NOVEL PROGNOSTIC FACTORS IN CHRONIC KIDNEY DISEASE

By

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Abstract

Chronic kidney disease (CKD) is a prevalent condition and is associated with an increased risk of serious adverse outcomes, including kidney failure and death. The identification of prognostic factors that improve our ability to predict an individual's risk of these adverse outcomes and identify potential targets for new treatments could bring significant benefits to the care of patients with CKD. In this work, data and samples from prospective cohort studies of participants with CKD were used to examine four potential prognostic factors: serum free light chains (FLC), urine FLC, monoclonal gammopathy, and serum endotrophin. Serum FLC and endotrophin concentrations were both associated with the risk of death in patients with CKD after adjustment for established prognostic factors, and serum FLC concentration was also independently associated with the risk of kidney failure. Urine FLC and monoclonal gammopathy were not associated with the risk of adverse outcomes. Possible explanations for the identified associations are discussed, as are suggestions for the next steps needed to assess the potential use of these prognostic factors in clinical practice with a view to improving the care of patients with CKD.

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Table of Contents

List of Tables

List of Figures

Abbreviations

Abbreviation	
ACEi	angiotensin-converting enzyme inhibitor
ACR	albumin-to-creatinine ratio
AER	albumin excretion rate
AGEs	advanced glycation end products
ANOVA	analysis of variance
APOL1	apolipoprotein L1
ARB	angiotensin II receptor blocker
BMI	body mass index
BP	blood pressure
BSA	body surface area
BTP	beta-trace protein
cFLC	combined free light chains
CGA	cause of CKD, GFR, albuminuria
CI	confidence interval
CINAHL	cumulative index to nursing and allied health literature
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COPD	chronic obstructive pulmonary disease
CRF	case report form
CRIB	Chronic Renal Impairment in Birmingham
CRISIS	Chronic Renal Insufficiency Standards Implementation Study
CRP	C-reactive protein
CVD	cardiovascular disease
DM	diabetes mellitus
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EDTA	ethylenediamine tetra-acetic acid
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EQ	EuroQol
ESKD	end-stage kidney disease
ESRD	end-stage renal disease
ESRF	end-stage renal failure
FLC	free light chains
FP	fractional polynomials

GFR	glomerular filtration rate
HR	hazard ratio
HRQL	health-related quality of life
IDI	integrated discrimination index
IDMS	isotope dilution mass spectrometry
IHD	ischaemic heart disease
IMD	index of multiple deprivation
IPD	individual participant data
IQR	interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
KFRE	kidney failure risk equation
KRT	kidney replacement therapy
LC-MG	light chain monoclonal gammopathy
MAP	mean arterial pressure
MDRD	Modification of Diet in Renal Disease
MG	monoclonal gammopathy
MGCS	monoclonal gammopathy of clinical significance
MGRS	monoclonal gammopathy of renal significance
MGUS	monoclonal gammopathy of undetermined significance
NHS	national health service
NICE	National Institute for Health and Care Excellence
NT-pro-BNP	N-terminal pro-brain natriuretic peptide
NURTuRE	National Unified Renal Translational Research Enterprise
PAD	peripheral artery disease
PCR	protein-to-creatinine ratio
PMH	past medical history
PROGRESS	Prognosis Research Strategy
PWV	pulse wave velocity
RAASi	renin-angiotensin-aldosterone system inhibitor
REC	research ethics committee
RIISC	Renal Impairment in Secondary Care
RR	relative risk
RRID	Renal Risk in Derby
SD	standard deviation
SHARP	Study of Heart and Renal Protection
SHR	subhazard ratio
SKS	Salford Kidney Study
SOP	standard operating procedure
SPEP	serum protein electrophoresis
STROBE	Strengthening the Reporting of Observational studies in Epidemiology

TGF	transforming growth factor
UK	United Kingdom
US	United States
USRDS	United States Renal Data System
vWF	von Willebrand factor

CHAPTER I: INTRODUCTION

Chronic kidney disease (CKD) is a common long-term condition, affecting over 9% of the global population, and is associated with an increased morbidity and mortality risk, which is directly related to the severity of CKD. Some individuals with CKD progress to kidney failure, a level of kidney function at which those affected will die without replacement of kidney function by dialysis treatment or kidney transplantation.

Understanding the factors associated with worse outcomes in CKD is crucial, for communication, risk stratification, and identification of targets for treatment. The work presented in this thesis is focused on novel risk factors in CKD. To provide the context for the experimental chapters reported in this thesis, this introductory chapter will provide an overview of the assessment of kidney function, markers of kidney disease, CKD, the concept of prognosis, and prognosis in patients with CKD. The chapter will conclude by making a case for more prognosis research in CKD.

1.1. Kidney function and markers of kidney disease

The kidneys perform multiple functions, and each particular function can be assessed in various ways. However, it is generally accepted that the best overall measure of kidney function is the glomerular filtration rate (GFR). Throughout this thesis, where the term 'kidney function' is used, it is used synonymously with GFR.

1.1.1. Glomerular filtration rate (GFR)

The GFR, expressed in ml/min, is the sum of the filtration rates across the glomeruli of all functioning nephrons. Thus, the GFR approximately reflects the total number of functioning nephrons. To account for differences in kidney size, which is proportional to body size, the GFR is adjusted for body surface area (BSA) and is expressed per 1.73 m^2 BSA (1.73 m^2 was the average adult BSA from historical data (1)). This scaling allows a comparison of the GFR between individuals or comparison with normal values.

The 'normal' GFR (in ml/min/1.73 m^2) varies by age and sex. It is approximately 100 ml/min/1.73 m^2 in young adults and then declines after 35 years of age (2). The decline is faster for females compared to males. Figure 1 shows the reference ranges for GFR by age and sex.

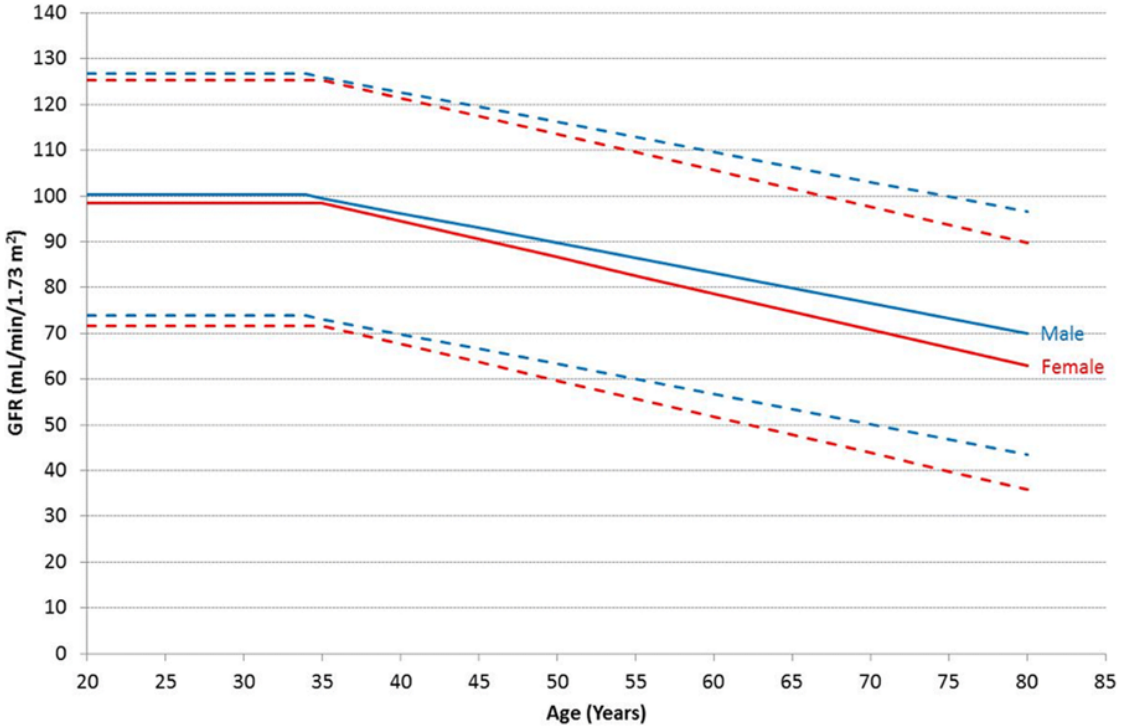


Figure 1.1. Reference ranges for GFR.

Age- and sex-specific ranges for GFR were developed based on measured GFRs from 2974 prospective living kidney donors. The solid lines represent the mean GFR for a given age and sex, and the interrupted lines are two standard deviations above and below the mean. From reference (2).

Assessment of the GFR involves the measurement of solutes, termed filtration markers, that undergo glomerular filtration and urinary excretion. The gold standard method is to administer an intravenous dose of an exogenous filtration marker (such as inulin or ethylenediamine tetra-acetic acid [EDTA]), and then to measure its clearance (measured GFR). There are several methods of doing this, but they are all relatively time-consuming, expensive, and cumbersome. Therefore, they are generally performed only in clinical situations where it is essential to have a precise measure of the GFR; for example, in prospective living kidney donors before proceeding to nephrectomy. In most situations,

however, the GFR is estimated using equations based on the serum concentration of endogenous filtration markers.

1.1.1.1. Estimation of the GFR

The endogenous filtration marker used routinely in clinical practice is creatinine. Creatinine is derived from the metabolism of creatine in skeletal muscle, after which it is released into the circulation and then freely filtered across the glomerulus to be excreted in the urine. The serum creatinine concentration itself was previously used as a surrogate for kidney function. However, its use in this way is limited by significant variation between individuals in the non-GFR determinants of serum creatinine concentration, particularly muscle mass. Equations have been developed to calculate an estimated GFR (eGFR) that, in addition to serum creatinine concentration, include variables that are surrogates for muscle mass (age, sex, and ethnicity), such that they improve upon serum creatinine alone. Until recently, the equation used in the UK and internationally has been the Modification of Diet in Renal Disease (MDRD) equation. The MDRD equation is now being replaced in clinical use by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

The Modification of Diet in Renal Disease (MDRD) equation.

The MDRD study was established to evaluate the effect of dietary protein restriction on the progression of kidney disease. In 1999 the study group used data from the study to develop an equation to estimate GFR from the serum creatinine concentration. This was a six-variable equation containing age, sex, ethnicity, serum creatinine, serum urea, and serum albumin (3). The equation was simplified to a four-variable equation (containing age, sex, ethnicity, and

serum creatinine) (4) and was later re-expressed for use with a standardized creatinine assay (5, 6). This MDRD equation for calculating eGFR is as follows:

$$eGFR = 175 \times Cr^{-1.154} \times age^{-0.203} \times 1.212\ (if\ black) \times 0.742\ (if\ female)$$

where eGFR is expressed in ml/min/1.73 m^2, Cr is the serum creatinine concentration in mg/dl (serum creatinine concentration can be converted from μmol/l to mg/dl by dividing it by 88.4), and age is in years.

The equation was developed using data from individuals with CKD and the accuracy of the MDRD formula for estimating the GFR in patients with CKD has been validated. However, in individuals with a normal or near-normal GFR, it is relatively imprecise and systematically underestimates the GFR (7-10). This issue prompted the development of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI).

The CKD-EPI equation was developed in 2009. Unlike the MDRD equation, it was developed using data from individuals both with and without CKD (11). It contains the same four variables as the four-variable MDRD equation (age, sex, ethnicity, and serum creatinine) but is more accurate in those with a GFR ≥ 60 ml/min/1.73 m^2 and is as accurate as the MDRD equation in those with a GFR < 60 ml/min/1.73 m^2 (11-14). The use of the CKD-EPI equation to estimate the GFR results in lower estimates of the prevalence of CKD and several studies have shown that those who are reclassified as not having CKD are at a lower risk of adverse health outcomes, suggesting the equation provides a more accurate discrimination of risk compared with the MDRD equation (15-21). The CKD-EPI equation for eGFR is as follows:

$$eGFR = 141 \times \min\left(\frac{Cr}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{Cr}{\kappa}, 1\right)^{-1.209} \times 0.993^{age} \times 1.018\ (if\ female) \times 1.159\ (if\ black)$$

where eGFR is expressed in ml/min/1.73 m^2, Cr is serum creatinine concentration in mg/dl (serum creatinine concentration can be converted from μmol/l to mg/dl by dividing it by 88.4), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Cr/κ or 1, and max indicates the maximum of Cr/κ or 1.

Because of the advantages of the CKD-EPI equation over the MDRD equation, the National Institute for Health and Care Excellence (NICE) CKD guideline recommends that clinical laboratories in the UK should use the creatinine-based CKD-EPI equation to calculate the eGFR (22).

Cystatin C based equations.

Given the issues around the non-GFR determinants of serum creatinine concentration, including the variation between individuals in muscle mass, several other endogenous filtration markers have been studied. Of these, cystatin C is the most established. Cystatin C is a cysteine protease inhibitor produced by all nucleated cells and is freely filtered at the glomerulus before being metabolised in the tubules. In 2012, the CKD-EPI group developed an equation to estimate the eGFR based on serum cystatin C concentration, as follows:

$$eGFR = 133 \times \min\left(\frac{Cys}{0.8}, 1\right)^{-0.499} \times \max\left(\frac{Cys}{0.8}, 1\right)^{-1.328} \times 0.996^{age} \times 0.932\ (if\ female)$$

where eGFR is expressed in ml/min/1.73 m^2, Cys is the serum cystatin C concentration in mg/l, min indicates the minimum of Cys/0.8 or 1, and max indicates the maximum of Cys/0.8 or 1.

Despite hopes that cystatin C-based equations may provide more accurate estimates of the GFR, the cystatin C-based CKD-EPI equation is not more accurate than the creatinine-based CKD-EPI equation (23). One reason for this is that the serum cystatin C concentration, like the serum creatinine concentration, has many non-GFR determinants (24-28).

However, cystatin C-based equations for estimating the GFR may be useful in certain situations. For example, the NICE CKD guidelines recommend the use of the cystatin C-based CKD-EPI equation to confirm or rule out CKD in individuals with a creatinine-based eGFR between 45 and 59 ml/min/1.73 m^2 but without any other markers of kidney disease (22). It may also provide a more accurate estimate of the GFR in individuals with extremes of muscle mass or with a diet unusually high in creatinine in whom a creatinine-based eGFR is likely to be inaccurate (23). Further, cystatin C may be useful in combination with creatinine; numerous estimating equations which incorporate both cystatin C and creatinine are more accurate than equations that use cystatin C or creatinine alone (29-33).

1.1.2. Decreased GFR as a marker of kidney disease

A GFR below a specific cut-off may be used as a marker of kidney disease. A GFR < 60 ml/min/1.73 m^2 is the cut-off used as part of the current definition for CKD (discussed below). However, there is some debate about the use of such a blanket cut-off, in part because GFR declines as part of normal healthy ageing such that the lower limit of the reference range falls below 60 ml/min/1.73 m^2 after the age of 55 years, as shown in Figure 1.1. A cut-off of 60 ml/min/1.73 m^2 could, therefore, result in an over-diagnosis of CKD in individuals older than 55 years. Further, some adults younger than 55 years may have a GFR ≥ 60 ml/min/1.73 m^2, but actually below the reference range for their age, and could, therefore, be missed by the current definition of CKD in the absence of other markers of kidney disease. However, the

cut-off of a GFR < 60 ml/min/1.73 m^2 was included in the definition of CKD on the basis that this level of GFR is associated with a higher risk of adverse health outcomes (although an interaction with age means that the excess risk associated with a lower GFR diminishes with increasing age).

1.1.3. Other markers of kidney disease

There are other markers of kidney disease, which may occur with or without a decreased GFR, the most commonly identified being increased albuminuria. It is increasingly recognised that albuminuria is a powerful marker of kidney damage and, independent of GFR, increased albuminuria is a strong risk factor for adverse clinical outcomes, including mortality and progression of CKD.

1.1.3.1. Albuminuria

Although the total level of proteinuria has played an important role in the assessment of kidney disease, and the term 'proteinuric' kidney disease may still be used where proteinuria is high, there is now a general shift towards measuring albuminuria.

The rate of urinary albumin excretion per 24 hours (albumin excretion rate, AER) is an essential parameter in the assessment of kidney health (or disease). In the glomerulus, a filtration barrier limits the filtration of albumin from the plasma into the urinary space based on its size and charge. In health, the AER is < 20 mg per 24 hours, and levels higher than this may reflect kidney disease, especially of the glomerulus where damage to or dysfunction of the filtration barrier results in increased albumin filtration.

The gold standard measure of the AER is from a 24-hour urine collection, calculated as the product of the urine volume and the albumin concentration. However, a 24-hour urine

collection is cumbersome to collect and is often performed incorrectly. Therefore in routine clinical practice, the concentrations of albumin and creatinine in an untimed single-void urine specimen are often used to estimate the AER. Based on the principle that the average urine excretion of creatinine in adults is 1 g (equivalent to 8.8 mmol) per 24 hours, the AER can be estimated from the untimed specimen by calculating the urine albumin-to-creatinine ratio (ACR). For example, a urine ACR of 500 mg/g (or 56.8 mg/mmol) would be approximately equivalent to an AER of 500 mg per 24 hours (thus suggesting kidney disease).

As with the serum creatinine concentration, urinary creatinine excretion correlates with muscle mass. Therefore, in individuals with unusually high or low levels of muscle mass, the urine ACR may not be an accurate estimate of the AER. For this reason, AER estimating equations have been developed which, similar to the eGFR equations, incorporate surrogates for muscle mass (age, sex, and ethnicity) in addition to the urine creatinine concentration. This allows the urine albumin concentration to be adjusted for the *expected* urinary creatinine excretion rather than the average 1 g (8.8 mmol) per 24 hours. However, such estimating equations are not currently used in routine clinical practice in the UK, and the ACR (expressed in mg/mmol) continues to be recommended by NICE as the preferred method for estimating the AER (22).

A urine ACR of 3 mg/mmol or higher (approximately equivalent to an AER ≥ 30 mg per 24 hours) is generally considered to represent increased albuminuria and is a marker of kidney disease.

1.1.3.2. Haematuria

Glomerular damage may also result in the passage of red blood cells into the urine, resulting in haematuria. This may be visible (previously termed “macroscopic haematuria”)

but is more often non-visible and detected on urinalysis (previously termed “microscopic haematuria”).

Haematuria may be a manifestation of disease in the urinary tract, rather than the glomerulus. However, haematuria in the presence of other markers of kidney disease such as a decreased GFR or increased albuminuria increases the likelihood that the haematuria is glomerular in origin.

1.1.3.3. Radiographic abnormalities of the kidneys

Multiple radiological techniques may be employed in the assessment of kidney disease, but ultrasound is the most commonly used. Common radiographic markers of kidney disease include decreased kidney size, thinning or scarring of the renal cortex, increased echogenicity of the renal parenchyma, and cysts, among others.

1.1.3.4. Others

There are many other potential markers of kidney disease, such as the presence of certain casts in the urine or histological abnormalities in those who undergo a kidney biopsy. These are described further in section 1.2.1.

1.2. Chronic kidney disease (CKD)

Numerous different disease pathways can result in a persistent alteration of the function or structure of the kidneys, ultimately resulting in CKD (34). CKD is usually irreversible and is manifest by the markers of kidney disease described above.

1.2.1. Definition of CKD

CKD was first defined in 2002, in the 'Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines for CKD', as the presence of kidney damage (resulting in structural or functional abnormalities) or a decreased GFR (< 60 ml/min/1.73 m^2) for at least three months, and this definition has been broadly accepted internationally (35). It is recognised, however, that not all persistent abnormalities of kidney structure or function are associated with adverse health consequences, and therefore the Kidney Disease: Improving Global Outcomes (KDIGO) group have recommended the addition of 'with implications for health' to the above definition (36). Thus, the current definition of CKD and that pertained to in this thesis is 'abnormalities of kidney structure or function, present for at least three months, with implications for health' (36). Abnormal kidney function is considered to be a GFR < 60 ml/min/1.73 m^2, and the myriad potential markers of kidney damage that may precede the development of a decreased GFR are shown in Table 1.1 (36).

Table 1.1. Criteria for the definition of CKD

Criteria	
Markers of kidney damage (one or more)	Albuminuria (ACR ≥ 3 mg/mmol)
	Urine sediment abnormalities
	Electrolyte and other abnormalities due to tubular disorders
	Abnormalities detected by histology
	Structural abnormalities detected by imaging
	History of kidney transplantation
Decreased GFR	GFR < 60 ml/min/1.73 m^2

One or more markers of kidney damage or a decreased GFR present for > 3 months. From (36).

1.2.2. Staging of CKD

Once CKD has been identified, disease staging may help guide management and provides information on prognosis. The original staging system consisted of five GFR stages (1 to 5) only. Later, stage 3 was divided into stages 3a and 3b, based on analysis of data on the risk of adverse clinical outcomes associated with the level of GFR, and more recently the staging system has been developed further to also include the cause of CKD and the level of albuminuria (CGA [cause of CKD, GFR, albuminuria] staging) (36).

The cause of CKD in an individual is most often inferred from the presence of co-morbid conditions that may cause kidney disease (e.g. diabetes mellitus, vascular disease, hypertension) and an assessment of the potential markers of kidney disease as described above. A minority of patients with CKD undergo a kidney biopsy which may more definitively establish the underlying cause. Specific causes of CKD are discussed in section 1.2.5, but many patients with CKD, especially those who present for the first time at a late stage of the disease, have CKD of unknown cause.

The GFR is categorized into six stages, as shown in Table 1.2. The associated terminology for each category is relative to the GFR expected in a young adult.

Table 1.2. GFR categories for the staging of CKD

GFR Category	GFR (ml/min/1.73 m^2)	Terms
G1	≥ 90	Normal or high
G2	60 to 89	Mildly decreased
G3a	45 to 59	Mildly to moderately decreased
G3b	30 to 44	Moderately to severely decreased
G4	15 to 29	Severely decreased
G5	< 15	Kidney failure

In the absence of other evidence of kidney damage, neither GFR category G1 nor G2 fulfils the criteria for CKD. From (36).

Albuminuria categories, based on the AER, are shown in Table 1.3. The approximate equivalent urine ACR levels are also shown. The terms here also describe the AER relative to that expected in a young adult.

Table 1.3. Albuminuria categories for the staging of CKD

Category	AER (mg/24 hours)	Urine ACR (mg/mmol)	Terminology
A1	< 30	< 3	Normal to mildly increased
A2	30 to 300	3 to 30	Moderately increased
A3	> 300	> 30	Severely increased*

**Including nephrotic syndrome (AER usually > 2.2g per 24 hours [ACR > 220 mg/mmol]). From (36).*

As an example of the use of the CGA staging system, an individual with a long history of DM and an eGFR of 24 ml/min/1.73 m^2 and an ACR of 18 mg/mmol would be classed as having CKD stage G4 A2 due to diabetic kidney disease.

1.2.3. Prevalence of CKD

CKD is common, with a prevalence in adults of approximately 10%. Differences in study populations, methods, and definitions have resulted in varying estimates of prevalence. Importantly, estimates have often been made based on single measures of kidney function or

structure that do not strictly meet the chronicity assumption for the accepted definition of CKD.

Most studies have identified CKD using only the GFR (and no other markers of kidney damage). Table 1.4 provides a summary of CKD prevalence estimates in the UK based on eGFR.

Table 1.4. Estimates of the UK prevalence of CKD stage G3 to G5

Years	Study population	*N*	Number of eGFRs	Prevalence (%)	Source
1998 to 2003	Primary care	38,262	1	8.5	(37)
2002 to 2008	Primary care	6,048,159	2	4.5	(38)
2004	Primary and secondary care	123,121	1	5.4	(39)
2005	Acute hospital admissions	6,073	1	17.7	(40)
2007 to 2010	Primary care	930,997	2	6.76	(41, 42)
2009 to 2010	Primary and secondary care	123,121	1	5.6	(39)
2009 to 2010	General population	6,046	1	6.1	(43)
2009 to 2011	Primary care	175,671	1	14.5	(44)
2010	Primary care	2,836,476	2	5.9	(45)

CKD defined as an eGFR < 60 ml/min/1.73 m^2.

There are few estimates of the prevalence of increased albuminuria or the other markers of CKD. However, in the UK, among over 20,000 individuals recruited from the general population between 1993 and 1997 (the EPIC-Norfolk Study), the estimated prevalence of a urine ACR of 2.5 to 25 mg/mmol was 11.8% and for a urine ACR of > 25 mg/mmol was 0.9% (based on a single urine specimen) (46). International estimates of CKD prevalence that include both the eGFR and albuminuria to define CKD, albeit based on single

assessments, include estimates of between 3% and 17% in the countries of Europe (47) and 14.8% in the US (not including those with established kidney failure) (48).

Globally, the prevalence of CKD has been estimated to be 13.4 % for all CKD stages and 10.6% for stages G3 to G5, based on a meta-analysis of 100 general population studies (49). The global burden of CKD may be increasing: data from the 'Global Burden of Disease' study showed that between 1990 and 2013 the rates of death and disability-adjusted life-years associated with CKD increased, in contrast to other non-communicable diseases, including in Western Europe (50).

Based on data obtained via the Global Burden of Disease Results Tool (51) (which incorporates CKD data from the Office for National Statistics and the UK Renal Registry), Figure 1.2 shows the incidence, prevalence, and deaths due to CKD in the UK from 2004 to 2017. The figure shows there is an increasing trend in all three parameters.

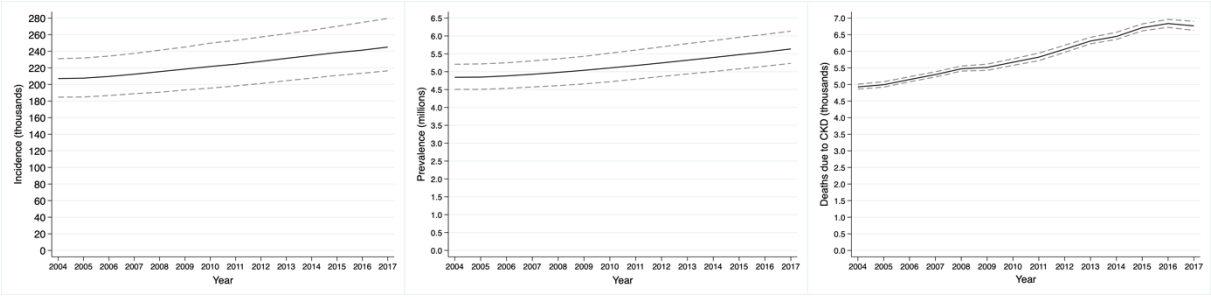


Figure 1.2. Incidence, prevalence, and deaths due to CKD in the UK

Estimates between 2004 and 2017, with 95% confidence intervals, based on data from the Office for National Statistics and the UK Renal Registry. Incidence and deaths due to CKD are expressed in thousands, and prevalence in millions.

1.2.4. Financial cost of CKD

There is considerable uncertainty as to the cost to the NHS of CKD. The total cost includes direct costs (associated with CKD itself and its progression, including kidney failure), and indirect costs incurred for non-renal care in cases where people with CKD have excess risk or consume excess health care resources relative to the non-CKD population such as excess length of hospital stay, CVD, and infection (52). The cost to the NHS of CKD in England in 2009–10 was estimated to be £1.45 billion, accounting for 1.3% of all NHS spending (53). As can be seen in the cost breakdown in Figure 1.3, the provision of KRT for those who have progressed to kidney failure is particularly expensive.

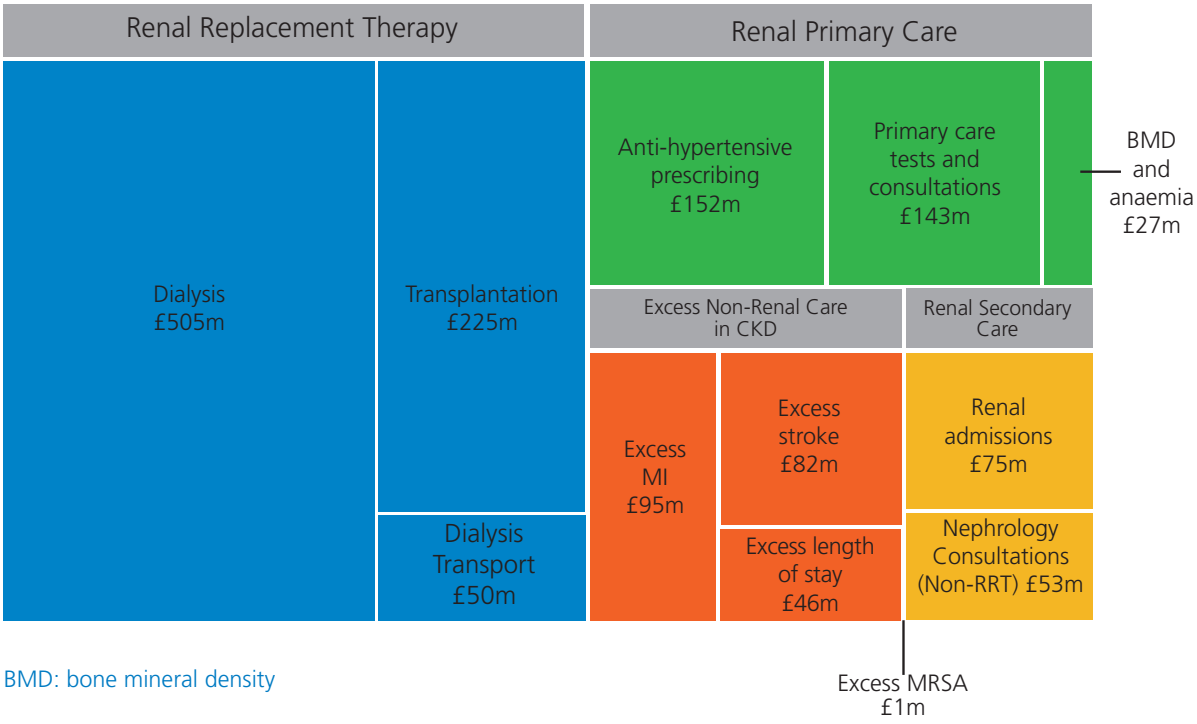


Figure 1.3. Financial costs to the NHS of CKD

Estimates of direct and indirect NHS expenditure on CKD in England, 2009-10. From (52).

There are no comparable estimates of current NHS expenditure on CKD that have been published, but it is highly likely to be higher than in 2009-10, especially given the increasing incidence and prevalence of CKD. The economic burden of CKD among individuals with DM in the UK is projected to rise markedly over time and has been forecasted at approximately £11.4 billion in 2025 (54).

1.2.5. Causes of CKD

Multiple heterogeneous disease pathways can result in CKD. The traditional way of classifying kidney disease has been to consider aetiologies that are pre-renal (reduced kidney perfusion), intrinsic to the kidneys (which can be further subdivided into diseases that primarily damage the vessels, the glomerulus, or the tubulointerstitium), and post-renal (urinary tract obstruction). Using this classification, the common causes of CKD are shown in Table 1.5.

Table 1.5. Common causes of CKD

	Causes of CKD
Pre-renal	Heart failure, cirrhosis
Intrinsic	
Vascular	Renal artery stenosis, hypertensive nephrosclerosis
Glomerular	Diabetic nephropathy, IgA nephropathy
Tubulointerstitial	Polycystic kidney disease, reflux nephropathy
Post-renal	Prostatic disease, abdominal or pelvic tumour

Causes of CKD by the traditional classification system of pre-renal, intrinsic, and post-renal causes.

Diabetes mellitus is the most common cause of CKD and the most common cause of kidney failure (that is, the need for dialysis or a kidney transplant). Table 1.6 shows the cause of CKD in patients with incident kidney failure in the UK in 2017.

Table 1.6. Causes of CKD in patients with incident kidney failure

Cause of CKD	**%**
Diabetes	29.4
Glomerulonephritis	14.1
Hypertension	6.3
Polycystic kidney disease	6.8
Pyelonephritis	5.7
Renal vascular disease	5.9
Other	16.9
Uncertain	14.9
Missing	14.4

Cause of CKD in adults with incident kidney failure in the UK in 2017, from the UK Renal Registry 21st Annual Report (55).

The underlying causes of CKD are different in the nature and site of the initial injury. For example, the immune-mediated injury to the glomeruli in glomerulonephritis compared to a genetic defect leading to cyst formation affecting the tubulointerstitium in polycystic kidney disease. Although the initial kidney insult may predominantly injure a particular kidney structure (i.e. the vessels, glomeruli, tubules, or interstitium), progression of CKD, irrespective of the primary cause, is associated with pathogenetic processes that result in damage and fibrosis to all components of the kidney resulting in altered structure and loss of function. Thus glomerulosclerosis, tubulointerstitial fibrosis, and vascular sclerosis are the pathological hallmarks of established CKD.

1.2.6. Common disease pathways and fibrosis in CKD

All primary causes of CKD share a common yet complex pathogenetic pathway of progressive injury and destruction of the normal kidney parenchyma due to fibrosis. There may be ongoing injury from the primary cause of CKD, but secondary maladaptive haemodynamic and metabolic factors play a pivotal role.

With nephron loss, there is an increase in pressure within the remaining glomeruli (intraglomerular hypertension) and an increase in filtration in the preserved nephrons (glomerular hyperfiltration). These adaptive responses allow the GFR to be preserved initially, even after nephron loss. However, intraglomerular hypertension and glomerular hyperfiltration are associated with increasing wall stress and damage to the glomerular endothelial cells (56). Further, intraglomerular hypertension leads to excessive expansion of the relatively elastic glomeruli and repetitive cycles of distension contraction, resulting in mechanical strain on mesangial cells which stimulates their production of cytokines (including transforming growth factor-beta [TGF-β]) and more extracellular matrix (56).

Intraglomerular hypertension and glomerular hyperfiltration are also associated with proteinuria, which itself has an important role in progressive fibrosis. Filtered proteins or albumin-bound factors (such as fatty acids) may cause tubular cell toxicity and local release of pro-inflammatory molecules and cytokines with the promotion of interstitial fibrosis (57, 58).

The development of interstitial fibrosis can be summarised by the response to injury of four cells: macrophages, myofibroblasts, tubular epithelial cells, and endothelial cells (59):

1. There is an interstitial inflammatory cell infiltrate composed primarily of macrophages. Depending upon local environmental cues, these macrophages can synthesise and secrete products that can influence fibrogenesis, such as cytokines (e.g. TGF-β), growth factors (e.g. platelet-derived growth factor), procoagulant factors, and matrix proteins (60).

   The macrophages can differentiate, depending upon local stimuli, into either pro-inflammatory (M1) or anti-inflammatory (M2) subtypes. M1 responses are associated with ‘maladaptive’ tissue repair with irreversible parenchymal loss and CKD, whereas

M2 responses are associated with ‘adaptive’ tissue repair, minimal scarring, and the restoration of normal parenchyma (59).

2. Myofibroblasts appear in the interstitium, derived primarily from resident kidney fibroblasts and pericytes. In severely damaged kidneys, they are also derived from the transdifferentiation of tubular epithelial cells and endothelial cells. These myofibroblasts are the primary source of scar-forming extracellular matrix proteins, and their presence is essential for scar formation (59).
3. Tubular epithelial cells can synthesise numerous products which can enter the interstitium such as reactive oxygen species, inflammatory chemokines, and profibrotic molecules (e.g. TGF-β) (59, 61). Proteinuria may be an important factor in this pathway, as filtered urinary proteins such as those of the complement cascade, cytokines, and biochemically modified or conjugated albumin may bind receptors expressed by tubular epithelial cells activating intracellular signalling pathways and cellular responses (57, 59). Tubular epithelial cells may also be stimulated to transdifferentiate into myofibroblasts. In severe fibrosis, the tubular epithelial cells lose their ability for regeneration, resulting in apoptosis, and non-functional atubular glomeruli (59).
4. Loss of interstitial capillary integrity with leakage into the interstitium of plasma proteins such as fibrinogen and albumin conjugates triggering an inflammatory and profibrotic response (59). There is also inadequate reparative angiogenesis and loss of the interstitial capillary network, compromised oxygen delivery, and hypoxia–oxidant stress, accentuating injury and fibrosis (59).

The importance of TGF-β as a molecular driver of fibrosis is well known. It is produced by tubular and interstitial cells and engages cellular receptors to stimulate

fibroblasts and myofibroblasts (59). However, many other fibrosis-promoting molecules contribute, and in particular angiotensin II, through activation of type 1 receptors on glomerular cells with the generation of various profibrotic factors, and cytokine- and chemokine-mediated recruitment of inflammatory cells into the kidney (62).

The processes described result in fibrotic tissue that is a sophisticated collection of multifunctional macromolecules that change in composition and structure over time (59). Collagen types I and III predominate, but other collagens and matrix molecules are important (59). The matrix molecules elicit cellular responses via cellular receptors that result in fibrosis-induced cellular loss and parenchymal destruction (59).

Remodelling and degradation of the fibrotic tissue can occur through multiple enzymatic pathways, such as the family of matrix metalloproteinases, and cellular endocytosis and proteolysis of collagen (59).

1.3. Prognosis

The primary focus of this thesis is on prognosis and prognostic factor research in CKD. The concept of prognosis is generally understood to entail a prediction about the likely outcome for somebody with a given disease (the original Greek word, *prognōsis*, was derived from *pro-* 'before' + *gignōskein* 'know'). This section introduces the context and importance of prognosis within clinical practice, the relevant definitions, and a framework for considering prognosis research.

1.3.1. The role of prognosis in clinical practice

Prognosis, along with diagnosis and treatment, is incorporated into the traditional model of clinical practice. It has a vital role in informing and guiding the decision-making of patients, healthcare providers, and policymakers (63, 64). Although the practice of prognostication has existed since prehistory, the value placed upon prognosis has varied greatly over the millennia.

In the time of Hippocrates (the fifth and fourth centuries BC), diagnostic tools and medical therapies were immature and therefore estimating prognoses was prominent in the role of the physician, as is evident from the well-known opening sentence of Hippocrates' *Prognostics* (64, 65):

> "It appears to me a most excellent thing for the physician to cultivate Prognosis; for by foreseeing and foretelling, in the presence of the sick, the present, the past, and the future, and explaining the omissions which patients have been guilty of, he will be the more readily believed to be acquainted with the circumstances of the sick; so that men will have confidence to intrust themselves to such a physician."

Making prognoses remained central to the role of the physician over the next two millennia, and were based primarily on the physician's cumulative observations of previous patients (64). However, from the 17th century, there were significant advances in biology and

the understanding of mechanisms of disease, and the perceived value of prognosis in the role of the physician declined (64). By the mid-19th century, further developments in the biomedical sciences and diagnostic tools such as the stethoscope, the microscope, and radiology, meant that diagnosis, rather than prognosis, reigned supreme in the role of the physician, and within this zeitgeist, prognoses were considered a characteristic of a disease rather than an individual (64, 66).

Recently, there has been a significant revival in the value placed upon prognosis. Scientific advances, in particular in the '-omics' (e.g. genomics, proteomics, and metabolomics) allow for the possibility of understanding, at the molecular level, why prognoses differ between individuals with the same disease and why there is variation in response to treatments (64).

Further, the availability of big data, incorporating not just routine demographic and health information but novel biological variables such as the '-omics' mentioned above, allows the discovery of characteristics which may be associated with variation in prognosis in certain health conditions (64).

These developments have coincided with an increasing interest in the practice of stratified (or personalised) medicine. This involves stratifying patients with a health condition by their likelihood of a particular outcome or response to a specific treatment. In contrast to a one-size-fits-all approach, treatments can be focused on those who will benefit, and the unnecessary costs and side effects associated with treating those who will not benefit can be avoided (64).

Finally, population-level prognosis information has played an increasingly important role at a managerial and political level to understand the performance of healthcare systems and the impact of changes in healthcare delivery and policy (64).

Despite a revival in the role of prognostication, there had been little progress in the development of its methodology over the centuries, the terminology used was inconsistent, and concepts were muddled (67). A group of healthcare professionals, researchers, and journal editors (the PROGnosis RESearch Strategy [PROGRESS] Partnership) addressed this by developing a framework which provides precise definitions and a clear framework for the understanding of prognosis and prognosis research (64).

1.3.2. The PROGRESS Framework

The PROGRESS framework was set out in a series of four papers published in 2013 (68-72). The framework provides standardised terminology and recommendations for the optimal study designs and statistical analyses of four distinct types of prognosis research, as outlined in this section (64).

Prognosis is the risk of future health outcomes in people with a given disease or health condition, and prognosis research is the investigation of the relations between future outcomes (endpoints) among people with a given baseline health state in order to improve health (68). The four types of prognosis research, as set out in the PROGRESS framework, are summarised in Table 1.8, and described in the following paragraphs.

Table 1.7. Types of prognosis research

Type of research	Objective
Fundamental prognosis research	Estimate the average outcome risk in a population with a given health condition in the context of the nature and quality of current healthcare
Prognostic factor research	Establish which characteristics are associated with changes in the average prognosis across individuals with a given health condition
Prognostic model research	The development, validation, and impact evaluation of models incorporating multiple prognostic factors to estimate an individual's outcome risk
Stratified medicine research	Establish which characteristics predict whether or not an individual responds to a particular treatment

Adapted from (72).

1.3.2.1. Fundamental prognosis research

Fundamental prognosis research (PROGRESS framework type I) provides an overall estimate of prognosis for a given health condition, i.e. an estimate of the average risk of a particular outcome among a group of individuals with a particular disease or health condition, in the context of the nature and quality of healthcare available at the time and place of the study (72). This is distinct from the natural history of a disease, which is the prognosis in the absence of care (68).

Examples of fundamental prognosis research include: (i) an estimated 22% of patients who sustain a wrist or hand fracture will have persistent pain at four months following the injury (73); (ii) among patients who have a spontaneous intracerebral haemorrhage, an estimated 46% will be alive at one year (74).

Information on overall prognosis from fundamental prognosis research can be essential to inform the decision-making of patients, clinicians, and healthcare planners. The overall prognosis (i.e. patient outcomes) for a particular health condition may be used as a measure of the performance of a health service, facilitating audit and the assessment of

change in response to particular measures, and may also allow a comparison between health services (e.g. between different countries).

1.3.2.2. Prognostic factor research

Prognostic factor research (PROGRESS framework type II) studies aim to identify prognostic factors. Prognostic factors are characteristics associated with differences in the outcome risk between individuals with a given health condition. A different value (or category) of a prognostic factor is associated with a different outcome risk, and prognostic factors, therefore, explain variation in outcomes across individuals with a given disease.

Examples of prognostic factor research include: (i) among patients admitted to hospital with an acute coronary syndrome, a higher serum uric acid concentration is associated with a higher risk of all-cause mortality (75); (ii) among patients with bipolar disorder, a higher level of physical activity is associated with a lower risk of requiring psychiatric hospitalisation (76).

Prognostic factors may provide information on pathophysiology, may identify targets for developing novel treatments, and may be used as a marker of treatment effect. Prognostic factors are also required to develop prognostic models.

1.3.2.3. Prognostic model research

Prognostic model research (PROGRESS framework type III) involves the development and validation of models which combine prognostic factors. A prognostic model incorporates multiple prognostic factors and allows the risk of a specific outcome to be calculated for individual patients, based on their values for the prognostic factors included in the model (70, 72). Synonyms for ‘prognostic model’ that are encountered in the medical

literature are prognostic (or prediction) index or rule, risk (or clinical) prediction model, and predictive model.

An example of prognostic model development was the derivation of a model which, for patients with primary melanoma, accurately predicts the risk of death at ten years from the point of diagnosis (77). The model allows the risk to be calculated based on the values of four readily available prognostic factors: patient age, sex, site of the primary melanoma, and tumour thickness (77).

Prognostic model research may also be performed to update a previously developed prognostic model (70, 72). This could be the recalibration of a prognostic model for use in a new setting, or the addition of new prognostic factors to the existing model (72). With regard to the latter, it may be expected that the addition of prognostic factors with a causal effect on the outcome results in models that perform better and are more generalisable since they are linked to biological pathways rather than merely based on statistical association (70). However, the inclusion of novel prognostic factors that are expensive or not readily available could be a barrier to the use of a prognostic model (70).

Where the information from a prognostic model leads to changes in clinical management, prognostic models can influence the patient outcome or the cost-effectiveness of care (positively or negatively). Prognosis research may, therefore, include clinical impact studies that aim to evaluate the impact of implementing a prognostic model on clinical practice and patient outcomes (70, 72). Although there are various potential designs for such clinical impact studies, it ideally includes a comparison of two cohorts, one in which usual care is provided and the other in which prognostic model predictions are made available to health professionals to guide treatment decisions (70).

1.3.2.4. Stratified medicine research

Information on the effectiveness of a particular therapeutic intervention can be obtained from randomised trials, but an individual's response to the intervention may deviate from average (71). Stratified medicine research (PROGRESS framework type IV) aims to identify factors that predict treatment effects (benefits or harms) in individuals with a particular health condition (72). These factors may then be used to practice stratified medicine. In contrast to 'all-comer' or 'empirical' medicine, stratified medicine seeks to target therapy to those who are predicted to benefit the most or sustain the least harm (71).

For example, in patients with breast cancer, human epidermal growth factor receptor 2 (HER-2) status, in addition to its baseline prognostic information, is used to determine whether a patient will respond to treatment with trastuzumab (an antibody against HER-2), such that trastuzumab is now given to patients who are HER-2 positive, but not to those testing negative (71, 78).

Stratified medicine may also be practised when the relative effect of a particular treatment is the same for all patients. In this situation, treatment may be targeted at those with the highest absolute risk and who will, therefore, have the largest absolute benefit from treatment (71). An example of this is the decision to give a statin to individuals with cardiovascular risk, estimated from a prognostic model, above a certain threshold (71, 79).

In part related to the enormous growth in '-omics' studies, and the availability of expensive new treatments, there is growing consensus that treatment decisions should be guided by stratified care and personalised medicine to maximise benefit and reduce harm and costs. As such, stratified medicine research is likely to play an increasingly important role in the coming decades.

1.4. Prognosis in CKD

There are many complications and adverse outcomes associated with CKD that can contribute to the overall burden of illness (80). There is an increased risk of early mortality, most often due to cardiovascular disease (CVD) (81-83), and an increased risk of adverse kidney outcomes, including progression to kidney failure (83-85). There is a myriad of other potential complications, including anaemia, mineral-bone disorder, malnutrition, infection (86), frailty (87), and impairment of learning and concentration (88).

The primary outcomes assessed in this thesis are kidney failure and death. While some patients with CKD will suffer an early cardiovascular death or have rapidly progressive CKD that results in kidney failure, some individuals have CKD that never progresses and who live to a healthy life expectancy. For kidney failure, death, and most other CKD complications, the risks vary depending on the cause of CKD, the GFR, the degree of albuminuria, and other factors such as co-morbid conditions (36). The most recent CKD staging approach (CGA staging) reflects the contribution that each component makes to prognosis, as each CGA component provides prognostic information independent of the other components (36). Figure 1.4 shows the risk of adverse outcomes such as death and kidney failure by the GFR and albuminuria categories.

			Albuminuria categories (mg/mmol)		
			A1	A2	A3
			< 3	3-30	> 30
GFR categories (ml/min/1.73 m^2)	G1	≥ 90			
	G2	60-89			
	G3a	45-59			
	G3b	30-44			
	G4	15-29			
	G5	< 15			

Figure 1.4. Prognosis of CKD by GFR and albuminuria category

The shaded areas reflect the risk of adverse outcomes, such as death and progression to kidney failure by GFR and albuminuria category: green is low risk, yellow is moderately increased risk, orange is high risk, and red is very high risk. Adapted from the KDIGO CKD guideline (89).

As an example, a patient with CKD with an eGFR of 24 ml/min/1.73 m^2 and an ACR of 18 mg/mmol should be considered to be at a very high risk of adverse outcomes.

1.4.1. Kidney failure

Kidney failure is defined as CKD with the requirement for KRT, i.e. either dialysis or kidney transplantation. Common synonyms for kidney failure in the medical literature are end-stage kidney disease (ESKD), end-stage renal disease (ESRD), and end-stage renal failure (ESRF).

It is well established that individuals with CKD are at a higher risk of kidney failure compared to those without CKD (82, 90, 91). The CKD Prognosis Consortium performed a meta-analysis of nine general population cohorts incorporating 845,125 participants and showed that having an eGFR < 60 ml/min/1.73 m^2 or increased albuminuria (a urine ACR > 3

mg/mmol), i.e. markers of CKD, are associated with a higher risk of kidney failure (82). Compared to those with an eGFR ≥ 60 ml/min/1.73 m^2, those with an eGFR of 45 to 59, 30 to 44, and 15 to 29 ml/min/1.73 m^2 had HRs (95% CI) for kidney failure of 9.6 (7.0 to 13.2), 98.1 (61.8 to 156), and 573 (241 to 1362), respectively (after adjustment for age, sex, race, CVD history, smoking status, DM, systolic blood pressure [BP], serum total cholesterol, and urine ACR) (82).

Compared to those with a urine ACR < 3 mg/mmol, those with a urine ACR of 3 to 29 or ≥ 30 mg/mmol had HRs for kidney failure of 12.0 (7.9 to 18.1) and 72.1 (43.0 to 121) respectively (after adjustment for age, sex, race, CVD history, smoking status, DM, systolic BP, serum total cholesterol, and eGFR) (82).

The same paper also included a separate meta-analysis of eight cohort studies of patients with DM, hypertension, or CVD (incorporating 173,892 participants) (82). This meta-analysis showed that in individuals with these co-morbidities, an eGFR < 60 ml/min/1.73 m^2 or a urine ACR > 3 mg/mmol is associated with a higher risk of kidney failure, with similar risk associations as those seen in the general population (82).

1.4.1.1. Prognostic factors for kidney failure

Each component of the CGA staging framework provides prognostic information concerning the risk of kidney failure in patients with CKD.

With regard to the cause of CKD, both polycystic kidney disease and diabetic nephropathy are associated with a higher risk of kidney failure compared to other causes of CKD. In a prospective cohort study incorporating 729 patients with CKD, compared to those with hypertensive nephropathy, those with CKD due to polycystic kidney disease had a five-

fold higher risk of kidney failure (adjusted HR 5.46 [2.28 to 10.6]), and there was also a higher risk in those with diabetic nephropathy (adjusted HR 1.96 [1.28 to 2.99]) (92).

The GFR and degree of albuminuria have also been shown in multiple studies of patients with CKD to be independently associated with the risk of progression to kidney failure (90, 93, 94). There is an independent inverse association between eGFR and the risk of kidney failure. In a meta-analysis of 13 prospective cohort studies incorporating 21,688 patients with CKD, a lower eGFR was associated with a higher risk of kidney failure (HR 6.24 [4.84 to 8.05] per 15 ml/min/1.73 m^2 lower eGFR, adjusted for age, sex, race, prior CVD, smoking status, DM, systolic BP, serum total cholesterol concentration and albuminuria) (95). A graphical representation of the association is shown in Figure 1.5.

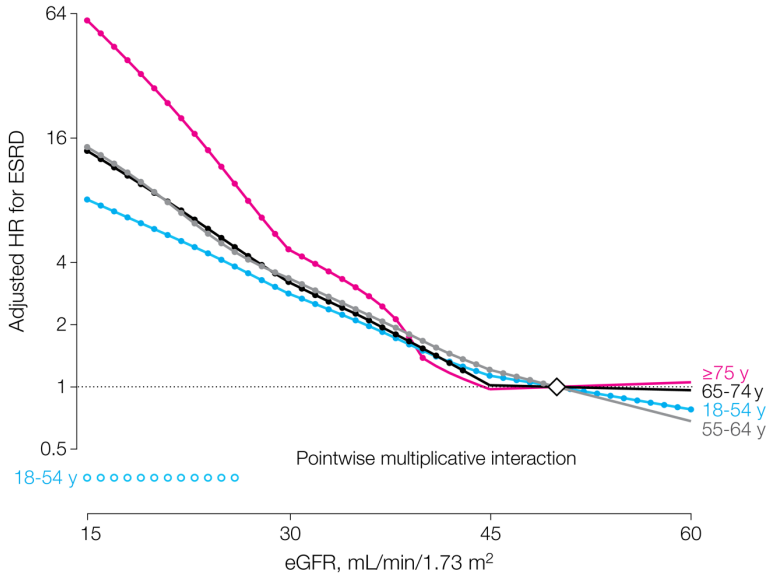


Figure 1.5. Association between eGFR and kidney failure in CKD

Relationship between eGFR and kidney failure, by age category, in a meta-analysis of 13 CKD cohort studies. Adjusted HR for kidney failure is relative to an eGFR of 50 ml/min/1.73 m^2, adjusted for sex, race, body mass index (BMI), systolic BP, total cholesterol, history of CVD, DM, smoking status, and albuminuria. From (96).

In the same meta-analysis, a higher urine ACR was also independently associated with a higher risk of kidney failure (HR 3.04 (95% CI 2.27 to 4.08) per eight-fold higher ACR, adjusted for age, sex, race, prior CVD, smoking status, DM, systolic BP, serum total cholesterol concentration and eGFR) (95). A graphical representation of the risk of kidney failure by urine ACR is shown in Figure 1.6.

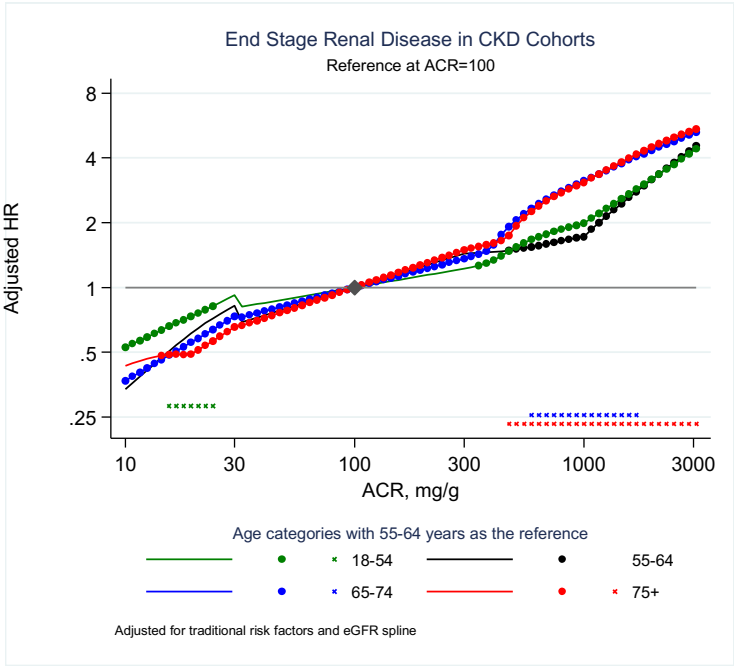


Figure 1.6. Association between urine ACR and risk of kidney failure in CKD

Relationship between urine ACR and kidney failure, by age category, in a meta-analysis of 13 CKD cohort studies. Adjusted HR for kidney failure by urine ACR, within age categories, compared to a urine ACR of 100mg/g (black diamond), adjusted for sex, race, BMI, systolic BP, total cholesterol, history of CVD, DM, smoking status, and eGFR. From (96).

Among the other prognostic factors for the risk of kidney failure, age is important: younger patients with CKD have a higher risk of kidney failure compared to older patients with CKD. In a community-based cohort study of nearly 2 million Canadian adults, the rate of

kidney failure was higher in younger age groups at all levels of eGFR (97). For example, among those with an eGFR of 15 to 29 ml/min/1.73 m^2, the adjusted rate of kidney failure for those aged 18 to 44 years was 24.0 per 1000 person-years, compared to 1.5 per 1000 person-years for those aged 85 years or older ($P < 0.001$) (97).

Examples of other reported prognostic factors for kidney failure in patients with CKD are given in Table 1.8.

Table 1.8. Examples of prognostic factors for kidney failure in CKD

Prognostic factor	Measure	Reference	Adjusted HR (95% CI)	Study
Ethnicity	Black	White	4.8 (2.9 to 8.4)	(98)
Systolic BP (mmHg)	> 157	≤ 128	1.28 (1.01 to 1.61)	(99)
	≥ 150	< 130	1.36 (1.02 to 1.85)	(100)
	Per +10		1.26 (1.18 to 1.34)	(101)
Diastolic BP (mmHg)	> 80	≤ 64	1.36 (1.07 to 1.73)	(99)
	≥ 90	60 to 74	1.81 (1.33 to 2.45)	(100)
Incident co-morbidities	Atrial fibrillation		3.2 (1.9 to 5.2)	(102)
	Major depressive episode		3.51 (1.77 to 6.97)	(103)
APOL1 gene variants	2 copies	0 copies	2.21 (1.56 to 3.14)	(104)

APOL1 = apolipoprotein L1; BP = blood pressure; CI = confidence interval; HR = hazard ratio.

1.4.1.2. Prognostic models for kidney failure

A prognostic model to predict the 5-year risk of kidney failure in patients with CKD was published in 2010 (105). It was developed in 382 patients with CKD stages G3a to G5 from the Chronic Renal Impairment in Birmingham (CRIB) prospective cohort study, and incorporates sex, serum creatinine, serum phosphate, and urine ACR. The 5-year risk (%) of kidney failure is calculated as:

$$5\ year\ risk\ (\%) = 1 - 0.73^{e^{2.90 \times ln\ (creatinine/2.8) + 1.45 \times ln\ (phosphate/4.0) + 0.26 \times ln\ (ACR/350) + 0.43\ (sex)}}$$

where serum creatinine and phosphate are in mg/dl, urine ACR is in mg/g, and sex is 0 for males and 1 for females.

External validation in a cohort of 213 patients with CKD suggested the model has moderate ability to predict kidney failure with a C statistic of 0.91 (95% CI 0.87 to 0.96), but the model was not taken up in routine clinical practice.

More recently, Tangri et al. have developed models that accurately predict the two- and five-year risk of kidney failure in patients with CKD. Multiple models were developed using data from patients referred to nephrology services in Canada with a GFR < 60 ml/min/1.73 m^2 (i.e. stages G3a to G5) (106). A four-variable equation (age, sex, eGFR, and urine ACR) and an eight-variable equation (the four variable plus serum calcium, phosphate, bicarbonate, and albumin) have both since been validated using data from 31 cohort studies incorporating over 700,000 individuals with CKD G3a-G5 in more than 30 countries worldwide (107).

The two-year risk (%) of kidney failure is calculated from the four-variable equation as follows:

$$1 - 0.9832^{e^{(-0.2201 \times (age/10 - 7.036) + 0.2467 \times (sex - 0.5642) - 0.5567 \times (eGFR/5 - 7.222) + 0.4510 \times (logACR - 5.137))}}$$

where age is in years, sex is 1 for males and 0 for females, and urine ACR is in mg/g.

This four-variable equation (known as the 'Kidney Failure Risk Equation' [KFRE]) is readily available as a web calculator (https://kidneyfailurerisk.com) and is likely to start being used more widely in clinical practice in the coming years. A planned update of the NICE CKD guideline is likely to include for the first time a recommendation that the KFRE should be used to aid patient decision making and prognostication (108). For example, when

deciding whether a patient with CKD should be managed in primary care or secondary care or when a patient should have dialysis access formed or transplant workup initiated, the prognostic information provided by KFRE may aid decision-making.

In 2018, Grams et al. developed models for patients with an eGFR < 30 ml/min/1.73 m^2 that are arguably even more sophisticated. Developed using data from 264,296 individuals in 30 countries from 29 cohorts participating in the CKD Prognosis Consortium, the models estimate not only the two- and four-year risk of kidney failure, CVD events, and death, but also the relative order of these outcomes (109). The models incorporate nine demographic and clinical prognostic factors: age, sex, ethnicity, history of CVD, smoking status, DM, systolic BP, eGFR, and urine ACR. Substantial risk factors for developing kidney failure as a first event included younger age, black ethnicity, higher systolic BP, lower eGFR, and higher urine ACR (109). The model demonstrated good calibration for estimating the risk of kidney failure and also showed good agreement with the KFRE for the prediction of kidney failure at two years (109).

This model has been made available as a web calculator making it readily accessible for use in clinical practice (http://ckdpcrisk.org/lowgfrevents/). As an example of the use of this prognostic model, a 60-year-old white man with a history of CVD, systolic BP of 140 mmHg, eGFR of 25 ml/min/1.73 m^2, and urine ACR of 3 mg/mmol but no DM and not a current smoker is predicted, at two years, to have a 74% chance of remaining event-free, a 17% chance of having a CVD event, a 9% chance of death, and a 5% chance of kidney failure. The prognostic information, in this case, may reinforce the relative importance of cardiovascular risk reduction, rather than dialysis preparation, for this particular patient.

1.4.1.3. Interventions to reduce the risk of kidney failure

Although patients with CKD have a higher risk of kidney failure compared to those without CKD, their risk may be reduced by measures to slow the rate of GFR decline. These measures may include specific therapy for treatable causes of CKD, such as immunosuppression for immune-mediated kidney disease. However, irrespective of the cause of CKD, therapies to achieve BP control and to achieve a reduction in proteinuria are the two main strategies shown to reduce the risk of kidney failure.

Elevated BP in patients with CKD is associated with a higher risk of kidney failure (see Table 1.8), and treatment to lower BP has been shown to reduce this risk, particularly in those with proteinuria (110-116). For example, in a meta-analysis incorporating data on over 5000 individuals from six cohorts, intensive BP control (< 130/80 mmHg), compared to standard BP control (< 140/90 mmHg), was associated with a lower risk of kidney failure (risk ratio 0.91 [95% CI 0.85 to 0.99]) (115). A separate meta-analysis, incorporating seven trials and 5308 participants, also showed that intensive BP control was associated with a lower risk of kidney failure (HR 0.79 [95% CI 0.67 to 0.93]) (113). However, a subgroup analysis showed that, while intensive BP lowering reduced the risk of kidney failure in people with proteinuria (HR 0.73 [95% CI 0.62 to 0.86]), it did not affect the risk of kidney failure in patients without proteinuria (HR 1.12 [95% CI 0.67 to 1.87]) (113). Both the NICE and KDIGO CKD guidelines recommend a BP target of < 140/90 mmHg and a lower target of < 130/80 mmHg in those with increased albuminuria (22, 36). The NICE CKD guideline also recommends the lower target of < 130/80 mmHg for those with DM (22).

In proteinuric kidney disease, reducing the level of proteinuria with renin-angiotensin-aldosterone system inhibitors (RAASi) such as angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB) has been shown to reduce the risk of GFR

decline and progression to kidney failure (117-121). For example, in meta-analyses examining the effects of ACEi and ARB in patients with moderately or severely increased albuminuria, treatment with an ACEi (9 studies, 7988 patients, relative risk [RR] 0.67 [95% CI 0.54 to 0.84] or an ARB (3 studies, 3298 patients, RR 0.78 [95% CI 0.66 to 0.90]) were associated with a lower risk of kidney failure compared to placebo or no treatment (121). In another meta-analysis, including 21 cohorts and 78,342 participants, a 30% reduction in albuminuria was associated with a 23.7% (95% CI 11.4 to 34.2%) lower risk of kidney failure (120).

Other measures that may reduce the risk of kidney failure in patients with CKD include:

- Treatment of the metabolic acidosis which commonly complicates CKD with supplemental bicarbonate. In a study of 134 patients with CKD and metabolic acidosis in which patients were randomized to either treatment with oral sodium bicarbonate or standard care, those treated with sodium bicarbonate had a lower risk of kidney failure (RR 0.13 [95% CI 0.04 to 0.40]) (122).
- In patients with DM, intensive glycaemic control has been shown to reduce the risk of CKD progression and kidney failure (123, 124). In a trial of intensive (target HbA1c < 6.5%) versus standard (target HbA1c based on local guidelines) glycaemic control in 11,140 patients with type 2 DM, the risk of kidney failure was approximately halved in the intensive control group (HR 0.54 [95% CI 0.34 to 0.85]) (123).

1.4.2. Mortality

There is a wealth of data showing that patients with CKD have a higher risk of death compared to those without CKD (81, 82, 90, 125-129). For example, in the 2018 United

States Renal Data System (USRDS) annual data report, mortality rates were 103 and 43.1 per 1,000 patient-years for those with and without CKD, respectively (adjusted for age, sex, and ethnicity) (130). In a cohort study involving 1,120,295 adults, an increased risk of death was evident at a GFR < 60 ml/min/1.73 m^2 and substantially increased at a GFR < 45 ml/min/1.73 m^2 (126). Compared to an eGFR ≥ 60 ml/min/1.73 m^2, the adjusted HR for death was 1.2 (95% CI 1.1 to 1.2) with an eGFR of 45 to 59 ml/min/1.73 m^2, 1.8 (95% CI 1.7 to 1.9) with an eGFR of 30 to 44 ml/min/1.73 m^2, 3.2 (3.1 to 3.4) with an eGFR of 15 to 29 ml/min/1.73 m^2, and 5.9 (95% CI 5.4 to 6.5) with an estimated GFR < 15 ml/min/1.73 m^2 (126).

In a CKD Prognosis Consortium meta-analysis of 14 general population cohorts with 105,872 participants, there was no increased mortality risk associated with having an eGFR of 60 to 89 ml/min/1.73 m^2 compared to a 'normal' eGFR of 90 to 104 ml/min/1.73 m^2 (81). However, having an eGFR < 60 ml/min/1.73 m^2 was associated with a significantly increased risk of all-cause mortality: an eGFR of 45 to 59, 30 to 44, and 15 to 29 ml/min/1.73 m^2 was associated with HRs for all-cause mortality of 1.28 (1.05 to 1.57), 1.97 (1.59 to 2.43), and 5.39 (3.30 to 8.80), respectively (adjusted for age, race, sex, CVD history, systolic BP, DM, smoking, and total cholesterol) (81).

In the same meta-analysis cited above, within the group with normal kidney function (eGFR 90 to 104 ml/min/1.73 m^2), the presence of increased albuminuria was also associated with an increased risk of mortality: compared to those with an ACR < 1.1 mg/mmol, an ACR of 1.1 to 3.3, 3.4 to 33.8, and ≥ 33.9 mg/mmol was associated with HRs for all-cause mortality of 1.48 (1.29 to 1.69), 1.61 (1.39 to 1.87), and 3.65 (2.13 to 6.27), respectively (adjusted for age, race, sex, CVD history, systolic BP, DM, smoking, and total cholesterol) (81).

Similar associations between the eGFR and level of albuminuria with the risk of death were also demonstrated in a meta-analysis of 10 cohort studies incorporating 266,975 individuals with a history of hypertension, DM, or CVD (82).

The higher mortality risk associated with CKD is due primarily to an excess of cardiovascular disease (CVD), as discussed in the following section.

1.4.2.1. Cardiovascular disease

Both the prevalence and the incidence of CVD are higher in patients with CKD compared to those without CKD. In the 2018 USRDS annual data report, a wide range of cardiovascular conditions were more common in patients with CKD compared to those without CKD, including stable coronary artery disease, acute myocardial infarction, heart failure, valvular heart disease, stroke, transient ischaemic attack, peripheral arterial disease (PAD), atrial fibrillation, sudden cardiac arrest, ventricular arrhythmias, venous thromboembolism, and pulmonary embolism (130). The overall prevalence of CVD among patients aged 66 years and older was 65.1% in those with CKD, compared to 32.6% in those without CKD (130).

There is also a large body of evidence showing that CKD is associated with a higher risk of incident CVD (90, 125-129, 131-136). The CKD Prognosis Consortium meta-analyses discussed above, demonstrating a higher risk of mortality associated with a lower eGFR or higher urine ACR also showed independent graded associations specifically with cardiovascular mortality (81, 82). Increased albuminuria, even if only moderate and in the presence of a normal GFR, is associated with CVD and cardiovascular death and adds to the cardiovascular risk in those with existing traditional cardiovascular risk factors such as DM or hypertension (90, 131, 137-139). A population-level cohort study from Canada suggested that

CKD is a stronger risk factor for an incident myocardial infarction than DM (135). It has been recommended therefore that CKD be considered a 'coronary heart disease risk equivalent', other examples of which include DM and PAD because the risk of a coronary event is at least as high as those who have known coronary heart disease (140).

The cardiovascular risk in patients with CKD is partly explained by an excess of traditional cardiovascular risk factors such as hypertension, DM, and dyslipidaemia. Therefore the management of individuals with CKD includes conventional cardiovascular risk management such as lifestyle measures, BP control, statin therapy, glycaemia control, and in some patients, antiplatelet therapy. However, even after adjustment for traditional risk factors, the presence of CKD is associated with a higher risk of CVD, and the non-traditional factors and underlying mechanisms for this association are the subjects of much research (126).

Until recently, there were no prognostic models available to accurately predict the risk of cardiovascular events in individuals with CKD (141). The Framingham risk score is a risk calculator used in clinical practice to estimate the 10-year cardiovascular risk of an individual that was developed in the general population (142). When its utility for risk prediction in patients with CKD was assessed using data from two CKD cohorts with nearly 1000 participants, it had poor discriminative and calibration ability, underestimating risk at five and ten years (143). The latest QRISK cardiovascular risk prediction score (QRISK3) includes the presence of CKD G3a to G5 as a risk factor, but only as a binary yes/no, which does not adequately take into account the graded association between the level of GFR and albuminuria with cardiovascular risk (144).

However, for patients with an eGFR < 30 ml/min/1.73 m^2, the prognostic models developed by Grams et al. in 2018 (discussed in section 1.4.1.2) may be used to predict the risk of cardiovascular events at two- and four-years and the relative order of such events in

relation to progression to kidney failure or death (109). In this model, the factors associated with having a CVD event as a first event include older age, a previous history of CVD, and DM (109).

1.4.2.2. Prognostic factors for mortality

As previously discussed, each component of the CGA staging system (cause of CKD, GFR, and level of albuminuria) provides prognostic information in patients with CKD, and this includes the risk of mortality.

With regard to the cause of CKD, diabetic nephropathy and atherosclerotic renovascular disease may be associated with a higher risk of death. In a Swedish prospective cohort study of nearly 1000 patients with CKD, relative to those with CKD due to glomerulonephritis, there was an independent higher risk of death associated with CKD due to diabetic nephropathy (adjusted HR 3.1 [2.3 to 4.3]) or atherosclerotic renovascular disease (adjusted HR 1.47 [1.23 to 1.76]) (145).

There is a wealth of evidence showing that, among patients with CKD, there is a graded and inverse relationship between the GFR and risk of death. For example, in a meta-analysis incorporating data from eight CKD cohorts, the adjusted HR for mortality was 1.47 (1.22 to 1.79) for a 15 ml/min/1.73 m^2 lower eGFR (95). The relationship can be seen in Figure 1.7.

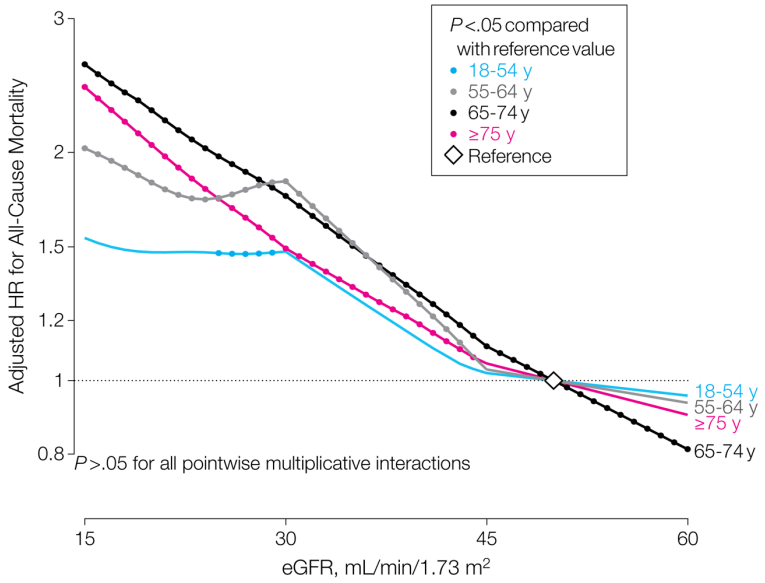


Figure 1.7. Association between eGFR and risk of death in CKD

From a meta-analysis of 13 CKD cohort studies. Hazard ratios, by categories of age, are relative to an eGFR of 50 ml/min/1.73 m^2, adjusted for sex, race, BMI, systolic BP, total cholesterol, history of CVD, DM, smoking status, and albuminuria. Reference (96).

There is also a significant independent association between level of albuminuria and risk of death in CKD. In the same meta-analysis, an eightfold higher urine ACR was associated with an adjusted HR for mortality of 1.40 (1.27 to 1.55) (95). In another cohort study of 920,985 patients, not included in the meta-analysis, among those with an eGFR of 45 to 59 ml/min/1.73 m^2, the adjusted rates of mortality per 1000 person-years were 7.0 (6.4 to 7.6), 11.9 (10.7 to 13.2), and 18.0 (15.6 to 20.9) for a urine ACR of < 3, 3 to 30, and > 30 mg/mmol, respectively (90). In those with an eGFR of 15 to 29 ml/min/1.73 m^2, the equivalent rates were 16.3 (13.0 to 25.0), 22.0 (18.5 to 26.0), and 24.6 (20.5 to 29.6), respectively (90).

As would be expected, age is also strongly associated with the risk of mortality in patients with CKD. In a prospective cohort of nearly 1000 patients with CKD, compared to

patients aged < 45 years, those 45 to 64 and those ≥ 65 had adjusted HRs for mortality of 2.8 (1.7 to 4.8) and 5.2 (3.1 to 9.0), respectively (145).

There are many other prognostic factors for mortality in CKD that have been reported in the literature, some examples of which are given in Table 1.9, and it will be noted that many of these are cardiovascular risk factors.

Table 1.9. Examples of prognostic factors for mortality in CKD

Prognostic factor	Measure	Reference	Adjusted HR	Study
Blood pressure (mmHg)	< 120/80	120 to 139/ 80 to 89	1.42 (1.41 to 1.43)	(146)
	140 to 159/ 90 to 99		0.95 (0.94 to 0.96)	
	> 160/100		1.05 (1.03 to 1.07)	
	≤ 130/	131 to 160/	1.22 (1.11 to 1.34)	(147)
	> 160/		1.06 (0.93 to 1.22)	
Body mass index (kg/m^2)	≤ 20	> 20	2.0 (1.4 to 2.8)	(145)
Serum phosphate (mg/dl)	per + 1		1.20 (1.05 to 1.37)	(148)
	per + 1		1.18 (1.12 to 1.25)	(149)
HbA1c (%)*	> 9	< 7	1.35 (1.20 to 1.53)	(150)
Plasma 1,25(OH)$_2$ D (pg/ml)	< 15	> 22	1.33 (1.01 to 1.74)	(151)
FGF-23 (RU/ml)	> 946	≤ 216	2.17 (1.56 to 3.08)	(152)

**in patients with DM.*

1.4.2.3. Prognostic models for mortality

A prognostic model to predict the risk of mortality by five years in individuals with CKD was developed using data from 382 participants with CKD stages G3 to G5 (but not receiving KRT) of the CRIB study (105). Of 44 candidate predictors, four were included in the final model: age, smoking status, and the cardiac markers NT-pro-BNP and Troponin T (TnT). The following equation gives the predicted 5-year risk (%) of mortality:

$$Risk\ (\%) = 1 - 0.84^{e^{0.044 \times (age - 60) + 0.34 \times ln\ (NT{-}pro{-}BNP/500) + 0.86\ (smoker) + 0.60\ (positive\ TnT)}}$$

where age is in years, NT-pro-BNP is in pg/ml, smoker is 1 for current smokers and 0 for others, and 'positive TnT' is 1 for those with a TnT ≥ 0.01 ng/ml and 0 for those with a TnT < 0.01 ng/ml.

The model was externally validated in a separate cohort of 213 patients with CKD stages G3 to G5. The C statistic of 0.82 suggested the model has moderate predictive ability, and the model has not been used in routine clinical practice.

As discussed above, the models developed in 2018 by Grams et al. may be used to predict the risk of death by two and four years in patients with CKD with a GFR < 30 ml/min/1.73 m^2 (109). Factors in the model that strongly predict death before experiencing kidney failure or a CVD event are older age and smoking.

1.4.2.4. Interventions to reduce the risk of mortality

A positive impact of nephrology care on mortality in patients with CKD has been inferred from multiple studies by comparison of early vs late (within six months of the need for dialysis) referral to nephrology. For example, a meta-analysis of 22 studies involving 12,749 patients with CKD showed a higher mortality rate in patients who were referred late (153), and a subsequent retrospective study of 39,031 patients showed that having at least two visits to a nephrology clinic was associated with a lower risk of mortality (154).

There are several therapeutic strategies employed to reduce the risk of death in patients with CKD, and they are principally aimed at reducing the risk of CVD events. The approach to reducing cardiovascular risk is as follows:

- Statin therapy. The Study of Heart and Renal Protection (SHARP) trial randomised 9270 patients with CKD (some on dialysis) and no known history of myocardial infarction or coronary revascularisation to either treatment with simvastatin plus

ezetimibe or placebo (155). In the subgroup of 6247 patients not on dialysis, those receiving simvastatin and ezetimibe had a significantly lower risk of major atherosclerotic events (risk ratio 0.78 [0.67 to 0.91]) (155). Subsequent meta-analyses have also shown that statin therapy reduces the risk of CVD events and death in non-dialysis CKD (156-158), and the NICE guideline on lipid modification recommends statin therapy for the primary or secondary prevention of CVD in patients with CKD (159).

- Control of hypertension. In a meta-analysis of 26 studies which included over 30,000 patients with CKD, ACEi therapy, compared to placebo therapy, reduced the risk of a major CVD event (HR 0.81 [0.73 to 0.89]), with a similar but statistically non-significant effect with calcium antagonists (HR 0.74 [0.53 to 1.03]) (160). Irrespective of antihypertensive drug class, a reduction in systolic BP was associated with a lower risk of a major CVD event (HR 0.83 [0.76 to 0.90] per 5 mmHg reduction) (160). The BP targets recommended by NICE are discussed in Section 1.4.1.3.
- Aspirin in some patients. A Cochrane review of antiplatelets in CKD found that, compared to placebo, antiplatelets reduced the risk of myocardial infarction (risk ratio 0.87 [0.76 to 0.99]) but not of stroke or death, and the risk of major bleeding was significantly increased (risk ratio 1.33 [1.10 to 1.65]) (161). The NICE CKD guideline suggests offering antiplatelet drugs to patients with CKD for secondary prevention of CVD, but being aware of the increased risk of bleeding (22).
- Other measures employed are those recommended in the general population and include smoking cessation, achieving and maintaining healthy body weight, regular exercise, and glycaemic control in patients with DM.

It is also important that patients with CKD receive adequate treatment for established CVD, but many studies show that patients with CKD are less likely to receive proven therapies for incident CVD. For example, therapies such as percutaneous coronary intervention, ACEi, and beta-blockers are less likely to be offered to patients with CKD compared to patients without CKD (162-165).

Although there is a focus on reducing cardiovascular risk, a holistic approach, including optimal management of co-morbid conditions and addressing other risks associated with mortality, is important. For example, patients with CKD have a higher risk of infection and infection-related death, and vaccination against influenza and pneumococcus may reduce these risks (166). Public Health England identifies CKD stages G3 to G5 as a clinical risk group that should be offered the influenza vaccination, and stages G4 to G5 as a group that should receive the pneumococcal immunisation (167).

CKD care should be multidisciplinary and ideally coordinated in a multidisciplinary clinic. Multidisciplinary care is associated with improved outcomes for patients with CKD, including reduced mortality (168, 169). In addition to a nephrologist, a multidisciplinary clinic may include health professionals with skills in patient education, dialysis vascular access, renal anaemia, clinical psychology, a dietician, and a social worker.

1.5. The need for more prognosis research in CKD

As described in previous sections of this chapter, the incidence and prevalence of CKD and deaths due to CKD are all increasing in the UK, as are common diseases that may be associated with CKD, such as DM, hypertension, and CVD. The increasing prevalence of CKD, the serious complications associated with it, and the financial costs, mean that CKD presents a significant and growing challenge for health services.

Further high-quality prognosis research in CKD may, therefore, be regarded as a priority within the field of nephrology. The importance of further prognosis research in CKD is demonstrated not only by a large number of prospective CKD cohort studies that have been established in the UK and globally, but also by impressive international collaborative efforts, in particular, the CKD Prognosis Consortium (170). The CKD Prognosis Consortium was established in 2009 by KDIGO as a group of investigators representing cohorts from around the world who share data for meta-analyses to study prognosis in CKD. This work has resulted in high quality and generalisable estimates of the association between routine kidney measures, such as eGFR and urine ACR, and adverse clinical outcomes in CKD.

Although eGFR and albuminuria are now well established prognostic factors in CKD, there is significant interest in identifying novel prognostic factors in CKD. This requires a different approach from the work done by the CKD Prognosis Consortium, which amalgamates data on routinely-collected variables that are available in multiple cohorts, because the investigation of novel prognostic factors is usually only feasible in one or a small number of studies initially.

Some of the potential benefits of further prognostic research and the identification of novel independent prognostic factors in CKD include:

- Accurate estimates of the average prognosis in CKD may allow the modelling of the population burden of CKD and provide a measure of the effectiveness of healthcare for CKD;
- Prognostic factors may be identified that predict treatment effects (e.g. high levels of albuminuria are associated with more benefit from RAASi);
- The identification of factors that provide a measure of the response to a particular treatment;
- The identification of prognostic factors that have a causal association with adverse outcomes would provide insight into the underlying pathophysiological mechanisms and identify potential therapeutic targets;
- Improved prognostic models would allow more accurate risk prediction, which may benefit patients, and aid clinicians in the practice of stratified medicine.

1.6. Summary and research aims

This introductory chapter has provided an overview of CKD and its importance with a particular focus on its prognostic implications. The case for more prognosis research in CKD has been made, and a useful framework for the conduct of prognosis research has been described. The work in this thesis aimed to assess four biomarkers in patients with CKD to determine whether they are independent prognostic factors and associated with the risk of kidney failure or death in CKD. The biological basis for assessing the specific biomarkers is discussed in each results chapter. They were selected based on pre-existing evidence that suggested either a demonstrable association in preliminary studies or evidence for a pathogenetic role in the progression of CKD. Hypotheses were prespecified and addressed using new data.

1.6.1. Hypotheses

The prespecified hypotheses that were tested for the work presented in this thesis comprised:

1. Higher levels of serum polyclonal light chains are independently associated with a higher risk of kidney failure and death in patients with CKD.
2. Higher levels of urinary free light chains are independently associated with a higher risk of kidney failure and death in patients with CKD.
3. The presence of a non-malignant monoclonal gammopathy is independently associated with a higher risk of kidney failure and death in patients with CKD.
4. Higher levels of serum endotrophin are independently associated with a higher risk of kidney failure and death in patients with CKD.

1.7. Thesis structure

Chapter II provides an overview of the methods employed in this thesis, including the statistical approach. Methods specific to each analysis are included in the relevant chapter.

Chapter III presents the assessment of serum polyclonal light chains as a prognostic factor in CKD, in the form of a meta-analysis incorporating data from four CKD cohorts (some published, and some new data).

Chapter IV reports an evaluation of urinary free light chains as a prognostic factor in CKD using a prospective cohort study.

Chapter V incorporates data from three CKD cohort studies to assess the prognostic significance of the presence of a non-malignant monoclonal gammopathy in patients with CKD.

Chapter VI presents the evaluation of a marker of collagen type VI formation, serum endotrophin, as a prognostic factor in CKD, using a prospective cohort study.

Chapter VII provides a summary of the results, draws conclusions from this research, and ends with a discussion of future research required.

CHAPTER II: GENERAL METHODS

This chapter provides an overview of the methods employed in the work presented in this thesis. Methods that are specific to a particular analysis are described in the relevant chapter.

All analyses were performed on samples and data from prospective cohort studies of patients with CKD. The Renal Impairment in Secondary Care (RIISC) study was the basis for the work presented in this thesis and is described in detail in the following sections. Several chapters also include data from other studies, including the Renal Risk in Derby (RRID) and Salford Kidney (SKS) studies, amongst others, and these studies are described in the relevant chapters.

2.1. The Renal Impairment in Secondary Care study

The RIISC study is a prospective cohort study of patients with CKD in secondary care that was established to assess prognosis and prognostic factors in patients with CKD. The study methodology was published in 2013 (171), and the study is registered in the ClinicalTrials.gov registry (identifier: NCT01722383). Details of the RIISC study follow with an emphasis on those aspects pertinent to the work presented in this thesis, and a brief description of the other aspects.

2.1.1. Ethics

The study protocol was approved by the South Birmingham Research Ethics Committee (REC) (reference 10/H1207/6) and University Hospitals Birmingham Research and Development department (reference RRK3917). The study was conducted in accordance with the Declaration of Helsinki. All participants provided written, informed, consent.

2.1.2. Setting

The study was conducted in nephrology clinics in two hospitals in Birmingham, UK: the Queen Elizabeth Hospital and Heartlands Hospital.

2.1.3. Participants

Adult patients with CKD who had been under follow-up in a general nephrology or CKD clinic for at least 12 months were invited to participate if they met the following eligibility criteria:

- Inclusion criteria
    - eGFR < 30 ml/min/1.73 m^2 or
    - eGFR 30 to 59 ml/min/1.73 m^2 with at least one of:
        - Urine ACR ≥ 70 mg/mmol on three occasions
        - eGFR decline of ≥ 5 ml/min/1.73 m^2 over a year
        - eGFR decline of ≥ 10 ml/min/1.73 m^2 over five years
- Exclusion criteria
    - Kidney replacement therapy (i.e. dialysis or kidney transplantation)
    - Immunosuppression for immune-mediated kidney disease

2.1.4. Baseline study visit

Recruitment occurred between October 2010 and December 2015, and eligible patients who consented to participate had their baseline study visit on the day of recruitment. Data collected during the baseline visit were recorded on a paper case report form (CRF) before being entered into an electronic study database. The data and samples collected during the baseline study visit are described below.

2.1.4.1. Demographic and lifestyle factors

A summary of the demographic and lifestyle variables that were collected and recorded is provided in Table 2.1.

Table 2.1. Demographic and lifestyle data collected at the baseline RIISC study visit

Variable	**Information**
Age	Years*
Sex	Male/female
Ethnicity	White/Black/South Asian/other
Education level	Highest qualification: none/GCSE/O level/NVQ/A level/undergraduate/postgraduate
Employment status	1. Currently employed: yes/no/retired 2. If employed or retired, job type: unskilled or manual/skilled or manual/clerical/managerial/professional
Deprivation	Index of Multiple Deprivation (IMD) 2010* (172), derived from participant's postcode. IMD provides an overall measure of relative deprivation experienced in the participant's area of residence
Smoking status	1. Current/previous/never 2. If current or previous: pack-years*
Alcohol intake	Units per week*

**recorded as continuous variables.*

2.1.4.2. Health-related quality of life

Health-related quality of life (HRQL) was assessed using the EuroQol EQ-5D-3L instrument, which has two components (173):

1. Descriptive system: a categorical self-assessment (no problems/some problems/extreme problems) in five domains:
    a. mobility
    b. self-care
    c. usual activities
    d. pain/discomfort
    e. anxiety/depression
2. Visual analogue scale: the participant rates their health on a continuous scale from 0 (labelled 'worst imaginable health state') to 100 (labelled 'best imaginable health state').

In a review of patient-reported outcome measures for patients with CKD, evidence for the EQ-5D was found to be more favourable compared to two other measures as it demonstrates good discriminative properties and the response rates for completion are high (174).

2.1.4.3. Clinical history

Factors related to the participant's clinical history were recorded as listed in Table 2.2.

Table 2.2. Clinical history recorded at the baseline RIISC study visit

Variable	**Notes**
Past medical history	1. Complete PMH recorded as free text 2. Yes/no for: DM, IHD, cerebrovascular disease, PAD, COPD, malignancy
Cause of CKD	Vascular/diabetes/glomerular/tubulointerstitial/cystic or congenital/other or unknown
Family history	Yes/no for: CKD, IHD, PAD, cerebrovascular disease, DM, COPD, malignancy
Current medications	All current drugs and their doses

PMH, past medical history.

2.1.4.4. Physical assessment

Variables recorded from the physical assessment that was performed at the baseline visit included anthropometric data (participants' height [cm], weight [kg], body mass index [kg/m^2], waist circumference [cm], hip circumference [cm], and thigh circumference [cm]) and blood pressure (BP). Blood pressure was recorded using the BpTRU™ device (BpTRU Medical Devices, Coquitlam, BC, Canada), an automated BP measuring device that, after a five minute rest period, records six readings at one-minute intervals. The first reading is discarded, and the average of the subsequent five readings is recorded as the BP. The standard

operating procedure (SOP) for BP measurement using BpTRU is presented in Appendix 1. In patients with CKD, clinic BP measurements by BpTRU are lower than manual BP measurements (which may be higher due to the 'white coat' effect) and similar to the daytime mean and overall mean from a 24-hour ambulatory BP monitor (the gold standard) (175).

Arterial stiffness was estimated by measuring carotid-to-femoral pulse wave velocity (PWV) using the Vicorder device (SMART Medical, Gloucestershire, UK), regarded as the gold-standard non-invasive technique for measurement of aortic stiffness (176). The SOP for PWV measurement using the Vicorder device is presented in Appendix 2. Increased arterial stiffness is associated with a higher risk of incident CVD and death in patients with CKD (177, 178).

An estimate of tissue advanced glycation end products (AGEs) was also obtained using the AGE Reader device (Diagnoptics Technologies, Groningen, Netherlands), which measures skin autofluorescence, based on the fluorescent properties of certain AGEs accumulated in dermal tissue. The SOP is presented in Appendix 3. AGEs are a heterogeneous group of compounds formed by the reaction of free amino groups on proteins, lipids, and nucleic acids with reactive carbonyl groups on reducing sugars (179, 180). In CKD, increased oxidation or decreased detoxification of carbonyl compounds results in increased concentrations of small carbonyl precursors and thus, the accumulation of AGEs (181). AGEs are pro-inflammatory and associated with endothelial dysfunction and arterial stiffness, and higher levels may be associated with a higher risk of CVD (182).

Finally, participants underwent a periodontal assessment. One of the primary hypotheses to be tested in RIISC was that chronic periodontitis in patients with CKD is associated with a higher risk of CKD progression and death. At the baseline visit, participants

underwent a periodontal assessment and plaque and saliva sampling, and the methodology and initial results from this have been published (183).

2.1.4.5. Samples

Samples of serum, plasma, and urine were collected at the baseline visit and all follow-up visits. For the routine clinical blood tests (full blood count, creatinine, eGFR, potassium, calcium, phosphate, albumin, parathyroid hormone, bicarbonate, glucose, HbA1c, and lipids) and urine ACR, samples of serum and urine were processed in the local hospital laboratory as per the current standard of care.

Further, extra blood and urine were collected specifically for the investigation of novel prognostic factors. For this purpose, serum, plasma, and urine were processed immediately after collection and stored at -80℃ until analysis. The SOP for the processing of serum, plasma, and urine is given in Appendix 4.

Saliva and DNA were also collected and stored, but not used in work presented in this thesis.

2.1.4.6. Assays

The assay methods used for each particular potential prognostic factor being assessed in this thesis are described within the relevant chapter.

Of particular importance in all multivariable analyses were the creatinine-based eGFR and the urine ACR. Serum creatinine assays were performed on a Roche cobas® 8000 modular analyser using the Jaffé method, calibrated to the isotope dilution mass spectrometry (IDMS) methodology, and eGFR was calculated using the CKD-EPI equation unless

otherwise stated. Urine ACR was measured using an immunoturbidimetric assay on a Roche Hitachi 702 analyser.

2.1.5. Follow-up

Participants were followed up with study visits at six months, 18 months, and 36 months, and after that 'remote' follow-up for outcomes. Participants were followed up until kidney failure, death, or ten years from the baseline study visit, and study follow-up is ongoing. Patients who withdrew from the study did not attend further study visits but gave consent for the remote collection of kidney failure and death outcome events.

2.1.5.1. Data and sample collection

At all follow-up visits, data were collected on lifestyle factors (current smoking status and alcohol intake), HRQL, current medications and their doses, and the following patient-reported outcomes sustained since the previous study visit:

- New diagnoses and the date of diagnosis
- Hospital admissions, with the reason for admission and dates
- Cardiovascular events (myocardial infarction/angina/stroke/transient ischaemic attack/PAD)
- Kidney failure

All elements of the physical assessment as described for the baseline visit were repeated at every follow-up visit other than the periodontal assessment, which was performed only at baseline and 36 months.

Further samples of serum, plasma, urine, and saliva were collected, processed, and stored at every follow-up visit in the same manner as at the baseline visit. DNA samples were not retaken at follow-up visits.

2.1.5.2. Outcomes

The events of interest in this work were kidney failure and death.

Kidney failure was defined as CKD with the requirement for KRT and was recorded as the time between the date of the baseline study visit and the date of dialysis treatment or kidney transplantation, whichever came first. In addition to patient-reported kidney failure events obtained at follow-up visits, the electronic database of each hospital's renal unit, which records all patients being treated with KRT, was regularly searched to identify new kidney failure events.

Deaths were defined as death from any cause and were identified through linkage with Lorenzo, an electronic health record, on which deaths are registered. The time between the date of the baseline study visit and the date of death was recorded.

For all analyses of RIISC data in this thesis, kidney failure and death events up to 31 December 2018 are included. Participants who had not experienced an endpoint by 31 December 2018 were censored on this date.

2.2. Role in the RIISC study

The RIISC study has been a collaborative effort, with many people involved in its design and conduct. This researcher's contributions to the conduct of the study have included:

- Screening potential participants for eligibility;
- Consenting and recruiting eligible patients into the study;
- Assessment of participants at baseline and follow-up study visits, collecting and recording the required data onto CRFs, and subsequent recording of data into the electronic study database;
- Management of samples, including storage, organisation, and arranging external sample transfers for assay;
- The writing and submission of major protocol amendments to the REC, including an update of the protocol, consent form, patient information sheet, and a patient study update.

2.3. Bias

Bias is defined as a systematic deviation in results from the truth. This is distinguished from random error which is a deviation in results from the truth caused by statistical fluctuations (in either direction) in the measured data (184). The primary sources of bias in prognosis research and how these have been addressed are described below:

2.3.1. Selection bias

The risk of selection bias is lower in prospective cohort studies such as RIISC compared to historical cohort studies, but biased results may still result from participation or attrition bias. These risks were minimised as follows.

- Participation bias:
    - Study visits (both baseline and follow-up) were aligned to participants' routine outpatient renal appointments such that the patient would receive their routine clinical review as part of the study visit and not need to come for a separate visit, reducing the risk of a low participation rate.
    - All eligible patients attending renal outpatient clinics were consecutively, rather than selectively, invited to participate in the study.
    - The eligibility criteria were clear and easy to apply, such that the risk of incorrectly inviting or excluding patients was low.
- Attrition bias:
    - The risk of participants missing follow-up study visits was reduced by aligning them with their routine clinic visits.
    - Electronic health sources were used to remotely capture kidney failure and death outcome events, such that if participants missed a follow-up visit or

withdrew from the study, there was a very low risk of missing these outcome data.

2.3.2. Information bias

The risk of information bias was reduced by:

- The outcome events (kidney failure and death) were clearly defined and not ambiguous or subjective.
- The majority of outcome events were captured using reliable electronic data sources, as described above, rather than patient-reported outcomes.
- A participant's baseline characteristics in no way influenced the above methods of outcome event capture.

2.4. Sample size

Issues concerning study sample size are described below.

2.4.1. RIISC

The aim was to recruit 1000 participants into RIISC as this would allow robust interpretation of the relationship between candidate prognostic factors and clinical outcomes, including subgroup analyses, e.g. by DM status. Recruitment was reviewed in December 2015, by which time 931 patients had been recruited. Based on the high number of observed kidney failure and death events (the most important factor influencing power for time-to-event analyses), the study ended recruitment with a final study population of 931 participants.

2.4.2. Other studies

Where individual participant data were included from studies other than RIISC, such as RRID and SKS, all recruited participants eligible for that analysis and for whom data were available were included.

2.5. Statistical methods

All statistical analyses were prespecified and not data-driven. Analyses deviated from the prespecified methods only where recommended in peer review.

2.5.1. Preliminary data cleaning and assessment

Data were assessed, cleaned, and prepared prior to each analysis. Duplicate cases were checked for and removed where identified, and implausible values for each variable were checked for by assessing the minimum and maximum values and distribution of each variable. Values were modified where there was a manifest error, e.g. height (cm) recorded as 1.66 was replaced with 166. Where there was still doubt about the plausibility of a value, the CRF was referred to, and the dataset corrected where possible. In six cases, both systolic and diastolic BP were recorded as 0 mmHg in the dataset and on the CRF, and these values were deleted and treated as missing data.

Categorical variables were coded numerically, and labels assigned to each value.

The distribution of each continuous variable was assessed with histograms to determine whether parametric or non-parametric statistical tests were appropriate and whether a transformation was required.

2.5.2. Description of the study population

For each analysis, the distributions of demographic characteristics (such as age, sex, and ethnicity), co-morbidities, established prognostic factors, and the prognostic factor being assessed are reported. These are presented in tabular form as the frequency and percentage for categorical variables, the mean and standard deviation (SD) for normally distributed continuous variables, and the median and interquartile range (IQR) for non-normally

distributed continuous variables. In the same table, the number of missing values for each variable are also presented.

Follow-up time is summarised as the median and interquartile range, estimated by the reverse Kaplan-Meier method (185). Outcomes are reported as both the number of events and as an event rate (e.g. events per 100 person-years of follow-up).

2.5.3. Relationship of a prognostic factor with other variables

The relationship of the prognostic factor being assessed with other baseline variables, including established prognostic factors such as eGFR and urine ACR, was evaluated both statistically and graphically, e.g. a scatter plot for the relationship between two continuous variables.

Fisher's exact test was used to assess for a significant relationship between two categorical variables.

The relationship between two normally distributed continuous variables was assessed statistically by Pearson's correlation coefficient (r), Spearman's rank correlation coefficient (ρ) where one variable was non-normally distributed, and Kendall's rank correlation coefficient (τ) where both variables were non-normally distributed. Correlation coefficients of 0.2, 0.5, and 0.8 were considered weak, moderate, and strong, respectively (186). Fractional polynomial transformations were also used to assess for non-linear relationships.

A statistical assessment of the relationship between a continuous variable and a binary categorical variable was by the t-test (for normally distributed continuous variables) or the Mann-Whitney U test (for non-normally distributed continuous variables).

The relationship between a continuous variable and a categorical variable with three or more categories was assessed by analysis of variance (ANOVA, for normally distributed

continuous variables) or the Kruskall-Wallis test (for non-normally distributed continuous variables).

2.5.4. Association between prognostic factor and outcomes

Kaplan-Meier analyses and curves, and regression models based on time-to-event data, were used to assess the association between potential prognostic factors and clinical outcomes.

Associations with the risk of death were assessed using Cox proportional hazards models (187), and are presented as a hazard ratio (HR) with a 95% confidence interval (CI). Log-log plots (ln(-ln(survival)) versus survival time) were assessed for each variable to ensure that the proportional-hazards assumption was not violated.

Associations with the risk of kidney failure were primarily assessed using competing-risks regression models, using the Fine and Gray method to model the subdistribution hazard (188). This method is appropriate for the analysis of time-to-event data in the presence of a competing risk, defined as an event that impedes the occurrence of the event of interest. When modelling time to kidney failure, death is a competing risk because patients who die cannot later proceed to develop kidney failure. The association between each variable and the risk of kidney failure is presented as a subhazard ratio (SHR) with a 95% CI.

An alternative approach to subdistribution hazard models in the presence of competing risks is to fit cause-specific hazard models. These were fitted and presented as supplemental results in tabular form in the appendices. Any tabulated results that differ significantly from the subdistribution hazard models are presented in bold type and discussed in Section 7.7.

2.5.4.1. Regression model development

First, univariable models were used to assess the association between the potential prognostic factor and the clinical outcome, i.e. without adjustment for additional variables. Univariable associations are also presented for all other baseline variables.

Multivariable regression models were then built to account for confounding and to assess the association of the factor with clinical outcomes after adjustment for established prognostic factors. Included variables were prespecified and not selected based on univariable analyses or automated stepwise variable selection procedures, avoiding biases caused by data-dependent model selection.

Given the issues associated with multiple testing and overfitting of models, interactions between the prognostic factor of interest and the other model covariates were not routinely tested for. In the few analyses where clinically plausible interactions were tested for, they are explicitly stated.

2.5.4.2. Continuous variables

Continuous variables were kept as continuous in all regression models to avoid the loss of information inherent in categorisation. Where it was felt to be potentially informative, models with categorised continuous variables are presented as a supplementary to, and not in place of, the primary models which retain the variable's continuous nature.

Potential non-linear associations between continuous variables and outcome were assessed by checking for an improvement in model fit using fractional polynomials (FP). In such models, for continuous variable x, powers (p) were selected from a set of eight (-2, -1, -0.5, 0 [ln], 0.5, 1, 2, 3) and the model with FP of degree 1 (FP1, where βx^p is substituted for βx) or degree 2 (FP2, where $\beta_1 x^{p_1} + \beta_2 x^{p_2}$ is substituted for βx) with the best fit was

selected (189). Given the additional complexity, FP2 models were only used if they provided a significantly better model fit compared to the best FP1 model.

Where FP models provide a better fit, graphs of risk (HR or SHR) versus the variable on its original scale are presented to aid the understanding of the non-linear relationship with outcome.

2.5.5. Analysis of data from multiple studies

Where analyses were performed on individual participant data amalgamated from multiple cohorts, a one-stage meta-analytic approach was used. Clustering was accounted for by stratifying the regression model by cohort, in which the baseline hazard is allowed to vary by cohort while the estimated coefficient for each predictor variable is assumed to be equal across cohorts (i.e. a fixed-effects model) (190).

2.5.6. Missing data

The number of missing values for each variable are reported in each chapter. Although commonly performed, complete case analysis, i.e. including only the cases with complete data, reduces the sample size available for analysis, and is statistically inefficient, especially in multivariable analyses where missing values in each of several variables can result in a large number of patients being excluded. In work presented in this thesis, data were missing in multiple variables, and in order to account for missing data in a way that allowed all patients to be included in the analyses, missing data were handled by multiple imputation.

Given that there were different types of variables with missing data, multiple imputation was performed using chained equations, in which each variable is imputed using its own imputation model (191). For continuous variables, on the basis of their skewed

distributions, predictive mean matching was used rather than linear regression alone, with imputed values drawn from the ten nearest neighbours as recommended by Morris et al. (192). All categorical variables with missing values had more than two categories, and therefore values were imputed using multinomial logistic regression (augmented to avoid perfect prediction as recommended by White et al. (193)).

The imputation models included all covariates to be included in the final Cox or competing-risks multivariable regression models, an outcome variable (for analyses of death this was a binary variable, and for kidney failure a categorical variable indicating censored, kidney failure, or death), and the cumulative hazard function (approximated by the Nelson-Aalen estimate (191)). Where multiple imputation was performed for individual-level data amalgamated from multiple cohorts, a categorical study variable was also included in the imputation models.

It has been recommended that the number of imputations created should be at least equal to the percentage of incomplete cases (191). Therefore, for the analyses presented in this thesis, the number of imputations was determined by the percentage of incomplete cases rounded up to the nearest five (e.g. if 17% participants had missing values in one or more variables, 20 imputations would be created).

Following imputation, analyses were performed on each imputed dataset, before the estimates of coefficients and their standard errors were combined using Rubin's rules (194).

2.5.7. Sensitivity analyses

The results of analyses from multiple imputation were compared to results from complete case analyses, and where there are significant differences, they are described and investigated.

2.5.8. Software

Stata 15 (StataCorp, College Station, Texas, US) was used to perform all statistical analyses and to create all graphs. Where user-written commands were used, they are referenced in the relevant chapter's methods section.

CHAPTER III: SERUM FREE LIGHT CHAINS

The work presented in this chapter aimed to address the hypotheses that higher concentrations of serum free light chains are associated with a higher risk of kidney failure and death in patients with chronic kidney disease (CKD).

This work has been published in the article 'The Association of Serum Free Light Chains With Mortality and Progression to End-Stage Renal Disease in Chronic Kidney Disease: Systematic Review and Individual Patient Data Meta-analysis,' in Mayo Clinic Proceedings in 2017 (195), and presented in poster format at the American Society of Nephrology Kidney Week, Chicago, 2016 and the UK Kidney Week, Liverpool, 2017.

3.1. Abstract

Objective

To clarify the associations between serum combined (κ + λ) free light chain (cFLC) concentration and risk of kidney failure and death in patients with chronic kidney disease (CKD), by conducting a systematic review and individual patient data meta-analyses.

Patients and Methods

On December 28, 2016, a search was conducted using four databases (MEDLINE, Embase, CINAHL, and PubMed) and conference proceedings for studies presenting independent analyses of associations between serum cFLC concentration and kidney failure or death in patients with CKD. Study quality was assessed in five domains: sample selection, measurement, attrition, reporting, and funding.

Results

Five prospective cohort studies were included, judged moderate to good quality. In multivariable meta-analyses, serum cFLC concentration was independently associated with the risk of kidney failure (three studies, 2092 participants, median 5.7 years follow-up), with a non-linear association suggesting increased risk up to 150 mg/l, beyond which the risk does not increase further. A higher serum cFLC concentration was also independently associated with a higher risk of death (five studies, 3851 participants, median 4.1 years follow-up), again with a non-linear association.

Conclusion

Higher concentrations of non-clonal serum FLCs are independently associated with a higher risk of death and kidney failure in patients with CKD. Future work is needed to explore the

biological role of serum FLCs in the adverse outcomes associated with CKD, and their use in risk stratification.

3.2. Introduction

There are prognostic factors measurable in the serum of patients with CKD that are associated with the risk of kidney failure and death, the strongest being measures of kidney function such as creatinine concentration and the eGFR derived from it. Beyond these markers of kidney function, there is a desire to identify independent serum prognostic factors that may add incremental value in risk prediction models and risk stratification. Serum factors that have a direct causal role in the adverse outcomes associated with CKD are perhaps more likely to be able to provide this information and may represent novel treatment targets.

For the various reasons outlined in this chapter, free light chains (FLC) deserve further investigation in this regard. Patients with CKD are exposed to relatively high serum concentrations of FLCs, which have numerous biological effects that are potentially deleterious and plausibly linked to kidney damage and the risk of death.

3.2.1. Structure and physiology of FLCs

Immunoglobulin (Ig) molecules consist of four chains: two identical heavy chains from one of five classes (gamma, alpha, mu, epsilon, or delta) and two identical light chains from one of two classes (kappa [κ] or lambda [λ]). Each heavy and light chain has a constant region and a variable region. The structure of an Ig molecule is illustrated in Figure 3.1.

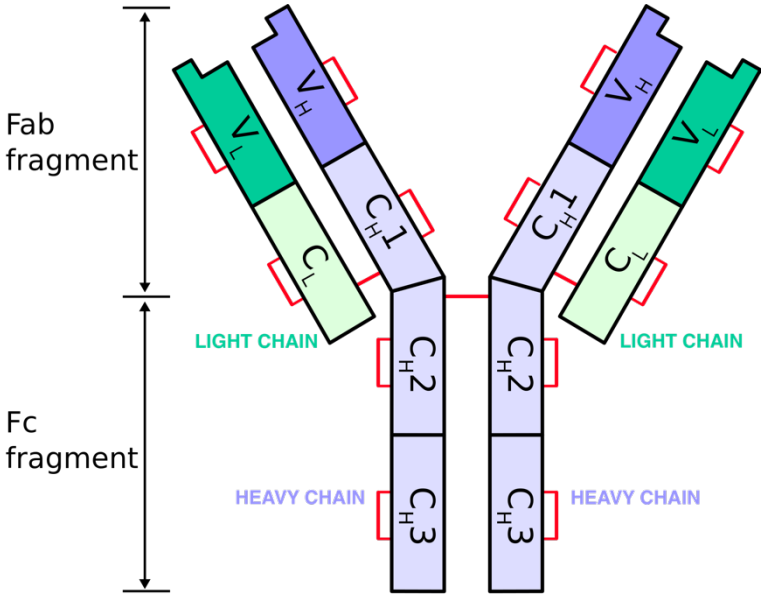


Figure 3.1. Structure of an immunoglobulin molecule

Each immunoglobulin molecule consists of two heavy chains (shown in purple) and two light chains (shown in green), and each chain consists of constant (C) and variable (V) regions.

The Fab portions of the Ig molecule bind antigen and the Fc portion binds to Fc receptors on effector cells such as B cells, natural killer cells, macrophages, neutrophils, and mast cells.

The Ig isotype is determined by the class of heavy chain (IgG, IgA, IgM, IgE, or IgD), and the designation of Ig molecules also includes the type of light chain with which the heavy chains are associated, e.g. IgG-κ, IgA-λ.

Immunoglobulin molecules are synthesised by plasma cells and other cells of the B cell lineage. Within B cells, light chains are produced in excess of heavy chains, such that only approximately 60% of synthesised light chains are incorporated into complete Ig molecules, and the remaining 40% are released into the blood as unbound FLCs (196). Approximately 500 mg per day of FLCs are produced, and there are approximately twice as

many κ-producing plasma cells than λ-producing cells (196). The clearance of FLCs is mainly renal, as described below, although there is a small contribution from the reticuloendothelial system (197).

In the blood, κ FLCs generally exist as monomers (~25 kDa) and λ FLCs as dimers (~50 kDa), and their size permits filtration at the glomerulus. After glomerular filtration, they enter the proximal tubule, from where they are endocytosed by proximal tubular cells (mediated by the cell surface receptors megalin and cubulin), before degradation within lysosomes into their constituent amino acids which are transported back into the circulation (198, 199). The renal handling of FLCs is illustrated in Figure 3.2.

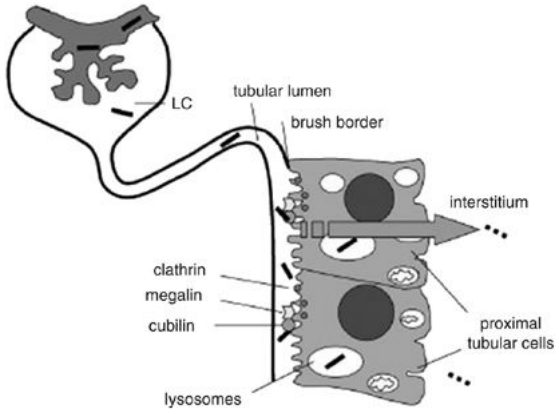


Figure 3.2. Renal handling of free light chains
Free light chains undergo glomerular filtration before being endocytosed and catabolized in proximal tubular cells. From reference (199).

Given the smaller size of monomeric κ FLCs compared to dimeric λ FLCs, κ FLCs have a higher filtration rate and rate of renal clearance and thus a shorter serum half-life. Therefore, despite an approximate 2:1 ratio of κ to λ FLC production, serum κ FLC concentration is usually lower than λ FLC concentration, with a median serum κ/λ FLC ratio

of approximately 0.6 (reference range 0.26 to 1.65) (200). A ratio outside of this reference range may signify monoclonal FLC production.

The proximal tubular pathway for FLC catabolism is thought to process all FLCs filtered at the glomerulus (197). Although between 1 and 10 mg of FLCs are excreted in the urine per day in healthy individuals, these are thought to be secreted in the urinary tract alongside IgA as part of the mucosal defence system rather than originating from glomerular filtration (197).

3.2.2. Serum FLCs in CKD

As GFR declines, so does the renal clearance of FLCs, such that the serum FLC concentration increases progressively with each stage of CKD, as is shown in Figure 3.3.

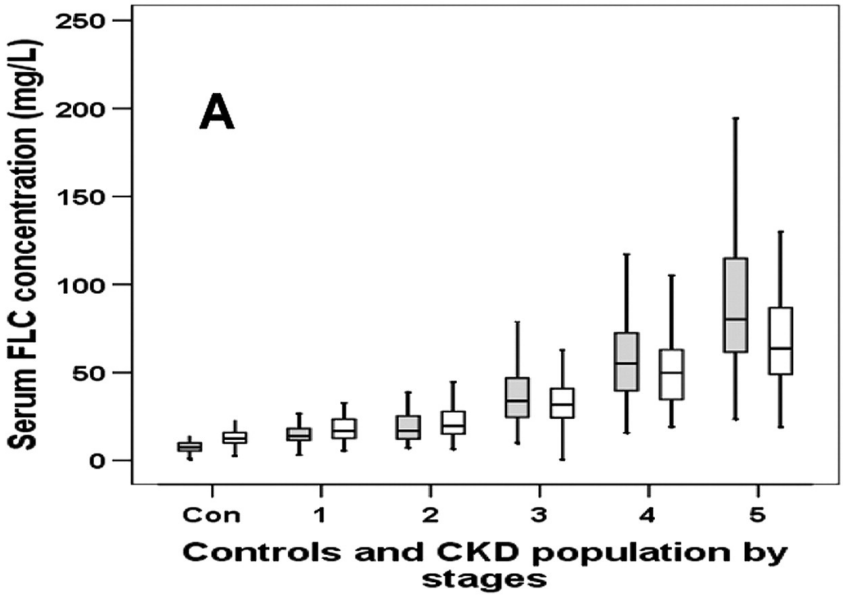


Figure 3.3. Box plot of serum FLC concentration by CKD stage

CKD stages refer to the G stage, with 3a and 3b combined. Grey boxes are κ FLC, and white boxes are λ FLC. Con = healthy control population. From reference (201).

As renal clearance declines, the clearance of FLCs becomes more dependent on the reticuloendothelial pathway. This pathway, unlike renal clearance, shows no size preference and clears both κ and λ FLCs at the same rate, so that the serum half-life of κ FLCs approaches that of λ FLCs. Thus, the relative FLC concentrations change to reflect more closely the higher rate of κ production, and the serum κ/λ FLC ratio progressively increases with CKD stage, as shown in Figure 3.4 (201).

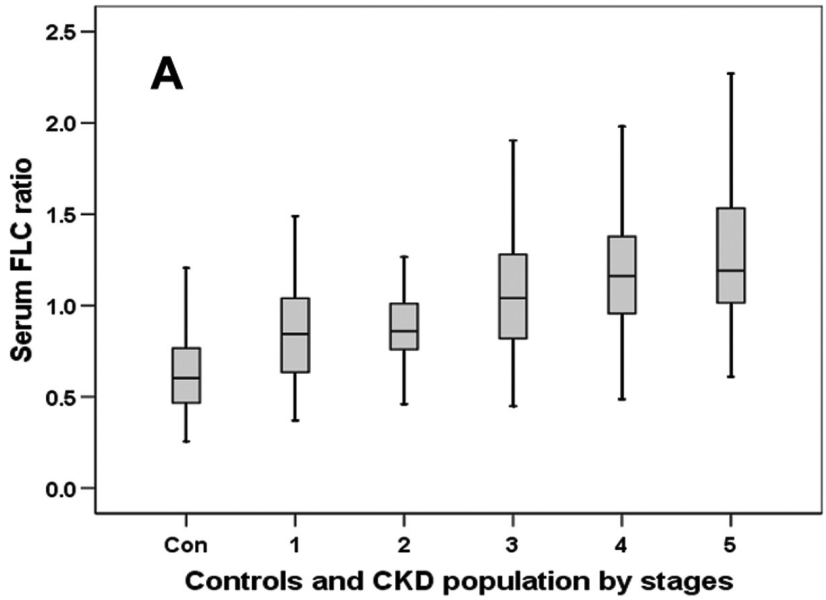


Figure 3.4. Serum κ\λ FLC ratio by stage of CKD

CKD stages refer to the G stage, with 3a and 3b combined. Con =healthy control population. From reference (201).

Because of this, some patients with CKD may have a serum κ/λ FLC ratio above the general population reference range, even in the absence of monoclonal FLC production (202). Therefore a 'renal reference range' for serum κ/λ FLC ratio of 0.37 to 3.10 has been proposed

for patients with CKD (201, 202). The renal reference range is more sensitive and specific for the diagnosis of monoclonal disorders in patients with CKD (202-204).

Serum FLCs may be measured as κ and λ FLCs separately or combined (κ + λ) FLC (cFLC). The serum κ\λ FLC ratio allows the detection of clonality. A rise in cFLC with a κ\λ ratio within the reference range is consistent with a non-clonal process, i.e. reduced clearance as is seen in CKD or increased production as is seen in various diseases associated with B cell activation and immune stimulation, such as infections, inflammation and autoimmune disease (205, 206). However, an abnormal κ\λ ratio with an increase in the involved FLC (i.e. a high ratio with increased κ FLC concentration, or a low ratio with increased λ FLC concentration) is a marker of a monoclonal process (monoclonal gammopathy).

3.2.3. Serum FLCs and prognosis

Serum cFLC concentration has been shown to have prognostic significance, including an independent association with the risk of death. There have been several studies showing that a higher non-clonal serum FLC concentration is associated with a higher risk of mortality in the general population. In nearly 16,000 individuals aged 50 years or older without a monoclonal disorder and with a median follow up of 12.7 years, those with a serum FLC concentration above the highest decile had a higher risk of death (risk ratio 2.07 [95% CI 1.91 to 2.24], after adjustment for age, sex, and serum creatinine) (207). The increased mortality was not restricted to any specific cause of death, with a higher risk of death observed in nearly all categories of the International Statistical Classification of Diseases. Another study in nearly 5000 individuals from the general population also showed a higher risk of death with a higher serum cFLC concentration, again without an association to any particular category of cause of death (208).

In addition to having prognostic significance in the general population, serum cFLC concentration has been shown to have an independent association with risk of death in various non-renal diseases, including heart failure (209, 210), rheumatoid arthritis (211), and COPD (212).

As described in the previous section, CKD is associated with higher serum FLC concentrations. Given the independent association between serum cFLC concentration and risk of death observed in the general population and other non-renal diseases, it is plausible that serum cFLC have a role in the higher mortality risk associated with CKD. Several studies have examined the association between serum cFLC concentration and risk of death in cohorts of patients with CKD but have produced conflicting results. Thus, further work is required to answer this question.

It is also possible that the higher serum FLC concentrations observed in CKD have a role in the risk of kidney failure. It is well established that high levels of monoclonal FLCs, present in diseases such as multiple myeloma, can cause kidney damage through various pathways but it is not known whether high levels of non-clonal FLCs are associated with kidney damage and the risk of kidney failure. Two studies have assessed this, but, again, the results are conflicting.

It is therefore still unknown whether higher concentrations of non-clonal serum FLCs are associated with a higher risk of kidney failure or death in patients with CKD. A systematic review and meta-analysis of individual patient data was performed to address these questions, with the inclusion of additional study data not included in the original published papers.

3.3. Hypotheses

The following pre-specified hypotheses were addressed:

1. Higher concentrations of non-clonal serum cFLC are associated with a higher risk of kidney failure in patients with CKD;
2. Higher concentrations of non-clonal serum cFLC are associated with a higher risk of death in patients with CKD.

3.4. Methods

This meta-analysis was prospectively registered on PROSPERO, an international database of prospectively registered systematic reviews (registration number: CRD42015025195) (213). Several stages of the meta-analysis, such as the literature search, data extraction, and study assessment process, were performed independently by two researchers, allowing a comparison of independently obtained results with discussion and agreement before moving on to the next stage. Where the term 'two researchers' is used, it refers to the author of this thesis and Dr Simon Fraser (Clinical Lecturer in Public Health, Academic Unit of Primary Care and Population Sciences, Faculty of Medicine, University of Southampton).

3.4.1. Eligibility criteria

Two researchers independently performed a literature search to identify quantitative studies (not case reports or qualitative studies) which had to contain all of the following to be included:

1. Participants with CKD. Participants were excluded at an individual level if they had received kidney replacement therapy (dialysis or a kidney transplant), or if they had a monoclonal gammopathy (e.g. multiple myeloma).
2. A measure of serum FLC concentration (κ and λ individually or cFLC).
3. Kidney failure or death as outcomes.
4. An estimate of the association between serum FLC concentration and the above outcomes.

No restrictions on language, publication date, or publication status were imposed on the search.

3.4.2. Search strategy

The search included MEDLINE, Embase, PubMed, CINAHL, the Cochrane library, the Centre for Reviews and Dissemination, the ClinicalTrials.gov register, the conference proceedings from three major nephrology conferences from 2012-2015 (UK Renal Association, European Renal Association/European Dialysis and Transplant Association, and the American Society of Nephrology Kidney Week), and the reference lists of identified eligible studies. The last search was performed on 28 December 2016.

The search strategy incorporated free text and MeSH (Medical Subject Headings) terms for CKD and FLCs, and excluded studies with myeloma in the title. As an example, the search strategy for MEDLINE (1946-present) is presented in Table 3.1.

Table 3.1. Search strategy for MEDLINE

Stage	Search terms
1	(Chronic kidney disease* OR CKD* OR chronic renal failure* OR renal failure* OR renal insufficiency, chronic OR renal insufficiency*) as free text words (.mp)
2	MeSH subject heading: exp Renal Insufficiency, Chronic/
3	1 OR 2
4	(light chain* OR immunoglobulin* OR light-chain* OR Ig* OR kappa-immunoglobulin* OR kappa immunoglobulin* OR lambda-immunoglobulin* OR lambda immunoglobulin*) as free text words (.mp)
5	MeSH subject heading: exp Immunoglobulin Light Chains
6	4 OR 5
7	free.mp
8	polyclonal.mp
9	7 OR 8
10	6 AND 9
11	3 AND 10
12	myeloma*.m_titl.
13	11 NOT 12
14	Limit to humans and remove duplicates
15	Screening titles and abstracts

The search strategy included MeSH (Medical Subject Headings) terms and free text.

Two researchers independently assessed each potentially eligible study, and any differences of opinion regarding eligibility were resolved by discussion.

3.4.3. Data collection

The corresponding author of each eligible study was contacted by email to request individual participant data (IPD) and anonymised IPD were obtained for all studies for the variables shown in Table 3.2. An assessment of the IPD integrity was performed as per Section 2.5.1.

Table 3.2. Variables collected for all eligible studies

Variable	**Notes**
Serum cFLC	mg/l
Age	Years
Sex	Male/female
Ethnicity	White/non-White
DM	Coded diagnosis: yes/no
CVD	Coded diagnosis: yes/no
Systolic BP	mmHg
Urine ACR	mg/mmol
eGFR	ml/min/1.73 m^2 (MDRD)
Serum albumin	g/dl
Serum calcium	mmol/l
Serum phosphate	mmol/l
Use of RAASi	Yes/no
Kidney failure	1.Yes/no 2.Time-to-kidney failure (months)
Death	1.Yes/no 2.Time-to-death (months)
Time to last follow-up	Months, for censoring

Variables included in the anonymised IPD collected for all eligible studies. RAASi = renin-angiotensin-aldosterone system inhibitors.

Of note, eGFR calculated by the four-variable MDRD equation was used in these analyses, as not all studies had eGFR calculated by the CKD-EPI equation.

3.4.4. Risk of bias assessment

Two researchers independently assessed the risk of bias in each study using a tool similar to that recommended in the Cochrane Handbook, attributing a low, moderate or high risk of bias based on sample selection, measurement, attrition, reporting and funding (214). This process was informed by systematically extracting data from and reviewing each study using a standardized form based on the STROBE Statement checklist, including study date, location, primary aim, participant characteristics (number, CKD stage), setting (e.g. primary or secondary care), main outcome, sampling method and potential sampling bias, potential confounders, presence of sample size calculation, main results (measure and magnitude of effect), method of serum FLC analysis, missing data, loss to follow up, and evidence of reporting bias including funding source (215). Final study quality status was then agreed by discussion.

3.4.5. Data synthesis

IPD were amalgamated, and patient characteristics were summarized in tabular form, as per Section 2.5.2, including the number of missing values for each variable. Relationships between serum cFLC concentration and other baseline characteristics were assessed statistically as per Section 2.5.3, with graphs presented for non-linear relationships.

The primary analyses were performed using a one-stage approach, i.e. the associations between serum cFLC concentration and kidney failure and death were estimated from all data in all studies simultaneously. This approach allows more modelling flexibility than the traditional two-stage approach, for example, fitting non-linear effects. All models were stratified by study, to account for clustering of patients within studies (Section 2.5.5). Fixed-effects univariable and multivariable models were fitted, using subdistribution hazard models

for the analysis of time to kidney failure, handling death as a competing risk. Cause-specific hazard models were also fitted and are presented in Appendix 5. Cox proportional hazards regression was used for the analysis of time to death. All multivariable models were pre-specified.

To allow the plotting of forest plots and assessment of statistical heterogeneity by I^2, a supplementary two-stage analysis was performed, in which estimates of the association between serum cFLC concentration and adverse outcomes were generated for each study separately, before combining these estimates using the fixed-effects inverse-variance method.

Missing data were managed by multiple imputation, as per section 2.5.6. As 23% of cases had missing data in at least one variable, 25 imputations were used.

3.4.6. Assays

All included studies measured serum FLC concentration using the Freelite® immunoassay (The Binding Site Group Ltd, Birmingham, UK).

3.5. Results

3.5.1. Study selection and IPD obtained

The numbers of studies screened, assessed for eligibility, and included, with reasons for exclusions, are shown in the flow diagram in Figure 3.5.

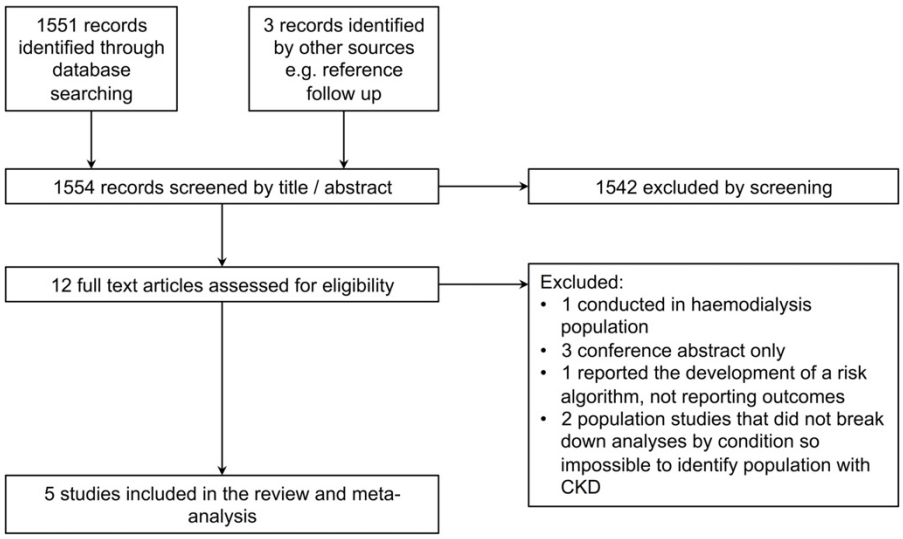


Figure 3.5. Flow diagram of studies screened, assessed, and included

Five studies were included, all of which were prospective cohort studies of patients with CKD (216-220). All five had presented an estimate of the association between serum FLC and the risk of death, and two presented an estimate of the association with kidney failure. IPD were sought and obtained from all five studies.

3.5.2. Study characteristics

The characteristics of each included study, including the number of participants, basic demographic data, and follow-up time, are presented in Table 3.3.

Table 3.3. Characteristics of each included study

First author, year	Number	Years of recruitment	Mean age (years)	Male sex (%)	CKD G stages	Follow-up (months)*
Assi at al. 2015 (216)	1695	2008-10	74	39	3	114.5
Desjardins et al. 2013 (217)	133 (89 non-dialysis)	2006-7	67	62	2-5	88.2
Haynes et al. 2011 (218)	364 (329 non-MGUS)	1997-99	61	65	3-5	72 for death 49.2 for kidney failure
Hutchison et al. 2014 (219)	848	2006-7	60	54	1-5	63
Ritchie et al. 2015 (220)	872	2004-10	66	62	3-5	41.4

**Average follow-up is presented as the median, except for Haynes et al., which is presented as the mean. MGUS = monoclonal gammopathy of undetermined significance.*

All studies were conducted in the UK apart from the study by Desjardins et al. which was conducted in France, and all studies recruited patients from secondary care apart from that by Assi et al. which recruited from primary care. The study by Haynes et al. included 35 participants with MGUS, and that by Desjardins et al. included 44 participants on dialysis. These participants were excluded from the meta-analyses.

3.5.3. Data integrity

The IPD obtained from each study were assessed as per Section 2.5.1, and there were no critical data issues identified.

3.5.4. Risk of bias within studies

Four studies were judged to have a moderate overall risk of bias, and one study was judged to have a low overall risk of bias. This risk of bias assessment across five domains and an overall judgement are shown in Figure 3.6.

Figure 3.6. Risk of bias within each study

Risk of bias in each domain and overall was judged to be low (green), moderate (yellow), or high (red).

3.5.5. Results of individual studies

The main results reported for each study are presented in Table 3.4. Two studies reported the association with kidney failure. An independent association between serum cFLC concentration and kidney failure was observed Ritchie et al. but not by Haynes et al. All five studies reported the association with death. The results obtained by Assi et al., Hutchison et al., and Ritchie et al. suggest an independent association between serum cFLC concentration and death whereas those by Desjardins et al. and Haynes et al. do not.

Table 3.4. Reported results from each study

First author, year	FLC analyses	Kidney failure			Death		
		Unadjusted HR (95% CI)	**Adjusted HR (95% CI)**	**Model covariates**	**Unadjusted HR (95% CI)**	**Adjusted HR (95% CI)**	**Model covariates**
Assi at al. 2015	Categorical: above/below 43.3 mg/l				**3.20 (2.34 to 4.36)**	**1.50 (1.04 to 2.16)**	Age, sex, CVD, DM, hypertension, smoking, eGFR, albuminuria, hsCRP, central obesity, PWV, serum albumin
Desjardins et al. 2013	Categorical: above/below the median for κ and λ separately				**κ: 3.05 (1.20 to 7.75)** λ: 1.35 (0.54 to 3.40)	κ: 1.22 (0.38 to 3.95) λ: 0.65 (0.22 to 1.92)	Age, eGFR
Haynes et al. 2011	Continuous: per +1SD ln(cFLC)	Not reported	1.05 (0.87 to 1.26)	Age, sex, eGFR	Not reported	1.15 (0.92 to 1.44)	Age, sex, eGFR, NT-proBNP, troponin, smoking
Hutchison et al. 2014	Continuous: per +1 ln(cFLC)				**3.21 (2.56 to 4.02)**	**2.71 (1.98 to 3.70)**	Age, ethnicity, CVD, hsCRP
Ritchie et al. 2015	Categorical: quartiles vs Q1 for death, vs Q1/2 for kidney failure	**Q3: 3.42 (2.20 to 5.30) Q4: 8.74 (5.85 to 13.06)**	**Q3: 1.72 (1.0 to 2.97) Q4: 3.73 (2.10 to 6.30)**	eGFR, PCR, phosphate	**Q3: 1.87 (1.30 to 2.69) Q4: 2.62 (1.84 to 3.71)**	**Q3: 1.49 (1.02 to 2.18) Q4: 1.99 (1.34 to 2.93)**	Age, eGFR, CVD

Statistically significant associations are in bold. Q = quartile.

3.5.6. Meta-analysis

This section reports the results obtained from the IPD meta-analysis.

3.5.6.1. Baseline characteristics

A summary of basic demographic characteristics, co-morbidities, serum cFLC concentration, and other standard prognostic variables from the amalgamated IPD is provided in Table 3.5, along with numbers of missing values.

Table 3.5. Summary of baseline data

Variable	Median (IQR) or *N*(%)	Missing data (*N*[%])
Age (years)	70 (60 to 77)	4 (0.1)
Sex		4 (0.1)
Male	1942 (50.5)	
Female	1905 (49.5)	
Ethnicity		108 (2.8)
White	3452 (92.2)	
Other	291 (7.8)	
Co-morbidities		
DM	853 (22.3)	23 (0.6)
CVD	1281 (33.5)	28 (0.7)
Systolic BP (mmHg)	137 (124 to 151)	151 (3.9)
Urine ACR (mg/mmol)	2.6 (0.3 to 23.1)	603 (15.7)
eGFR (ml/min/1.73 m^2)	43.3 (27.7 to 55.3)	19 (0.5)
Serum albumin (g/dl)	42 (39 to 44)	49 (1.3)
Serum calcium (mmol/l)	2.30 (2.22 to 2.39)	49 (1.3)
Serum phosphate (mmol/l)	1.16 (1.02 to 1.32)	139 (3.6)
RAASi	2343 (62.0)	69 (1.8)
Serum cFLC (mg/l)	49.4 (33.7 to 80.0)	16 (0.4)

Categorical factors are summarised as N with percentage, and continuous factors as the median with interquartile range.

The median serum cFLC concentration was 49.4 (IQR 33.7 to 80.0) mg/l, and the distribution is shown in Figure 3.7.

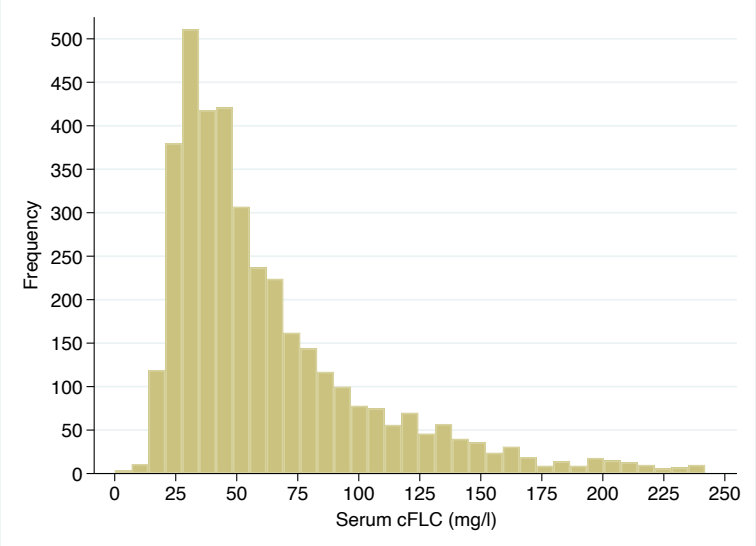


Figure 3.7. Histogram of serum cFLC concentration

Histogram illustrating the skewed distribution of serum cFLC concentration.

3.5.6.2. Relationship between cFLC and other prognostic factors

Statistical assessment of the relationships between serum cFLC concentration and other baseline factors are shown in Table 3.6.

Table 3.6. Relationships between cFLC and other baseline factors

Associations	Kendall's τ or Median (IQR)	*P*
Age	-0.004	0.73
Sex		<0.001
Female	42.9 (29.7 to 66.9)	
Male	57.8 (39.7 to 90.1)	
Ethnicity		<0.001
White	47.5 (33.1 to 75.1)	
Non-White	71.3 (44.5 to 121.8)	
DM		<0.001
Yes	63.7 (43.1 to 97.8)	
No	46.2 (32.0 to 73.6)	
CVD		<0.001
Yes	54.9 (38.1 to 89.5)	
No	46.7 (32.0 to 75.4)	
Systolic BP	0.122	<0.001
Urine ACR	0.413	<0.001
eGFR	-0.546	<0.001
Serum albumin	-0.069	<0.001
Serum calcium	-0.136	<0.001
Serum phosphate	0.193	<0.001
RAASi		0.90
Yes	49.4 (34.6 to 76.7)	
No	49.2 (32.2 to 84.6)	

The relationship between serum cFLC concentration and other continuous variables is presented as Kendall's τ with associated P-value. Relationships with categorical variables are shown as the median for each category with a P-value from a Mann-Whitney U test.

Serum cFLC concentrations were significantly higher in males, those of non-White ethnicity, and those with DM or CVD. There was a weak positive correlation with urine ACR and very weak positive correlations with systolic BP and serum phosphate. There was a moderate negative correlation with eGFR and very weak negative correlations with serum albumin and serum calcium.

In a multivariable analysis, variables that had an independent relationship with serum cFLC concentration were sex, ethnicity, serum albumin, eGFR, and urine ACR. Male sex (males 59.8 [45.3 to 83.3] mg/l; females 42.2 [32.0 to 61.8] mg/l; *P*<0.001), non-White ethnicity (non-White 77.5 [59.6 to 111.5] mg/l; White 49.0 [36.2 to 71.0]; *P*<0.001), lower

serum albumin (Figure 3.8), lower eGFR (Figure 3.9), and higher urine ACR (Figure 3.10) were associated with a higher serum cFLC concentration.

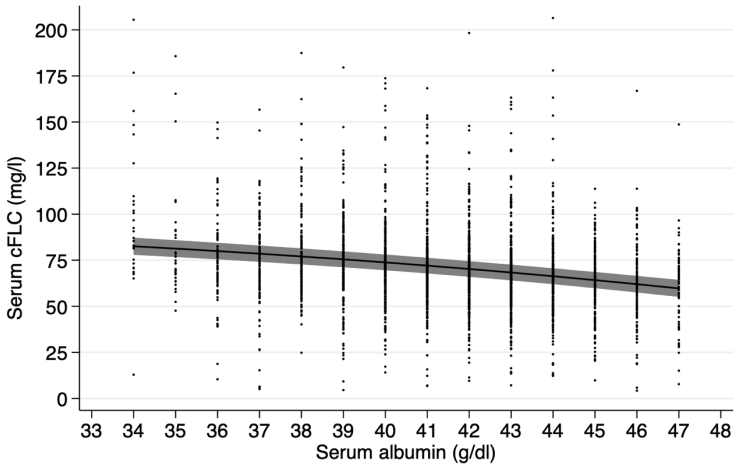


Figure 3.8. Relationship between serum albumin and serum cFLC concentration

Scatter plot. The line represents the predicted serum cFLC concentration with a 95% CI from an FP1 model with power 3.

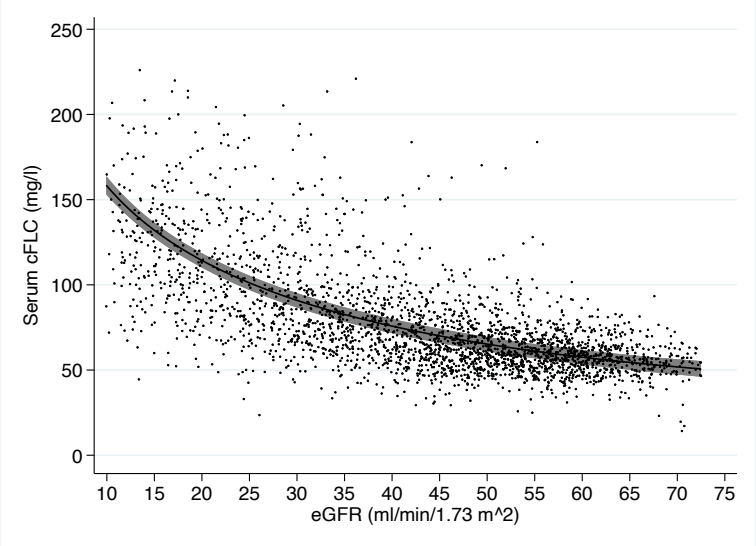


Figure 3.9. Relationship between eGFR and serum cFLC concentration

Scatter plot. The line represents the predicted serum cFLC concentration with a 95% CI from an FP2 model with powers 0 and 1.

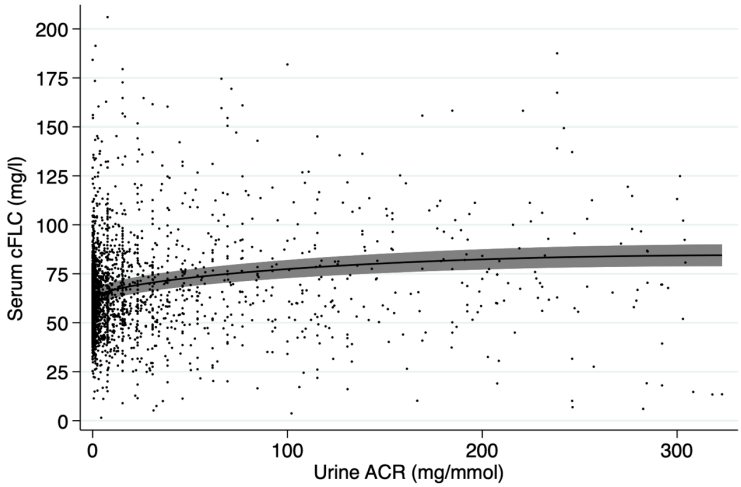


Figure 3.10. Relationship between urine ACR and serum cFLC concentration

Scatter plot. The line represents the predicted serum cFLC concentration with a 95% CI from an FP2 model with powers 0.5 and 2.

3.5.6.3. Kidney failure

Although only two studies had reported progression to kidney failure (Haynes et al. and Ritchie et al.), a third study (Hutchison et al.) had also collected outcome data for kidney failure, and these data were included in the meta-analysis.

Therefore, IPD for 2092 participants from three studies were included. During a median follow-up time of 5.7 years, 492 (23.5%) participants experienced kidney failure, with an overall kidney failure rate of 5.9 per 100 person-years.

The univariable associations and the multivariable model for kidney failure are shown in Table 3.7.

Table 3.7. Univariable and multivariable associations between baseline factors and risk of kidney failure

Variable	Univariable			Multivariable		
	SHR	**95% CI**	***P***	**SHR**	**95% CI**	***P***
Age	1.00[a]	1.00 to 1.00	<0.001	1.00[a]	1.00 to 1.00	<0.001
Male sex	0.78	0.65 to 0.93	0.007	0.92	0.76 to 1.11	0.37
Non-White ethnicity	1.55	1.21 to 1.99	0.001	1.17	0.89 to 1.54	0.27
DM	1.22	0.99 to 1.49	0.06	0.94	0.76 to 1.17	0.60
CVD	0.83	0.69 to 1.01	0.07	0.98	0.80 to 1.21	0.86
Systolic BP	1.15	1.05 to 1.27	0.004	1.11	1.00 to 1.24	0.048
Urine ACR	15.18[b] 0.12[c]	9.49 to 24.30 0.06 to 0.22	<0.001 <0.001	1.39[h] 1.01[i]	1.25 to 1.55 1.01 to 1.02	<0.001 <0.001
eGFR	0.00[d] 127669[e]	0.00 to 0.00 8940 to 1823193	<0.001 <0.001	0.00[b]	0.00 to 0.00	<0.001
Serum albumin	0.99[a]	0.98 to 0.99	<0.001	1.18	1.05 to 1.31	0.004
Serum calcium	0.47[a] 1.77[f]	0.39 to 0.56 1.55 to 2.02	<0.001 <0.001	0.86	0.78 to 0.96	0.005
Serum phosphate	73.21[d] 0.58[e]	38.85 to 137.96 0.50 to 0.67	<0.001 <0.001	1.30	1.15 to 1.48	<0.001
RAASi	1.28	1.06 to 1.54	0.011	1.06	0.86 to 1.31	0.58
Serum cFLC	0.19[g]	0.16 to 0.23	<0.001	1.76[h] 0.51[i]	1.33 to 2.31 0.38 to 0.69	<0.001 <0.001

For continuous variables with a linear association, SHR is per +1 SD. Two rows for a continuous variable indicate the SHR for each power of the degree-2 fractional polynomial transformation. Fractional polynomial transformations are denoted by: $a = x^3$; $b = x^{0.5}$; $c = x^{0.5}\ln(x)$; $d = x$; $e = x^2$; $f = x^3\ln(x)$; $g = x^{-1}$; $h = \ln(x)$; $i = (\ln(x))^2$.

On univariable analysis, a higher serum cFLC concentration was associated with a higher risk of kidney failure. The relationship was non-linear and is illustrated in Figure 3.11.

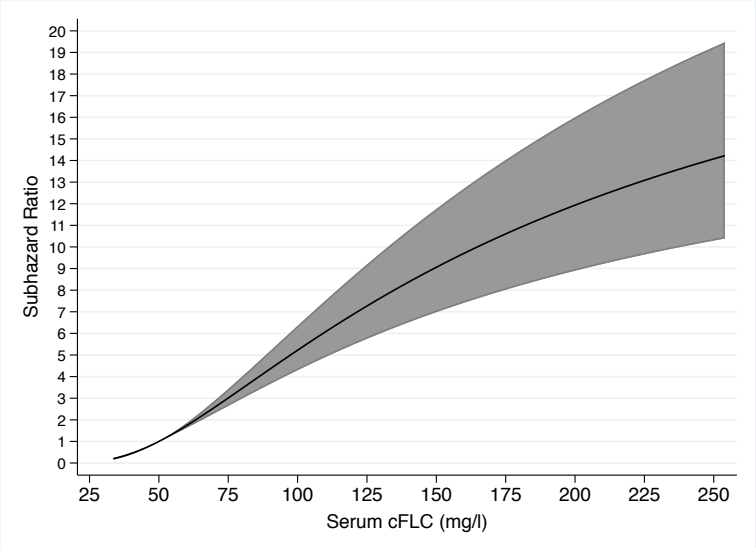


Figure 3.11. Unadjusted SHR for kidney failure according to serum cFLC concentration

Subhazard ratio with 95% CI, relative to 50 mg/l.

Other variables associated with a higher risk of kidney failure on univariable analysis were younger age (non-linear, Figure 3.12), female sex, non-White ethnicity, higher systolic BP, higher urine ACR (non-linear, Figure 3.13), lower eGFR (non-linear, Figure 3.14), lower serum albumin (non-linear, Figure 3.15), lower serum calcium (non-linear, Figure 3.16), lower serum phosphate (non-linear, Figure 3.17), and the use of RAASi. Diagnoses of DM and CVD were not associated with the risk of kidney failure.

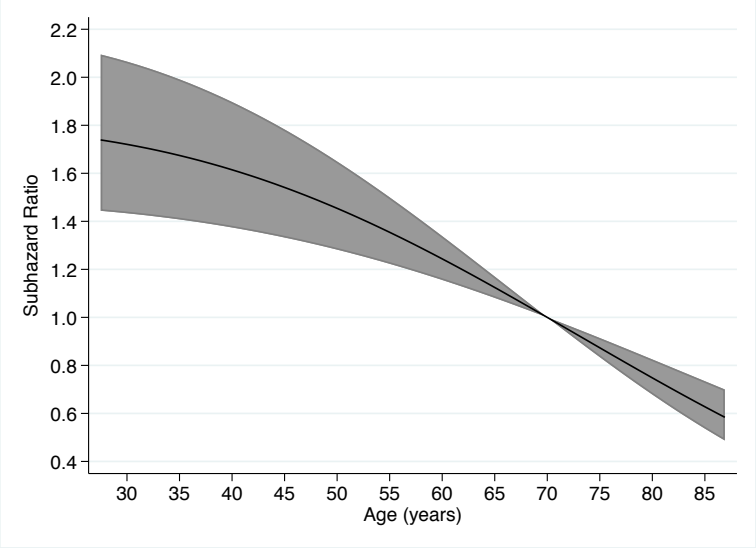


Figure 3.12. Unadjusted SHR for kidney failure according to age

SHR with 95% CI, relative to 70 years.

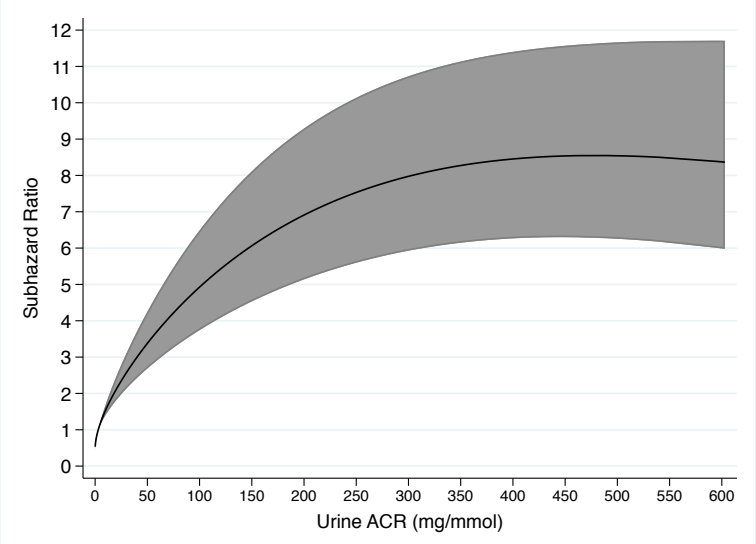


Figure 3.13. Unadjusted SHR for kidney failure according to urine ACR

SHR with 95% CI, relative to 3 mg/mmol.

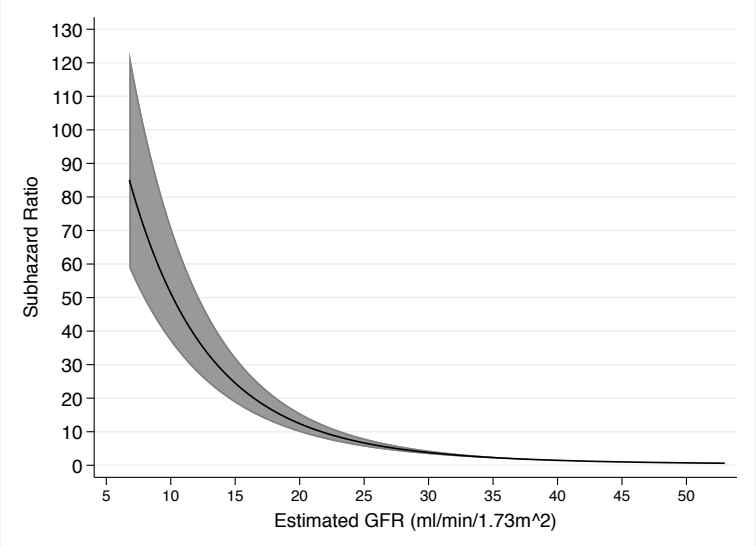


Figure 3.14. Unadjusted SHR for kidney failure according to eGFR

SHR with 95% CI, relative to 45 ml/min/1.73 m^2.

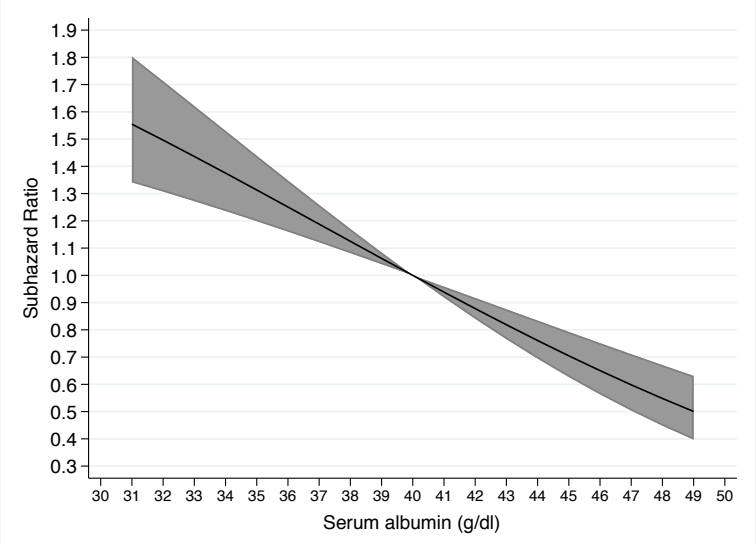


Figure 3.15. Unadjusted SHR for kidney failure according to serum albumin

SHR with 95% CI, relative to 40 g/dl.

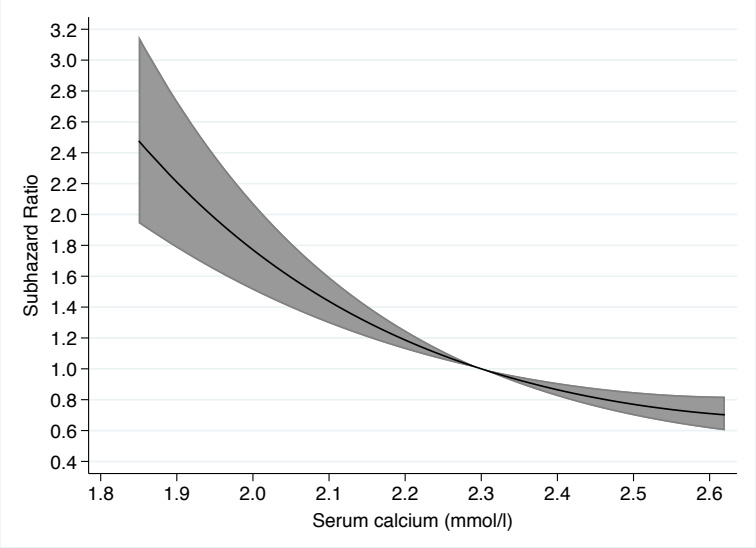


Figure 3.16. Unadjusted SHR for kidney failure according to serum calcium

SHR with 95% CI, relative to 2.3 mmol/l.

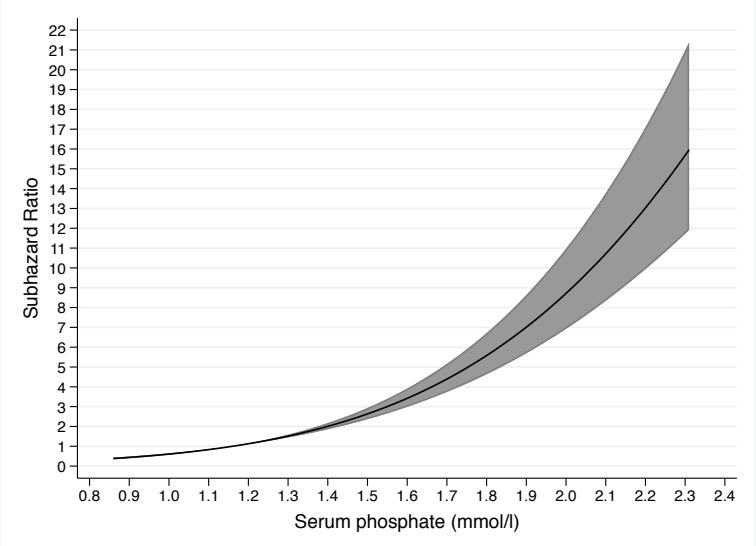


Figure 3.17. Unadjusted SHR for kidney failure according to serum phosphate

SHR with 95% CI, relative to 1.16 mmol/l.

In the multivariable model (Table 3.7), a higher serum cFLC concentration remained independently associated with an increased risk of kidney failure after adjustment for age, sex, ethnicity, DM, CVD, systolic BP, urine ACR, eGFR, serum albumin, serum calcium, serum phosphate, and use of RAASi. The association was non-linear, as shown in Figure 3.18, with a graded increase in the risk of kidney failure up to a serum cFLC concentration of approximately 150 mg/l. Beyond 150 mg/l, the risk does not appear to increase further.

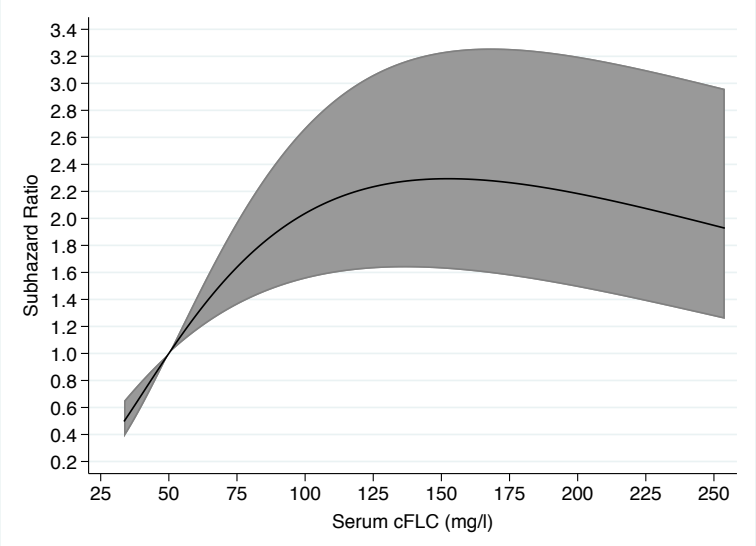


Figure 3.18. Adjusted SHR for kidney failure according to serum cFLC concentration

SHR with 95% CI, relative to 50 mg/l, from the multivariable model in Table 3.7.

A forest plot showing the risk of kidney failure associated with a higher serum cFLC concentration by study and overall is presented in Figure 3.19.

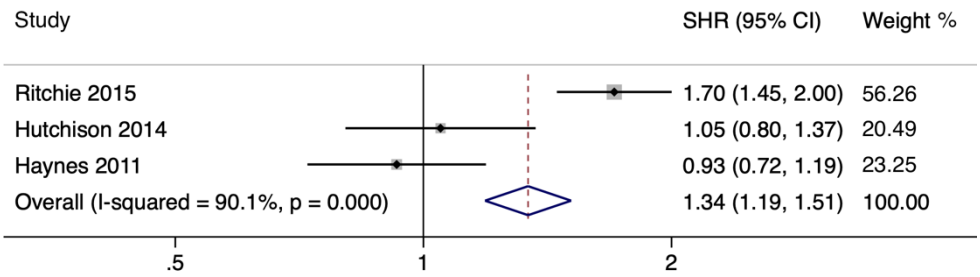


Figure 3.19. Forest plot for risk of kidney failure

Risk of kidney failure is per +1 SD in serum cFLC concentration, adjusted for age, sex, ethnicity, DM, CVD, systolic BP, urine ACR, eGFR, serum albumin, serum calcium, serum phosphate, and use of RAASi.

Other baseline factors associated with a higher risk of kidney failure in the multivariable model (Table 3.7) were younger age (non-linear, Figure 3.20), higher systolic BP, higher urine ACR (non-linear, Figure 3.21), lower eGFR (non-linear, Figure 3.22), higher serum albumin, lower serum calcium, and higher serum phosphate. Sex, ethnicity, DM, CVD, and use of RAASi were not significantly associated with the risk of kidney failure.

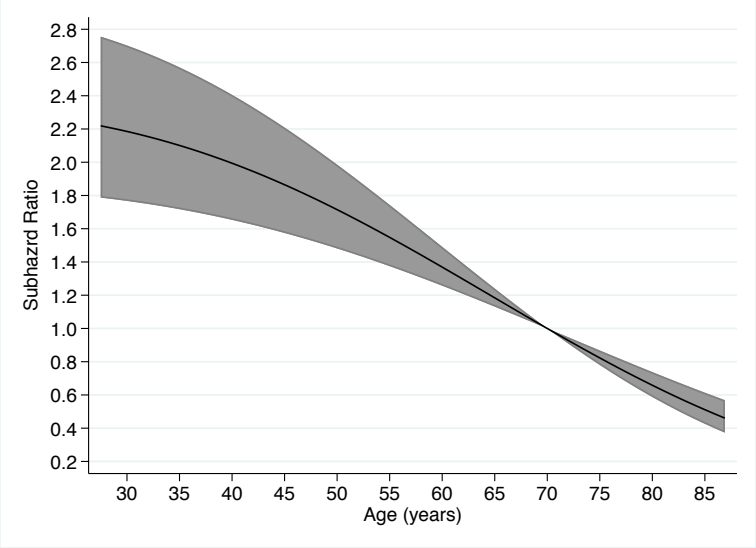


Figure 3.20. Adjusted SHR for kidney failure according to age

SHR with 95% CI, relative to 70 years, from the multivariable model in Table 3.7.

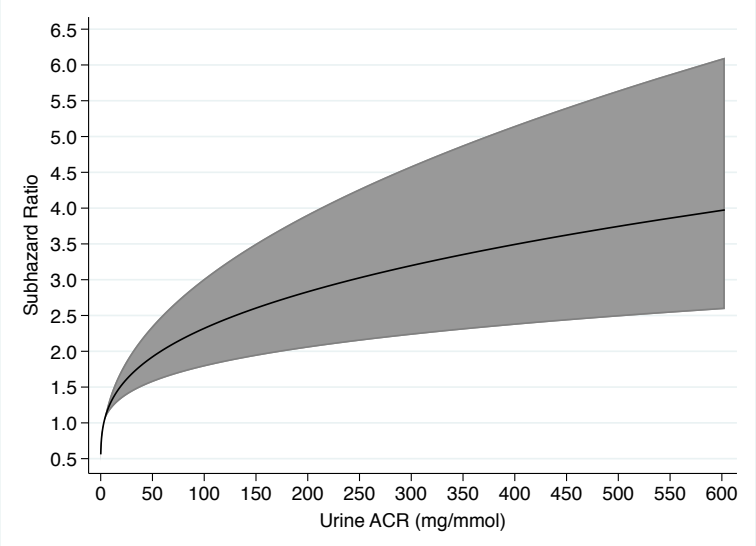


Figure 3.21. Adjusted SHR for kidney failure according to urine ACR

SHR with 95% CI, relative to 3 mg/mmol, from the multivariable model in Table 3.7.

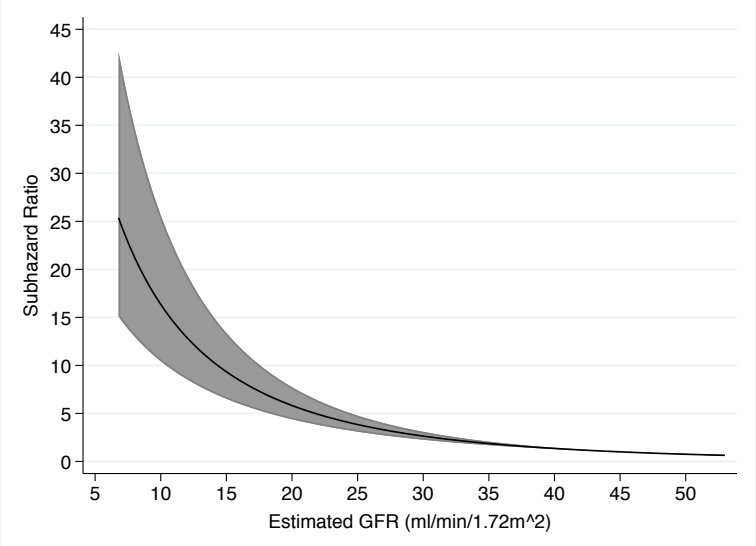


Figure 3.22. Adjusted SHR for kidney failure according to eGFR

SHR with 95% CI, relative to 45 ml/min/1.73 m^2, from the multivariable model in Table 3.7.

3.5.6.4. Death

Data for 3851 participants from all five studies were included in the analyses for death, with a median follow-up time of 4.1 years. 628 (16.31%) participants died, with an overall death rate of 4.3 per 100 person-years. Mean survival was estimated to be 26.1 years.

The univariable associations and the multivariable model for the risk of death are presented in Table 3.8.

Table 3.8. Univariable and multivariable associations between baseline factors and risk of death

Variable	Univariable			Multivariable		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age	2.89	2.57 to 3.25	<0.001	2.28	2.01 to 2.60	<0.001
Male sex	1.61	1.36 to 1.91	<0.001	1.31	1.10 to 1.57	0.003
Non-White ethnicity	0.68	0.47 to 0.97	0.033	0.79	0.55 to 1.15	0.22
DM	1.63	1.38 to 1.94	<0.001	1.26	1.06 to 1.51	0.009
CVD	2.77	2.35 to 3.26	<0.001	1.68	1.42 to 1.99	<0.001
Systolic BP	0.00[a] 74002[b]	0.00 to 0.00 216 to 2.53e+07	<0.001 <0.001	0.99	0.91 to 1.07	0.74
Urine ACR	1.05[c]	1.01 to 1.08	0.004	0.89	0.79 to 1.02	0.09
eGFR	0.00[d]	0.00 to 0.01	<0.001	0.77	0.66 to 0.90	0.001
Serum albumin	0.99[e]	0.98 to 0.99	<0.001	0.55[f] 2.12e+08[g]	0.00 to 92.34 2039 to 2.21e+13	0.82 0.001
Serum calcium	0.84	0.78 to 0.92	<0.001	0.99	0.91 to 1.07	0.75
Serum phosphate	1.22	1.13 to 1.32	<0.001	1.13	1.02 to 1.24	0.022
RAASi	0.85	0.72 to 1.00	0.054	0.93	0.78 to 1.10	0.40
Serum cFLC	3.82[c] 0.97[e]	3.15 to 4.63 0.94 to 0.99	<0.001 0.010	5.83[a] 0.96[e]	3.28 to 10.36 0.93 to 0.99	<0.001 0.009

For continuous variables with a linear association, HR is per +1 SD. Two rows for a continuous variable indicate the HR for each power of the degree-2 fractional polynomial transformation. Fractional polynomial transformations are denoted by: $a = x^{0.5}$; $b = x^{0.5}ln(x)$; $c = ln(x)$; $d = x^2$; $e = x^3$; $f = x^{-2}$; $g = x^{-2}ln(x)$.

A higher serum cFLC concentration was associated with a higher risk of death on univariable analysis, with a non-linear association, as shown in Figure 3.23.

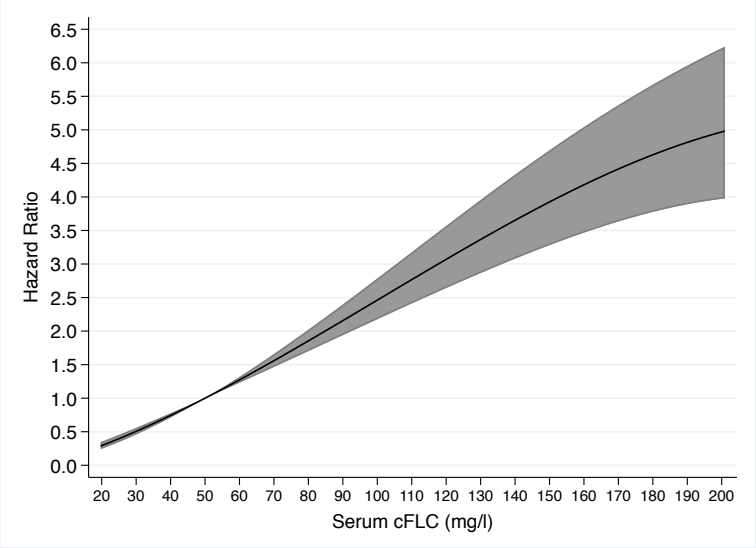


Figure 3.23. Unadjusted HR for death according to serum cFLC concentration

Hazard ratio with 95% CI, relative to 50 mg/l.

Other factors associated with a higher risk of death on univariable analysis were older age, male sex, White ethnicity, DM, CVD, higher systolic BP (non-linear, Figure 3.24), higher urine ACR (non-linear, Figure 3.25), lower eGFR (non-linear, Figure 3.26), lower serum albumin (non-linear, Figure 3.27), lower serum calcium, and higher serum phosphate. The use of RAASi was not associated with the risk of death on univariable analysis.

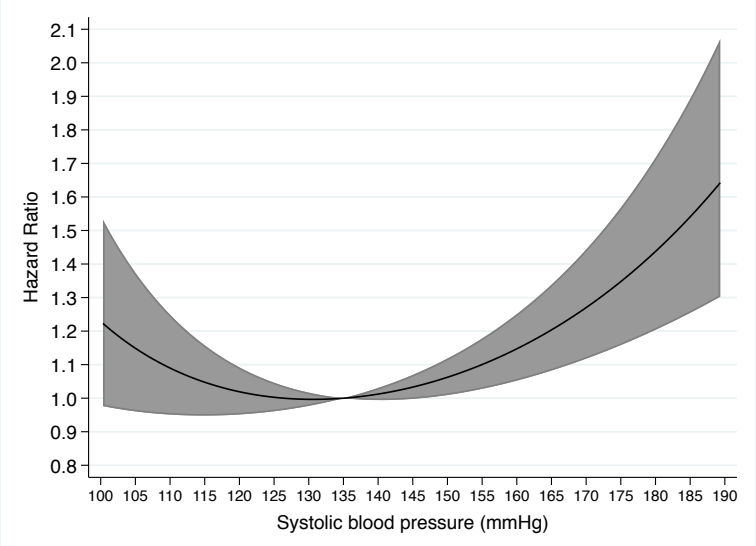


Figure 3.24. Unadjusted HR for death according to systolic BP

HR with 95% CI, relative to 135 mmHg.

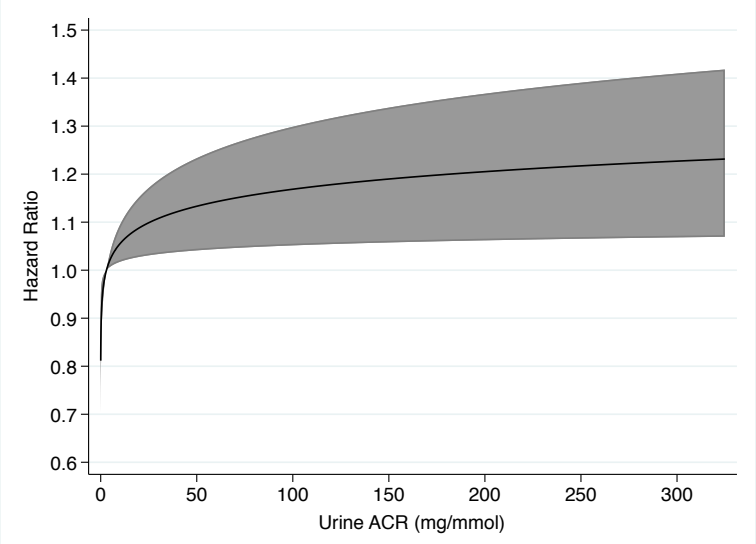


Figure 3.25. Unadjusted HR for death according to urine ACR

HR with 95% CI, relative to 3 mg/mmol.

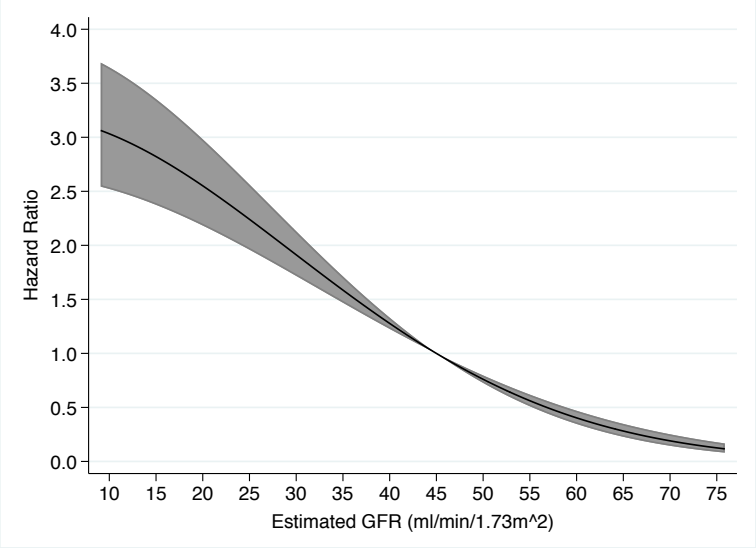


Figure 3.26. Unadjusted HR for death according to eGFR

HR with 95% CI, relative to 45 ml/min/1.73 m^2.

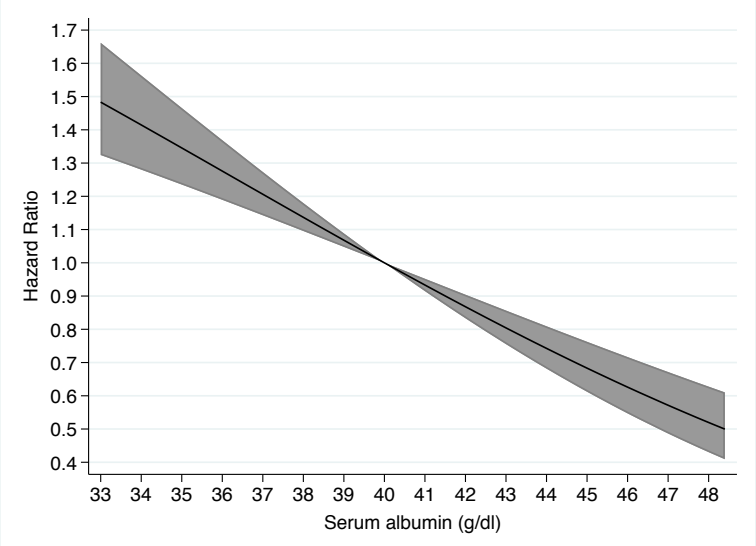


Figure 3.27. Unadjusted HR for death according to serum albumin

HR with 95% CI, relative to 40 g/dl.

In the multivariable model (Table 3.8), a higher serum cFLC concentration remained independently associated with a higher risk of death after adjustment for age, sex, ethnicity, DM, CVD, systolic BP, urine ACR, eGFR, serum albumin, serum calcium, serum phosphate, and the use of RAASi. Again, the association was non-linear and is shown in Figure 3.28.

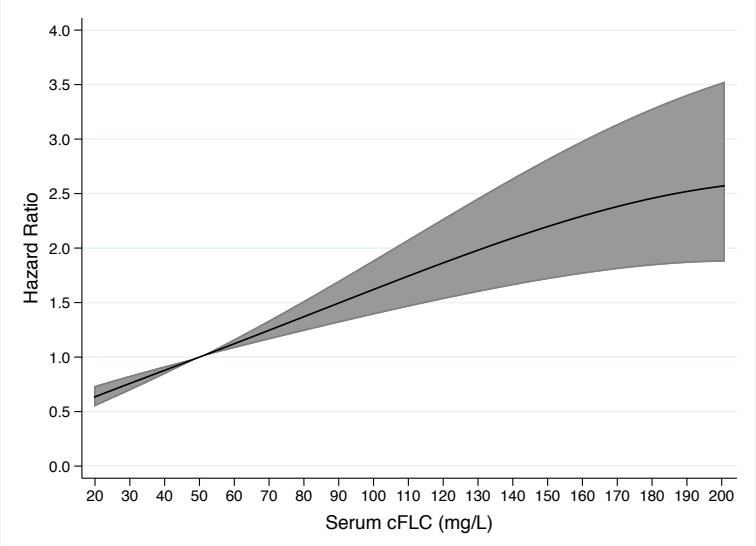


Figure 3.28. Adjusted HR for death according to serum cFLC concentration

HR with 95% CI, relative to 50 mg/l, from the multivariable model in Table 3.8.

A forest plot showing the risk of death associated with a higher serum cFLC concentration by study and overall is shown in Figure 3.29.

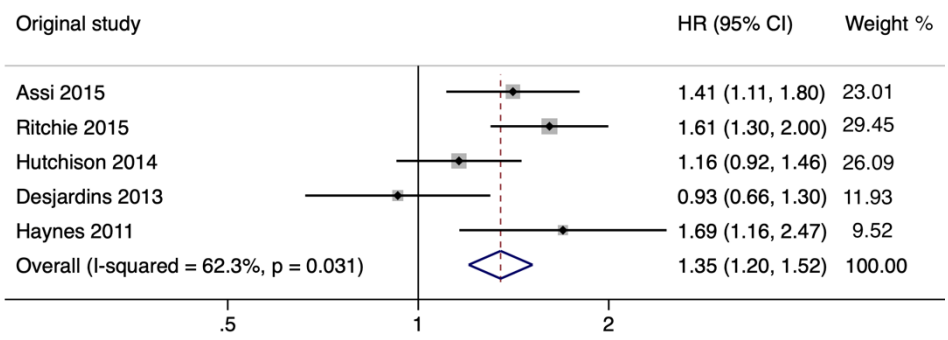


Figure 3.29. Forest plot for risk of death

Risk of death per +1 SD in serum cFLC, adjusted for age, sex, ethnicity, DM, CVD, systolic BP, urine ACR, eGFR, serum albumin, serum calcium, serum phosphate, and use of RAASi.

Other factors significantly associated with a higher risk of death in the multivariable model (Table 3.8), were older age, male sex, DM, CVD, lower eGFR, lower serum albumin (non-linear, Figure 3.30), and higher serum phosphate. Ethnicity, systolic BP, urine ACR, serum calcium, and the use of RAASi were not associated with the risk of death.

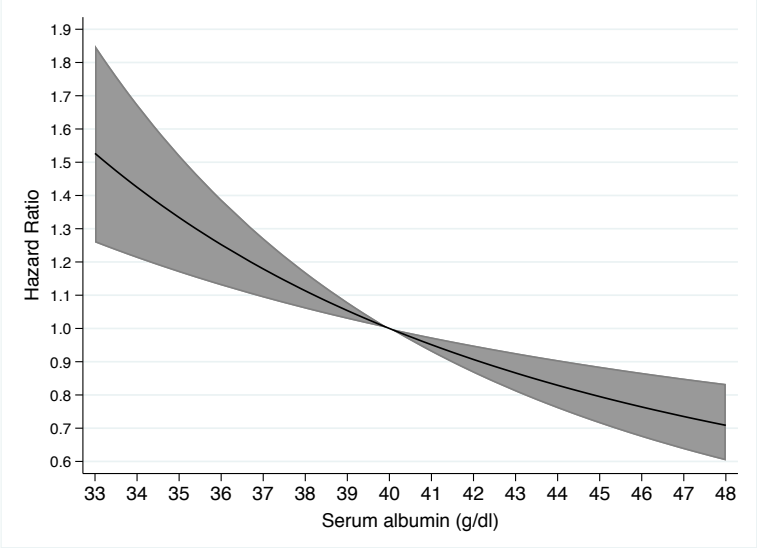


Figure 3.30. Adjusted HR for death according to serum albumin

HR with 95% CI, relative to 40 g/dl, from the multivariable model in Table 3.8.

3.6. Discussion

This systematic review and meta-analysis of IPD examined the hypotheses that higher serum cFLC concentrations in patients with CKD are associated with a higher risk of kidney failure and death, and the results are consistent with these hypotheses.

Five moderate-to-good quality prospective cohort studies were included that incorporated patients across the full spectrum of pre-dialysis CKD (216-220). There was an independent association between serum cFLC concentration and the risk of both kidney failure and death in analyses that included established prognostic factors.

Analysis of the data showed that eGFR was a significant determinant of serum cFLC concentration, which increased as eGFR decreased, consistent with previously published results (201, 216-218, 220). Sex, ethnicity, serum albumin, and urine ACR were also shown to be independent determinants of serum cFLC concentration, relationships that have been demonstrated in these data in their original studies (216, 218, 220).

3.6.1. Kidney failure

Two studies had reported an estimate of the association between serum cFLC concentration and the risk of kidney failure (218, 220). However, these studies reported inconsistent results, and it remained unknown whether an independent association existed. By incorporating additional IPD from a third study and applying a uniform pre-specified analysis across the data from all cohorts, this meta-analysis provides the most persuasive evidence to date on serum cFLC concentration as a risk factor for kidney failure in CKD.

The results of the meta-analysis show that a higher serum cFLC concentration is independently associated with a higher risk of kidney failure in patients with CKD after

adjustment for age, sex, ethnicity, DM, CVD, systolic BP, urine ACR, eGFR, serum albumin, serum calcium, serum phosphate, and use of RAASi. Further, the risk appears to increase with serum cFLC concentration up to a concentration of approximately 150 mg/l, beyond which the risk plateaus.

The results of this meta-analysis do not prove that high serum cFLC concentrations have a causal role in the risk of kidney failure, and to date, there are no published studies that report a direct mechanism for non-clonal FLCs in kidney injury. However, there are biologically plausible mechanisms by which high concentrations of serum FLCs may exacerbate kidney damage in CKD, thus increasing the risk of progression to kidney failure.

First, it is well established that monoclonal FLCs can cause direct kidney injury in multiple myeloma, B-cell lymphoproliferative disorders, or monoclonal gammopathy of renal significance. In these disorders, the FLCs can cause kidney disease through various pathways that can result in deposition diseases, cast formation, or tubular toxicity. The latter may be caused by the induction of pathways linked with inflammation, apoptosis, and fibroblastic differentiation (221-224).

Second, in CKD, it has been shown that non-clonal FLCs can bind with uromodulin to form tubular casts, and the number of FLC-containing casts positively correlates with indices of chronic kidney damage and interstitial macrophage numbers, and inversely correlates with capillary density (225). It has been suggested that non-clonal FLCs in CKD might promote cast formation in the distal tubules, leading to interactions between uromodulin and macrophages and the promotion of fibrosis (225). Another study observed that in patients with CKD, there is a significant deposition of FLCs in the renal tubules, the degree of which correlates with the degree of renal impairment and interstitial fibrosis (226).

Third, FLCs are biologically active molecules, with enzymatic activity, binding of various intra- and extra-cellular proteins, and binding to various cells such as mesangial cells, B-cells, and mast cells (227). Again, although there is no direct evidence to show any of these biological activities of FLCs play a specific role in the risk of kidney failure, there are plausible mechanisms by which they might, such as the activation of mast cells which can contribute to the development of interstitial fibrosis (228).

3.6.2. Death

All five included studies presented an estimate of the association between serum FLCs and the risk of death. Three studies observed an independent association with death, while two studies did not. Therefore it remained unknown whether non-clonal serum FLC concentration is associated with mortality in patients with CKD.

The meta-analysis of IPD from the five studies demonstrated that a higher serum cFLC concentration was associated with a higher risk of death after adjustment for age, sex, ethnicity, DM, CVD, systolic BP, urine ACR, eGFR, serum albumin, serum calcium, serum phosphate, and the use of RAASi.

Higher serum cFLC concentrations may reflect changes in the non-renal determinants of its concentration, such as B-cell stimulation and activation and reticuloendothelial health, which may themselves be associated with the risk of death. However, there are plausible mechanisms through which FLCs themselves, through their multitude of biological activities, may have a causal role in the higher risk of death. Serum FLCs isolated from patients with kidney disease have been shown to abrogate essential functions of neutrophils, including chemotaxis (229, 230). They also inhibit neutrophil apoptosis which may interfere with the resolution of inflammation, thus perpetuating a chronic inflammatory state, which is

associated with adverse outcomes in patients with CKD (229-231). Further, FLCs activate mast cells which may accelerate both atherosclerosis and myocardial fibrosis, and indeed serum cFLC concentration has previously been shown to correlate with cardiovascular risk in both type 1 and type 2 DM (227, 232-234).

The association between serum cFLC concentration and risk of death observed in this meta-analysis is consistent with general population studies identifying an association between elevated serum FLCs and mortality in individuals without CKD (207, 235). However, contrary to our findings in patients with pre-dialysis CKD, a study of patients with kidney failure being treated with haemodialysis found an inverse relationship between serum cFLC concentration and death, i.e. a higher serum cFLC concentration was associated with a lower risk of death (236). The authors of that study speculated that higher serum cFLC concentrations may reflect less uraemia-related bone marrow dysfunction and that increased serum FLC concentrations may be associated with improved defence against infection. Further work is needed in the haemodialysis population to validate that study's findings.

The renal clearance of FLCs and the association between serum cFLC concentration and kidney function was described in Section 3.2.2. The associations between serum cFLC concentration and adverse outcomes demonstrated in this meta-analysis may reflect a residual confounding effect of kidney function. However, there is good supportive evidence for a truly independent association between serum FLCs with adverse outcomes. First, the significant association between serum cFLC concentration and adverse outcomes remained after adjustment for creatinine-based eGFR. Although it has been suggested that other filtration markers such as cystatin C, beta-trace protein (BTP), and beta-2-microglobulin (B2M) may provide more accurate estimates of GFR, a patient-level meta-analysis showed limited

additional value for these markers for outcome assessment in analyses that included creatinine-based eGFR (237).

Second, while serum cFLC concentration is in part determined by kidney function, other factors are important, such as B-cell and plasma cell stimulation and activation, and non-renal clearance through the reticuloendothelial system, which accounts for a greater proportion of clearance in CKD as kidney function declines (238). The degree of correlation with kidney function, and the large variability that remains in serum cFLC concentration after adjustment for kidney function, provides evidence for the significant contribution of these non-renal factors in the determination of serum cFLC concentration.

Third, the association between serum cFLC concentration and death has been demonstrated in studies of the general population with normal kidney function and studies of non-renal disease, supporting the theory that FLCs have adverse effects via mechanisms other than through an association with kidney impairment (207, 209, 212, 235, 239). It remains possible that even in these groups without kidney disease, the serum cFLC concentration partially reflects the spectrum of kidney function.

Finally, there are biologically plausible mechanisms for a relationship between serum FLCs and adverse outcomes, such as through their association with inflammation and reticuloendothelial system health.

3.6.3. Strengths and limitations

The strengths of this meta-analysis include a broad search strategy, the use of robust methods for study selection and quality assessment, and the inclusion of IPD from all eligible studies. Models were pre-specified and measures to reduce the risk of bias, including robust

statistical methods such as including non-linear associations and the use of multiple imputation to address missing data, were used.

Limitations include a limited search of grey literature, such that studies may have been missed if they were only reported as conference abstracts. Further, data were not available for other markers of systemic inflammation, such as C-reactive protein, or other prognostic factors such as the cause of CKD.

3.6.4. Future research

Further research is needed to identify the biological basis for the associations between serum FLCs and adverse outcomes in CKD. If evidence emerges of a causal role in the risk of kidney failure and death, then FLCs as a treatment target may be explored.

Further, the utility of serum cFLC concentration as a biomarker for enhanced risk prediction and risk stratification should be assessed. Serum FLC concentration, unlike many other biomarkers assessed as prognostic factors, are now routinely available for measurement in clinical practice. An initial assessment of the incremental value of serum cFLC concentration, when added to existing prognostic factors, could be performed in existing data. However, a robust assessment to produce generalisable results and a cost-benefit analysis would require a multi-centre validation study.

3.7. Conclusion

The serum cFLC concentration in patients with CKD without monoclonal disease is an independent prognostic factor for the risks of kidney failure and death. The nature of the associations, in particular, whether there is a causal relationship, in which case FLCs may ultimately be assessed as treatment targets, requires further research. Further work is also required to assess the potential use of serum cFLC concentration in risk prediction and stratification in patients with CKD.

CHAPTER IV: URINE FREE LIGHT CHAINS

The work presented in this chapter aimed to address the hypotheses that higher concentrations of urine free light chains (FLC) are associated with a higher risk of kidney failure and death in patients with chronic kidney disease (CKD). It has previously been shown that serum FLC concentration correlates with urine FLC excretion and following on from the results of Chapter III, an assessment of urine FLC as an independent prognostic factor in CKD was conducted.

This work has been published in the article 'Association between urinary free light chains and progression to end stage renal disease in chronic kidney disease,' in PLOS ONE in 2018 (240), and presented in poster format at the UK Kidney Week, Harrogate, 2018.

4.1. Abstract

Background

Urine free light chain (FLC) excretion correlates with serum FLC concentration, which is an independent prognostic factor in CKD. Further, urinary FLCs may reflect tubular exposure to potentially nephrotoxic FLCs. As assessment was made of the association between urine FLC and kidney failure and death in patients with CKD. Further, the incremental value of urine FLCs when added to an established model for the prediction of kidney failure was assessed.

Materials and Methods

Five hundred fifty-six patients with CKD and urine FLC measurements from a prospective cohort study were included, with a median follow-up time of 6.1 years. The association between urine kappa/creatinine (κCR) and lambda/creatinine (λCR) ratios and development of kidney failure was assessed by competing-risks regression (to account for the competing risk of death). The change in C-statistic and integrated discrimination improvement were used to assess the incremental value of adding urine κCR or λCR to the Kidney Failure Risk Equation (KFRE). Cox proportional hazards regression was performed to assess the association with death.

Results

One hundred ninety-one participants developed kidney failure, and 129 participants died. Higher urine κCR and λCR were associated with a higher risk of kidney failure, but the associations lost significance after adjustment for standard prognostic factors. Neither κCR nor λCR provided incremental value when added to the KFRE for estimating the risk of kidney failure at two years. Similarly, higher urine κCR and λCR were associated with a

higher risk of death on univariable analysis, but not after adjustment for standard prognostic factors for mortality.

Conclusions

Despite a correlation with serum FLCs, urine FLC excretion is not independently associated with the risk of kidney failure or death in patients with CKD. Further, they do not improve upon a current model for risk stratification.

4.2. Introduction

There are prognostic factors measurable in the urine of patients with CKD that are associated with the risk of kidney failure or death, the most significant by far being albumin. Urinary albumin is a powerful prognostic factor because, not only is it a marker of glomerular damage, but it may also itself be involved in the pathogenesis of progressive CKD, exacerbating kidney damage in the tubulointerstitium.

The identification of urinary factors that provide prognostic information over and above that provided by standard prognostic factors, including urinary albumin, has the potential to bring numerous benefits to clinical practice, including improved risk stratification and the identification of targets for new therapies. The search for these urinary factors is more likely to be fruitful when focused on markers that are of a different size to albumin, undergo different renal handling, and which have a direct causal association with the adverse outcomes being assessed.

Urine FLCs meet these criteria. FLC molecules are smaller than albumin and processed differently in the kidneys, such that urine FLC concentrations are likely to be a reflection of tubular, rather than glomerular, health. Further, urine FLCs are determined in part by serum FLC concentration which, as shown in Chapter III is an independent prognostic factor in CKD. Finally, FLCs are known to have direct pathogenetic properties in the kidney, and therefore urine FLCs merit study as potential prognostic factors in CKD, and in particular whether they provide prognostic information with regard to the risk of kidney failure beyond that provided by standard prognostic factors including urinary albumin.

It is also possible that urinary FLCs may be associated with mortality risk. Besides a possible correlation with serum FLC, several urinary markers of tubular injury have previously been shown to be associated with mortality.

4.2.1. Urine FLCs

An overview of FLC biology was presented in Section 3.2. Free light chains filtered at the glomerulus are endocytosed and catabolized by proximal tubular cells, such that in health no FLCs from glomerular filtration are excreted in the urine. A small quantity of FLCs is excreted in the urine but is thought to originate further down the urinary tract alongside secretory IgA for mucosal defence (197).

The presence of significant FLC in the urine implies either concentrations in the proximal tubule greater than can be reabsorbed, usually due to excess monoclonal FLC production in plasma cell dyscrasias, or renal disease with glomerular hyperfiltration or tubular dysfunction (198).

As with albumin and other urinary markers, the urinary excretion of FLCs may be expressed as a ratio with urine creatinine concentration to give a urine κ/creatinine ratio (κCR) and urine λ/creatinine ratio (λCR). This adjusts for variable urine concentration and would be expected to correlate with daily urine FLC excretion.

4.2.2. Urine FLCs in CKD

There is a negative correlation between urine FLC/creatinine ratios and eGFR, such that in patients with CKD, urine κCR and λCR progressively increases as GFR declines (201). This relationship was demonstrated in a study of 338 patients with CKD and is illustrated in Figure 4.1.

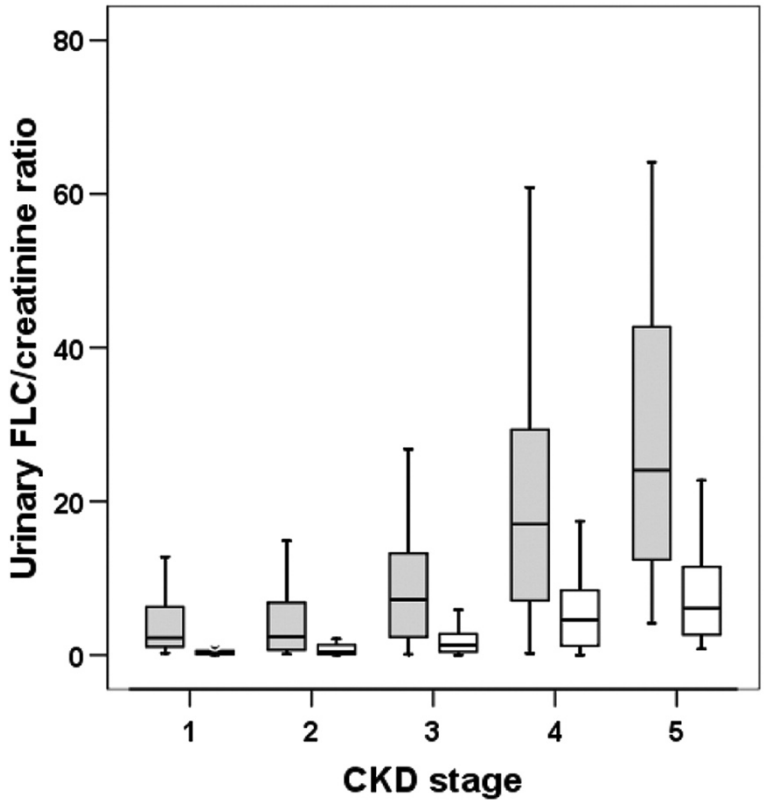


Figure 4.1. Urine FLC excretion by stage of CKD

Urine κCR (grey) and λCR (white) increase with worsening CKD G stage. From (201).

The proportion of patients with abnormal urine FLC/creatinine ratios, relative to a reference range derived in a healthy population, increased with each CKD stage (36, 50, 74, 89, and 100%, for CKD G stages 1 through 5, respectively; $P < 0.001$) (201).

Further, the proportion of patients with abnormal urine FLC/creatinine ratios also increased with each higher category of albuminuria (45, 61, 89, and 93% for urine ACR < 2, 2 to 10, 10 to 20, and > 20 mg/mmol, respectively; $P < 0.001$), as shown in Figure 4.2 (201).

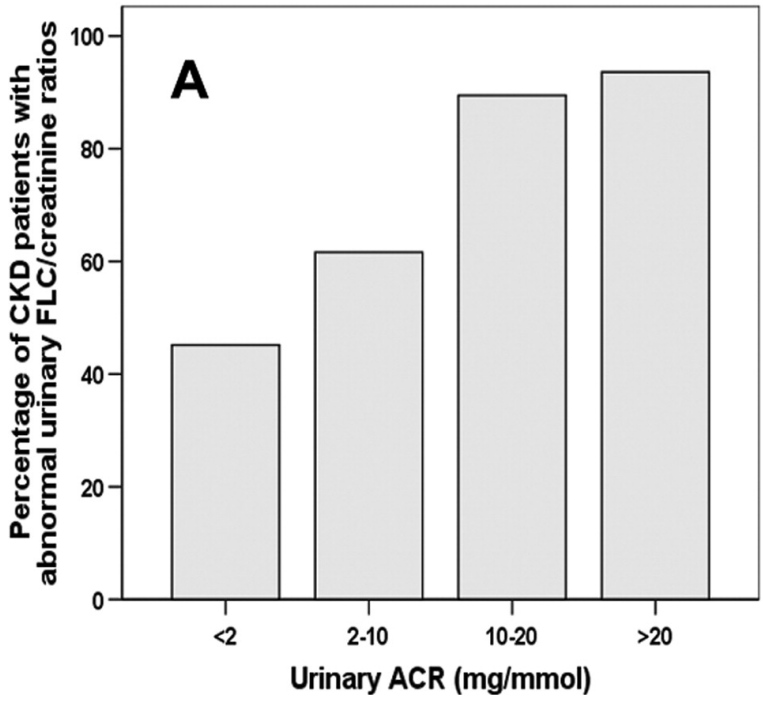


Figure 4.2. Abnormal urine FLC/creatinine ratios according to urine ACR

Abnormal defined as urine κCR > 4.0 or λCR > 0.45, taken as the upper limits of the 95% confidence intervals from a healthy population. From (201).

It has also been demonstrated that there is a correlation between urinary κCR and λCR and their corresponding serum FLC concentrations (R = 0.55 and 0.57 respectively, $P <$ 0.001, controlling for urine ACR) (201).

Several studies suggest that urinary FLCs may be more sensitive than albuminuria as a marker of early CKD. Two studies observed that urine κCR and λCR are raised before the development of increased albuminuria in patients with type 2 diabetes mellitus (DM) (241, 242). Urine FLC concentrations have also been shown to correlate with disease activity in IgA nephropathy and lupus nephritis (243, 244).

Urine FLCs have not been studied for their use in the diagnosis of CKD or prognosis. Given that early CKD is associated with higher urinary excretion of FLCs, urine FLCs may

have a role in the early detection of kidney disease. Further, FLC molecules have properties quite different from albumin, including their size, renal handling, and pathogenetic properties within the kidney, and their role as prognostic factors merits study. The work presented in this chapter aimed to address whether urinary FLC excretion in patients with CKD is independently associated with the risk of kidney failure or death and, further, whether they may have a potential role in risk stratification.

4.3. Hypotheses

The following pre-specified hypotheses were addressed:

1. Higher urine FLC/creatinine ratios are associated with a higher risk of kidney failure in patients with CKD;
2. Higher urine FLC/creatinine ratios add incremental value to the Kidney Failure Risk Equation for the prediction of kidney failure by two years in patients with CKD;
3. Higher urine FLC/creatinine ratios are associated with a higher risk of death in patients with CKD.

4.4. Methods

4.4.1. Patients

Data and samples from the Renal Impairment in Secondary Care (RIISC) Study, a prospective cohort study of patients with CKD, were used. The RIISC study is described in detail in Section 2.1.

All eligibility criteria described in Section 2.1.3 applied, but for this analysis, participants with a monoclonal gammopathy were also excluded, defined as:

- a known diagnosis of myeloma, MGUS, AL amyloidosis, or another monoclonal gammopathy of renal significance, or
- a serum κ/λ FLC ratio outside of the renal reference range (0.37-3.1) with an increased concentration of the involved light chain.

4.4.2. Urine FLCs

Urine concentrations of κ and λ FLCs were measured by turbidimetry on a Roche Modular P analyser using the Freelite® immunoassay (The Binding Site Group Ltd, Birmingham, UK). Urine κ and λ concentrations were divided by urine creatinine concentration, measured using a Roche Hitachi 702 analyser, to obtain urine κCR and λCR (in mg/mmol).

4.4.3. Other variables

The measurement of serum creatinine concentration and urine ACR and the calculation of eGFR by the CKD-EPI equation were all performed as stated in Section 2.1.4.6. Mean arterial pressure (MAP) was calculated as $((2 \times DBP) + SBP) \div 3$.

4.4.4. Follow-up

Patients were recruited between October 2010 and December 2015, and data up to December 2018 were collected for the following outcomes:

- Kidney failure, defined as the initiation of kidney replacement therapy (dialysis or kidney transplantation)
- Death, from any cause.

4.4.5. Statistical methods

The distributions of baseline characteristics, including urine κCR and λCR, are presented in tabular form with the number of missing values reported for each variable. Histograms are plotted to show the distribution of urine κCR and λCR.

The relationships between urine κCR and λCR and other baseline variables were assessed statistically. Relationships with continuous variables are expressed as Pearson's *r* (after log transformation of both variables) with its corresponding *P*, and fractional polynomials were used to assess for non-linear relationships and presented graphically. For categorical variables, median and interquartile ranges are shown with between-group differences assessed using the Mann-Whitney U or Kruskal-Wallis tests.

Univariable and multivariable regression models were fitted to show the association between urine κCR and λCR and other variables with adverse outcomes. Subdistribution hazard models were used to assess the association with kidney failure (handling death as a competing risk) and presented as a subhazard ratio (SHR) with a 95% confidence interval (CI). Cause-specific hazard models were also fitted and are presented in Appendix 6. Cox proportional hazards regression was used to assess associations with death and are presented

as a hazard ratio (HR) with 95% CI. Multivariable models were prespecified and non-linear associations were assessed, as per Section 2.5.4.2.

Missing data were handled by multiple imputation as per Section 2.5.6. For the kidney failure model, 19% of participants had missing data in at least one variable, and therefore 20 imputations were used. For the death model, 11% of participants had missing data, and therefore 15 imputations were used.

4.4.5.1. Risk stratification

To examine whether urine κCR or λCR provide incremental value in risk stratification, the four-variable Kidney Failure Risk Equation (KFRE) (106) (Section 1.4.1.2) was used as the baseline model for comparison. The KFRE estimates an individuals' risk of kidney failure at two and five years. Binary logistic regression models were fitted for the outcome of kidney failure at two years. The baseline model contained only the KFRE-calculated two-year risk of kidney failure, calculated as:

$$1 - 0.9832^{e^{(-0.2201 \times (age/10 - 7.036) + 0.2467 \times (male - 0.5642) - 0.5567 \times (eGFR/5 - 7.222) + 0.4510 \times (logACR - 5.137))}}$$

(the four-variable, non-North America, two-year risk equation from eAppendix 2 of (107); ACR was converted to mg/g before being entered into the model by dividing by 0.113).

Urine κCR and λCR (separately) were added to the baseline KFRE model and the models compared. Overall model performance was estimated by pseudo R^2, discrimination was assessed by the C-statistic, and calibration by the Hosmer-Lemeshow goodness-of-fit test. The incremental value of adding urine κCR or λCR to the baseline model was assessed by the change in C-statistic and by the reclassification measure Integrated Discrimination Index (IDI). The IDI is a measure of the extent to which adding a new marker to a model correctly revises upward the predicted risk of individuals who experience an event and

correctly revises downward the predicted risk of individuals who do not experience an event (245, 246).

4.5. Results

Urinary FLCs were measured in 636 participants of the RIISC study. Forty-one participants were excluded because they had evidence of a monoclonal gammopathy (21 with a serum κ/λ FLC ratio outside the renal reference range, 15 with MGUS, and 5 with multiple myeloma). Further, 39 patients had urine FLC or creatinine concentrations above or below the limits of detection and were excluded because urine κCR and λCR could not be calculated. Therefore, 556 participants were included for analysis. Median follow-up time was 6.1 years, and there were 191 kidney failure events and 129 deaths.

4.5.1. Baseline characteristics

The baseline characteristics of the study population are shown in Table 4.1.

Table 4.1. Baseline characteristics of the study population

Characteristic	Median (IQR) or *N* (%)	Data completeness (%)
Age (years)	64 (51 to 76)	100
Male gender	351 (63.1)	100
Ethnicity		100
White	380 (68.3)	
South Asian	117 (21.0)	
Black	56 (10.1)	
Other	3 (0.5)	
Co-morbidities		100
DM	196 (35.3)	
IHD	120 (21.6)	
Cerebrovascular disease	53 (9.5)	
PAD	53 (9.5)	
COPD	57 (10.3)	
Malignancy	71 (12.8)	
Cause of CKD		90
Ischaemic/hypertensive	145 (28.9)	
Glomerulonephritis	72 (14.3)	
Diabetic kidney disease	65 (12.9)	
Polycystic kidney disease	29 (5.8)	
Interstitial nephropathy	29 (5.8)	
Reflux nephropathy	12 (2.4)	
Other/uncertain	150 (29.9)	
eGFR (ml/min/1.73 m^2)	24.9 (19.3 to 34.1)	98
Urine ACR (mg/mmol)	28.1 (5.7 to 103.5)	92
Blood pressure (mmHg)		
Systolic	128 (116 to 144)	99
Diastolic	76 (68 to 85)	99
Serum κ (mg/l)	44.9 (29.4 to 67.0)	99
Serum λ (mg/l)	32.5 (23.4 to 47.0)	99
Urine κCR (mg/mmol)	14.6 (7.1 to 27.7)	100
Urine λCR (mg/mmol)	2.1 (1.0 to 5.1)	100

Categorical variables summarised as the number and %, and continuous variables as the median and interquartile range.

Median urine κCR was 14.6 (IQR 7.1 to 27.7) mg/mmol and median λCR was 2.1 (IQR 1.0 to 5.1) mg/mmol. Their distributions are shown in Figure 4.3.

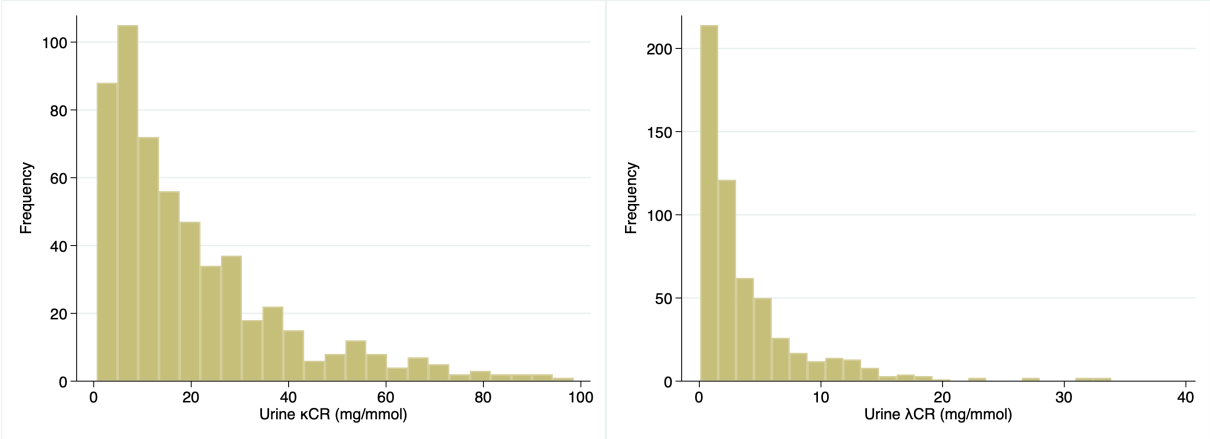


Figure 4.3. Histograms of urine κCR and λCR

Histograms illustrating the skewed distributions of urine κCR and λCR.

4.5.2. Relationships with other baseline variables

The relationships of urine κCR and λCR with other baseline variables are shown in Table 4.2. For comparison, the relationships of urine ACR are also given.

Table 4.2. Relationships of urine κCR, λCR, and ACR with other baseline variables

Variable	Urine κCR		Urine λCR		Urine ACR	
	Median (IQR) or *r*	***P***	**Median (IQR) or *r***	***P***	**Median (IQR) or *r***	***P***
Age	0.109	0.010	0.012	0.77	-0.321	<0.001
Gender		0.028		0.007		0.038
Male	16.2 (7.4 to 29.3)		2.3 (1.1 to 5.2)		32.5 (7.3 to 111.3)	
Female	12.3 (6.3 to 26.5)		1.9 (0.7 to 4.3)		20.0 (4.4 to 83.2)	
Ethnicity		0.007		0.001		<0.001
White	13.1 (7.1 to 24.5)		1.9 (1.0 to 4.3)		16.9 (4.2 to 76.4)	
South Asian	20.0 (8.4 to 37.2)		3.4 (1.5 to 7.9)		78.2 (22.8 to 156.6)	
Black	12.9 (4.0 to 28.9)		1.8 (0.6 to 5.2)		39.0 (9.5 to 88.7)	
Other	12.7 (8.3 to 39.9)		2.0 (1.9 to 7.4)		237.3 (187.1 to 302.4)	
Co-morbidities						
Diabetes Mellitus		<0.001		0.001		0.28
Yes	18.7 (8.1 to 34.9)		2.9 (1.2 to 6.4)		23.8 (4.2 to 86.2)	
No	12.6 (6.6 to 24.3)		1.8 (0.9 to 4.3)		29.3 (6.9 to 108.7)	
Cardiovascular disease		0.37		0.89		0.048
Yes	16.3 (7.1 to 30.3)		2.2 (1.0 to 5.2)		22.6 (3.7 to 82.6)	
No	14.3 (7.1 to 27.3)		2.0 (1.0 to 5.0)		29.1 (6.9 to 117.0)	
Malignancy		0.46		0.35		0.015
Yes	16.0 (7.5 to 25.9)		1.8 (1.1 to 3.6)		12.0 (2.9 to 83.0)	
No	14.3 (7.0 to 27.8)		2.2 (1.0 to 5.1)		29.9 (6.5 to 106.6)	
Cause of CKD		<0.001		<0.001		<0.001
Ischaemic/hypertensive	13.6 (7.3 to 27.7)		2.1 (1.0 to 4.7)		12.4 (2.6 to 56.0)	
Glomerulonephritis	8.2 (4.9 to 17.2)		1.4 (0.8 to 2.7)		70.5 (30.4 to 159.3)	
Diabetic kidney disease	20.9 (10.4 to 51.8)		4.7 (1.6 to 8.1)		64.9 (23.0 to 237.3)	
Polycystic kidney disease	12.0 (5.5 to 19.0)		1.5 (0.5 to 3.1)		10.2 (6.1 to 18.7)	
Interstitial nephropathy	15.0 (8.1 to 27.3)		3.1 (1.1 to 5.0)		10.4 (3.6 to 35.0)	
Reflux nephropathy	8.2 (3.7 to 22.8)		1.8 (0.6 to 3.9)		87.8 (29.3 to 141.0)	
Other/uncertain	16.1 (7.4 to 31.0)		2.3 (1.0 to 5.7)		32.9 (6.9 to 113.5)	
eGFR	-0.387	<0.001	-0.340	<0.001	0.100	0.027
Urine ACR	0.400	<0.001	0.516	<0.001	N/A	
Systolic BP	0.184	<0.001	0.179	<0.001	0.227	<0.001
Diastolic BP	0.079	0.06	0.111	0.009	0.231	<0.001
Serum κ	0.513	<0.001	0.479	<0.001	0.175	<0.001
Serum λ	0.494	<0.001	0.563	<0.001	0.221	<0.001
Urine κCR	N/A		0.925	<0.001	0.400	<0.001
Urine λCR	0.925	<0.001	N/A		0.516	<0.001

Urine FLC/creatinine ratios were higher in males, those of South Asian ethnicity, and those with DM or diabetic kidney disease, and lower in those with glomerulonephritis. Their strongest correlations, other than with each other, were moderate positive correlations with their counterpart serum FLC. There were weak-to-moderate positive correlations with urine ACR, very weak positive correlations with systolic BP, and weak negative correlations with eGFR.

In a multivariable analysis, factors independently associated with urine FLC/creatinine ratios were their counterpart serum FLC concentration (Figure 4.4), urine ACR (Figure 4.5), and a renal diagnosis of glomerulonephritis (associated with a 9.3 [5.5 to 13.0] mg/mmol lower urine κCR, $P < 0.001$, and a 1.3 [0.3 to 2.2] mg/mmol lower urine λCR, $P = 0.007$).

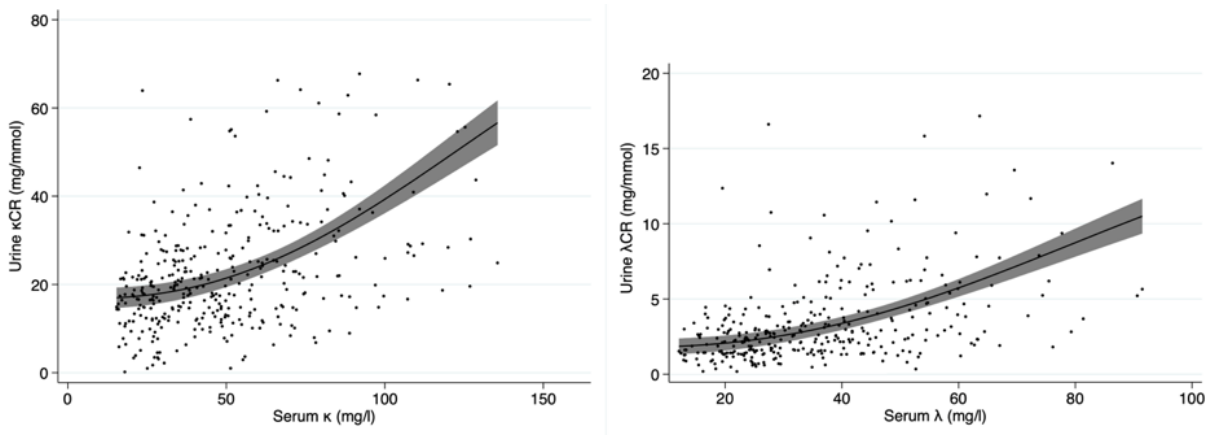


Figure 4.4. Relationship between urine κCR and λCR and their counterpart serum FLC

Both relationships are non-linear. The data were best fit using FP2 models with powers 0 and 1 for κCR, and 3 and 3 for λCR.

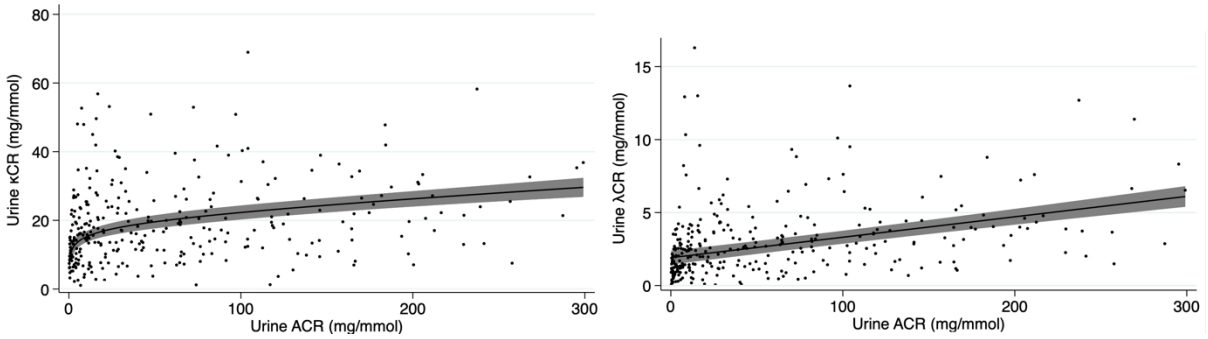


Figure 4.5. Relationship between urine κCR and λCR and urine ACR

The relationship with κCR was fit using an FP2 model with powers 0 and 1. The relationship with λCR is linear.

4.5.3. Kidney failure

During the median follow-up time of 6.1 years, 191 (34.4%) participants progressed to kidney failure, with an overall event rate of 8.1 per 100 person-years. The univariable associations between urine κCR and λCR and other baseline factors with the risk of kidney failure are shown in Table 4.3.

Table 4.3. Univariable associations between baseline factors and kidney failure

Variable	SHR	95% CI	*P*
Age	1.00[a]	1.00 to 1.00	<0.001
Male gender	0.93	0.70 to 1.25	0.64
Ethnicity			
White	Ref		
South Asian	1.72	1.24 to 2.38	0.001
Black	1.78	1.16 to 2.72	0.008
Other	0.00	0.00 to 0.00	<0.001
Co-morbidities			
DM	0.94	0.69 to 1.27	0.67
IHD	0.68	0.47 to 1.00	0.05
Cerebrovascular disease	1.07	0.66 to 1.73	0.79
PAD	0.72	0.41 to 1.27	0.26
COPD	0.44	0.23 to 0.85	0.014
Malignancy	0.44	0.25 to 0.78	0.005
Cause of CKD			
Ischaemic/hypertensive	Ref		
Glomerulonephritis	1.14	0.69 to 1.89	0.61
Diabetic kidney disease	1.87	1.14 to 3.07	0.014
Polycystic kidney disease	3.71	2.39 to 5.75	<0.001
Interstitial nephropathy	0.81	0.36 to 1.80	0.60
Reflux nephropathy	1.07	0.41 to 2.77	0.89
Other/uncertain	1.12	0.73 to 1.71	0.60
eGFR	0.42	0.31 to 0.56	<0.001
Urine ACR	1.48[b]	1.34 to 1.64	<0.001
Systolic BP	1.22	1.06 to 1.40	0.005
Diastolic BP	1.27	1.10 to 1.47	0.001
MAP	1.30	1.13 to 1.51	<0.001
Serum κ	2.64[b]	2.07 to 3.37	<0.001
Serum λ	0.23[c]	0.16 to 0.34	<0.001
Serum κ + λ	3.09[b]	2.37 to 4.03	<0.001
Urine κCR	1.80[b]	1.53 to 2.11	<0.001
Urine λCR	1.72[b]	1.51 to 1.95	<0.001

For continuous variables with a linear association, SHR is per +1 SD. Non-linear fractional polynomial transformations are denoted by: a = x^3; b = ln(x); c = $x^{-0.5}$.

On univariable analysis, higher urine κCR and λCR concentrations were both associated with a higher risk of kidney failure. The relationships are non-linear and are illustrated in Figure 4.6.

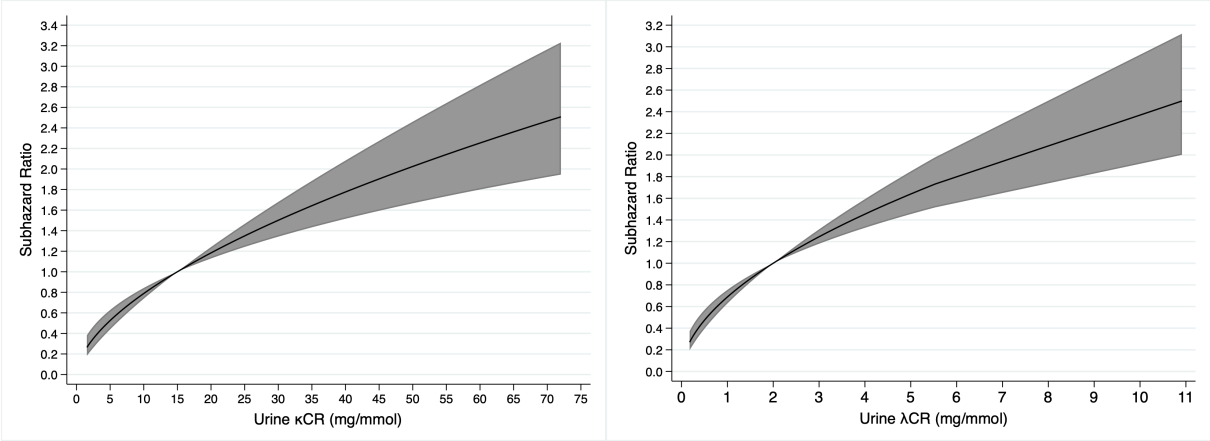


Figure 4.6. Unadjusted SHR for kidney failure according to urine κCR and λCR

Subhazard ratio with 95% CI, relative to 15 mg/mmol for urine κCR and 2 mg/mmol for urine λCR.

Other variables associated with a higher risk of kidney failure on univariable analysis were younger age (non-linear, Figure 4.7), non-White ethnicity, CKD due to polycystic kidney disease or diabetic kidney disease, lower eGFR, higher urine ACR (non-linear, Figure 4.8), higher BP, and higher serum FLCs (non-linear, Figure 4.9). Having COPD or malignancy were associated with a lower risk of kidney failure.

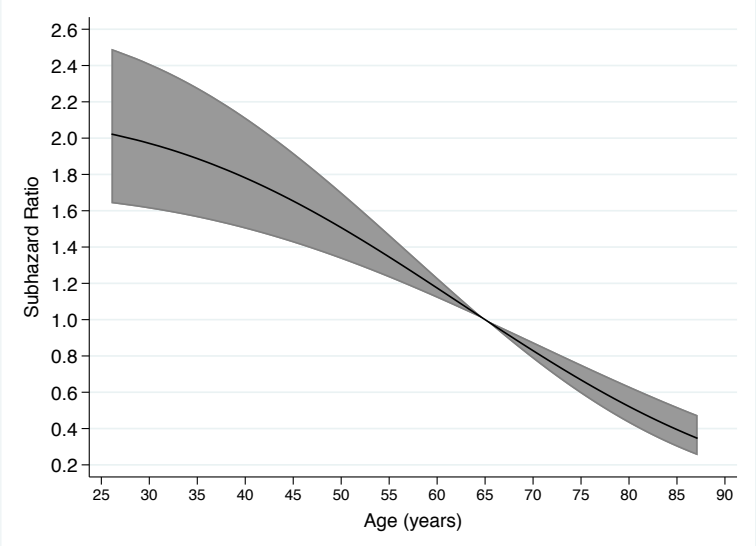


Figure 4.7. Unadjusted SHR for kidney failure according to age

SHR with 95% CI, relative to 65 years.

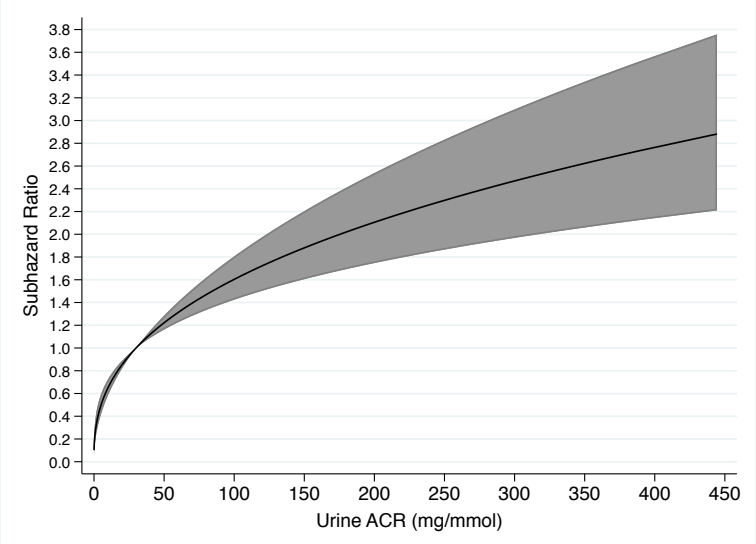


Figure 4.8. Unadjusted SHR for kidney failure according to urine ACR

SHR with 95% CI, relative to 30 mg/mmol.

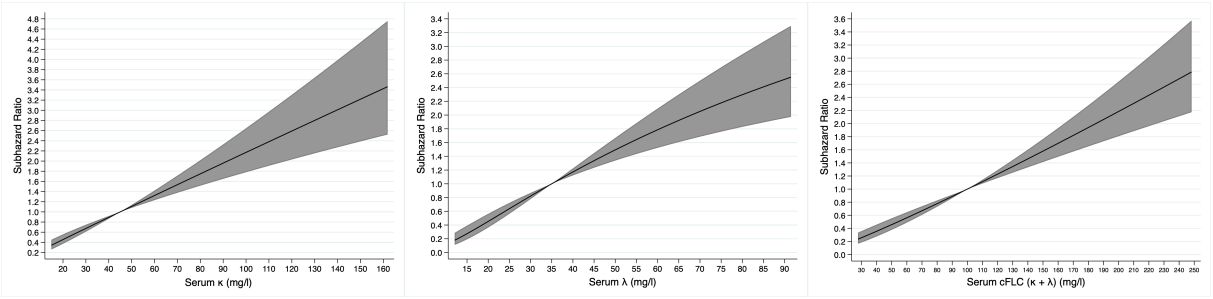


Figure 4.9. Unadjusted SHR for kidney failure according to serum FLCs

Subhazard ratio with 95% CI (κ relative to 45 mg/l, λ relative to 35 mg/l, cFLC relative to 100 mg/l).

The multivariable models for urine κCR and λCR (separately) are shown in Table 4.4.

Table 4.4. Multivariable models for the risk of kidney failure

Variable	Urine κCR			Urine λCR		
	SHR	**95% CI**	***P***	**SHR**	**95% CI**	***P***
Age	0.41	0.33 to 0.51	<0.001	0.41	0.33 to 0.50	<0.001
Male sex	1.42	1.01 to 2.01	0.046	1.42	1.01 to 2.00	0.045
Ethnicity						
White	Ref			Ref		
South Asian	0.98	0.67 to 1.44	0.92	0.95	0.65 to 1.39	0.79
Black	1.63	1.00 to 2.66	0.05	1.60	0.98 to 2.62	0.06
Other	0.00	.	.	0.00	.	.
Cause of CKD						
Ischaemic/hypertensive	Ref			Ref		
Diabetic kidney disease	0.95	0.55 to 1.63	0.84	0.94	0.55 to 1.61	0.82
Glomerulonephritis	0.80	0.45 to 1.44	0.46	0.80	0.45 to 1.44	0.46
Polycystic kidney disease	7.02	3.84 to 12.9	<0.001	7.19	3.90 to 13.2	<0.001
Interstitial nephropathy	0.44	0.17 to 1.12	0.09	0.44	0.17 to 1.11	0.08
Reflux nephropathy	0.31	0.10 to 0.93	0.037	0.31	0.10 to 0.94	0.038
Other/uncertain	0.84	0.52 to 1.34	0.46	0.82	0.51 to 1.31	0.40
MAP	0.86	0.72 to 1.03	0.09	0.86	0.72 to 1.02	0.09
eGFR	0.97[a]	0.96 to 0.98	<0.001	0.97[a]	0.96 to 0.98	<0.001
	66.0[b]	24.5 to 178	<0.001	64.9[b]	24.4 to 173	<0.001
Urine ACR	5.53[c]	3.48 to 8.77	<0.001	5.48[c]	3.48 to 8.62	<0.001
	0.99[d]	0.99 to 1.00	<0.001	0.99[d]	0.99 to 1.00	<0.001
Urine κCR	1.08	0.90 to 1.31	0.41			
Urine λCR				1.15	0.96 to 1.38	0.13

For continuous variables with a linear association, SHR is per +1 SD. The two rows for eGFR and urine ACR indicate the SHR for each power of the degree-2 fractional polynomial transformation. Fractional polynomial transformations are denoted by: $a = x^{-2}$; $b = x^{-0.5}$; $c = x^{0.5}$; $d = x^{3}$.

After adjustment for age, sex, ethnicity, cause of CKD, MAP, eGFR, and urine ACR, neither urine κCR (SHR 1.08 [0.90 to 1.31] per +1 SD) or urine λCR (SHR 1.15 [0.96 to 1.38] per +1 SD) had independent associations with the risk of kidney failure.

Baseline factors that were associated with a higher risk of kidney failure in the multivariable models were younger age, male sex, CKD caused by polycystic kidney disease, lower eGFR (non-linear, Figure 4.10), and higher urine ACR (non-linear, Figure 4.11). CKD caused by reflux nephropathy was associated with a lower risk of kidney failure.

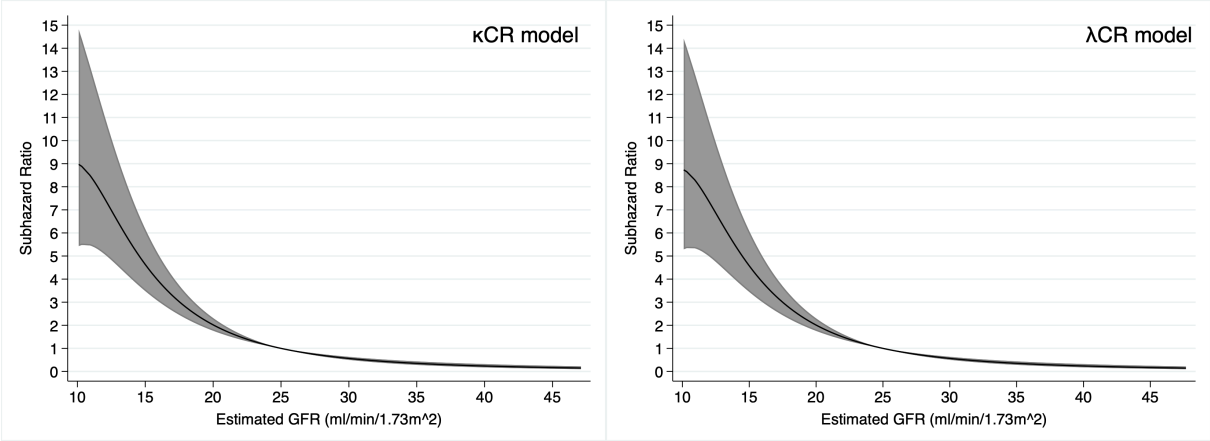


Figure 4.10. Adjusted SHR for kidney failure according to eGFR

SHR with 95% CI, relative to 25 ml/min/1.73 m^2, from the multivariable models in Table 4.4.

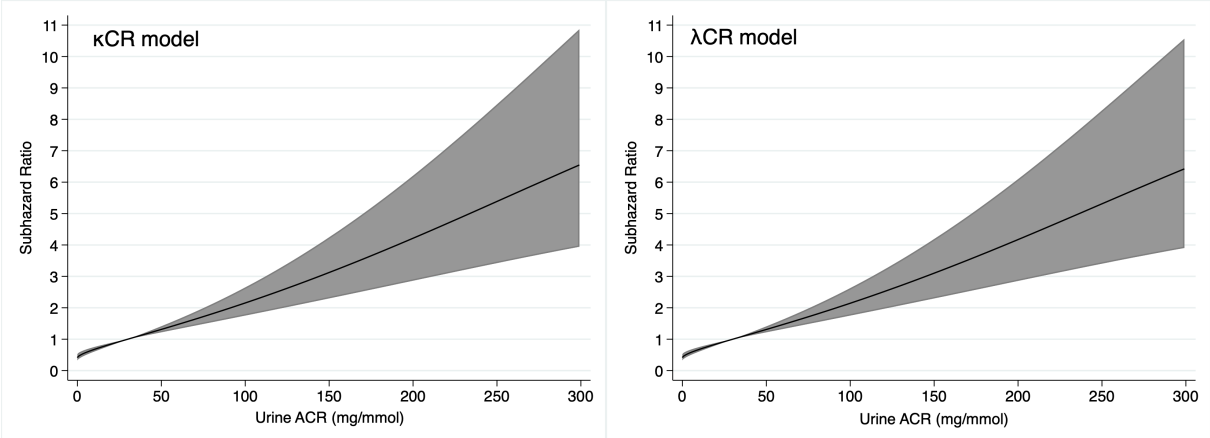


Figure 4.11. Adjusted SHR for kidney failure according to urine ACR

SHR with 95% CI, relative to 30 mg/mmol, from the multivariable models in Table 4.4.

When these analyses were repeated in participants with a urine ACR < 30 mg/mmol (*N*=265), the results were similar, i.e. higher urine κCR and urine λCR were associated with a significantly increased risk of kidney failure on univariable analysis, but not in multivariable models.

4.5.3.1. Risk stratification

After excluding those who died without kidney failure within two years (*N*=39), 517 participants had data on the outcome of kidney failure by two years. Of these, 62 (12.0%) had experienced kidney failure within two years from baseline. The logistic regression models for the prediction of kidney failure by two years, with measures of model performance and the incremental value of adding urine κCR or λCR to the KFRE, are shown in Table 4.5.

Table 4.5. Logistic regression models for the prediction of kidney failure at two years

Statistic	**Model**		
	KFRE	**KFRE + urine κCR**	**KFRE + urine λCR**
Odds ratio (95% CI)			
KFRE	1.10 (1.08 to 1.13)	1.10 (1.08 to 1.13)	1.10 (1.08 to 1.13)
κCR or λCR		1.04 (0.73 to 1.49)	1.05 (0.73 to 1.52)
Pseudo R^2	0.345	0.345	0.345
Hosmer-Lemeshow (*P*)	6.71 (0.57)	5.23 (0.73)	5.22 (0.73)
C-statistic	0.891	0.891	0.890
Δ C-statistic		0.00 (-0.01 to 0.01)	0.00 (-0.01 to 0.01)
IDI		0.00 (-0.01 to 0.02)	0.00 (-0.01 to 0.01)

The odds ratios for urine κCR and λCR are per +1 SD.

The baseline model, containing only KFRE, had a strong predictive ability for kidney failure at two years (C-statistic 0.891) and was well calibrated (Hosmer-Lemeshow statistic 6.71, *P*=0.57). Urine κCR or urine λCR were not significantly associated with kidney failure in the models containing KFRE, and their addition to KFRE provided no improvement in model performance. Neither model showed any change in the C-statistic, suggesting no improvement in model discrimination between those who did and did not develop kidney failure, nor any significant improvement in the reclassification of risk based on the IDI.

4.5.4. Death

During the median follow-up time of 6.1 years, 129 (23.2%) participants died, and the overall death rate was 5.5 per 100 person-years. The univariable associations between urine κCR and λCR and other baseline factors with the risk of death are shown in Table 4.6.

Table 4.6. Univariable associations between baseline factors and risk death

Variable	Hazard ratio	95% CI	*P*
Age	3.34	2.56 to 4.35	<0.001
Male gender	1.13	0.78 to 1.62	0.52
Ethnicity			
White	Ref		
South Asian	0.65	0.40 to 1.08	0.10
Black	0.75	0.39 to 1.43	0.38
Other	1.03	0.14 to 1.37	0.98
Co-morbidities			
DM	1.48	1.04 to 2.10	0.028
IHD	2.14	1.49 to 3.07	<0.001
Cerebrovascular disease	1.75	1.06 to 2.89	0.027
PAD	2.46	1.58 to 3.81	<0.001
COPD	1.39	0.84 to 2.28	0.20
Malignancy	2.05	1.35 to 3.11	0.001
Smoking status			
Never	Ref		
Previous	1.68	1.16 to 2.44	0.006
Current	0.99	0.52 to 1.86	0.97
eGFR	0.34	0.24 to 0.48	<0.001
Urine ACR	1.00	0.80 to 1.24	0.99
Systolic BP	1.25	1.05 to 1.48	0.011
Diastolic BP	0.64	0.53 to 0.76	<0.001
MAP	0.85	0.71 to 1.02	0.08
Urine κCR	1.23	1.03 to 1.44	0.021
Urine λCR	1.19	1.01 to 1.40	0.042

Associations for continuous variables were all linear, and the HR is per +1 SD.

On univariable analysis, higher urine κCR and λCR concentrations were both associated with a higher risk of death (urine κCR: HR 1.23 [1.03 to 1.44] per +1 SD; urine λCR: HR 1.19 [1.01 to 1.40] per + 1SD).

Other variables associated with a higher risk of death on univariable analysis were older age, a diagnosis of DM, IHD, cerebrovascular disease, PAD, or malignancy, being a previous smoker, lower eGFR, higher systolic BP, and lower diastolic BP.

The multivariable models for urine κCR and λCR (separately) are shown in Table 4.7.

Table 4.7. Multivariable models for the risk of death

Variable	Urine κCR			Urine λCR		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age	2.93	2.10 to 4.08	<0.001	2.96	2.12 to 4.14	<0.001
Male sex	1.00	0.66 to 1.52	1.00	0.97	0.64 to 1.46	0.87
Ethnicity						
White	Ref			Ref		
South Asian	1.23	0.71 to 2.14	0.45	1.21	0.70 to 2.10	0.49
Black	1.02	0.51 to 2.02	0.96	1.03	0.52 to 2.04	0.93
Other	1.73	0.20 to 14.7	0.62	1.46	0.17 to 12.3	0.73
Co-morbidities						
DM	1.11	0.75 to 1.63	0.61	1.09	0.74 to 1.60	0.67
IHD	1.23	0.82 to 1.82	0.32	1.19	0.81 to 1.77	0.38
CVD	1.13	0.67 to 1.90	0.66	1.14	0.68 to 1.92	0.61
PVD	1.64	1.00 to 2.69	0.048	1.64	1.00 to 2.69	0.048
COPD	1.25	0.74 to 2.12	0.41	1.29	0.76 to 2.18	0.34
Malignancy	1.88	1.18 to 2.98	0.007	1.80	1.14 to 2.84	0.011
Smoking status						
Never smoked	Ref			Ref		
Previous smoker	1.00	0.66 to 1.51	0.99	0.97	0.64 to 1.48	0.90
Current Smoker	1.37	0.67 to 2.81	0.39	1.35	0.66 to 2.78	0.41
eGFR	0.51	0.34 to 0.76	0.001	0.53	0.36 to 0.80	0.002
Urine ACR	1.49	1.21 to 1.84	<0.001	1.44	1.16 to 1.78	0.001
Systolic BP	1.02	0.85 to 1.23	0.80	1.02	0.85 to 1.23	0.84
Urine κCR	0.91	0.73 to 1.13	0.41			
Urine λCR				1.01	0.81 to 1.28	0.90

All relationships between continuous variables and death were linear.

After adjustment for age, sex, ethnicity, co-morbidities, smoking status, eGFR, urine ACR, and systolic BP, neither urine κCR (HR 0.91 [0.73 to 1.13] per +1 SD) or urine λCR (HR 1.01 [0.81 to 1.28] per +1 SD) had independent associations with the risk of death.

Baseline factors that were associated with a higher risk of death in the multivariable models were older age, a diagnosis of PAD or malignancy, lower eGFR, and higher urine ACR.

4.6. Discussion

The work presented in this chapter examined the hypotheses that higher urine FLC/creatinine ratios would be associated with a higher risk of kidney failure in patients with CKD and improve risk stratification when added to a current prognostic model based on standard prognostic factors. Further, it addressed the hypothesis that higher urinary FLC/creatinine ratios are associated with a higher risk of death in CKD. However, the results were not consistent with these hypotheses and suggested that urine FLC/creatinine ratios are not independent prognostic factors for kidney failure or death in patients with CKD.

The most important determinant of urine FLC/creatinine ratios was their counterpart serum FLC concentration. This correlation in patients with CKD has been reported previously (201). There are no published data on these correlations in healthy individuals. However, one would expect either no correlation or a very weak one because the capacity of the proximal tubules to catabolise FLCs is such that no filtered FLCs reach the urine, and therefore the urine FLC concentration should not reflect the serum concentration. The moderate correlations observed in this CKD study population are likely to reflect tubular disease, with impairment of FLC endocytosis and catabolism in the proximal tubule, or, as nephrons are lost in CKD, hyperfiltration of the remaining functional glomeruli and increased FLC delivery that exceeds the capacity of the proximal tubule to reabsorb and metabolise them. These mechanisms lead to the urinary escape of FLCs, which more closely reflects serum concentration.

Urine FLC/creatinine ratios were also positively correlated with urine ACR. In CKD, glomerular damage (associated with increased albuminuria and possibly with increased filtration of FLCs) often coexists with tubulointerstitial fibrosis (and thus possibly reduced

tubular FLC reabsorption). The correlation between urinary albumin and FLC is, therefore, not unexpected.

The finding of differences in urine FLC/creatinine ratios by cause of CKD has also been previously reported (201). In this cohort, those with glomerulonephritis had significantly lower urine FLC/creatinine ratios, consistent with the results of a previous study (201). The reason for this is not apparent, although as previously described, unlike urine ACR, higher urine FLC/creatinine ratios are likely to be more reflective of tubular rather than glomerular pathology.

4.6.1. Kidney failure

It was postulated that urine FLC/creatinine ratios may reflect kidney damage in a different way to urine ACR, being more reflective of tubular damage and that they may also reflect tubular exposure to FLCs where they may have deleterious effects. Thus, it was hypothesised that urine FLC/creatinine ratios would provide prognostic information on the risk of kidney failure in patients with CKD. Urine κCR and λCR were associated with the risk of kidney failure, but after adjustment for standard prognostic factors, the associations were significantly attenuated and became non-significant.

The lack of an independent association may reflect their lack of specificity for renal damage. As described, urine κCR and λCR correlated most strongly with their counterpart serum FLC, which may be determined by factors other than kidney function/damage, such as systemic inflammation and reticuloendothelial function. Further, urine κCR and λCR may also be influenced by mucosal secretion of FLCs in the urinary tract rather than solely reflecting renal loss.

Adding urine κCR or λCR to the KFRE, an established model for the estimation of the two-year risk of kidney failure, did not improve model performance in this cohort. Given the lack of an independent association with the risk of kidney failure identified in the previous step, it was not expected that urine κCR or λCR would improve risk stratification. However, these analyses were pre-specified and were conducted as had been planned before the assessment of multivariable associations.

4.6.2. Death

It was hypothesised that urine FLC/creatinine are independent prognostic factors for the risk of death in patients with CKD, but the results are not consistent with this hypothesis. Although urine κCR and λCR were associated with the risk of death, neither had a significant association after adjusting for standard prognostic factors for mortality in CKD. This is despite a significant correlation with serum FLC concentration, itself a marker of the risk of death as established in Chapter III.

Previous studies have identified several urinary markers of tubular damage that are independently associated with mortality (247-249). Markers of tubular injury reflect kidney disease and tubular dysfunction, which may be associated with death through pathways involving mineral metabolism, erythropoiesis, acid-base regulation, and urinary concentrating ability (249). They may also reflect systemic deleterious processes, as kidney injury or fibrosis may parallel similar processes in other organs such as the heart and lungs (249). Despite their reflection of kidney damage and tubular dysfunction, urine FLCs were not independently associated with the risk of death in patients with CKD.

4.6.3. Strengths and limitations

This work was performed in a well-characterised cohort of patients with prospective follow-up and a significant number of outcome events, and a rigorous approach to statistical modelling that incorporated standard prognostic factors. However, it was an observational study, without mechanistic data, and performed in a single cohort of individuals with advanced CKD that limits generalisability.

4.6.4. Future research

While no independent association was found with the risk of kidney failure or death in this cohort, further study in a larger cohort of patients may be justified. Further, the use of urine FLCs in detecting tubular dysfunction and in the diagnosis of CKD, especially in early disease, may be explored.

This study population had relatively advanced CKD (median eGFR 25 ml/min/1.75 m^2, median urine ACR 28 mg/mmol), and the results may not be generalisable to all patients with CKD. Given the previous findings that increased urine FLC levels are detectable before the development of increased albuminuria, the use of urinary FLCs to stratify risk in early CKD could be examined in other CKD cohorts with populations made up of less severe CKD.

4.7. Conclusion

Neither urine κCR or urine λCR are independently associated with the risk of kidney failure or death in patients with CKD, and they do not improve upon the KFRE for predicting this risk of kidney failure by two years. Future work may explore the role of urine FLCs in the diagnosis and prognosis of early CKD.

CHAPTER V: MONOCLONAL GAMMOPATHY

The work presented in this chapter aimed to address the hypotheses that the presence of a monoclonal gammopathy (MG) is associated with a higher risk of kidney failure and death in patients with chronic kidney disease (CKD).

Chapter III demonstrated that increased serum concentrations of non-clonal free light chains (FLC) are associated with a higher risk of kidney failure and death in patients with CKD. Further, it is well known that patients with monoclonal diseases, such as multiple myeloma, are at risk of kidney damage, kidney failure, and death. The association between non-malignant MG and adverse outcomes in patients with CKD was, therefore, assessed.

At the time of thesis submission, this work has been accepted for publication in PLOS Medicine. It was presented as an oral presentation at the UK Kidney Week, Brighton, 2019.

5.1. Abstract

Background

Malignant monoclonal gammopathy (MG) and increased non-clonal serum FLC concentration are both associated with an increased risk of kidney failure and death in patients with CKD. The association between the presence of a non-malignant MG and the risk of kidney failure or death in individuals with CKD was assessed.

Methods

Data were used from three prospective cohorts of individuals with CKD (not on dialysis or with a kidney transplant): 1. Renal Impairment in Secondary Care (RIISC, Queen Elizabeth and Heartlands Hospitals, Birmingham, UK, *N*=878), 2. Salford Kidney Study (SKS, Salford Royal Hospital, Salford, UK, *N*=861), 3. Renal Risk in Derby (RRID, Derby, UK, *N*=1739). Participants were excluded if they had multiple myeloma or any other B cell lymphoproliferative disorder with end-organ damage.

Results

All non-malignant MG was identified in the baseline serum of participants of RIISC only. Further, light-chain (LC) MG was identified and studied in participants of all three studies. One hundred two (11.6%) of the 878 RIISC participants had an MG. During a median follow-up time of 6.2 years, there were 324 kidney failure events and 202 deaths. The presence of MG was not independently associated with risk of kidney failure (adjusted SHR 1.16 [95% CI 0.80 to 1.69]) or death (adjusted HR 1.37 [95% CI 0.93 to 2.00]). Fifty-five (1.6%) of the 3478 participants from all three studies had LC-MG. During the median follow-up time of 5.2 years, 564 participants progressed to kidney failure, and 803 died. As with all MG, LC-MG was not independently associated with the risk of kidney failure (adjusted SHR 1.42 [0.78 to 2.57]) or death (adjusted HR 1.49 [0.93 to 2.39]).

Conclusions

The prevalence of MG was higher in this CKD cohort than that reported in the general population. However, the presence of an MG was not independently associated with a higher risk of kidney failure or, unlike in the general population, risk of death.

5.2. Introduction

The monoclonal gammopathies (MG) are a group of disorders characterized by the proliferation of a single clone of plasma cells that produces a monoclonal immunoglobulin (termed a paraprotein) in an amount that can be detected by serum or urine immunofixation, or by the serum FLC assay (250). The paraprotein can consist of either an intact immunoglobulin or just FLCs.

Monoclonal gammopathies are common, with a paraprotein detectable in the serum of approximately 1% of the population overall (251). The MGs are associated with a spectrum of diseases, from asymptomatic non-malignant disorders through to life-threatening malignant disease, as described in the following paragraphs.

5.2.1. Malignant monoclonal gammopathies

In some MGs, the clonal process producing the paraprotein is malignant with neoplastic disease infiltrating bone, lymph nodes, liver, spleen, or other organs. Examples of malignant MGs are multiple myeloma and Waldenström's macroglobulinemia. Overall median survival in multiple myeloma is approximately five years (252). Further, nearly 50% of patients with multiple myeloma develop kidney disease, with approximately 10% requiring dialysis, and the presence of kidney disease is associated with worse survival (253, 254).

5.2.2. Non-malignant monoclonal gammopathies

In most MGs, the clonal expansion of plasma cells is small and limited, and there is no evidence of neoplastic disease. Most individuals with an MG fall into this category and have no symptoms and no demonstrable organ damage. This is termed monoclonal gammopathy of undetermined significance (MGUS). MGUS is common, affecting around

3% of those aged over 50 years (255), although the prevalence has been reported to be higher in patients with CKD (218, 220). MGUS requires monitoring but no specific treatment.

However, non-malignant MGs can occasionally be associated with disease due to the adverse properties of the secreted paraprotein itself (monoclonal gammopathy of clinical significance, MGCS) (256). For example, there may be tissue deposition of the paraprotein or the paraprotein may have autoantibody activity. Monoclonal gammopathy of renal significance (MGRS) represents a group of disorders in which a paraprotein secreted by a non-malignant B cell or plasma cell clone causes kidney damage (257). In MGRS, specific targeted therapy is indicated to preserve kidney function.

5.2.3. Kidney disease in monoclonal gammopathies

Paraproteins can directly cause kidney injury in both malignant MGs, such as multiple myeloma, and in non-malignant MGs (MGRS). There are various mechanisms by which paraproteins cause kidney disease, which tends to be mediated by FLCs, including intratubular cast formation, direct tubular toxicity, or paraprotein deposition within different compartments of the kidney.

When large amounts of monoclonal FLCs are produced such that the capacity of the proximal tubule to endocytose them is exceeded, FLC binding with uromodulin leads to precipitation and cast formation within the tubules. The casts may cause tubular obstruction, rupture, and interstitial inflammation (254, 258).

FLCs may also cause direct tubular toxicity, especially in the proximal tubule. Intracellular accumulation of endocytosed monoclonal FLCs is associated with the formation of reactive oxygen species such as hydrogen peroxide, and the initiation of apoptotic, pro-inflammatory and fibrotic pathways (259-261).

Paraproteins may also be associated with deposition diseases, characterized by deposits of light chain or heavy chain fragments in various kidney compartments. Examples are light chain deposition disease, AL amyloidosis (where the light chain fragments form amyloid fibrils that deposit in the kidney), and heavy chain deposition disease (where heavy chains, with or without light chains, are deposited in the kidney).

Patients with established kidney disease due to myeloma or MGRS have a risk of kidney failure, requiring dialysis, that varies by the particular form of MGRS. It is common to detect a paraprotein in patients with CKD, in part because the prevalence of both MGUS and CKD increase with age. The clinician needs to consider whether the finding of a paraprotein reflects incidental MGUS, or whether the paraprotein has a causal role in the kidney disease (MGRS, or a malignant MG such as multiple myeloma). Often, the probability of MGRS is felt to be low (for example, if the patient has another clear cause for kidney disease), and a kidney biopsy, which would exclude MGRS definitively, is not performed. The renal prognosis for a patient with (presumed) MGUS is not known. There has been only one small study, by Haynes et al., that assessed the risk of kidney failure associated with MGUS in patients with CKD (218). In the cohort of 364 patients with CKD, the 35 (9.6%) patients with MGUS had a higher rate of kidney failure, but not after adjustment for age, sex, and eGFR. Further research is needed in cohorts with more patients and events to examine this association.

5.2.4. Non-malignant MG and survival

Although MGUS is defined by the absence of organ damage, several general population studies have shown that the presence of MGUS is associated with shorter survival (262, 263). This is in part related to malignant transformation of the MGUS to multiple

myeloma or other plasma-cell or lymphoid disorder, which occurs at a rate of approximately 1% per year (262).

The effect of MGUS on survival in patients with CKD is not known. Given the already increased risk of death in CKD, particularly from cardiovascular disease, it is not clear that an MGUS would be associated with a similar effect on survival to that seen in the general population. Indeed, in the study by Haynes et al., the presence of MGUS was not associated with a higher risk of death in patients with CKD (218). No other published studies have examined the association between MGUS and risk of death in patients with CKD.

The detection of a non-malignant MG (often assumed to be MGUS) in a patient with CKD is common, but despite the known potential pathogenetic properties of paraproteins and the higher rate of death seen in the general population, there has been little study of the prognostic significance of non-malignant MG in patients with CKD. The work presented in this chapter assessed whether the presence of a non-malignant MG is associated with a higher risk of kidney failure or death in patients with CKD.

5.3. Hypotheses

The following pre-specified hypotheses were addressed:

1. the presence of a non-malignant MG is independently associated with a higher risk of kidney failure in patients with CKD;
2. the presence of a non-malignant MG is independently associated with a higher risk of death in patients with CKD.

5.4. Methods

5.4.1. Patients

Patients from three prospective UK cohorts of individuals with CKD who had not received kidney replacement therapy (KRT, i.e. dialysis or kidney transplant) were included: the Renal Impairment in Secondary Care (RIISC) study, the Salford Kidney Study (SKS, previously termed Chronic Renal Insufficiency Standards Implementation Study [CRISIS]), and the Renal Risk in Derby (RRID) study.

Each study had research ethics committee (REC) approval (RIISC: West Midlands South Birmingham REC, ref 10/H1207/6; SKS: North West GM South REC; ref 15/NW/0818; RRID: East Midlands Nottingham 1 REC). All participants in all three studies provided written informed consent, and all studies were conducted in accordance with the Declaration of Helsinki.

The RIISC study has been described in Section 2.1, and details of the SKS and RRID studies cohorts have been published (180, 264). The study inclusion and exclusion criteria are summarised in Table 5.1, but for this analysis, participants were also excluded at an individual level if they had a malignant MG (multiple myeloma or another malignant B cell lymphoproliferative disorder).

Table 5.1. Number of participants included and characteristics of each cohort study

Study	No. included	Setting	Inclusion criteria	Exclusion criteria	Years of recruitment	End of follow-up	Median (IQR) follow-up (months)
RIISC	878	Secondary care	1. eGFR < 30 or 2. eGFR 30-59 with a. eGFR decline* or b. Urine ACR > 70	1. Previous dialysis or kidney transplant 2. Immunosuppression for immune-mediated kidney disease	2010 to 2015	End of 2018	74 (64 to 83)
SKS	861	Secondary care	eGFR >10 to <60	1. Previous dialysis or kidney transplant	2002 to 2010	End of 2017	139 (110 to 161)
RRID	1739	Primary care	eGFR 30-59	1. Expected survival < 1 year 2. Previous solid organ transplant	2008 to 2010	End of 2015	61 (60 to 63)

**eGFR decline defined as > 5 ml/min/1.73 m^2 per year, or > 10 ml/min/1.73 m^2 over 5 years.*

5.4.2. Definition of monoclonal gammopathy

Although the majority of included participants who were identified to have an MG will have had MGUS, the more general term non-malignant MG is used throughout to reflect the fact that only a minority of participants had kidney biopsies to exclude MGRS definitively. Two forms of non-malignant MG were assessed:

1. Any non-malignant MG (assessed in the RIISC cohort only), defined as:
    a. A monoclonal protein on serum protein electrophoresis (SPEP) confirmed by serum immunofixation or
    b. A serum κ/λ FLC ratio < 0.37 or > 3.10 with an increased level of the involved light chain;
2. Light-chain (LC) MG (assessed in all three cohorts) defined as a serum κ/λ FLC ratio < 0.37 or > 3.10 with an increased level of the involved light chain.

In the RIISC cohort, SPEP and immunofixation (using standard laboratory procedures) and serum FLC concentrations were measured, allowing the detection of any non-malignant MG. In the SKS and RRID cohorts, only serum FLC concentration was measured, and therefore only LC-MG could be detected in these two cohorts.

The Freelite® assay (The Binding Site Group Ltd, Birmingham, UK) was used to measure κ and λ FLC concentration in all three cohorts. The serum κ/λ FLC ratio 'renal reference range' of 0.37 to 3.10 was used, as has been recommended in patients with kidney impairment to account for the associated change in FLC clearance (201, 202).

5.4.3. Study design

Patients were recruited prospectively in all three cohorts, and data and biological samples collected at baseline visits were used for this analysis. Years of recruitment, end of

follow-up, and median follow-up time for each study are given in Table 5.1. Time-to-event data were collected for kidney failure (defined as the initiation of KRT) and death from any cause.

Individual participant data were available for the following variables: age, sex, ethnicity (White, Black, South Asian, or other), smoking status (current smoker, previous smoker, never smoked), co-morbidities (DM, IHD, cerebrovascular disease, PAD, COPD, and malignancy), cause of CKD (vascular, diabetes, glomerular, tubulointerstitial, cystic or congenital, or other or unknown), mean arterial pressure (MAP), eGFR (calculated using the four-variable MDRD formula), and urine ACR.

No formal sample size calculations were carried out for these analyses which were performed using the available specimen collections and data sets.

5.4.4. Statistical analysis

Missing data were assumed to be missing at random and multiple imputation using chained equations was performed as per Section 2.5.6.

Continuous variables all had skewed distributions as assessed by histograms. The relationships between MG or LC-MG status with other categorical baseline variables were assessed using Fisher's exact test, and relationships with continuous variables were assessed using the Wilcoxon rank-sum test.

The prognostic significance of an MG or LC-MG for risk of kidney failure was estimated using subdistribution hazard models (accounting for the competing risk of death) and expressed as a subhazard ratio (SHR) with a 95% confidence interval (CI). Cause-specific hazard models were also fitted and are presented in Appendix 7. The associations with risk of death were estimated using Cox proportional hazards models and expressed as a

hazard ratio (HR) with a 95% CI. Log-log plots were assessed for each variable to ensure that the proportional-hazards assumption was not violated.

The analyses of LC-MG included amalgamated data from all three cohorts, and clustering was accounted for by the use of stratified models as per Section 2.5.5.

All variables included in multivariable models were pre-specified. Fractional polynomials were used to explore the presence of non-linear relationships between continuous predictors and each outcome, and where they provided a better model fit, plots of risk against the variable on its original scale are presented.

5.5. Results

Assessment of the association between any non-malignant MG and adverse outcomes included only RIISC data and is presented first, followed by analyses for LC-MG, which included data from all three cohorts.

5.5.1. Any non-malignant MG

Eight hundred seventy-eight participants from the RIISC cohort were included, and 102 (11.6%) of these had an MG. Types of MG were as follows: 63 (61.8%) were IgG, 8 (7.8%) were IgM, 5 (4.9%) were IgA, 1 (1.0%) was biclonal (IgG and IgM), and 25 (24.5%) were LC-MG. Median follow-up time was 6.2 years. Study population characteristics and the relationship between MG status and other baseline variables are shown in Table 5.2.

Table 5.2. Baseline characteristics by MG status

Variable	All	MG +ve	MG -ve	Completeness of data (%)
***N* (%)**	878	102 (11.6)	776 (88.4)	100
Age (years)	64.6 (51.7 to 76.0)	73.8 (59.8 to 81.4)	63.7 (50.2 to 75.5)	100
Sex (male)	542 (61.7)	66 (64.7)	476 (61.3)	100
Ethnicity				100
White	598 (68.1)	68 (66.7)	530 (68.3)	
South Asian	188 (21.4)	24 (23.5)	164 (21.1)	
Black	84 (9.6)	9 (8.9)	75 (9.7)	
Other	8 (0.9)	1 (1.0)	7 (0.9)	
Co-morbidities				100
DM	341 (38.8)	48 (47.1)	293 (37.8)	
IHD	208 (23.7)	30 (29.4)	178 (22.9)	
Cerebrovascular disease	102 (11.6)	15 (14.7)	87 (11.2)	
PAD	93 (10.6)	14 (13.7)	79 (10.2)	
COPD	89 (10.1)	8 (7.8)	81 (10.4)	
Malignancy	128 (14.6)	22 (21.6)	106 (13.7)	
Smoking status				98.2
Never	416 (48.3)	47 (47.0)	369 (48.4)	
Previous	333 (38.6)	40 (40.0)	293 (38.5)	
Current	113 (13.1)	13 (13.0)	100 (13.1)	
Cause of CKD				91.2
Vascular	230 (28.7)	34 (36.2)	196 (27.7)	
Diabetes	125 (15.6)	20 (21.3)	105 (14.9)	
Glomerular	109 (13.6)	7 (7.4)	102 (14.4)	
Tubulointerstitial	89 (11.1)	6 (6.4)	83 (11.7)	
Cystic or congenital	66 (8.2)	4 (4.3)	62 (8.8)	
Other or unknown	182 (22.7)	23 (24.5)	159 (22.5)	
MAP (mmHg)	93 (85 to 102)	92 (83 to 103)	93 (86 to 102)	97.6
eGFR (ml/min/1.73 m^2)	31 (23 to 42)	28 (22 to 42)	31 (23 to 42)	96.8
Urine ACR (mg/mmol)	33.4 (6.3 to 130.0)	32.7 (5.6 to 161.2)	33.4 (6.5 to 122.7)	94.0

Categorical variables are shown as a frequency (percentage) and continuous variables as the median (interquartile range).

Compared to those without an MG, those with an MG were on average older (P<0.001) and a higher proportion had a history of malignancy (P=0.037). For all other baseline variables, there were no statistically significant differences between those with and those without an MG.

5.5.1.1. Kidney failure

Three hundred twenty-seven (37.2%) participants progressed to kidney failure, with rates per 100 person-years of 10.5 and 9.3 for those with and without MG, respectively. The univariable associations between baseline variables and the risk of kidney failure are shown in Table 5.3.

Table 5.3. Association between baseline variables and risk of kidney failure and death

Variable	Kidney failure						Death					
	Univariable			Multivariable			Univariable			Multivariable		
	SHR	95% CI	*P*	SHR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
MG+	0.97	0.68 to 1.38	0.85	1.16	0.80 to 1.69	0.43	2.13	1.49 to 3.02	<0.001	1.37	0.93 to 2.00	0.11
Age	1.00[a]	1.00 to 1.00	<0.001	1.00[a]	0.99 to 1.00	<0.001	3.36	2.73 to 4.12	<0.001	2.83	2.21 to 3.64	<0.001
Male sex	0.99	0.79 to 1.23	0.92	0.55	0.44 to 0.69	<0.001	1.27	0.95 to 1.69	0.11	0.88	0.62 to 1.24	0.46
Ethnicity												
White	Ref			Ref			Ref			Ref		
South Asian	2.02	1.58 to 2.57	<0.001	1.29	0.98 to 1.69	0.07	0.51	0.33 to 0.78	0.002	0.91	0.58 to 1.42	0.67
Black	1.98	1.42 to 2.76	<0.001	1.77	1.32 to 2.38	<0.001	0.80	0.48 to 1.33	0.39	1.13	0.67 to 1.90	0.65
Other	2.64	1.07 to 6.55	0.036	1.82	0.91 to 3.62	0.09	0.56	0.08 to 3.86	0.56	0.66	0.16 to 2.72	0.57
Co-morbidities												
DM	0.92	0.73 to 1.15	0.46				1.64	1.25 to 2.16	<0.001	1.27	0.94 to 1.72	0.12
IHD	0.85	0.65 to 1.11	0.22				2.44	1.83 to 3.24	<0.001	1.44	1.05 to 1.96	0.022
Cerebrovascular disease	0.77	0.53 to 1.13	0.18				1.97	1.38 to 2.81	<0.001	1.27	0.87 to 1.85	0.21
PAD	0.86	0.59 to 1.27	0.45				2.21	1.57 to 3.11	<0.001	1.27	0.85 to 1.91	0.24
COPD	0.45	0.28 to 0.72	0.001				1.46	0.99 to 2.16	0.06	1.14	0.74 to 1.77	0.55
Malignancy	0.51	0.35 to 0.76	0.001				2.16	1.56 to 2.99	<0.001	1.56	1.10 to 2.22	0.013
Smoking status												
Never	Ref						Ref			Ref		
Previous	0.69	0.54 to 0.88	0.003				1.73	1.28 to 2.34	<0.001	1.06	0.76 to 1.49	0.74
Current	1.07	0.78 to 1.47	0.71				1.14	0.71 to 1.84	0.58	1.25	0.70 to 2.24	0.45
Cause of CKD												
Vascular	Ref			Ref			Ref					
Diabetes	1.92	1.33 to 2.78	0.001	1.05	0.69 to 1.60	0.81	0.81	0.52 to 1.26	0.35			
Glomerular	1.19	0.81 to 1.76	0.38	1.00	0.66 to 1.51	1.00	0.22	0.11 to 0.41	<0.001			
Tubulointerstitial	0.89	0.57 to 1.38	0.59	0.63	0.37 to 1.06	0.08	0.31	0.16 to 0.59	<0.001			
Cystic or congenital	2.85	2.01 to 4.04	<0.001	3.99	2.74 to 5.83	<0.001	0.26	0.10 to 0.63	0.003			
Other or unknown	1.24	0.89 to 1.73	0.21	1.21	0.85 to 1.73	0.28	0.82	0.57 to 1.17	0.27			
MAP	1.39	1.25 to 1.54	<0.001	0.93	0.83 to 1.06	0.28	0.79	0.68 to 0.93	0.005			
eGFR	1.18[b]	1.14 to 1.22	<0.001	0.94[b]	0.93 to 0.96	<0.001	0.45	0.36 to 0.56	<0.001	0.67	0.53 to 0.86	0.002
	1.06[c]	1.05 to 1.07	<0.001	3.26[e]	2.73 to 3.91	<0.001						
Urine ACR	1.48[d]	1.37 to 1.59	<0.001	3.30[f]	2.61 to 4.17	<0.001	0.78[g]	0.63 to 0.96	0.018	1.24	1.07 to 1.45	0.005
				1.00[a]	1.00 to 1.00	<0.001	1.01[a]	1.00 to 1.01	<0.001			

Continuous variables are linear per +1 SD unless indicated. Two rows for a continuous variable indicate the SHR or HR for each power from an FP2 model. Fractional polynomial transformations are indicated by: $a = x^3$; $b = x^{-2}$; $c = x^{-2}\ln(x)$; $d = \ln(x)$; $e = x^{-1}$; $f = x^{0.5}$; $g = x$.

On univariable analysis, the presence of an MG did not have a significant association with the risk of kidney failure (SHR 0.97 [95% CI 0.68 to 1.38], *P*=0.85; Figure 5.1).

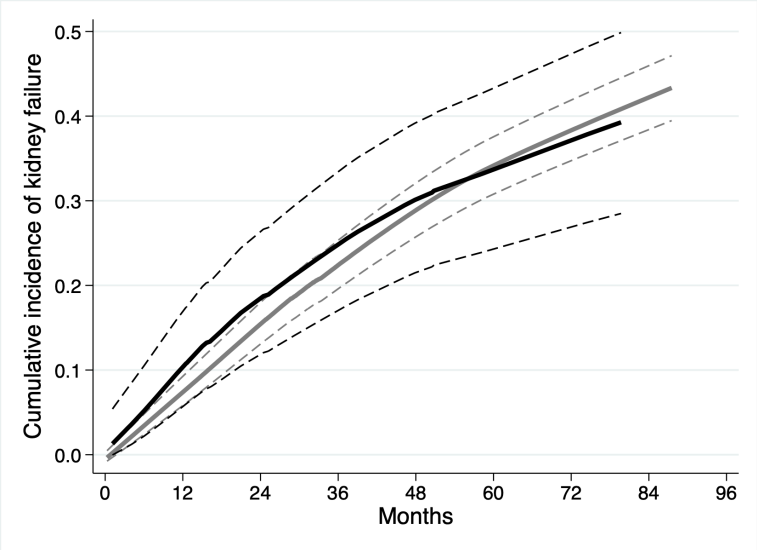


Figure 5.1. Cumulative incidence of kidney failure by MG status

Black line = MG+ with interrupted black lines representing the 95% CI; grey line = MG- with interrupted grey lines representing the 95% CI.

Age, eGFR and urine ACR had non-linear relationships with the risk of kidney failure on univariable analysis, as shown in Figure 5.2.

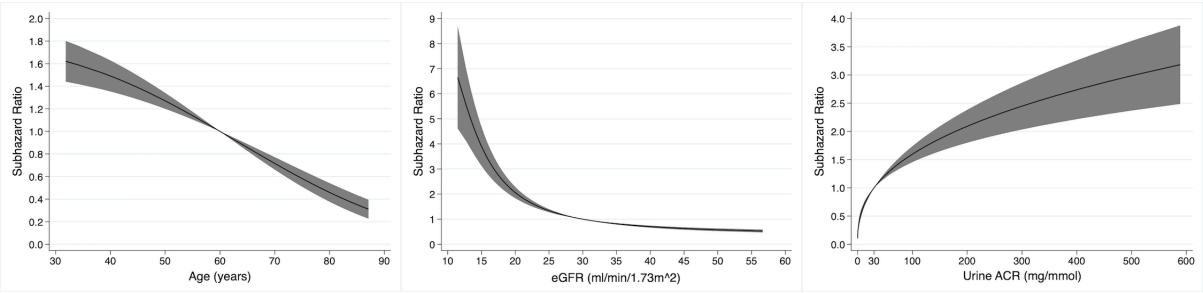


Figure 5.2. Unadjusted SHR for kidney failure, according age, eGFR and urine ACR

SHR represents risk relative to 60 years for age, 30 ml/min/1.73 m^2 for eGFR, and 30 mg/mmol for urine ACR.

The multivariable model for kidney failure is shown in Table 5.3. After adjustment for age, sex, ethnicity, cause of CKD, MAP, eGFR, and urine ACR, the presence of an MG was not significantly associated with risk of kidney failure (SHR 1.16 [95% CI 0.80 to 1.69], *P*=0.43).

Younger age (non-linear, Figure 5.3), female sex, Black ethnicity, a cystic or congenital cause of CKD, lower eGFR (non-linear, Figure 5.3), and higher urine ACR (non-linear, Figure 5.3) were all associated with a higher risk of kidney failure in the multivariable model.

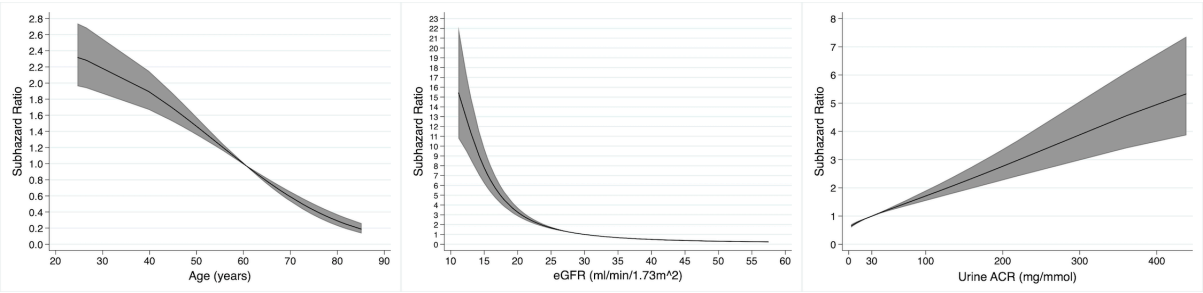


Figure 5.3. Adjusted SHR for kidney failure according to age, eGFR, and urine ACR

SHR relative to 60 years for age, 30 ml/min/1.73 m^2 for eGFR, and 30 mg/mmol for urine ACR, from the multivariable model in Table 5.3.

5.5.1.2. Death

Two hundred two (23.0%) participants died. The death rates per 100 person-years were 10.8 and 5.3 for those with and without MG, respectively. The univariable associations with death are shown in Table 5.3. The presence of an MG was associated with a higher risk of death (HR 2.13 [95% CI 1.49 to 3.02], P<0.001), as shown in Figure 5.4.

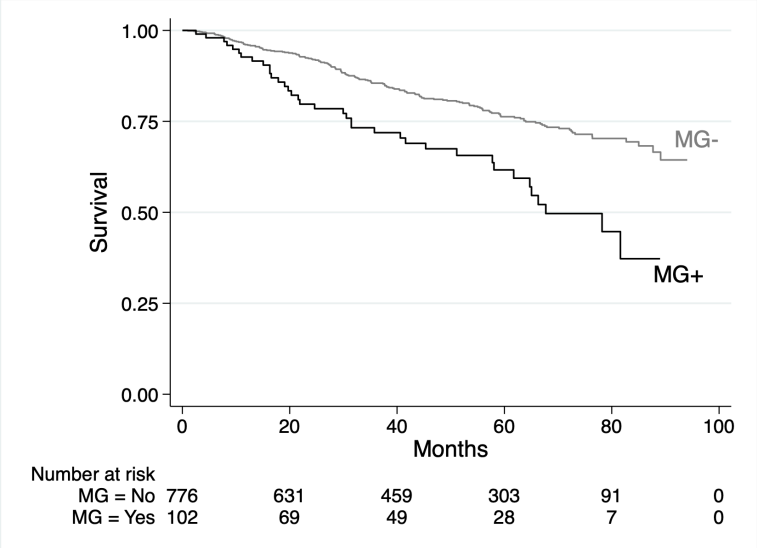


Figure 5.4. Kaplan-Meier survival curves by MG status

However, as shown in the multivariable model in Table 5.3, after adjusting for age, sex, ethnicity, co-morbidities, smoking status, eGFR and urine ACR, the presence of an MG no longer had a statistically significant association with death (HR 1.37 [95% CI 0.93 to 2.00], *P*=0.11).

Older age, a history of IHD or malignancy, lower eGFR and higher urine ACR were associated with a higher risk of death in the multivariable model.

5.5.2. Non-malignant LC-MG

Three thousand four hundred seventy-eight participants from the three cohorts were included, and 55 (1.6%) of these had an LC-MG. Median follow-up time was 5.2 years. Table 5.4 shows the study population characteristics and the relationship between LC-MG status and other baseline variables.

Table 5.4. Baseline characteristics by LC-MG status

Variable	All	LC-MG +ve	LC-MG -ve	Data completeness (%)
N (%)	3478	55 (1.6)	3423 (98.4)	
Age (years)	71.0 (61.2 to 78.0)	77.8 (71.0 to 82.0)	71.0 (61.1 to 78.0)	100
Sex (male)	1760 (50.6)	38 (69.1)	1722 (50.3)	100
Ethnicity				100
White	3126 (89.9)	44 (80.0)	3082 (90.0)	
South Asian	237 (6.8)	5 (9.1)	232 (6.8)	
Black	96 (2.8)	6 (10.9)	90 (2.6)	
Other	19 (0.6)	0 (0.0)	19 (0.6)	
Co-morbidities				100
DM	914 (26.3)	16 (29.1)	898 (26.2)	
IHD	1347 (38.7)	10 (18.2)	1337 (39.1)	
Cerebrovascular	395 (11.4)	3 (5.5)	392 (11.5)	
PAD	879 (25.3)	5 (9.1)	874 (25.5)	
Smoking status				99.5
Never	1486 (43.0)	26 (47.3)	1460 (42.9)	
Previous	1667 (48.2)	28 (50.9)	1639 (48.1)	
Current	307 (8.9)	1 (1.8)	306 (9.0)	
MAP (mmHg)	93 (86 to 102)	92 (85 to 99)	93 (86 to 102)	99.3
eGFR (ml/min/1.73 m^2)	42.3 (26.2 to 54.4)	40.4 (24.3 to 54.2)	42.3 (26.3 to 54.4)	99.2
Urine ACR (mg/mmol)	3.4 (0.3 to 27.3)	4.7 (0.4 to 76.6)	3.4 (0.2 to 26.7)	95.5

Categorical variables are shown as a frequency (percentage) and continuous variables as the median (interquartile range).

Compared to those without an LC-MG, those with an LC-MG were on average older (P<0.001), a higher proportion were male (P=0.006) and of Black ethnicity (P=0.004), and a lower proportion had a history of IHD (P=0.001) or PAD (P=0.004). There were no statistically significant differences between those with and those without LC-MG for all other baseline variables.

5.5.2.1. Kidney failure

Five hundred sixty-four (16.2%) patients progressed to kidney failure, with rates per 100 person-years of 4.9 and 3.2 for those with and without an LC-MG, respectively. The

univariable associations between baseline variables and the risk of kidney failure are shown in Table 5.5.

Table 5.5. Association between baseline variables and risk of kidney failure and death

Variable	Kidney failure						Death					
	Univariable			Multivariable			Univariable			Multivariable		
	SHR	**95% CI**	***P***	**SHR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
LC-MG+	1.07	0.58 to 1.96	0.82	1.42	0.78 to 2.57	0.26	2.51	1.59 to 3.96	<0.001	1.49	0.93 to 2.39	0.10
Age	1.01[a] 1.00[b]	1.00 to 1.02 0.99 to 1.00	0.20 0.050	1.00[a]	1.00 to 1.00	<0.001	2.88	2.60 to 3.19	<0.001	2.76	2.48 to 3.08	<0.001
Male sex	0.95	0.81 to 1.12	0.53	1.14	0.96 to 1.37	0.13	1.59	1.37 to 1.84	<0.001	1.27	1.09 to 1.49	0.002
Ethnicity												
White	Ref			Ref			Ref			Ref		
South Asian	1.94	1.56 to 2.41	<0.001	1.15	0.89 to 1.48	0.30	0.67	0.47 to 0.94	0.022	1.11	0.79 to 1.56	0.56
Black	1.84	1.35 to 2.49	<0.001	1.71	1.29 to 2.27	<0.001	0.77	0.48 to 1.25	0.30	1.10	0.70 to 1.73	0.69
Other	2.80	1.34 to 5.86	0.006	1.42	0.55 to 3.62	0.47	0.48	0.12 to 1.94	0.30	0.79	0.29 to 2.14	0.64
Co-morbidities												
DM	0.92	0.77 to 1.09	0.31				1.71	1.48 to 1.97	<0.001	1.42	1.22 to 1.65	<0.001
IHD	1.03	0.85 to 1.24	0.78				1.64	1.35 to 1.99	<0.001	1.31	1.10 to 1.56	0.002
Cerebrovascular	0.84	0.64 to 1.10	0.20				1.97	1.65 to 2.36	<0.001	1.39	1.15 to 1.69	0.001
PAD	1.10	0.88 to 1.36	0.41				0.93	0.70 to 1.22	0.58	0.93	0.76 to 1.14	0.49
Smoking status												
Never	Ref						Ref			Ref		
Previous	0.72	0.60 to 0.86	<0.001				1.71	1.47 to 2.00	<0.001	1.26	1.07 to 1.48	0.005
Current	1.14	0.91 to 1.44	0.26				1.34	1.04 to 1.74	0.026	1.56	1.16 to 2.09	0.003
MAP	1.33	1.23 to 1.44	<0.001	1.07	0.97 to 1.18	0.16	0.00[h] 71.39[i]	0.00 to 0.00 6.10 to 835.26	<0.001 0.001			
eGFR	1.17[c] 1.06[d]	1.15 to 1.20 1.05 to 1.06	<0.001 <0.001	0.96[c] 2.4[g]	0.95 to 0.97 2.10 to 2.80	<0.001 <0.001	0.00[f]	0.00 to 0.01	<0.001	0.65	0.58 to 0.74	<0.001
Urine ACR	10.97[e] 0.80[f]	7.20 to 16.70 0.68 to 0.95	<0.001 0.008	3.58[e]	2.62 to 4.90	<0.001	1.68[e] 0.40[j]	1.18 to 2.40 0.29 to 0.55	0.005 <0.001	1.15[h] 1.01[i]	1.09 to 1.22 1.00 to 1.01	<0.001 <0.001

Continuous variables are linear per +1 SD unless indicated. Two rows for a continuous variable indicate the SHR or HR for each power from an FP2 model. Fractional polynomial transformations are indicated by: a = x^3; b = $x^3 ln(x)$; c = x^{-2}; d = $x^{-2} ln(x)$; e = $x^{0.5}$; f = x^2; g = x^{-1}; h = $ln(x)$; i = $(ln(x))^2$; j = $x^{0.5} ln(x)$.

The presence of an LC-MG did not have a significant association with the risk of kidney failure in univariable analysis (SHR 1.07 [95% CI 0.58 to 1.96], *P*=0.82; Figure 5.5).

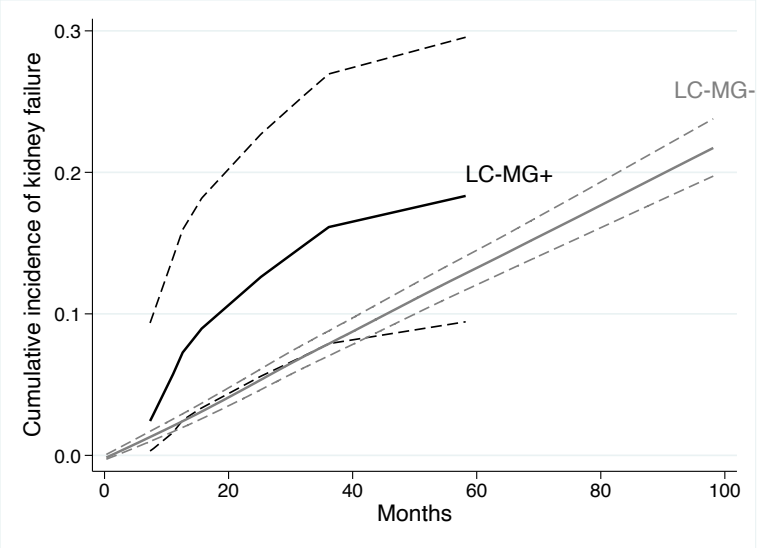


Figure 5.5. Cumulative incidence of kidney failure by LC-MG status

Black line = LC-MG+; grey line = LC-MG-.

Age, eGFR, and urine ACR had non-linear associations with risk of kidney failure in the univariable analyses, as shown in Figure 5.6.

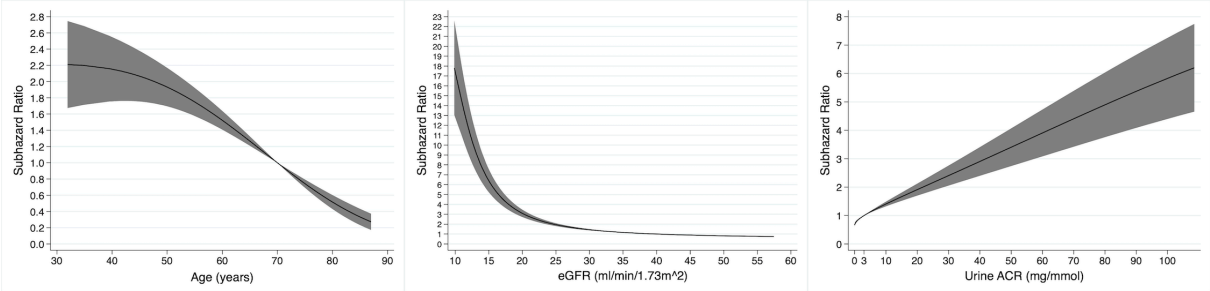


Figure 5.6. Unadjusted SHR for kidney failure according to age, eGFR, and urine ACR

SHR relative to 70 years for age, 40 ml/min/1.73 m^2 for eGFR, and 3 mg/mmol for urine ACR.

The multivariable model for kidney failure is shown in Table 5.5. After adjusting for age, sex, ethnicity, cause of CKD, MAP, eGFR, and urine ACR, the presence of an LC-MG did not have a statistically significant association with risk of kidney failure (SHR 1.42 [95% CI 0.78 to 2.57], *P*=0.26).

In this multivariable model, younger age (non-linear, Figure 5.7), Black ethnicity, lower eGFR (non-linear, Figure 5.7), and higher urine ACR (non-linear, Figure 5.7) were associated with a higher risk of kidney failure.

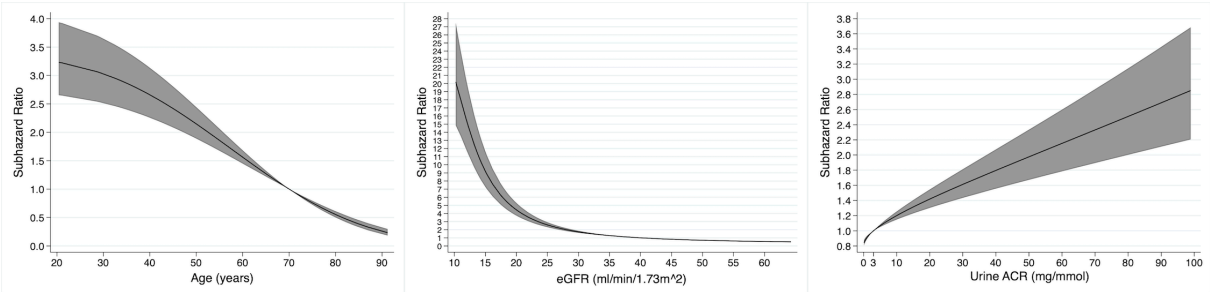


Figure 5.7. Adjusted SHR for kidney failure according to age, eGFR, and urine ACR

SHR relative to 70 years for age, 40 ml/min/1.73 m^2 for eGFR, and 3 mg/mmol for urine ACR, from the multivariable model.

5.5.2.2. Death

Eight hundred three (23.1%) participants died. Death rates were 9.3 and 4.5 per 100 person-years for those with and without an LC-MG, respectively. The univariable associations between baseline factors and death are shown in Table 5.5. LC-MG was associated with a higher risk of death (HR 2.51 [95% CI 1.59 to 3.96], *P*<0.001) and Figure 5.8 shows Kaplan-Meier survival curves by LC-MG status.

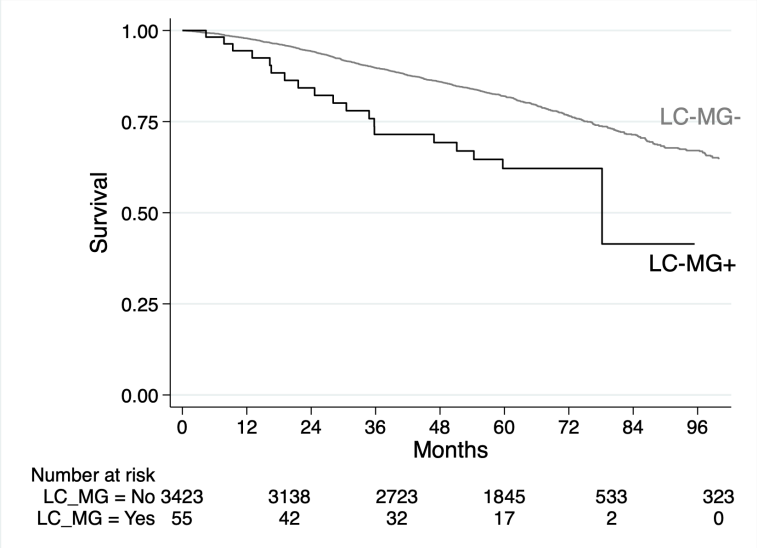


Figure 5.8. Kaplan-Meier survival curves by LC-MG status

The univariable analyses showed that MAP, eGFR, and urine ACR had non-linear associations with risk of death (Figure 5.9).

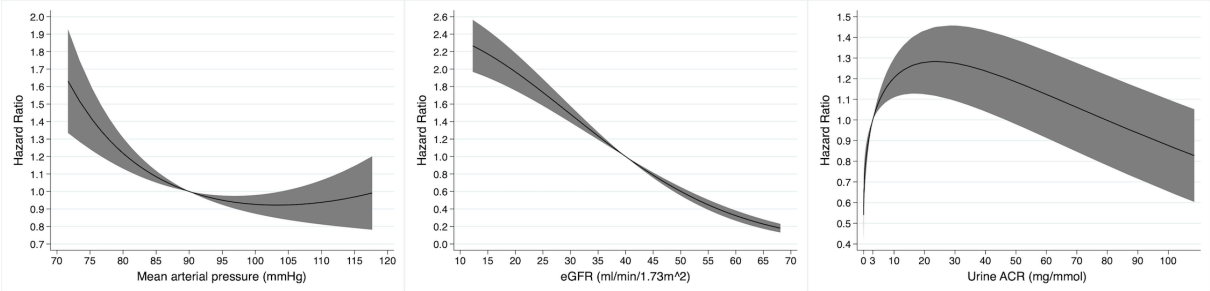


Figure 5.9. Unadjusted HR for death according to MAP, eGFR, and urine ACR

HR with 95% CI relative to 90 mmHg for MAP, 40 ml/min/1.73 m^2 for eGFR, and 3 mg/mmol for urine ACR.

In the multivariable model (Table 5.5), after adjustment for age, sex, ethnicity, co-morbidities, smoking status, eGFR, and urine ACR, an LC-MG did not have a statistically significant association with death (HR 1.49 [95% CI 0.93 to 2.39], *P*=0.10).

In this multivariable model, older age, male sex, a history of DM, IHD, or cerebrovascular disease, being a previous or current smoker, lower eGFR, and higher urine ACR (non-linear association, Figure 5.10) were associated with a higher risk of death.

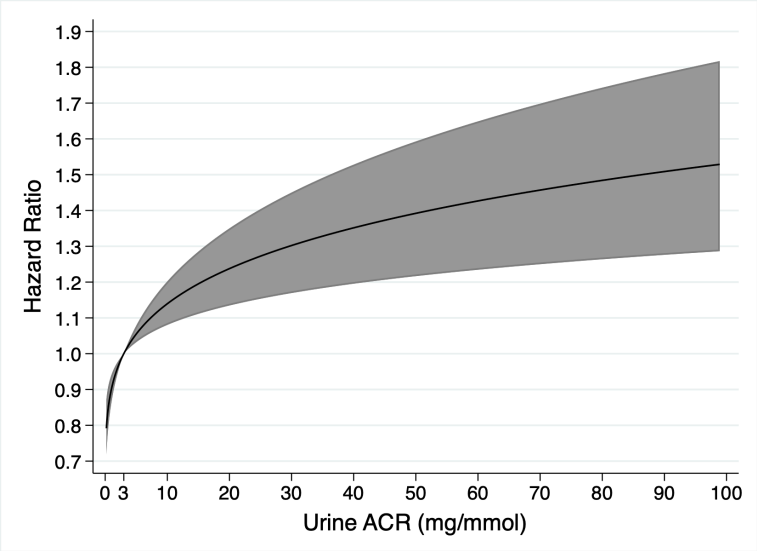


Figure 5.10. Adjusted HR for death according to urine ACR

HR relative to 3 mg/mmol, from the multivariable model in Table 5.5.

5.6. Discussion

It was hypothesised that, in patients with CKD, the presence of a non-malignant MG would be associated with a higher risk of kidney failure and death, based on the known pathogenetic properties of paraproteins in the kidney, and on the knowledge that MGUS is associated with reduced survival in the general population. Only one other study, by Haynes et al. (218), has assessed the relationship between MG and clinical outcomes in patients with CKD. That study had far fewer participants (382, of whom 35 had MGUS) and event rates than the work presented here and therefore there is likely to be less bias in the estimates presented in this chapter. While patients with CKD are already at a higher risk of kidney failure and death compared to the general population, the results of this work suggest that the presence of a non-malignant MG does not add to these risks.

The prevalence of non-malignant MG in this CKD population was significantly higher than the reported estimates of prevalence for the general population (255). This was also the case in the study by Haynes et al. (218), and it appears attributable to an increased prevalence of both intact Ig MG and LC-MG. The prevalence of total MGUS in the Olmsted County cohort in individuals aged 70 to 79 was 5.9%, and the prevalence of LC-MGUS was 1.1% (265); in this study, the crude prevalence was 11.6% for total MGUS (median age 73.8 years) and 1.6% for LC-MGUS (median age 77.8 years).

5.6.1. Kidney failure

The presence of a non-malignant MG was not associated with a higher risk of kidney failure in this study. This is consistent with the results of the study by Haynes et al. (218). This may be reassuring for patients with CKD and a non-malignant MG and their clinicians. Paraproteins are known to have potentially pathogenetic properties that can directly cause

kidney damage in MGRS and malignant MGs. If a significant association with kidney failure had been detected, it might have suggested that there are undiagnosed cases of MGRS. It is common to detect a paraprotein during the assessment of a patient with CKD, and in many patients, a kidney biopsy is foregone, and a presumed diagnosis of MGUS is made. Consensus guidelines have recently been published and recommend that a kidney biopsy be performed in those with MG and unexplained kidney disease, those with known risk factors for CKD but an atypical clinical course, and those with kidney disease and MG aged younger 50 years (266).

5.6.2. Death

The results of this study and the study by Haynes et al. (218) suggest that the shorter survival associated with MGUS in the general population is not seen in patients with CKD. It is possible that neither study was large enough to detect a small increase in the risk of death, or that follow-up was not long enough to detect an increase in mortality due to malignant transformation which occurs at a rate of approximately 1% per year. However, it may be that the already significantly increased rate of death in individuals with CKD, particularly due to CVD, renders any risk associated with an MG negligible.

5.6.3. Strengths and limitations

A significant strength of this study was the inclusion of participants from multiple cohorts from both primary and secondary care and that it is the largest cohort to date of patients with MGUS and CKD.

A significant limitation was the absence of SPEP and immunofixation data from the SKS and RRID cohorts. In these two cohorts, only LC-MG could be detected, and many

patients with an intact Ig MG would not have been identified. However, in the RIISC study, where SPEP and immunofixation were performed on serum from all participants, the presence of any non-malignant MG was not associated with a higher risk of kidney failure or death.

Further, other clinically important outcomes associated with MGUS in the general population were not assessed, such as cardiovascular events, infections, or the evolution of an MG to multiple myeloma or other paraprotein-related diseases.

Finally, CKD progression by the change in eGFR with time was not assessed, which would likely be a more sensitive marker for MG-associated kidney damage than the outcome of kidney failure.

5.6.4. Future research

Further research is required concerning the prognostic implications of non-malignant MG in patients with CKD, particularly for outcomes other than kidney failure and death, such as CKD progression, CVD events, and evolution into a malignant disease.

5.7. Conclusion

In conclusion, the prevalence of non-malignant MG appears to be higher in patients with CKD than in the general population, but these patients and their healthcare providers may be reassured that the MG does not significantly add to the risks of kidney failure or death.

CHAPTER VI: SERUM ENDOTROPHIN

The work presented in this chapter aimed to address the hypotheses that a higher serum concentration of endotrophin is associated with a higher risk of kidney failure and death in patients with chronic kidney disease (CKD).

Endotrophin is a fragment of collagen type VI, cleaved off after microfibril formation, and its concentration in the serum has been used as a marker of collagen type VI expression. As it is known that CKD is associated with a greater fibrotic burden in the kidney and cardiovascular system, it was hypothesised that serum endotrophin concentration, as a marker of this fibrotic load, would be associated with adverse outcomes in patients with CKD.

This work has been published in the article 'Serum endotrophin, a type VI collagen cleavage product, is associated with increased mortality in chronic kidney disease,' in PLOS ONE in 2017 (267), and was presented in poster format at the American Society of Nephrology Kidney Week, Chicago, 2016 and the UK Kidney Week, Liverpool, 2017.

6.1. Abstract

Background

Patients with CKD are thought to have dysregulation of extracellular matrix formation with accelerated systemic and renal fibrosis. The relationship between serum endotrophin concentration, a marker of collagen type VI formation, and the risk of kidney failure and death in a cohort of patients with CKD was assessed.

Methods

Serum endotrophin concentration was measured in 500 patients from the RIISC study, a prospective cohort study of patients with CKD. Patients were followed up until kidney failure or death. The association between serum endotrophin and kidney failure was assessed by competing risks regression (handling death as a competing event), and the association with death was assessed by Cox proportional hazards regression.

Results

Median follow-up time was 37 months, and there were 104 kidney failure events and 66 deaths. Serum endotrophin concentration was not significantly associated with the risk of kidney failure (adjusted SHR 1.04 [0.85 to 1.27] per +1 SD) but did have an independent association with the risk of death (adjusted HR 1.59 [1.24 to 2.04] per +1 SD).

Conclusions

Serum endotrophin concentration is not independently associated with the risk of kidney failure in patients with CKD but is independently associated with mortality. This may reflect increased cardiovascular fibrosis, but further work is required for validation and exploration of the nature of the association.

6.2. Introduction

Kidney fibrosis is the common final pathological manifestation of CKD, irrespective of the original cause of kidney disease, as described in Section 1.2.6, and its strong association with renal prognosis has been shown in many studies (268, 269). As described below, CKD is also associated with increased cardiovascular fibrosis. Endotrophin is a marker of collagen type VI deposition, and its use as a potential non-invasive marker of fibrosis and as a prognostic factor in patients with CKD merits further study.

6.2.1. Collagen type VI

Collagen type VI forms a network of beaded microfilaments in the ECM of most connective tissues, where it interacts with other ECM molecules and provides structural support for cells. In addition to a mechanical role, collagen type VI has cytoprotective functions such as the inhibition of apoptosis and oxidative damage, and the regulation of cell differentiation and autophagy (270-272). It is an important protein within the healthy kidney, being one of the most abundant proteins of the glomerular ECM, localised within the glomerular basement membrane and the mesangial matrix (273), and it also forms part of the reticular structure of the renal interstitium (274).

Nearly all forms of CKD are associated with renal collagen deposition and fibrosis. The deposition of collagen type VI is markedly increased in the fibrotic lesions seen in the glomerulus and interstitium in patients with CKD (275-277). The degree of tubulointerstitial fibrosis is strongly associated with long-term renal prognosis. The degree of interstitial collagen type VI expression, specifically, has been shown to be associated with the risk of kidney failure in patients with membranous nephropathy (278).

In addition to kidney fibrosis, patients with CKD have increased collagen deposition and fibrosis in other organs. Diffuse myocardial fibrosis has been demonstrated even in early CKD (279, 280) and, as in the kidney, collagen type VI forms part of the healthy myocardial ECM and increased deposition is demonstrated in myocardial fibrosis (281-285). Further, increased collagen deposition and fibrosis of arterial walls is observed in patients with CKD, which, in addition to arterial wall calcification, is associated with increased arterial stiffness (286-288).

Collagen type VI is composed of three chains: α1, α2, and α3 (289). Each chain contains a short collagenous region between domains at the N and C termini, as shown in Figure 6.1 (289, 290).

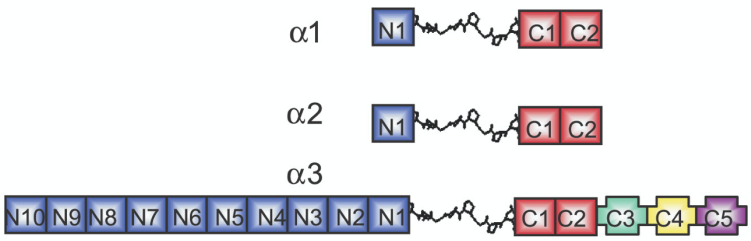


Figure 6.1. Organisation of domains in the α1, α2 and α3 chains of collagen type VI

The collagenous region is shown in black, with domains at the N and C termini. Note the C5 domain of α3 (purple), termed endotrophin. From (290).

Intracellularly, collagen type VI monomers form dimers and then tetramers before secretion into the ECM. The secreted tetramers then associate end-to-end to form beaded microfibrils, as shown in Figure 6.2 (289-291).

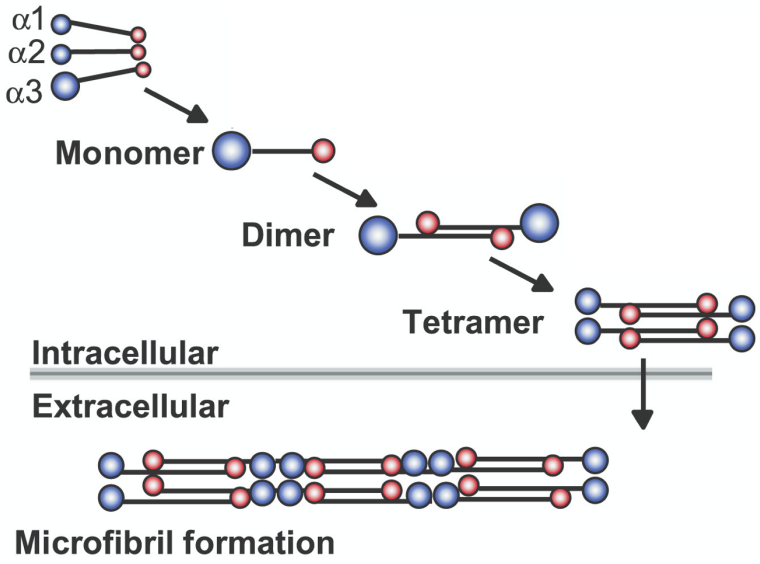


Figure 6.2. The assembly of collagen type VI microfibrils from the three α chains

The formation of monomers, dimers, and tetramers occurs intracellularly, while microfibril assembly occurs in the extracellular space. From (290).

The C5 domain of the α3 chain, termed endotrophin (shown in purple in Figure 6.1), plays a crucial role in microfibril assembly, but following microfibril formation is immediately cleaved off, and its measurement has thus been used as a marker for collagen type VI expression (289, 291, 292).

6.2.2. Endotrophin

Because endotrophin is cleaved off mature collagen type VI microfibrils shortly after their assembly, serum endotrophin concentration has been measured as a surrogate marker of collagen type VI formation.

In addition to playing a vital role in collagen type VI microfibril formation, released endotrophin has important biological effects in its own right. It appears to be particularly

abundant in adipose tissues, where it has pro-fibrotic effects and is pro-inflammatory, acting as a potent chemoattractant to macrophages (293).

To date, few published studies have assessed the prognostic significance of serum endotrophin levels. Given that CKD is associated with increased kidney collagen type VI deposition and endotrophin expression (294) and that plasma endotrophin levels correlate strongly with kidney transplant dysfunction (295) and failure (Nordic Bioscience, unpublished data), it was hypothesised that serum endotrophin concentration would correlate with kidney damage in CKD, and thus be associated with the risk of kidney failure.

Further, given the association between CKD and organ fibrosis, particularly cardiovascular fibrosis, and the potentially deleterious systemic effects of endotrophin, it was hypothesised that higher serum endotrophin levels would be associated with a higher risk of death.

,

6.3. Hypotheses

The work presented in this chapter aimed to address the following pre-specified hypotheses:

1. Higher serum endotrophin concentration is associated with a higher risk of kidney failure in patients with CKD;
2. Higher serum endotrophin concentration is associated with a higher risk of death in patients with CKD.

6.4. Methods

6.4.1. Patients

For sample availability reasons, data and samples from the six-month follow-up visit, rather than baseline visit, for the first 500 patients recruited into the Renal Impairment in Secondary Care (RIISC) Study were used. The RIISC study is described in detail in Section 2.1. All eligibility criteria, as described in Section 2.1.3, applied.

6.4.2. Assay

Serum endotrophin concentration was measured using the 'Pro-C6' competitive ELISA (Nordic Bioscience, Herlev, Denmark) (296).

6.4.3. Follow-up

The six-month visits occurred between April 2011 and September 2014. Time-to-event data were calculated from the date of the participant's six-month visit, and outcomes up to 31 December 2018 were included for the following:

- Kidney failure, defined as the initiation of kidney replacement therapy (dialysis or kidney transplantation)
- Death, from any cause.

6.4.4. Statistical methods

The distributions of baseline characteristics, including serum endotrophin concentration, are presented in tabular form with the number of missing values reported for each variable. The distribution of serum endotrophin concentration is illustrated by a histogram.

The relationships between serum endotrophin concentration and other baseline variables were assessed statistically. Given the possibility that serum endotrophin concentration may reflect cardiovascular fibrosis, the association with pulse wave velocity (PWV), a measure of arterial stiffness (see Section 2.1.4.4), was assessed. Relationships with continuous variables are expressed as Kendall's τ with its corresponding *P*, and fractional polynomials were used to assess for non-linear relationships and presented graphically. For categorical variables, median and interquartile ranges are shown with between-group differences assessed using the Mann-Whitney U or Kruskal-Wallis tests.

Univariable and multivariable regression models were fitted to show the association between serum endotrophin and other variables with outcomes. Subdistribution hazard models were used to assess the association with kidney failure (handling death as a competing risk) and presented as a subhazard ratio (SHR) with a 95% confidence interval (CI). Cause-specific hazard models were also fitted and are presented in Appendix 8. Cox proportional hazards regression was used to assess associations with death and are presented as a hazard ratio (HR) with 95% CI. Multivariable models were prespecified and non-linear associations were assessed, as per Section 2.5.4.2.

Missing data were handled by multiple imputation as per Section 2.5.6. For the kidney failure analyses, 13% of participants had missing data in at least one variable, and therefore 15 imputations were used. For the death analyses, 3% of participants had missing data, and therefore five imputations were used.

6.5. Results

The 500 participants had a median follow-up time of 6.4 years, during which there were 170 kidney failure events and 109 deaths.

6.5.1. Study population characteristics

The characteristics of the study population at the six-month visit (the time point at which serum endotrophin was measured) are shown in Table 6.1.

Table 6.1. Characteristics of the study population

Variable	Median (IQR) or *N*(%)	Data completeness (%)
Age	64 (50 to 76)	100
Sex (male)	308 (61.6)	100
Ethnicity		100
White	361 (72.2)	
South Asian	90 (18.0)	
Black	44 (8.8)	
Other	5 (1.0)	
Cause of CKD		89.6
Vascular	130 (29.0)	
Diabetes	48 (10.7)	
Glomerular	82 (18.3)	
Cystic and congenital	38 (8.5)	
Tubulointerstitial	52 (11.6)	
Other/uncertain	98 (21.9)	
Co-morbidities		100
Cerebrovascular disease	54 (10.8)	
COPD	60 (12.0)	
DM	183 (36.6)	
IHD	112 (22.4)	
Malignancy	72 (14.4)	
PAD	51 (10.2)	
Smoking status		100
Never	218 (43.6)	
Previous	215 (43.0)	
Current	67 (13.4)	
Systolic BP (mmHg)	124 (114 to 139)	100
Diastolic BP (mmHg)	75 (67 to 83)	100
MAP (mmHg)	91 (84 to 99)	100
PWV (m/s)	9.7 (8.4 to 11.3)	82.2
eGFR (ml/min/1.73 m^2)	27 (19 to 35)	99.4
Urine ACR (mg/mmol)	32.4 (6.1 to 128.3)	97.6
Serum endotrophin (ng/ml)	23.1 (16.8 to 30.1)	99.6

From the six-month visit.

Median serum endotrophin concentration was 23.1 ng/ml (IQR 16.8 to 30.1), and its distribution is illustrated in Figure 6.1.

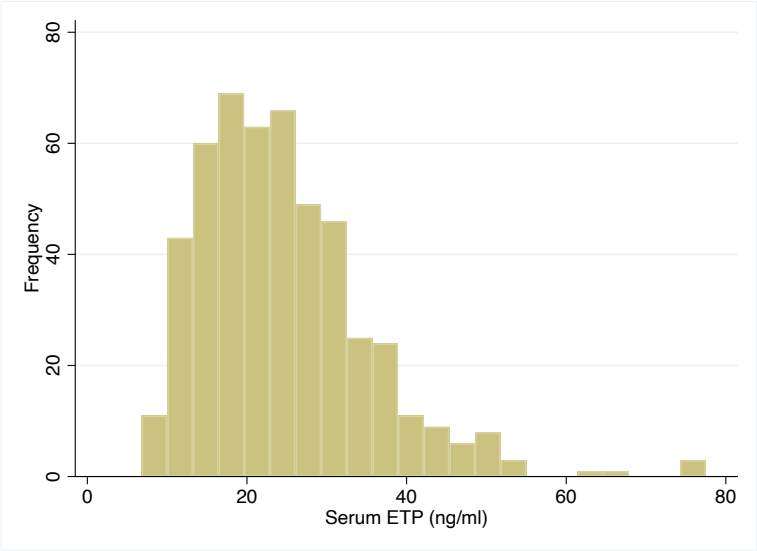


Figure 6.1. Histogram of serum endotrophin concentration

The histogram illustrates the skewed distribution of serum endotrophin concentration.

6.5.2. Relationships between endotrophin and other variables

The relationship of serum endotrophin concentration with other variables is shown in Table 6.2.

Table 6.2. Relationship between serum endotrophin and other variables

Variable	Kendall's τ or Median (IQR)	*P*
Age	0.174	<0.001
Sex		0.028
Female	24.6 (18.2 to 31.4)	
Male	22.0 (16.2 to 29.4)	
Ethnicity		0.23
White	23.1 (16.7 to 29.8)	
South Asian	24.3 (18.5 to 31.9)	
Black	21.5 (15.2 to 29.4)	
Other	21.8 (11.5 to 27.9)	
Cause of CKD		<0.001
Vascular	25.3 (20.3 to 31.9)	
Diabetes	31.7 (23.3 to 38.0)	
Glomerular	17.1 (12.6 to 24.8)	
Cystic and congenital	19.9 (14.8 to 26.7)	
Tubulointerstitial	22.2 (18.2 to 29.6)	
Other/uncertain	23.4 (16.5 to 28.4)	
Co-morbidities		
Cerebrovascular disease		0.29
Yes	23.2 (17.3 to 31.6)	
No	23.1 (16.6 to 29.8)	
COPD		0.84
Yes	23.1 (17.0 to 30.6)	
No	23.1 (16.7 to 29.9)	
Diabetes mellitus		<0.001
Yes	26.1 (18.6 to 32.9)	
No	21.4 (16.2 to 28.0)	
Ischaemic heart disease		0.017
Yes	25.0 (19.0 to 31.1)	
No	22.2 (16.5 to 29.4)	
Malignancy		0.97
Yes	22.9 (16.9 to 29.2)	
No	23.1 (16.7 to 30.2)	
Peripheral artery disease		0.38
Yes	24.0 (19.4 to 29.4)	
No	23.0 (16.5 to 30.2)	
Smoking status		0.18
Never	23.3 (16.8 to 30.9)	
Previous	23.3 (18.0 to 29.4)	
Current	21.1 (15.5 to 28.8)	
Systolic BP	0.135	<0.001
Diastolic BP	-0.129	<0.001
MAP	0.002	0.96
PWV	0.099	0.003
eGFR	-0.537	<0.001
Urine ACR	0.061	0.045

Serum endotrophin concentration was higher in females, those with DM or diabetic kidney disease, and those with IHD. Any other relationships, including that with PWV, were very weak, except for the relationship with eGFR.

In a multivariable analysis, the only variables with significant independent associations with serum endotrophin concentration were sex (2.1 [0.5 to 3.8] ng/ml higher in females, P=0.013), cause of CKD (7.2 [4.6 to 9.9] ng/ml higher in diabetic kidney disease, P<0.001), and eGFR, which had a non-linear relationship as shown in Figure 6.2.

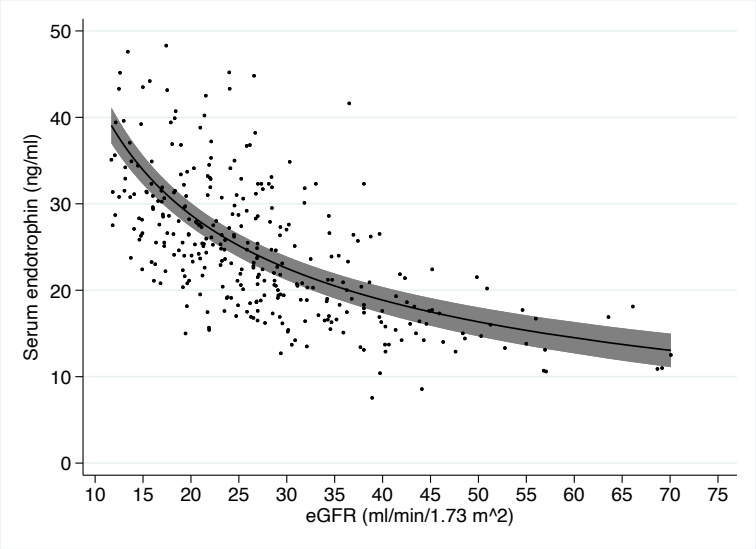


Figure 6.2. Relationship between eGFR and serum endotrophin concentration

The non-linear relationship is best fit with the fractional polynomial transformation of eGFR $x^{-0.5}$.

6.5.3. Kidney failure

During the median follow-up time of 6.4 years, 170 (34.0%) participants progressed to kidney failure, with an overall event rate of 7.3 per 100 person-years. The univariable

associations between serum endotrophin concentration and other baseline factors with the risk of kidney failure are shown in Table 6.3.

Table 6.3. Univariable and multivariable associations with kidney failure

Variable	Univariable			Multivariable		
	SHR	**95% CI**	***P***	**SHR**	**95% CI**	***P***
Age	1.00[a]	1.00 to 1.00	<0.001	0.99[3]	0.99 to 1.00	<0.001
Sex (male)	0.86	0.63 to 1.16	0.32	1.09	0.78 to 1.53	0.62
Ethnicity						
White	Ref			Ref		
South Asian	1.90	1.34 to 2.68	<0.001	1.25	0.84 to 1.86	0.28
Black	1.52	0.92 to 2.52	0.10	1.30	0.75 to 2.23	0.35
Other	1.31	0.38 to 4.58	0.67	1.07	0.25 to 4.50	0.93
Cause of CKD						
Vascular	Ref			Ref		
Diabetes	2.00	1.12 to 3.57	0.020	1.01	0.56 to 1.82	0.98
Glomerular	1.36	0.81 to 2.28	0.24	0.82	0.46 to 1.45	0.49
Cystic and congenital	3.07	1.89 to 4.98	<0.001	3.19	1.80 to 5.64	<0.001
Tubulointerstitial	1.11	0.62 to 1.98	0.72	0.81	0.43 to 1.55	0.53
Other/uncertain	1.33	0.83 to 2.15	0.24	1.01	0.61 to 1.66	0.97
Co-morbidities						
Cerebrovascular disease	0.76	0.45 to 1.30	0.32			
COPD	0.41	0.21 to 0.77	0.006			
DM	0.75	0.54 to 1.04	0.09			
IHD	0.83	0.57 to 1.22	0.35			
Malignancy	0.48	0.28 to 0.83	0.008			
PAD	0.62	0.34 to 1.13	0.12			
Smoking status						
Never	Ref					
Previous	0.77	0.56 to 1.07	0.12			
Current	0.85	0.53 to 1.37	0.51			
MAP	1.35	1.17 to 1.56	<0.001	1.04	0.87 to 1.24	0.64
eGFR	5.17[b]	3.72 to 7.19	<0.001	13.03[b]	7.67 to 22.16	<0.001
Urine ACR	1.47[c]	1.33 to 1.62	<0.001	3.19[d]	2.22 to 4.59	<0.001
				1.00[a]	0.99 to 1.00	0.014
Serum endotrophin	0.01[b]	0.00 to 0.03	<0.001	1.04	0.85 to 1.27	0.69

SHR for continuous variables are per +1 SD, unless fractional polynomial transformation provided better model fit, which are denoted by: a = x^3; b = $x^{-0.5}$; c = ln(x); d = $x^{0.5}$.

On univariable analysis, a higher serum endotrophin concentration was associated with a higher risk of kidney failure, as shown in Figure 6.3.

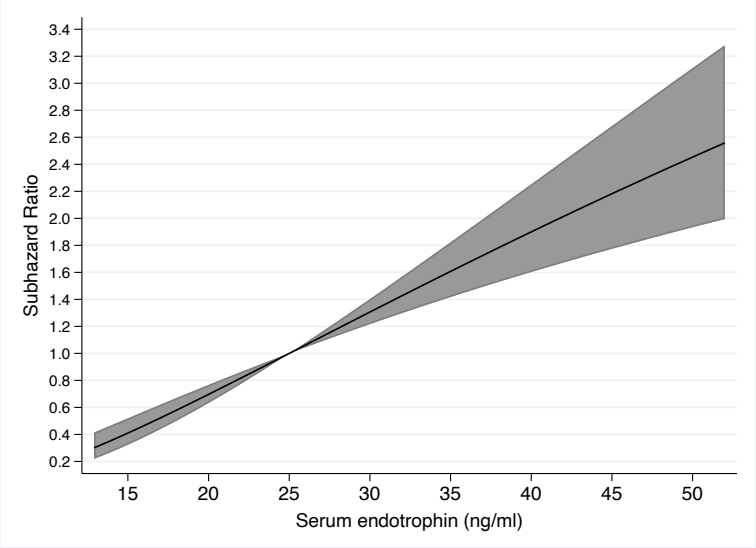


Figure 6.3. Unadjusted SHR for kidney failure according to serum endotrophin

SHR with 95% CI, relative to 25 ng/ml.

Other variables associated with a higher risk of kidney failure on univariable analysis were younger age (non-linear, Figure 6.4), South Asian ethnicity, CKD due to diabetes or a cystic or congenital disease, higher MAP (non-linear, Figure 6.5), lower eGFR (non-linear, Figure 6.6), and higher urine ACR (non-linear, Figure 6.7). Diagnoses of COPD or malignancy were associated with a lower risk of kidney failure.

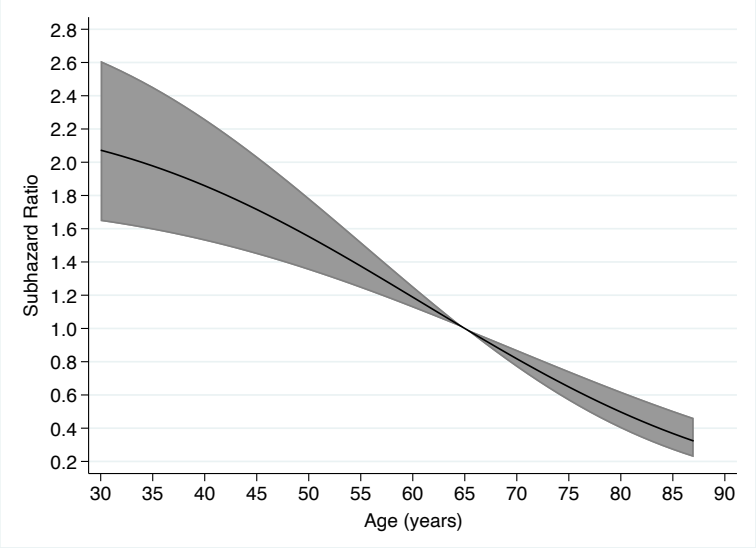


Figure 6.4. Unadjusted SHR for kidney failure according to age

SHR with 95% CI, relative to 65 years.

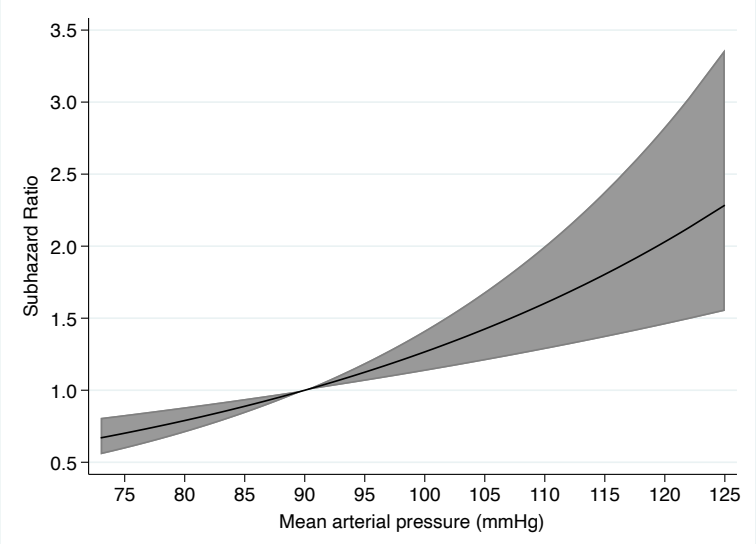


Figure 6.5. Unadjusted SHR for kidney failure according to MAP

SHR with 95% CI, relative to 90 mmHg.

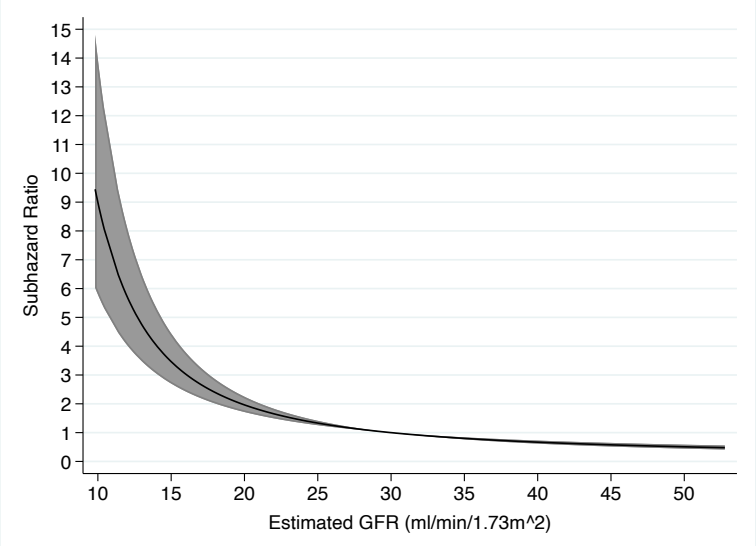


Figure 6.6. Unadjusted SHR for kidney failure according to eGFR

SHR with 95% CI, relative to 30 ml/min/1.73 m^2.

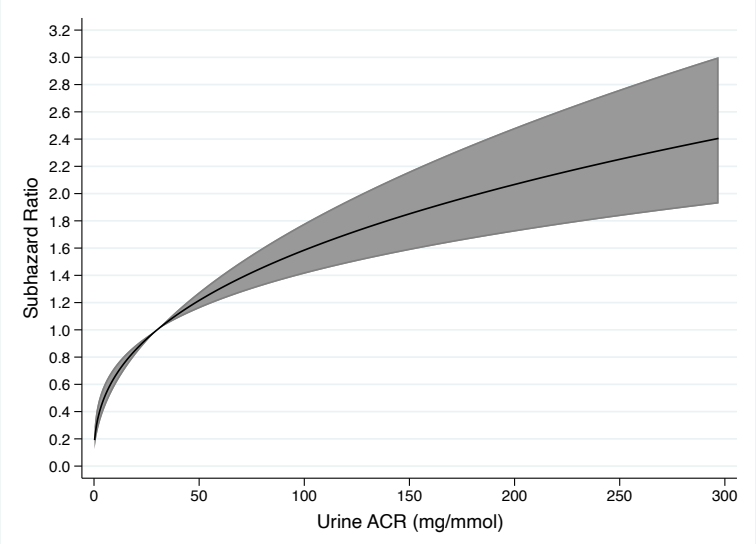


Figure 6.7. Unadjusted SHR for kidney failure according to urine ACR

SHR with 95% CI, relative to 30 mg/mmol.

The multivariable model for kidney failure is shown in Table 6.3. After adjusting for age, sex, ethnicity, cause of CKD, MAP, eGFR, and urine ACR, serum endotrophin concentration was no longer associated with the risk of kidney failure (SHR 1.04 [0.85 to 1.27] per +1 SD).

Factors associated with a higher risk of kidney failure in the multivariable model were younger age (non-linear, Figure 6.8), a cystic or congenital cause of CKD, lower eGFR (non-linear, Figure 6.9), and higher urine ACR (non-linear, Figure 6.10).

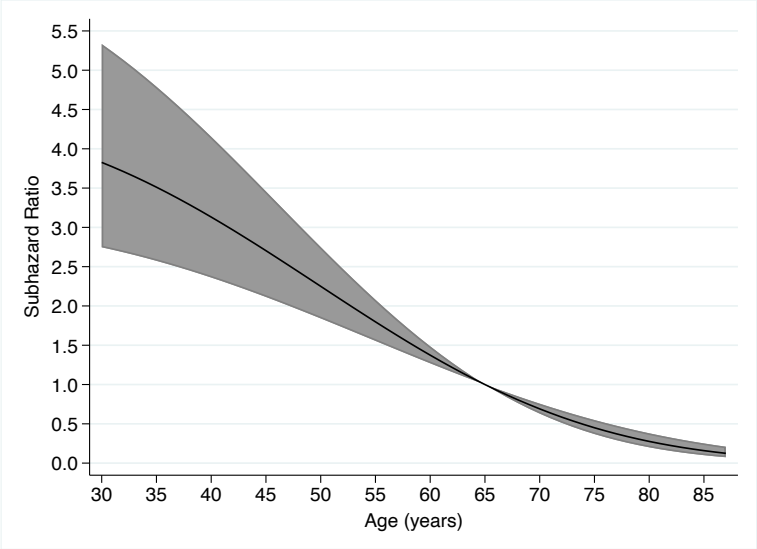


Figure 6.8. Adjusted SHR for kidney failure according to age

SHR with 95% CI, relative to 65 years, from the multivariable model in Table 6.3.

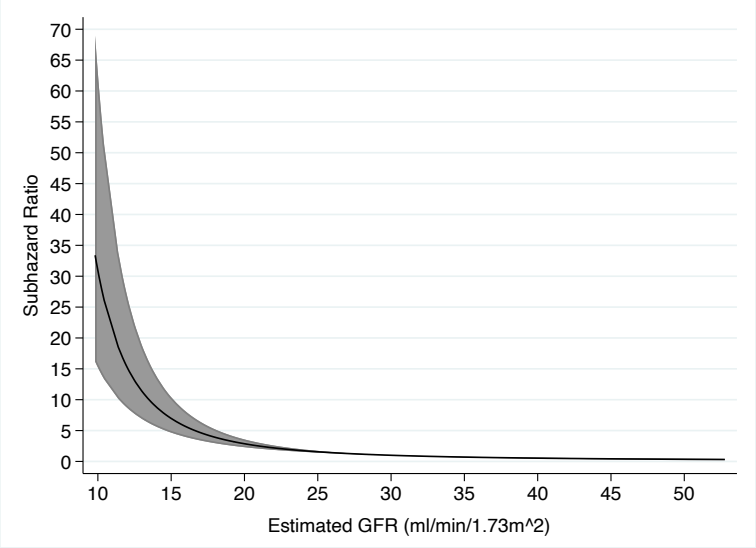


Figure 6.9. Adjusted SHR for kidney failure according to eGFR

SHR with 95% CI, relative to 30 ml/min/1.73 m^2 from the multivariable model in Table 6.3.

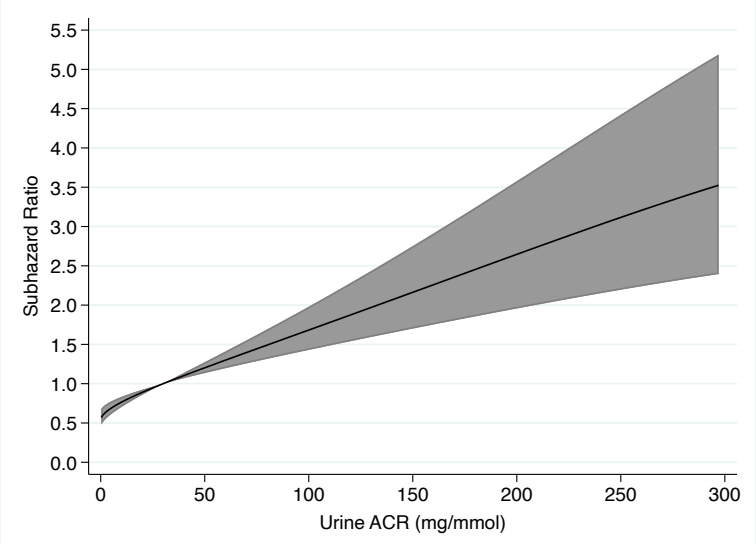


Figure 6.10. Adjusted SHR for kidney failure according to urine ACR

SHR with 95% CI, relative to 30 mg/mmol from the multivariable model in Table 6.3.

6.5.4. Death

During the median follow-up time of 6.4 years, 109 (21.8%) participants died, and the overall death rate was 4.7 per 100 person-years. The univariable associations between serum endotrophin concentration and other variables with the risk of death are shown in Table 6.4.

Table 6.4. Univariable and multivariable associations with death

Variable	Univariable			Multivariable		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age	3.63	2.71 to 4.86	<0.001	3.51	2.47 to 4.98	<0.001
Sex (male)	1.11	0.75 to 1.64	0.60	1.06	0.67 to 1.66	0.81
Ethnicity						
White	Ref			Ref		
South Asian	0.82	0.48 to 1.43	0.49	1.28	0.68 to 2.38	0.44
Black	0.78	0.38 to 1.60	0.49	1.21	0.56 to 2.64	0.63
Other	0.84	0.12 to 6.07	0.87	0.82	0.10 to 6.63	0.86
Cause of CKD						
Vascular	Ref					
Diabetes	0.94	0.51 to 1.77	0.86			
Glomerular	0.21	0.09 to 0.46	<0.001			
Cystic and congenital	0.26	0.08 to 0.84	0.024			
Tubulointerstitial	0.30	0.13 to 0.70	0.006			
Other/uncertain	0.58	0.34 to 0.98	0.041			
Co-morbidities						
Cerebrovascular disease	1.88	1.15 to 3.09	0.012	1.20	0.69 to 2.09	0.52
COPD	1.36	0.82 to 2.26	0.23	1.29	0.76 to 2.20	0.35
DM	2.05	1.40 to 2.99	<0.001	1.33	0.87 to 2.02	0.19
IHD	2.52	1.71 to 3.71	<0.001	1.46	0.96 to 2.22	0.08
Malignancy	1.32	0.82 to 2.13	0.26	1.03	0.62 to 1.70	0.91
PAD	2.40	1.50 to 3.83	<0.001	1.32	0.79 to 2.22	0.29
Smoking status						
Never	Ref			Ref		
Previous	1.53	1.02 to 2.29	0.041	1.15	0.71 to 1.85	0.57
Current	0.94	0.49 to 1.80	0.85	1.44	0.68 to 3.04	0.34
MAP	5.e+224[a] 0.00[b]	7.e+107 to . 0.00 to 0.00	<0.001 <0.001	1.17	0.95 to 1.44	0.14
eGFR	0.34	0.23 to 0.50	<0.001	0.77	0.50 to 1.19	0.24
Urine ACR	0.97	0.76 to 1.24	0.83	1.39	1.08 to 1.78	0.009
Serum endotrophin	0.01[a]	0.00 to 0.03	<0.001	1.59	1.24 to 2.04	<0.001

HR for continuous variables are per +1 SD, unless fractional polynomial transformation provided better model fit, which are denoted by: a = x^{-2}; b = $x^{-0.5}$.

On univariable analysis, a higher serum endotrophin concentration was associated with a higher risk of death. The association was non-linear, as shown in Figure 6.11.

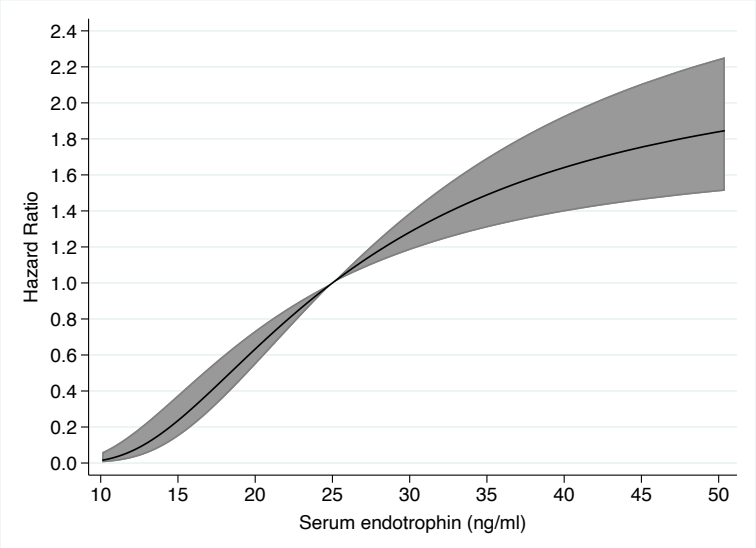


Figure 6.11. Unadjusted HR for death according to serum endotrophin concentration

HR with 95% CI, relative to 25 ng/ml.

Other factors associated with a higher risk of death on univariable analysis were older age, a history of cerebrovascular disease, DM, IHD, or PAD, being a previous smoker, a MAP < 78 or > 108 mmHg (Figure 6.12), and lower eGFR. Having a non-vascular or non-diabetes cause of CKD was associated with a lower risk of death.

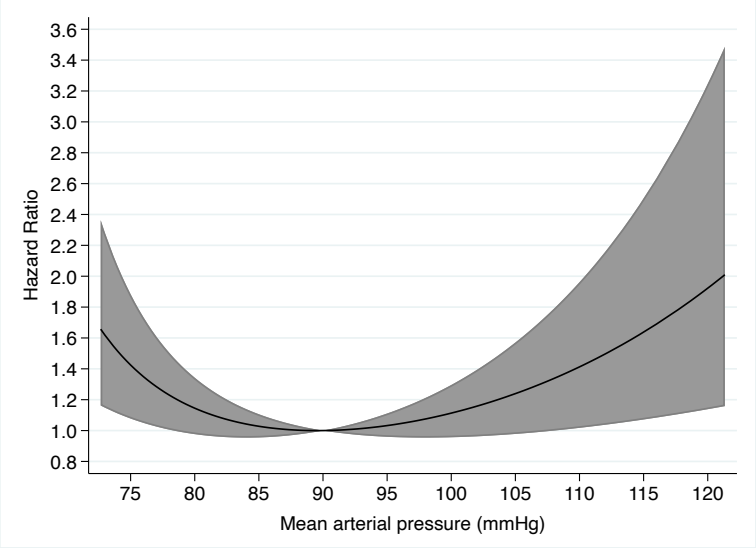


Figure 6.12. Unadjusted HR for death according to MAP

HR with 95% CI, relative to 90 mmHg.

After adjusting for age, sex, ethnicity, co-morbidities, smoking status, MAP, eGFR, and urine ACR, a higher serum endotrophin concentration remained significantly associated with a higher risk of death (HR 1.59 [1.24 to 2.04] per +1 SD).

Other factors associated with a higher risk of death in the multivariable model were older age and higher urine ACR. It is particularly notable that eGFR is not associated with death in this model. When serum endotrophin concentration is removed from the multivariable model, eGFR becomes significantly associated with mortality (HR 0.53 [0.35 to 0.80] per +1 SD, P=0.003; the HRs for all other variables remain similar).

6.6. Discussion

Tissue fibrosis and remodelling have been implicated in both CKD progression and the increased risk of death associated with CKD. The work presented in this chapter demonstrates an independent association between serum endotrophin concentration, a marker of collagen type VI formation, and mortality in a cohort study of participants with CKD.

As has previously been demonstrated in kidney transplant recipients (295), there was an inverse relationship between eGFR and serum endotrophin concentration, for which there are several possible explanations. First, the correlation may reflect a reduction in renal clearance of endotrophin as kidney function declines. Although the estimated molecular weight of endotrophin is approximately 10 to 15 kDa (297), the renal clearance of endotrophin is not known. Second, it may reflect the increased abundance of fibrotic tissue, and therefore collagen type VI and endotrophin generation, in patients with CKD: renal fibrosis may be a contributing source of endotrophin, but the elevated serum levels are also likely to reflect a greater systemic fibrotic burden in patients with more advanced CKD. These data do not allow a determination of the relative contributions of these potential explanations for the relationship described.

6.6.1. Kidney failure

Based on the hypothesis that serum endotrophin concentration may reflect increased kidney endotrophin expression, which has been shown to co-localise with collagen type VI in kidney fibrosis (294), and thus kidney damage, the association between serum endotrophin concentration and the risk of kidney failure in patients with CKD was explored. Although there was a higher risk of kidney failure associated with a higher endotrophin concentration,

after adjustment for eGFR and other standard prognostic factors, there was no independent association.

It is likely that serum endotrophin level is highly influenced by systemic, particularly cardiovascular, fibrosis, and therefore not specific enough as a marker of kidney fibrosis. Urine endotrophin-creatinine ratio, meanwhile, has been shown to be an independent predictor of CKD progression, suggesting that urine endotrophin is a more specific marker of kidney fibrosis than serum endotrophin (294). The search for non-invasive, particularly urinary, markers of kidney fibrosis is an active area of nephrology research, as discussed in the final chapter.

6.6.2. Death

The results in this chapter suggest an independent association between serum endotrophin concentration and the risk of death in patients with CKD. After adjustment for eGFR and other standard prognostic factors, a higher serum endotrophin concentration was independently associated with a higher risk of death (HR 1.59 [1.24 to 2.04] per +1 SD, $P<0.001$). Furthermore, the association between serum endotrophin concentration and risk of death appears to be stronger than for eGFR, which is one of the most important prognostic factors for mortality in CKD.

Despite the lack of an association between PWV, a marker of arterial stiffness, and serum endotrophin concentration, the association between endotrophin and mortality may still represent the effect of systemic, particularly cardiovascular, collagen type VI deposition and fibrosis. Previous studies have demonstrated increased diffuse myocardial fibrosis in patients with CKD (279, 280) and collagen type VI deposition has been demonstrated in myocardial fibrosis (281-285). Further, increased collagen deposition and fibrosis of the vascular wall is

observed in patients with CKD (286-288). The presence of collagen type VI has also been demonstrated in atherosclerotic lesions (298). Serum endotrophin concentration may reflect cardiovascular collagen type VI expression, thus a surrogate marker for cardiac and arterial fibrosis or atherosclerosis, and mortality risk. In patients with DM, serum endotrophin concentration correlates with markers of atherosclerosis severity and with the risk of cardiovascular events (299).

Collagen type VI itself has also been shown to have various deleterious effects. For example, it has a significant role in platelet adhesion, which is intimately involved in atherosclerosis and microvascular pathology. Collagen type VI binds platelets both directly and via von Willebrand factor (vWF), and of the multiple subendothelial collagens to which vWF binds, collagen type VI appears to be especially important (300-302). Collagen type VI may also have deleterious effects on the myocardium. Collagen type VI deletion in knockout mice is associated with improved cardiac function, structure and remodelling after myocardial infarction (303).

Endotrophin has also been demonstrated to have various adverse biological effects. For example, it has been shown to activate cardiac fibroblasts from healthy adult donors and induces fibrogenesis (304). It also plays a pivotal role in shaping a metabolically unfavourable microenvironment within adipose tissue, where it triggers fibrosis and inflammation and ultimately results in systemic elevation of pro-inflammatory cytokines, insulin resistance, and the metabolic syndrome (293). Further, endotrophin has been shown to play a role in promoting tumour growth and metastasis (270, 271, 305).

The underlying nature of the relationship between serum endotrophin concentration and risk of death requires further work.

6.6.3. Strengths and limitations

The strengths of this work include the utilisation of a large prospective CKD cohort with detailed bio-clinical phenotyping incorporating multiple prognostic factors for adverse outcomes. The limitations include it being single-centre, with no validation cohort, and the lack of experimental data to explore the mechanisms underlying the association between serum endotrophin and mortality. In particular, markers of cardiovascular fibrosis other than PWV, urinary endotrophin excretion, and causes of death, would have helped significantly to explain the associations described.

6.6.4. Future research

The independent association between serum endotrophin concentration and risk of death requires validation in a separate cohort of patients with CKD. Ideally, additional data, particularly on cardiovascular health and fibrosis, such as cardiac magnetic resonance imaging, cardiovascular events, and cause of death, would be collected to further the understanding of the nature of the relationship. Following validation, its role in risk prediction for mortality in patients with CKD could be investigated.

6.7. Conclusions

In conclusion, serum endotrophin concentration is not independently associated with the risk of kidney failure in patients with CKD but is independently associated with the risk of death after adjustment for standard prognostic factors. Further work is required to validate this finding and to understand the nature of the association.

CHAPTER VII: GENERAL DISCUSSION

7.1. Introduction

Chronic kidney disease (CKD) has an estimated prevalence of approximately 10% in adults in the UK, and the prevalence is increasing. It is associated with an increased risk of serious adverse health outcomes, including kidney failure and death. However, the prognosis for an individual patient with CKD will fall on a spectrum, from asymptomatic disease with no progression and a lifespan equivalent to that expected in the healthy population, to rapid progression with kidney failure or early cardiovascular death.

Identifying the likely prognosis for an individual patient with CKD provides important information for both the patient and their clinicians, helping guide management decisions in the individual patient's care. Prognostic factors help to stratify risk and may be combined in prognostic models to predict an individual's risk of adverse outcomes such as kidney failure.

There are established prognostic factors in CKD that are associated with the risk of adverse outcomes, including age, cause of CKD, eGFR, and urine ACR. Risk prediction models, such as the Kidney Failure Risk Equation, are increasingly being employed in routine clinical practice to guide decision-making. For example, decisions around whether a patient needs follow-up in secondary care nephrology rather than primary care, or whether a patient should begin preparation for kidney replacement therapy can be aided by accurate prognostic information. An upcoming revision of the NICE CKD guideline is likely to recommend the use of the KFRE in routine care of patients with CKD. An assessment of the impact of introducing the model into clinical practice on clinical outcomes and the cost-effectiveness of care will be an essential consideration.

The identification of new factors that provide added prognostic information above and beyond that provided by established prognostic factors has the potential to improve risk stratification and risk prediction, and the potential to identify targets for new treatments, and thus improve the care of patients with CKD.

In work presented in this thesis, four potential prognostic factors in CKD were examined for independent associations with the risk of kidney failure or death, as summarised in the following paragraphs.

7.2. Serum free light chains

Five studies had published estimates of the association between serum cFLC concentration and the risks of kidney failure or death in patients with CKD, but their results were inconsistent. A meta-analysis of individual participant data was conducted, incorporating additional data not previously reported, to examine these associations.

A higher serum cFLC concentration was independently associated with a higher risk of kidney failure. For the first time, it was shown that the relationship between serum cFLC concentration and risk of kidney failure is non-linear, with an increasing risk up to a serum cFLC concentration of approximately 150 mg/l, above which the risk plateaus. Given the known nephrotoxic effects of FLCs, such as their ability to cause tubular toxicity or form casts, the association may be causal, although this cannot be proven from these data and requires further research.

Serum cFLC concentration was also independently associated with the risk of death. Again, the relationship is non-linear, with a relatively smaller increase in risk at higher levels of cFLC concentration. The association may reflect confounding, such as inflammatory processes not measured in these data, but a causal association is possible given the potentially

deleterious effects of FLCs. Future work that incorporates measures of inflammation, such as C-reactive protein and cytokines, is required to explore the nature of the association further.

Serum FLCs are routinely measured in clinical practice in the assessment of monoclonal disorders, but there is currently no role for the routine assessment of non-clonal serum FLC concentrations. Now that the association with kidney failure has been established, the potential incremental value of adding serum cFLC concentration to prognostic models for the prediction of kidney failure, such as the KFRE, should be assessed. Further, prognostic models may be developed and assessed that incorporate serum cFLC concentration for the prediction of risk of death in patients with CKD.

Should evidence of a causal role in the association between serum FLCs and the risk of adverse outcomes be established, an assessment may be made of their potential as a treatment target. For example, rituximab, a monoclonal antibody targeted against CD20 expressed on most B cells, leads to B cell depletion and a reduction in serum cFLC concentration when used in conditions such as systemic lupus erythematosus and rheumatoid arthritis (306, 307).

7.3. Urine free light chains

Urine FLC excretion in patients with CKD is in part determined by serum FLC concentration. Given the results of Chapter III, and the supposition that urine FLC excretion may reflect kidney exposure to potentially nephrotoxic FLCs, an assessment was made of the association between urine FLCs and the risk of kidney failure and death in a prospective cohort of patients with CKD.

A significant correlation between serum FLC concentration and urine FLC/creatinine concentrations was confirmed. However, urine FLC/creatinine concentrations were not

independently associated with the risk of kidney failure or death and did not provide any improvement when added to the KFRE for the prediction of kidney failure at two years.

The detection of monoclonal urine FLCs (Bence Jones protein) is still used in clinical practice in the assessment for monoclonal disorders, but there is no evidence to date that the measurement of urine non-clonal FLCs is clinically useful. Given previous work showing that urine FLC excretion increases early in CKD before the development of increased albuminuria, however, the use of urine FLCs for the early diagnosis of CKD may be explored.

7.4. Monoclonal gammopathy

The presence of a malignant monoclonal gammopathy (MG) may be causally associated with kidney failure, and with death. Chapter III showed a higher serum concentration of non-clonal FLCs is also associated with a higher risk of kidney failure and death in patients with CKD. However, there has been little study of the prognostic significance of non-malignant MG in patients with CKD.

One study, by Haynes et al., found no independent association between the presence of an MGUS and the risk of kidney failure (218). Further, unlike in the general population, MGUS was not associated with worse survival (218). However, this was a relatively small study, and given the common finding of a non-malignant MG in patients with CKD, an assessment was made using data from three cohort studies of the association between non-malignant MG and kidney failure and death in CKD.

As observed in other CKD cohorts, the prevalence of an MG was higher than the prevalence in the general population. However, the presence of an MG was not independently associated with the risk of kidney failure or death. These results are consistent with the findings of Haynes et al. (218).

This information is of significant importance in clinical practice. It is common to detect a non-malignant MG in patients with CKD and based on these results these patients and their clinicians may be reassured that the MG does not add to their risk of kidney failure or death. However, the association between the presence of an MG and other important outcomes, such as cardiovascular events or malignant transformation, were not studied and may be studied in future research.

7.5. Serum endotrophin

Finally, the prognostic significance of serum endotrophin concentration in patients with CKD was assessed. CKD is associated not only with kidney fibrosis but also with accelerated cardiac and arterial fibrosis. It was hypothesized that the serum concentration of endotrophin, a marker of collagen type VI deposition, may reflect this fibrotic burden and be associated with the risk of adverse outcomes.

While there was no independent association between serum endotrophin concentration and the risk of kidney failure, a higher serum endotrophin concentration was independently associated with a higher risk of death. Of particular interest was the finding that this association was stronger than the association between eGFR and risk of death in this cohort.

This finding requires validation in a separate cohort of patients with CKD, ideally with additional cardiovascular phenotyping and data on causes of death to explore the association between endotrophin and death. Blood pressure and PWV were assessed in this cohort and did not have strong relationships with endotrophin. Should the significant association between endotrophin and mortality be validated, its role in the risk prediction for mortality in patients with CKD may be assessed.

Despite a lack of association between serum endotrophin and the risk of kidney failure, recent work has demonstrated an association between urinary endotrophin excretion and CKD progression, perhaps suggesting that urine endotrophin is a more specific and reliable marker of kidney fibrosis than serum concentration (294).

7.6. Strengths and limitations

The work in this thesis has all been performed using data and samples from prospective cohort studies of patients with CKD, and methods were employed to reduce the risk of bias, such as remote outcome event capture to supplement patient-reported events and robust pre-specified statistical analyses.

However, the data are observational. The association between each prognostic factor and kidney failure and death were estimated, but the underlying nature of the associations and in particular whether they were causal relationships, could only be speculated upon. The lack of mechanistic data, such as that from kidney biopsy specimens, is a significant limitation and is common to most observational CKD cohort studies. The availability of kidney biopsy tissue in the recently-established NURTuRE (the National Unified Renal Translational Research Enterprise)-CKD prospective study, described below, is one of its particular advantages.

7.7. Cause-specific hazard models

In addition to the primary analyses for kidney failure in each chapter in which the subdistribution hazard was modelled, cause-specific hazards were also modelled and presented in Appendices 5 to 8. It has been suggested that subdistribution hazard models are preferable to estimate the future risk of an outcome and prognosis (308). In contrast, cause-

specific hazard models allow an estimation of the association between a factor on the hazard, e.g. of kidney failure, and are preferable when considering whether a factor has a causal association with the outcome (308).

There were no significant differences between the results of the subdistribution hazard models and the cause-specific hazard models with regard to the novel prognostic factors being assessed. In both types of modelling, a higher serum cFLC concentration was associated with a higher risk of kidney failure, but urine FLC/creatinine ratios, monoclonal gammopathy, and serum endotrophin concentration were not.

The higher risk of kidney failure associated with a higher serum cFLC concentration in the cause-specific hazard models would be consistent with but does not prove, a causal association. Interestingly, the graph in Appendix 5 suggests that the hazard associated with a higher serum cFLC concentration continues to increase even above 150 mg/l, despite there not being a further increase in incidence above this concentration (based on the subdistribution hazard model), likely due to the higher risk of the competing event of death associated with high concentrations of serum cFLC. The potential pathogenetic properties of FLCs seen in monoclonal disorders such as multiple myeloma and MGRS, and the other plausible mechanisms by which FLCs might be nephrotoxic, as discussed in Chapter III, lend weight to the hypothesis of a causal relationship. However, further research is needed to explore the nature of the association.

7.8. Future research

Several findings from work presented in this thesis may form the basis of further research. First, serum cFLC concentration has been shown, using data from five prospective CKD cohort studies, to be independently associated with the risk of kidney failure and death.

The role of serum FLC concentration in risk prediction may now be examined. This would preferably involve assessing the incremental value of serum FLC concentration when added to pre-existing models predicting the risk of kidney failure and death in patients with CKD, rather than the development of new models. A preliminary assessment could be undertaken using the existing data.

Further laboratory-based research may also be undertaken to examine the nature of the underlying association, for example by assessing the effect on cells of the kidney, heart, and vasculature to exposure to high concentrations of FLCs. Should evidence for a causal role be demonstrated, the use of treatments targeted against FLCs or FLC-producing B cells may be explored.

The finding of an independent association between serum endotrophin concentration and the risk of death in patients with CKD first requires validation in a separate cohort of patients. If the association is replicated, an assessment should be made of its role in risk prediction, and further exploration of the nature of the association may be accomplished through detailed cardiovascular phenotyping and associations with incident cardiovascular disease and causes of death. Endotrophin measurement is not currently available in routine clinical practice, and further work is required to assess whether it may have a future role in the management of patients with CKD.

In the UK, the NURTuRE-CKD study, which recently completed recruitment, has collected and stored serum, urine, DNA and kidney biopsy tissue from over 3000 patients with CKD from 18 NHS trusts, with linked clinical and outcome data. With the accrual of patient follow-up and outcome events, this biobank will provide the basis for the further development of risk prediction in CKD and the identification of further prognostic factors.

Further, international collaborations, such as the CKD Prognosis Consortium and iNET-CKD (International Network of Chronic Kidney Disease cohort studies), the availability of big data updated in real-time, developments in -omics research, and novel methods of prognosis research such as machine learning, may all have a role in the future conduct of high-quality prognosis research in CKD with the goal of improving outcomes for patients.

APPENDIX 1.

SOP: BLOOD PRESSURE MEASUREMENT USING THE BPTRU DEVICE

Purpose

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

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

Preparation and Method

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

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

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

APPENDIX 2.

SOP: MEASUREMENT OF ARTERIAL STIFFNESS USING THE VICORDER DEVICE

Purpose

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

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

Method

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

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

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

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

Ensure that all data collected is stored in spreadsheet.

APPENDIX 3.

SOP: MEASUREMENT OF AGES USING THE AGE READER

Purpose

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

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

Intended Use

Measurements should be done on the dominant arm on healthy undamaged skin without birthmarks or excessive hair growth, tattoos or scars. Self tanning agents must not be used for at least 2 days. If patient has used self tanning agents document and inform the patient not to use next time 2 days before the appointment. Sun-blockers and other skin care products should be removed before measurement.

Pigmented skin

The device and its software have been validated in patients with Fitzpatrick class 1-4 skin colour. For measurements on patients with Fitzpatrick class 5-6 (dark brown or black), users should check with the manufacturer or distributor for the correct software version in order to avoid unreliable results. If a measurement is performed on a skin type that is too dark to give a reliable result, the AGE Reader CU will give a warning.

UV-Radiation

Using the guidelines of the ICNIRP it is concluded that during AGE Reader CU measurements, as intended, even when repeated up to a 100 times on the same skin site within an 8-hour period, the local radiation exposure on the skin of the patients, and to the eyes of patients and operators remain considerably below the maximum allowed values for that period. Radiation exposure to the eyes normally does not occur. Exposure of the eyes longer than 60 seconds per 8-hour period should be avoided (ie do not look directly into the UV light)

Procedure and method

Follow the instructions as set out in AGE reader operator manual 2010 to be found with equipment.

APPENDIX 4.

SOP: PLASMA, SERUM, AND URINE SAMPLE HANDLING AND PROCESSING

Purpose

The purpose of this SOP is to ensure standardised operating procedures, when collecting blood and urine samples for the purpose of this study.

Blood, urine and saliva samples will be collected from all participants at all time-points

Introduction/Method

. **Collect blood samples using vacutainers (order of draw: 2 x red, 1 x EDTA, 1 x Paxgene)**
. **Tubes should be completely filled by the vacuum in order to obtain the correct ratio of blood to additive. Over and under filing alters the ration and changes results.**
. **Thoroughly mix by inverting the tube 8-10 times**
. **Leave serum (2 x red top) to clot for 1 hour at room temperature**
. **Spin at 2500rpm for 10 minutes at 4°C**
. **Spin the EDTA samples immediately at 2500rpm for 10 minutes at 4°C**
. **Urine collected as midstream clean catch. Where possible ask the patient to provide a fresh sample. Urine samples collected more than 2 hours ago should be discarded.**
. **Spin at 3000rpm for 15 minutes at 4°C**
. **After spinning of all samples aliquot and transfer to a -80°C freezer**

APPENDIX 5.

RESULTS FROM CAUSE-SPECIFIC HAZARD MODELS (CHAPTER III)

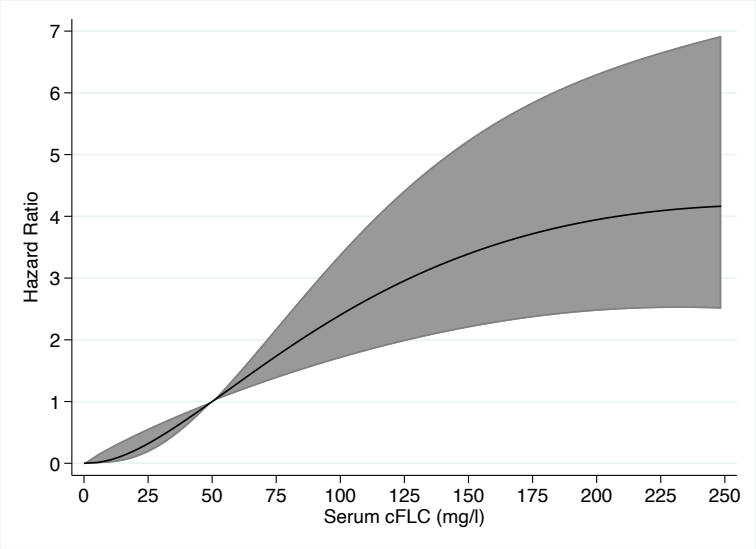
Univariable and multivariable cause-specific hazards of kidney failure using data from Chapter III.

Results in bold differ from the subdistribution hazard model.

Variable	Univariable			Multivariable		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age	0.86	0.78 to 0.94	0.001	0.79	0.71 to 0.88	<0.001
Male sex	0.81	0.68 to 0.97	0.021	0.86	0.71 to 1.05	0.14
Non-White ethnicity	1.48	1.15 to 1.91	0.002	1.05	0.79 to 1.39	0.76
DM	**1.31**	**1.07 to 1.60**	**0.010**	1.11	0.89 to 1.37	0.35
CVD	0.93	0.77 to 1.13	0.46	1.01	0.82 to 1.25	0.91
Systolic BP	1.17	1.06 to 1.28	0.001	1.11	1.00 to 1.24	0.043
Urine ACR	16.3[a]	10.2 to 26.0	<0.001	1.40[g]	1.26 to 1.55	<0.001
	0.12[b]	0.06 to 0.24	<0.001	1.01[h]	1.01 to 1.02	<0.001
eGFR	0.00[c]	0.00 to 0.00	<0.001	0.00[a]	0.00 to 0.00	<0.001
	606168[d]	52199 to 7.0e+06	<0.001	32.5[e]	5.50 to 192	<0.001
Serum albumin	0.98[e]	0.98 to 0.99	<0.001	1.14	1.02 to 1.27	0.018
Serum calcium	0.44[e]	0.37 to 0.52	<0.001	0.87	0.80 to 0.95	0.001
	1.84[f]	1.62 to 2.10	<0.001			
Serum phosphate	115[c]	59.9 to 221	<0.001	1.24	1.12 to 1.37	<0.001
	0.54[d]	0.47 to 0.63	<0.001			
RAASi	1.23	1.01 to 1.48	0.035	1.03	0.83 to 1.28	0.78
Serum cFLC	198[g]	66.6 to 590	<0.001	32.0[g]	9.01 to 113	<0.001
	0.00[a]	0.00 to 0.01	<0.001	0.01[a]	0.00 to 0.05	<0.001

For continuous variables with a linear association, HR is per +1 SD. Two rows for a continuous variable indicate the HR for each power of the degree-2 fractional polynomial transformation. Fractional polynomial transformations are denoted by: $a = x^{0.5}$; $b = x^{0.5}ln(x)$; $c = x$; $d = x^2$; $e = x^3$; $f = x^3ln(x)$; $g = ln(x)$; $h = (ln(x))^2$.

Adjusted HR with 95% CI for kidney failure according to serum cFLC concentration (relative to 50 mg/l), from the multivariable cause-specific hazard model shown in the above table, to demonstrate the non-linear association.



APPENDIX 6.

RESULTS FROM CAUSE-SPECIFIC HAZARD MODELS (CHAPTER IV)

Univariable and multivariable cause-specific hazards of kidney failure using data from Chapter IV.

Results in bold differ from the subdistribution hazard model.

Variable	Univariable			Multivariable (κCR)			Multivariable (λCR)		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age	0.74	0.65 to 0.84	<0.001	0.50	0.41 to 0.61	<0.001	0.50	0.40 to 0.61	<0.001
Male gender	0.95	0.71 to 1.28	0.75	**1.31**	**0.94 to 1.84**	**0.11**	**1.31**	**0.94 to 1.83**	**0.12**
Ethnicity									
White	Ref			Ref			Ref		
South Asian	1.67	1.20 to 2.31	0.002	0.94	0.64 to 1.40	0.77	0.92	0.62 to 1.36	0.67
Black	1.70	1.11 to 2.60	0.015	**1.71**	**1.03 to 2.82**	**0.037**	**1.68**	**1.01 to 2.77**	**0.044**
Other	0.00	0.00 to .	1.00	0.00	.	.	0.00	.	.
Co-morbidities									
DM	0.98	0.73 to 1.32	0.90						
IHD	0.81	0.55 to 1.18	0.27						
Cerebrovascular disease	1.16	0.72 to 1.86	0.55						
PAD	0.82	0.47 to 1.41	0.46						
COPD	0.47	0.25 to 0.89	0.020						
Malignancy	0.53	0.30 to 0.93	0.027						
Cause of CKD									
Ischaemic/hypertensive	Ref			Ref			Ref		
Glomerulonephritis	0.93	0.57 to 1.54	0.79	0.84	0.47 to 1.51	0.57	0.84	0.47 to 1.50	0.56
Diabetic kidney disease	1.88	1.16 to 3.04	0.010	0.88	0.50 to 1.54	0.64	0.88	0.50 to 1.54	0.65
Polycystic kidney disease	3.06	1.83 to 5.12	<0.001	7.13	3.77 to 13.5	<0.001	7.23	3.77 to 13.9	<0.001
Interstitial nephropathy	0.66	0.30 to 1.48	0.32	0.37	0.14 to 1.02	0.06	0.37	0.13 to 1.01	0.05
Reflux nephropathy	0.83	0.29 to 2.32	0.72	**0.35**	**0.12 to 1.06**	**0.06**	**0.36**	**0.12 to 1.07**	**0.07**
Other/uncertain	1.02	0.67 to 1.55	0.93	0.84	0.52 to 1.34	0.46	0.81	0.51 to 1.30	0.38
eGFR	0.97[a]	0.95 to 0.98	<0.001	0.97[a]	0.96 to 0.98	<0.001	0.97[a]	0.96 to 0.98	<0.001
	2.08[b]	1.70 to 2.53	<0.001	77.7[d]	30.2 to 200	<0.001	75.0[d]	29.0 to 194	<0.001
Urine ACR	1.51[c]	1.36 to 1.67	<0.001	6.91[e]	4.26 to 11.2	<0.001	6.74[e]	4.15 to 11.0	<0.001
				0.99[f]	0.99 to 1.00	<0.001	0.99[f]	0.99 to 1.00	<0.001
Systolic BP	1.25	1.09 to 1.43	0.002						
Diastolic BP	1.20	1.04 to 1.39	0.012						
MAP	1.27	1.10 to 1.46	0.001	0.86	0.72 to 1.03	0.10	0.86	0.73 to 1.03	0.10
Serum κ	3.42[c]	2.66 to 4.40	<0.001						
Serum λ	4.25[c]	3.19 to 5.65	<0.001						
Serum κ + λ	4.18[c]	3.17 to 5.50	<0.001						
Urine κCR	1.94[c]	1.65 to 2.29	<0.001	1.05	0.89 to 1.25	0.54			
Urine λCR	1.83[c]	1.60 to 2.10	<0.001				1.12	0.96 to 1.31	0.16

For continuous variables with a linear association, HR is per +1 SD. Non-linear fractional polynomial transformations are denoted by: $a = x^{-2}$; $b = x^{-1}$; $c = ln(x)$; $d = x^{-0.5}$; $e = x^{0.5}$; $f = x^3$.

APPENDIX 7.

RESULTS FROM CAUSE-SPECIFIC HAZARD MODELS (CHAPTER V)

Univariable and multivariable cause-specific hazards of kidney failure using data from Chapter V. The first table incorporates data from the RIISC study only, and the second table incorporates data from all three studies. Results in bold differ from the subdistribution hazard model.

Variable	Univariable			Multivariable		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
MG+	1.13	0.80 to 1.59	0.50	1.19	0.82 to 1.74	0.36
Age	1.00[a]	1.00 to 1.00	<0.001	0.60	0.52 to 0.70	<0.001
Male sex	1.04	0.83 to 1.29	0.76	0.48	0.37 to 0.61	<0.001
Ethnicity						
White	Ref			Ref		
South Asian	1.87	1.46 to 2.40	<0.001	1.15	0.86 to 1.53	0.35
Black	1.93	1.38 to 2.69	<0.001	1.95	1.37 to 2.77	<0.001
Other	**2.43**	**1.00 to 5.90**	**0.05**	1.66	0.61 to 4.52	0.32
Co-morbidities						
DM	0.99	0.79 to 1.23	0.90			
IHD	1.02	0.78 to 1.33	0.88			
Cerebrovascular disease	0.88	0.61 to 1.27	0.50			
PAD	0.98	0.68 to 1.43	0.94			
COPD	0.48	0.30 to 0.77	0.002			
Malignancy	0.61	0.42 to 0.90	0.012			
Smoking status						
Never	Ref					
Previous	0.75	0.58 to 0.95	0.019			
Current	1.09	0.79 to 1.49	0.60			
Cause of CKD						
Vascular	Ref			Ref		
Diabetes	1.89	1.33 to 2.70	<0.001	0.99	0.64 to 1.54	0.97
Glomerular	0.98	0.66 to 1.44	0.91	0.86	0.54 to 1.35	0.51
Tubulointerstitial	0.76	0.48 to 1.19	0.23	**0.52**	**0.32 to 0.84**	**0.008**
Cystic or congenital	2.46	1.70 to 3.55	<0.001	3.92	2.60 to 5.91	<0.001
Other or unknown	1.20	0.86 to 1.68	0.28	1.18	0.81 to 1.70	0.38
MAP	1.35	1.21 to 1.50	<0.001	1.00	0.88 to 1.14	0.98
eGFR	1.23[b]	1.18 to 1.27	<0.001	0.94[b]	0.92 to 0.95	<0.001
	1.08[c]	1.06 to 1.09	<0.001	3.93[e]	3.23 to 4.77	<0.001
Urine ACR	1.49[d]	1.39 to 1.60	<0.001	4.21[f]	3.20 to 5.54	<0.001
				1.00[a]	1.00 to 1.00	0.011

Continuous variables are linear per +1 SD unless indicated. Two rows for a continuous variable indicate the HR for each power from an FP2 model. Fractional polynomial transformations are indicated by: a = x^3; b = x^{-2}; c =$x^{-2}ln(x)$; d = $ln(x)$;e = x^{-1};f = $x^{0.5}$.

Variable	Kidney failure					
	Univariable			Multivariable		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
LC-MG+	1.49	0.80 to 2.80	0.21	1.24	0.66 to 2.35	0.50
Age	1.00[a]	1.00 to 1.00	<0.0001	0.74	0.68 to 0.80	<0.001
Male sex	1.00	0.85 to 1.18	0.99	1.12	0.94 to 1.34	0.20
Ethnicity						
White	Ref			Ref		
South Asian	1.82	1.44 to 2.29	<0.001	1.08	0.84 to 1.39	0.56
Black	1.80	1.31 to 2.49	<0.001	1.94	1.38 to 2.74	<0.001
Other	2.54	1.20 to 5.38	0.015	1.27	0.58 to 2.78	0.55
Co-morbidities						
DM	1.01	0.84 to 1.20	0.95			
IHD	1.13	0.91 to 1.41	0.26			
Cerebrovascular	0.96	0.72 to 1.27	0.76			
PAD	1.08	0.84 to 1.39	0.56			
Smoking status						
Never	Ref					
Previous	0.78	0.65 to 0.94	0.008			
Current	1.18	0.93 to 1.51	0.17			
MAP	1.28	1.18 to 1.38	<0.001	1.07	0.98 to 1.17	0.15
eGFR	1.21[b]	1.18 to 1.24	<0.001	0.96[b]	0.95 to 0.97	<0.001
	1.07[c]	1.06 to 1.08	<0.001	2.56[f]	2.25 to 2.91	<0.001
Urine ACR	1.00[d]	1.00 to 1.00	<0.001	1.55[e]	1.42 to 1.70	<0.001
	1.58[e]	1.49 to 1.67	<0.001	1.01[g]	1.01 to 1.02	<0.001

Continuous variables are linear per +1 SD unless indicated. Two rows for a continuous variable indicate the HR for each power from an FP2 model. Fractional polynomial transformations are indicated by: $a = x^3$; $b = x^{-2}$; $c = x^{-2}\ln(x)$; $d = x^{-0.5}$; $e = \ln(x)$; $f = x^{-1}$; $g = (\ln(x))^2$.

APPENDIX 8.

RESULTS FROM CAUSE-SPECIFIC HAZARD MODELS (CHAPTER VI)

Univariable and multivariable cause-specific hazards of kidney failure using data from Chapter VI.

Results in bold differ from the subdistribution hazard model.

Variable	Univariable			Multivariable		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age	0.72	0.62 to 0.83	<0.001	0.52	0.43 to 0.64	<0.001
Sex (male)	0.87	0.64 to 1.18	0.37	0.95	0.69 to 1.31	0.76
Ethnicity						
White	Ref			Ref		
South Asian	1.91	1.34 to 2.71	<0.001	1.17	0.79 to 1.73	0.44
Black	1.46	0.89 to 2.41	0.14	**1.77**	**1.04 to 3.03**	**0.037**
Other	1.30	0.32 to 5.27	0.71	1.19	0.28 to 5.04	0.81
Cause of CKD						
Vascular	Ref			Ref		
Diabetes	2.13	1.22 to 3.69	0.007	0.95	0.52 to 1.72	0.87
Glomerular	1.14	0.69 to 1.88	0.60	0.83	0.47 to 1.46	0.51
Cystic and congenital	2.69	1.61 to 4.49	<0.001	3.46	1.96 to 6.11	<0.001
Tubulointerstitial	0.97	0.54 to 1.76	0.93	0.76	0.39 to 1.48	0.42
Other/uncertain	1.26	0.78 to 2.01	0.34	1.16	0.71 to 1.91	0.55
Co-morbidities						
Cerebrovascular disease	0.85	0.50 to 1.45	0.56			
COPD	0.43	0.23 to 0.81	0.009			
DM	0.85	0.61 to 1.17	0.32			
IHD	1.00	0.69 to 1.46	0.99			
Malignancy	0.51	0.30 to 0.89	0.017			
PAD	0.71	0.39 to 1.28	0.25			
Smoking status						
Never	Ref					
Previous	0.82	0.59 to 1.13	0.23			
Current	0.85	0.53 to 1.35	0.49			
MAP	1.40	1.21 to 1.61	<0.001	1.13	0.96 to 1.33	0.14
eGFR	7.64[a]	5.42 to 10.8	<0.001	17.9[a]	10.8 to 29.7	<0.001
Urine ACR	1.50[b]	1.36 to 1.66	<0.001	4.15[c]	3.02 to 5.70	<0.001
Serum endotrophin	0.00[a]	0.00 to 0.01	<0.001	1.14	0.95 to 1.35	0.16

HR for continuous variables are per +1 SD, unless fractional polynomial transformation provided better model fit, which are denoted by: a = $x^{-0.5}$; b = ln(x); c = $x^{0.5}$.

REFERENCES

1. McIntosh JF, Moller E, Van Slyke DD. STUDIES OF UREA EXCRETION. III: The Influence of Body Size on Urea Output. J Clin Invest. 1928;6(3):467-83.
2. Fenton A, Montgomery E, Nightingale P, Peters AM, Sheerin N, Wroe AC, et al. Glomerular filtration rate: new age-and gender-specific reference ranges and thresholds for living kidney donation. BMC nephrology. 2018;19(1):336.
3. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130(6):461-70.
4. Levey AS, Greene T, Kusek J, Beck G. A simplified equation to predict glomerular filtration rate from serum creatinine. J Am Soc Nephrol. 2000;11:155A.
5. Levey A, Coresh J, Greene T, Marsh J, Stevens L, Kusek J, et al. Expressing the MDRD study equation for estimating GFR with IDMS traceable (gold standard) serum creatinine values. J Am Soc Nephrol. 2005;16:69A.
6. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med. 2006;145(4):247-54.
7. Poggio ED, Wang X, Greene T, Van Lente F, Hall PM. Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. J Am Soc Nephrol. 2005;16(2):459-66.
8. Lewis J, Agodoa L, Cheek D, Greene T, Middleton J, O'Connor D, et al. Comparison of cross-sectional renal function measurements in African Americans with hypertensive nephrosclerosis and of primary formulas to estimate glomerular filtration rate. Am J Kidney Dis. 2001;38(4):744-53.
9. Rule AD, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Ann Intern Med. 2004;141(12):929-37.
10. Froissart M, Rossert J, Jacquot C, Paillard M, Houillier P. Predictive performance of the modification of diet in renal disease and Cockcroft-Gault equations for estimating renal function. J Am Soc Nephrol. 2005;16(3):763-73.
11. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.
12. Earley A, Miskulin D, Lamb EJ, Levey AS, Uhlig K. Estimating equations for glomerular filtration rate in the era of creatinine standardization: a systematic review. Ann Intern Med. 2012;156(11):785-95, W-270, W-1, W-2, W-3, W-4, W-5, W-6, W-7, W-8.
13. Stevens LA, Schmid CH, Greene T, Zhang YL, Beck GJ, Froissart M, et al. Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m2. Am J Kidney Dis. 2010;56(3):486-95.
14. Kilbride HS, Stevens PE, Eaglestone G, Knight S, Carter JL, Delaney MP, et al. Accuracy of the MDRD (Modification of Diet in Renal Disease) study and CKD-EPI (CKD

Epidemiology Collaboration) equations for estimation of GFR in the elderly. Am J Kidney Dis. 2013;61(1):57-66.
15. Matsushita K, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, et al. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. JAMA. 2012;307(18):1941-51.
16. Levey AS, Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. Am J Kidney Dis. 2010;55(4):622-7.
17. Matsushita K, Selvin E, Bash LD, Astor BC, Coresh J. Risk implications of the new CKD Epidemiology Collaboration (CKD-EPI) equation compared with the MDRD Study equation for estimated GFR: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Kidney Dis. 2010;55(4):648-59.
18. White SL, Polkinghorne KR, Atkins RC, Chadban SJ. Comparison of the prevalence and mortality risk of CKD in Australia using the CKD Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) Study GFR estimating equations: the AusDiab (Australian Diabetes, Obesity and Lifestyle) Study. Am J Kidney Dis. 2010;55(4):660-70.
19. Skali H, Uno H, Levey AS, Inker LA, Pfeffer MA, Solomon SD. Prognostic assessment of estimated glomerular filtration rate by the new Chronic Kidney Disease Epidemiology Collaboration equation in comparison with the Modification of Diet in Renal Disease Study equation. Am Heart J. 2011;162(3):548-54.
20. Stevens LA, Li S, Kurella Tamura M, Chen SC, Vassalotti JA, Norris KC, et al. Comparison of the CKD Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) study equations: risk factors for and complications of CKD and mortality in the Kidney Early Evaluation Program (KEEP). Am J Kidney Dis. 2011;57(3 Suppl 2):S9-16.
21. Matsushita K, Tonelli M, Lloyd A, Levey AS, Coresh J, Hemmelgarn BR, et al. Clinical risk implications of the CKD Epidemiology Collaboration (CKD-EPI) equation compared with the Modification of Diet in Renal Disease (MDRD) Study equation for estimated GFR. Am J Kidney Dis. 2012;60(2):241-9.
22. National Institute for Health and Care Excellence (2014) Chronic kidney disease in adults: assessment and management. NICE guideline (CG182).
23. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. Am J Kidney Dis. 2008;51(3):395-406.
24. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int. 2004;65(4):1416-21.
25. Groesbeck D, Kottgen A, Parekh R, Selvin E, Schwartz GJ, Coresh J, et al. Age, gender, and race effects on cystatin C levels in US adolescents. Clin J Am Soc Nephrol. 2008;3(6):1777-85.
26. Macdonald J, Marcora S, Jibani M, Roberts G, Kumwenda M, Glover R, et al. GFR estimation using cystatin C is not independent of body composition. Am J Kidney Dis. 2006;48(5):712-9.
27. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney Int. 2009;75(6):652-60.

28. Liu X, Foster MC, Tighiouart H, Anderson AH, Beck GJ, Contreras G, et al. Non-GFR Determinants of Low-Molecular-Weight Serum Protein Filtration Markers in CKD. Am J Kidney Dis. 2016;68(6):892-900.
29. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367(1):20-9.
30. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Improved GFR estimation by combined creatinine and cystatin C measurements. Kidney Int. 2007;72(12):1535-42.
31. Anderson AH, Yang W, Hsu CY, Joffe MM, Leonard MB, Xie D, et al. Estimating GFR among participants in the Chronic Renal Insufficiency Cohort (CRIC) Study. Am J Kidney Dis. 2012;60(2):250-61.
32. Eriksen BO, Mathisen UD, Melsom T, Ingebretsen OC, Jenssen TG, Njolstad I, et al. Cystatin C is not a better estimator of GFR than plasma creatinine in the general population. Kidney Int. 2010;78(12):1305-11.
33. Schaeffner ES, Ebert N, Delanaye P, Frei U, Gaedeke J, Jakob O, et al. Two novel equations to estimate kidney function in persons aged 70 years or older. Ann Intern Med. 2012;157(7):471-81.
34. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. Lancet. 2017;389(10075):1238-52.
35. National Kidney Foundation. KDOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification. American Journal of Kidney Diseases. 2002;39(2):S1-S266.
36. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney International Supplements. 2013;3:1-150.
37. Stevens PE, O'Donoghue DJ, de Lusignan S, Van Vlymen J, Klebe B, Middleton R, et al. Chronic kidney disease management in the United Kingdom: NEOERICA project results. Kidney Int. 2007;72(1):92-9.
38. Denburg MR, Haynes K, Shults J, Lewis JD, Leonard MB. Validation of The Health Improvement Network (THIN) database for epidemiologic studies of chronic kidney disease. Pharmacoepidemiol Drug Saf. 2011;20(11):1138-49.
39. Gifford FJ, Methven S, Boag DE, Spalding EM, Macgregor MS. Chronic kidney disease prevalence and secular trends in a UK population: the impact of MDRD and CKD-EPI formulae. QJM. 2011;104(12):1045-53.
40. Annear NM, Banerjee D, Joseph J, Harries TH, Rahman S, Eastwood JB. Prevalence of chronic kidney disease stages 3-5 among acute medical admissions: another opportunity for screening. QJM. 2008;101(2):91-7.
41. de Lusignan S, Tomson C, Harris K, van Vlymen J, Gallagher H. Creatinine fluctuation has a greater effect than the formula to estimate glomerular filtration rate on the prevalence of chronic kidney disease. Nephron Clin Pract. 2011;117(3):c213-24.
42. de Lusignan S, Tomson C, Harris K, van Vlymen J, Gallagher H. UK Prevalence of Chronic Kidney Disease for the Adult Population Is 6.76% Based on Two Creatinine Readings. Nephron Clinical Practice. 2012;120(2):107-.
43. Public Health England. Chronic kidney disease prevalence model. 2014.
44. O'Callaghan CA, Shine B, Lasserson DS. Chronic kidney disease: a large-scale population-based study of the effects of introducing the CKD-EPI formula for eGFR reporting. BMJ Open. 2011;1(2):e000308.

45. Jameson K, Jick S, Hagberg KW, Ambegaonkar B, Giles A, O'Donoghue D. Prevalence and management of chronic kidney disease in primary care patients in the UK. Int J Clin Pract. 2014;68(9):1110-21.
46. Yuyun MF, Khaw KT, Luben R, Welch A, Bingham S, Day NE, et al. Microalbuminuria, cardiovascular risk factors and cardiovascular morbidity in a British population: the EPIC-Norfolk population-based study. Eur J Cardiovasc Prev Rehabil. 2004;11(3):207-13.
47. Bruck K, Stel VS, Gambaro G, Hallan S, Volzke H, Arnlov J, et al. CKD Prevalence Varies across the European General Population. J Am Soc Nephrol. 2016;27(7):2135-47.
48. Saran R, Robinson B, Abbott KC, Agodoa LY, Albertus P, Ayanian J, et al. US Renal Data System 2016 Annual Data Report: Epidemiology of Kidney Disease in the United States. Am J Kidney Dis. 2017;69(3 Suppl 1):A7-A8.
49. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. PLoS One. 2016;11(7):e0158765.
50. Jager KJ, Fraser SDS. The ascending rank of chronic kidney disease in the global burden of disease study. Nephrol Dial Transplant. 2017;32(suppl_2):ii121-ii8.
51. Institute for Health Metrics and Evaluation, Global Health Data Exchange, Global Burden of Disease Results Tool. Accessible at http://ghdx.healthdata.org/gbd-results-tool.
52. Kerr M. NHS Kidney Care. Chronic Kidney Disease in England: The Human and Financial Cost. 2012.
53. Kerr M, Bray B, Medcalf J, O'Donoghue DJ, Matthews B. Estimating the financial cost of chronic kidney disease to the NHS in England. Nephrol Dial Transplant. 2012;27 Suppl 3:iii73-80.
54. Nguyen NTQ, Cockwell P, Maxwell AP, Griffin M, O'Brien T, O'Neill C. Chronic kidney disease, health-related quality of life and their associated economic burden among a nationally representative sample of community dwelling adults in England. PLoS One. 2018;13(11):e0207960.
55. UK Renal Registry (2019) UK Renal Registry 21st Annual Report, Bristol, UK. Available from https://www.renalreg.org/publications-reports/.
56. Cortes P, Riser BL, Yee J, Narins RG. Mechanical strain of glomerular mesangial cells in the pathogenesis of glomerulosclerosis: clinical implications. Nephrol Dial Transplant. 1999;14(6):1351-4.
57. Burton C, Harris KP. The role of proteinuria in the progression of chronic renal failure. Am J Kidney Dis. 1996;27(6):765-75.
58. Arici M, Chana R, Lewington A, Brown J, Brunskill NJ. Stimulation of proximal tubular cell apoptosis by albumin-bound fatty acids mediated by peroxisome proliferator activated receptor-gamma. J Am Soc Nephrol. 2003;14(1):17-27.
59. Eddy AA. Overview of the cellular and molecular basis of kidney fibrosis. Kidney Int Suppl (2011). 2014;4(1):2-8.
60. Nikolic-Paterson DJ, Lan HY, Hill PA, Atkins RC. Macrophages in renal injury. Kidney Int Suppl. 1994;45:S79-82.
61. Zeisberg M, Neilson EG. Mechanisms of tubulointerstitial fibrosis. J Am Soc Nephrol. 2010;21(11):1819-34.
62. Ruiz-Ortega M, Ruperez M, Esteban V, Rodriguez-Vita J, Sanchez-Lopez E, Carvajal G, et al. Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases. Nephrol Dial Transplant. 2006;21(1):16-20.

63. Croft P, Altman DG, Deeks JJ, Dunn KM, Hay AD, Hemingway H, et al. The science of clinical practice: disease diagnosis or patient prognosis? Evidence about "what is likely to happen" should shape clinical practice. BMC Med. 2015;13:20.
64. Croft P, Riley RD, van der Windt DA, Moons KG. Prognosis in healthcare. Prognosis Research in Healthcare: Concepts, Methods, and Impact. Oxford: Oxford University Press; 2019.
65. Thomas JM, Cooney LM, Jr., Fried TR. Prognosis Reconsidered in Light of Ancient Insights-From Hippocrates to Modern Medicine. JAMA Intern Med. 2019;179(6):820-3.
66. Christakis NA. The ellipsis of prognosis in modern medical thought. Soc Sci Med. 1997;44(3):301-15.
67. Riley RD, van der Windt DA, Croft P, Moons KG. Prologue. Prognosis Research in Healthcare: Concepts, Methods, and Impact. Oxford: Oxford University Press; 2019.
68. Hemingway H, Croft P, Perel P, Hayden JA, Abrams K, Timmis A, et al. Prognosis research strategy (PROGRESS) 1: a framework for researching clinical outcomes. BMJ. 2013;346:e5595.
69. Riley RD, Hayden JA, Steyerberg EW, Moons KG, Abrams K, Kyzas PA, et al. Prognosis Research Strategy (PROGRESS) 2: prognostic factor research. PLoS Med. 2013;10(2):e1001380.
70. Steyerberg EW, Moons KG, van der Windt DA, Hayden JA, Perel P, Schroter S, et al. Prognosis Research Strategy (PROGRESS) 3: prognostic model research. PLoS Med. 2013;10(2):e1001381.
71. Hingorani AD, Windt DA, Riley RD, Abrams K, Moons KG, Steyerberg EW, et al. Prognosis research strategy (PROGRESS) 4: stratified medicine research. BMJ. 2013;346:e5793.
72. Croft P, Riley RD, van der Windt DA, Moons KG. A framework for prognosis research. Prognosis Research in Healthcare: Concepts, Methods, and Impact. Oxford: Oxford University Press; 2019.
73. Cashin AG, Traeger AC, Hubscher M, Moseley GL, di Pietro F, Parkitny L, et al. Persistent Pain After Wrist or Hand Fracture: Development and Validation of a Prognostic Model. J Orthop Sports Phys Ther. 2019;49(1):28-35.
74. Poon MT, Fonville AF, Al-Shahi Salman R. Long-term prognosis after intracerebral haemorrhage: systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2014;85(6):660-7.
75. Lopez-Pineda A, Cordero A, Carratala-Munuera C, Orozco-Beltran D, Quesada JA, Bertomeu-Gonzalez V, et al. Hyperuricemia as a prognostic factor after acute coronary syndrome. Atherosclerosis. 2018;269:229-35.
76. Melo MCA, Garcia RF, de Araújo CFC, Rangel DM, de Bruin PFC, de Bruin VMS. Physical activity as prognostic factor for bipolar disorder: An 18-month prospective study. Journal of affective disorders. 2019;251:100-6.
77. Schuchter L, Schultz DJ, Synnestvedt M, Trock BJ, Guerry D, Elder DE, et al. A prognostic model for predicting 10-year survival in patients with primary melanoma. The Pigmented Lesion Group. Ann Intern Med. 1996;125(5):369-75.
78. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol. 2018;36(20):2105-22.
79. Hingorani AD, Hemingway H. How should we balance individual and population benefits of statins for preventing cardiovascular disease? BMJ. 2010;342:c6244.

80. Uhlig K, Levey AS. Developing guidelines for chronic kidney disease: we should include all of the outcomes. Ann Intern Med. 2012;156(8):599-601.
81. Chronic Kidney Disease Prognosis C, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet. 2010;375(9731):2073-81.
82. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney Int. 2011;79(12):1341-52.
83. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int. 2011;80(1):17-28.
84. Ishani A, Grandits GA, Grimm RH, Svendsen KH, Collins AJ, Prineas RJ, et al. Association of single measurements of dipstick proteinuria, estimated glomerular filtration rate, and hematocrit with 25-year incidence of end-stage renal disease in the multiple risk factor intervention trial. J Am Soc Nephrol. 2006;17(5):1444-52.
85. Gansevoort RT, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, et al. Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. Kidney Int. 2011;80(1):93-104.
86. James MT, Quan H, Tonelli M, Manns BJ, Faris P, Laupland KB, et al. CKD and risk of hospitalization and death with pneumonia. Am J Kidney Dis. 2009;54(1):24-32.
87. Wilhelm-Leen ER, Hall YN, M KT, Chertow GM. Frailty and chronic kidney disease: the Third National Health and Nutrition Evaluation Survey. Am J Med. 2009;122(7):664-71 e2.
88. Hailpern SM, Melamed ML, Cohen HW, Hostetter TH. Moderate chronic kidney disease and cognitive function in adults 20 to 59 years of age: Third National Health and Nutrition Examination Survey (NHANES III). J Am Soc Nephrol. 2007;18(7):2205-13.
89. Foley RN, Collins AJ. The USRDS: what you need to know about what it can and can't tell us about ESRD. Clin J Am Soc Nephrol. 2013;8(5):845-51.
90. Hemmelgarn BR, Manns BJ, Lloyd A, James MT, Klarenbach S, Quinn RR, et al. Relation between kidney function, proteinuria, and adverse outcomes. JAMA. 2010;303(5):423-9.
91. Hallan SI, Ritz E, Lydersen S, Romundstad S, Kvenild K, Orth SR. Combining GFR and albuminuria to classify CKD improves prediction of ESRD. J Am Soc Nephrol. 2009;20(5):1069-77.
92. De Nicola L, Provenzano M, Chiodini P, Borrelli S, Garofalo C, Pacilio M, et al. Independent Role of Underlying Kidney Disease on Renal Prognosis of Patients with Chronic Kidney Disease under Nephrology Care. PLoS One. 2015;10(5):e0127071.
93. Ruggenenti P, Perna A, Mosconi L, Pisoni R, Remuzzi G. Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. "Gruppo Italiano di Studi Epidemiologici in Nefrologia" (GISEN). Kidney Int. 1998;53(5):1209-16.
94. Keane WF, Zhang Z, Lyle PA, Cooper ME, de Zeeuw D, Grunfeld JP, et al. Risk scores for predicting outcomes in patients with type 2 diabetes and nephropathy: the RENAAL study. Clin J Am Soc Nephrol. 2006;1(4):761-7.

95. Astor BC, Matsushita K, Gansevoort RT, van der Velde M, Woodward M, Levey AS, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. Kidney Int. 2011;79(12):1331-40.
96. Hallan SI, Matsushita K, Sang Y, Mahmoodi BK, Black C, Ishani A, et al. Age and association of kidney measures with mortality and end-stage renal disease. JAMA. 2012;308(22):2349-60.
97. Hemmelgarn BR, James MT, Manns BJ, O'Hare AM, Muntner P, Ravani P, et al. Rates of treated and untreated kidney failure in older vs younger adults. JAMA. 2012;307(23):2507-15.
98. Hsu CY, Lin F, Vittinghoff E, Shlipak MG. Racial differences in the progression from chronic renal insufficiency to end-stage renal disease in the United States. J Am Soc Nephrol. 2003;14(11):2902-7.
99. Palit S, Chonchol M, Cheung AK, Kaufman J, Smits G, Kendrick J. Association of BP with Death, Cardiovascular Events, and Progression to Chronic Dialysis in Patients with Advanced Kidney Disease. Clin J Am Soc Nephrol. 2015;10(6):934-40.
100. Peralta CA, Norris KC, Li S, Chang TI, Tamura MK, Jolly SE, et al. Blood pressure components and end-stage renal disease in persons with chronic kidney disease: the Kidney Early Evaluation Program (KEEP). Arch Intern Med. 2012;172(1):41-7.
101. Anderson AH, Yang W, Townsend RR, Pan Q, Chertow GM, Kusek JW, et al. Time-updated systolic blood pressure and the progression of chronic kidney disease: a cohort study. Ann Intern Med. 2015;162(4):258-65.
102. Bansal N, Xie D, Tao K, Chen J, Deo R, Horwitz E, et al. Atrial Fibrillation and Risk of ESRD in Adults with CKD. Clin J Am Soc Nephrol. 2016;11(7):1189-96.
103. Hedayati SS, Minhajuddin AT, Afshar M, Toto RD, Trivedi MH, Rush AJ. Association between major depressive episodes in patients with chronic kidney disease and initiation of dialysis, hospitalization, or death. JAMA. 2010;303(19):1946-53.
104. Parsa A, Kao WH, Xie D, Astor BC, Li M, Hsu CY, et al. APOL1 risk variants, race, and progression of chronic kidney disease. N Engl J Med. 2013;369(23):2183-96.
105. Landray MJ, Emberson JR, Blackwell L, Dasgupta T, Zakeri R, Morgan MD, et al. Prediction of ESRD and death among people with CKD: the Chronic Renal Impairment in Birmingham (CRIB) prospective cohort study. Am J Kidney Dis. 2010;56(6):1082-94.
106. Tangri N, Stevens LA, Griffith J, Tighiouart H, Djurdjev O, Naimark D, et al. A predictive model for progression of chronic kidney disease to kidney failure. JAMA. 2011;305(15):1553-9.
107. Tangri N, Grams ME, Levey AS, Coresh J, Appel LJ, Astor BC, et al. Multinational Assessment of Accuracy of Equations for Predicting Risk of Kidney Failure: A Meta-analysis. JAMA. 2016;315(2):164-74.
108. National Institute for Health and Care Excellence (2017) Surveillance report 2017 – Chronic kidney disease (stage 4 or 5): management of hyperphosphataemia (2013) NICE guideline CG157, Chronic kidney disease in adults: assessment and management (2014) NICE guideline CG182 and Chronic kidney disease: managing anaemia (2015) NICE guideline NG8.
109. Grams ME, Sang Y, Ballew SH, Carrero JJ, Djurdjev O, Heerspink HJL, et al. Predicting timing of clinical outcomes in patients with chronic kidney disease and severely decreased glomerular filtration rate. Kidney Int. 2018;93(6):1442-51.
110. Jafar TH, Stark PC, Schmid CH, Landa M, Maschio G, de Jong PE, et al. Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-

converting enzyme inhibition: a patient-level meta-analysis. Ann Intern Med. 2003;139(4):244-52.
111. Pohl MA, Blumenthal S, Cordonnier DJ, De Alvaro F, Deferrari G, Eisner G, et al. Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. J Am Soc Nephrol. 2005;16(10):3027-37.
112. Bakris GL, Weir MR, Shanifar S, Zhang Z, Douglas J, van Dijk DJ, et al. Effects of blood pressure level on progression of diabetic nephropathy: results from the RENAAL study. Arch Intern Med. 2003;163(13):1555-65.
113. Lv J, Ehteshami P, Sarnak MJ, Tighiouart H, Jun M, Ninomiya T, et al. Effects of intensive blood pressure lowering on the progression of chronic kidney disease: a systematic review and meta-analysis. CMAJ. 2013;185(11):949-57.
114. Ku E, Gassman J, Appel LJ, Smogorzewski M, Sarnak MJ, Glidden DV, et al. BP Control and Long-Term Risk of ESRD and Mortality. J Am Soc Nephrol. 2017;28(2):671-7.
115. Tsai WC, Wu HY, Peng YS, Yang JY, Chen HY, Chiu YL, et al. Association of Intensive Blood Pressure Control and Kidney Disease Progression in Nondiabetic Patients With Chronic Kidney Disease: A Systematic Review and Meta-analysis. JAMA Intern Med. 2017;177(6):792-9.
116. Sarnak MJ, Greene T, Wang X, Beck G, Kusek JW, Collins AJ, et al. The effect of a lower target blood pressure on the progression of kidney disease: long-term follow-up of the modification of diet in renal disease study. Ann Intern Med. 2005;142(5):342-51.
117. Jafar TH, Schmid CH, Landa M, Giatras I, Toto R, Remuzzi G, et al. Angiotensin-converting enzyme inhibitors and progression of nondiabetic renal disease. A meta-analysis of patient-level data. Ann Intern Med. 2001;135(2):73-87.
118. Ruggenenti P, Perna A, Gherardi G, Garini G, Zoccali C, Salvadori M, et al. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet. 1999;354(9176):359-64.
119. Ruggenenti P, Perna A, Gherardi G, Benini R, Remuzzi G. Chronic proteinuric nephropathies: outcomes and response to treatment in a prospective cohort of 352 patients with different patterns of renal injury. Am J Kidney Dis. 2000;35(6):1155-65.
120. Heerspink HJ, Kropelin TF, Hoekman J, de Zeeuw D, Reducing Albuminuria as Surrogate Endpoint C. Drug-Induced Reduction in Albuminuria Is Associated with Subsequent Renoprotection: A Meta-Analysis. J Am Soc Nephrol. 2015;26(8):2055-64.
121. Maione A, Navaneethan SD, Graziano G, Mitchell R, Johnson D, Mann JF, et al. Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and combined therapy in patients with micro- and macroalbuminuria and other cardiovascular risk factors: a systematic review of randomized controlled trials. Nephrol Dial Transplant. 2011;26(9):2827-47.
122. de Brito-Ashurst I, Varagunam M, Raftery MJ, Yaqoob MM. Bicarbonate supplementation slows progression of CKD and improves nutritional status. J Am Soc Nephrol. 2009;20(9):2075-84.
123. Zoungas S, Chalmers J, Neal B, Billot L, Li Q, Hirakawa Y, et al. Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. N Engl J Med. 2014;371(15):1392-406.
124. Group DER, de Boer IH, Sun W, Cleary PA, Lachin JM, Molitch ME, et al. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med. 2011;365(25):2366-76.

125. Wen CP, Cheng TY, Tsai MK, Chang YC, Chan HT, Tsai SP, et al. All-cause mortality attributable to chronic kidney disease: a prospective cohort study based on 462 293 adults in Taiwan. Lancet. 2008;371(9631):2173-82.
126. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004;351(13):1296-305.
127. Astor BC, Hallan SI, Miller ER, 3rd, Yeung E, Coresh J. Glomerular filtration rate, albuminuria, and risk of cardiovascular and all-cause mortality in the US population. Am J Epidemiol. 2008;167(10):1226-34.
128. Mann JF, Gerstein HC, Pogue J, Bosch J, Yusuf S. Renal insufficiency as a predictor of cardiovascular outcomes and the impact of ramipril: the HOPE randomized trial. Ann Intern Med. 2001;134(8):629-36.
129. Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, et al. Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol. 2006;17(7):2034-47.
130. United States Renal Data System. 2018 USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 2018.
131. Hallan S, Astor B, Romundstad S, Aasarod K, Kvenild K, Coresh J. Association of kidney function and albuminuria with cardiovascular mortality in older vs younger individuals: The HUNT II Study. Arch Intern Med. 2007;167(22):2490-6.
132. Brantsma AH, Bakker SJ, Hillege HL, de Zeeuw D, de Jong PE, Gansevoort RT, et al. Cardiovascular and renal outcome in subjects with K/DOQI stage 1-3 chronic kidney disease: the importance of urinary albumin excretion. Nephrol Dial Transplant. 2008;23(12):3851-8.
133. Weiner DE, Tighiouart H, Amin MG, Stark PC, MacLeod B, Griffith JL, et al. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. J Am Soc Nephrol. 2004;15(5):1307-15.
134. Weiner DE, Tighiouart H, Stark PC, Amin MG, MacLeod B, Griffith JL, et al. Kidney disease as a risk factor for recurrent cardiovascular disease and mortality. Am J Kidney Dis. 2004;44(2):198-206.
135. Tonelli M, Muntner P, Lloyd A, Manns BJ, Klarenbach S, Pannu N, et al. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: a population-level cohort study. Lancet. 2012;380(9844):807-14.
136. McCullough PA, Li S, Jurkovitz CT, Stevens L, Collins AJ, Chen SC, et al. Chronic kidney disease, prevalence of premature cardiovascular disease, and relationship to short-term mortality. Am Heart J. 2008;156(2):277-83.
137. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, Jensen G, Clausen P, Scharling H, et al. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. Circulation. 2004;110(1):32-5.
138. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA. 2001;286(4):421-6.
139. Ibsen H, Wachtell K, Olsen MH, Borch-Johnsen K, Lindholm LH, Mogensen CE, et al. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE Study. Kidney Int Suppl. 2004(92):S56-8.
140. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood

Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation. 2003;108(17):2154-69.
141. Taal MW. Chronic kidney disease: towards a risk-based approach. Clin Med (Lond). 2016;16(Suppl 6):s117-s20.
142. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117(6):743-53.
143. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS, et al. The Framingham predictive instrument in chronic kidney disease. J Am Coll Cardiol. 2007;50(3):217-24.
144. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. BMJ. 2017;357:j2099.
145. Evans M, Fryzek JP, Elinder CG, Cohen SS, McLaughlin JK, Nyren O, et al. The natural history of chronic renal failure: results from an unselected, population-based, inception cohort in Sweden. Am J Kidney Dis. 2005;46(5):863-70.
146. Kovesdy CP, Bleyer AJ, Molnar MZ, Ma JZ, Sim JJ, Cushman WC, et al. Blood pressure and mortality in U.S. veterans with chronic kidney disease: a cohort study. Ann Intern Med. 2013;159(4):233-42.
147. Weiss JW, Johnson ES, Petrik A, Smith DH, Yang X, Thorp ML. Systolic blood pressure and mortality among older community-dwelling adults with CKD. Am J Kidney Dis. 2010;56(6):1062-71.
148. Da J, Xie X, Wolf M, Disthabanchong S, Wang J, Zha Y, et al. Serum Phosphorus and Progression of CKD and Mortality: A Meta-analysis of Cohort Studies. Am J Kidney Dis. 2015;66(2):258-65.
149. Palmer SC, Hayen A, Macaskill P, Pellegrini F, Craig JC, Elder GJ, et al. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. JAMA. 2011;305(11):1119-27.
150. Shurraw S, Hemmelgarn B, Lin M, Majumdar SR, Klarenbach S, Manns B, et al. Association between glycemic control and adverse outcomes in people with diabetes mellitus and chronic kidney disease: a population-based cohort study. Arch Intern Med. 2011;171(21):1920-7.
151. Kendrick J, Cheung AK, Kaufman JS, Greene T, Roberts WL, Smits G, et al. Associations of plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations with death and progression to maintenance dialysis in patients with advanced kidney disease. Am J Kidney Dis. 2012;60(4):567-75.
152. Kendrick J, Cheung AK, Kaufman JS, Greene T, Roberts WL, Smits G, et al. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. J Am Soc Nephrol. 2011;22(10):1913-22.
153. Chan MR, Dall AT, Fletcher KE, Lu N, Trivedi H. Outcomes in patients with chronic kidney disease referred late to nephrologists: a meta-analysis. Am J Med. 2007;120(12):1063-70.
154. Tseng CL, Kern EF, Miller DR, Tiwari A, Maney M, Rajan M, et al. Survival benefit of nephrologic care in patients with diabetes mellitus and chronic kidney disease. Arch Intern Med. 2008;168(1):55-62.
155. Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic

kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet. 2011;377(9784):2181-92.
156. Palmer SC, Craig JC, Navaneethan SD, Tonelli M, Pellegrini F, Strippoli GF. Benefits and harms of statin therapy for persons with chronic kidney disease: a systematic review and meta-analysis. Ann Intern Med. 2012;157(4):263-75.
157. Navaneethan SD, Pansini F, Perkovic V, Manno C, Pellegrini F, Johnson DW, et al. HMG CoA reductase inhibitors (statins) for people with chronic kidney disease not requiring dialysis. Cochrane Database Syst Rev. 2009(2):CD007784.
158. Upadhyay A, Earley A, Lamont JL, Haynes S, Wanner C, Balk EM. Lipid-lowering therapy in persons with chronic kidney disease: a systematic review and meta-analysis. Ann Intern Med. 2012;157(4):251-62.
159. National Institute for Health and Care Excellence (2016) Cardiovascular disease: risk assessment and reduction, including lipid modification. NICE guideline (CG181).
160. Blood Pressure Lowering Treatment Trialists C, Ninomiya T, Perkovic V, Turnbull F, Neal B, Barzi F, et al. Blood pressure lowering and major cardiovascular events in people with and without chronic kidney disease: meta-analysis of randomised controlled trials. BMJ. 2013;347:f5680.
161. Palmer SC, Di Micco L, Razavian M, Craig JC, Perkovic V, Pellegrini F, et al. Antiplatelet agents for chronic kidney disease. Cochrane Database Syst Rev. 2013(2):CD008834.
162. Han JH, Chandra A, Mulgund J, Roe MT, Peterson ED, Szczech LA, et al. Chronic kidney disease in patients with non-ST-segment elevation acute coronary syndromes. Am J Med. 2006;119(3):248-54.
163. Freeman RV, Mehta RH, Al Badr W, Cooper JV, Kline-Rogers E, Eagle KA. Influence of concurrent renal dysfunction on outcomes of patients with acute coronary syndromes and implications of the use of glycoprotein IIb/IIIa inhibitors. J Am Coll Cardiol. 2003;41(5):718-24.
164. Patel UD, Ou FS, Ohman EM, Gibler WB, Pollack CV, Jr., Peterson ED, et al. Hospital performance and differences by kidney function in the use of recommended therapies after non-ST-elevation acute coronary syndromes. Am J Kidney Dis. 2009;53(3):426-37.
165. Fox CS, Muntner P, Chen AY, Alexander KP, Roe MT, Cannon CP, et al. Use of evidence-based therapies in short-term outcomes of ST-segment elevation myocardial infarction and non-ST-segment elevation myocardial infarction in patients with chronic kidney disease: a report from the National Cardiovascular Data Acute Coronary Treatment and Intervention Outcomes Network registry. Circulation. 2010;121(3):357-65.
166. Gilbertson DT, Unruh M, McBean AM, Kausz AT, Snyder JJ, Collins AJ. Influenza vaccine delivery and effectiveness in end-stage renal disease. Kidney Int. 2003;63(2):738-43.
167. Public Health England (2020) Immunisation of individuals with underlying medical conditions: The Green Book, Chapter 7.
168. Fenton A, Sayar Z, Dodds A, Dasgupta I. Multidisciplinary care improves outcome of patients with stage 5 chronic kidney disease. Nephron Clin Pract. 2010;115(4):c283-8.
169. Curtis BM, Ravani P, Malberti F, Kennett F, Taylor PA, Djurdjev O, et al. The short- and long-term impact of multi-disciplinary clinics in addition to standard nephrology care on patient outcomes. Nephrol Dial Transplant. 2005;20(1):147-54.
170. Matsushita K, Ballew SH, Astor BC, Jong PE, Gansevoort RT, Hemmelgarn BR, et al. Cohort profile: the chronic kidney disease prognosis consortium. Int J Epidemiol. 2013;42(6):1660-8.

171. Stringer S, Sharma P, Dutton M, Jesky M, Ng K, Kaur O, et al. The natural history of, and risk factors for, progressive chronic kidney disease (CKD): the Renal Impairment in Secondary care (RIISC) study; rationale and protocol. BMC Nephrol. 2013;14:95.
172. Department for Communities and Local Government. The English Indices of Deprivation 2010.
173. EuroQol G. EuroQol--a new facility for the measurement of health-related quality of life. Health Policy. 1990;16(3):199-208.
174. Gibbons E, Fitzpatrick R. A structured review of patient-reported outcome measures for adults with chronic kidney disease. University of Oxford. 2010.
175. Brothwell S, Dutton M, Ferro C, Stringer S, Cockwell P. Optimising the accuracy of blood pressure monitoring in chronic kidney disease: the utility of BpTRU. BMC Nephrol. 2013;14:218.
176. Lioufas N, Hawley CM, Cameron JD, Toussaint ND. Chronic Kidney Disease and Pulse Wave Velocity: A Narrative Review. Int J Hypertens. 2019;2019:9189362.
177. Zoungas S, Cameron JD, Kerr PG, Wolfe R, Muske C, McNeil JJ, et al. Association of carotid intima-medial thickness and indices of arterial stiffness with cardiovascular disease outcomes in CKD. Am J Kidney Dis. 2007;50(4):622-30.
178. Baumann M, Wassertheurer S, Suttmann Y, Burkhardt K, Heemann U. Aortic pulse wave velocity predicts mortality in chronic kidney disease stages 2-4. J Hypertens. 2014;32(4):899-903.
179. Noordzij MJ, Lefrandt JD, Smit AJ. Advanced glycation end products in renal failure: an overview. J Ren Care. 2008;34(4):207-12.
180. McIntyre NJ, Fluck RJ, McIntyre CW, Taal MW. Skin autofluorescence and the association with renal and cardiovascular risk factors in chronic kidney disease stage 3. Clin J Am Soc Nephrol. 2011;6(10):2356-63.
181. Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW. Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. Kidney Int. 1999;55(2):389-99.
182. Stinghen AE, Massy ZA, Vlassara H, Striker GE, Boullier A. Uremic Toxicity of Advanced Glycation End Products in CKD. J Am Soc Nephrol. 2016;27(2):354-70.
183. Sharma P, Dietrich T, Sidhu A, Vithlani V, Rahman M, Stringer S, et al. The periodontal health component of the Renal Impairment In Secondary Care (RIISC) cohort study: a description of the rationale, methodology and initial baseline results. J Clin Periodontol. 2014;41(7):653-61.
184. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. PLoS Med. 2007;4(10):e297.
185. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996;17(4):343-6.
186. Zou KH, Tuncali K, Silverman SG. Correlation and simple linear regression. Radiology. 2003;227(3):617-22.
187. Cox DR. Regression Models and Life-Tables. Journal of the Royal Statistical Society: Series B (Methodological). 1972;34(2):187-202.
188. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999;94(446):496-509.
189. Royston P, Sauerbrei W. Multivariable model-building: a pragmatic approach to regression anaylsis based on fractional polynomials for modelling continuous variables: John Wiley & Sons; 2008.

190. Smith CT, Williamson PR, Marson AG. Investigating heterogeneity in an individual patient data meta-analysis of time to event outcomes. Stat Med. 2005;24(9):1307-19.
191. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. Statistics in Medicine. 2011;30(4):377-99.
192. Morris TP, White IR, Royston P. Tuning multiple imputation by predictive mean matching and local residual draws. BMC Med Res Methodol. 2014;14:75.
193. White IR, Daniel R, Royston P. Avoiding bias due to perfect prediction in multiple imputation of incomplete categorical variables. Comput Stat Data Anal. 2010;54(10):2267-75.
194. Rubin DB. Multiple imputation for nonresponse in surveys: John Wiley & Sons; 2004.
195. Fraser SDS, Fenton A, Harris S, Shardlow A, Liabeuf S, Massy ZA, et al. The Association of Serum Free Light Chains With Mortality and Progression to End-Stage Renal Disease in Chronic Kidney Disease: Systematic Review and Individual Patient Data Meta-analysis. Mayo Clin Proc. 2017;92(11):1671-81.
196. Waldmann TA, Strober W, Mogielnicki RP. The renal handling of low molecular weight proteins. II. Disorders of serum protein catabolism in patients with tubular proteinuria, the nephrotic syndrome, or uremia. J Clin Invest. 1972;51(8):2162-74.
197. Wikilite.com [Internet]. The biology of immunoglobulins. The Binding Site Group Ltd. Available from: https://www.wikilite.com/the-biology-of-immunoglobulins.
198. Solomon A. Light chains of human immunoglobulins. Methods Enzymol. 1985;116:101-21.
199. Dimopoulos MA, Kastritis E, Rosinol L, Blade J, Ludwig H. Pathogenesis and treatment of renal failure in multiple myeloma. Leukemia. 2008;22(8):1485-93.
200. Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, et al. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem. 2002;48(9):1437-44.
201. Hutchison CA, Harding S, Hewins P, Mead GP, Townsend J, Bradwell AR, et al. Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. Clin J Am Soc Nephrol. 2008;3(6):1684-90.
202. Hutchison CA, Plant T, Drayson M, Cockwell P, Kountouri M, Basnayake K, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrol. 2008;9:11.
203. Park JW, Kim YK, Bae EH, Ma SK, Kim SW. Combined analysis using extended renal reference range of serum free light chain ratio and serum protein electrophoresis improves the diagnostic accuracy of multiple myeloma in renal insufficiency. Clin Biochem. 2012;45(10-11):740-4.
204. Palladini G, Milani P, Foli A, Basset M, Russo F, Bosoni T, et al. The impact of renal function on the clinical performance of FLC measurement in AL amyloidosis. Clin Chem Lab Med. 2016;54(6):939-45.
205. Hutchison CA, Landgren O. Polyclonal immunoglobulin free light chains as a potential biomarker of immune stimulation and inflammation. Clin Chem. 2011;57(10):1387-9.
206. Brebner JA, Stockley RA. Polyclonal free light chains: a biomarker of inflammatory disease or treatment target? F1000 Med Rep. 2013;5:4.
207. Dispenzieri A, Katzmann JA, Kyle RA, Larson DR, Therneau TM, Colby CL, et al. Use of nonclonal serum immunoglobulin free light chains to predict overall survival in the general population. Mayo Clin Proc. 2012;87(6):517-23.

208. Eisele L, Dürig J, Huttmann A, Dührsen U, Führer A, Kieruzel S, et al. Polyclonal Free Light Chain Elevation and Mortality In the German Heinz Nixdorf Recall Study. Blood. 2010;116(21):3903-.
209. Jackson CE, Haig C, Welsh P, Dalzell JR, Tsorlalis IK, McConnachie A, et al. Combined Free Light Chains Are Novel Predictors of Prognosis in Heart Failure. JACC Heart Fail. 2015;3(8):618-25.
210. Shantsila E, Wrigley B, Lip GY. Free light chains in patients with acute heart failure secondary to atherosclerotic coronary artery disease. Am J Cardiol. 2014;114(8):1243-8.
211. Deng X, Crowson CS, Rajkumar SV, Dispenzieri A, Larson DR, Therneau TM, et al. Elevation of serum immunoglobulin free light chains during the preclinical period of rheumatoid arthritis. J Rheumatol. 2015;42(2):181-7.
212. Hampson JA, Stockley RA, Turner AM. Free light chains: potential biomarker and predictor of mortality in alpha-1-antitrypsin deficiency and usual COPD. Respir Res. 2016;17:34.
213. Simon Fraser, Maarten Taal, Adam Shardlow, Lakhvir Assi, Paul Cockwell, Anthony Fenton, Phil Kalra, Scott Harris, Anne Burmeister. Systematic review and meta-analysis of the performance of serum free light chains as risk predictors in people with chronic kidney disease. PROSPERO 2015 CRD42015025195 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42015025195.
214. Higgins J, Green S, eds. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration website. http://www.handbook.cochrane.org/. Updated March 2011. Accessed August 24, 2017.
215. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370(9596):1453-7.
216. Assi LK, McIntyre N, Fraser S, Harris S, Hutchison CA, McIntyre CW, et al. The Association between Polyclonal Combined Serum Free Light Chain Concentration and Mortality in Individuals with Early Chronic Kidney Disease. PLoS One. 2015;10(7):e0129980.
217. Desjardins L, Liabeuf S, Lenglet A, Lemke HD, Vanholder R, Choukroun G, et al. Association between free light chain levels, and disease progression and mortality in chronic kidney disease. Toxins (Basel). 2013;5(11):2058-73.
218. Haynes R, Hutchison CA, Emberson J, Dasgupta T, Wheeler DC, Townend JN, et al. Serum free light chains and the risk of ESRD and death in CKD. Clin J Am Soc Nephrol. 2011;6(12):2829-37.
219. Hutchison CA, Burmeister A, Harding SJ, Basnayake K, Church H, Jesky MD, et al. Serum polyclonal immunoglobulin free light chain levels predict mortality in people with chronic kidney disease. Mayo Clin Proc. 2014;89(5):615-22.
220. Ritchie J, Assi LK, Burmeister A, Hoefield R, Cockwell P, Kalra PA. Association of Serum Ig Free Light Chains with Mortality and ESRD among Patients with Nondialysis-Dependent CKD. Clin J Am Soc Nephrol. 2015;10(5):740-9.
221. Pote A, Zwizinski C, Simon EE, Meleg-Smith S, Batuman V. Cytotoxicity of myeloma light chains in cultured human kidney proximal tubule cells. Am J Kidney Dis. 2000;36(4):735-44.
222. Sengul S, Zwizinski C, Batuman V. Role of MAPK pathways in light chain-induced cytokine production in human proximal tubule cells. Am J Physiol Renal Physiol. 2003;284(6):F1245-54.

223. Sengul S, Zwizinski C, Simon EE, Kapasi A, Singhal PC, Batuman V. Endocytosis of light chains induces cytokines through activation of NF-kappaB in human proximal tubule cells. Kidney Int. 2002;62(6):1977-88.
224. Batuman V. Proximal tubular injury in myeloma. Contrib Nephrol. 2007;153:87-104.
225. Basnayake KI. The biology of immunoglobulin free light chains in kidney disease: a study of Monoclonal and Polyclonal light chains: University of Birmingham; 2012.
226. Parasuraman R, Wolforth SC, Wiesend WN, Dumler F, Rooney MT, Li W, et al. Contribution of polyclonal free light chain deposition to tubular injury. Am J Nephrol. 2013;38(6):465-74.
227. Thio M, Blokhuis BR, Nijkamp FP, Redegeld FA. Free immunoglobulin light chains: a novel target in the therapy of inflammatory diseases. Trends Pharmacol Sci. 2008;29(4):170-4.
228. Holdsworth SR, Summers SA. Role of mast cells in progressive renal diseases. J Am Soc Nephrol. 2008;19(12):2254-61.
229. Cohen G, Horl WH. Free immunoglobulin light chains as a risk factor in renal and extrarenal complications. Semin Dial. 2009;22(4):369-72.
230. Cohen G, Haag-Weber M, Mai B, Deicher R, Horl WH. Effect of immunoglobulin light chains from hemodialysis and continuous ambulatory peritoneal dialysis patients on polymorphonuclear leukocyte functions. J Am Soc Nephrol. 1995;6(6):1592-9.
231. Cohen G, Rudnicki M, Horl WH. Uremic toxins modulate the spontaneous apoptotic cell death and essential functions of neutrophils. Kidney Int Suppl. 2001;78:S48-52.
232. Quinn LM, Mcwilliam S, Campbell JP, Wang Y, Hughes D, Drayson MT, et al. Elevated serum free light chains predict cardiovascular risk in type 1 diabetes mellitus. British Journal of Diabetes. 2016;16(4):176-8.
233. Bellary S, Faint JM, Assi LK, Hutchison CA, Harding SJ, Raymond NT, et al. Elevated serum free light chains predict cardiovascular events in type 2 diabetes. Diabetes Care. 2014;37(7):2028-30.
234. van der Heijden M, Kraneveld A, Redegeld F. Free immunoglobulin light chains as target in the treatment of chronic inflammatory diseases. Eur J Pharmacol. 2006;533(1-3):319-26.
235. Anandram S, Assi LK, Lovatt T, Parkes J, Taylor J, Macwhannell A, et al. Elevated, combined serum free light chain levels and increased mortality: a 5-year follow-up, UK study. J Clin Pathol. 2012;65(11):1036-42.
236. Thilo F, Caspari C, Scholze A, Tepel M. Higher serum levels of free k plus lambda immunoglobulin light chains ameliorate survival of hemodialysis patients. Kidney Blood Press Res. 2011;34(5):344-9.
237. Inker LA, Coresh J, Sang Y, Hsu CY, Foster MC, Eckfeldt JH, et al. Filtration Markers as Predictors of ESRD and Mortality: Individual Participant Data Meta-Analysis. Clin J Am Soc Nephrol. 2017;12(1):69-78.
238. Hutchison CA, Basnayake K, Cockwell P. Serum free light chain assessment in monoclonal gammopathy and kidney disease. Nat Rev Nephrol. 2009;5(11):621-8.
239. Eisele L, Dürig J, Huttmann A, Dührsen U, Führer A, Kieruzel S, et al. Polyclonal Free Light Chain Elevation and Mortality In the German Heinz Nixdorf Recall Study. Blood. 2015;116(21):3903.
240. Fenton A, Jesky MD, Webster R, Stringer SJ, Yadav P, Chapple I, et al. Association between urinary free light chains and progression to end stage renal disease in chronic kidney disease. PLoS One. 2018;13(5):e0197043.

241. Hassan SB, Hanna MO. Urinary kappa and lambda immunoglobulin light chains in normoalbuminuric type 2 diabetes mellitus patients. J Clin Lab Anal. 2011;25(4):229-32.
242. Hutchison CA, Cockwell P, Harding S, Mead GP, Bradwell AR, Barnett AH. Quantitative assessment of serum and urinary polyclonal free light chains in patients with type II diabetes: an early marker of diabetic kidney disease? Expert Opin Ther Targets. 2008;12(6):667-76.
243. Hanaoka M, Gono T, Kawaguchi Y, Uchida K, Koseki Y, Katsumata Y, et al. Urinary free light chain is a potential biomarker for ISN/RPS class III/IV lupus nephritis. Rheumatology (Oxford). 2013;52(12):2149-57.
244. Rocchetti MT, Papale M, d'Apollo AM, Suriano IV, Di Palma AM, Vocino G, et al. Association of urinary laminin G-like 3 and free K light chains with disease activity and histological injury in IgA nephropathy. Clin J Am Soc Nephrol. 2013;8(7):1115-25.
245. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(2):157-72; discussion 207-12.
246. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation. 2009;119(17):2408-16.
247. Jotwani V, Katz R, Ix JH, Gutierrez OM, Bennett M, Parikh CR, et al. Urinary Biomarkers of Kidney Tubular Damage and Risk of Cardiovascular Disease and Mortality in Elders. Am J Kidney Dis. 2018;72(2):205-13.
248. Nohara-Shitama Y, Adachi H, Enomoto M, Fukami A, Kumagai E, Nakamura S, et al. Twenty-four-Hour Urinary Potassium Excretion, But Not Sodium Excretion, Is Associated With All-Cause Mortality in a General Population. J Am Heart Assoc. 2018;7(1).
249. Sarnak MJ, Katz R, Newman A, Harris T, Peralta CA, Devarajan P, et al. Association of urinary injury biomarkers with mortality and cardiovascular events. J Am Soc Nephrol. 2014;25(7):1545-53.
250. Bird J, Behrens J, Westin J, Turesson I, Drayson M, Beetham R, et al. UK Myeloma Forum (UKMF) and Nordic Myeloma Study Group (NMSG): guidelines for the investigation of newly detected M-proteins and the management of monoclonal gammopathy of undetermined significance (MGUS). Br J Haematol. 2009;147(1):22-42.
251. Axelsson U, Bachmann R, Hallen J. Frequency of pathological proteins (M-components) om 6,995 sera from an adult population. Acta Med Scand. 1966;179(2):235-47.
252. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2016, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2016/, based on November 2018 SEER data submission, posted to the SEER web site, April 2019.
253. Gavriatopoulou M, Terpos E, Kastritis E, Dimopoulos MA. Current treatments for renal failure due to multiple myeloma. Expert Opin Pharmacother. 2016;17(16):2165-77.
254. Finkel KW, Cohen EP, Shirali A, Abudayyeh A, American Society of Nephrology Onco-Nephrology F. Paraprotein-Related Kidney Disease: Evaluation and Treatment of Myeloma Cast Nephropathy. Clin J Am Soc Nephrol. 2016;11(12):2273-9.
255. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of monoclonal gammopathy of undetermined significance. N Engl J Med. 2006;354(13):1362-9.

256. Fermand JP, Bridoux F, Dispenzieri A, Jaccard A, Kyle RA, Leung N, et al. Monoclonal gammopathy of clinical significance: a novel concept with therapeutic implications. Blood. 2018;132(14):1478-85.
257. Leung N, Bridoux F, Hutchison CA, Nasr SH, Cockwell P, Fermand JP, et al. Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. Blood. 2012;120(22):4292-5.
258. Sprangers B. Aetiology and management of acute kidney injury in multiple myeloma. Nephrol Dial Transplant. 2018;33(5):722-4.
259. Wang PX, Sanders PW. Immunoglobulin light chains generate hydrogen peroxide. J Am Soc Nephrol. 2007;18(4):1239-45.
260. Hutchison CA, Batuman V, Behrens J, Bridoux F, Sirac C, Dispenzieri A, et al. The pathogenesis and diagnosis of acute kidney injury in multiple myeloma. Nat Rev Nephrol. 2011;8(1):43-51.
261. Doshi M, Lahoti A, Danesh FR, Batuman V, Sanders PW, American Society of Nephrology Onco-Nephrology F. Paraprotein-Related Kidney Disease: Kidney Injury from Paraproteins-What Determines the Site of Injury? Clin J Am Soc Nephrol. 2016;11(12):2288-94.
262. Kyle RA, Larson DR, Therneau TM, Dispenzieri A, Kumar S, Cerhan JR, et al. Long-Term Follow-up of Monoclonal Gammopathy of Undetermined Significance. N Engl J Med. 2018;378(3):241-9.
263. Kristinsson SY, Bjorkholm M, Andersson TM, Eloranta S, Dickman PW, Goldin LR, et al. Patterns of survival and causes of death following a diagnosis of monoclonal gammopathy of undetermined significance: a population-based study. Haematologica. 2009;94(12):1714-20.
264. Hoefield RA, Kalra PA, Baker P, Lane B, New JP, O'Donoghue DJ, et al. Factors associated with kidney disease progression and mortality in a referred CKD population. Am J Kidney Dis. 2010;56(6):1072-81.
265. Dispenzieri A, Katzmann JA, Kyle RA, Larson DR, Melton LJ, 3rd, Colby CL, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. Lancet. 2010;375(9727):1721-8.
266. Leung N, Bridoux F, Batuman V, Chaidos A, Cockwell P, D'Agati VD, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. Nat Rev Nephrol. 2019;15(1):45-59.
267. Fenton A, Jesky MD, Ferro CJ, Sorensen J, Karsdal MA, Cockwell P, et al. Serum endotrophin, a type VI collagen cleavage product, is associated with increased mortality in chronic kidney disease. PLoS One. 2017;12(4):e0175200.
268. Madaio MP. Renal biopsy. Kidney Int. 1990;38(3):529-43.
269. Alexopoulos E. How important is renal biopsy in the management of patients with glomerular diseases? Nephrol Dial Transplant. 2001;16 Suppl 6:83-5.
270. Park J, Scherer PE. Adipocyte-derived endotrophin promotes malignant tumor progression. J Clin Invest. 2012;122(11):4243-56.
271. Karousou E, D'Angelo ML, Kouvidi K, Vigetti D, Viola M, Nikitovic D, et al. Collagen VI and hyaluronan: the common role in breast cancer. Biomed Res Int. 2014;2014:606458.
272. Cescon M, Gattazzo F, Chen P, Bonaldo P. Collagen VI at a glance. J Cell Sci. 2015;128(19):3525-31.

273. Lennon R, Byron A, Humphries JD, Randles MJ, Carisey A, Murphy S, et al. Global analysis reveals the complexity of the human glomerular extracellular matrix. J Am Soc Nephrol. 2014;25(5):939-51.
274. Lemley KV, Kriz W. Anatomy of the renal interstitium. Kidney Int. 1991;39(3):370-81.
275. Groma V. Demonstration of collagen type VI and alpha-smooth muscle actin in renal fibrotic injury in man. Nephrol Dial Transplant. 1998;13(2):305-12.
276. Nerlich AG, Schleicher ED, Wiest I, Specks U, Timpl R. Immunohistochemical localization of collagen VI in diabetic glomeruli. Kidney Int. 1994;45(6):1648-56.
277. Mohan PS, Carter WG, Spiro RG. Occurrence of type VI collagen in extracellular matrix of renal glomeruli and its increase in diabetes. Diabetes. 1990;39(1):31-7.
278. Wu Q, Jinde K, Nishina M, Tanabe R, Endoh M, Okada Y, et al. Analysis of prognostic predictors in idiopathic membranous nephropathy. Am J Kidney Dis. 2001;37(2):380-7.
279. Edwards NC, Moody WE, Yuan M, Hayer MK, Ferro CJ, Townend JN, et al. Diffuse interstitial fibrosis and myocardial dysfunction in early chronic kidney disease. Am J Cardiol. 2015;115(9):1311-7.
280. Lopez B, Gonzalez A, Hermida N, Laviades C, Diez J. Myocardial fibrosis in chronic kidney disease: potential benefits of torasemide. Kidney Int Suppl. 2008(111):S19-23.
281. Bashey RI, Martinez-Hernandez A, Jimenez SA. Isolation, characterization, and localization of cardiac collagen type VI. Associations with other extracellular matrix components. Circ Res. 1992;70(5):1006-17.
282. Shimizu M, Umeda K, Sugihara N, Yoshio H, Ino H, Takeda R, et al. Collagen remodelling in myocardia of patients with diabetes. J Clin Pathol. 1993;46(1):32-6.
283. Heeneman S, Cleutjens JP, Faber BC, Creemers EE, van Suylen RJ, Lutgens E, et al. The dynamic extracellular matrix: intervention strategies during heart failure and atherosclerosis. J Pathol. 2003;200(4):516-25.
284. Dinh W, Bansemir L, Futh R, Nickl W, Stasch JP, Coll-Barroso M, et al. Increased levels of laminin and collagen type VI may reflect early remodelling in patients with acute myocardial infarction. Acta Cardiol. 2009;64(3):329-34.
285. Mall G, Huther W, Schneider J, Lundin P, Ritz E. Diffuse intermyocardiocytic fibrosis in uraemic patients. Nephrol Dial Transplant. 1990;5(1):39-44.
286. London GM, Marchais SJ, Guerin AP. Arterial stiffness and function in end-stage renal disease. Adv Chronic Kidney Dis. 2004;11(2):202-9.
287. Chue CD, Townend JN, Steeds RP, Ferro CJ. Arterial stiffness in chronic kidney disease: causes and consequences. Heart. 2010;96(11):817-23.
288. Moody WE, Edwards NC, Chue CD, Ferro CJ, Townend JN. Arterial disease in chronic kidney disease. Heart. 2013;99(6):365-72.
289. Sun S, Karsdal MA. Chapter 6 - Type VI Collagen. In: Karsdal MA, editor. Biochemistry of Collagens, Laminins and Elastin: Academic Press; 2016. p. 49-55.
290. Baldock C, Sherratt MJ, Shuttleworth CA, Kielty CM. The supramolecular organization of collagen VI microfibrils. J Mol Biol. 2003;330(2):297-307.
291. Lamande SR, Morgelin M, Adams NE, Selan C, Allen JM. The C5 domain of the collagen VI alpha3(VI) chain is critical for extracellular microfibril formation and is present in the extracellular matrix of cultured cells. J Biol Chem. 2006;281(24):16607-14.
292. Aigner T, Hambach L, Soder S, Schlotzer-Schrehardt U, Poschl E. The C5 domain of Col6A3 is cleaved off from the Col6 fibrils immediately after secretion. Biochem Biophys Res Commun. 2002;290(2):743-8.

293. Sun K, Park J, Gupta OT, Holland WL, Auerbach P, Zhang N, et al. Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction. Nat Commun. 2014;5:3485.
294. Rasmussen DGK, Fenton A, Jesky M, Ferro C, Boor P, Tepel M, et al. Urinary endotrophin predicts disease progression in patients with chronic kidney disease. Sci Rep. 2017;7(1):17328.
295. Stribos EGD, Nielsen SH, Brix S, Karsdal MA, Seelen MA, van Goor H, et al. Non-invasive quantification of collagen turnover in renal transplant recipients. PLoS One. 2017;12(4):e0175898.
296. Sun S, Henriksen K, Karsdal MA, Byrjalsen I, Rittweger J, Armbrecht G, et al. Collagen Type III and VI Turnover in Response to Long-Term Immobilization. PLoS One. 2015;10(12):e0144525.
297. Bu D, Crewe C, Kusminski CM, Gordillo R, Ghaben AL, Kim M, et al. Human endotrophin as a driver of malignant tumor growth. JCI Insight. 2019;5.
298. Katsuda S, Okada Y, Minamoto T, Oda Y, Matsui Y, Nakanishi I. Collagens in human atherosclerosis. Immunohistochemical analysis using collagen type-specific antibodies. Arterioscler Thromb. 1992;12(4):494-502.
299. Holm Nielsen S, Goncalves I, Shore A, Natali A, Khan F, Genovese F, et al. 3043Endotrophin, a fragment of collagen type VI, is correlated to IMT and associated with cardiovascular events in patients with atherosclerosis and diabetes: the IMI-SUMMIT cohort. European Heart Journal. 2019;40(Supplement_1).
300. Wu XX, Gordon RE, Glanville RW, Kuo HJ, Uson RR, Rand JH. Morphological relationships of von Willebrand factor, type VI collagen, and fibrillin in human vascular subendothelium. Am J Pathol. 1996;149(1):283-91.
301. Ross JM, McIntire LV, Moake JL, Rand JH. Platelet adhesion and aggregation on human type VI collagen surfaces under physiological flow conditions. Blood. 1995;85(7):1826-35.
302. Ohneberg K, Schumacher M, Beyersmann J. Modelling two cause-specific hazards of competing risks in one cumulative proportional odds model? Stat Med. 2017;36(27):4353-63.
303. Luther DJ, Thodeti CK, Shamhart PE, Adapala RK, Hodnichak C, Weihrauch D, et al. Absence of type VI collagen paradoxically improves cardiac function, structure, and remodeling after myocardial infarction. Circ Res. 2012;110(6):851-6.
304. Reese-Petersen AL, Karsdal M, Genovese F. P5001Acute cardiac fibrogenic response triggered by endotrophin, a type VI collagen signalling molecule. European Heart Journal. 2019;40(Supplement_1).
305. Park J, Scherer PE. Endotrophin - a novel factor linking obesity with aggressive tumor growth. Oncotarget. 2012;3(12):1487-8.
306. Chiche L, Cournac JM, Mancini J, Bardin N, Thomas G, Jean R, et al. Normalization of serum-free light chains in patients with systemic lupus erythematosus upon rituximab treatment and correlation with biological disease activity. Clin Rheumatol. 2011;30(5):685-9.
307. Kormelink TG, Tekstra J, Thurlings RM, Boumans MH, Vos K, Tak PP, et al. Decrease in immunoglobulin free light chains in patients with rheumatoid arthritis upon rituximab (anti-CD20) treatment correlates with decrease in disease activity. Ann Rheum Dis. 2010;69(12):2137-44.
308. Austin PC, Lee DS, Fine JP. Introduction to the Analysis of Survival Data in the Presence of Competing Risks. Circulation. 2016;133(6):601-9.