stimulates tubular epithelial cells to a pro-inflammatory phenotype (271). Serum tryptase concentrations have been shown to increase with worsening renal impairment (152), but the relationship between tryptase concentration and clinical outcomes in patients with CKD has not been explored to date. Chymase, the other major protease produced by mast cells, can convert angiotensin (AT)I to ATII and activate transforming growth factor beta (TGFβ) (153, 154).

I therefore analysed data from the RIISC study to explore the relationship between serum tryptase concentration, other independent prognostic factors for progression of CKD, and clinical outcomes. Given the potential involvement of mast cell proteases in the RAAS pathway, the cohort was stratified by ACEi/ARB use.

5.4 Methods

Information regarding the RIISC study can be found in Chapter 2 (Section 2.1). The first 503 individuals who were recruited into the RIISC study were evaluated (73). RIISC commenced recruitment in October 2010 and the censor date used in these analyses was March 24th 2014.

5.4.1 Assays Specific to this Chapter

Serum tryptase concentration was measured by the ImmunoCAP Tryptase sandwich immunoassay (Phadia AB, Uppsala, Sweden). Baseline tryptase levels in healthy individuals are reported by the manufacturer of the assay as 1-15 μg/L (214). Sample analysis was performed by the Clinical Immunology Service at the University of Birmingham (Birmingham, UK).

C-reactive protein (CRP) was measured using the Full Range C-Reactive Protein Kit on a SPA™ automated PLUS turbidimeter (The Binding Site Group Ltd, Birmingham, UK). The normal range for CRP is between 0.1 and 9 mg/L, with 90 percent below 3 mg/L (213).

Serum kappa (κ) and lambda (λ) free light chain (FLC) concentrations were measured by nephelometry on a Dade-Behring BNTMII Analyser (Siemens AG, Erlangen, Germany) using particle enhanced high-specificity homogenous immunoassays (Freelite™; The Binding Site Group Ltd, Birmingham, UK). The normal reference

ranges for serum FLC concentrations have been previously described as κ: 3.3–19.4 mg/L and λ: 5.7–26.3 mg/L, with the assay sensitivity being demonstrated as <1 mg/L. Kappa and Lambda FLC concentrations were combined to calculate the combined FLC (cFLC) concentration. For the purpose of analysis, data are presented as cFLC and Kappa-Lambda FLC ratio.

Patients with a potential monoclonal gammopathy, as defined by the presence of an abnormal κ-λ FLC ratio using the renal reference range (0.37-3.1), were excluded (n=12) (131).

CRP and serum FLC analyses were performed by technicians at The Binding Site Group Ltd (Birmingham, UK).

5.4.2 Specific Statistical Analyses Related to this Chapter

Analyses were performed using Stata 13.1 (Statacorp, College Station, Texas, USA).

Techniques for the analysis of descriptive statistics and survival analyses are described in Section 2.4. For the survival analyses in this manuscript, tryptase, CRP and FLC concentrations have been divided into three groups (tertiles).

Variables significant to $P<0.1$ by univariable analysis were subsequently included in a multivariable analyses together with *a priori* variables (age, gender, eGFR and ACR), chosen as the variables strongly associated with ESRD (Section 1.4).

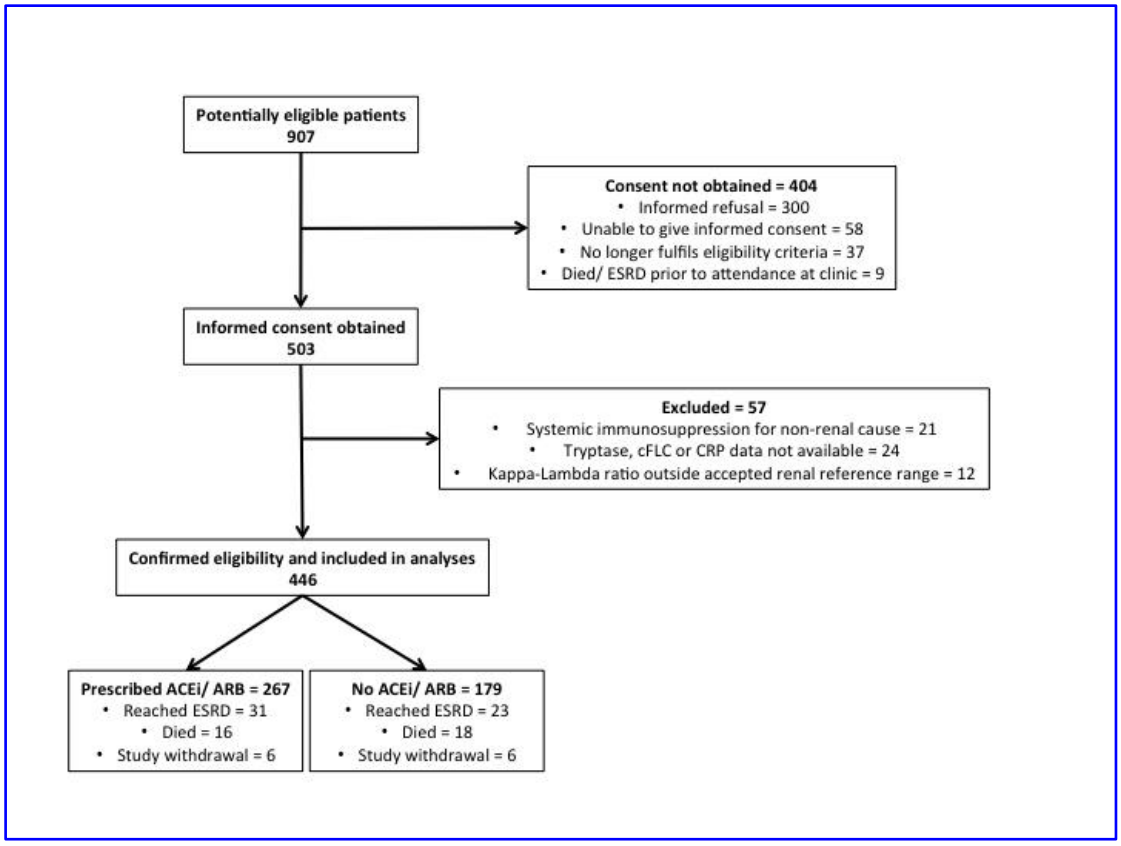
5.5 Results

Five hundred and three participants had tryptase concentrations measured. Figure 5-1 indicates the number of individuals at each stage of evaluation. Participants were excluded from further analyses if they were taking systemic immunosuppression for a non-renal cause (n=21), did not have tryptase, cFLC or CRP data available (n=24) or if their κ-λ FLC ratio fell outside of the accepted renal reference range (n=12). Therefore 446 individuals were included in the study. The aetiology of participants' kidney disease are stated in Table 5-1.

The baseline characteristics of the cohort are presented in Table 5-2. The median follow-up was 628 days (IQR 470-857.3 days) with a follow up period of 3-1243 days. At recruitment, the median age was 65 years (IQR 50.8-77 years); 60.8% were male and 31.9% were non-white (South Asian 20.1%, Black 9.4%, other 2.4%). Almost half (46.3%) of participants were in the most deprived SES quintile nationally as defined by IMD 2010 score. No difference in SES was seen when analysed by CKD stage with IMD Score (*P*=0.48), IMD rank (*P*=0.35) or percentage in most deprived quintile (*P*=0.75). Comorbidity was common and increased with advancing CKD stage (Table 5-3).

The median eGFR at recruitment was 25.8 ml/min/1.73m^2 (IQR 19.6-33.7). 56.6% of individuals had stage G4 CKD. Median ACR was 33 mg/mmol (IQR 6.6-130.3 mg/mmol).

Figure 5-1. Flow diagram of the participants in the study.



Abbreviations; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ESRD, end-stage renal disease.

Table 5-1. Aetiology of Chronic Kidney Disease.

Renal Diagnosis	N (%)
Ischaemic/ hypertensive nephropathy	118 (26.5)
Glomerulonephritis (primary)	70 (15.7)
Diabetic Nephropathy	49 (11.0)
Polycystic Kidney Disease	26 (5.8)
Interstitial nephropathies	21 (4.7)
Reflux nephropathy	16 (3.6)
Obstructive uropathy	11 (2.5)
Hereditary (not otherwise stated)	5 (1.1)
Other cystic renal disease	4 (0.9)
Glomerulonephritis (secondary)	2 (0.5)
Unknown	103 (23.1)
Not Stated	21 (4.7)

Table 5-2. Patient demographics and baseline descriptive statistics.

	All	No ACE/ARB	ACEi/ARB	*P*-value	Data completeness (%)
n (%)	**446 (100)**	**179 (40.1)**	**267 (59.9)**		
Renal Function					
Creatinine (µmol/L) *	208 (164-262)	214 (170-268.5)	202 (155-258)	0.0981	99.3
eGFR (ml/min/1.73m2) *	26.8 (20.7-34.9)	25.4 (18.8-31.8)	27.5 (21.6-37.0)	0.0086	99.3
ACR (mg/mmol) *	28.8 (6-109.7)	22.6 (5.3-95.1)	36 (6.4-119.7)	0.1131	95.5
Demographics					
Age (years) *	65 (50.8-77)	71 (57-80)	62 (48-74)	0.0001	100
Gender - Female (%)	39.5	41.3	38.2	0.506	100
Ethnicity					
White (%)	71.1	76.5	67.4	0.166	100
South Asian (%)	17.7	15.1	19.4		
Black (%)	8.3	5.6	10.1		
Other (%)	2.9	2.8	3		
Smoking Status					
Never (%)	44.6	38	49.1	0.069	100.0
Current (%)	14.1	35.4	12.7		
Previous (%)	41.3	45.8	38.2		

Table 5-2 continued…

	All	No ACE/ARB	ACEi/ARB	*P*-value	Data completeness (%)
Alcohol Consumption					
None (%)	54.3	55.3	53.6	0.484	100.0
1-10 unit (%)	33.0	33.5	32.6		
11-20 units (%)	8.1	8.4	7.9		
>20 units (%)	4.7	2.8	6		
SES					
Score *	31.2 (17.8-46.3)	33.2 (18.6-47)	28.9 (17.6-45.4)	0.3494	100
Rank *	7694.5 (2894.3-15913)	6829 (2775-15140)	8695 (3120-15934)	0.3145	
% in most deprived national quintile	46.9	49.7	44.9	0.322	
Comorbidity					
Cerebrovascular disease (%)	12.3	16.8	9.4	0.02	100.0
COPD (%)	11.0	11.2	10.9	0.918	
Diabetes (%)	38.6	37.4	39.3	0.687	
IHD (%)	24.0	29.1	20.6	0.04	
Malignancy (%)	15.2	23.5	15.2	<0.001	
PVD (%)	11.7	12.8	10.9	0.521	
CCI	3 (1-4)	3 (2-5)	2 (0-4)	0.0002	99.8
Anthropometrics					
BMI (kg/m^2) *	28.6 (24.9-33)	27.9 (23.8-31.8)	29.4 (26-33.9)	0.0023	99.6
Blood Pressure					
Systolic BP (mmHg) **	129.7 (21.2)	131.2 (21.7)	128.7 (20.9)	0.2168	98.7
Diastolic BP (mmHg) **	76.0 (12.7)	75.1 (13.3)	76.5 (12.3)	0.2535	98.7

Table 5-2 continued…

	All	No ACE/ARB	ACEi/ARB	*P*-value	Data completeness (%)
Medication					
Alpha Blockers (%)	26.2	25.7	26.2	0.833	100
Beta Blockers (%)	30.7	33	29.2	0.4	100
Calcium Antagonists (%)	44.4	44.1	60.1	0.928	100
Diuretics (%)	48	49.2	47.2	0.683	100
Statins (%)	63	65.4	63	0.398	100
Number of antihypertensive medications (n)	2 (1-3)	2 (1-2)	3 (2-3)	0.0001	100
Biomarkers - Traditional					
Albumin (g/L) *	44 (41-46)	44 (41-46)	44 (41-46)	0.8858	98.9
Bicarbonate (mmol/L) **	25.0 (3.2)	25.1 (3.2)	25.0 (3.2)	0.7315	97.5
Calcium (mmol/L) **	2.3 (0.2)	2.3 (0.2)	2.3 (0.1)	0.8015	98.4
Cholesterol (mmol/L) *	4.5 (3.8-5.4)	4.5 (3.7-5.3)	4.5 (3.8-5.5)	0.92	98.0
Corrected Calcium (mmol/L) **	2.2 (0.1)	2.2 (0.1)	2.2 (0.1)	0.9447	98.4
Ferritin (ng/mL) *	115 (61-218)	119 (64.5-236)	110 (57.8-210.3)	0.5488	96.6
Glucose (mmol/L) *	5.2 (4.7-6.6)	5.3 (4.7-7.0)	5.1 (4.6-6.5)	0.1196	96.6
Haemoglobin (g/L) *	124.8 (16.3)	122.9 (15.3)	126.1 (16.8)	0.0398	97.3
HbA1c (IFCC; mmol/mol) *	43 (39-55)	43 (39-56)	43.5 (39-55)	0.8756	94.8
HDL (mmol/L) *	1.2 (1.0-1.5)	1.3 (1.1-1.6)	1.2 (1.0-1.5)	0.0607	100
Phosphate (mmol/L) **	1.14 (0.2)	1.2 (0.2)	1.1 (0.2)	0.5883	98.0
Potassium (mmol/L) **	4.7 (0.6)	4.6 (0.6)	4.8 (0.6)	<0.0001	98.0
Triglycerides (mmol/L) *	1.5 (1.1-2.3)	1.5 (1.1-2.3)	1.6 (1.1-2.3)	0.4836	97.5
Uric Acid (micromol/L) **	467.2 (119.7)	472.6 (122.1)	463.2 (118.2)	0.4427	97.3

Table 5-2 continued…

	All	No ACE/ARB	ACEi/ARB	*P*-value	Data completeness (%)
Biomarkers - Non-traditional					
Tryptase (microgram/L) *	8.2 (5.5-11.3)	8.1 (5.3-11.8)	8.2 (5.7-11)	0.9647	100
cFLC (mg/L) *	73.9 (50.4-102.9)	80.2 (51.1-107.0)	69.9 (50.3-96.8)	0.1209	100
Kappa (mg/L) *	41.5 (27.1-61.1)	45.8 (26.9-65.7)	38.8 (27.2-57.0)	0.0571	100
Lambda (mg/L) *	31.1 (22.2-44.4)	32.5 (20.8-45.8)	30.0 (22.4-42.9)	0.5731	100
Kappa-Lambda Ratio **	1.4 (0.4)	1.4 (0.4)	1.3 (0.4)	0.0099	100
CRP (mg/L) *	2.9 (1.4-7.3)	3.4 (1.8-9.5)	2.6 (1.2-5.7)	0.0009	100

* median (interquartile range) ** mean (standard deviation)

Abbreviations: ACR, albumin creatinine ratio; BP, blood pressure; CCI, Charlson comorbidity index; cFLC, combined serum free light chains; COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; IHD, ischaemic heard disease; PVD, peripheral vascular disease; SES, socioeconomic status

Table 5-3. Comorbidity, split by KDIGO eGFR CKD classification

	All	Stage G1&G2	Stage G3a	Stage G3b	Stage G4	Stage G5	*P*-value
Cerebrovascular disease (%)	12.3	16.7	6.5	9.6	13.6	15.4	0.615
COPD (%)	11.0	8.3	16.1	12.3	10.8	5.1	0.615
Diabetes (%)	38.6	33.3	37.6	36.8	42.0	30.8	0.446
IHD (%)	24.0	16.7	19.3	24.6	25.2	20.5	0.878
Malignancy (%)	15.2	0.0	16.1	16.6	16.0	10.3	0.522
PVD (%)	11.7	8.3	9.5	13.2	11.6	12.8	0.871
CCI	3 (1-4)	0.5 (0-1.8)	1 (0-3)	2 (0-4)	3 (1-4)	4 (3-6)	0.0001

Abbreviations: CCI; Charlson comorbidity index; COPD, chronic obstructive pulmonary disease; IHD, ischaemic heart disease; PVD, peripheral vascular disease

Fifty nine point nine percent (n=267) of individuals were prescribed an ACEi/ARB. Comparisons between those receiving or not receiving ACEi/ARB are shown in Table 5-2. Patients prescribed ACEi/ARB were typically younger, had less comorbidity and had a higher BMI. Median eGFR, mean potassium and mean haemoglobin were higher in the ACEi/ARB prescribed group. Median serum tryptase concentrations were similar for those prescribed (median 8.2μg/L, IQR 5.7-11.0) and not prescribed (median 8.1μg/L, IQR 5.3-11.8) ACEi/ARB (*P*=0.9647).

Individuals were prescribed a median of two antihypertensive medications (IQR 1-3) with no significant difference between CKD stages in the number of antihypertensive agents prescribed (*P*=0.38).

5.5.1 Laboratory variables

The results of the laboratory variables are shown in Table 5-2. Serum concentrations of tryptase, CRP and cFLC increased with advancing CKD stage (Table 5-4). Kappa-Lambda FLC ratio increased with CKD stage (Table 5-4).

Table 5-4. Novel Biomarkers, split by KDIGO eGFR CKD classification

	All	Stage G1&G2	Stage G3a	Stage G3b	Stage G4	Stage G5	*P*-value
Tryptase (microgram/L) *	8.2 (5.5-11.3)	4.0 (2.6-5.7)	6.0 (4.2-9.4)	6.7 (5.0-9.1)	8.7 (6.2-11.8)	11.3 (6.6-14.4)	0.0001
cFLC (mg/L) *	73.9 (50.4-102.9)	35.3 (23.2-61.6)	42.4 (34.0-54.4)	56.5 (39.7-82.2)	84.7 (60.0-106.0)	138.5 (106.5-165.2)	0.0001
Kappa (mg/L) *	41.5 (27.1-61.1)	17.1 (11.3-31.4)	23.5 (16.0-29.5)	29.8 (20.7-46.9)	48.3 (33.5-65.2)	76.5 (56.5-104.9)	0.0001
Lambda (mg/L) *	31.1 (22.2-44.4)	18.7 (11.4-27.9)	19.1 (15.5-24.8)	24.4 (19.4-35.0)	34.0 (25.1-45.7)	54.5 (43.3-67.0)	0.0001
Kappa-Lambda Ratio **	1.4 (0.4)	1.1 (0.2)	1.2 (0.4)	1.3 (0.5)	1.4 (0.4)	1.4 (0.4)	0.0025
CRP (mg/L) *	2.9 (1.4-7.3)	1.7 (0.9-2.7)	1.6 (0.8-5.0)	3.0 (1.1-6.0)	3.1 (1.5-8.5)	3.5 (2.4-5.9)	0.0031

* median (interquartile range) ** mean (standard deviation)

Abbreviations: cFLC, combined serum free light chains; CRP, C reactive protein

5.5.2 Relationship between markers of inflammation

There were statistically significant but weak correlations between tryptase and cFLC (R=0.28, *P*<0.001) and between cFLC and CRP (R=0.24, *P*<0.001). There was no significant correlation between CRP and tryptase (R=0.08, *P*=0.11).

The association between tryptase as the dependent variable and cFLC (both variables log transformed due to distribution) was maintained in a linear regression model incorporating renal function. This model explained 9.4% of the variation of tryptase (R^2 0.094).

5.5.3 Outcomes

Fifty-four participants (12.1%) reached ESRD during the follow-up period, at a median time of 446 days (IQR 251.3-745.3 days). Thirty-four participants (7.6%) died at a median follow-up of 373 days (IQR 197.3-664.3 days).

Time-independent relationships of the biomarkers and outcomes of progression to ESRD and death are shown in Table 5-5. In univariable analyses tryptase and cFLC were associated with ESRD whilst cFLC and CRP were associated with death.

Time dependent survival analysis for tryptase was subsequently performed with the variables divided into tertiles. Lower and upper limits for boundaries for each tertile are provided in Table 5-6.

Table 5-5. Time independent analyses of inflammatory biomarkers (tryptase, C-reactive protein (CRP), combined free light chains (cFLC) and Kappa-Lambda FLC ratio) and outcomes of progression to End Stage Renal Disease (ESRD) and Mortality

End Stage Renal Disease: Categorical Data*				
	n (%)			
	1	**2**	**3**	***P*-value**
Tryptase	10 (6.6)	14 (9.3)	30 (20.4)	0.001
CRP	17 (11.4)	19 (12.8)	18 (12.2)	0.939
cFLC	2 (1.3)	16 (10.7)	36 (24.3)	<0.0001
Kappa Lambda Ratio	21 (14.1)	14 (9.4)	19 (12.8)	0.437

End Stage Renal Disease: Continuous Data**				
		95% CI		
	Beta	**lower**	**Upper**	***P*-value**
Tryptase §	0.26	0.092	0.429	0.003
CRP §	-0.032	-0.377	0.314	0.857
cFLC §	0.525	0.385	0.666	<0.001
Kappa Lambda Ratio	-0.016	-0.14	0.108	0.798

Mortality: Categorical Data*				
	n (%)			
	1	**2**	**3**	***P*-value**
Tryptase	11 (7.4)	14 (9.3)	9 (6.1)	0.575
CRP	8 (5.4)	12 (8.1)	14 (9.5)	0.402
cFLC	6 (4.2)	10 (6.7)	18 (12.1)	0.027
Kappa Lambda Ratio	12 (8.1)	5 (3.4)	17 (11.5)	0.03

Mortality: Continuous Data**				
		95% CI		
	Beta	**lower**	**Upper**	***P*-value**
Tryptase §	0.034	-0.175	0.243	0.751
CRP §	0.468	0.049	0.887	0.029
cFLC §	0.309	0.129	0.49	0.001
Kappa Lambda Ratio	0.099	-0.053	0.252	0.201

Abbreviations: cFLC, combined serum free light chains; CI, confidence interval; CRP, C reactive protein

* Comparison performed with Chi-squared test
** Comparison performed with linear regression
§ Transformed on a natural log scale due to distribution

Table 5-6. Category Boundaries for variables divided into tertiles

	Lower Tertile		Middle Tertile		Upper Tertile	
	Lower	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**
Whole cohort						
Tryptase (microgram/L)	0	6.35	6.37	10.2	10.3	49.5
cFLC (mg/L)	20.18	57.16	57.56	93.09	93.18	297.27
CRP (mg/L)	0	1.95	1.95	5.15	5.15	137.84
Prescribed ACEi/ARB						
Tryptase (microgram/L)	0.44	6.35	6.37	9.92	10.1	49.5
cFLC (mg/L)	20.18	56.67	56.94	88.56	89.17	245.57
CRP (mg/L)	0	1.53	1.54	4.07	4.09	115.571
No ACEi/ARB						
Tryptase (microgram/L)	0	6.29	6.4	10.6	10.7	30.6
cFLC (mg/L)	20.65	57.74	60.88	99.99	100.18	297.27
CRP (mg/L)	0	2.34	2.40	7.07	7.08	137.84

Abbreviations: ARB, angiotensin II receptor blocker; ACEi, angiotensin converting enzyme inhibitor; cFLC, combined serum free light chains; CI, confidence interval; CRP, C reactive protein

5.5.3.1 End Stage Renal Disease

No association was identified between aetiology of renal disease and progression to ESRD.

Table 5-7 shows the results of univariable and multivariable Cox proportional hazard analyses. In univariable analysis, younger age, lower eGFR, higher ACR, highest tryptase tertile and highest and middle cFLC tertiles were associated with an increased risk of ESRD. In the multivariate analyses, lower age, male gender, lower eGFR and higher ACR remained significant. Significance was lost for all tryptase and cFLC tertiles.

Individuals were then dichotomised according to ACEi/ARB prescription. For those participants not prescribed ACEi/ARB, lower age, male gender, lower eGFR, higher ACR and highest cFLC tertile was significant but tryptase was not. Combined FLC lost significant in the multivariable analysis (Table 5-7).

By univariable analysis of participants prescribed ACEi/ARB, the highest tryptase tertile (see Figure 5-2 for Kaplan Meier plot) and middle and highest cFLC tertiles were associated with an increased risk of progression to ESRD. In the multivariate model with *a priori* variables, the middle tryptase tertile (HR 5.78, 95% CI 1.19-28.03, *P*=0.03) and highest tryptase tertile (6.19, 95% CI 1.49-25.69, *P*=0.01) were significant for progression to ESRD, in addition to all four *a priori* variables (Table 5-7). End stage renal disease was reached in 3.4%, 10.5% and 21.4% of the lowest, middle and highest tryptase tertiles respectively. Combined FLC tertiles were not significantly associated with progression to ESRD.

Table 5-7. Cox proportional hazard analysis. Variables associated with progression to end-stage renal disease.

		Univariable HR (95% CI)	*P*-Value	Multivariable HR (95% CI)	*P*-Value
Whole cohort					
Tryptase	**Lowest Tertile**	ref		ref	
	Middle Tertile	1.35 (0.60-3.04)	0.468	1.40 (0.58-3.38)	0.45
	Highest Tertile	3.44 (1.68-7.05)	0.001	1.58 (0.74-3.38)	0.242
cFLC	**Lowest Tertile**	ref		ref	
	Middle Tertile	8.47 (1.95-36.85)	0.004	3.38 (0.75-15.21)	0.112
	Highest Tertile	24.35 (5.85-101.29)	<0.001	3.19 (0.68-14.96)	0.141
CRP	**Lowest Tertile**	ref		.	
	Middle Tertile	1.18 (0.61-2.28)	0.614	.	.
	Highest Tertile	1.34 (0.69-2.31)	0.392	.	.
Age (per 10 year increase)		0.83 (0.71-0.97)	0.021	0.70 (0.58-0.85)	<0.001
Gender (female as reference)		1.24 (0.71-2.18)	0.442	2.05 (1.09-3.87)	0.027
eGFR (per 5ml/min increase)		0.47 (0.38-0.58)	<0.001	0.47 (0.37-0.60)	<0.001
ACR (per 10mg/mmol rise)		1.04 (1.03-1.05)	<0.001	1.04 (1.03-1.05)	<0.001

Table 5-7 continued…

		Univariable HR (95% CI)	*P*-Value	Multivariable HR (95% CI)	*P*-Value
Prescribed ACEi/ARB					
Tryptase	**Lowest Tertile**	ref		ref	
	Middle Tertile	3.02 (0.82-11.17)	0.097	5.78 (1.19-28.03)	0.029
	Highest Tertile	7.79 (2.30-26.36)	0.001	6.19 (1.49-25.69)	0.012
cFLC	**Lowest Tertile**	ref		ref	
	Middle Tertile	8.48 (1.06-67.82)	0.044	2.27 (0.26-19.76)	0.457
	Highest Tertile	28.53 (3.84-211-97)	0.001	1.42 (0.15-13.21)	0.756
CRP	**Lowest Tertile**	ref		.	
	Middle Tertile	1.30 (0.57-2.97)	0.531	.	.
	Highest Tertile	0.88 (0.35-2.23)	0.788	.	.
Age (per 10 year increase)		0.83 (0.67-1.03)	0.087	0.67 (0.51-0.89)	0.005
Gender (female as reference)		1.13 (0.54-2.35)	0.75	4.04 (1.52-10.69)	0.005
eGFR (per 5ml/min)		0.44 (0.33-0.57)	<0.001	0.32 (0.21-0.49)	<0.001
ACR (per 10mg/mmol rise)		1.04 (1.02-1.05)	<0.001	1.04 (1.02-1.06)	<0.001

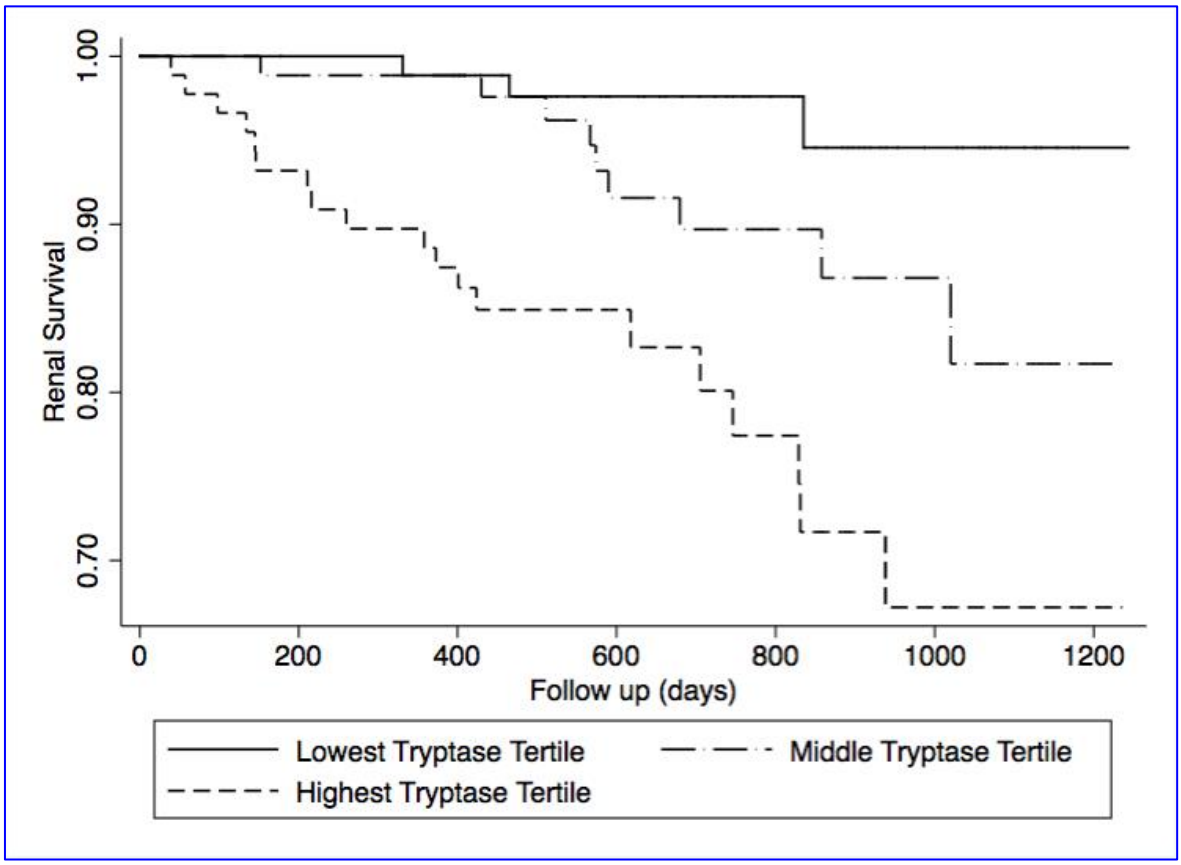
Table 5-7 continued…

		Univariable HR (95% CI)	*P*-Value	Multivariable HR (95% CI)	*P*-Value
No ACEi/ARB					
Tryptase	**Lowest Tertile**	ref		.	
	Middle Tertile	0.65 (0.21-2.05)	0.461	.	.
	Highest Tertile	1.48 (0.56-3.90)	0.426	.	.
cFLC	**Lowest Tertile**	ref		ref	
	Middle Tertile	9.42 (1.19-74.40)	0.033	3.05 (0.33-28.23)	0.325
	Highest Tertile	15.56 (2.02-119.89)	0.008	3.90 (0.44-34.83)	0.223
CRP	**Lowest Tertile**	ref		.	
	Middle Tertile	0 (0-.)	1	.	.
	Highest Tertile	1.19 (0.51-2.79)	0.688	.	.
Age (per 10 year increase)		0.73 (0.57-0.95)	0.017	0.64 (0.46-0.88)	0.005
Gender (female as reference)		1.38 (0.58-3.29)	0.468	1.43 (0.57-3.62)	0.446
eGFR (per 5ml/min)		0.49 (0.34-0.69)	<0.001	0.53 (0.36-0.77)	0.001
ACR (per 10mg/mmol rise)		1.05 (1.02-1.08)	0.001	1.06 (1.02-1.09)	0.001

Abbreviations: ARB, angiotensin II receptor blocker; ACEi, angiotensin converting enzyme inhibitor; ACR, albumin creatinine ratio; cFLC, combined serum free light chains; CI, confidence interval; CRP, C reactive protein; eGFR, estimated glomerular filtration rate

Figure 5-2. Kaplan-Meier plot demonstrating increased risk for progression to ESRD by Tryptase tertile for individuals prescribed ACEi/ARB.

The y-axis shows probability of event free survival. The x axis shows time in days from study recruitment. Statistical significance was determined using the log-rank test (*P*=0.002).



	Subjects remaining in follow up Time from Recruitment (days)												
	0	**100**	**200**	**300**	**400**	**500**	**600**	**700**	**800**	**900**	**1000**	**1100**	**1200**
Lowest Tertile	89	89	89	88	85	74	59	48	36	24	16	8	1
Middle Tertile	89	89	87	86	81	70	55	48	37	21	17	11	3
Upper Tertile	89	86	81	78	72	54	39	32	28	18	13	11	5

5.5.3.2 Death

No relationship was seen between tryptase concentration and death, both when analysed as a whole cohort or according to ACEi/ARB usage.

The highest cFLC tertile was associated with an increased HR for death in univariable Cox regression analysis for the whole cohort (HR 4.13 95% CI 1.63-10.47 *P*=0.003) and both the ACEi/ARB subset (HR 3.99 95% CI 1.08-14.82 *P*=0.039) and no ACEi/ARB subset (HR 4.23 95% CI 1.16-15.41 *P*=0.029).

The relationship between cFLC tertiles and mortality was lost in multivariable analysis with *a priori* variables for complete cohort; increasing age and elevated ACR were the only significant *a priori* variables associated with an increased HR for death. These data are presented in Table 5-8. Multivariable analyses were not performed with the group separated into ACEi/ARB usage due to limited event numbers (16 in ACEi/ARB group, 23 in non ACEi/ARB group).

Table 5-8. Cox proportional hazard analysis. Variables associated with mortality.

		Univariable HR (95% CI)	*P*-Value	Multivariable HR (95% CI)	*P*-Value
Whole cohort					
Tryptase	**Lowest Tertile**	ref		.	
	Middle Tertile	1.23 (0.56-2.71)	0.611	.	.
	Highest Tertile	0.89 (0.37-2.16)	0.799	.	.
cFLC	**Lowest Tertile**	ref		ref	
	Middle Tertile	1.71 (0.62-4.73)	0.299	0.96 (0.29-3.18)	0.941
	Highest Tertile	4.13 (1.63-10.47)	0.003	1.64 (0.50-5.42)	0.417
CRP	**Lowest Tertile**	ref		ref	
	Middle Tertile	1.57 (0.64-3.83)	0.327	1.14 (0.41-3.13)	0.806
	Highest Tertile	2.16 (0.90-5.17)	0.084	1.63 (0.64-4.20)	0.307
Age (per 10 year increase)		2.42 (1.70-3.46)	<0.001	2.64 (1.72-4.05)	<0.001
Gender (female as reference)		1.41 (0.69-2.89)	0.351	1.30 (0.54-3.14)	0.553
eGFR (per 5ml/min increase)		0.75 (0.61-0.91)	0.005	0.83 (0.64-1.08)	0.159
ACR (per 10mg/mmol rise)		1.02 (0.99-1.04)	0.199	1.04 (1.01-1.06)	0.003

Table 5-8 continued…

		Univariable HR (95% CI)	*P*-Value
Prescribed ACEi/ARB			
Tryptase	**Lowest Tertile**	ref	
	Middle Tertile	1.73 (0.50-5.91)	0.385
	Highest Tertile	1.42 (0.37-5.34)	0.608
cFLC	**Lowest Tertile**	ref	
	Middle Tertile	1.33 (0.30-5.98)	0.707
	Highest Tertile	3.99 (1.08-14.82)	0.039
CRP	**Lowest Tertile**	ref	
	Middle Tertile	1.78 (0.52-6.07)	0.36
	Highest Tertile	1.35 (0.36-5.05)	0.652
Age (per 10 year increase)		2.63 (1.56-4.45)	<0.001
Gender (female as reference)		0.80 (0.30-2.14)	0.653
eGFR (per 5ml/min)		0.71 (0.53-0.96)	0.027
ACR (per 10mg/mmol rise)		1.02 (1.00-1.05)	0.064

Table 5-8 continued…

		Univariable HR (95% CI)	*P*-Value
No ACEi/ARB			
Tryptase	**Lowest Tertile**	ref	
	Middle Tertile	0.80 (0.27-2.37)	0.681
	Highest Tertile	0.74 (0.24-2.35)	0.613
cFLC	**Lowest Tertile**	ref	
	Middle Tertile	1.79 (0.43-7.51)	0.425
	Highest Tertile	4.23 (1.16-15.41)	0.029
CRP	**Lowest Tertile**	ref	
	Middle Tertile	2.21 (0.52-9.40)	0.281
	Highest Tertile	4.95 (1.34-18.30)	0.016
Age (per 10 year increase)		2.08 (1.25-3.46)	0.005
Gender (female as reference)		2.79 (0.92-8.48)	0.071
eGFR (per 5ml/min)		0.81 (0.61-1.07)	0.135
ACR (per 10mg/mmol rise)		1.00 (0.95-1.05)	0.982

Abbreviations: Abbreviations: ARB, angiotensin II receptor blocker; ACEi, angiotensin converting enzyme inhibitor; ACR, albumin creatinine ratio; cFLC, combined serum free light chains; CI, confidence interval; CRP, C reactive protein; eGFR, estimated glomerular filtration rate

5.6 Discussion

The identification of CKD patients at high risk of progression to ESRD enables timely preparation for renal replacement therapy and accurate allocation of resources (88, 272). Furthermore, identifying the mechanisms associated with high risk of progression may lead to the development of better targeted therapies.

In these analyses of participants recruited in the RIISC observational study, higher baseline tryptase concentrations were associated with increased risk of progression to ESRD for individuals prescribed an ACEi/ARB. Furthermore, this association (HR for middle tertile 5.78, upper tertile 6.19) remained after correction for age, gender, eGFR and ACR. Whilst previous studies have described a rise in tryptase concentration by CKD stage, this is the first study to explore an association between higher serum concentrations of tryptase and progression to ESRD.

Tryptase is released from mast cells and the number of these cells increase at tissue sites of chronic inflammation (273). Few mast cells are found in the renal parenchyma of *normal* kidneys and the concentration of mast cells increases in diseases associated with chronic inflammation and have been associated with the severity of interstitial fibrosis in patients with progressive CKD due to a variety of aetiologies (140-149) and renal allograft dysfunction (274).

There are a number of putative mechanisms for an association between mast cells and progression of CKD; these include the release of proteases that promote inflammation, fibrosis and haemodynamic stress within the kidney. Tryptase activates

PAR2 expression, upregulating TGF-beta and protein expression within mesangial and tubular epithelial cells to induce synthesis of pro-fibrotic cytokines and extracellular matrix deposition resulting in interstitial fibrosis (149, 271).

Multivariable analysis demonstrated significance in the group prescribed ACEi/ARB despite both groups having similar median tryptase concentrations. ACEi/ARB exhibit class effects over and above their BP lowering properties for proteinuric CKD and cardiovascular disease (275), likely linked to RAAS blockade and the inhibition of angiotensin II formation. Chymase, the other major mast cell protease, acts as the main ACE independent pathway of angiotensin II production (276, 277). Mast cell activation as measured by mast cell protease levels, in patients receiving ACEi/ARB, may define patients at increased risk of progression to ESRD as a consequence of pathways that bypass the protective effects of ACEi/ARB.

The results presented in this chapter support the development of early phase studies to assess whether mast cell directed therapies have the potential to slow CKD progression in patients at high risk of ESRD. Animal models indicate that targeting specific mast cell pathways can attenuate renal fibrosis (150). In particular, chymase inhibition has been demonstrated to prevent myocardial fibrosis and preserve cardiac function after cardiac left ventricular repair surgery in rats (278). Additionally, Wei and colleagues, found that combined chymase and ACE inhibition, compared to ACE inhibition alone, improved left ventricular function, decreased adverse cardiac remodelling, and improved survival after myocardial infarction in hamsters (279). Inhibitors of tryptase and chymase have been developed, but have not been trialled to date in patients with CKD (280-282).

5.6.1 Strengths and Weaknesses

A major strength of this study is the use of the RIISC study for the recruitment of a socioeconomically diverse advanced CKD cohort. Median MDRD eGFR was 25.8 ml/min/1.73m^2 and median ACR was 33 mg/mmol at recruitment and, during the observation period, 12.1% people reached ESRD. Data, including SES and medication use, were collected prospectively and laboratory samples collected according to strict standard operating procedures (73). This enabled the investigation of serum tryptase and other non-traditional biological markers including CRP and polyclonal cFLCs. Notably, both these are routinely available laboratory tests that have been shown, in other cohorts, to be independently associated with progression to ESRD (134) and death (133, 283). Exploring the relationship between cFLC and tryptase was of particular interest as mast cells can be activated by immunoglobulin light chains (284, 285); recent studies have consistently show that in patients with CKD, high cFLC are an independent determinant of progression to ESRD and mortality (133, 134, 286). This work showed a similar HR for these end-points for cFLC, but recruited smaller numbers and was therefore not powered to confirm this relationship.

Whilst a substantial proportion of participants reached end-points of ESRD or death, the absolute numbers limited the range of dependent variables that could be studied. Recognising this we focused on the *a priori* variables of age, gender, eGFR and ACR; which are the cornerstone of risk prediction models for both progression to ESRD and mortality (44, 85, 86). The addition of further biological markers may have increased the possibility of overfitting.

As with all observational studies this work assessed association rather than causation. Tryptase was used as a marker of mast cell activation. As discussed earlier, there are biologically plausible pathways linking mast cell proteases to tissue fibrosis and CKD progression. However, I acknowledge there are other substances released by mast cells whose impact on progression of ESRD is unclear (287). This study, in conjunction with available animal data, provides a basis for further elucidation of mechanisms linking tryptase to CKD progression and consideration of studies investigating the therapeutic benefits of mast cell protease inhibition.

5.7 Conclusion to Chapter 5

In summary this chapter presents the first data to demonstrate serum tryptase concentration is an independent prognostic factor for progression to ESRD in patients with CKD receiving treatment with an ACEi or ARB.

6 RESULTS 4. The impact of Health Related Quality of Life on mortality and progression to end-stage renal disease in pre-dialysis chronic kidney disease.

6.1 Preface

The previous chapters have investigated the impact of ethnicity (Chapters 3 and 4) on outcomes and, using a stratified medicine approach, have identified elevated serum tryptase concentration an independent prognostic factor for progression to end-stage renal disease (ESRD) in patients with chronic kidney disease (CKD) who are receiving treatment with an angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blockers (ARB) (Chapter 5).

Whilst the end points of death and progression to ESRD are of key importance to patients and clinicians, discussion and management of health related quality of life (HRQL) are, arguably, as important: impaired HRQL profoundly influences patient well-being. As an article by Kimmel and colleagues states *'survival is not enough'* (288).

I therefore investigated the impact of CKD on HRQL and whether there was an association between HRQL on the clinical end-points. These analyses utilised the RIISC cohort and the HRQL information gathered using the EuroQol EQ-5D questionnaire (Section 2.4.2).

6.2 Abstract

Background. Chronic kidney disease is associated with reduced HRQL. However, the relationship between pre-dialysis CKD, HRQL and clinical outcomes, including mortality and progression to ESRD is unclear.

Methods. All 745 participants recruited into the Renal Impairment In Secondary Care study to end March 2014 were included. Demographic, clinical and laboratory data were collected at baseline including an assessment of HRQL using the Euroqol EQ-5D-3L. Health states were converted into an EQ-$5D_{index}$ score using a set of weighted preferences specific to the UK population. Multivariable Cox proportional hazards regression and competing risk analyses were undertaken to evaluate the association of HRQL with progression to ESRD or all-cause mortality. Regression analyses were then performed to identify variables associated with the significant HRQL components.

Results. Median eGFR was 25.8 ml/min/1.73 m^2 (IQR 19.6-33.7ml/min) and median ACR was 33 mg/mmol (IQR 6.6-130.3 mg/mmol). Five hundred and fifty five participants (75.7%) reported problems with one or more EQ-5D domains. When adjusted for age, gender, comorbidity, eGFR and ACR, both reported problems with self-care (hazard ratio (HR) 2.54, 95% confidence interval (CI) 1.22-5.29, *P*=0.013) and reduced EQ-$5D_{index}$ score (HR 0.28, 95% CI 0.10-0.81, *P*=0.019) were significantly associated with an increase in all-cause mortality. Similar findings were observed for competing risk analyses. Reduced HRQL was not a risk factor for progression to ESRD in multivariable analyses.

Conclusions. Impaired HRQL is common in the pre-dialysis CKD population. Reduced HRQL, as demonstrated by problems with self-care or a lower EQ-$5D_{index}$ score, is associated with a higher risk for death but not ESRD. Multiple factors influence these aspects of HRQL but renal function, as measured by eGFR and ACR, are not among them.

6.3 Introduction

There is increasing evidence of an association between pre-dialysis CKD and impaired HRQL as assessed by a variety of patient reported outcome measures (PROMs) (75-78).

Health related quality of life can be assessed using disease specific or generic instruments. The use of different PROMs within different populations to evaluate HRQL means that it is difficult to assess the relevance of the results reported. Furthermore, there are limited quantifiable data on the relationship between HRQL scores and clinical outcomes, including mortality and progression to ESRD. Previous studies have either been small, investigating these outcomes in a Taiwanese population (289), or have focused on individuals of black ethnicity with hypertensive CKD in the United States (290). A recently published study investigated the impact of HRQL using a kidney disease specific tool (KDQOL-36) and found that low HRQL was independently association with CV events and death, but not CKD progression (291).

A systematic review of PROMs in CKD supported the use of preference-based utility measures, favouring the EuroQol, EQ-5D due to ease of use for patients and for the ability to derive utility values for health economic evaluation (192).

To date, there have been few studies investigating the relationship between pre-dialysis CKD and HRQL as measured by EQ-5D (75-77), and no studies examining the relationship between EQ-5D scores and clinical outcomes. To address this, this chapter presents the results of the evaluation of HRQL within the RIISC study, where EQ-5D was collected at recruitment, to assess the relationship between HRQL and CKD stage, and the impact of HRQL on risk of death or progression to ESRD.

6.4 Methods

The methodology, including information about the RIISC cohort, the ethical permissions and the study design have been described in detail in the methods section of this thesis (Section 2.2).

6.4.1 Quality of life

Data were collected from participants using the EQ-5D-3L (abbreviated to EQ-5D throughout this chapter). Whilst information is provided in Section 2.3.2 of the methods, a succinct summary is provided below.

The EQ-5D is a validated, generic preference-based measure of health status. It comprises a 5-question multi-attribute questionnaire and a visual analogue self-rating scale (VAS) (193). Respondents are asked to rate severity of their current problems

(level 1=no problems, level 2=some/moderate problems, level 3=severe/extreme problems) for five dimensions of health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression).

Health states were converted into an EQ-$5D_{index}$ score ranging from −0.594 to 1.0 (where 1 is full health and lower values indicate worse HRQL) using a set of weighted preferences produced from the United Kingdom (UK) population (194). The EQ VAS asks respondents to rate their own health state relative to full health (score=100) or worst imaginable health state (score=0). A copy of the EQ-5D is provided in Appendix 5.

6.4.2 Specific Statistical Analyses Related to this Chapter

Analyses were performed using Stata 13.1 (Statacorp, College Station, Texas, USA).

Techniques for the analysis of descriptive statistics and survival analyses, including competing risks, are described in Section 2.4. The censor date for the analyses was March 24th 2014.

6.4.2.1 Univariable and Multivariable Analyses

Individual constituents of the EQ-5D were analysed (univariable analyses). Any components demonstrating $P<0.1$ were then included in multivariable analyses together with *a priori* variables (age, gender, comorbidity assessed by Charlson comorbidity index (CCI), eGFR and ACR).

A priori variables were selected for the reasons discussed in Section 4.4.2.2.

6.4.2.2 Regression analyses to assess the impact of demographic, clinical and laboratory variables on HRQL

Logistic regression was performed to analyse the relationship between problems in each of the five domains with clinical, demographic and laboratory variables using dichotomised data (patients with moderate and severe problems in a domain were combined and compared to those with no problems). Odds ratios (ORs) with 95% CI and two-tailed *P*-values are presented.

Linear regression was utilised for the calculated EQ-$5D_{index}$ score and the EQ VAS (coefficient with 95% CI and *P*-value). Residual plots were evaluated to determine appropriateness of linear regression models.

Data were entered into multivariable analyses if $P<0.1$ and a backwards selection model performed until remaining variables had a $P<0.05$. Goodness-of-fit is indicated by pseudo R^2 (logistic regression) or R^2 (linear regression) values.

6.5 Results

6.5.1 Descriptive Statistics

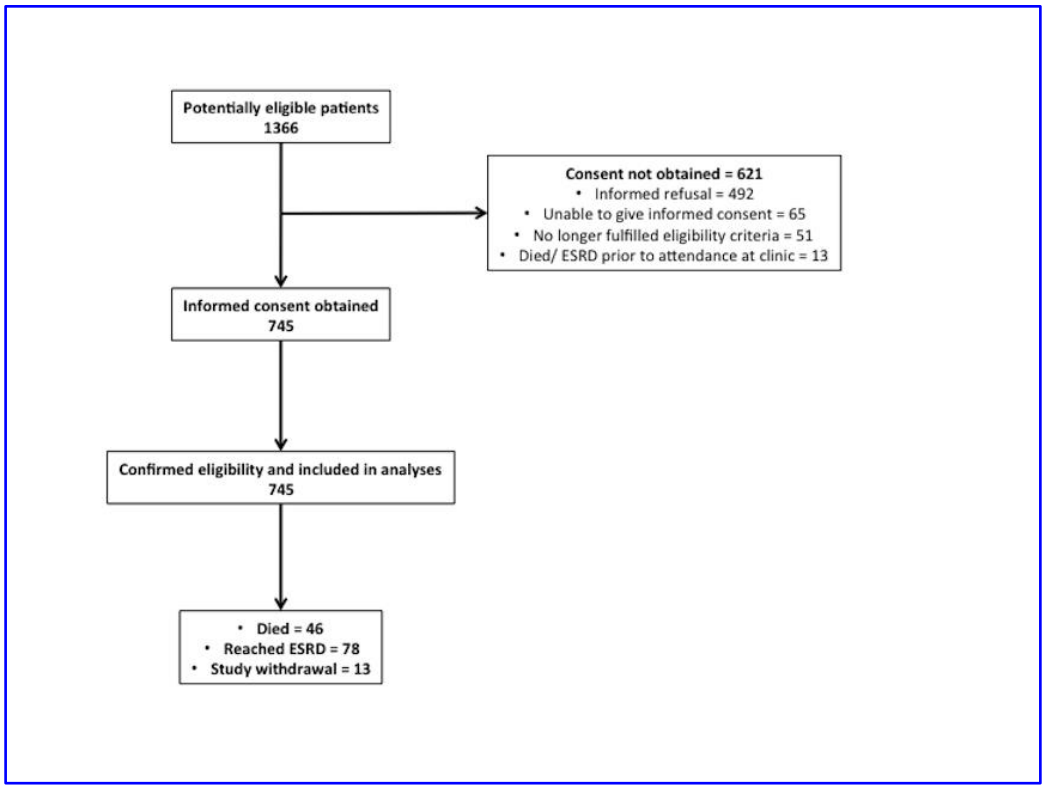
All participants recruited to end March 2014 (n=745) were included in the study. Figure 6-1 indicates the number of individuals at each stage of evaluation. Baseline demographic, clinical and laboratory data are shown in Table 6-1. Median age at

recruitment was 64 years (IQR 50-76 years) and 60.8% were male. The proportion of male participants decreased with lower CKD stage (*P*=0.045). Sixty eight point one percent were of white ethnicity, 20.1% South-Asian, 9.4% black ethnicity, and 2.4% from other ethnic groups. There was a borderline difference in ethnicity by CKD stage (*P*=0.052).

Forty-six point three percent of participants were in the most deprived quintile nationally (IMD 2010). No difference in SES was seen when analysed by CKD stage for IMD score (*P*=0.517) or comparing the percentage in the most deprived quintile (*P*=0.351). Comorbidity was common and increased with advancing CKD stage, both as assessed by individual comorbidities and the CCI (*P*=0.007).

Table 6-2 illustrates the study population using the KDIGO CKD classification (292). Median eGFR was 25.8 ml/min/1.73 m^2 (IQR 19.6 - 33.7ml/min/1.73 m^2) and Median ACR was 33 mg/mmol (IQR 6.6-130.3 mg/mmol).

Figure 6-1. Flow diagram of the participants in the study.



Abbreviation: ESRD, end stage renal disease

Table 6-1. Demographic, clinical and laboratory data.
Categorical variables are expressed as number (%), and continuous variables as mean (SD) or median (IQR).

eGFR (ml/min/1.73m^2)	**All**	**Stage G1/G2 ≥60**	**Stage G3a 45-59**	**Stage G3b 30-44**	**Stage G4 15-29**	**Stage G5 <15**	***P*-value**	**Data completeness (%)**
Number in each group (%)	745 (100)	29 (3.9)	45 (6.0)	173 (23.2)	423 (56.8)	75 (10.1)		
Age (years) *	64 (50-76)	41 (34.5-55.5)	55 (45-66.5)	61.5 (48.3-73.8)	69 (54-75.5)	64 (53.5-75.5)	<0.001	100
Gender - Female (%)	39.2	34.5	28.9	33.1	41.0	50.6	0.045	100
Ethnicity								
White (%)	68.1	44.8	71.1	68.0	70.9	59.7	0.052	100
South Asian (%)	20.1	27.6	15.6	20.3	18.0	31.2		
Black (%)	9.4	17.2	11.1	9.3	9.0	7.8		
Other (%)	2.4	10.3	2.2	2.3	2.1	1.3		
SES (IMD 2010)								
Score *	31.9 (46.7-35.1)	35.1 (15.2-44.4)	31.9 (16.1-49)	29 (17.1-44.4)	34.1 (18.6-47.3)	30 (17.4-46.0)	0.615	99.2
% in most deprived national quintile	46.3	51.7	51.1	43.9	49.5	42.1	0.351	99.5
Educational Attainment								
No formal qualifications (%)	46.5	17.2	18.2	40.7	54.9	40.5	<0.001	100
GCSE/ O'level (%)	21.8	41.4	27.3	25.6	18.4	21.6		
NVQ (%)	9.1	3.4	13.6	7.6	9.3	10.8		
A'Level (%)	7.5	13.8	20.5	8.7	5.0	8.1		
Undergraduate (%)	10.0	13.8	13.6	12.2	9.1	6.8		
Postgraduate (%)	5.1	10.3	6.8	5.2	3.3	12.2		

Table 6-1 continued…

eGFR (ml/min/1.73m^2)	All	Stage G1/G2 ≥60	Stage G3a 45-59	Stage G3b 30-44	Stage G4 15-29	Stage G5 <15	*P*-value	Data completeness (%)
Current Employment Status								
Not currently in employment (%)	19.1	17.2	20.0	18.5	17.7	28.0	**<0.001**	**100**
In Employment (%)	28.5	65.5	53.3	34.7	21.3	25.3		
Retired (%)	52.5	17.2	26.7	46.8	61.0	46.7		
Job Type (when last working)								
None (%)	0.2	0.0	0.0	0.6	0.0	0.0	**0.128**	**100**
Unskilled manual (%)	21.4	20.0	18.6	18.1	23.4	18.3		
Skilled manual (%)	38.2	24.0	30.2	39.4	40.3	30.5		
Clerical (%)	12.9	4.0	14.0	12.9	13.6	10.7		
Managerial (%)	10.0	8.0	11.6	10.3	9.5	10.7		
Professional (%)	17.4	44.0	25.6	18.7	13.1	21.4		
Smoking Status								
Never (%)	48.0	75.9	38.6	45.9	48.1	46.8	0.042	99.3
Current (%)	12.8	10.3	18.2	17.1	11.0	11.7		
Previous (%)	39.2	13.8	43.2	37.1	41.0	41.6		

Table 6-1 continued…

eGFR (ml/min/1.73m^2)	All	Stage G1/G2 ≥60	Stage G3a 45-59	Stage G3b 30-44	Stage G4 15-29	Stage G5 <15	*P*-value	Data completeness (%)
Alcohol Consumption								
None (%)	57.7	58.6	42.2	52.9	59.7	66.2	0.034	100
1-10 unit (%)	29.7	13.8	44.4	34.3	28.4	23.4		
11-20 units (%)	8.6	20.7	4.4	8.1	8.5	7.8		
>20 units (%)	4.0	6.9	8.9	4.7	3.3	2.6		
Anthropometrics								
BMI (kg/m^2) *	28.6 (24.9-33.21)	30 (25.3-33.6)	27.2 (24.6-31.7)	28.5 (24.8-31.9)	28.7 (24.9-34)	28.6 (24.7-33.2)	0.786	98.5
Individual Comorbidities								
Malignancy (%)	14.0	14.3	17.6	16.1	13.8	9.1	0.494	100
Diabetes (%)	34.0	33.3	32.4	30.6	35.4	33.8	0.043	
COPD (%)	10.0	9.5	14.7	12.8	9.5	5.2	0.383	
CVD (%)	11.3	19.0	8.8	8.9	11.3	15.6	0.429	
IHD (%)	21.2	14.3	17.6	21.1	21.4	23.4	0.219	
PVD (%)	9.5	9.5	8.8	10.6	8.6	13.0	0.788	
Comorbidity Score								
CCI *	3 (1-5)	1 (0-2)	1 (0-3)	2 (0-4)	3 (2-5)	5 (3-6)	<0.001	99.9

Table 6-1 continued…

eGFR (ml/min/1.73m^2)	All	Stage G1/G2 ≥60	Stage G3a 45-59	Stage G3b 30-44	Stage G4 15-29	Stage G5 <15	*P*-value	Data completeness (%)
Blood Pressure								
Systolic BP (mmHg) **	130.5 (20.5)	127.7 (20.2)	124 (18.3)	128.9 (20.6)	130.7 (20.2)	137.5 (21.7)	0.004	98.7
Diastolic BP (mmHg) **	76.5 (12.6)	81.9 (11.7)	77.7 (10.7)	77.6 (12.5)	75.5 (12.4)	76.4 (14.9)	0.057	98.7
Biological Markers								
Creatinine (µmol/L) *	212 (166.5-271.5)	91 (66-106.5)	135 (111-145)	167 (145.3)	235 (205-272)	378 (328.3-434)	<0.001	98.4
eGFR (ml/min/1.73m^2) *	25.8 (19.6-33.7)	75.6 (70.5-96.1)	49.3 (46.9-52.6)	35.8 (32.5-39.3)	22.8 (19.4-26.5)	12.7 (11.0-14.2)	<0.001	98.4
ACR (mg/mmol) *	33 (6.6-130.3)	92.4 (47.9-207.4)	56.3 (6.7-259.7)	24.4 (5.0-140.3)	28 (4.8-98.9)	79.6 (16.0-163.3)	<0.001	94.5
Haemoglobin (g/L) **	124.3 (17.2)	139.0 (17.5)	136.3 (16.0)	129.1 (17.4)	121.8 (15.8)	114.6 (14.9)	<0.001	95.6
Bicarbonate (mmol/L) **	24.0 (3.6)	26.0 (2.8)	25.2 (3.3)	24.9 (3.4)	23.8 (3.6)	21.6 (3.6)	<0.001	96.8
Albumin (g/L) *	43 (43-46)	41 (36.5-45)	44 (41-46.5)	44 (41-46)	43 (40-46)	42 (48-44)	0.002	98
CRP (mg/L) **	3.0 (1.4-7.2)	2.2 (1.1-4.6)	1.6 (0.8-3.7)	3.0 (1.3-5.9)	3.4 (1.6-7.9)	3.5 (2.0 - 10.3)	<0.001	93.8

* median (interquartile range) ** mean (standard deviation)

Abbreviations: ACR, Albumin Creatinine Ratio; BMI, Body Mass Index; BP, Blood Pressure; CCI, Charlson Comorbidity index; CRP, C-reactive protein; eGFR, estimated Glomerular Filtration Rate; SES, Socio-economic status

Table 6-2. Study population by Kidney Disease Improving Global Outcomes (KDIGO) classification.

			ACR categories (mg/mmol)			
			<3 **A1** **N (%)**	**3-30** **A2** **N (%)**	**>30** **A3** **N (%)**	**ACR not stated** **N (%)**
eGFR (ml/min/1.73m^2)	**≥60**	**G1/G2**	1 (0.1)	1 (0.1)	25 (3.4)	2 (0.3)
	45-59	**G3a**	8 (1.1)	13 (1.7)	24 (3.2)	0 (0)
	30-44	**G3b**	28 (3.8)	59 (7.9)	78 (10.5)	8 (1.1)
	15-29	**G4**	66 (8.9)	138 (18.5)	192 (24.8)	27 (3.6)
	<15	**G5**	5 (0.7)	19 (2.6)	47 (6.3)	4 (0.5)

Abbreviations: ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate

6.5.2 Self-Reported HRQL

Complete HRQL data were available for 733 participants (98.4%). Proportions of individuals reporting problems with each of the five domains are shown in Figure 6-2. One hundred and seventy eight participants (24.3%) reported no problems within any domain. Problems with one, two, three, four and five domains were reported by 136 (18.6%), 129 (17.6%), 153 (20.9%), 91 (12.4%) and 46 (6.3%) participants, respectively.

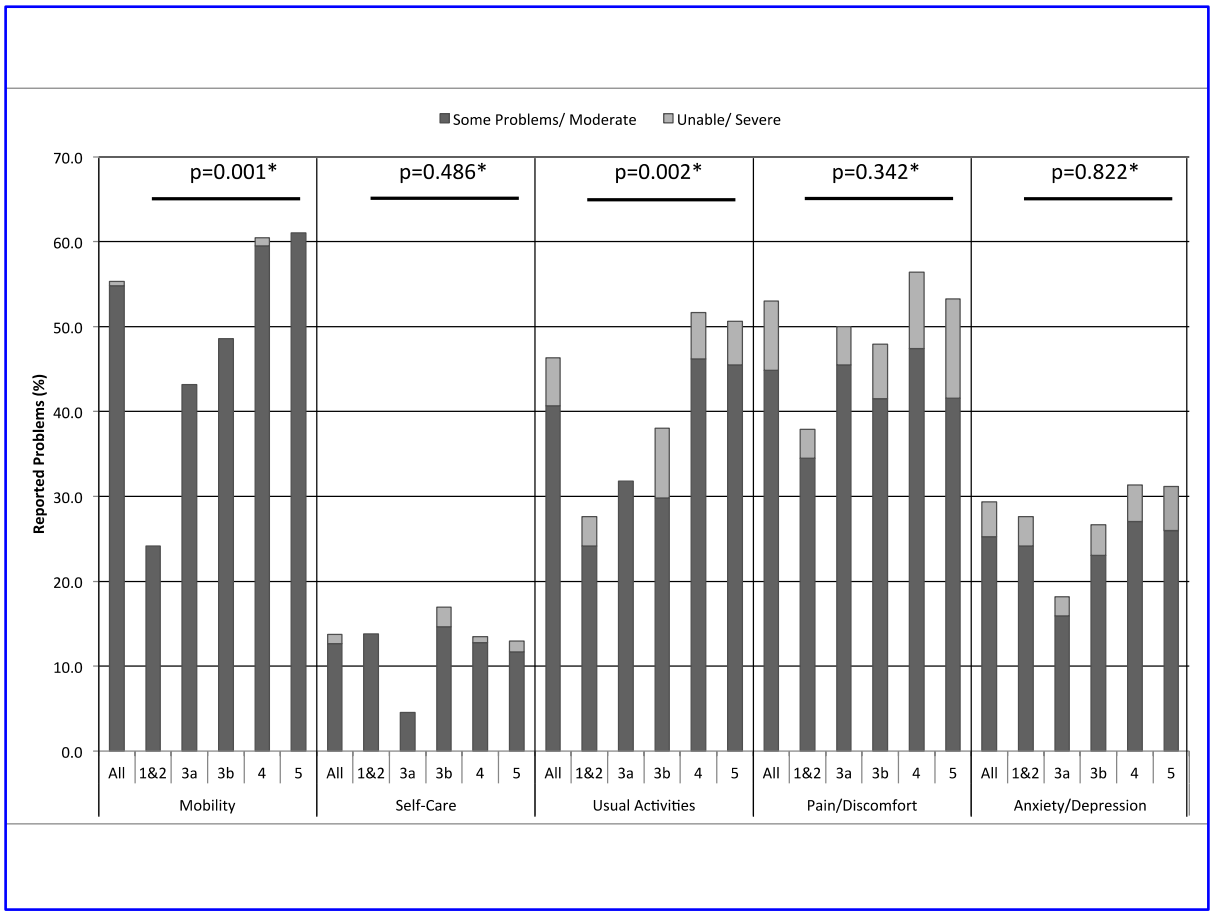
6.5.3 Associations between HRQL and CKD

As illustrated in Figure 6-2, statistically significant differences between CKD stages were seen in the mobility (P=0.001) and usual activity (P=0.002) domains, with more problems reported with a worse CKD stage. No significant difference was found between CKD stages and the other domains.

Only a small number of participants described problems in the unable/severe category, therefore data were dichotomised to combine the respondents who reported moderate problems with those in the severe or unable category.

Health related quality of life for the EQ VAS and calculated EQ-$5D_{index}$ score are shown in Table 6-3. The EQ-$5D_{index}$ score decreased (worsened) with more advanced CKD stage (P=0.017). No significant difference was seen between CKD stage and the EQ VAS.

Figure 6-2. Reported HRQL Problems by EQ-5D domain. Data presented as whole cohort (All) and categorised by chronic kidney disease stage (determined by eGFR).



* *P*-value for Chi-squared test comparing each EQ-5D domain by CKD stage.

Table 6-3. Calculated EQ-5D Index Score and Visual Analogue Scale by chronic kidney disease stage (determined by eGFR).

	EQ-5D Index Score	**Visual Analogue Scale**
All	0.74 (0.66-0.88)	65 (50-80)
Stage G1/G2	0.85 (0.70-1)	50 (75-82.5)
Stage G3a	0.80 (0.69-1)	70 (50-80)
Stage G3b	0.80 (0.68-1)	70 (50-80)
Stage G4	0.74 (0.62-0.85)	60 (50-80)
Stage G5	0.73 (0.62-1)	55 (50-80)
***P*-value**	0.0165	0.094

6.5.4 Association between HRQL and Clinical end-points

6.5.4.1 Death

By March 24th 2014, 46 (6.2%) participants had died. Univariable cox regression analysis demonstrated that reported problems with mobility, self-care (Figure 6-3), usual activities, lower EQ-$5D_{index}$ score, and lower EQ VAS, were all associated with an increased risk of death. Table 6-4 indicates univariable cox regression analyses for *a priori* variables and EQ-5D components.

In multivariable analysis, each significant EQ-5D variable was combined with age, gender, comorbidity assessed by CCI, eGFR and ACR. Self-care (HR 2.54, 95% CI 1.22-5.29, *P*=0.013, Figure 6-4, Table 6-5) and the EQ-$5D_{index}$ score (HR 0.28, 95% CI 0.10-0.81, *P*=0.019, Table 6-5) were independently associated with an increased risk of death. Fourteen out of 102 (13.7%) participants who reported problems with self-care died compared to 32/641 (5.0%) participants who reported no problems (chi-squared *P*=0.001).

To adjust the HR associated for death for the competing end-point of ESRD, a competing risk analysis was performed. Problems with self-care (sub-distribution hazard ratio (SHR) 2.61, 95% CI 1.26-5.60, *P*=0.01) and a lower EQ-$5D_{index}$ score (SHR 0.32, 95% CI 0.11-0.96, *P*=0.042) remained significant in the multivariable analysis with age, gender, comorbidity, eGFR and ACR (Table 6-5).

Figure 6-3. Cox Proportional Hazards Regression for reported problems with self-care and death. Univariable Analyses.

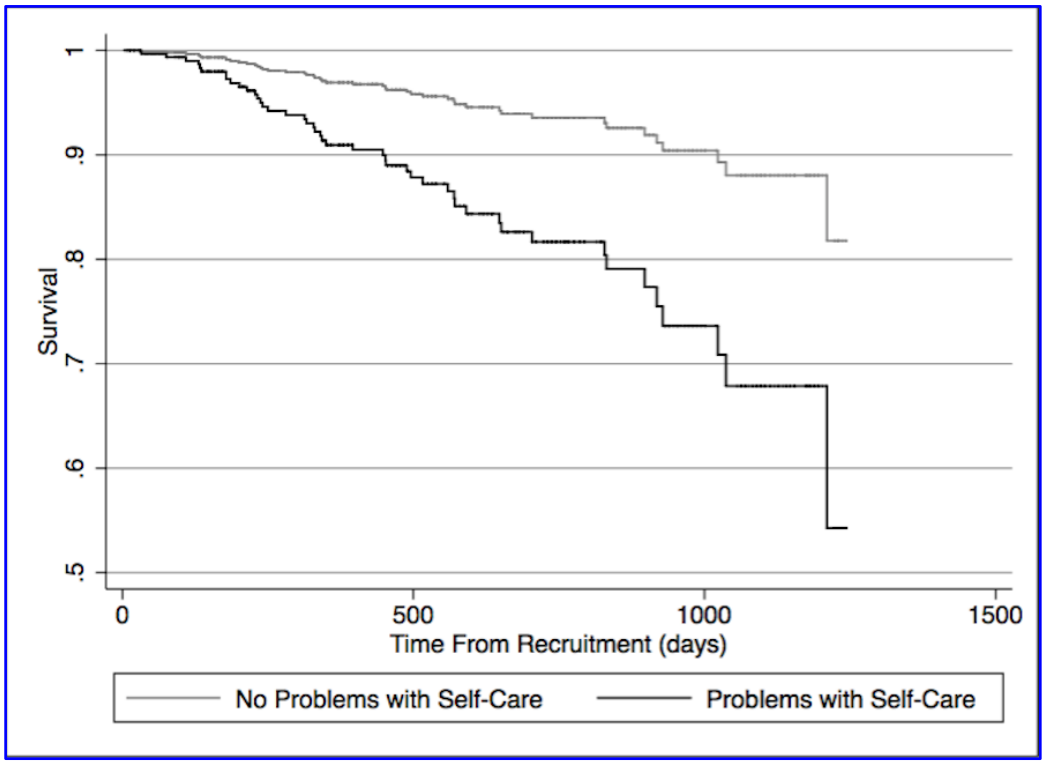


Table 6-4. Univariable Survival Analyses (Cox regression) for hazard ratio (HR) for death. A priori variables and all components of EQ-5D shown.

	HR (95% CI)	*P*-Value
A Priori Variables		
Age (per 10 year increase)	2.42 (1.79-3.28)	<0.001
Gender (female as reference)	1.38 (0.74-2.55)	0.311
Charlson Comorbidity Index	1.41 (1.25-1.58)	<0.001
eGFR (per 5ml/min increase)	0.81 (0.69-0.96)	0.015
ACR (per 10mg/mmol rise)	1.02 (1.00-1.03)	0.065
ED5D Components		
Mobility	3.72 (1.79-7.73)	<0.001
Self-Care	3.04 (1.62-5.71)	0.001
Usual Activities	3.02 (1.59-5.75)	0.001
Pain/Discomfort	1.11 (0.62-1.98)	0.727
Anxiety/ Depression	1.56 (0.86-2.84)	0.146
EQ-5D Index Score	0.20 (0.09-0.45)	<0.001
Visual Analogue Scale	0.98 (0.96-0.99)	<0.001

Abbreviations: ACR, albumin creatinine ratio; CI, confidence interval, eGFR, estimated glomerular filtration rate; HR, hazard ratio.

Figure 6-4. Cox Proportional Hazards Regression for reported problems with self-care and death. Multivariable Analyses.

Covariates included age, gender, Comorbidity (assessed by Charlson Comorbidity Index) and renal function (eGFR and ACR).

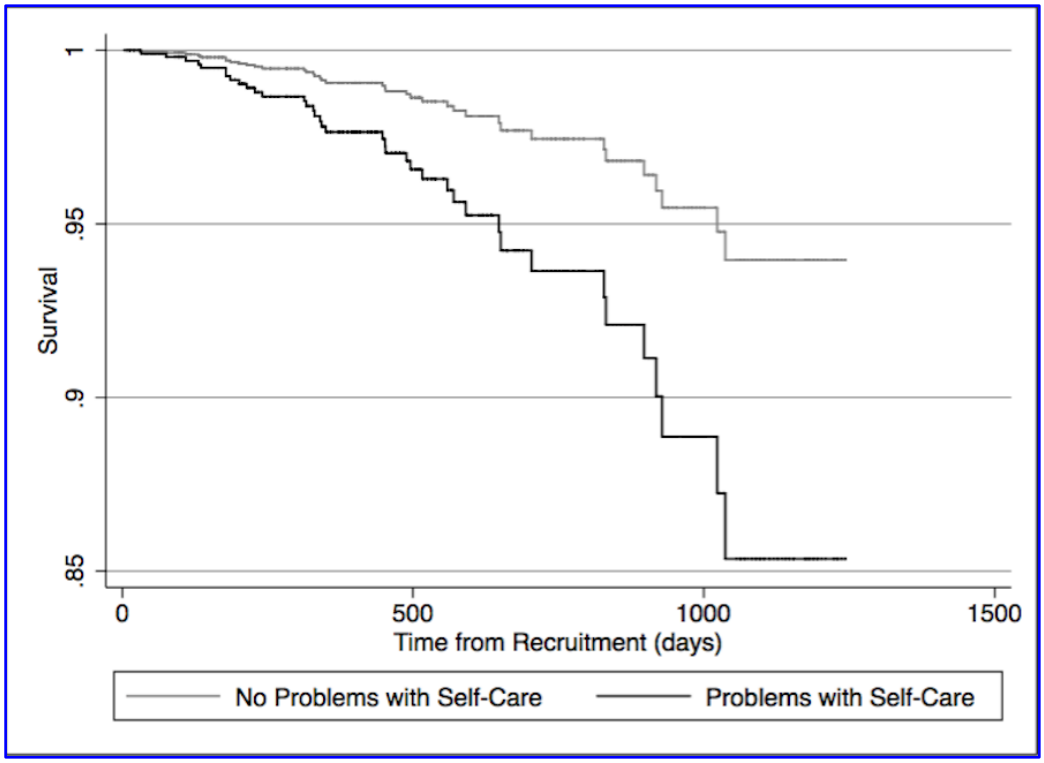


Table 6-5. Multivariable Survival Analyses (Cox regression and Competing risk) for hazard ratio (HR) and subdistribution hazard ratio (SHR) for death.

	Cox Regression Analyses		Competing Risk Analyses	
	HR (95% CI)	***P*-Value**	**SHR (95% CI)**	***P*-Value**
Identified problem with self care	2.54 (1.22-5.29)	0.013	2.61 (1.26-5.40)	0.01
Age (per 10 year increase)	2.04 (1.44-2.88)	<0.001	2.24 (1.59-3.16)	<0.001
Gender (female as reference)	1.50 (0.68-3.29)	0.311	1.44 (0.68-3.07)	0.341
Charlson Comorbidity Index	1.24 (1.06-1.44)	0.006	1.19 (1.04-1.37)	0.01
eGFR (per 5ml/min increase)	0.85 (0.70-1.05)	0.128	0.90 (0.75-1.10)	0.306
ACR (per 10mg/mmol rise)	1.02 (1.00-1.03)	0.013	1.02 (1.00-1.03)	0.045
EQ-5D index score	0.28 (0.10-0.81)	0.019	0.32 (0.10-0.96)	0.042
Age (per 10 year increase)	2.09 (1.48-2.95)	<0.001	2.31 (1.66-1.66)	<0.001
Gender (female as reference)	1.57 (0.73-3.37)	0.247	1.43 (0.70-2.91)	0.324
Charlson Comorbidity Index	1.20 (1.03-1.40)	0.02	1.15 (1.01-1.32)	0.032
eGFR (per 5ml/min increase)	0.85 (0.69-1.05)	0.126	0.91 (0.75-1.11)	0.344
ACR (per 10mg/mmol rise)	1.02 (1.01-1.04)	0.002	1.02 (1.00-1.03)	0.016

Abbreviations: ACR, albumin creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate

These analyses also identify increasing age, comorbidity and higher ACR as being associated with death. Estimated GFR was not significant; however a creatinine greater than 265 μmol/L (3mg/dL) *scores* 2 points in the CCI. Reanalysing the data for the CCI *without* the renal disease points results in eGFR demonstrating significance in Cox regression but not competing risk analyses (Table 6-6).

Table 6-6. Multivariable Survival Analyses (Cox regression and Competing risk) for death – Charlson comorbidity index without renal component.

	Cox Regression Analyses		**Competing Risk Analyses**	
	HR (95% CI)	***P*-Value**	**SHR (95% CI)**	***P*-Value**
Identified problem with self care	2.49 (1.19-5.20)	0.015	2.57 (1.23-5.36)	0.012
Age (per 10 year increase)	2.00 (1.41-2.84)	<0.001	2.20 (1.55-3.11)	<0.001
Gender (female as reference)	1.82 (0.85-3.92)	0.124	1.68 (0.82-3.44)	0.159
Charlson Comorbidity Index without renal component*	1.23 (1.04-1.44)	0.013	1.19 (1.02-1.38)	0.03
eGFR (per 5ml/min increase)	0.78 (0.63-0.97)	0.025	0.84 (0.68-1.04)	0.108
ACR (per 10mg/mmol rise)	1.02 (1.00-1.03)	0.02	1.02 (1.00-1.03)	0.061
EQ-5D index score	0.29 (0.10-0.84)	0.023	1.32 (0.10-0.99)	0.048
Age (per 10 year increase)	2.06 (1.46-2.92)	<0.001	2.27 (1.63-3.16)	<0.001
Gender (female as reference)	1.86 (0.88-3.92)	0.103	1.62 (0.82-3.22)	0.167
Charlson Comorbidity Index without renal component*	1.18 (1.00-1.39)	0.051	1.14 (0.98-1.33)	0.095
eGFR (per 5ml/min increase)	0.79 (0.63-0.98)	0.033	0.86 (0.69-1.06)	0.151
ACR (per 10mg/mmol rise)	1.02 (1.01-1.04)	0.002	1.02 (1.00-1.03)	0.015

Abbreviations: ACR, albumin creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; SHR, subdistribution hazard ratio

* Calculated using all Charlson Comorbidity Index components EXCEPT moderate/severe renal disease (creatinine >265mmol/L)

6.5.4.2 End-Stage Renal Disease

Seventy-eight participants (10.5%) had reached ESRD by the censor date. Lower EQ VAS score was the only component of the EQ-5D associated with an increased HR for progression to ESRD (Table 6-7). Significance was lost when the *a priori* variables of age, gender, comorbidity, eGFR and ACR were included in a multivariable analysis. Similarly, competing risk analysis indicated an association with a lower VAS and ESRD in univariable but not multivariable analysis.

Table 6-7. Univariable survival analyses (Cox regression) for end-stage renal disease (ESRD).

A priori variables and all components of EQ-5D shown.

	HR (95% CI)	***P*-Value**
A Priori Variables		
Age (per 10 year increase)	0.84 (0.74-0.96)	0.011
Gender (female as reference)	1.01 (0.64-1.60)	0.955
Charlson Comorbidity Index	1.20 (1.09-1.31)	<0.001
eGFR (per 5ml/min increase)	0.41 (0.34-0.49)	<0.001
ACR (per 10mg/mmol rise)	1.03 (1.03-1.04)	<0.001
ED5D Components		
Mobility	1.00 (0.64-1.56)	0.998
Self-Care	0.59 (0.26-1.35)	0.211
Usual Activities	1.25 (0.80-1.96)	0.317
Pain/Discomfort	1.09 (0.70-1.71)	0.695
Anxiety/ Depression	1.17 (0.72-1.89)	0.519
EQ-5D Index Score	1.02 (0.44-2.35)	0.958
Visual Analogue Scale	0.99 (0.98-1.00)	0.016

Abbreviations: ACR, albumin creatinine ratio; CI, confidence interval, eGFR, estimated glomerular filtration rate; HR, hazard ratio.

6.5.5 The impact of demographic, clinical and laboratory variables on HRQL

The analyses above demonstrate the two HRQL factors associated with death in the survival analyses were problems with self-care and a lower EQ-$5D_{index}$ score. In order to explore factors predictive of these two elements, further exploratory analyses were performed for self-care (logistic regression) and the EQ-$5D_{index}$ score (linear regression).

6.5.5.1 Self-care:

Table 6-8 shows the factors that were associated with (P<0.1) reported problems with self-care.

Ethnicity classified as other or not stated, people who were not currently working, higher body mass index (BMI), higher bicarbonate concentration, and higher C reactive protein (CRP) were statistically significantly associated with reported problems with self-care in multivariable analysis (Table 6-8). This model explained 16.5% of variability with self-care (pseudo R^2 0.165). Of note, age and renal function did not influence this aspect of HRQL.

6.5.5.2 EQ-$5D_{index}$ score:

Table 6-9 shows factors (P<0.1) associated with a higher EQ-5D score (i.e. better HRQL).

Multivariable analysis found the following variables remained associated with better HRQL: male gender; currently in employment; not smoking in comparison to current

smoking; lower BMI; less comorbidity; and lower CRP (Table 6-9). This linear regression model explained 20.8% of the variability in HRQL as assessed by the EQ-$5D_{index}$ score (adjusted R^2 0.208). Again age and renal function were not associated with this assessment of overall HRQL.

Table 6-8. Variables predictive of reported problems with self-care by logistic regression.

	Univariable Analyses			Multivariable Analyses*		
	OR (95% CI)	**SE**	***P*-value**	**OR (95% CI)**	**SE**	***P*-value**
Age (per 10 year increase)	1.29 (1.12-1.49)	0.09	<0.001			
Ethnicity (white as reference)						
South Asian	1.46 (0.88-2.41)	0.38	0.146	1.97 (1.06-3.68)	0.63	0.032
Black	1.36 (0.68-2.72)	0.48	0.391	1.04 (0.41-2.61)	0.49	0.938
Other/ Not stated	2.80 (0.96-8.13)	1.52	0.058	4.30 (1.25-14.76)	2.71	0.021
SES (most deprived Quintile)	1.51 (0.99-2.30)	0.33	0.058			
Academic Qualifications (none versus some)	2.16 (1.40-3.32)	0.47	<0.001			
Employment status (currently employed as reference)						
Not employed	26.02 (6.09-111.21)	19.28	<0.001	15.22 (3.42-67.79)	11.60	<0.001
Retired	23.77 (5.77-97.94)	17.17	<0.001	19.14 (4.57-80.20)	13.99	<0.001
Weekly Alcohol Consumption (none as reference)						
Under 10 units	0.54 (0.32-0.91)	0.14	0.021			
11-10 units	0.97 (0.47-2.00)	0.36	0.935			
More than 20 units	0.18 (0.02-1.32)	0.18	0.092			
BMI (kg/m^2)	1.07 (1.04-1.11)	0.02	<0.001	1.06 (1.03-1.10)	0.02	0.001
Charlson Comorbidity Index	1.21 (1.11-1.32)	0.05	<0.001			
Diabetes	2.41 (1.58-3.68)	0.52	<0.001			
Chronic obstructive pulmonary disease	2.61 (1.50-4.52)	0.73	0.001			
Ischaemic Heart disease	1.79 (1.14-2.83)	0.42	0.012			
Peripheral Vascular Disease	2.15 (1.21-3.83)	0.63	0.009			
SBP (mmHg)	1.01 (1.00-1.02)	0.01	0.06			
DBP (mmHg)	0.98 (0.96-1.00)	0.01	0.019			

Table 6-8 continued…

	Univariable Analyses			Multivariable Analyses*		
	OR (95% CI)	**SE**	***P*-value**	**OR (95% CI)**	**SE**	***P*-value**
Haemoglobin (g/L)	0.98 (0.97-1.00)	0.01	0.015			
Bicarbonate (mmol/L)	1.10 (1.04-1.17)	0.03	0.002	1.10 (1.03-1.18)	0.04	0.008
Albumin (g/L)	0.95 (0.91-0.99)	0.02	0.012			
CRP (mg/L)	1.02 (1.00-1.03)	0.01	0.007			
log CRP	1.53 (1.27-1.83)	0.14	<0.001	1.27 (1.03-1.57)	0.14	0.027

Abbreviations; BMI, body mass index; CRP, C-reactive protein; CI, confidence interval; DBP, diastolic blood pressure; OR, odds ratio; SBP, systolic blood pressure; SE, standard error; SES, socio-economic status;

* Significant variables removed in a backwards stepwise technique until remaining variables had a $P<0.05$.

Table 6-9. Variables predictive of higher EQ-5D index score by linear regression.

	Univariable Analyses			Multivariable Analyses*		
	Coefficient (95% CI)	**SE**	***P*-value**	**Coefficient (95% CI)**	**SE**	***P*-value**
Age (per 10 year increase)	-0.03 (-0.05--0.02)	0.01	<0.001			
Gender (male as reference)	0.04 (0.00-0.08)	0.02	0.048	0.05 (0.00-0.10)	0.02	0.042
SES (most deprived Quintile)	-0.06 (-0.10--0.02)	0.02	0.004			
Academic Qualifications (none versus some)	-0.01 (-0.14--0.06)	0.02	<0.001			
Employment status (currently employed as reference)						
Not employed	-0.26 (-0.32--0.21)	0.03	<0.001	-0.19 (-0.26--0.13)	0.03	<0.001
Retired	-0.21 (-0.25--0.16)	0.02	<0.001	-0.13 (-0.19--0.08)	0.03	<0.001
Smoking status (non smoker as reference)						
Current	-0.07 (-0.13-0.00)	0.03	0.037	-0.11 (-0.18--0.04)	0.03	0.001
Previous	-0.07 (-0.11--0.02)	0.02	0.003	-0.03 (-0.08-0.02)	0.02	0.235
Weekly Alcohol Consumption (none as reference)						
Under 10 units	0.07 (0.03-0.12)	0.02	0.002	0.03 (-0.02-0.07)	0.02	0.254
11-10 units	0.04 (-0.03-0.12)	0.04	0.261	0.01 (-0.07-0.08)	0.04	0.887
More than 20 units	0.17 (0.07-0.28)	0.05	0.001	0.11 (0.01-0.22)	0.05	0.033
BMI (kg/m^2)	-0.01 (-0.01--0.01)	0.00	<0.001	-0.01(-0.01-0.00)	0.00	<0.001
Charlson Comorbidity Index	-0.03 (-0.04--0.02)	0.00	<0.001	-0.02 (-0.03--0.01)	0.01	0.003
Diabetes	-0.11 (-0.15--0.07)	0.02	<0.001			
Chronic obstructive pulmonary disease	-0.10 (-0.17--0.04)	0.03	0.001			
Cerebrovascular disease	-0.09 (-0.26--0.03)	0.03	0.003			
Ischaemic Heart disease	-0.13(-0.18--0.08)	0.03	<0.001			
Peripheral Vascular Disease	-0.14 (-0.21--0.08)	0.42	<0.001			

Table 6-9 continued…

	Univariable Analyses			Multivariable Analyses*		
	Coefficient (95% CI)	**SE**	***P*-value**	**Coefficient (95% CI)**	**SE**	***P*-value**
SBP (mmHg)	0.00 (0.00-0.00)	0.00	0.001			
DBP (mmHg)	0.00 (0.00-0.00)	0.00	0.011			
eGFR (per 5ml/min)	0.01 (0.00-0.02)	0.00	0.006			
Haemoglobin (g/L)	0.00 (0.00-0.00)	0.00	<0.001			
Albumin (g/L)	0.01 (0.00-0.01)	0.00	0.001			
CRP (mg/L)	-0.05 (-0.07--0.29)	0.01	<0.001			
log CRP	0.00 (-0.01-0.00)	0.00	<0.001	-0.02 (-0.04-0.00)	0.01	0.03

Abbreviations; BMI, body mass index; CRP, C-reactive protein; CI, confidence interval; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; SE, standard error; SES, socio-economic status;

* Significant variables removed in a backwards stepwise technique until remaining variables had a $P<0.05$.

6.6 Discussion

The relationship between pre-dialysis CKD, HRQL and clinical outcomes is an important aspect of nephrology practice. These analyses, conducted in a cohort of people with advanced and/or progressive CKD, demonstrated that reported problems with HRQL, as measured by the EQ-5D, were common; only 24.3% of participants reported no problem in any EQ-5D domain.

Impaired HRQL was a risk factor for death; problems with self-care and overall HRQL, assessed by the EQ-$5D_{index}$ score, were associated with an increased HR for death when analysed with age, gender, comorbidity, eGFR and ACR. This association was present in both cox proportional hazard regression and competing risk regression (with ESRD as the competing risk). No element of HRQL was independently associated with risk of progression to ESRD.

Until recently, previous studies investigating HRQL in patients with pre-dialysis CKD had focused on specific populations (Taiwanese (289) or individuals of black ethnicity with hypertensive CKD in the United States(290)). Whilst the generalisability of these studies to a multi-ethnic United Kingdom CKD population is questionable, both studies identified an association with HRQL and death in similarity to the findings presented here. However, the association with HRQL and CKD progression was conflicting; Tsai and colleagues identified an association (289) but Porter and colleagues only noted an association in a composite of death and CKD progression (290).

A combined analysis of the Chronic Renal Insufficiency Cohort and Hispanic Chronic Renal Insufficiency Cohort has recently been published (291). The disease specific KDQOL-36 questionnaire was completed by 3837 patients (of a total of 3939 enrolled). Consistent with the data presented here, they found that low HRQL was independently associated with a higher risk of death but not CKD progression in several KDQOL-36 subscales (physical component summary, mental component subscale, effects and symptoms). The KDQOL-36 questionnaire is a detailed HRQL survey based on a chronic disease core, with added items relevant to patients with kidney disease (293). Compared to the EQ-5D, it is more time consuming to complete, has some components that apply more to those undergoing RRT than the pre-dialysis population, and its utility in health economic evaluations is not as established.

To explore further the factors that influence the components of HRQL associated with death (self-care and the EQ-$5D_{index}$ score), I assessed the relationship between these components and demographic, clinical and laboratory variables utilizing regression analysis. Not being currently employed, whether young and not working or retired, conveyed the highest HR associated with impaired HRQL. Other significant factors for a lower HRQL included higher BMI, a higher CRP, and multimorbidity. Further research is warranted.

Interestingly, no association was identified between these aspects of HRQL and SES, increasing age of the participants or renal function, as measured by eGFR or ACR. This lack of association between HRQL and renal function, is a finding variably supported by previous studies (76, 294, 295).

This work, and that of others, demonstrate that reported problems with HRQL are common in this population (192) and I have found an association between impaired HRQL and death. It is therefore important to consider what strategies could be used to improve HRQL; improving HRQL will not only improve patient well-being but may convey a survival advantage. Previous studies have demonstrated that optimisation of haemoglobin, psychological interventions and physical exercise may be of benefit (296-298). However, the majority of these studies have focused on patients who have reached ESRD rather than pre-dialysis CKD. Therefore, the transferability of these, or of interventions instigated for other chronic disease states, requires evaluation.

In this study of patients with pre-dialysis CKD, problems with self-care and the EQ-$5D_{index}$ score (194) were both of prognostic significance; in clinical practice problems with self-care may be the more useful HRQL screening question to identify patients with CKD at an increased risk of death. There are analogies here to the findings of O'Hare and colleagues who quantified the impact of age on outcomes in individuals with CKD (299); similarly, knowledge about impaired HRQL may help inform clinicians and patients about the relative risk of death compared to ESRD. This information could inform decision making including when to discuss renal replacement therapy options and to help ensure such individuals are adequately supported when counselled about their higher mortality risk. Additionally it may provide a trigger to enable social care resources to be targeted to those most in need and may enable identification of a high-risk group where interventions to improve outcomes can be studied.

6.6.1 Strengths and Weaknesses

The major strength of this study is the use of a prospectively recruited, socio-economically and ethnically diverse cohort of patients with advanced and/or progressive pre-dialysis dependent CKD. Detailed demographic and clinical data were collected at initial recruitment and the participants were tracked longitudinally to record outcomes, including death and ESRD. HRQL was assessed by the EQ-5D tool, which is recommended as the preference based measure for HRQL evaluation in CKD (192). Survival analyses were performed using both Cox proportional hazard analyses and competing risk analyses. The latter is important, though rarely used, as it enabled the competing risk of ESRD to be taken into account when assessing death and vice versa: both end-points are (separately) of key interest to patients, their families and clinicians (222, 300).

A weakness, as with all observational studies, is that these analyses have assessed association rather than causation. Whilst the analyses for factors associated with an increased risk of death included baseline renal function and progression to ESRD (in competing risk analyses), I did not include any other measure of CKD progression.

In addition, whilst considerable demographic and clinical information was collected, there was no formal assessment of frailty, depression or nutritional status of the participants. These factors have been associated with impairment of HRQL (78, 301-306).

6.7 Conclusion to Chapter 6

In summary, data presented in this chapter demonstrate that impaired HRQL is common in a diverse pre-dialysis CKD population and that impaired HRQL, as demonstrated by problems with self-care or a lower EQ-$5D_{index}$ score, is associated with a higher risk for death but not ESRD. Multiple factors influence these aspects of impaired HRQL but renal function, as measured by eGFR and ACR, are not among them.

Further studies are recommended to evaluate interventions that may improve HRQL within the pre-dialysis CKD population and to investigate whether any improvements in HRQL are associated with a survival advantage.

7 DISCUSSION

In the results chapters I present data from a series of studies focusing on high-risk patients with chronic kidney disease (CKD). In this final chapter, I discuss the key findings from each study, highlight the common themes identified throughout this thesis and describe potential future directions leading from this research. I examine the key strengths and limitations of the work before drawing final conclusions.

7.1 Chapter 3 Summary: The impact of ethnicity, chronic kidney disease and cardiovascular comorbidity on mortality in a multi-ethnic primary care population.

This chapter investigated the determinants of mortality in an ethnically diverse primary care population, using enhanced electronic data collected from 31,254 people in 62 primary care practices within central Birmingham. The analyses identified ethnicity as a risk factor for mortality; the risk of death in South Asian and black individuals were lower when compared to white individuals. The difference was, in part, independent of age, gender, socioeconomic status (SES), renal function and comorbidities.

The analyses identified renal function, both estimated glomerular filtration rate (eGFR) and albumin creatinine ratio (ACR), and comorbidity as significant risk factors for mortality. Additionally, the data showed measurement of ACR in high-risk populations was by no means universal; only 11,205 of the 31,254 (35.9%) individuals had an ACR recorded.

7.2 Chapter 4 Summary: The impact of ethnicity on progression to end-stage renal disease in pre-dialysis chronic kidney disease.

Whilst Chapter 3 focused on a multi-ethnic primary care population, Chapter 4 investigated the impact of ethnicity in a secondary care nephrology population, the Renal Impairment in Secondary Care (RIISC) study, where over 30% of individuals were of black and minority ethnic backgrounds.

I demonstrated that individuals of South Asian and black ethnicity were at higher risk of end stage renal disease (ESRD) than their white counterparts, and that this persisted in competing risk analyses (where death was the competing risk). The difference between risk of ESRD was explained by known risk factors (age, gender, eGFR and ACR); median ACR was highest in South Asians and I identified albuminuria as the principal modifiable risk factor.

7.3 Chapter 5 Summary: Serum tryptase concentration and progression to end-stage renal disease.

The work in Chapter 5 analysed participants in the RIISC study to investigate the association of serum tryptase to progression of ESRD, and whether this relationship was influenced by use of angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB).

Analyses identified serum tryptase concentration as an independent prognostic factor (after adjustment of the *a priori* variables of age, gender, eGFR and ACR) for

progression to ESRD in those receiving treatments with ACEi/ARB. These data suggest the potential of using tryptase to assess pathways that bypass the protective effects of ACEi/ARB which could be utilised in the era of stratified medicine.

7.4 Chapter 6 Summary: The Impact of Health Related Quality of Life on mortality and progression to end-stage renal disease in pre-dialysis chronic kidney disease.

Whilst the earlier chapters concentrated on the endpoints of death and progression to ESRD, I was keen to recognise and investigate the impact of CKD on health related quality of life (HRQL) and any association between HRQL and clinical endpoints. I therefore analysed data on HRQL, assessed with the EuroQol EQ-5D questionnaire, within the RIISC study cohort.

I demonstrated that impaired HRQL was common in pre-dialysis CKD and that reduced HRQL was associated with a higher risk for death but not ESRD. The components associated with an increased risk of death were difficulties with self care and a lower EQ-$5D_{index}$ score (a global assessment of HRQL where the five individual health states from the EQ-5D questionnaire are converted into a score using a set of weighted preferences specific to the UK population (194)). I then investigated these two aspects (problems with self care and the EQ-$5D_{index}$ score) and found that although renal function did not influence these components, multiple other factors did.

7.5 Common themes and future directions

Within this thesis, there are several common themes which are discussed below. I also highlight possible directions for future research.

7.5.1 Increasing comorbidity is associated with death

I assessed the impact of comorbidity in primary and secondary care cohorts by investigating the impact of individual and combined comorbidity. The technique used to assess combined comorbidity varied by the population studied, principally due to the availability of data; I used cumulative cardiovascular comorbidity in primary care (Chapter 3) and the Charlson comorbidity index (CCI) (216) in the RIISC cohort (Chapters 4-6). Both were associated with death in multivariable analyses.

This finding is important given the prevalence of comorbidity, especially in older individuals (307). An understanding of the impact of comorbidity, and its potential to influence the balance between risk of death versus progression to ESRD, is important in routine primary care and nephrology practice and the data I present highlights this. A discussion regarding *silo working* by different secondary care specialties is beyond the scope of this thesis but increasing comorbidity is likely to require a more collaborative approach to healthcare.

Whilst the Charlson comorbidity score is used routinely by health researchers, it was developed in 1984 and focused on conditions which influenced mortality within one year from hospital discharge. Management of many of the conditions in the Charlson scoring system (Table 2.2) has changed dramatically and, whilst attempts to update

the scoring system have been proposed (308), their use is not routine. As discussed earlier in this thesis, a limitation of the use of the CCI in nephrology research is that renal impairment is one factor in the Charlson score, and therefore has the potential to minimise the significance of eGFR in multivariable analyses; indeed this was one of the findings I report in Chapter 6.

Future work 1: An evaluation of current comorbidity scoring systems and their utility in nephrology.

7.5.2 The significance of albuminuria

Albuminuria, assessed in this work as a urine ACR, has been demonstrated to add prognostic information in addition to measurements of eGFR, both in terms of risk of ESRD and death. This is in agreement with multiple previous studies, perhaps most notably the work of the Chronic Kidney Disease Prognosis Consortium (43, 44, 46, 309) which analysed the available data in response to the KDIGO controversies conference (42) and resulted in the 2012 update of the international CKD staging system (54).

The results in this thesis highlight two pertinent issues

7.5.2.1 Primary care underutilises testing for albuminuria

Chapter 3 demonstrated the significance of elevated ACR and its association with mortality in a primary care setting. However, only 35.9% of individuals within the cohort had their ACR tested. People were more likely to have it tested if they were of

South Asian descent, male gender, had diabetes or had greater vascular comorbidity. The use of ACR in screening for microalbuminuria in diabetes has been established practice for many years (40, 41) and these analyses were based on data collected before the 2012 update of the CKD staging system; it would therefore be important to evaluate whether the use of ACR in primary care has improved since the publication of both international and national (e.g. NICE CKD (33)) guidelines. Strategies to increase the ACR measurement in routine clinical practice should be encouraged and the utility of ACR for risk stratification and for enhanced treatment (e.g. with ACEi/ARB and to more rigorous blood pressure targets) highlighted.

Previously, one of the assessment measures for CKD in the Quality and Outcome Framework (QOF) primary care pay for performance model was '*the percentage of patients on the CKD register whose notes have a record of a urine albumin creatinine ratio (or protein creatinine ratio) test in the preceding 12 months'* (64). This component has now been removed and, in QOF 2015-2016, the only stated outcome for CKD is *'The contractor establishes and maintains a register of patients aged 18 or over with CKD with classification of categories G3a to G5'* (64). Thus, there is no longer a financial incentive to assess urine ACR.

Future work 2: To develop strategies to ensure that ACR is being routinely measured within primary care for those with CKD, diabetes or vascular comorbidity.

7.5.2.2 Albuminuria is the principal modifiable risk factor for ESRD in progression of ESRD in people of South Asian ethnicity

The research presented in Chapter 4 confirms a higher risk of ESRD in individuals of South Asian or black ethnicity and that the difference in ESRD risk between ethnicities is explained by conventional risk factors of age, gender, eGFR and ACR. Importantly this thesis identifies a significant difference in median ACR between ethnic groups at baseline and proposes ACR as the key modifiable risk factor. As discussed in Chapter 4, prior work has identified higher levels of proteinuria in paediatric and adolescent South Asian populations (265, 266), suggesting potential genetic or environmental factors may influence albuminuria.

In addition to other serum, plasma, urine and saliva samples collected as part of the RIISC study, we collected DNA samples using the The PAXgene tube system (Quiagen, Venlo, Netherlands) (Section 2.4.5). This provides an opportunity to investigate any genetic basis explaining the increased ACR in non-white ethnic groups.

Future work 3: To investigate a potential genetic basis of increased albuminuria in South Asian individuals.

Blood pressure control, especially ACEi/ARB usage in those with albuminuria, is a key component to slowing CKD progression. A review of the current literature (discussed in Chapter 4) acknowledged there are limited data on response to antihypertensive treatment, including renin angiotensin aldosterone system (RAAS) blockade, in South Asian individuals. It is therefore important to explore the response

to treatment by ethnicity, as this may provide potential to tailor treatment to those most likely to respond. I was unable to incorporate ethnicity into the analyses with tryptase (Chapter 5) as I needed to balance the number of *a priori* variables with the number of events to reduce the likelihood of overfitting, but it would be important to rerun the analyses in the future when more events have occurred.

Future work 4: To assess response to key interventions, especially RAAS blockade, within different ethnicities.

7.5.3 Ethnicity should be viewed as a non-traditional risk factor for death and ESRD.

The ethnically diverse population within the West Midlands, provided an excellent opportunity to explore the relationships between ethnicity, kidney disease and clinical outcomes. Both primary and secondary care cohorts demonstrated white participants tended to be older and tended to have more comorbidities than those of South Asian or black ethnicity. In the analysis of primary care data (Chapter 3), the risk of death was lower for South Asian and black participants than for white participants, including in the multivariable analyses which included age, renal function and comorbidity. In the analysis of the secondary care RIISC cohort (Chapter 4), I did not identify a mortality difference between ethnicities. However, there was a difference in progression to ESRD (discussed above in Section 7.2) including in competing risk analyses.

7.5.4 Socioeconomic status was not significantly associated with adverse outcomes.

In contrast to previous work (see Section 1.5.2), the analyses presented in this thesis showed no association between SES and any of the outcomes studied, including death, progression to ESRD or reduced HRQL.

Whilst this may be due to the universal healthcare system operating in the UK, other explanations are possible. Both primary and secondary care cohorts had a high percentage of people in the most deprived national SES quintile (calculated using index of multiple deprivation scores (186, 310)) with over 70% in primary care and approximately 50% in secondary care studies. We therefore have not been able to comprehensively compare the highest to the least socioeconomically deprived. Additionally, significant resources have been spent on health promotion programmes targeted at inner-city Birmingham (121); the work presented may suggest the lack of association between SES and outcomes is related to the success of such programmes.

7.5.5 Serum tryptase is independently associated with ESRD in people receiving treatment with ACEi or ARB

Chapter 5 presents the first data demonstrating serum tryptase concentration as an independent prognostic factor for progression to ESRD in patients receiving treatment with ACEi/ARB. The chapter provides information regarding potential putative mechanisms for this association; perhaps the most compelling are the role of mast cell proteases as the main ACE independent pathway of angiotensin II production, and therefore may identify individuals who have incomplete RAAS blockade, and the role

of proteases promoting renal disease through transforming growth factor beta (TGF-β) (by stimulating TGF-β directly and via promotion of angiotensin II) (139).

Combined with studies which have investigated inhibitors of tryptase and chymase in animal studies, including chymase inhibition attenuating renal disease in animal models (311), the data presented provides further justification for investigation of the concept of RAAS breakthough and the potential for trials of mast cell protease inhibitors in humans.

Future work 5: To investigate mechanisms and the concept of RAAS escape/ breakthough through a mast cell protease dependent pathway.

7.5.6 Impaired health related quality of life is common in CKD and associated with increased risk of death

This thesis explored the relationship between HRQL, CKD and outcomes. The data presented show that impaired HRQL is common and therefore should be explored routinely in clinical consultations to identify its presence. The results I have presented indicate that a comprehensive and time-consuming assessment may not be necessary; a question regarding self-care could potentially be used as a screening tool.

Studies are needed to investigate whether specific interventions can improve patient well being and whether these interventions may alter an individual's risk of death. National studies are starting to investigate HRQL in CKD (for example, 'Think Kidneys' Transforming Participation in Chronic Kidney Disease; TP-CKD (312)) but

it is vital the applicability of findings in the ethnically diverse population can be demonstrated.

Future work 6: To evaluate interventions which, if applied to those with non-dialysis dependent CKD with impaired HRQL, may lead to an improvement in patient well being.

7.6 Key Strengths and Limitations

I have discussed strengths and weaknesses as a component of individual result chapters. Here I summarise the general strengths and weaknesses of my research presented in this thesis.

The key strength of the studies presented has been the ability to perform statistically robust analyses on high-risk, ethnically diverse primary and secondary care cohorts, and present the findings in clinically relevant ways. The results have direct relevance to the population managed by nephrologists and primary care physicians in Birmingham, other multicultural cities in the UK and beyond. As previously highlighted, this is a population under-represented in other national or international cohort studies.

The main limitations of the studies presented is their observational nature and a recognition that, for the chapters focusing on the RIISC study, the results may not be generalisable to a CKD population comprising less advanced renal impairment. Additionally, whilst the aetiology of renal disease (see Section 2.4.7.1 for

classification) is described in the chapters utilising the RIISC cohort, I recognise the majority of these diagnoses are 'presumed' rather than based on definite histology. Indeed, under 30 percent (201/727 in Chapter 4) of patients underwent a renal biopsy. Whilst the large majority of patients with significant renal disease do not have a kidney biopsy, as in most cases knowledge of histology will not alter management (313), it is important to recognised that the presumed diagnosis is not always correct (314). This limitation makes it more challenging to assess any association between renal diagnosis with outcomes, especially for aetiologies such as diabetic kidney disease. Chapter 4 shows a high prevalence of diabetes (36.5%) within the cohort but a more modest prevalence of diabetic kidney disease (11.3%). Work is needed on increasing the accuracy of diagnoses in nephrology, either through increased use of kidney biopsies or through novel biomarkers with high diagnostic discrimination for the underlying renal disease. (315).

The chapters utilising the RIISC study had different numbers of participants due to the selection criteria stated in each chapter. Chapter 5 had the fewest participants due to the requirement to analyse samples for tryptase, and this was limited by laboratory and assay capacity. Additionally, the censor dates used in each chapter reflected the timing of the analyses. However I believe that, despite these limitations, the detailed phenotyping of both primary and secondary care cohorts, and the univariable and multivariable analyses performed, provide a sound footing from which to explore the future work I have proposed.

7.7 Executive Conclusions

This thesis describes the impact of CKD on individuals and society and has discussed the previously identified risk factors associated with adverse events, including ESRD, death and impaired HRQL.

Though a series of studies, in both primary and secondary care cohorts, I have focused on areas of uncertainty which have implications for patient care.

I found that: (i) comorbidity has a profound impact at a population level on survival in CKD; (ii) albuminuria is the principle modifiable risk factor for progression to ESRD in people of South Asian ethnicity; (iii) serum tryptase is an independent prognostic factor for ESRD in patients with CKD receiving treatment with an ACEi or ARB; and (iv) Low HRQL is common in CKD and reduced HRQL is associated with a higher risk for death.

The findings from this thesis contribute to the understanding of CKD in ethnically diverse, high-risk populations and form the basis for further studies.

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