Predictors of Renal and Patient Outcomes in Chronic Kidney Disease

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Submitted in fulfilment of the requirements for the degree of MD

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Acknowledgements

I would like to thank my principal supervisor Dr Mark MacGregor for the opportunity to undertake this research. His knowledge, support, energy, vision and guidance have been invaluable. I would also like to acknowledge my co-supervisor Professor Alan Jardine for his support throughout this project.

I am grateful to Dr Jamie Traynor, Dr Christopher Deighan and Dr Denis O'Reilly for their expert help and input to the nephrology clinic proteinuria database. I would like to thank Dr Mario Hair of the University of the West of Scotland for his statistical expertise and Dr Bruce MacKinnon, Dr Lilian Murray and Professor Gordon McInnes for their assistance with the hypertension database. I would also like to acknowledge Dr Fiona Gifford's input to the laboratory database analysis. I am grateful to Marilyn Roth from the Department of Epidemiology and Public Health, University College London for generously supplying data from the Health Survey for England, 2009.

I would like to acknowledge staff from the seven general practices around Ayrshire that welcomed me into their premises and supported me during study recruitment:

Beith Health Centre, Beith
Stewarton Medical Centre
Kilwinning Medical Practice
Townhead Surgery, Irvine
Old Irvine Road Surgery, Kilmarnock
Portland Surgery, Troon
Racecourse Road Medical Group, Ayr

I am grateful to my husband, family and friends for their support throughout my studies, especially Dr Elaine Spalding who provided invaluable moral support. Finally I am exceptionally grateful to the patients of Ayrshire and Arran who enthusiastically participated in this research. Without them it would not have been possible.

Author's declaration

The work presented in this thesis is that of the author and her supervisors, Dr Mark MacGregor and Professor Alan Jardine. All clinical research work was carried out by the author. All statistical analyses were carried out by the author with some input from Dr Fiona Gifford (Chapter 6 only) and statistical advice form Dr Mario Hair University of the West of Scotland (Chapters 3 and 4) and Dr Lilian Murray (Chapter 7).

I declare that this thesis has been composed by myself and is a record of work performed by me. It has not been previously submitted for a higher degree.

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September 2012

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Publications arising from this work:

Original manuscripts:

Methven S, MacGregor MS, Traynor J, O'Reilly DStJ, Deighan CJ.
Assessing proteinuria in chronic kidney disease: albumin:creatinine ratio versus total protein:creatinine ratio. Nephrol Dial Transplantation, 2010. 25;9:2991-2996

Methven S, MacGregor MS, Traynor J, O'Reilly D StJ, Deighan CJ
Comparison of urinary albumin and urinary total protein as predictors of patient outcomes in CKD. Am J Kidney Dis 2011. 57;1: 21-28

Accompanied by editorial form Prof De Jong entitled "What to measure- albuminuria or total proteinuria?"

Methven S, Traynor J,Hair MD, O'Reilly DStJ, Deighan CJ, MacGregor MS
Stratifying risk in chronic kidney disease: an observational study of UK guidelines for measuring total proteinuria and albuminuria
QJM 2011. 104; 663 - 670

Gifford F**, Methven S,** Boag D, 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; 1045-53

Letter to the editor:

Methven S, MacGregor MS, Traynor J, O'Reilly DStJ, Deighan CJ
Reply to "Proteinuria or albuminuria?"
Nephrol Dial Transplant 2010. 25:10; 3455. Letter.

Methven S, MacGregor MS, Traynor J, O'Reilly DStJ, Deighan CJ
Reply to "Improving the interpretation of protein:creatinine ratios. The impact of creatinine excretion"
Nephrol Dial Transplant 2011. 26; 1109 - 1110. Letter

Publications related to this work

Reviews:
Methven S, MacGregor MS.
Clinical Management of Chronic Kidney Disease. Clin Med 2009. 9;3:269 – 272

MacGregor MS, **Methven S**.
Assessing kidney function. In: *Handbook of Chronic Kidney Disease Management.* Ed Daugirdas JT. Philadelphia, PA: Lippincott Williams & Wilkins. 2011. Book chapter.

Letter to the editor:
Methven S, Traynor J,Hair MD, O'Reilly DStJ, Deighan CJ, MacGregor MS
Urine albumin: protein ratio as a predictor of patient outcomes in CKD (Letter to the Editor)
Nephrol Dial Transplant 2012. 27 (8); 3372 - 3373

Definitions/Abbreviations

ABW	Actual body weight
ACE	Angiotensin converting enzyme
ACEi	Angiotensin converting enzyme inhibitor
ACR	Albumin: creatinine ratio
Adj Ca	Adjusted calcium
ANOVA	Analysis of variance
ARB	Angiotensin receptor blocker
AUC	Area under the curve
BB	Beta blocker therapy
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
C&G	Cockcroft and Gault
CCB	Calcium channel blocker
CHI	Community health index
Chol:HDL	Cholesterol to high density lipoprotein ratio
CKD	Chronic kidney disease
CKD-EPI	CKD Epidemiology Group equation
CKD-MBD	CKD mineral bone disorders
CrCl	Creatinine clearance
CRP	C-reactive protein
CV	Cardiovascular
CV	Co-efficient of variation
DBP	Diastolic blood pressure
ECE	Estimated creatinine excretion
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EPO	Erythropoetin
ESRD	End stage renal disease
EQ-5D	European Quality of Life – 5 Dimensions
FGF-23	Fibroblast growth factor 23
GBM	Glomerular basement membrane
GBPC	Glasgow Blood Pressure Clinic
GFR	Glomerular filtration rate
GN	Glomerulonephritis
GP	General Practitioner
Hb	Haemoglobin
HDL	High density lipoprotein

HR	Heart rate
IBW	Ideal body weight
ICIQ-UI	International Consultation on Incontinence Questionnaire – urinary incontinence form
IDMS	Isotope Dilution Mass Spectrometry
IHD	Ischaemic heart disease
IPSS	International prostate symptom score
IQR	Interquartile range
K	Potassium
KDIGO	Kidney Disease: Improving Global Outcomes
LBW	Lean body weight
LDL	Low density lipoprotein
LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
LUTS	Lower urinary tract symptoms
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
MCE	Measured creatinine excretion
MDRD	Modification of diet in renal disease
mmHg	Millimetres of mercury
MRB	Mineralocorticoid receptor blocker
NAP	Non-albumin protein
NAPCR	Non-albumin protein: creatinine ratio
NICE	National Institute for Health and Clinical Excellence
NKDEP	National Kidney Disease Education Project
NKF-KDOQI	National Kidney Foundation - Kidney Disease Outcome Quality Initiative
NPV	Negative predictive value
NRI	Net reclassification index
OHA	Oral hypoglycaemic agent
PO4	Phosphate
PP	Pulse pressure
PPV	Positive predictive value
PRD	Primary renal disease
PTH	Parathyroid hormone
QOF	Quality Outcomes Framework
RAAS	Renin-angiotensin-aldosterone system
ROC	Receiver operator characteristic
RRT	Renal replacement therapy
SBP	Systolic blood pressure
SCr	Serum creatinine
SERPR	Scottish electronic renal patient record

SD	Standard Deviation
SIGN	Scottish Intercollegiate Guidelines Network
SIMD	Scottish index of multiple deprivation
Sn	Sensitivity
Sp	Specificity
Statin	HMG Co-A reductase inhibitor
Trigs	Triglyceride
TPCR	total protein: creatinine ratio
UAC	Urinary albumin concentration
UAE	Urinary albumin excretion
ULN	Upper limit of normal
UPE	24-hour urine total protein excretion
US	United States
VAS	Visual analogue scale

Abstract

Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular disease and end stage renal failure. Accurate identification of those with a reduced glomerular filtration rate and significant proteinuria facilitates early diagnosis and risk stratification.

This thesis explores the optimal measure of proteinuria, to accurately quantify proteinuria and as a predictor of renal and patient outcomes. We examine the prevalence of CKD in a general population cohort and assess the impact of different estimated glomerular filtration rate (eGFR) formulae. We explore the prognostic role of reduced eGFR and proteinuria in patients with hypertension and present the baseline characteristics of a community cohort study of patients with predominantly early CKD. They will be followed for ten years to identify predictors of cardiovascular and renal outcome.

Urine total protein:creatinine ratio (TPCR) and albumin:creatinine ratio (ACR) have largely replaced 24-hour urine collections for proteinuria quantification. The performance of these spot measures to identify significant proteinuria is compared in a cohort of 6842 patients attending a general nephrology clinic. Both tests perform well overall but TPCR is statistically significantly superior as a predictor of 24-hour total proteinuria than ACR (as measured by the area under the receiver operator characteristic (ROC) curve to predict 1g/day total proteinuria). On sub-group analysis the performance of the spot samples is poorer in women and the elderly, likely as a result of low muscle mass and low urine creatinine (the denominator in TPCR/ACR).

The performance of TPCR and ACR were then compared as predictors of outcome in a similar cohort of 5586 CKD patients using a hierarchical Cox survival model. TPCR and

ACR both performed well as independent predictors of death, commencement of renal replacement therapy (RRT) and doubling of serum creatinine. Notably TPCR performed well at low levels where albuminuria has been considered superior. These findings are novel. The spot samples performed as well as 24-hour collections in the sub-group with timed urine collections.

The National Institute for Health and Clinical Excellence in England recommend ACR to monitor all patients with CKD; the Scottish Intercollegiate Guidelines Network recommend TPCR for non-diabetic renal disease. Therefore, we investigated the implications of these recommendations using survival modelling. The same cohort was divided into 5 groups: no proteinuria, low proteinuria (using TPCR and ACR), high proteinuria (TPCR and ACR) and two groups where TPCR and ACR were discordant (i.e. TPCR above the diagnostic threshold but ACR below it and vice versa) using the recommended thresholds (ACR 30mg/mmol/TPCR 50mg/mmol to predict 0.5g/day total proteinuria and ACR 70mg/mmol/TPCR 100mg/mmol to predict 1g/day total proteinuria). Using univariate survival analysis the discordant group had significantly poorer outcomes (using the same outcomes as previously) than those with significant proteinuria as measured by both tests. The discordant group was older with poorer renal function and some of the excess risk was abolished on multivariate analysis, however the risk did not return to the level of those without detectable proteinuria. TPCR, but not ACR, measures non-albumin proteins and these may have pathophysiological roles in progression. This requires further study. However this analysis confirmed that TPCR identifies patients at high risk of adverse outcomes.

TPCR and ACR may vary as a result of muscle mass. We adjusted TPCR and ACR for estimated creatinine excretion (ECE) (calculated using the Cockcroft and Gault formula) and performed cross-sectional and longitudinal analyses. Adjusting TPCR and ACR for

ECE improves prediction of significant proteinuria in sub-groups with poor baseline test performance (such as women and the elderly) using ROC curve analysis. However when adjusted and unadjusted values were compared as predictors of outcome (using a net reclassification index analysis) adjusted values were significantly inferior. Urine creatinine is an independent predictor of mortality and hence may be directly contributing to the predictive value of TPCR and ACR rather than simply correcting for urine flow rate. As such, adjusting for ECE may act to remove the effect of a second independent predictor, leading to inferior test performance. Therefore the decision to adjust TPCR and ACR for ECE depends on the test application: to predict significant proteinuria adjustment of TPCR and ACR is of benefit, but adjustment leads to inferior performance as a prognostic test.

The prevalence of CKD stages 3-5 was assessed using a general population laboratory database. Overall population prevalence was 5.63% using the modification of diet in renal disease (MDRD) formula and fell to 4.94% when the CKD-Epidemiology group (CKD-EPI) formulae were applied. Those reclassified to an earlier stage of CKD were predominantly middle aged women. Prevalence over a five year period was found to be stable using the CKD-EPI formulae but rose slightly according to MDRD.

Proteinuria and eGFR were assessed as predictors of outcome in a large specialist hypertension clinic cohort. On multivariate survival analysis both baseline dipstick proteinuria and an eGFR<60ml/min/1.73m^2 remained strong independent predictors of cardiovascular and all-cause mortality, despite intensive specialist intervention to control blood pressure. These simple tests should be advocated for risk stratification in these patients.

Lastly the baseline characteristics of a community CKD cohort are presented. We recruited 411 participants from seven general practices around Ayrshire and a detailed baseline

clinical and biochemical assessment was performed. Patients were invited to participate if they were included in the primary care register of CKD stages 3-5. Over a quarter had an eGFR>60ml/min/1.73m^2 on the meat-fasted study sample. Proteinuria was of notably low prevalence and the cohort had a large burden of cardiovascular disease. Complications of renal disease were uncommon. The characteristics of the cohort differ from those under hospital follow-up. Their long term outcomes should contribute to refining risk stratification in this population.

Proteinuria and eGFR are key aspects of diagnosis and monitoring in CKD. Identification of the optimal measures of both is essential and findings presented here contribute to that. There is a need to refine risk stratification in CKD, to identify those who require intensive intervention, and to reassure the rest. The findings of this thesis also contribute to that. Further study is required to refine the core aspects of diagnosis and investigation of CKD.

1 Chapter 1: Introduction

1.1 Background

The importance of renal disease has been recognised since Hippocrates made the association between bubbles in the urine and disease of the kidneys in 400 BC (1). In comparison, the epidemiological study of early kidney disease is a recent area of interest. Initially a lack of a consensus definition hindered research and clinical practice in this field. However, over the past 15 years, recognition of early renal disease has been improved by the advent of formulae derived from demographic studies to calculate the estimated glomerular filtration rate (eGFR) (1999), the publication of an international classification of chronic kidney disease (CKD) based on eGFR (2002), the introduction of widespread eGFR reporting and the implementation of primary care CKD registers in the UK (both 2006).

The management of patients with end stage renal disease (ESRD) has been improved over the past 50 years with increased availability of renal replacement therapy (RRT), and technological and immunological advances in dialysis and transplantation respectively. However quality of life for patients receiving dialysis is significantly inferior to that of the general population (2), and the burden of premature cardiovascular disease and excess infections and cancers results in shortened life expectancy, with the greatest impact amongst the youngest patients (3). Therefore, improved diagnosis and risk stratification in early CKD remains essential, in order to allow early intervention and hopefully prevent, or reduce the rate, of progression to ESRD.

This is a rapidly evolving field, and the developments to date will be reviewed in detail in this introduction (chapter 1). In subsequent chapters, key aspects of CKD diagnosis are considered. In chapter 2, the relationship between total proteinuria and albuminuria is explored and their roles in prognostication are examined in chapters 3 and 4. Chapter 5

considers whether modification of the total protein: creatinine ratio (TPCR) and albumin: creatinine ratio (ACR) would improve their prognostic ability. Chapter 6 compares eGFR prediction formulae and the impact of their use on a general population cohort and chapter 7 presents the baseline findings from a primary care longitudinal cohort study of early CKD. Chapter 8 assesses the role of eGFR and proteinuria measurement in risk stratification in hypertension and the implications of these findings are explored in the discussion (chapter 9).

1.2 Epidemiology of CKD

1.2.1 Classification of Chronic Kidney Disease

Proteinuria and reduced excretory capacity are cardinal features of kidney disease. Chronic kidney disease is defined as a persistent reduction in GFR with the presence of kidney damage, or kidney damage alone. The abnormality must be present for ≥90 days to be defined as persistent. The criteria used to define kidney damage are outlined in Table 1-1 (4). Proteinuria is the most common marker of kidney damage (5).

Table 1-1 - Criteria for the definition of chronic kidney disease

Structural or functional abnormalities of the kidneys for at least 90 days, as manifested by either:
(1) Kidney damage, with or without decreased glomerular filtration rate (GFR), as defined by: • pathologic abnormalities • markers of kidney damage – urinary abnormalities (proteinuria and/ or haematuria) – blood abnormalities (renal tubular syndromes) – imaging abnormalities – kidney transplantation • kidney transplant recipients
(2) GFR <60 mL/min/1.73 m^2, with or without kidney damage

If any of the above criteria are fulfilled, CKD is then classified according to the staging system proposed by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) in 2002, and endorsed by Kidney Disease: Improving Global Outcomes (KDIGO) in 2004 (5, 6). In the United Kingdom, the classification system has been endorsed in modified form by the Scottish Intercollegiate Guidelines Network (SIGN), National Institute for Health and Clinical Excellence (NICE) and Joint Specialty Committee on Renal Disease (7-9). The stages are described in Table 1-2 (5).

Table 1-2 – International Staging System of Chronic Kidney Disease

Stage	Definition	eGFR ($mL/min/1.73m^2$)
1	Presence of kidney damage, with normal or raised GFR	≥90
2	Presence of kidney damage, with mildly reduced GFR	60-89
3	Moderately reduced GFR	30-59
4	Severely reduced GFR	15-29
5	End-stage kidney disease	<15

Since the introduction of the classification system in 2002, there have been proposals to modify it, as more prognostic evidence became available (10). An international controversies conference was held, which proposed three modifications. Firstly, to add the cause of kidney disease (if known) to the stage of CKD. Secondly, to subdivide Stage 3 ($30 \leq eGFR \leq 59$), into 2 levels, 3A and 3B, based on eGFR; 3A when $45 < eGFR < 59$, and 3B when $30 < eGFR < 44$). Thirdly, to add the stage of albuminuria to the stage of CKD, according to eGFR. These changes seek to improve the assessment of overall prognosis by including these accepted prognostic indicators in the classification (11). New international CKD guidelines are currently under development by KDIGO, and are expected to incorporate these modifications in the form of the CGA classification (cause, eGFR and albuminuria).

1.2.2 Heterogeneity of Chronic Kidney Disease

Chronic kidney disease is an "umbrella term" which includes numerous specific renal diseases, some of which are more well-defined than others. The term was introduced to facilitate recognition and classification of kidney disease as described above, but could obscure the importance of distinct renal pathologies with differing natural history and prognosis. For instance, compare two glomerular diseases; minimal change nephropathy characterized by nephrotic syndrome but no progressive loss of excretory renal function and idiopathic membranous nephropathy with variable levels of proteinuria and where around a third will develop progressive renal disease. Furthermore interstitial diseases have a different natural history such as patients with adult polycystic kidney disease who often suffer a linear decline in GFR without proteinuria. Subsequently the therapeutic approaches to these distinct conditions differ in some important aspects. The expected recommendation of the upcoming KDIGO guidelines to add cause to the classification is in recognition of this important feature of renal disease.

1.2.3 Incidence and Prevalence of Chronic Kidney Disease

The true incidence and prevalence of CKD, according to the definition outlined above, is difficult to ascertain. Prior to the CKD classification being introduced, studies used varying definitions of kidney disease and its severity which made comparisons of prevalence difficult. The incidence and prevalence of patients receiving dialysis treatment for established renal failure is well documented in the UK and around the world by a number of organisations: Scottish Renal Registry; UK Renal Registry; European Renal Registry; Australia and New Zealand Dialysis and Transplant Registry; and the United States Renal Data System (12-16). The incidence of new patients starting RRT in Scotland in 2006 – 2010 was 10.7/100,000 population (12). However this reflects prevalence of treatment as opposed to prevalence of the disease. The prevalence and incidence of CKD itself is more

poorly documented. The majority of studies have relied on single measurements of serum creatinine and proteinuria in general population cohorts which may over-estimate the prevalence, compared to using a reduced eGFR on two occasions >90 days apart to define CKD (as recommended by the CKD classification). Laboratory studies of populations gathered during routine clinical care assume absence of disease in the unsampled population, which will underestimate prevalence. However there is also likely to be oversampling of patients being tested during an acute illness, when kidney function may have deteriorated acutely. This will overestimate prevalence (especially if a single measure is used). The relative contribution of these conflicting factors to the overall prevalence estimate is unknown but these studies still provide valuable information.

In the past, studies often focussed on advanced kidney disease, in order to predict need for renal replacement therapy (17-19). More recently there has been a paradigm shift in nephrologists' approach to kidney disease, exemplified by an increased recognition of the importance of early kidney disease as a potential time for intervention in order to prevent progression, late presentation and the recognition of the excess burden of cardiovascular disease in this group (20). Recent studies of incidence and prevalence reflect this, with more attention being paid to earlier disease. The identification of this patient group has also been improved greatly by routine eGFR reporting (21).

The Health Survey of Nord-Trondelag County (HUNT II) in Norway found an overall prevalence of Stages 1 – 5 CKD of 10.2% (4.3% Stages 3 – 5) in a representative sample of 15,625 adults (22). The Ausdiab study found a prevalence of Stage 3 – 5 CKD of 11.2% in a sample of 11,247 non-institutionalised adults ≥ 25years of age in Australia (23). In the USA, prevalence estimates of CKD are derived from the National Health and Nutrition Examination Survey (NHANES). The most recent survey contains data from 13,233 non-institutionalised adults ≥20 years during 1999 – 2004. The prevalence of Stages 1 -4 CKD

was 13.1%. This was compared to the same survey from 1988 – 1994 where the prevalence was 10%, with the largest increment being in Stage 3 CKD which rose from 5.4% to 7.7%. This is one of the few publications to have assessed secular trends, and showed an overall relative increase of 1.3 (24). The authors suggest that this may be partly accounted for by the increasing prevalence of diabetes and obesity. Proteinuria was also common in this cohort, with frank proteinuria in 1.3%, and microalbuminuria in 8.2% of subjects.

There is increasing evidence from the UK in recent years. The Health Survey in England (HSE) is a regular survey of a selection of the population. It assessed kidney disease for the first time in 2009. Combining 2009 and 2010 data it now includes over 6000 participants aged ≥16 years and is a nationally representative sample in England. A single measurement of blood and urine was taken and 6% of men and 7% of women were found to have Stage 3 – 5 CKD, according to eGFR measurement, with marked differences according to age. Less than 1% of men and women aged 16-24 had stage 3-5, rising to 29% of men and 35% of women aged 75 and over. Albuminuria was found in 9% of men and 8% of women. In the majority, this was microalbuminuria (8% in men and women) and only 1% (or less) had macroalbuminuria. Again, there was marked variation according to age: around 5%-6% in the younger age groups, rising to 26% of men and 19% of women aged 75 and over. Taking these parameters together, overall estimates for Stages 1-5 CKD were produced – 13% in men and women (25). However these estimates will be subject to change depending on the age distribution in a given population because of the strong relationship between age and prevalence of CKD described above. A slightly older study from England, the NEOERICA project, utilised primary care computer records in three regions of England to identify those with CKD. A valid creatinine was available in 30% of the total adult cohort (aged ≥18 years), and the study reported an age-standardised prevalence of stage 3 – 5 CKD of 10.6% for females and 5.8% for males (26). This study suffers from selection bias, as the 30% of the cohort with available results had serum creatinine

measured for a clinical indication and were not selected randomly. A summary of the findings of these prevalence studies is shown in table 1-3.

Table 1-3 - Summary of studies of prevalence of CKD

Study	Year	Country	Number	Stage of CKD	Prevalence (%)
HUNT-II	2006	Norway	15,625	3 – 5	4.3
AusDiab	2003	Australia	11,247	3 – 5	11.2
NHANES	1999-2004	USA	13,233	1 – 4	13.1
HSE	2009-10	England	>6000	1 – 5	13.0
NEOERICA	1998-2003	England (laboratory database)	130,226	3 – 5	Males 5.8 Females 10.6

A reduced eGFR and proteinuria do not necessarily co-exist, and this was demonstrated in the Prevention or Renal and Vascular End Stage Disease (PREVEND) general population cohort study from the Netherlands (27). Both these measures identify at risk populations and the overlap is a relatively small proportion of the total, as shown in Figure 1-1. This was one of the few studies that assessed the presence of non-visible haematuria and none of the studies above took account of the other criteria for CKD such as structural or histological abnormalities.

Figure 1-1 - Venn diagram indicating the prevalence of macroalbuminuria, erythrocyturia, and impaired renal function in a population of 8592 participants of general population screening (27)

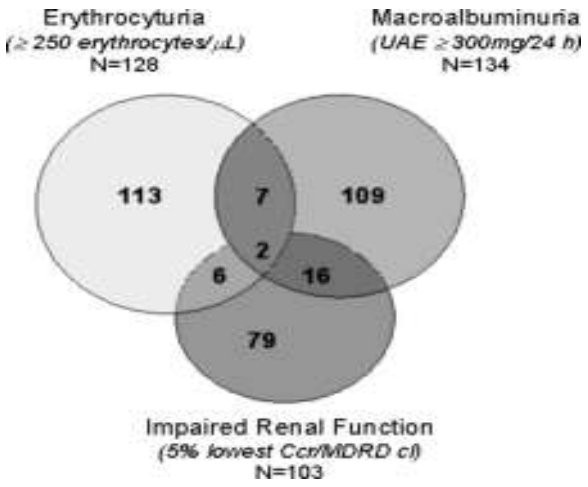


Figure reproduced with permission from the American Society of Nephrology.

In summary, the estimated prevalence of CKD is 4.3 – 13.1% with significant international differences. The prevalence may be rising, at least in the USA. True estimates have been hampered by methodological problems. Given the marked differences in prevalence between age-groups and gender, the actual prevalence in any defined geographical area will be dependant upon its demographic composition.

1.3 Assessment of Kidney Function

In order to define the severity of kidney disease according to the international classification we must be able to estimate GFR and quantify proteinuria accurately. Section 1.3 summarises the key issues surrounding the measurement of these variables.

1.3.1 Measurement of glomerular filtration rate

One of the key measures of kidney function in current use is the glomerular filtration rate (GFR) (the sum of the filtration by all of the glomeruli). GFR is approximately proportional to the total number of nephrons and to the size of the glomeruli. Therefore it is lower in children and small adults. GFR typically declines from the fourth decade onwards, at around 1 mL/min per year (28). In clinical practice, GFR is normalized to body surface area to take account of size differences. Usually body surface area (BSA) is calculated from an equation proposed by Dubois and Dubois (1916) that depends on height and weight, but not age or gender (29). When GFR is normalized to BSA, GFR/1.73 m^2 in young adult men and women is similar and is in the range of 100-120 mL/min.

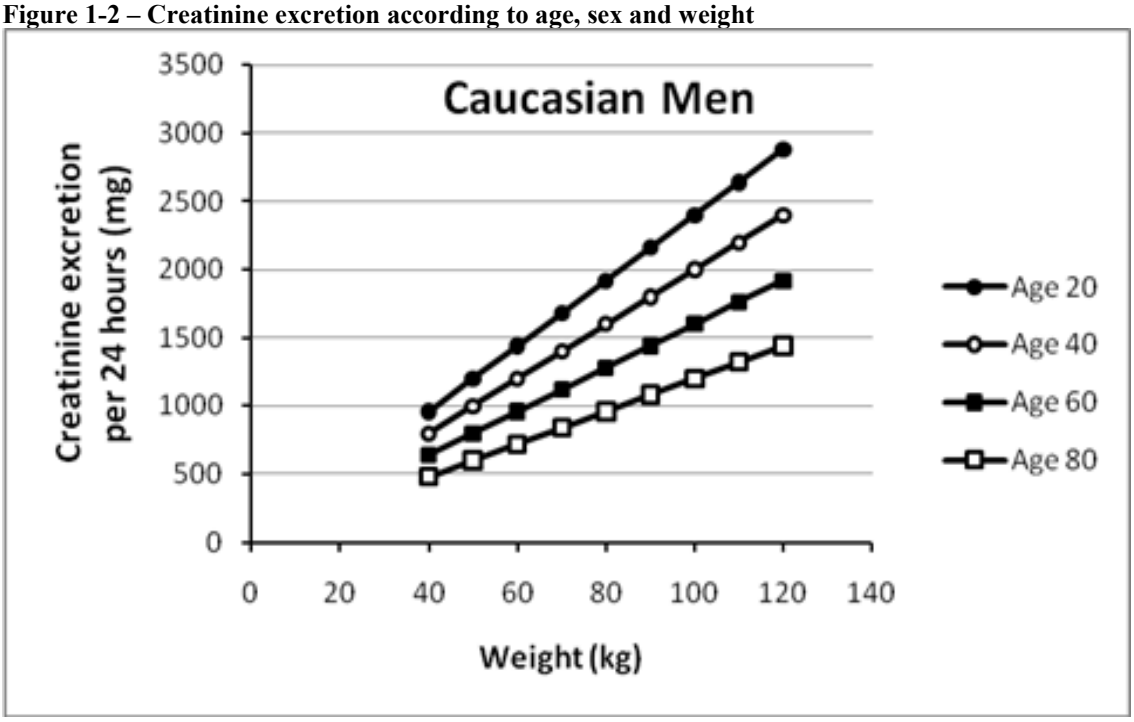
1.3.2 Use of serum creatinine as an endogenous marker of GFR

GFR cannot be measured directly. It can be measured indirectly using the clearance of an exogenous marker substance such as inulin which is the recognised gold standard but is not widely used because of cost and inconvenience. Other exogenous markers are used occasionally in clinical practice but are limited by cost, inconvenience and exposure to radioactivity or iodinated contrast.

Serum creatinine (SCr) is the most widely used endogenous marker of GFR. It is a low molecular weight organic cation (113 Daltons) which is produced at a relatively constant rate in each individual by the non-enzymatic degradation of creatine in muscle. Creatinine

is also derived from dietary intake of meat (either in the form of creatine, or creatinine itself) (30). The plasma level of creatinine is dictated by intake, generation, metabolism and excretion. Creatinine generation varies markedly between individuals mostly depending on their muscle mass (31). Muscle mass depends on body weight, and for any given weight, is higher in men than in women, and African Americans then Caucasians (32). Muscle mass decreases markedly as we age (33): creatinine excretion rate in an 80-year old is approximately half that in a 20-year-old of the same body weight, as shown in figure 1-2. Patients with cachexia, such as those with cirrhosis, will also have a very low creatinine excretion rate (34).

Figure 1-2 – Creatinine excretion according to age, sex and weight



Creatinine is distributed throughout total body water, and is freely filtered by the glomerulus and actively secreted by the tubular cells. For this reason, total creatinine clearance is the sum of GFR and tubular secretion, and so overestimates the GFR. The percentage of creatinine removal by tubular secretion varies with kidney function. When GFR is high, this percentage is relatively small (10 – 40%), but when GFR is low, the contribution of tubular secretion to creatinine clearance becomes more important (50-60%) (35). Tubular secretion of creatinine can be competitively inhibited by the administration

of drugs such as trimethoprim and cimetidine allowing a more accurate estimation of true GFR (36). Extra-renal elimination of creatinine is negligible in individuals with normal renal function, but when GFR is significantly reduced, there is appreciable creatinine removal by other routes such as degradation by intestinal flora (37).

Various equations have been developed over the past 35 years to predict creatinine excretion (as opposed to GFR) based on sex, age and weight, and more recent equations have added race, as follows:

1. Cockcroft and Gault formula (1976) (32):

- 24-hr creatinine excretion (g) = (140-age) x weight (kg) x 0.0002 [x 0.85 if female]

2. Walser formula (1987) (38):

- Male: (28.2-0.172 x age) x weight (kg)
- Female: (21.9-0.115 x age) x weight (kg)

3. Goldwasser formula (1997) (39):

- [23.6-(age/8.3)(+1.9 if black)] x weight (kg)

4. Rule (Mayo Quadratic) Formula (2004) (40)

- {exp[7.26-0.26(if female) – (0.011 x (age – 55) if age>55)]} xBSA/1.73

5. Ix (equation D) (2011) (41):

- 879.89+12.51 x weight (kg) – 6.19 x age + (34.51 if black) – (379.42 if female)

1.3.3 Serum creatinine assays

Most serum creatinine assays are based on the Jaffe colorimetric reaction with alkaline picrate. Various endogenous and exogenous substances (e.g. ketones, glucose, bilirubin) interfere with these colorimetric reactions, giving a falsely high, or less commonly, low serum creatinine. The degree of interference relates to the assay used and cannot be easily corrected for. The non-creatinine chromagens affect the assay most at lower levels of creatinine, when they contribute up to 20% (30). Serum creatinine can be measured using enzymatic methods which have fewer problems with interference, but are more expensive (42). Efforts are being made to adjust all creatinine assays to give results closer to the true serum creatinine concentration by using reference creatinine preparations and reference methods of measurement (isotope dilution mass spectroscopy – IDMS) (43). IDMS-calibrated serum creatinine values tend to be lower (by about 6%) than serum creatinine measured using many of the older methods.

1.3.4 GFR prediction equations

GFR can be estimated from creatinine by a number of methods. Creatinine clearance can be calculated from the creatinine content of a 24-hour urine specimen and a serum creatinine level (measured during the collection period), but this is cumbersome. Therefore various authors have developed equations that, using surrogates for lean body mass (body size, gender, and age, and sometimes race) attempt to predict the GFR from serum creatinine measurement only (as opposed to the estimated creatinine excretion). The ones in common clinical use are shown in Table 1-4. The Cockcroft and Gault (C&G) formula estimates creatinine clearance (32). As creatinine clearance overestimates GFR because of tubular secretion of creatinine, some multiply the estimated creatinine clearance by 0.8 to remove the contribution of tubular secretion and obtain a value closer to the true GFR. It also requires a body weight measurement as it is not normalized to BSA.

The Modification of Diet in Renal Disease (MDRD) formula was derived during the clinical study of the same name, which studied patients primarily with a GFR less than 60 mL/min (per 1.73 m^2) (44). GFR was measured using isotopically tagged iothalamate; iothalamate is a substance that is filtered by the glomerulus but is *not* secreted by the tubules. For a given value of SCr, the GFR was found to be about 26% lower in women than in men, and about 18% lower in Caucasians (men or women) than in African Americans. The race effect is thought to be due to the fact that muscle mass in African Americans tends to be higher and so their creatinine excretion rate is also increased, but altered tubular handling of creatinine may also play a role (45). There are several forms of the MDRD equation as shown below. Few patients with GFR values higher than 60mL/min/1.73m^2 were included in the study cohort, and the MDRD equation is increasingly unreliable if GFR ≥60mL/min/1.73m^2. Above 60mL/min/1.73m^2 the MDRD equation underestimates GFR and has decreased precision. For this reason, some recommend that eGFR values greater than 60mL/min/1.73m^2 estimated using the MDRD equation be reported simply as being >60ml/min/1.73m^2 (46). The MDRD equation is normalized to 1.73 m^2 of body surface area, unlike the Cockcroft and Gault equation, and this must be borne in mind when comparing values obtained using the two methods.

The most recent equation to estimate GFR from serum creatinine was developed by the CKD-Epidemiology Group and was based on a large patient sample that included many patients with GFR >60mL/min/1.73m^2 (47). This CKD-EPI estimate of eGFR is actually a set of 8 equations; the choice of which equation to use depends on whether the patient is male or female, African American or Caucasian, and whether the SCr is in a lower or higher range (see Table 1-4). When eGFR is below 50mL/min/1.73m^2, the MDRD and CKD-EPI equations give very similar results. Above this level, CKD-EPI may be more reliable.

Table 1-4 – Formulae to predict creatinine clearance and estimated GFR

		Year	IDMS Traceable	Equation
Cockcroft and Gault (Cr Cl)		1976	No	(140-age) × (Wt in kg) × (0.85 if female) / (72 × SCr in mg/dL)
MDRD4 (eGFR)		1999	Yes	eGFR=175 x $(0.011312 \times sCr)^{-1.154}$ x $age^{-0.203}$ x 0.742 *(if female)* x 1.212 *(if black)*
MDRD4 (eGFR)		1999	No	eGFR=186 x $(0.011312 \times sCr)^{-1.154}$ x $age^{-0.203}$ x 0.742 *(if female)* x 1.212 *(if black)*
CKD-EPI (eGFR)		2009	Yes	
White/ Other	**Female**			If sCr ≤ 62µmol/L eGFR = 144 x $(Scr/0.7)^{-0.329}$ x $(0.993)^{age}$ If sCr > 62µmol/L eGFR = 144 x $(Scr/0.7)^{-1.209}$ x $(0.993)^{age}$
White/ Other	**Male**			If sCr ≤80µmol/L eGFR = 141 x $(Scr/0.9)^{-0.411}$ x $(0.993)^{age}$ If sCr > 80µmol/L eGFR = 141 x $(Scr/0.9)^{-1.209}$ x $(0.993)^{age}$
Black	**Female**			If sCr ≤ 62µmol/L eGFR = 166 x $(Scr/0.7)^{-0.329}$ x $(0.993)^{age}$ If sCr > 62µmol/L eGFR = 166 x $(Scr/0.7)^{-1.209}$ x $(0.993)^{age}$
Black	**Male**			If sCr ≤80µmol/L eGFR = 163 x $(Scr/0.9)^{-0.411}$ x $(0.993)^{age}$ If sCr > 80µmol/L eGFR = 163 x $(Scr/0.9)^{-1.209}$ x $(0.993)^{age}$

Cockcroft and Gault (32), MDRD formula: IDMS-traceable (48), not IDMS-traceable (44), CKD-EPI Equation (47):

Cystatin C is an alternative endogenous marker of GFR. It is a 13 kDalton protein which is produced at a constant rate by all nucleated cells, freely filtered at the glomerulus, and is not secreted, but is reabsorbed within the tubules where it is completely metabolized (49). It does not vary with muscle mass, gender or age so may be a superior marker of GFR in select groups (50). However it is more costly, assays have not been standardized and is not yet in widespread clinical use.

1.3.5 Limitations of GFR prediction equations

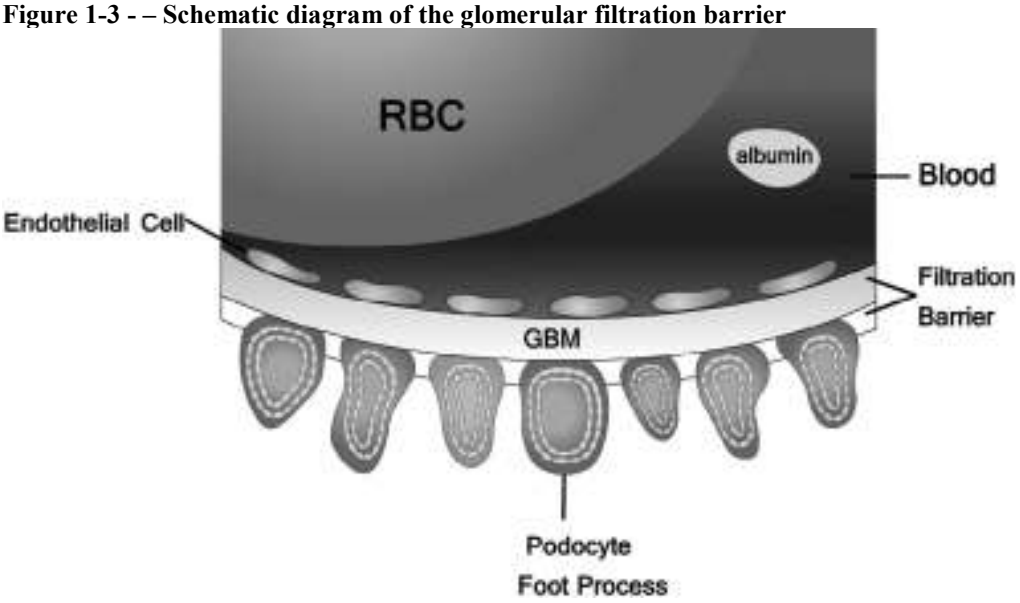
All the prediction equations rely on the assumption that muscle mass can be predicted from demographics such as age, sex and race. They cannot take inter-individual differences in muscle mass into consideration and as such will always remain more-or-less precise estimates of the true GFR. Whether based on serum creatinine or cystatin C, the eGFR prediction equations assume that kidney function is stable at the time it is measured and are unsuitable for use during periods of acute kidney injury. Also, in very lean or cachectic patients with body mass index < 18.5 kg/m^2, both MDRD and C&G tend to overestimate eGFR and creatinine clearance, respectively (51). Obese patients present a particular problem for creatinine-based estimates of GFR. The muscle mass is a lower percentage of total weight than in normal individuals, but is greater than would be predicted by height. If actual body weight is used in the C&G formula, GFR is markedly overestimated, whereas if ideal body weight is used (as recommended by C&G), GFR will be underestimated. Lean body weight gives the closest estimate and can be calculated from height and weight. Cockcroft and Gault, but not MDRD, tends to overestimate clearances in obese patients with BMI > 30 kg/m^2 (52). In the United Kingdom, laboratories currently measure serum creatinine, using either a colorimetric or enzymatic assay, calculate an IDMS traceable value and subsequently an eGFR, using the MDRD formula (21, 43). The significant issues with this technique should be borne in mind when interpreting the results.

1.3.6 Measurement of proteinuria

In health, the glomerulus has a negatively charged, selective filtration barrier, composed of the glomerular capillary endothelium and glycocalyx, the glomerular basement membrane (GBM) and podocytes which prevent most proteins entering the urine (as shown in figure 1-3) (53). Small proteins (typically molecules <4nm diameter) are freely filtered, large proteins are not, and in between the proportion filtered is determined by molecular size, conformation and charge. Large, negatively charged molecules such as albumin (molecular weight 67,000 Daltons) only penetrate the filtration barrier in very small amounts. Subsequently, filtered protein is then almost completely reabsorbed in the proximal tubule, via megalin and cubulin mediated endocytosis (54). A small quantity of protein is actively secreted by the tubular cells. Some proteins, such as uromodulin have physiological roles in urine, including urothelial defence against infection and a potential protective role in interstitial inflammation (55, 56). Normally the total daily urine protein excretion is up to 150-200 mg, and for albumin 30 mg, but most healthy adults excrete substantially less.

Some glomerular diseases affect the function of components of the filtration barrier (such as the podocyte). As this barrier is compromised, increasing amounts of protein appear in the filtrate, overwhelming the tubular reabsorption capacity, thus producing proteinuria.

Figure 1-3 - – Schematic diagram of the glomerular filtration barrier



In disease, the amount of proteinuria may increase dramatically. This may be described as glomerular, tubular or overflow proteinuria. The generally accepted paradigm is that once proteinuria reaches >1 g/day it is the result of glomerular damage with subsequent leakage through the glomerular barrier, and is predominantly albumin. Lower levels of proteinuria may also be albuminuria of glomerular origin but can also be caused by tubular damage, with resultant failure of re-absorption of filtered small physiological proteins ("tubular proteinuria"). Excess circulating protein can overwhelm tubular reabsorptive capabilities (e.g. free light chains in myeloma), and this is termed overflow proteinuria. However there is some emerging evidence of a tubular origin for albuminuria. An American research group studied rats with diabetic nephropathy and controls, using a relatively new technique called 2-photon microscopy. They found the glomerular sieving co-efficient of albumin to be 50 times higher than previously recorded, with no difference between the rats with nephropathy and controls. However, only the diabetic rats had albuminuria, supporting the hypothesis of a tubular source (57). This finding has been fiercely contested by other research groups (58). The terminology to describe the degree of proteinuria, and the equivalent values for albumin and total protein are shown in Table 1-5.

Table 1-5 - Measures of albumin and protein excretion in the urine. The albumin and total protein columns are only approximate equivalents as there is a non-linear relationship between albuminuria and total proteinuria.

	Albumin: creatinine ratio (mg/mmol)	**Albumin Excretion Rate (mg/day)**	**Protein: creatinine ratio (mg/mmol)**	**Protein Excretion Rate (mg/day)**
Normal	<2.5 men * <3.5 women	<30	<15	<150
Microalbuminuria	2.5-30 men 3.5-30 women	30–300	-	-
Proteinuria	>30	>300	50-300	500-3500
Nephrotic range	-	-	>300	>3500

* some recommend a single threshold for males and females of 3mg/mmol

1.3.7 Quantification of urine protein

Urine protein can be quantified by a variety of techniques, including urine dipsticks, laboratory quantification of timed urine collections (typically 24-hour collections or fractions of this) and spot urine samples (which may be first morning void or a random sample). Laboratory quantification may be of total protein or albumin, and there are a number of assays in use worldwide. There are a number of aspects to be considered when evaluating these different techniques.

Firstly, urine dipstick tests for total protein or albumin are cheap and easy to use. They utilize chemical or immune reactions to generate color changes in reagent pads. The colour is assessed by the operator, which has been shown to be operator dependant, or read by a machine (which improves reliability but raises cost) (59, 60). Dipsticks have four main disadvantages. Firstly, they measure concentration rather than quantity. Urine volume varies widely according to hydration status and osmotic load. Therefore, if the urine flow rate is high, significant proteinuria may be missed or, conversely, over-diagnosed at low urine flow rates. In order to improve this intra-individual variation, a creatinine test pad has been added to some dipsticks to give a measure of urine concentration. This appears promising, but requires further study (61). The second shortcoming is that total protein dipsticks typically detect a protein concentration of >0.15 g/L, but are less sensitive to some non-albumin proteins such as immunoglobulin light chains (Bence-Jones protein) (62, 63). The major manufacturers of these urine dipsticks (Roche and Siemens) use different cut-offs and a different number of concentration categories, which further confuses interpretation of the results. Thirdly the recording of dipstick urinalysis findings is less formal than a laboratory based record and may not be available for subsequent comparison. And lastly the use of dipstick quantification of urine protein is not subject to the same rigorous quality control as laboratory methods. Currently, they are not

recommended for routine screening for proteinuria in clinical practice because of a significant high false negative rate (7, 8, 64).

They do have two main advantages. They do not require laboratory equipment or electricity so can be used in remote or resource-poor settings. Also, they provide an assessment of non-visible haematuria which can be an important additional diagnostic feature of glomerular disease.

In the laboratory, total proteinuria is generally measured by a colorimetric or turbidimetric method, because of the variety of proteins being measured. Each assay has differing sensitivities for different proteins, making comparison between assays difficult. Total protein assays have a lower precision than albumin assays and are difficult to standardize. Urinary albumin is usually measured by immunoassays which measure albumin specifically. Urine is a hostile and highly variable biochemical environment with a wide range of pH and osmotic concentration. Consequently, there is a wide variety of albumin species and fragments within urine, which make assay standardization a challenge, particularly as the prognostic impact of these different moieties is not well-defined. High-performance liquid chromatography measures consistently higher values for albumin than immunoassays, as it also measures some albumin fragments, and may therefore allow earlier detection of microalbuminuria (65). Nevertheless, currently there is no international reference method or reference material for urine albumin assays (66). A reference method for albuminuria based on liquid chromatography mass spectrometry is being developed by the U.S.-based National Kidney Disease Education Project (NKDEP) and other groups.

Protein excretion varies diurnally and with posture, being lowest overnight and when supine. Some adolescents have transient proteinuria when upright, which disappears when supine, known as orthostatic proteinuria, which usually resolves (67). Transient proteinuria

can also develop in response to fever, exercise and other stressors. A 24-hour urine collection is traditionally the accepted gold standard for measuring proteinuria, as it is unaffected by diurnal variation. Other timed urine collections (e.g. overnight) have also been used. However, timed urine collections have a number of drawbacks. Firstly, they are very inconvenient for patients leading to poor collection rates; in one clinical study only 59% of patients provided the desired overnight sample, and in general clinical practice the collection rate may well be lower (68). Secondly, they are often poorly performed, with incomplete collections leading to substantial inaccuracy (69, 70). Lastly, they are considerably more time consuming to analyse in the laboratory and therefore more expensive, with one study finding the cost of analysing a spot sample to be only 15% of that of a 24-hour collection (71).

Spot urine samples can be analyzed for total protein or albumin. Although more accurate than dipsticks, this still generates a concentration rather than a quantity. Creatinine is excreted in urine at a relatively constant rate (30). Thus, if creatinine concentration is also measured, a TPCR or ACR can be calculated to adjust for urine concentration. There are small studies over the past 20 years assessing the correlation between TPCR and 24-hour urinary protein excretion in a variety of populations, including patients with CKD, rheumatology out-patients and kidney transplant recipients, which show that TPCR performs reliably to quantify significant proteinuria (69, 72-76). The use of TPCR in pregnant women has also been studied and found to be a reliable test (77, 78). A systematic review, published in 2005 supported the use of TPCR in place of 24-hour urine collections to “rule-out” significant proteinuria, however 10 of the 16 included studies were of pregnant women, and only 216 patients with CKD were included in total (79). ACR also correlates well with 24-hour urinary albumin excretion, though the research focuses almost exclusively on diabetic renal disease (68, 80-82). Spot samples from the first micturition after rising are recommended as results from them correlate well with 24-hour excretion

(83). However, a random daytime sample will usually give acceptable accuracy (84). Inadequate urine volume and freezer storage of specimens may lead to under- and over-estimation of urine albumin respectively (85). As discussed above, creatinine excretion rate varies markedly with muscle mass. In particular, women and the elderly have a lower creatinine generation rate and this artificially inflates the ratio of protein or albumin to creatinine. For example, an elderly woman with low muscle mass and consequently low urine creatinine excretion will have a substantially higher albumin: creatinine ratio than a bodybuilder with the same 24-hour albuminuria because his urine creatinine excretion will be 2 – 3 times higher. Some use a higher diagnostic threshold for ACR in women to partially address this. A similar issue is likely to occur with different races, but there is less evidence available (86). Therefore in patients with abnormal muscle mass and resultant abnormal creatinine generation, ACR or TPCR may give misleading results, and a 24-hour urine estimation should be considered.

1.3.8 Clinical applications of urine protein quantification

Accurate measurement of proteinuria may be desirable for a number of reasons. It is a core feature of renal disease and as such may be utilized in the diagnosis of kidney disease and, in particular, glomerular disease (87). Repeated measurements of proteinuria may be useful to monitor the natural history of glomerular disease. Proteinuria is also an indication for kidney biopsy, and total proteinuria >1 g/day is commonly used as a threshold. This threshold will be affected by the overall clinical picture e.g. if associated with haematuria, a lower threshold of 450 mg/day may be used (9). Proteinuria thresholds (>0.5-1 g/day) may also be used as indications for treatment with angiotensin-converting enzyme inhibitors (discussed below in detail), or immunosupression in specific glomerular disease. Subsequent response to these therapeutic strategies can also be monitored by quantifying ongoing proteinuria.

In summary, proteinuria is a cardinal sign of kidney disease, and accurate quantification is essential for the diagnosis, monitoring and treatment of renal disease. There are multiple current methods by which this may be achieved.

1.3.9 Recommendations on the measurement of proteinuria

In diabetic kidney disease, albuminuria is used for screening, diagnosis and monitoring. Studies of the natural history of diabetic nephropathy promoted the concept of microalbuminuria as an early marker of nephropathy and subsequent intervention studies measured albuminuria, which has resulted in it becoming the accepted marker in patients with diabetes. However the early studies did not examine the utility of TPCR levels below the laboratory reference range for predicting outcomes (88, 89).

In terms of the measurement of albuminuria, a spot sample for ACR is most convenient, but albumin concentration or 24-hour urine excretion is still used by some. The ACR appears to be a better predictor of renal outcomes in diabetics than urinary albumin concentration or 24h urinary albumin excretion (90). First morning voids are more reliable than random spot urines to monitor microalbuminuria (83).

In non-diabetic kidney disease, it is controversial whether total proteinuria or albuminuria is the more appropriate test to screen for kidney disease. Most research studies have measured 24-hour urinary total protein excretion, and thresholds for risk, investigation and intervention have arisen from these studies (91). Measurement of albuminuria has the theoretical, technical and clinical advantages outlined above. The theoretical paradigm underpinning this is that by using ACR one can measure albumin as a marker of risk (i.e. the signal), without measuring physiological proteins (i.e. noise), thus increasing sensitivity by minimising the noise: signal ratio. This assumes that the quantity of non-albumin proteinuria adds no additional information to albumin, and that the quantity of

physiological proteinuria is irrelevant to risk. It is not known what level of risk is carried by proteinuric patients with low levels of albuminuria but high levels of non-albumin proteinuria. Microalbuminuria may not be reliably detected by total protein assays but there is limited evidence about which non-diabetic patients should be screened for microalbuminuria, and what treatments should be used.

There are various guidelines that make recommendations about the measurement of proteinuria. All of them accept the use of spot samples, corrected for urine creatinine, as screening tests for proteinuria, but this is not universally accepted in the literature (92, 93). The NICE guidelines for England and Wales and the NKF-KDOQI guidelines in the United States of America both recommend the universal use of ACR for diabetic and non-diabetic CKD. They add the caveat that TPCR may be used at elevated levels of ACR assuming that the predominant urine protein at these higher levels is albumin (5, 8). Conversely, the Scottish Intercollegiate Guidelines Network, the UK CKD guidelines, the Welsh Renal National Services Framework recommend TPCR for non-diabetic kidney disease, and reserve the use of ACR for diabetic kidney disease only (7, 9, 94).

There are few data directly comparing ACR and TPCR in a kidney disease population. The AusDiab study assessed both in a large cross-section of the general population, Collier at al assessed the performance in a small sample of 117 clinic patients and Birmingham et al focused exclusively on a lupus nephritis cohort (95-97). They found a non-linear relationship between albuminuria and total proteinuria. None of these studies reported renal or patient outcomes.

A recent study took a different approach and assessed the utility of the albumin: protein ratio (APR), derived by dividing the ACR by TPCR. The hypothesis was that a low APR (i.e. a high proportion of non-albumin proteins) is predictive of tubulo-interstitial disease

on renal biopsy; they found the cut-point to be 0.4, below which the test had an excellent sensitivity and specificity for interstitial disease (98).

1.4 Progression of CKD

In the preceding sections the definition and classification of CKD, along with issues regarding optimal measurement of eGFR and proteinuria have been discussed. These aspects are essential for the correct diagnosis of CKD, however the most important purpose of these measurements is to inform prognosis, both renal outcome and patient mortality. In this section the important predictors of renal progression are discussed, and the following section deals with cardiovascular and all-cause mortality in patients with CKD.

The most commonly reported outcome measure of renal disease is the commencement of RRT. This has strengths and weaknesses. It is easy to define and is recorded accurately and therefore easy to document, but it is a treatment not a clinical state and therefore cannot take account of those who do not undergo RRT when clinically indicated because of extenuating circumstances. The initiation of RRT is also clinician and patient dependant and therefore not reproducible. An alternative measure of renal progression is doubling of serum creatinine, which correlates with a fall in eGFR of 50% within the individual. The third frequently used measure is eGFR slope. The measurement of eGFR slope and doubling of serum creatinine are both vulnerable to over interpretation in the context of acute kidney injury, eGFR slope more so. This will be more problematic in clinical databases than clinical trials with pre-specified measurement points. The ascertainment of all-cause mortality is the least problematic (assuming complete recording of deaths, as in the UK (99)), however the cause of death may bear no relationship to kidney disease (a high noise: signal ratio). Cardiovascular mortality has a stronger correlation with renal disease, as described in the following section, but may be inaccurately recorded (100).

1.4.1 Proteinuria

Proteinuria is the single strongest predictor of progressive renal disease (101). It usually reflects primary glomerular injury and subsequent tubular toxicity due to exposure to large amounts of filtered protein. This leads to interstitial fibrosis and atrophy, reduced nephron mass, subsequent intra-glomerular hypertension in the remaining glomeruli and progressive injury (102). The role of albuminuria and proteinuria in the progression of renal disease has been studied in a variety of populations. The presence of albuminuria is an independent predictor of the development of de-novo renal impairment (eGFR<60ml/min/1.73m^2) in the general population (103). In a non-diabetic, hypertensive population microalbuminuria was also found to be an independent predictor of development of chronic renal insufficiency with a relative risk of 7.61 (95% CI 3.19 – 8.16) (104). Macroalbuminuria is a superior predictor of renal progression, than a reduced baseline eGFR itself (27). In the multiple risk factor intervention trial (MRFIT) of 12,866 men at high risk of heart disease, dipstick proteinuria of ≥2 + was associated with an adjusted hazard ratio of 14.21 (95% CI 9.16 – 22.05) for developing established renal failure, and 1+ protein on dipstick with an adjusted hazard ratio of 2.30 (95% CI 1.28 – 4.13) (105). This finding was supported in a general population screening study in Japan, of 107,192 participants which found an adjusted odds ratio of 14.9 (95% CI 10.9 – 20.2) for dipstick proteinuria to predict development of end stage renal disease (106). An analysis of renal outcomes in the Norwegian HUNT II study found that using eGFR criteria alone identified 4.7% of the general population at risk of ESRD and correctly identified 69.4% of those progressing to ESRD, whereas combining eGFR and albuminuria refined the predictive ability with only 1.4% of the population identified at risk but without a significant loss of sensitivity (107). The CKD Prognosis Consortium performed a meta-analysis of 13 studies (21,688 participants) of cohorts with CKD and found albuminuria to be a strong independent predictor of ESRD, adding additional information to that derived

from eGFR measurement (108). This evidence has contributed to the decision to add albuminuria to the staging of the CKD classification system.

There is also strong evidence of the role of albuminuria in the progression of diabetic kidney disease. Baseline proteinuria is an independent predictor of renal outcome in nephropathy associated with Type 1 diabetes mellitus (109) and Type 2 diabetes mellitus (110). The presence of microalbuminuria has been found to predict the development of clinical proteinuria (88) and is now widely used as a screening test in diabetes (111). However the relationship between albuminuria and progressive renal decline in patients with diabetes is not uniform. Varying patterns of renal injury have been observed in patients with microalbuminuria: in a study of renal biopsies of diabetic patients with preserved excretory renal function and a median albumin excretion rate of 44micrograms/min, only one third were found to have the "typical" histological features of diabetic nephropathy, one third showed normal or near normal biopsy appearances and the remaining third were described as atypical with only mild glomerular changes but severe tubulo-interstitial changes (112). Another report described the phenomenon of non-albuminuric renal insufficiency in Type 2 diabetes mellitus; in their cohort 39% of those with an eGFR<60ml/min/1.73m^2 had normoalbuminuria but developed progressive renal decline at the same rate as those with micro- and macroalbuminuria (113).

There is abundant evidence of a pivotal role for albuminuria and proteinuria play in the progression of renal disease, as outlined above. However there are very sparse data comparing them as predictors of outcome. A recent post-hoc analysis of the Reduction of End Points in Non-insulin-dependant Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) study compared 24-hour urine total protein excretion (UPE), 24-hour urine albumin excretion (UAE), urine albumin concentration (UAC) and albumin:creatinine ratio (ACR) as predictors of a renal event (composite of doubling of serum creatinine and end-stage renal disease). The hazard ratios are shown in Table 1-6.

Disappointingly urine total protein concentration was not measured on the spot samples so total protein:creatinine ratio could not be included in the analysis. No mortality data were included (90).

Table 1-6 - Hazard ratios for renal event according to the type of urine protein measurement (90)

Measurement	Hazard ratio for renal event
UPE	3.02 (2.53 – 3.62)
UAE	3.16 (2.60 – 3.86)
UAC	3.23 (2.67 – 3.91)
ACR	4.36 (3.50 – 5.45)

1.4.2 Use of proteinuria as a surrogate end point

The progression of CKD can be slow therefore hard end points such as development of established renal failure may be very distant events from disease onset and their use as trial end points may prevent early identification of preventative strategies. Given the strong relationship between proteinuria and progression of renal disease, it has been suggested that the reduction in proteinuria achieved by medical intervention should be used as a surrogate end point for established renal failure in clinical trials. However, the United States Food and Drug Administration (FDA) has refused to accept proteinuria as a surrogate endpoint. It accepts doubling of serum creatinine as a surrogate endpoint, using the rationale that this predicts the onset of established renal failure. In contrast, proteinuria is not a necessary intermediate step in the path to ESRD: a patient can develop advanced kidney disease requiring dialysis without ever having had proteinuria so its modification may not be considered a valid surrogate endpoint. Others argue that in proteinuric renal disease, modification of proteinuria is the single strongest predictor of outcome (114), and therefore is valid. This has been challenged in studies of diabetes, where the assumption was that reduction of microalbuminuria was roughly equivalent to the reduction in risk of ESRD (115). In the ACCORD microvascular study of type 2 diabetes (114), microalbuminuria was reduced 21% by improved glycaemic control, but ESRD incidence fell by only 5% (114). The use of surrogate markers in other areas of medicine has also come under close scrutiny recently, prompted by the withdrawal of rosiglitazone from the market, which had performed well when glycated haemoglobin was used as the surrogate endpoint rather than survival (116). A scientific work group was established under the auspices of the National Kidney Foundation and the FDA and concluded that proteinuria could not be used as a surrogate endpoint currently, with a small number of specific exceptions (117).

1.4.3 Interventions to reduce proteinuria

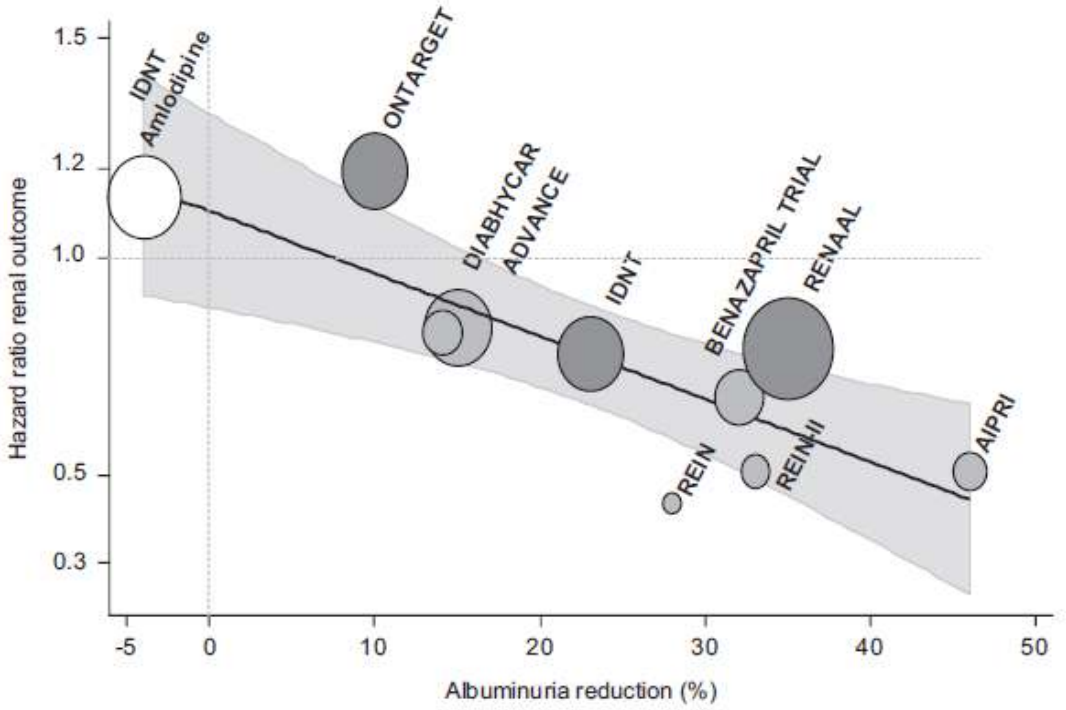
There is a strong relationship between hypertension and proteinuria. Reducing systemic blood pressure results in a reduction in urine protein excretion and both are major therapeutic targets. Blood pressure will be discussed in more detail in the next section. The use of agents that interfere with the renin angiotensin aldosterone system reduce proteinuria to a greater extent than is accounted for by their blood pressure lowering ability alone. The administration of angiotensin converting enzyme inhibitors (ACEi) has been shown to reduce progression of renal disease in a large number of randomised control trials in non-diabetic renal disease and diabetic nephropathy associated with Type 1 and Type 2 diabetes (118-121). There are racial differences in the effect of blockade of the renin angiotensin aldosterone system, but ACEi have been shown to be effective in reducing proteinuria and renal decline in African Americans with renal disease (122). The greatest benefit is seen in those with the highest baseline urine protein excretion (118), and the residual level of proteinuria, achieved following initiation of therapy, predicts subsequent renal outcome (110, 123). However low risk patients, with proteinuria quantified as <0.5g/24 hours, do not seem to derive any additional benefit from the administration of ACEi, in terms of renal progression (124).

Another class of drugs targeting the renal angiotensin aldosterone system was introduced more recently: angiotensin receptor blockers (ARB). They have been shown to have blood pressure-independent renoprotective effects in the RENAAL trial and Irbesartan Diabetic Nephropathy Trial (IDNT) (125, 126) and be as effective as ACEi in reducing proteinuria and progression of renal disease (127). A post hoc analysis of the RENAAL trial specifically assessed the interplay of the acute haemodynamic effect of the drug on glomerular filtration rate and long-term outcome. It found that the greater the initial fall in eGFR, the slower the rate of long-term eGFR decline, independent of blood pressure or albuminuria (128). It has also been shown that increasing the dose of ACEi to doses higher

than those usually recommended for blood pressure lowering can have additional anti-proteinuric effects (129-131) but only one study had sufficient follow-up to show a reduction in the rate of ESRD (132).

There is a linear relationship between the reduction in albuminuria achieved by inhibitors of the renin angiotensin system and the reduction in risk of an adverse renal outcome, as demonstrated in figure 1-4 (133).

Figure 1-4 - Relationship between reduction in albuminuria and subsequent risk of renal outcome, compared with placebo in a number of landmark trials of renin angiotensin system blockade (133)**.**



Dark grey; ARB trial or combination ACEi and ARB, Light grey; ACEi trial, White; calcium channel blocker trial, grey area represents 95% confidence intervals.

The following trials are included in this figure: IDNT Amlodipine (126), ONTARGET (134), DIABHYCAR (135), ADVANCE (136), IDNT (126), Benazapril trial (132), REIN (137), REIN-II (138), RENAAL (125), AIPRI (139). (132)

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The combined use of ACEi and ARB should be of theoretical benefit as it should result in more complete blockade of the renin angiotensin aldosterone system. This has been assessed in a meta-analysis which confirmed an overall additional 25% reduction in albuminuria (140). However these studies did not titrate to the maximal anti-albuminuric dose of ACEi or ARB prior to introducing the second agent. Therefore the same effect may have been achieved by larger doses of one or other agent rather than the combination (133). Furthermore, the greatest benefit from dual blockade is derived in those who respond well to a single agent; adding a second agent in poor responders is of limited utility. During the conduct of the meta-analysis, the published results of one of the major studies of dual renin angiotensin blockade in non-diabetic renal disease (the COOPERATE study published in the Lancet (141)) were found to be inconsistent and the study has subsequently been retracted (142). This cast some doubt on the utility of the combination of these agents, and this has been further undermined by the findings of the Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET). This is a large study of 25,620 participants at high cardiovascular risk who were randomised equally to ramipril (ACEi), telmisartan (ARB) or combination therapy. There was no reduction of cardiovascular outcomes in the combination group, but a larger number reached the primary and secondary renal endpoints (composite endpoint of death, dialysis and doubling of serum creatinine or dialysis and doubling of serum creatinine respectively) (134, 143). There was a particular increase in the need for dialysis for acute kidney injury. Proponents of dual renin-angiotensin blockade have highlighted the fact that this was a study of patients at high cardiovascular risk, and not specifically a study of patients with kidney disease, that the mean albuminuria at baseline was only 0.81 – 0.83mg/mmol and that there was a trend towards benefit in the subgroup with macroalbuminuria. Does this study indicate that the loss of renal auto-regulation with dual renin angiotensin system blockade outweighs the anti-proteinuric benefits in those at low renal risk? Should we limit the use of dual blockade to the patients with kidney disease and

proteinuria or does this trial have more far-reaching consequences? Further studies addressing this issue in patients with CKD and diabetes are ongoing (144, 145).

The use of ACEi and ARB prevents suppression of renin via the negative feedback loop mechanism. This results in increased plasma renin activity and can cause activation of the renin angiotensin system. As a result of this observation, a new class of drugs called direct renin inhibitors has been developed. Aliskerin has been shown to reduce albuminuria in combination with losartan, independently of blood pressure lowering effects, in patients with diabetic nephropathy (146). In the aliskerin trial in type 2 diabetes using cardio-renal disease endpoints (ALTITUDE), aliskerin was added to ACEi or ARB therapy in patients with type 2 diabetes and either albuminuria or both reduced eGFR *and* cardiovascular disease (147). However safety concerns have been raised and treatment in ALTITUDE was discontinued on the recommendation of the data monitoring committee following a statistically significant excess of strokes in the aliskerin group. All events have not yet been collected and adjudicated and the full results are awaited (148).

Other strategies that have been shown to work in conjunction with ACEi or ARB to reduce proteinuria include mineralocorticoid receptor blockade (MRB), a low to moderate sodium diet or diuretic therapy (149, 150). One trial comparing anti-proteinuric strategies found the combination of an ACEi and MRB (spironolactone) resulted in a greater reduction in proteinuria than an ACEi plus ARB (42% versus 16%) (151) There was also an arm in this trial including an ACEi, ARB and MRB, which resulted in a 48% reduction in proteinuria, however 2 of 11 participants developed marked hyperkalaemia ($K \geq 6.0$mmol/L). Eplerenone (a selective aldosterone antagonist) has also been shown to be effective at reducing proteinuria, in conjunction with an ACEi, in patients with diabetes (152). Combinations of these strategies result in further reductions in albuminuria, such as the combination of losartan plus low sodium diet plus hydrochlorothiazide led to a 70%

reduction in proteinuria in one trial and reduction in dietary sodium can transform a non-responder to ACEi or ARB into a good responder (150).

Non-dihydropyridine calcium channel blockers may also have a beneficial effect on proteinuria when used in conjunction with an ACEi (153). They are effective anti-hypertensive agents in renal disease and have a superior anti-proteinuric effect when compared to dihydropyridine calcium channel blockers (such as amlodipine) and some advocate their greater use in renal disease (154).

There are a number of other agents including endothelin antagonists and transforming growth factor beta antagonists that are currently being investigated in clinical trials and animal models (155).

1.4.4 Hypertension

Hypertension is common in patients with CKD, and is associated with poorer outcomes (156). Experimental studies have shown that systemic hypertension is transmitted to the glomeruli and subsequent glomerular hypertension is damaging to the kidney (157) This increased glomerular capillary pressure leads to accelerated decline in kidney function. Tight blood pressure control slows progression of renal disease in proteinuric patients (156). In particular, the use of renin-angiotensin system blockade normalises the intra-glomerular hypertension, by attenuating the vasoconstrictive effects of angiotensin II on the efferent arteriole. These drugs also reduce proteinuria to a greater extent than their blood pressure lowering abilities alone, as described in the preceding section. The relationship between systemic hypertension and urine protein losses is strong, and the presence of significant proteinuria requires tighter blood pressure targets.

Guidelines recommend maintaining systolic blood pressure (SBP) at 120-139 mmHg, and diastolic blood pressure (DBP) <90 mmHg in all patients with CKD (8). In patients with proteinuria (TPCR ≥100 mg/mmol; ACR ≥70 mg/mmol), and/or diabetes mellitus, SBP should be kept at 120-129 mmHg and DBP <80 mmHg (7, 8).Reducing SBP below 100-110 mmHg may be detrimental (156).

1.4.5 Dyslipidaemia

Low levels of high density lipoprotein (HDL) have been shown to be an independent predictor of the development of CKD (158), although a post-hoc analysis of the MDRD study showed that the relationship between low HDL and development of kidney failure was attenuated on multi-variate modelling (159). A meta-analysis of statins and albuminuria found that they may have a beneficial effect on pathologic albuminuria, but the quality of the evidence was poor (160). Treating dyslipidaemia in CKD, using an HMG Co-A reductase inhibitor (statin), with or without a selective cholesterol absorption inhibitor (e.g. ezetimibe), has not been shown to retard the progression of CKD in the study of heart and renal protection (SHARP) and the protection against nephropathy in diabetes with atorvastatin (PANDA) respectively (161, 162).

1.4.6 Glycaemic control

Diabetic nephropathy is a leading cause of established renal failure requiring dialysis (12). Optimal glycaemic control in Type 1 diabetes mellitus has been shown to retard the onset and progression of kidney disease in the Diabetes Control and Complications Trial (DCCT) (163). This effect has been sustained during the observational follow-up period following the formal end of the trial (the Epidemiology of Diabetes Interventions and Complications study) (164). A similar effect has been shown in patients with type 2 diabetes mellitus in the UK Prospective Diabetes Study (UKPDS) (115). No other

therapeutic intervention has been shown to be as effective for the primary prevention of diabetic nephropathy (165).

1.4.7 Obesity

Obesity is associated with the development of CKD, independent of possible confounders. There is some disagreement as to the optimal measure of obesity; some investigators have shown an elevated body mass index to be associated with CKD (166), while others have demonstrated that waist: hip ratio is a superior measure of obesity as a predictor of progressive CKD (167). Surgical and non-surgical weight loss seems to improve blood pressure, reduce proteinuria and reduce hyperfiltration in the kidney. It is not yet known if these improvements in surrogate outcomes will translate into long term benefits (168).

1.4.8 Anaemia

Anaemia is commonly associated with CKD as a result of reduced production of erythropoietin (EPO) from the peritubular cells. It is thought to cause tissue hypoxia and promote fibrosis. Studies using recombinant human EPO or erythropoietin stimulating agents to correct the anaemia of CKD have not shown any beneficial effect on progression (169-171).

1.4.9 Metabolic acidosis

Metabolic acidosis in renal disease results from reduced ammonia production and proton excretion in the tubules. It is associated with progression of renal disease (172). Recently it has been shown that correcting metabolic acidosis using oral bicarbonate supplementation slows the progression of CKD. This may be as a result of reduced maladaptive compensatory changes in the remnant tubules of the reduced renal mass (such as increased

ammonia production and subsequent complement activation causing tubulo-interstitial damage) (173).

1.4.10 Uric acid

In healthy individuals uric acid is excreted by the kidney; consequently, serum levels rise in kidney disease. There has been much debate if raised serum uric acid concentration is merely a marker of reduced glomerular filtration, or implicated in the causal pathway. An elevated uric acid has been shown to be an independent risk factor for the development of chronic kidney disease in general population cohorts (174, 175). However a post hoc analysis of the MDRD study found that uric acid was not associated with progression of established CKD (in this analysis it was not an independent predictor for developing established renal failure) (176). This finding has been confirmed in other studies (177).

However a recent trial of allopurinol did find that its administration slowed the progression of renal disease in a small cohort of patients with established CKD (178). Further data are needed to confirm this finding.

1.5 Cardiovascular disease, survival and CKD

The incidence of cardiovascular disease in patients with established kidney disease requiring dialysis is 20 – 100 times higher than in the general population and a leading cause of death (3). There is also an increasingly recognised relationship between less severe CKD and cardiovascular disease (20, 179). This increased risk may reflect a clustering of traditional risk factors such as hypertension and hyperlipidaemia, or may be related to factors unique to renal disease including proteinuria and CKD mineral bone disorders (CKD MBD).

There is abundant evidence of the efficacy of interventions to prevent cardiovascular events in the general population. Given the paucity of such evidence in CKD, it is tempting to extrapolate from general population evidence and conclude that patients with CKD, a group at high cardiovascular risk, will derive great benefit from these interventions. However, to date, clinical trials have given conflicting results, and adequately powered trials of interventions in specific kidney disease cohorts are essential to guide our future management. A number of these factors are discussed in the sections below.

1.5.1 Traditional risk factors for cardiovascular disease: Hypertension

The relationship between hypertension and increased risk of cardiovascular disease has been clearly demonstrated in the general population (180). Hypertension is more common in patients with CKD, compared to the general population (age and gender adjusted odds ratio 2.1 [95% CI 2.0 – 2.2]) (26) In patients with CKD the relationship with blood pressure is complex, as uncontrolled hypertension causes proteinuria and progression of renal disease, both of which are associated with increased cardiovascular risk. Therefore control of hypertension is a major priority in CKD. It is also assumed to be beneficial in reducing cardiovascular risk in proteinuric and non-proteinuric CKD, although there is little direct evidence.

1.5.2 Dyslipidaemia

Hyperlipidaemia is a well recognised cardiovascular risk factor in the general population, and treatment with a statin has been shown to reduce cardiovascular death (181). The response to statin therapy in CKD is more complex. Neither atorvastatin nor rosuvastatin has been shown to be beneficial in patients requiring dialysis in the Deutsche Diabetes Dialyse Studie (4D) and a study to evaluate the use of rosuvastatin in subjects on regular haemodialysis (AURORA) respectively (182, 183). However the recently published

SHARP showed a 17% relative risk reduction in first major atherosclerotic event using simvastatin plus ezetimibe in patients with pre-dialysis and dialysis-dependant CKD (161).

1.5.3 Diabetes Mellitus

Diabetes mellitus is a major cause of cardiovascular disease, and a patient with diabetes has an overall risk of death from myocardial infarction three times that of the general population (184). While patients with diabetes (especially type 2) often have a clustering of cardiovascular risk factors, hyperglycaemia itself has been shown to be directly linked to macrovascular disease with a linear relationship (185). The presence of diabetic nephropathy (with albuminuria) is associated with an even greater risk of death (186). In patients with diabetes, control of blood pressure is the most important intervention to reduce cardiovascular events, while glycaemic control improves microvascular complications as described above (187). Once a patient with diabetes has developed established renal failure, the risk of cardiovascular mortality can be reduced by kidney transplantation, and it appears that survival may be improved further by a simultaneous kidney and pancreas transplant (188).

1.5.4 Cigarette smoking

There are few studies assessing cigarette smoking as a modifiable risk factor for cardiovascular disease in patients with CKD. An American study of incident dialysis patients who were current smokers found smoking to be associated with a 37% increase in mortality, after adjustment (189).

1.5.5 Obesity and Physical inactivity

There is a plethora of evidence regarding the link between obesity, physical inactivity and cardiovascular disease in the general population (190, 191). However there is no direct

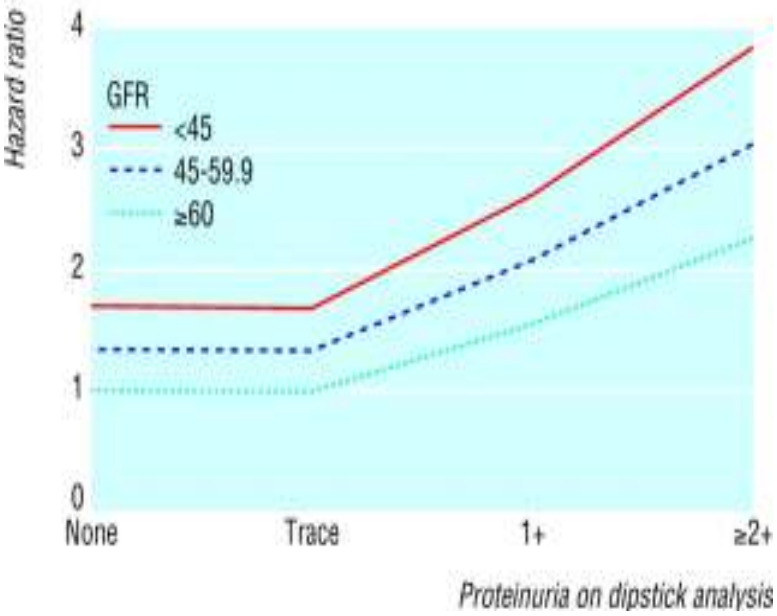
evidence regarding the impact of these factors, and any subsequent interventions, on the development of cardiovascular disease in patients with CKD. There is a paradoxical relationship between weight and mortality in patients receiving dialysis therapy, with obese patients demonstrating a survival advantage (192). There has been a large amount of speculation regarding causation, but it most likely reflects obesity as a marker of relative health, while weight loss occurs in the unwell patients (for instance those with frequent infections or inflammatory conditions).

However given the prevalence of obesity and cardiovascular disease in patients with CKD not requiring dialysis, and the limited number of effective interventions, it is widely accepted that healthy eating and weight control are desirable (8).

1.5.6 Novel risk factors for cardiovascular disease in CKD: Proteinuria

Studies have demonstrated the strong independent relationship between proteinuria and cardiovascular disease and mortality. The Framingham general population cohort showed that dipstick proteinuria in a casual urine specimen was an independent predictor of cardiovascular and all-cause mortality (193, 194). The MRFIT cohort of men at high cardiovascular risk also measured dipstick proteinuria on casual urine specimens and found the same relationship (195). Tonelli et al demonstrated the additive mortality effect of dipstick proteinuria at any level of eGFR in people with coronary disease, as shown in figure 1-5. However a study of 13,177 community dwelling adults over 75 years old in the UK, found dipstick proteinuria to be independently associated with all-cause but not cardiovascular mortality (196).

Figure 1-5 - Adjusted risk of all cause mortality according to proteinuria and kidney dysfunction (197)



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Other studies have used laboratory quantification of albuminuria, rather than dipstick. The Alberta Kidney Disease Network used province-wide laboratory data to assess the relationship and found ACR to be an independent predictor of myocardial infarction and all-cause mortality (198). They also studied dipstick proteinuria and found the same relationship. Other studies of laboratory measures of albuminuria have shown the same independent relationship with vascular disease (199), and confirmed the relationship in subgroups such as the elderly (200). A meta-analysis of proteinuria and coronary risk, including 26 studies found a risk ratio of 1.47 (95% CI 1.23 – 1.74) for all proteinuria, with a significant dose-dependant effect for micro- versus macroalbuminuria (201).

Recently the CKD Prognosis Consortium published a meta-analysis of the relationship of albuminuria and eGFR with all-cause and cardiovascular mortality in general population cohorts. Over 1.2 million participants were included from 22 studies, with >100,000 urine ACR measurements (the remainder being dipstick). This confirmed the independent gradated linear relationship between albuminuria (using both ACR and dipstick) and cardiovascular mortality. There was no significant interaction with eGFR. The threshold value of ACR above which there was an association with increased risk was notably low at 1.1mg/mmol (20). The CKD prognosis consortium went on to confirm this independent relationship between albuminuria and cardiovascular mortality in a further meta-analysis of CKD cohorts (108).

There are few data available assessing the relationship between urine total protein and outcomes. One Finnish study of 1056 patients with type 2 diabetes mellitus and 1375 non-diabetic subjects demonstrated an association between spot urine concentration of total protein (i.e. not adjusted for creatinine concentration) and subsequent cardiovascular and all-cause mortality (202).

In summary, there are a wealth of data describing the strong independent relationship between dipstick proteinuria or albuminuria and cardiovascular disease, cardiovascular mortality and all-cause mortality. However there are few data about the relationship between these outcomes and total proteinuria, and no literature comparing these measurements as predictors of outcome.

1.5.7 Anaemia

The anaemia of CKD is associated with left ventricular hypertrophy (LVH) and fibrosis, with one study showing the independent risk of LVH being 32% higher for every 0.5g/dL decrease in haemoglobin (203). Foley et al studied the impact of anaemia in a cohort of patients commencing dialysis therapy and found a strong association between anaemia and cardiac abnormalities with a 1g/dL fall in haemoglobin being associated with a 46% higher risk of left ventricular dilatation and a 55% higher risk of poor left ventricular ejection fraction, and a 14% increase in the likelihood of death after commencing RRT (204).

Therefore it was hypothesised that correction of anaemia with recombinant human EPO or erythropoietin stimulating agents would prevent cardiovascular events. Two major studies of correction of anaemia in CKD were published simultaneously in 2006; the CREATE study compared a high versus a low target haemoglobin in patients with CKD Stages 3 and 4, using a composite primary end point of cardiovascular events; a haemoglobin of approximately 13.5g/dL versus 11.5g/dL was achieved but there was no outcome difference between the groups (169). The CHOIR study had a similar design of high versus low target haemoglobin (achieved haemoglobin 13g/dL versus 11.3g/dL) and demonstrated an increased event rate in the high haemoglobin group, using a different composite primary endpoint of death, myocardial infarction, hospitalisation for congestive cardiac failure or stroke (170). Notably the high haemoglobin groups required larger doses of EPO and had more hypertension.

More recently, a study of darbepoetin (an erythropoietin stimulating agent) in patients with CKD not requiring dialysis, anaemia and type 2 diabetes mellitus was published. This study compared treatment with darbepoetin to a target haemoglobin of 13g/dL versus placebo (with rescue therapy for Hb<9.0g/dL) and found no improvement in the composite primary endpoint of death, myocardial infarction, unstable angina, heart failure and stroke,

but a statistically significant increase in stroke in the treatment group(171). This has cast further doubt on the use of agents to correct anaemia in patients with CKD not requiring dialysis.

1.5.8 CKD associated mineral bone disorders

CKD is associated with a number of disturbances in calcium and phosphate metabolism collectively known as CKD associated mineral bone disorders. These include hypovitaminosis D leading to hypocalcaemia, hyperphosphatemia secondary to reduced nephron mass, secondary hyperparathyroidism and elevated levels of the phosphaturic hormone fibroblast growth factor 23 (FGF-23). This disruption in normal bone mineral metabolism may promote vascular calcification and arterial stiffness, mediating cardiovascular events (205). There is also increasing evidence of "off target" effects such as the relationship between FGF-23 and left ventricular hypertrophy (206).

There are therapeutic interventions available, including activated vitamin D supplementation, phosphate binders (both calcium based and non-calcium containing binders) and calcimimetic agents. There is a wealth of evidence of the beneficial effects of these agents on biochemical markers of CKD-MBD, but very little in relation to hard cardiovascular endpoints. Cinacalcet has been shown to reduce cardiovascular admissions in addition to reducing rates of fracture and parathyroidectomy (207).

1.5.9 Metabolic acidosis

Metabolic acidosis causes increased protein catabolism, decreased protein synthesis, and negative nitrogen and total body protein balance. It is associated with a number of adverse prognostic indicators, such as hypoalbuminaemia, and is implicated in protein energy wasting and malnutrition which is linked to mortality in dialysis patients (208, 209).

However the relationship between metabolic acidosis and outcome in maintenance dialysis patients is complex, and takes the form of a J-shaped curve. This is thought to be an example of reverse epidemiology, with healthier dialysis patients maintaining intake of protein, the catabolism of which leads to a mild metabolic acidosis (208).

The metabolic acidosis associated with CKD in non-dialysis patients may be less severe, and there is less evidence about its adverse effects. A recent clinical trial assessed nutritional parameters following bicarbonate supplementation in CKD patients, and found an improvement in the intervention group, but no survival endpoints have yet been reported (173). There are potential downsides of bicarbonate supplementation with increased prevalence of volume overload and hypertension and potential vascular calcification so further studies are needed, but there was no significant increase in blood pressure during the study which was reassuring (172, 173).

1.5.10 Uric acid

The relationship between uric acid and cardiovascular disease is complex and not well elucidated. There is increasing evidence that hyperuricaemia causes hypertension. This may be one explanation for the conflicting results of laboratory versus clinical studies assessing the independent effects of uric acid on cardiovascular outcomes. If uric acid has a role in the causal pathway by mediating hypertension, and the study corrects for hypertension in the multivariate analysis this effect may be lost (210). There is also evidence of a role for uric acid in development of the metabolic syndrome: lowering uric acid levels in animal models can prevent or reverse its features (210).

Patients with clinical gout receive treatment with allopurinol to reduce serum uric acid levels. There is insufficient evidence at present to support treating patients with

asymptomatic hyperuricaemia, however there are clinical trial data emerging that may inform decision making in the future (211).

1.6 Management of CKD

The prevalence of CKD is high, as described above, and the majority of these patients will have uncomplicated CKD Stage 3. In the UK this group is managed in the community by the general practice team of doctors and nurses. The identification of this group has been facilitated by the widespread reporting of an eGFR measurement along with each creatinine measurement in an adult, so increasing recognition of patients with early CKD (21). Guidelines have been produced that recommend specific strategies for the identification, management and referral of patients with CKD in primary care (9, 87). The Quality Outcomes Framework (QOF) of the General Medical Services contract in the UK has a structured payment system for primary care for a number of chronic illnesses. For patients with CKD the QOF rewards the following; the identification of those with CKD; establishing a register of such patients to facilitate regular monitoring; regular monitoring of kidney function, proteinuria and blood pressure; and the management of blood pressure to specific targets (212). The QOF indicators have evolved over time since its introduction in 2006 and the inclusions and exclusions, and financial incentives are shown in table 1-7 (213). The financial incentives are performance related with the lower percentage being the threshold at which some payment is received by the practice and the higher percentage is the threshold at which the full available payment will be given. The threshold for the upper payment for most QOF indicators in other conditions is 90%.

Table 1-7 - Quality Outcomes Framework Indicators for CKD in Primary Care

Indicator	Year included	Year Removed	Points	Payment stages
Records				
CKD1: The practice can produce a register of patients aged 18 years and over with CKD (US National Kidney Foundation: Stage 3 to 5 CKD)	2006		6	
Initial Management				
CKD2: The percentage of patients on the CKD register whose notes have a record of blood pressure in the previous 15 months	2006		6	40-90%
Ongoing Management				
CKD3: The percentage of patients on the CKD register in whom the last blood pressure reading, measured in the previous 15 months, is 140/85 or less	2006		11	40-70%
CKD4: The percentage of patients on the CKD register with hypertension who are treated with an angiotensin converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB) (unless a contraindication or side effects are recorded)	2006	2008	4	40-80%
CKD5: The percentage of patients on the CKD register with hypertension and proteinuria who are treated with an angiotensin converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB) (unless a contraindication or side effect are recorded)	2008		4	40-80%
CKD6: The percentage of patients on the CKD register whose notes have a record of a urine albumin: creatinine ratio (or protein: creatinine ratio) test in the previous 15 months	2009		6	40-80%

Specific therapeutic targets and recommendations for the management of patients with CKD in primary care have been extrapolated from secondary care populations as there is comparatively little specific evidence in this group of patients. This is unwise as the risk profiles of these groups are unlikely to be comparable, and specific study of patients with CKD in primary care is warranted.

1.7 Summary

Chronic kidney disease is a major public health problem with the associated burden of renal replacement therapy and premature cardiovascular disease. Early identification of patients at risk is essential to prevent the progression of renal disease and attenuate the cardiovascular risk. In this chapter I have reviewed what is currently known about the accurate measurement of excretory renal function and proteinuria and the factors that influence renal and mortality risk.

1.8 Aims of this project

The overall aim of this project is to explore the optimal predictors of renal outcome and survival for patients with chronic kidney disease.

1.8.1 Principal aims:

- To identify the optimal type of proteinuria measurement as a predictor of renal and patient outcomes
- To assess the prevalence of reduced eGFR and examine the optimal measure of eGFR
- To characterise patients in the community with CKD Stage 3
- To identify predictors of renal disease progression
- To identify predictors of cardiovascular disease

1.8.2 Secondary aims:

- To identify the optimal measurement of proteinuria in a CKD population to predict significant proteinuria
- To assess secular trends in prevalence of a reduced eGFR
- To assess the prevalence of CKD in a hypertension cohort

1.9 Hypotheses

The roles of total proteinuria, albuminuria and non-albumin proteinuria as predictors of renal and cardiovascular outcome remain unknown. It is hypothesised that non-albumin proteinuria plays an important role in the progression of CKD.

It is hypothesised that renal and cardiovascular risk in CKD Stage 3 (predominantly managed in the community), are not homogeneous and can be predicted from other clinical factors.

2 Chapter 2: Proteinuria: A retrospective cross-sectional study of protein: creatinine ratio versus albumin: creatinine ratio in chronic kidney disease

2.1 Introduction

Accurate identification and quantification of proteinuria are core elements in the diagnosis and management of CKD. The choice of TPCR or ACR to quantify proteinuria remains controversial, as discussed in chapter 1 (section 1.3.9). Timed urine collections (usually performed over 24 hours) are still considered the gold standard for quantification of proteinuria but have major limitations as a result of incomplete or inaccurate collections (69, 70) and are no longer recommended as a first line test by national guidelines (5, 7-9, 214).

The biochemistry laboratory in Glasgow Royal Infirmary routinely analyses urine for both ACR and TPCR in samples received from the kidney unit. The aim of this study was to examine the relationship between TPCR, ACR and 24-hour urinary protein in a population of patients attending a secondary care kidney clinic, and compare the diagnostic performance of TPCR and ACR at various thresholds, in order to investigate the optimal test to identify significant proteinuria.

2.2 Methods

2.2.1 Laboratory assays

Random spot urine samples are sent from all patients attending the renal clinics. The Glasgow Royal Infirmary biochemistry laboratory routinely measures ACR and TPCR, and has done consistently since 29th November 1999. Twenty four hour urine collections are performed on request, and assayed for volume, protein, albumin and creatinine concentration. The electronic patient record (Proton, Clinical Computing UK Ltd, Brentford, UK) calculates an eGFR using the four-variable MDRD equation (44, 215).

Prior to August 2006, urine albumin was measured on a Bayer Advia 1650 analyser using an immunoturbidimetric method with anti-human albumin antiserum with a mean between batch co-efficient of variation (CV) of 4.4% at a concentration of 0.54 g/L. The urine total protein assay was performed on the same analyser using the pyrogallol red colorimetric method, with a mean between batch CV of 8.32% at a concentration of 0.56 g/L. From August 2006, an Abbott Architect 2000 was used. Urinary albumin was measured using an immunoturbidimetric method using anti-human albumin antiserum with a mean between batch CV of 5.1% at a concentration of 0.111 g/L. Urinary total protein was analysed using a turbidimetric method with benzethonium precipitation, with a mean between batch CV of 1.8% at a concentration of 0.58 g/L. Urine creatinine was assayed using a reaction rate Jaffe method with Abbott reagents. The mean between batch CV is 3.4% at a concentration of 5.9 mmol/L. In-house comparison was made between the Bayer Advia 1650 and the Abbott Architect 2000 results, and no significant differences were found, in precision and accuracy between the results obtained before and after the change in instrumentation for these analytes. Returns to the United Kingdom External Quality Assurance Scheme showed no change in accuracy, precision or bias in the laboratory's results during this period. The laboratory is fully accredited by Clinical Pathology Accreditation (UK) Ltd.

2.2.2 Study Population

Patients attending the renal clinics are entered into the electronic patient record, which also receives laboratory data electronically. We retrospectively searched for patients with TPCR and ACR measured on the same date. The most recent paired results were used, in order to maximise the number of samples analysed using the more recent assays. Patients were excluded if they were under 18 years old, on RRT, or the sample was performed before 29th November 1999. The following data were also obtained: gender, age at time of urine sample, primary renal disease, use of ACEi or ARB, weight, height, blood pressure, serum creatinine, eGFR, and contemporaneous 24-hour urine protein (if available).

2.2.3 Ethical Permission

For the last decade, written consent has been obtained, which states that the data will be used for audit and research, in addition to routine clinical care. For this audit, data were downloaded and patient identifiers removed prior to further analysis. As this was an audit formal ethical approval was not required.

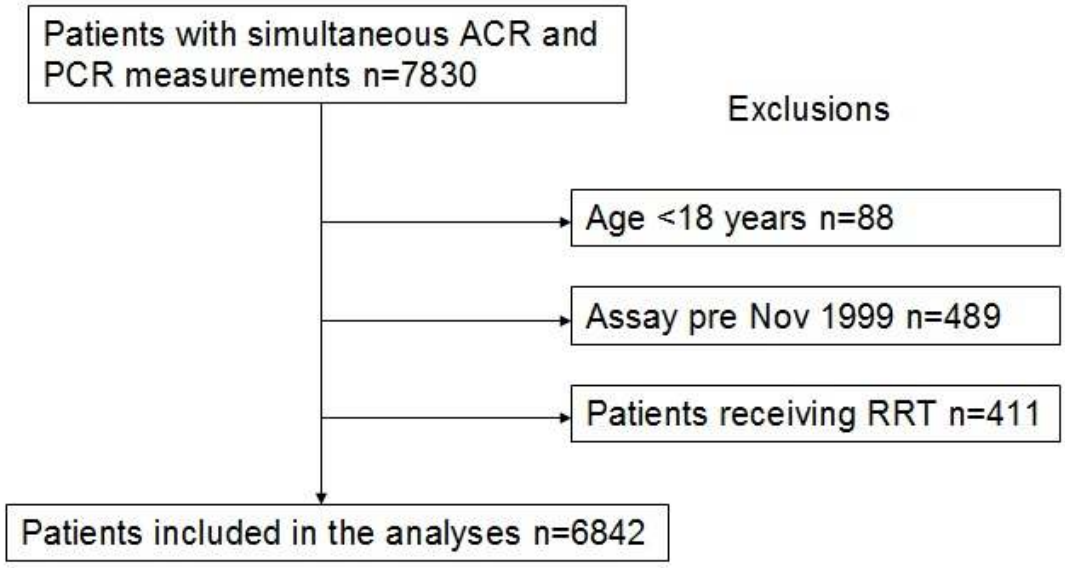
2.2.4 Statistical analysis

Data were analysed using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA). All data were assessed for normality, and appropriate summary statistics are presented. Total proteinuria, albuminuria, TPCR and ACR data were log-transformed given the large range of values. Correlation was assessed using Spearman's rho. Bland and Altman's method was used to compare different measures of proteinuria. Receiver-operator characteristic (ROC) curves were constructed to allow comparison of assays for key threshold values of proteinuria, and comparison of the area under the curve (AUC) was made using Hanley and McNeil's method (216) (MedCalc 10.4 Software, Mariakerke, Belgium). Significance testing was performed using the Mann-Whitney-U test. All tests are two-tailed.

2.3 Results

We identified 7830 patients with simultaneous ACR and TPCR results. We excluded 489 with samples analysed prior to 29th November 1999, as these were performed intermittently from 1991, and laboratory assay details were unavailable. We excluded 88 children <18 years old and 411 patients receiving renal replacement therapy, as shown in Figure 2-1.

Figure 2-1 - Flowchart of exclusions



Background data for the remaining 6842 patients are presented (Table 2-1). The data regarding race are incomplete, but race is relatively homogeneous in our population: 95.5-98.9% white, 0.74-3.71% Indo-Asian, 0.09-0.23% black and 0.3-0.59% other minority ethnic groups (217). In this cohort, the prevalence of Indo-Asian and black patients is 0.52% and 0.15% respectively, similar to local population prevalence. 3484 samples were analysed prior to August 2006 (date assays changed) and 3358 afterwards.

Table 2-1 - Background data. Normally distributed data are presented as mean ± standard deviation. Other data are presented as median (interquartile range). The primary renal diagnosis (PRD) is categorised according to the European Dialysis and Transplantation Association

Variable	6842 patients	Percentage with available data
Age (years)	61±17 (range 18-97)	100%
Gender	51% male	100%
Primary Renal Disease		65.3%
Primary glomerulonephritis	16.1%	
Interstitial disease	21.4%	
Multisystem diseases	16.7%	
Diabetic nephropathy	10.3%	
Other	0.2%	
CKD; aetiology unknown	35.3%	
On ACEi and/or ARB	30.7%	100%
Weight (kg)	77.2±19	83%
Height (cm)	165 ± 11	87.7%
Body Surface Area (m^2)	1.84 ± 0.24	36.1%
Systolic blood pressure (mmHg)	140±24	80.0%
Diastolic blood pressure (mmHg)	76±13	79.9%
Serum creatinine (µmol/L)	134 (IQR 105-175)	91.4%
eGFR (mL/min/1.73m^2)	41.3 (IQR 31.4-58.5)	91.4%
24-hour urine protein (g/day)	0.34 (IQR 0.15-0.92)	24.8%
TPCR (mg/mmol)	35 (IQR 17-106)	100%
ACR (mg/mmol)	10 (IQR 2-48)	100%

The lower limit of detection for ACR changed during the study; at various times ACR was reported as <3 or <10 mg/mmol. These values have been analysed as 3 or 10 mg/mmol respectively. To ensure this did not affect our findings, the data were re-analysed excluding any pair of results that included ACR <10 mg/mmol. This second population was 4462 patients, and the results were essentially unchanged.

2.3.1 Relationship between ACR and TPCR

The relationship between ACR and TPCR is non-linear (Figures 2-2 and 2-3). As expected, ACR is almost always less than TPCR. The relationship between ACR and non-albumin protein: creatinine ratio (NAPCR) (derived by subtracting ACR from TPCR) is presented in Figure 2-4. For NAPCR of 10-100 mg/mmol there is a poor correlation with ACR, demonstrated by the wide scatter, making it difficult to predict TPCR from ACR.

Figure 2-2 - Relationship between urine TPCR and ACR. Note both axes are on a logarithmic scale. The diagonal line is the line of identity.

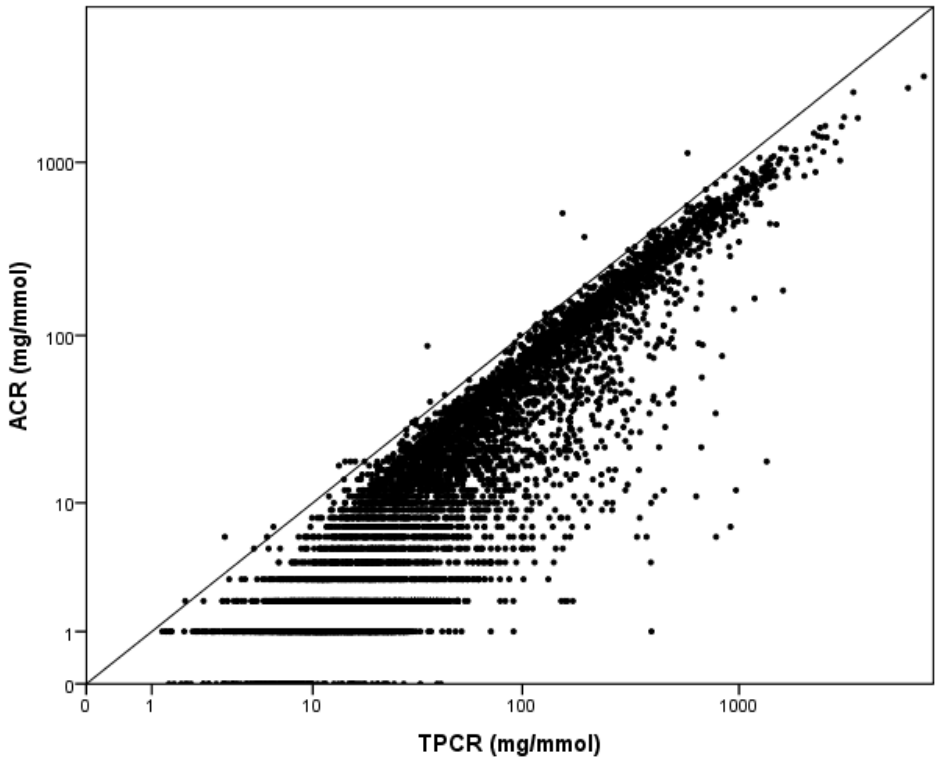


Figure 2-3 - Bland Altman plot of urine TPCR and ACR. The difference is expressed as a percentage. Note the abcissa is a logarithmic scale.

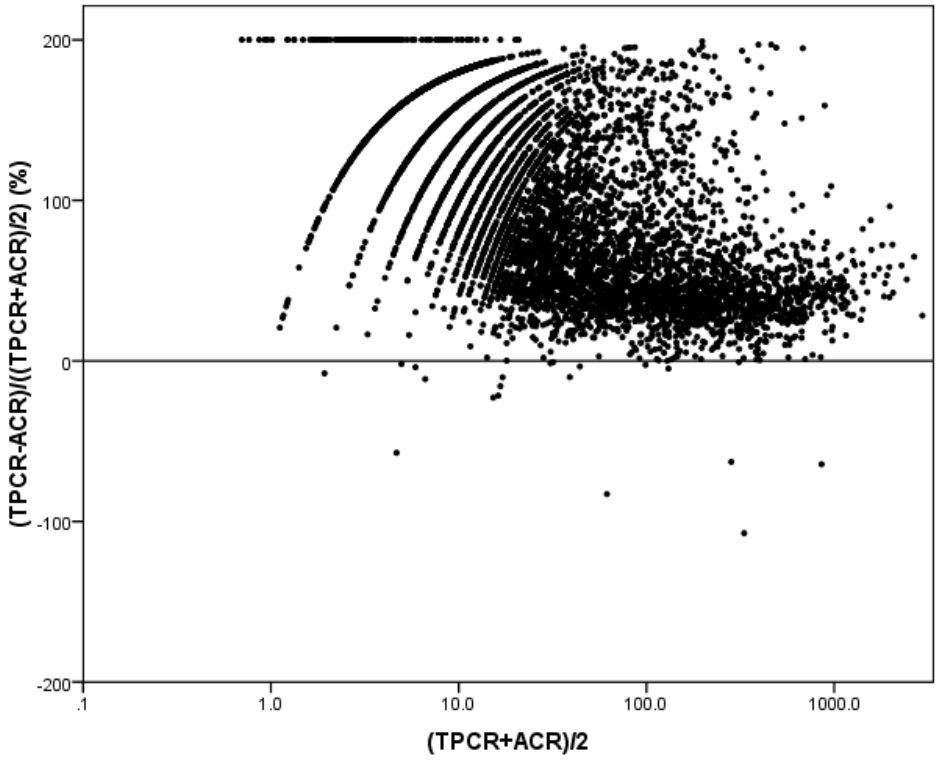
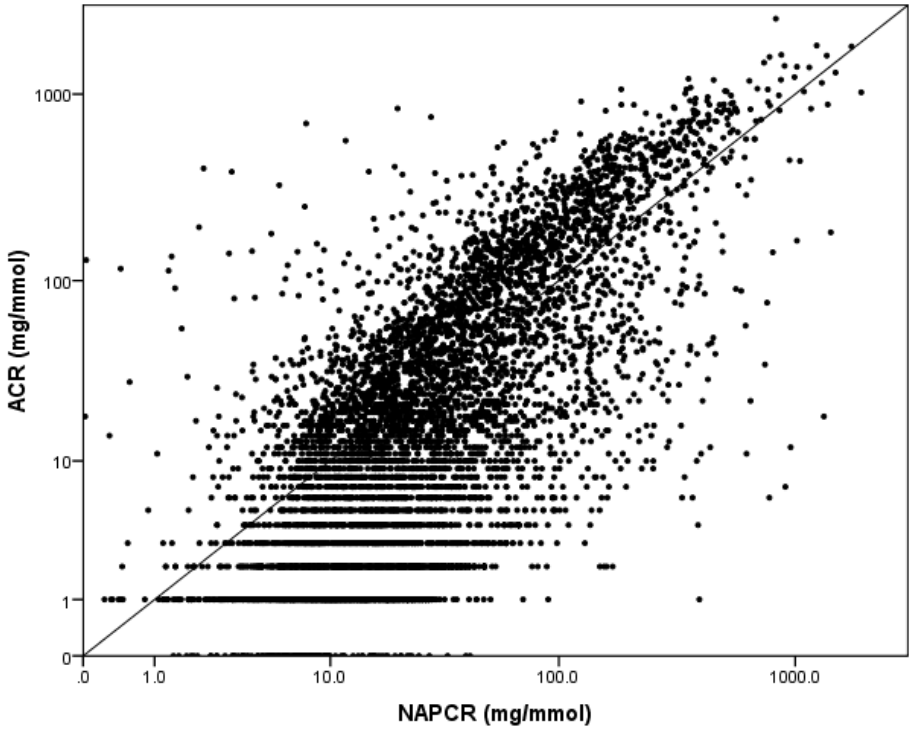


Figure 2-4 - Relationship between urine NAPCR and ACR. The diagonal is the line of identity. Note both axes are a logarithmic scale. Spearman's rho is 0.674 (p<0.001).



2.3.2 TPCR, ACR and 24-hour urine protein

Contemporaneous ACR, TPCR and 24-hour urine protein results were available in 1696 patients. TPCR is more highly correlated with 24-hour urine protein though ACR also performs well (Figures 2-5 and 2-6). In the range 300-1000 mg/day where clinical decisions are made, there is considerably greater scatter with ACR.

Figure 2-5 - Relationship between TPCR and 24-hour urine protein. A simple linear regression line is shown. Spearman's rho is 0.91 (p<0.001) for TPCR. Both axes are logarithmic.

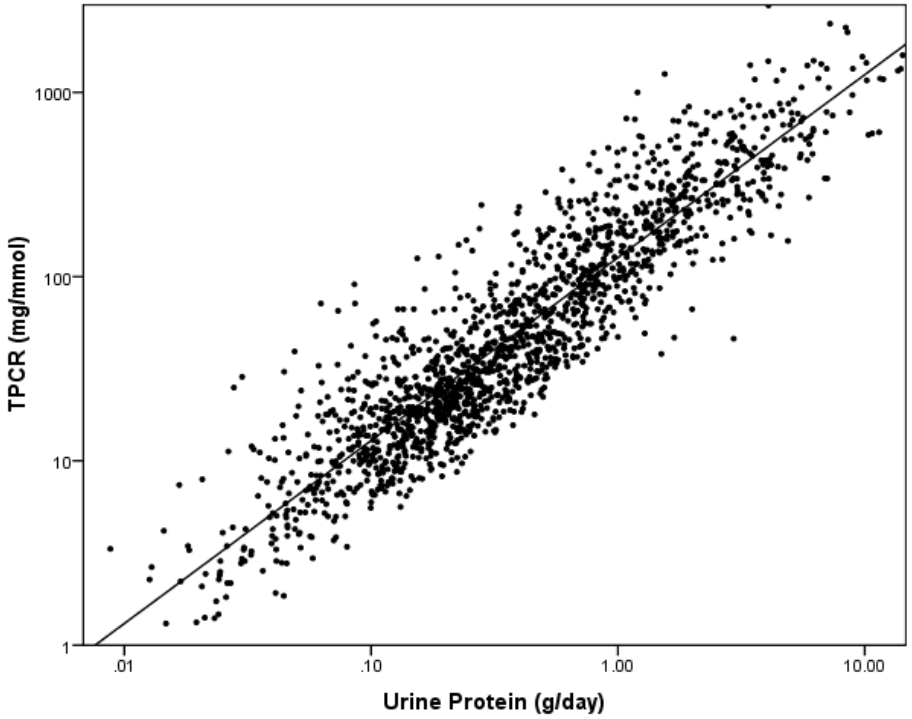
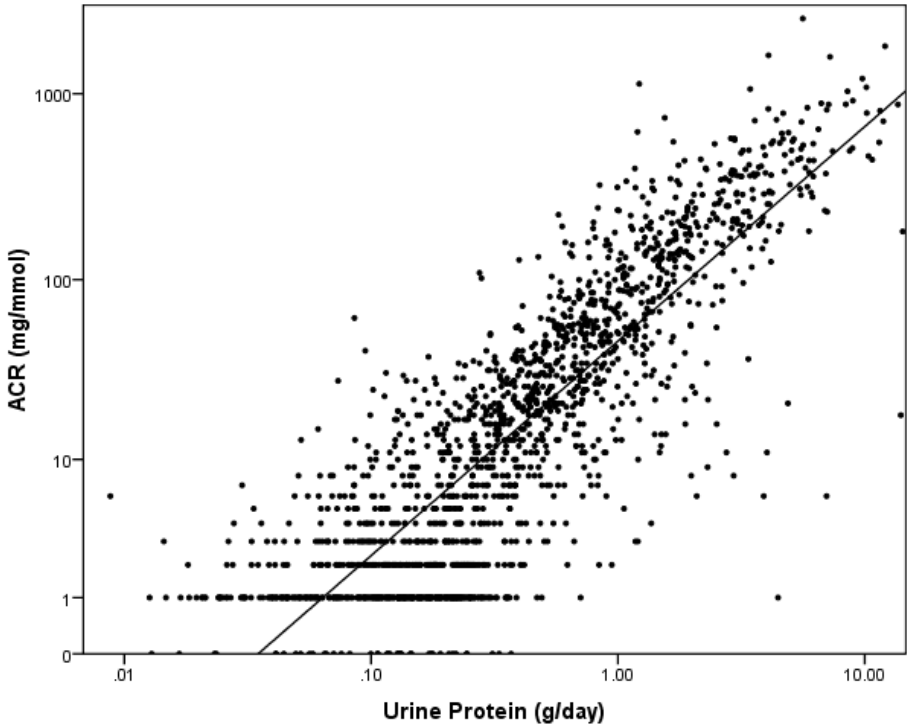


Figure 2-6 - Relationship between ACR and 24-hour urine protein. A simple linear regression line is shown. Spearman's rho is 0.84 (p<0.005) for ACR. Both axes are logarithmic.



We assessed the ability of ACR and TPCR to predict a 24-hour urine protein of >1g/day and >500mg/day. For >1g/day of total proteinuria, both ROC curves have highly significant AUCs (TPCR: 0.968, p<0.001; ACR: 0.947, p<0.001), however the performance of TPCR is significantly superior (p=0.004). Similar AUCs were found for 0.5g/day (TPCR: 0.967, p<0.001; ACR: 0.951, p<0.001), and again TPCR is significantly superior (p=0.001). Guidelines suggest cut-points of TPCR 100 mg/mmol (7, 8) and ACR 70 mg/mmol (8) to predict proteinuria of >1g/day, and TPCR of 50mg/mmol and ACR 30mg/mmol to predict >0.5g/day. The performance of these cut-points is presented in table 2-2 and figures 2-7 and 2-8. TPCR is substantially more sensitive than ACR, but less specific. To achieve comparable sensitivity to predict 1g/day using ACR, the cut-point fell to 17.5 mg/mmol, with specificity falling below TPCR at 69.8%, and for 0.5g/day an ACR of 14.5 mg/mmol must be used, with specificity falling below TPCR at 80.5%.

Table 2-2 - ROC curve analysis of TPCR and ACR to predict proteinuria of 1g/day and 0.5g/day (p<0.005 for all). Using US units of 1g/g (equivalent to a TPCR of 113.6 mg/mmol): sensitivity 91.2%, specificity 90.5%.

	Sn (%)	Sp (%)	PPV(%)	NPV(%)	LR+	LR-
To predict 1 g/day proteinuria						
TPCR (100 mg/mmol)	93.9	88.5	71.0	98.0	8.2	0.07
ACR (70 mg/mmol)	79.0	95.2	83.5	93.8	16.4	0.06
To predict 0.5 g/day proteinuria						
TPCR (50 mg/mmol)	91.3	87.5	82.5	94.0	7.3	0.1
ACR (30 mg/mmol)	78.2	94.6	90.0	87.5	14.5	0.23

Figure 2-7 – ROC curves for ACR and TPCR to predict a 24-hour urine protein of >1g/ day

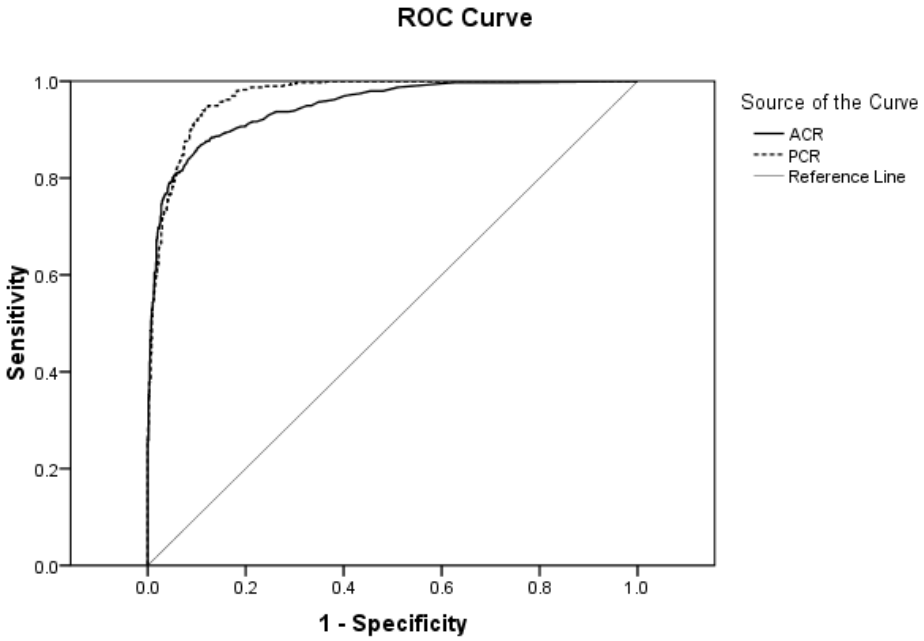
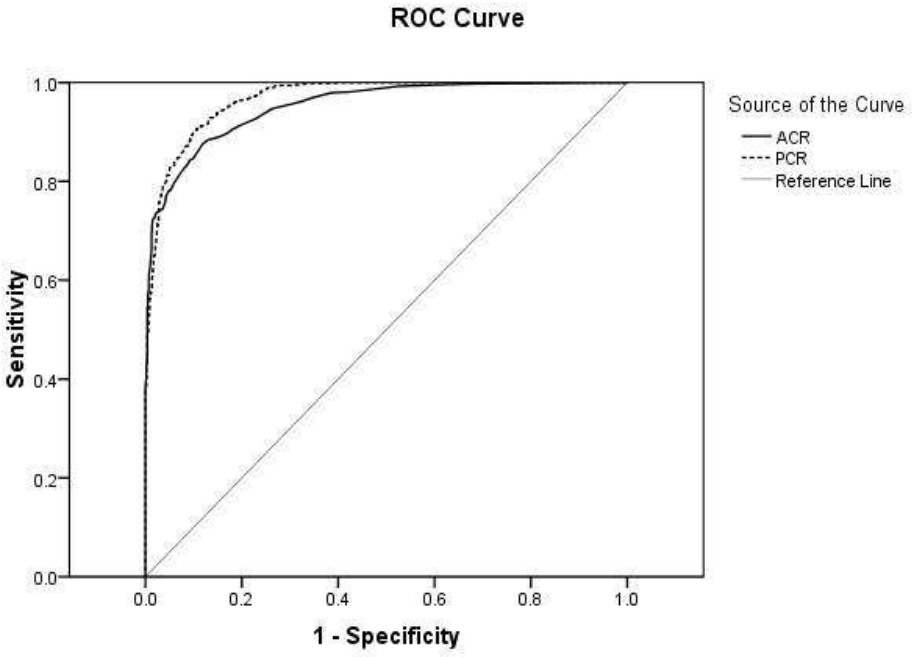


Figure 2-8 - ROC curves for ACR and TPCR to predict a 24-hour urine protein of >0.5g/ day.



The urinary total protein assay changed in August 2006. However only 293 24-hour urine collections (17%) were performed after this date, so direct comparison between pre- and post- assay change was not feasible

2.3.3 Completeness of 24-hour urine collections

The ROC curves rely on 24-hour urine collections as a "gold standard" measure of proteinuria. To ensure that incomplete urine collections were not influencing the results, we re-analysed the data after excluding low volume collections (<500mls/day) or creatinine excretion rates below the laboratory reference range (9-17 mmol/day for males; 7.5-12.5 mmol/day for females). These exclusions had no major impact on the findings.

2.3.4 Sub-group analysis

The performance of ACR and TPCR was assessed according to gender, age group, eGFR, and use of ACEi/ARB (table 2-3). ACR and TPCR yield higher sensitivity and lower specificity in females than males. To achieve 95% sensitivity of TPCR predicting proteinuria >1g/day, a cut-point of 78 mg/mmol is required in males (specificity 86%), and 118 mg/mmol in females (specificity 89%).

With advancing age, TPCR (and to a lesser extent ACR) becomes a more sensitive and less specific test; to achieve 95% sensitivity for prediction of total proteinuria >1 g/day requires a TPCR of 74 mg/mmol in those <49 years old (specificity 91%) compared to 132 mg/mmol in those >74 years old (specificity 83%).

When age and sex are combined the differences are amplified. To achieve 95% sensitivity for prediction of 1g/day of proteinuria in a man <49 years old, a TPCR of 65 mg/mmol is required (specificity 93%), compared to 179 mg/mmol in a woman >74 years.

With decreasing eGFR, there is a trend towards falling specificity of TPCR and ACR to predict 1 g/day of proteinuria. This is not clearly associated with any change in sensitivity, nor is it replicated for proteinuria of 0.5 g/day.

2.3.5 Use of ACEi/ARB

The sensitivity of TPCR to predict 1.0 g/day of proteinuria is considerably lower in patients receiving ACEi /ARB, falling towards the same level as ACR (Table 2-3). No fall is seen with ACR (Table 2-3). TPCR is very similar between the groups, but is composed of different proportions of non-albumin proteinuria (NAP) and albumin (Table 2-4). The NAP level and the proportion of NAP to total proteinuria are significantly lower in the ACEi/ARB treated group (Table 2-4). The Bland-Altman plots (Figure 2-9 and 2-10) show the difference between TPCR and ACR is less in the ACEi/ARB treated group, especially at higher levels of mean TPCR/ACR.

Table 2-3 - ROC curve analysis of performance of TPCR and ACR to predict proteinuria of 1g/ day according to patient sub-groups

TPCR	n	Sn (%)	Sp (%)	LR+	LR-
All	1696	93.9	88.5	8.2	0.07
Male	877	91.0	91.2	10.3	0.10
Female	819	98.7	86.4	7.3	0.02
Age ≤49	550	86.8	95.4	18.9	0.14
Age 49 – 64	430	94.2	92.0	11.8	0.06
Age 64 – 74	381	96.5	83.6	5.9	0.04
Age >74	335	97.7	76.1	4.1	0.03
eGFR<15	167	95.2	45.2	1.7	0.11
eGFR 15-29	198	94.6	76.1	4.0	0.07
eGFR 30-59	430	93.2	92.7	12.8	0.07
eGFR ≥ 60	498	81.8	98.7	62.9	0.18
ACEi/ARB	233	82.4	96.7	25.0	0.18
No ACEi/ARB	1463	95.7	87.4	7.6	0.05
ACR					
All	1696	79.0	95.2	16.4	0.06
Male	877	77.5	97	25.8	0.23
Female	819	81.6	93.7	13.0	0.20
Age ≤49	550	75.8	98.0	37.9	0.25
Age 49 – 64	430	79.8	98.5	31.9	0.21
Age 64 – 74	381	81.4	92.5	10.9	0.20
Age >74	335	78.4	89.9	7.8	0.24
eGFR<15	167	77.1	81.0	4.1	0.28
eGFR 15-29	198	76.8	89.4	7.2	0.26
eGFR 30-59	430	83.0	97.1	28.6	0.18
eGFR ≥ 60	498	70.5	99.0	70.5	0.30
ACEi/ARB	233	82.4	96.7	25.0	0.18
No ACEi/ARB	1463	78.6	95.1	16.0	0.23

Table 2-4 - Baseline demographics of patients receiving angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) versus those who are not.

	ACEi/ARB group	Non-ACEi/ARB group	Z value (Mann-Whitney U test)	p value
SBP (mmHg)	142 ± 23	139 ±25	-5.419	<0.001
DBP (mmHg)	76 ± 13	76 ± 13	-0.039	0.995
sCr (mmol)	142 (108 – 190)	139 (95 – 235)	-0.239	0.811
eGFR (ml/min/1.73m^2)	38.6 (27.5 – 57.3)	41 (22.1 – 66.6)	-0.912	0.362
TPCR (mg/mmol)	35.7 (18.5 – 85.4)	34.5 (15.6 – 118.9)	-0.285	0.775
ACR (mg/mmol)	13 (3 – 51)	9 (2 – 47)	-4.893	<0.001
NAPCR (mg/mmol)	20.2 (11.7 – 37.5)	22.3 (10.8 – 57.2)	-4.045	<0.001
NAPCR:TPCR (%)	56.2 (35.9 – 83.8)	71.1 (45.5 – 88.5)	-11.197	<0.001

Figure 2-9 - Bland-Altman plots of patients receiving angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Note the ordinate is expressed as a percentage.

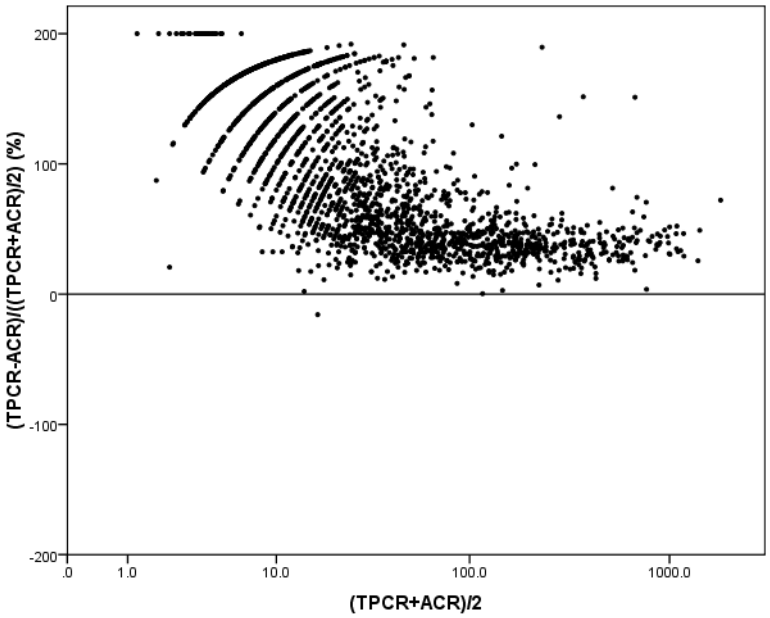
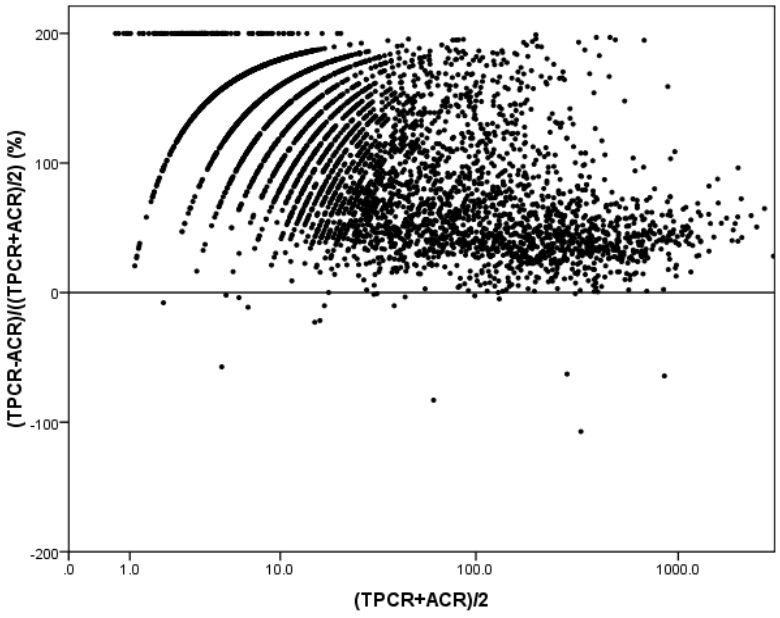


Figure 2-10 - Bland-Altman plots of patients not receiving angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Note the ordinate is expressed as a percentage.



2.4 Discussion

2.4.1 Findings of this study

These data show that TPCR is a highly sensitive and reasonably specific test for detection of significant proteinuria (total proteinuria >0.5 g/day or >1 g/day) in unselected patients attending a hospital kidney clinic. ACR performs significantly less well by ROC curve analysis, and is substantially less sensitive, though this is not entirely surprising given that TPCR is more closely related to 24-hour total proteinuria than ACR. To improve the sensitivity of ACR requires very low thresholds with poorer specificity than TPCR. In addition to the use of TPCR and ACR for monitoring glomerular disease and prognostication, they are often used as screening tests, so sensitivity is of prime importance to avoid under-diagnosis of those at risk of progressive renal decline. Total proteinuria cannot be reliably predicted from albuminuria, because of the variable proportion of non-albumin proteins, particularly in the clinically relevant range of 0.3-1 g/day.

A single cut-point for TPCR or ACR will lead to varying sensitivity and specificity according to patient characteristics, and our results quantify the impact. Sensitivity is higher with increasing age, and in females, whilst specificity is lower. This is likely due to lower muscle mass in these groups (33), resulting in lower creatinine excretion, and thus higher TPCR (or ACR) for a given concentration of urinary protein. The findings with reducing eGFR were less consistent, but one would expect low muscle mass in CKD stages 4-5 (218). To predict 1g/day of proteinuria with 95% sensitivity a TPCR threshold of 65mg/mmol in a young man and 179mg/mmol in an elderly woman is needed. This almost three-fold difference means that use of universal cut-points may lead to under-recognition and under-treatment of young men with proteinuria. While gender and age specific cut-points could mitigate this (86), this would undermine the simplicity of TPCR. Clinicians should be aware of these limitations and judiciously use 24-hour collections where doubt

remains. Similar differences in sensitivity and specificity are likely for different racial groups, but we were unable to assess these in our predominantly white population.

2.4.2 Non-albumin proteinuria

The proportion of NAP is significantly lower in those receiving ACEi/ARB. This suggests that ACEi/ARB selectively reduce NAP, or that these drugs are being utilised in patients with pre-existing low levels of NAP (for instance if ACR alone is being used to identify patients with significant proteinuria). We cannot define the correct option in our cross-sectional study. However, enalapril reduces the large non-selective pore size in the glomerular basement membrane, reducing urinary loss of proteins with a large molecular radius, in diabetic and non-diabetic kidney disease (219, 220). This supports the former theory, that ACEi/ARBs selectively reduce NAP. The magnitude of decline in proteinuria with ACEi therapy predicts the degree of renoprotection (221). In our population, ACR failed to identify 22% of patients with significant proteinuria (i.e. those with TPCR >100 mg/mmol, but ACR <70 mg/mmol). These patients with a high proportion of NAP, may gain the largest reduction in proteinuria and thus the largest benefit from ACEi/ARB.

2.4.3 Limitations

This study has several limitations. It was retrospective, and therefore undetected bias may be present. There may have been drift in assays during the study period, but this will affect all assays and we have no reason to expect systematic bias. The relationships demonstrated may only apply to assays used in our study. A variety of assays are used to measure total protein, and even albumin immunoassays have considerable inter-assay and inter-laboratory variation (70). The vast majority of 24-hour urine collections were performed before August 2006, therefore the former assays are over-represented in these results. However the pyrogallol red method remains well established and widely used globally.

Formal comparison of this technique (used pre-2006) with the turbidimetric method (post-2006) have shown them to yield comparable results. For the analyses involving 24-hour urine samples, the majority of the ACR and TPCR results were calculated from the 24-hour samples. Our main aim was to compare the relative performance of ACR and TPCR, rather than to demonstrate the utility of spot samples, which has been shown before. This approach will artificially improve the correlation of TPCR and 24h urine protein, but as the assay CV is only 1.8-8.3%, will have a relatively small impact, compared to the dramatic variations seen with ACR (e.g. Fig 2-2). Furthermore, as this is a random effect it will not systematically bias the calculations of sensitivity and specificity. Lastly ACR was reported by the laboratory with no decimal place, whereas TPCR was reported to one decimal place. This resulted in loss of granularity at low levels of ACR as seen in figure 2-2, 2-4 and 2-6. The strengths of this study are the large numbers, and the representative nature of the unselected adult population attending a general nephrology clinic. It may not be representative of primary care-based CKD populations.

2.4.4 Guideline recommendations on the monitoring of proteinuria

Guideline recommendations on measuring proteinuria differ. KDOQI recommends monitoring proteinuria using ACR, unless ACR exceeds 500-1000 mg/g (56-113 mg/mmol), when TPCR is acceptable (5). In England and Wales, the National Institute for Health and Clinical Excellence recommends that urine should be analysed for ACR (8). The Scottish Intercollegiate Guideline Network (7) recommends TPCR in patients with non-diabetic kidney disease, reserving ACR for diabetic patients. Our findings suggest cautious use of ACR in all patients, as a significant number with proteinuria >1 g/day will not be identified. Given our data, and the additional cost of ACR the converse strategy to KDOQI's recommendations would be more logical: only testing ACR if TPCR is not elevated (for instance <50mg/mmol).

2.4.5 Conclusion

In conclusion, TPCR is a more sensitive screening test than ACR to predict clinically significant proteinuria (as defined using 24-hour total proteinuria). The diagnostic performances of both tests vary substantially with age, gender and to some extent eGFR, an effect that is probably related to muscle mass. These results suggest that in order to correctly identify significant proteinuria, clinicians should interpret the result with the patient's muscle mass in mind, rather than dutifully observing a single cut-point. Total proteinuria cannot be adequately predicted from ACR (as a result of variable levels of non-albumin proteins which are measured using both TPCR and 24-hour total proteinuria), and our results suggest caution is appropriate before utilising ACR in patients with non-diabetic CKD.

3 Chapter 3: Comparison of urinary albumin and urinary total protein as predictors of patient outcomes in chronic kidney disease

3.1 Introduction

There is extensive evidence that dipstick proteinuria and albuminuria are associated with adverse patient outcomes including end stage renal disease, cardiovascular disease and mortality, as discussed in the introduction (section 1.4.1 and 1.5.6 respectively). In chapter 2 TPCR was found to be superior to ACR as a predictor of significant proteinuria. However the impact of total proteinuria on mortality has been less well characterised, and ACR and TPCR have not been compared as predictors of renal and patient outcomes.

Using the results of a similar cohort of patients from Glasgow Royal Infirmary to those described in chapter 2, who routinely had their urine samples analysed for both albumin and total protein, we assessed whether TPCR was superior to ACR as a predictor of renal outcomes and mortality in CKD patients.

3.2 Methods

3.2.1 Laboratory assays

The assays used are described in detail in Chapter 2. The biochemistry laboratory has measured ACR and TPCR in all samples from the renal service since 29th November 1999.

3.2.2 Study population

The details of the overall cohort are described in Chapter 2. However the method used to extract this cohort from the database was slightly, but importantly, different from chapter 2. As before we retrospectively searched our database for all patients who had total protein, albumin and creatinine measured on a urine sample on the same date. For the majority of patients, this was measured in a spot sample, however for the minority who performed a 24-hour urine collection, the ACR and TPCR were calculated from an aliquot of the 24-hour urine collection. The earliest available paired results for ACR and TPCR were used (unlike the previous analysis in which the most recent paired results for each patient were used). The search strategy was changed from that used in chapter 2 in order to maximise the period of follow-up available for analysis. The majority of the exclusion criteria were the same (samples pre-1999 were excluded, patients under 18 years old and those receiving renal replacement therapy). In addition, those who had less than one year's follow-up available were excluded (on the basis that there was insufficient exposure to the variable of interest). The same baseline data were also obtained: gender, age at time of urine sample, primary renal disease, use of ACEi/ARB, weight, height, blood pressure, serum creatinine, eGFR, and contemporaneous 24-hour urine protein (if available). For this longitudinal analysis, subsequent measurements of serum creatinine and eGFR were obtained. The following outcomes were also recorded: date of death and date of commencing RRT, for established renal failure (RRT for acute kidney injury was excluded from this analysis).

3.2.3 Ethical Permission

For the last decade, written consent for use of the electronic patient record has been obtained from patients, and the consent specifically states that the data will be used for the purposes of audit and research, in addition to routine clinical care. Data were downloaded with patient identifiers removed prior to further analysis. The National Health Service National Research Ethics Service confirmed that ethical approval was not required for this analysis (correspondence available).

3.2.4 Statistical Analyses

Data were analysed using SPSS 16.0 for Windows (SPSS Inc, www.spss.com). All data were assessed for normality, and appropriate summary statistics are presented. A hierarchical Cox regression survival analysis was constructed for the outcomes of all cause mortality, commencement of renal replacement therapy and doubling of serum creatinine. The follow-up was censored at the time that the first outcome was reached for each patient. The co-variates of age, gender, blood pressure and serum creatinine were entered in the first block, and either ACR, TPCR, 24-hour urine albumin or 24-hour urine total protein entered in the second block. TPCR, ACR, 24-hour urine total protein and 24-hour urine albumin were converted to a log scale and ACR and TPCR were standardised in order to facilitate a fair comparison. TPCR results tend to be higher and this would bias the comparison without prior standardisation. The hazard ratios presented for ACR and TPCR are for one standard deviation difference (on the log scale). Cases were excluded from the Cox regression survival analysis if any of the variables were missing (mostly blood pressure). The analyses were repeated with missing variables imputed using regression, to ensure there was no influence on the model.

The linearity of each continous predictor was tested by calculating Martingale residuals for the Cox regression model without the predictor and then plotting these against the predictor using lowess smoothing. The proportional hazards assumption was tested by creating time dependent covariates for each predictor and including them in the model if the interaction was significant. The albumin assay changed in August 2006, therefore a sensitivity analysis was performed for the samples prior to the assay change.

Proteinuria was also analysed as a categorical variable, divided into 3 clinically relevant groups. Although there is no reliable conversion factor for ACR and TPCR, we defined ACR and TPCR groups with approximately equivalent values using recommendations from recent guidelines, shown in Table 3-1 (8). A sensitivity analysis was performed, by splitting the reference group and using the lower half of normoalbuminuria as the new reference group.

Table 3-1 - Equivalent values for 24-hour urine protein excretion, total protein: creatinine ratio and albumin: creatinine ratio

Proteinuria (g/day)	TPCR (mg/mmol)	ACR (mg/mmol)
<0.15	<15	<3
0.15 - 0.5	15 – 50	3 - 30
0.5 – 0.999	50 – 99	30 – 69
1.0 – 3.499	100 – 349	70 - 244
≥3.5	≥350	≥245

3.3 Results

We identified 8457 patients with ACR and TPCR measured on the same day between 24th November 1999 and 28th May 2008. A flow diagram of the population and exclusions is shown in Figure 3-1. Background data for the remaining 5586 patients are presented in Table 3-2. The baseline demographic data of the 1808 patients who performed a 24-hour urine collection were not significantly different to the overall group. The data regarding race are incomplete, however the prevalence of black patients in this cohort is recorded at 0.15%, which is similar to the overall prevalence in the local population of 0.09 – 0.23%. Additionally the prevalence of Indo-Asian patients is 0.52%, again similar to the local population (217). 4402 of the baseline samples were analysed prior to August 2006 (the date our laboratory assays changed) and 1184 afterwards. Patients were followed up for a median of 3.5 years (interquartile range IQR 2.1 – 6.0 years).

Figure 3-1 - Flow diagram of the cohort, exclusions and sub-groups

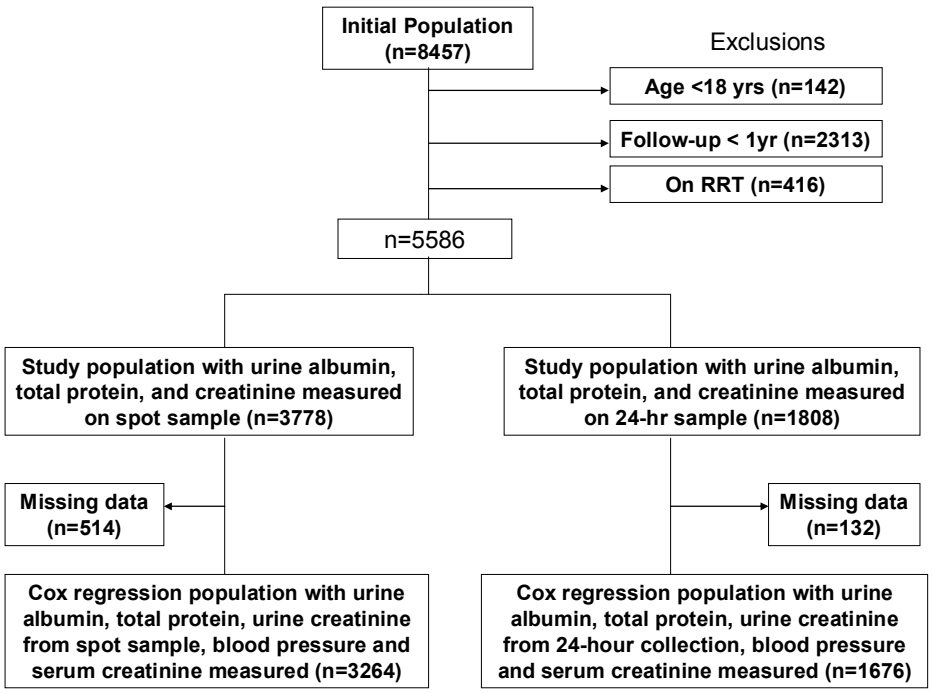


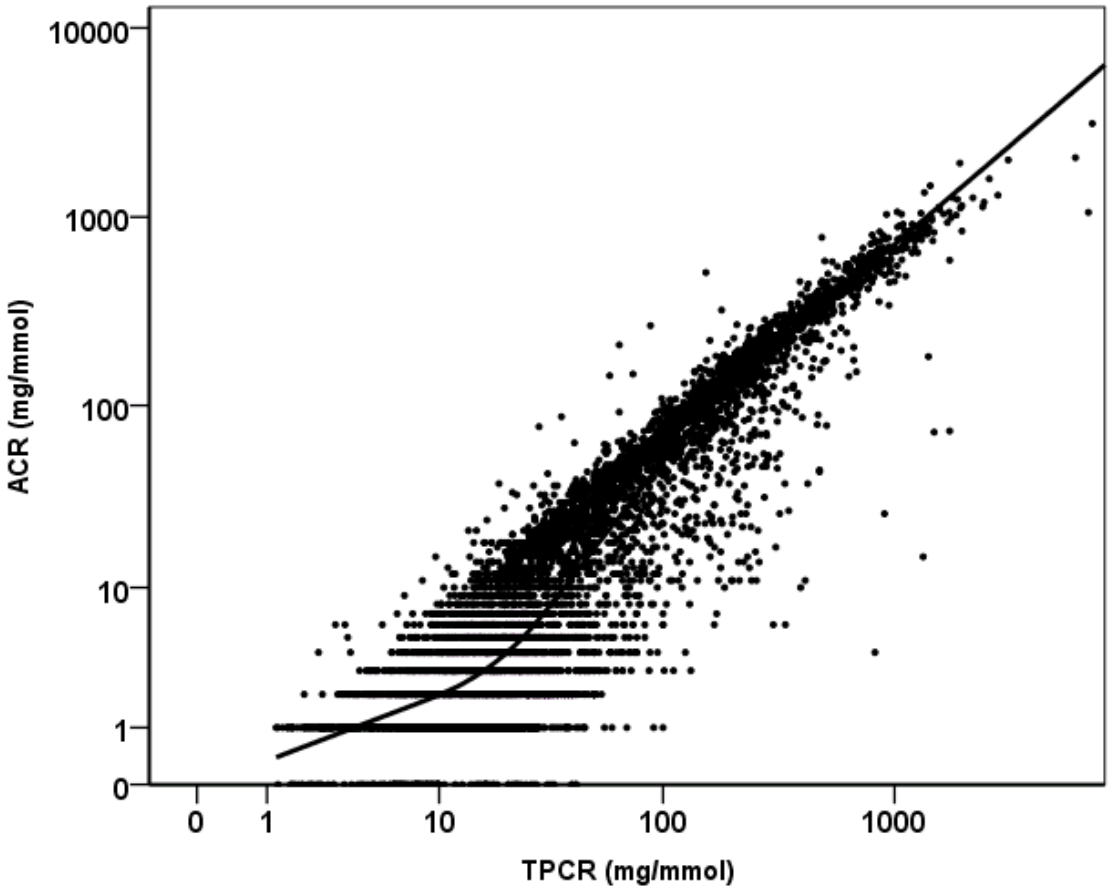
Table 3-2 - Baseline descriptive data for 5586 patients with chronic kidney disease attending an outpatient clinic. Primary renal disease is classified according to the European Dialysis and Transplantation Association primary renal disease codes. Data are presented as mean (standard deviation) or where specified, median (interquartile range [IQR]).

Variable	Total cohort (n=5586)	Available data	24-hour collection group (n=1808)	Available data
Age (years)	59 (16) (range 18-97)	100%	56 (16)	100
Gender	50% male	100%	48%	100
Primary Renal Disease		68.0%		54.2%
Primary glomerulonephritis	17.0%		26.3%	
Interstitial disease	22.5%		26.6%	
Multisystem diseases	16.3%		20.5%	
Diabetic nephropathy	11.1%		11.1%	
Other	0.1%		0.1%	
CKD; aetiology unknown	33.1%		15.4%	
On ACEi and/or ARB	22.1%	100%	20.8%	100%
Weight (kg)	77.8 (18.3)	90.5%	78.1 (18.4)	94.8%
Height (m)	1.65 (0.1)	91.2%	1.65 (0.1)	95.9%
Body Surface Area (m^2)	1.9 (0.2)	84.7%	1.9 (0.2)	90.2%
Systolic blood pressure (mmHg)	144 (27)	91.2%	143 (28)	95.2%
Diastolic blood pressure (mmHg)	78 (14)	91.2%	79 (13)	95.2%
Serum creatinine (µmol/L)	140 (IQR 100-190)	97.0%	130 (IQR 100 – 180)	97.8%
eGFR (mL/min/1.73m^2)	41.9 (IQR 28.2-62.8)	97.0%	46.7 (IQR 30.8 – 64.1)	97.8%
24-hour urine protein (mg/day)	-	-	340 (IQR 150-920)	32.4%
24-hour urine albumin (mg/day)	-	-	117.6 (IQR 16.9-743.6)	32.4%
TPCR (mg/mmol)	35 (IQR 17-106)	100%	32 (IQR 14 – 132)	100%
ACR (mg/mmol)	10 (IQR 2-48)	100%	12 (IQR 2 – 77)	100%

3.3.1 Relationship between ACR and TPCR in this cohort

A scatterplot of ACR versus TPCR is shown in Figure 3-2.

Figure 3-2 - Relationship between baseline urine total protein:creatinine ratio (TPCR) and albumin: creatinine ratio (ACR) in 5586 patients with chronic kidney disease.



Note that both axes are on a logarithmic scale. The line is a regression line using lowess smoothing. The values shown in this graph include TPCR and ACR derived from timed urine collections and spot urines.

3.3.2 Outcomes

There were 844 deaths during follow-up (15% of the population). Median time to death from the time of the baseline urine sample was 3.0 years (IQR 1.8 – 4.7 years). RRT was commenced in 468 patients (8%). The median time to commencement of RRT was 1.7 years (IQR 0.6 – 3.4 years). The serum creatinine of 999 patients (18%) doubled during the follow-up period. The median time to doubling of serum creatinine was 2.2 years (1.1 – 3.8 years).

3.3.3 Comparison of patient outcomes for urine albumin and urine total protein: ACR and TPCR derived from spot samples versus ACR and TPCR derived from timed urine collections

Cox regression analyses for death, RRT and doubling serum creatinine are shown in table 3-3. There are no significant differences between the results for ACR and TPCR derived from a spot urine sample when compared with the ACR and TPCR derived from a timed urine collection. Adjusted hazard ratios for TPCR were higher than for ACR for all-cause mortality and doubling of serum creatinine, and higher for ACR for commencement of RRT but the 95% confidence intervals overlapped. Sensitivity analysis of the pre-August 2006 results (when the assay changed), yielded similar results, and are therefore not shown. Repeat analyses with imputed data (using regression) for any missing variables did not alter the results significantly. Given the similar predictive ability for ratios derived from a random urine sample and timed urine sample, these data were combined and ROC curve analyses were performed to compare ACR and TPCR as predictors of mortality, renal replacement therapy and doubling of serum creatinine; these are shown in figure 3-3, 3-4 and 3-5 respectively, and are not significantly different.: the performance of ACR and TPCR was almost identical with similar areas under the curve.

Table 3-3 - Association of baseline urinary albumin: creatinine ratio (ACR) and total protein: creatinine ratio (TPCR) with subsequent patient outcomes in 5586 patients with chronic kidney disease

	Death		RRT		Doubled sCr	
	HR	**aHR**	**HR**	**aHR**	**HR**	**aHR**
Spot ACR (n=3264)	1.42 (1.30 – 1.55)	1.49 (1.34- 1.66)	3.28 (2.87 – 3.76)	2.41 (2.06 – 2.83)	1.90 (1.75 – 2.06)	1.95 (1.78 – 2.08)
Spot TPCR (n=3264)	1.53 (1.40 – 1.66)	1.54 (1.38 - 1.71)	2.99 (2.69 – 3.33)	2.03 (1.77 – 2.32)	1.92 (1.78 – 2.07)	2.01 (1.82 – 2.21)
24-hr ACR (n=1676)	1.48 (1.31 – 1.66)	1.26 (1.11- 1.42)	2.82 (2.37 – 3.35)	2.24 (1.83 – 2.74)	2.06 (1.85 – 2.30)	1.91 (1.69 – 2.16)
24-hr TPCR (n=1676)	1.51 (1.37 – 1.66)	1.28 (1.14 - 1.44)	2.68 (2.32 – 3.09)	1.88 (1.59 – 2.23)	2.03 (1.85 – 2.23)	2.12 (1.84 – 2.45)
Spot and 24-hr ACR (combined) (n=4940)	1.41 (1.31 – 1.51)	1.38 (1.28- 1.50)	3.00 (2.69 – 3.36)	2.33 (2.06 - 3.01)	1.94 (1.81 – 2.08)	1.92 (1.78 – 2.08)
Spot and 24-hr TPCR (combined) (n=4940)	1.53 (1.43 – 1.63)	1.41 (1.31 - 1.53)	2.84 (2.59 – 3.11)	1.96 (1.76 - 2.18)	1.96 (1.84 – 2.08)	2.03 (1.87 – 2.19)

Hazard ratios and adjusted hazard ratios (with 95% confidence intervals) from multivariate Cox regression analyses are presented, per one standard deviation difference in the variable. Age, gender, blood pressure and serum creatinine are co-variates in all models. Serum creatinine is a time-dependent co-variate for renal replacement therapy. Age is a time-dependent co-variate for doubling serum creatinine.

Figure 3-3 - Receiver operator characteristic curve for baseline urinary albumin: creatinine ratio (ACR) and total protein: creatinine ratio (TPCR) to predict all-cause mortality. Area under curve for ACR 0.647 (95% CI 0.628 – 0.667; p<0.001), and for TPCR 0.664 (95% CI 0.644 – 0.683; p<0.001)

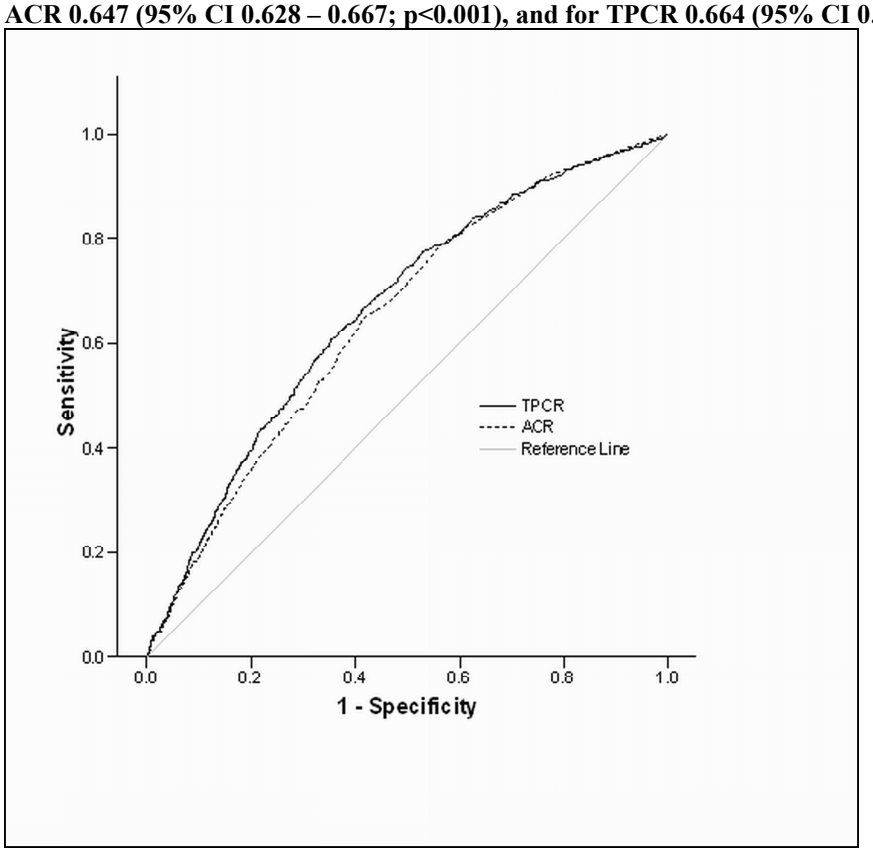


Figure 3-4 - Receiver operator characteristic curve for baseline urinary albumin: creatinine ratio (ACR) and total protein: creatinine ratio (TPCR) to predict commencement of renal replacement therapy. Area under curve for ACR 0.815 (95% CI 0.796 – 0.833; p<0.001), and for TPCR 0.826 (95% CI 0.807 – 0.844; p<0.001)

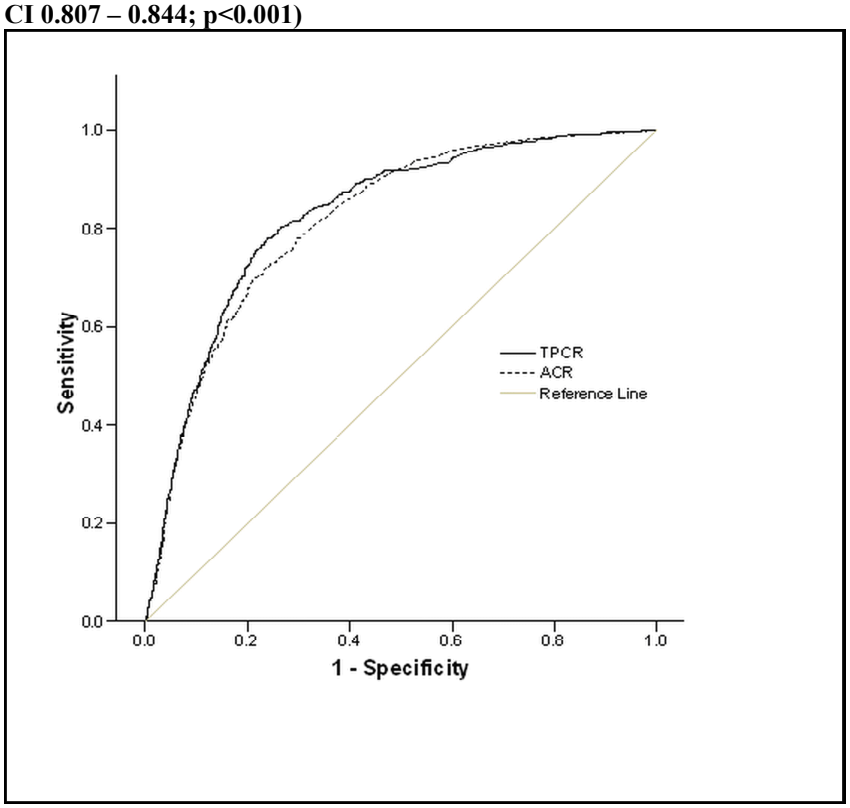
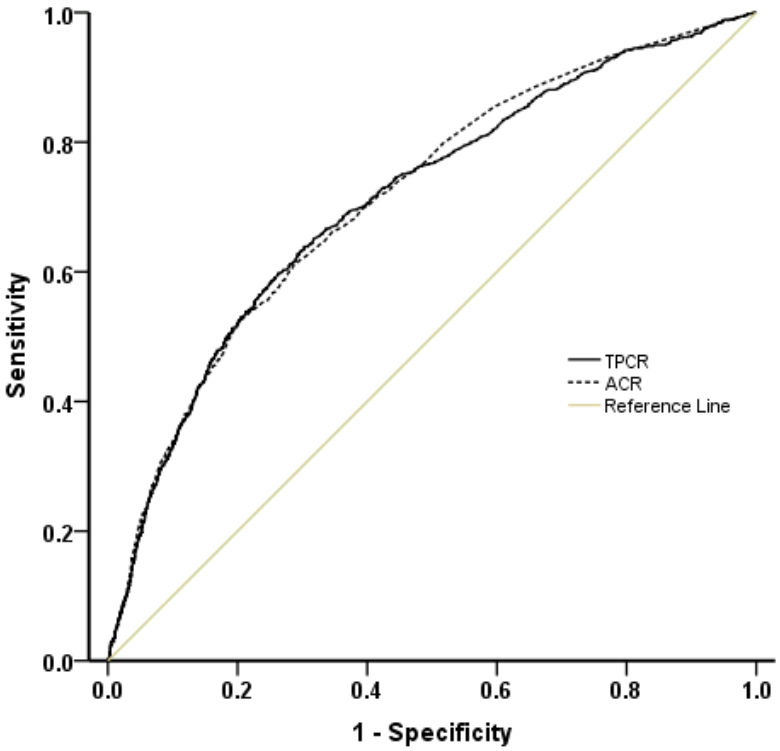


Figure 3-5 - Receiver operator characteristic curve for baseline urinary albumin: creatinine ratio (ACR) and total protein: creatinine ratio (TPCR) to predict doubling of serum creatinine. Area under curve for ACR 0.715 (95% CI 0.697 – 0.732; p<0.001), and for TPCR was 0.713 (95% CI 0.695 – 0.731; p<0.001)



The receiver operator characteristic curves include the ACR and TPCR derived from timed urine collections and spot urines.

TPCR and ACR were also analysed as categorical variables (using the combined results from random urines and timed urine collections), comparing microalbuminuria (ACR 3 – 30mg/mmol) and macroalbuminuria (ACR>30mg/mmol) (and TPCR equivalent) to normoalbuminuria (ACR<3mg/mmol - the reference group) as described in the methods. The performance of ACR and TPCR was similar for all three patient outcomes with overlapping 95% confidence intervals. This applied across all the categories examined, including microalbuminuria. The Cox regression analyses are shown in table 3-4. A sensitivity analysis was performed using 4 categories, with the lower half of normoalbuminuria as the reference group (ACR<1.5mg/mmol and TPCR<7.5mg/mmol). Again there was no significant difference in performance between TPCR and ACR in these categories (table 3-5).

Table 3-4 – Association of different categories of baseline urinary TPCR and ACR with subsequent patient outcomes (3 groups)

TPCR	n	Death	RRT	Doubled sCr
<15 mg/mmol	1470	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
15.1 – 50 mg/mmol	1687	1.40 (1.11 – 1.77)	2.15 (1.25 – 3.71)	1.49 (1.18 – 1.89)
>50 mg/mmol	1783	2.24 (1.78 – 2.80)	6.46 (3.90 – 10.69)	3.73 (3.01 – 4.63)
ACR				
<3.0 mg/mmol	1754	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
3.1 – 30 mg/mmol	1562	1.47 (1.19 – 1.83)	2.51 (1.48 – 4.25)	1.70 (1.36 – 2.13)
>30 mg/mmol	1624	2.12 (1.72 – 2.63)	7.19 (4.36 – 11.88)	3.87 (3.14 – 4.77)

Adjusted hazard ratios (with 95% confidence intervals) from multivariate Cox regression analyses are presented. Age, gender, blood pressure and serum creatinine are co-variates in all models.

Table 3-5 - Association of different categories of baseline urinary TPCR and ACR with subsequent patient outcomes, using the lower half of the laboratory normal range as the reference group

TPCR	n	Death	RRT	Doubled sCr
<7.5 mg/mmol	534	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
7.5 – 15 mg/mmol	929	1.50 (0.99 – 2.29)	2.21 (0.72 – 6.78)	1.49 (1.00 – 2.23)
15.1 – 50 mg/mmol	1687	1.84 (1.26 – 2.70)	3.71 (1.34 – 10.26)	1.93 (1.34 – 2.78)
>50 mg/mmol	1779	2.94 (2.02 – 4.28)	11.14 (4.11 – 30.19)	4.83 (3.40 – 6.85)
ACR				
<1.5mg/mmol	1007	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
1.5 – 3.0mg/mmol	740	1.30 (0.92 – 1.84)	1.80 (0.67 – 4.88)	1.22 (0.85 – 1.74)
3.1 – 30mg/mmol	1562	1.69 (1.27 – 2.25)	3.53 (1.54 – 8.13)	1.87 (1.40 – 2.51)
>30mg/mmol	1620	2.44 (1.83 – 3.25)	10.12 (4.47 – 22.93)	4.26 (3.22 – 5.64)

Adjusted hazard ratios (with 95% confidence intervals) from multivariate Cox regression analyses are presented. Age, gender, blood pressure and serum creatinine are co-variates in all models.

3.3.4 Comparison of patient outcomes for urine albumin and urine total protein: 24-hour urinary albumin versus 24-hour urinary total protein

Table 3-6 shows the Cox regression analyses for 24-hour urine albumin compared to 24-hour urine protein. Adjusted hazard ratios for total proteinuria were not significantly different to albuminuria for death and RRT, but was significantly higher for doubling of serum creatinine. There was no significant difference between the ROC curve analysis for the three outcome measures (figures 3-6 to 3-8).

Table 3-6 - Association of baseline measures of proteinuria with subsequent patient outcomes in a subset of 1808 chronic kidney disease patients with 24-hour urine samples

	n	Death	RRT	Doubled sCr
24-hour urine albumin	1676	1.17 (1.02 – 1.34)	2.22 (1.77 – 2.78)	1.91 (1.66 – 2.20)
24-hour urine total protein	1676	1.27 (1.05 – 1.54)	2.46 (1.86 – 3.25)	3.04 (2.40 – 3.85)
24-hour ACR	1676	1.26 (1.11 – 1.42)	2.24 (1.83 – 2.74)	1.91 (1.69 – 2.16)
24-hour TPCR	1676	1.28 (1.14 – 1.44)	1.88 (1.59 – 2.23)	2.12 (1.84 – 2.45)

Adjusted hazard ratios (with 95% confidence intervals) from multivariate Cox regression analyses are presented. The adjusted hazard ratios for ACR and TPCR are per one standard deviation difference in the variable. Age, gender, blood pressure and serum creatinine are co-variates in all models. Serum creatinine is a time-dependent co-variate for renal replacement therapy (RRT). Age is a time-dependent co-variate for doubling serum creatinine (sCr)

Figure 3-6 - Receiver operator characteristic curves for baseline 24-hour albuminuria and 24-hour total proteinuria to predict all-cause mortality. Area under the curve for 24-hour albuminuria was 0.608 (95%CI 0.576 – 0.640; p<0.001) and for 24-hour total proteinuria was 0.623 (95%CI 0.591 – 0.655; p<0.001).

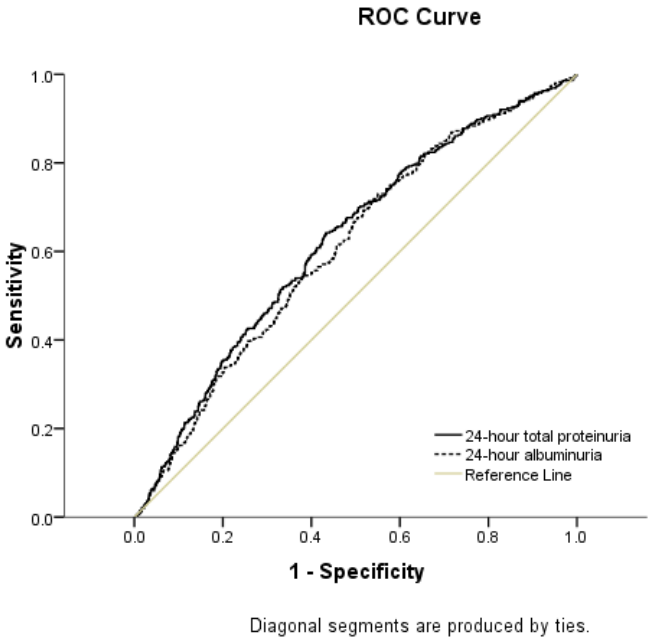


Figure 3-7 - Receiver operator characteristic curves for baseline 24-hour albuminuria and 24-hour total proteinuria to predict commencement of renal replacement therapy. Area under the curve for 24-hour albuminuria was 0.760 (0.727 – 0.792; p<0.001) and for 24-hour total proteinuria was 0.772 (0.740 – 0.804; p<0.001).

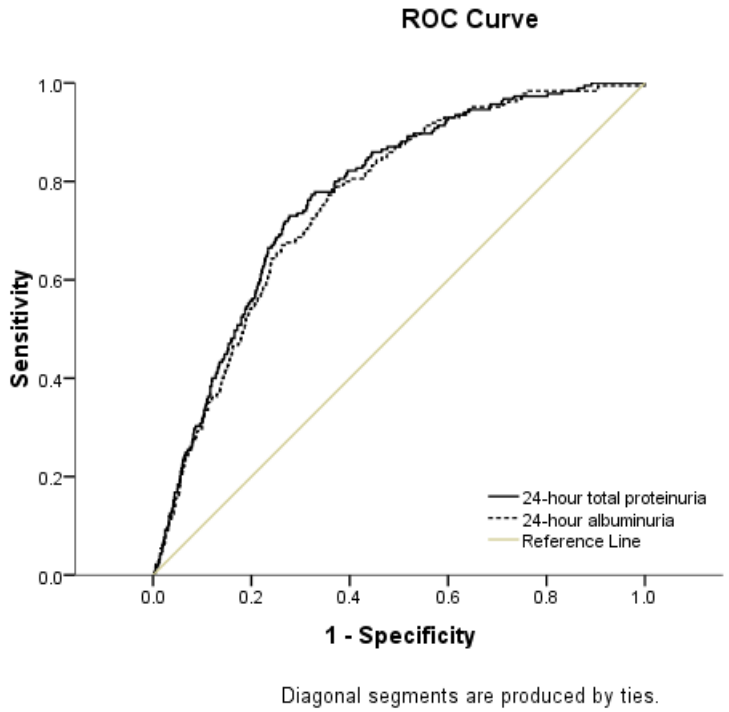
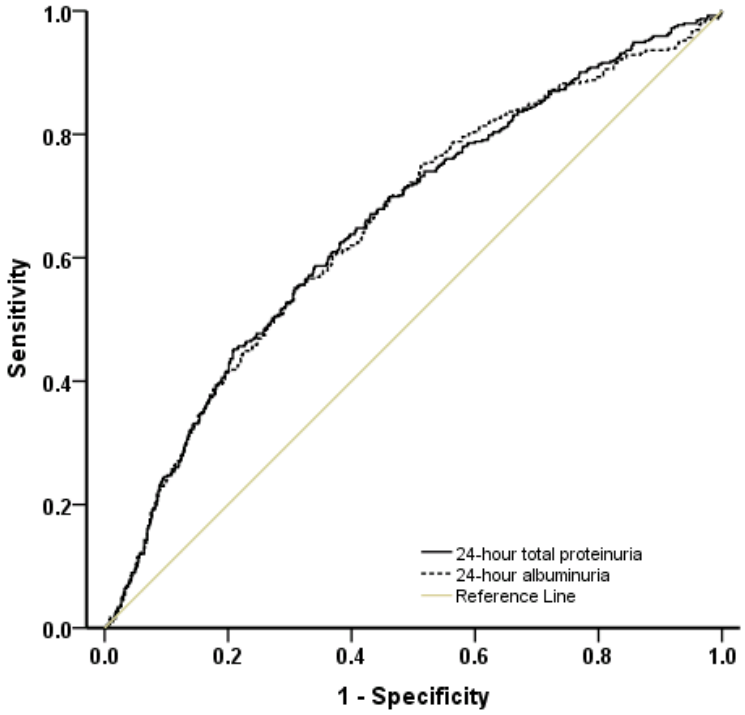


Figure 3-8 - Receiver operator characteristic curves for baseline 24-hour albuminuria and 24-hour total proteinuria to predict doubling of serum creatinine. The area under the curve for 24-hour albuminuria was 0.652 (95% CI 0.619 – 0.686; p<0.001) and for 24-hour total proteinuria was 0.655 (95% CI 0.622 – 0.688; p<0.001).



3.3.5 Comparison of patient outcomes for urine albumin and urine total protein: Urine Ratios Indexed to Creatinine versus 24-hour Urine Samples

We also compared the performance of ACR and TPCR (derived from the 24-hour samples) to the 24-hour urinary albumin and total protein results in this subset of 1808 patients. The results of the Cox regression analyses are in table 3-6. The adjusted hazard ratios for ACR and TPCR derived from a timed urine collection were similar to the aHR of the 24-hour albumin or total protein excretion. The ROC curves comparing ACR and TPCR (derived from a timed urine collection) to 24-hour urine albumin and total protein showed no significant difference for either analyte, so have not been shown.

3.4 Discussion

3.4.1 Findings of the study

Our study shows that total urinary protein and albumin are equally powerful predictors of all-cause mortality and renal outcomes in patients with CKD attending a hospital kidney clinic. We demonstrated this when assessing albuminuria or total proteinuria with ACR and TPCR respectively, derived from a random urine sample. This was confirmed it in a large subgroup with both ACR and TPCR derived from a timed urine collection, and 24-hour urine albumin and total protein excretion results. The analysis held true whether TPCR and ACR were assessed as continuous or categorical variables. Unexpectedly, TPCR also performed well at low levels (TPCR of 15-50 mg/mmol, equivalent to 0.15-0.5 g/day of total proteinuria), where albuminuria has traditionally been seen as the superior marker of risk and this finding persisted when the lower half of the normal range was used as the reference group in the survival analysis.

3.4.2 Albuminuria, total proteinuria and outcomes

The literature comparing albuminuria and total proteinuria is discussed in the introduction (chapter 1). It is limited and none of the studies assessed patient outcomes in a CKD population. A post-hoc analysis of the RENAAL study compared 24-hour total proteinuria, albuminuria and ACR to predict a composite renal end-point in patients with Type 2 diabetes mellitus and found that ACR was superior. TPCR was not measured and mortality data were not included (90). There are 2 studies in the renal transplant population; the first compared albuminuria and non-albumin proteinuria as a predictor of graft loss (222) and found both were independent predictors and provided different information. The second study compared 24-hour albuminuria and proteinuria and found albuminuria to be the superior predictor of graft loss (223). The study presented here demonstrates that both are equally predictive of patient outcomes in chronic kidney disease, at all levels of

proteinuria, in a mixed population of patients attending a general nephrology clinic, who predominantly have non-diabetic kidney disease.

There is extensive literature linking albuminuria with cardiovascular disease and mortality in both renal and general populations (88, 199-201). The CKD prognosis consortium performed large meta-analyses of renal and general population studies of albuminuria and dipstick proteinuria (which also predominantly measures albuminuria (70)) and showed a strong association with cardiovascular and all-cause mortality (20, 108). Several other studies (193, 195-197) have also demonstrated a link between dipstick proteinuria and mortality. The relationship between total proteinuria and all cause mortality, demonstrated in this chapter, is less well documented. One Finnish study demonstrated an association between spot urine concentration of total protein (i.e. not adjusted for creatinine concentration) and subsequent cardiovascular and all-cause mortality (202).

3.4.3 The role of non-albumin proteinuria

In urine, total protein is comprised predominantly of albumin, but also of physiological proteins (such as uromodulin) and other non-albumin proteins of various molecular weights. The proportions of these proteins vary widely in pathological states, and non-albumin proteins are less well-defined compared to albumin. There is less inter- and intra-laboratory variation in albumin assays than total protein assays (70), and efforts are underway to standardise the albumin assay across laboratories (66), however ACR is 2 – 10 times more costly than TPCR. Given the technical challenges, it is perhaps surprising that TPCR performed as well as ACR in predicting risk in our study. High molecular weight proteinuria has been shown to correlate more strongly with rate of progression of renal disease than intermediate molecular weight, low molecular weight or even total proteinuria. This is thought to be as a result of increased tubular toxicity, though an alternative hypothesis would be that this finding is simply a consequence of loss of

glomerular size selectivity (224). The fractional excretion of Immunoglobulin G (a high molecular weight protein) has also been shown to be a strong predictor of adverse outcomes in patients with CKD, as a result of loss of glomerular size selectivity (225). Other specific non-albumin proteins (low molecular weight proteins) can be measured in the urine such as retinol binding protein, and alpha-1 and beta-2-microglobulin, the former being more stable at a range of pH (226). Alpha-1-microglobulin has been shown to be a marker of tubular pathology and can rise to significant levels in disease states (227). However these are specialist immunoassays that are not widely available for screening purposes, so are not currently a viable alternative to the total protein assay for the detection of non-albumin proteinuria.

There is substantial variation in the amount of non-albumin proteinuria between individuals at clinically significant levels of albuminuria. We have shown in chapter 2 that using only ACR to identify patients with significant proteinuria (>1g/ day) would lead to over one fifth of patients (22%) being undetected, who would otherwise have been identified using TPCR as a result of high proportions of non-albumin proteinuria. The non-albumin proteinuria may carry some additional prognostic significance which is not captured by measuring albumin alone.

3.4.4 24-hour urine collections – the gold standard?

A subsidiary finding in our study was that TPCR and ACR were as powerful as 24-hour urine protein and albumin respectively at predicting patient outcomes. Traditionally, 24-hour urine samples have been seen as the "gold standard" method to measure total proteinuria or albuminuria, but spot urine samples are more convenient for patients, clinicians and laboratories. The ability of spot urine TPCR and ACR to predict 24-hour total proteinuria and albuminuria respectively has been investigated, and shown to be accurate, reliable and reproducible (79). One study has examined the comparative

performance of ACR and 24-hour albuminuria and proteinuria to predict renal outcomes, and found ACR to be superior (90). The study did not include TPCR measurements. There are two possible explanations for the excellent performance of TPCR and ACR: 24-hour urine collections are difficult for patients to collect accurately, and so spot urines may represent a more accurate estimate of true 24-hour urine protein excretion; second, the urinary creatinine may also be contributing to the predictive power of the test. Creatinine excretion correlates with muscle mass (218), so malnutrition or muscle wasting would lead to a higher TPCR or ACR for any given level of urinary protein excretion and may contribute to the risk. Urine creatinine excretion has been shown to be an independent predictor of cardiovascular and all-cause mortality in the general population (228). In a retrospective study we cannot differentiate between these explanations. Furthermore, this analysis should be replicated in a study comparing random spot urine samples to 24-hour urine samples.

3.4.5 Limitations

Our study has several limitations, some of which were outlined in relation to the study in chapter 2 given that they were derived from a similar cohort. It was retrospective in nature. Our lack of complete data on race and primary renal disease has hampered our ability to produce subgroup analyses. Twenty-four hour urine collections were available in approximately one third of patients. There may have been drift in the assays over such a prolonged period, but this will affect all assays and we have no reason to expect a systematic bias. These issues affect our assessment of ACR and TPCR equally. The relationships demonstrated may only apply to the assays used in our study. However, strengths of this study include the large number of patients, and the representative nature of the population; an unselected adult population attending a general nephrology clinic. It is not representative of primary care-based CKD populations, which have a lower prevalence of proteinuria, and a different age distribution.

3.4.6 Conclusion

Total proteinuria and albuminuria perform equally as predictors of renal outcomes and mortality in patients with CKD. ACR and TPCR were as effective as 24-hour urine samples at predicting outcomes, and are more convenient for patients, clinicians and laboratories. TPCR also performed well in the "microalbuminuria" range. Both ACR and TPCR are useful tools to stratify risk in CKD.

4 Chapter 4: Stratifying Risk in Chronic Kidney Disease: an Observational Study of UK Guidelines for Measuring Total Proteinuria and Albuminuria

4.1 Introduction

We have shown in Chapter 2 that TPCR is a superior predictor of 24-hour total proteinuria. In Chapter 3 we have demonstrated that proteinuria is associated with adverse outcomes, and spot urines predict this equally well as 24-hour urine collections. TPCR and ACR perform equally as predictors of renal and patient outcome in a mixed population attending a nephrology clinic. However the optimal method to measure proteinuria remains uncertain. Intervention studies in diabetic kidney disease have traditionally measured albuminuria (146, 229, 230) while those in non-diabetic kidney disease have used total proteinuria (124). Two key thresholds have been identified in the management of proteinuria; 1g/day of total proteinuria, above which aggressive blood pressure control has been demonstrated to reduce progression to end-stage kidney disease (156) and 0.5g/day of total proteinuria above which the use of angiotensin converting enzyme inhibitors have been found to be specifically beneficial, over and above their blood pressure lowering effects, to retard progression of kidney disease (221).

The NICE guidelines in England and Wales and NKF-KDOQI guidelines in the United States recommend quantifying proteinuria using ACR in all patients with CKD, whereas the Scottish guidelines (SIGN) recommend TPCR in non-diabetic patients (5, 7, 8). The relationship between total protein and albumin in the urine is non-linear, but this study uses the same equivalent levels as outlined in chapter 3: >1 g/day proteinuria (equivalent to ACR >70 mg/mmol or TPCR >100 mg/mmol) and >0.5 g/day proteinuria (equivalent to ACR >30 mg/mmol or TPCR >50 mg/mmol) (8).

The aim of this study was to compare the outcomes of patients identified as having significant proteinuria, according to the thresholds described above by ACR and TPCR (as recommended by the differing national guidelines).

4.2 Methods

4.2.1 Laboratory Assays

The laboratory assays used in this analysis are described in section 2.2.1.

4.2.2 Study population

The population studied in this analysis are the same patient cohort described in section 3.2.2.

4.2.3 Ethical Permission

This is described in section 3.2.3.

4.2.4 Statistical Analysis

Data were analysed using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA, www.spss.com). All data were assessed for normality, and appropriate summary statistics are presented. As before, TPCR and ACR data were log-transformed given the skewed distribution of values. Comparison of the groups was performed using 2 sample T test, analysis of variance, Mann-Whitney U test and the Kruskall Wallis test as appropriate.

Proteinuria was defined as significant using the two thresholds of 0.5 g/day or 1g/day (0.5g/day being equivalent to ACR ≥30 mg/mmol, and TPCR ≥50 mg/mmol and 1g/day being equivalent to ACR ≥70 mg/mmol and TPCR ≥100 mg/mmol). Mild proteinuria was defined as below the thresholds described, and above the laboratory reference range (ACR 3-29mg/mmol and TPCR 15-49mg/mmol (i.e. microalbuminuria) for the 0.5g/day threshold and ACR 3-69mg/mmol and TPCR 15-99mg/mmol for the 1g/day threshold). No proteinuria was defined as less than the laboratory reference range (ACR <3mg/mmol and TPCR <15mg/mmol). Kaplan-Meier survival plots were constructed. A similar statistical technique was employed as for chapter 3, namely hazard ratios were calculated for the main outcome measures (all-cause mortality, commencement of RRT and doubling of serum creatinine) using a hierarchical Cox regression survival analysis with age, gender,

blood pressure and serum creatinine as co-variates entered in the first block, and either ACR or TPCR entered in the second block. The hazard ratios presented are for a 10-fold increase in the variable measured (due to the use of a logarithmic scale). Cases were excluded from the Cox regression survival analysis if any of the variables were missing (mostly blood pressure). The analyses were repeated with missing variables imputed using regression, to ensure there was no influence on the model. The linearity of each continous predictor was tested by calculating martingale residuals for the Cox regression model without the predictor and then plotting these against the predictor using lowess smoothing. The proportional hazards assumption was tested by creating time dependent co-variates for each predictor and including them in the model if the interaction was significant.

4.3 Results

4.3.1 Demographics

The cohort and overall demographic data is identical to that described in Chapter 3. A flow diagram of the population, exclusions and grouping according to proteinuria for this analysis is shown in Figure 4-1. Baseline data for the 5586 patients are presented in Table 4-1, divided according to level of proteinuria. The primary renal disease was available in 68% of the total cohort as described in chapter 3 (defined according to the European Renal Association- European Dialysis and Transplantation Association codes). Of the patients in Group 4 (discordant group) 72% had a PRD recorded. The proportion of primary glomerulonephritis was lower (7.2% v 17% in the overall cohort), interstitial disease was considerably higher (39.5% v 22.5% in the overall cohort) multisystem disease and diabetic nephropathy lower (4.8% v 16.3% and 8.4% v 11.1% respectively) and CKD cause unknown was higher (40.1% v 33.1%). Of note 26 patients in the total cohort had a PRD of myelomatosis and of these 5 patients were in Group 4 (discordant group). Patients were followed up for a median of 3.5 years (IQR 2.1 – 6.0 years).

Figure 4-1 – Flowchart of population and exclusions, showing the cohort divided into groups according to degree of proteinuria

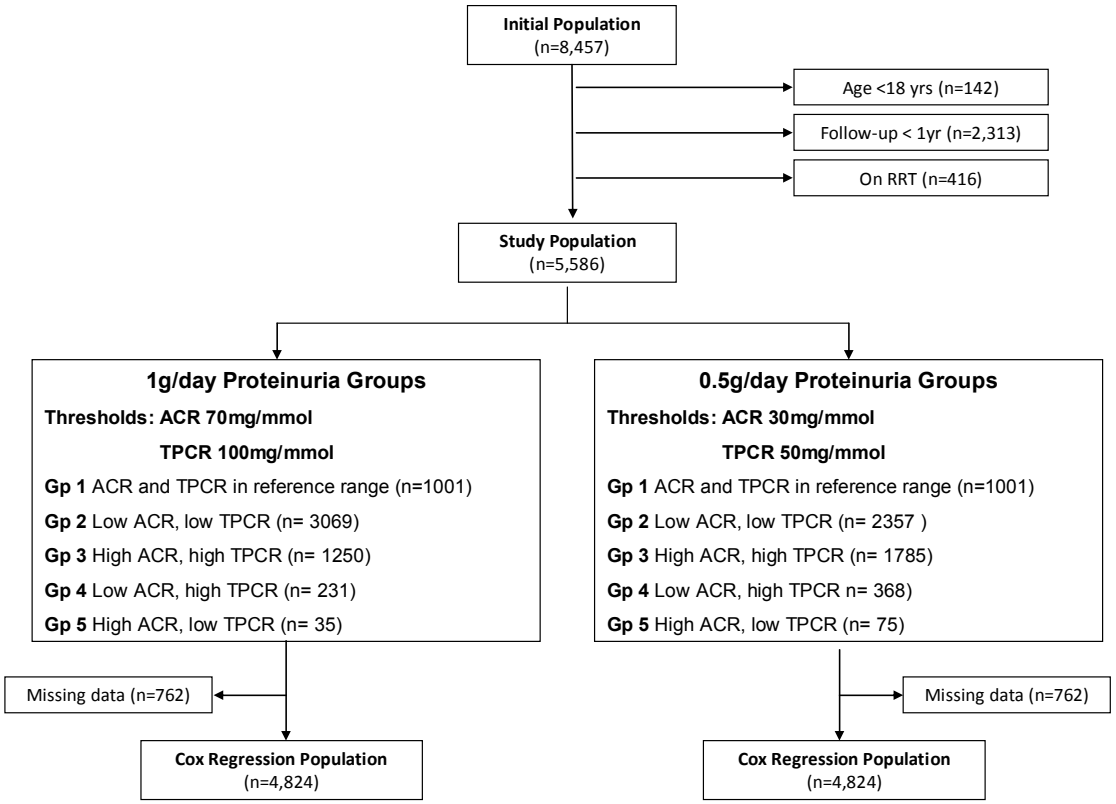


Table 4-1 - Demographics for the population, divided according to the described groups (as per the thresholds for 1g/day)

	Group 1 No proteinuria (ACR/ TPCR within reference range)	Group 2 Mild proteinuria (low ACR, low TPCR)	Group 3 Significant proteinuria (High ACR, high TPCR)	Group 4 Discordant (Low ACR, high TPCR)	Group 5 Discordant (High ACR, low TPCR)	P value
Number	1001	3069	1250	231	35	-
Age (years)	53.2 (± 17)	60.6 (±16.4)	58.3 (±15.5)	64.4 (±14.3)	51.7 (±16.1)	p<0.001
Sex	49% male	48% male	46% male	46% male	63% male	p=0.001
sCr (µmol/l)	107 (90 – 138)	155 (100 – 180)	170 (120 – 257)	250 (174 – 380)	130 (100 – 200)	p<0.001
MDRD (ml/min/ 1.73m^2)	59.8 (42.4 – 73.3)	41.8 (29.4 – 61.8)	33.3 (20.7 – 51.9)	20.8 (12.4 – 32.0)	46.8 (28.4 – 68.4)	p<0.001
SBP (mmHg)	134 (±23)	143 (±26)	154 (±29)	145 (±28)	147 (±25)	p<0.001
DBP (mmHg)	76 (±12)	77 (±14)	81 (±14)	77 (±14)	86 (±15)	p<0.001
ACR (mg/mmol)	1 (1 – 2)	4.6 (3 – 18)	188 (120-351)	44 (21 – 57)	79 (75 – 92)	p<0.001
TPCR (mg/mmol)	8.4 (5.7 – 11.1)	26.3 (18.2 – 44.4)	275.0 (181.8 – 500.0)	142.9 (117.7 – 200.0)	92.4 (87.0 – 97.0)	p<0.001
NAPCR (mg/mmol)	7.4 (4.3 – 9.9)	17.8 (11.0 – 26.6)	81.3 (45.3 – 153.1)	108.1 75.0 – 169.1)	10.9 (-2 – 19.8)	p<0.001
ACEi/ARB use (%)	5.2	22.6	24.3	12.1	28.6	p<0.001

Demographics expressed as mean (± standard deviation) or median (interquartile range).Significance testing performed using analysis of variance and Kruskall Wallis test as appropriate.

4.3.2 Patient Outcomes

The patient outcomes of the cohort are described in chapter 3.

4.3.3 Cohort Subgroups: Clinically important thresholds of proteinuria: 1g/day

The cohort was divided into three groups with concordant ACR and TPCR results:

- Group 1: no proteinuria (within laboratory reference range of ACR<3mg/mmol and TPCR<15mg/mmol) (n=1001)
- Group 2: mild proteinuria (<1g/day equivalent) (n=3069)
- Group 3: significant proteinuria (>1g/day equivalent) (n=1250).

Two groups with discordant results by ACR and TPCR were also defined:

- Group 4: significant proteinuria by TPCR but not ACR (urine total protein over 1g/day equivalent, but low urine albumin) (n=231)
- Group 5: significant proteinuria by ACR but not TPCR (urine total protein <1g/day equivalent, but high urine albumin) (n=35).

The numbers in Group 5 are very small and have therefore been excluded from the results presented here. However when Group 5 was included, the results did not alter significantly. The demographics of Groups 3 and 4 were compared using a 2 sample T test and Mann-Whitney U test, as appropriate. Group 4 (discordant proteinuria) was significantly older with lower eGFRs ($p<0.001$), while Group 3 (significant proteinuria) had significantly higher blood pressures and proteinuria, measured by ACR and TPCR ($p<0.001$). There was no difference in gender between the groups ($p=0.936$). Kaplan-Meier survival plots were constructed for all-cause mortality (Figure 4-2), renal survival (Figure 4-3) and doubling of serum creatinine (Figure 4-4). Patient survival of Group 4 (discordant proteinuria) was significantly worse than Groups 2 (mild proteinuria) and 3 (significant proteinuria) (log rank test, $p<0.001$). Renal survival for Group 4 (discordant proteinuria) is similar to Group 3 (significant proteinuria), and significantly worse than Group 2 (mild proteinuria) ($p<0.001$).

Figure 4-2 - Kaplan Meier survival plot for all-cause mortality for the groups according to ACR and TPCR

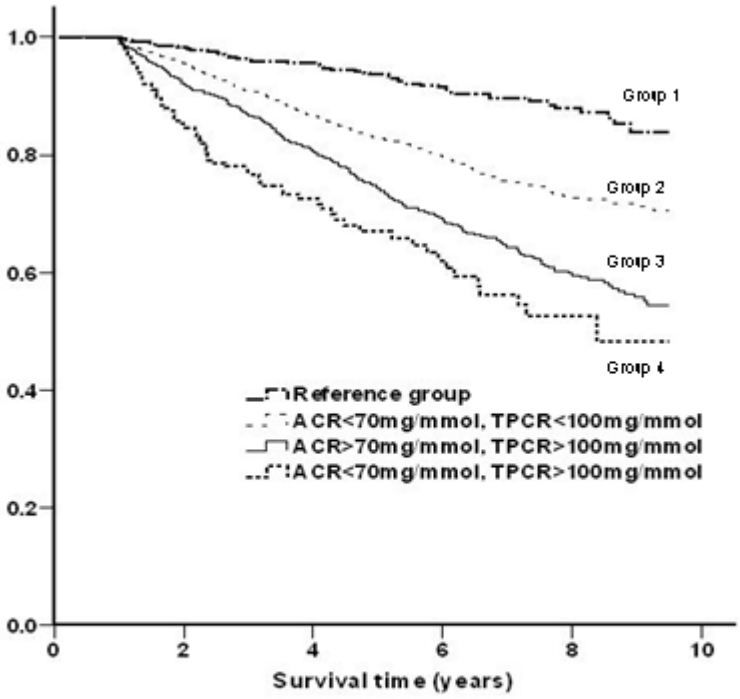
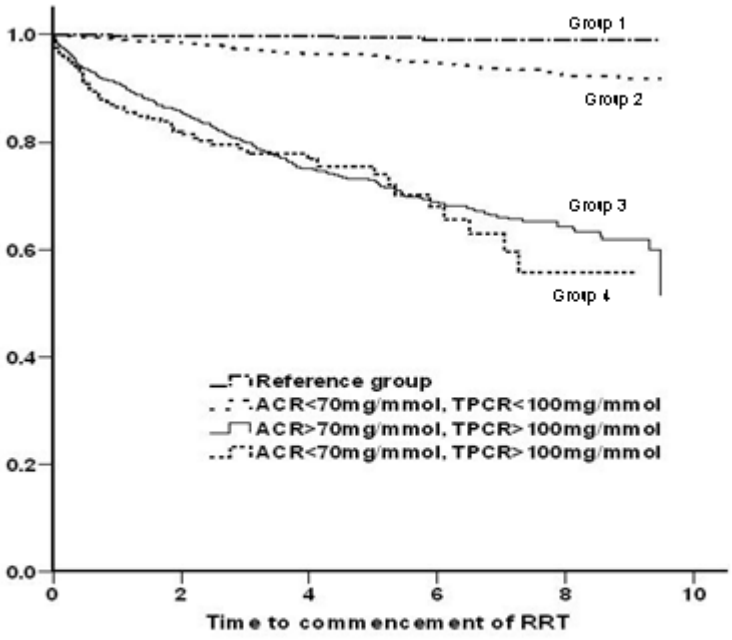
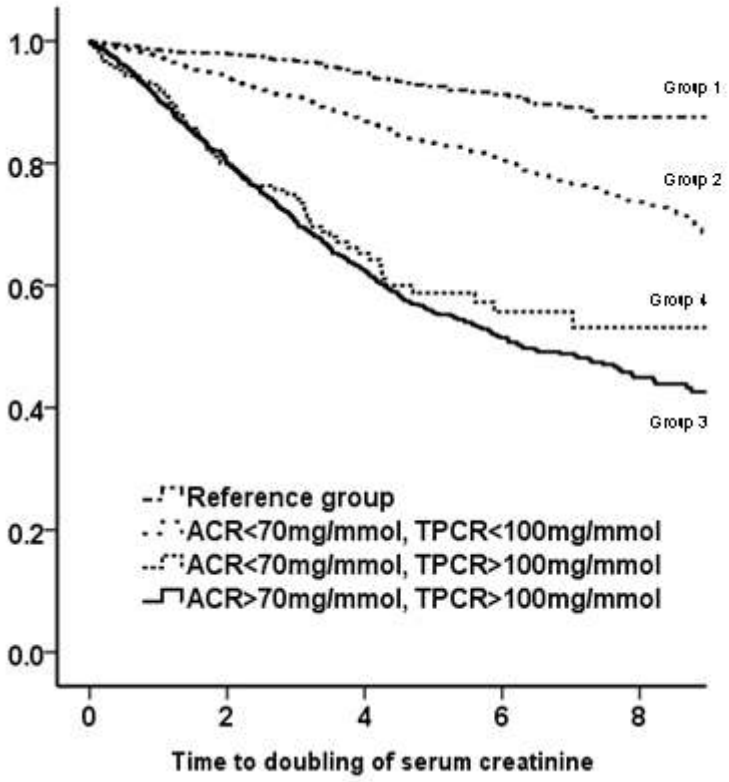


Figure 4-3 - Kaplan Meier survival plot for renal survival for the groups according to ACR and TPCR



Reference Group ACR<3mg/mmol and TPCR<15mg/mmol.

Figure 4-4 - Kaplan Meier survival plot for doubling of serum creatinine for the groups according to ACR and TPCR



Reference Group ACR<3mg/mmol and TPCR<15mg/mmol.

A multi-variate analysis was performed using Cox regression analyses for all-cause mortality, RRT and doubling serum creatinine, with age, sex, kidney function and blood pressure as co-variates (Table 4-2). The risk of all cause mortality for Group 4 (discordant proteinuria) compared to Group 3 (significant proteinuria) is attenuated by the multi-variate analysis, but the risk does not fall to that of Group 2 (mild proteinuria). The same pattern is seen for commencement of RRT and doubling of serum creatinine. Repeat analyses with imputed data (using regression) for any missing variables did not alter the results significantly, and are therefore not shown.

Table 4-2 – Association of baseline urinary ACR and TPCR with subsequent patient outcomes in 4824 patients with CKD, (using thresholds approximately equivalent to 1g/day of proteinuria)

	Group 1 No proteinuria (ACR and TPCR within reference range)	Group 2 Mild proteinuria (low ACR, low TPCR)	Group 3 Significant proteinuria (High ACR, high TPCR)	Group 4 Discordant proteinuria (Low ACR, high TPCR)
Death	1.00	1.57 (1.18 – 2.09)	2.59 (1.91 – 3.50)	1.91 (1.29 – 2.83)
RRT	1.00	2.06 (1.07 – 3.97)	7.91 (4.15– 15.08)	4.40 (2.17 – 8.91)
Doubled sCr	1.00	1.70 (1.28 – 2.25)	5.07 (3.82 – 6.74)	3.56 (2.43 – 5.20)

Adjusted hazard ratios (with 95% confidence intervals) from multivariate Cox regression analyses are presented, for a ten-fold increase in the variable measured. Age, gender, blood pressure and serum creatinine are co-variates in all models. Serum creatinine is a time-dependent co-variate for RRT. Age is a time-dependent co-variate for doubling sCr.

4.3.4 Cohort subgroups: Clinically important thresholds of proteinuria: 0.5g/day

The same process was applied to the 0.5g/day threshold, using the appropriate cut-points (see Figure 4-1 for a description of Groups 1 - 5). There were 2228 patients with total proteinuria >0.5g/day using either measure (i.e. the patients with ACR≥30mg/mmol or TPCR≥50mg/mmol) and 2153 patients with TPCR≥50mg/mmol, of which 368 had an ACR <30mg/mmol, i.e. the discordant group (16.5% and 17.1% of the total respectively depending on the denominator). Kaplan Meier plots were also constructed for the three outcome measures using a proteinuria threshold of 0.5g/day (see figure 4-5, 4-6 and 4-7 respectively). For the outcome of all-cause mortality, Group 4 (discordant proteinuria) had a significantly worse outcome than Groups 2 (mild proteinuria) and 3 (significant proteinuria); ($p<0.001$), and for renal survival and doubling of serum creatinine, Group 4 had a significantly worse outcome than Group 2 (mild proteinuria), but performed better than Group 3 (significant proteinuria) ($p<0.001$).

Figure 4-5 - Kaplan Meier survival plot for all-cause mortality for the groups according to ACR and TPCR to predict 0.5g/day of total proteinuria

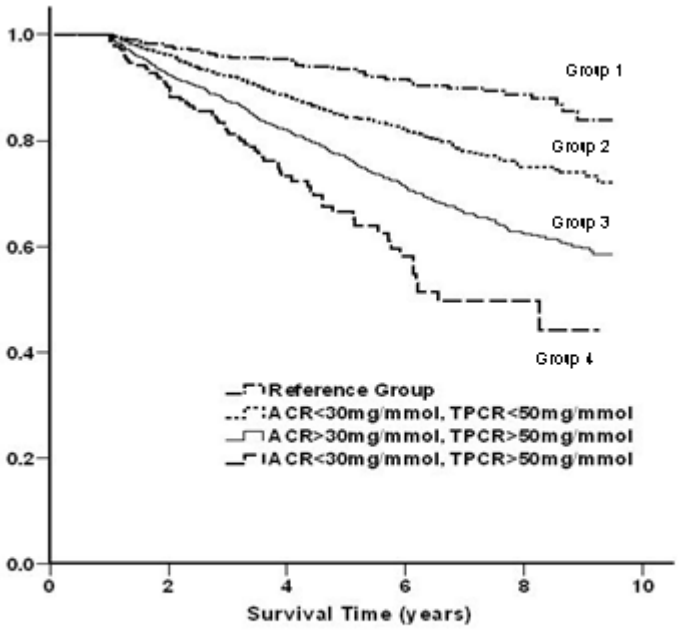
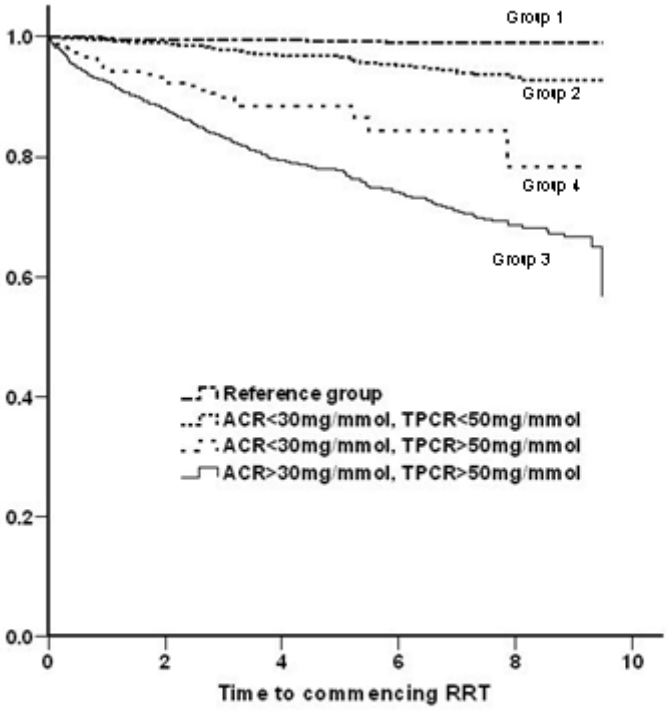
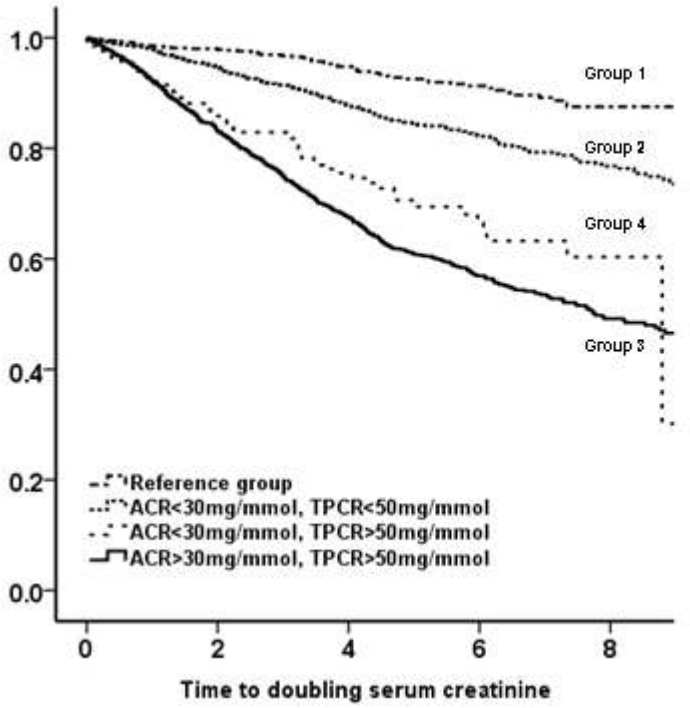


Figure 4-6 - Kaplan Meier survival plot for renal survival for the groups according to ACR and TPCR to predict 0.5g/day of total proteinuria



Reference Group ACR<3mg/mmol and TPCR<15mg/mmol.

Figure 4-7 - Kaplan Meier survival plot for doubling of serum creatinine for the groups according to ACR and TPCR to predict 0.5g/day of total proteinuria



Reference Group ACR<3mg/mmol and TPCR<15mg/mmol.

A multi-variate analysis was also performed for the 0.5g/day proteinuria threshold using Cox regression analyses for all-cause mortality, RRT and doubling serum creatinine, with age, sex, kidney function and blood pressure as co-variates, (Table 4-3). The same pattern of results is seen as for the 1g/day threshold. Repeat analyses with imputed data (using regression) for any missing variables did not alter the results significantly.

Table 4-3 – Association of baseline urinary ACR and TPCR with subsequent patient outcomes in 4824 patients with CKD (using thresholds approximately equivalent to 0.5g/day of proteinuria)

	Group 1 No proteinuria (ACR and TPCR within reference range)	Group 2 Mild proteinuria (low ACR, low TPCR)	Group 3 Significant proteinuria (High ACR, high TPCR)	Group 4 Discordant proteinuria (Low ACR, high TPCR)
Death	1.00	1.49 (1.11 – 1.99)	2.48 (1.86 – 3.32)	2.34 (1.63 – 3.35)
RRT	1.00	2.28 (1.08 – 4.77)	8.46 (4.14 – 17.26)	2.90 (1.31 – 6.43)
Doubled sCr	1.00	1.47 (1.11 – 1.95)	4.11 (3.14 – 5.38)	2.35 (1.62 – 3.40)

Adjusted hazard ratios (with 95% confidence intervals) from multivariate Cox regression analyses are presented, for a ten-fold increase in the variable measured. Age, gender, blood pressure and serum creatinine are co-variates in all models. Serum creatinine is a time-dependent co-variate for RRT. Age is a time-dependent co-variate for doubling sCr.

4.4 Discussion

4.4.1 Findings of the study

In this cohort of patients attending a hospital kidney clinic, ACR failed to identify 231 patients with significant proteinuria who were identified with TPCR. This represents 15% of patients with significant proteinuria (defined as TPCR *and/or* ACR >1g/day equivalent of urine protein, n=1516) or 16% of patients who would have been identified by TPCR alone (231/ 1481). The same pattern was seen when using the 0.5g/ day total proteinuria threshold with the unidentified proportion using ACR alone being 16.5% and 17% respectively. This subgroup of patients (with significant proteinuria by TPCR but not ACR) had a higher rate of interstitial disease and lower rate of glomerular disease than the overall cohort. This supports TPCR detecting non-albumin proteins ("tubular proteinuria"), undetected by measuring ACR alone. This group has a high risk of renal events and death, with comparable renal survival and poorer patient survival than those with significant proteinuria by both TPCR and ACR. This increased risk for the subgroup of patients with significant proteinuria by TPCR but not ACR remains when the lower threshold of 0.5 g/day of proteinuria was used.

With multivariate analysis, some of the excess risk is abolished, with the risk of all-cause mortality, commencement of RRT and doubling of serum creatinine falling below that of the significant proteinuria group (Group 3), but remaining higher than the low proteinuria group, (Group 2). This can be explained, in part, by the differences in the demographics of the groups, with Group 4 being significantly older and with a lower eGFR. However, Group 4 (discordant proteinuria) still represents a high risk group that would be identified using an appropriate total proteinuria threshold, but not using an equivalent albuminuria threshold.

4.4.2 Guideline recommendations on the measurement of proteinuria

NICE currently recommends that ACR should be used to screen all patients with CKD annually, with thresholds for action of 30 and 70 mg/mmol (8). NKF-KDOQI similarly recommends the use of ACR, although allows the use of TPCR when ACR is >56 mg/mmol equivalent (5). However, SIGN recommends using TPCR for CKD patients without diabetic nephropathy using action thresholds of 50 and 100 mg/mol (7). Our data illustrate the potential impact of these differing recommendations on an unselected adult population attending a general nephrology clinic. Simply reducing the albuminuria threshold to improve sensitivity is ineffective, as it leads to unacceptably low specificity as shown in chapter 2. Microalbuminuria (around 30-300mg of urine albumin per day) has an established role in detecting early diabetic nephropathy (88), and has been shown to predict cardiovascular mortality in the general population (20). However we have shown in chapter 3 that total proteinuria is also predictive at equivalent levels in a mixed population (albeit predominantly non-diabetic patients) with CKD. The arguments put forward to justify the recommendations of the NICE guidelines are firstly of simplicity; the same test should be used for diabetic and non-diabetic CKD to aid implementation and interpretation across medical disciplines (diabetologists, other physicians and primary care). Secondly, there is less inter- and intra-laboratory variation in albumin assays than total protein assays and efforts are underway to standardise the albumin assay across laboratories (70). However ACR is 2 – 10 times more costly than TPCR, and also has methodological shortcomings such as fragmentation and the variable measurement of non-immunoreactive albumin in the urine. Also the evidence is lacking regarding the role of albuminuria in the progression of non-diabetic CKD. Therefore it is incumbent upon the proponents of ACR to justify its use and associated extra costs, rather than vice versa. Only TPCR takes account of the non-albumin protein component of urine that consists of a less well-defined group of proteins compared to albumin. Furthermore, there is substantial variation in the

amount of non-albumin proteinuria between individuals at clinically significant levels of albuminuria, as we have shown in chapter 2.

4.4.3 Limitations

Our study has several limitations. It was retrospective, and the number of patients in Group 4 was relatively small. There may have been drift in the assays over such a prolonged period, but this will affect all assays and we have no reason to expect a systematic bias. As before, the relationships demonstrated may only apply to the assays used in our study, which is of particular importance in an analysis of this nature as it may limit its generalisability. However strengths of this study include the large numbers of patients, and the representative nature of the population, namely an unselected adult population attending a general nephrology clinic. Although our study population is based on a secondary care cohort of patients there are clear lessons from this study for both primary and secondary care practitioners who adhere to a referral pattern based solely on level of ACR.

Prospective studies are required to clarify the roles of total proteinuria and albuminuria as predictors of patient outcomes. Interventional studies in CKD should also assess the impact on both ACR and TPCR. Further research should examine the importance of specific non-albumin proteins in the urine both for prognostication, and to shed light on underlying pathophysiology.

4.4.4 Conclusion

In conclusion, screening with ACR alone will fail to identify 16% of patients with significant levels of proteinuria who would be identified by TPCR. This subgroup is at higher risk of death and renal outcomes than those with low proteinuria (low ACR, low TPCR) and merit identification. The current approach to measuring proteinuria recommended by guidelines should be reconsidered. The non-albumin component of proteinuria may have pathophysiological significance, and both should be taken into account.

5 The Impact of Muscle Mass on the Assessment of Proteinuria in Chronic Kidney Disease

5.1 Introduction

In chapter 2 we showed that TPCR and ACR correlate well with 24-hour urine protein or albumin, but their predictive ability markedly varies with age and gender. In chapter 3 and 4 we showed that TPCR and ACR both identify patients at high risk of progressive renal disease and death.

Urine total protein:creatinine ratio and albumin:creatinine ratio are derived values: urine protein or albumin concentration is divided by urine creatinine concentration. As creatinine is generated at a relatively constant rate in individuals, it can act as a correction for urine flow rate. The observed differences in performance of the tests between subgroups (e.g. age, gender) is thought to relate to differing creatinine excretion as a consequence of muscle mass. For any given level of protein excretion per litre, the TPCR or ACR will be correspondingly higher in patients with lower muscle mass. There are three possible consequences of this:

1. muscle mass may confound the ability of TPCR or ACR to predict 24-hr urine protein excretion and subsequent outcomes;
2. muscle mass may add to the prognostic ability of the test either by acting as a surrogate of general health or by correcting for body size;
3. muscle mass may have a neutral effect, as a consequence of a combination of these factors, or neither.

To investigate this, we assessed the impact of adjusting TPCR or ACR for creatinine excretion in a large cohort of patients with chronic kidney disease. We examined the ability of the adjusted spot samples to quantify proteinuria accurately (by predicting 1 g/day of total urine protein). We then examined prediction of outcomes relevant to patients, including renal survival and patient survival.

5.2 Methods

5.2.1 Laboratory assays

The laboratory assays used in this analysis, are described in section 2.2.1.

5.2.2 Study Population

The population studied in this analysis are the same patient cohort described in section 3.2.2.

5.2.3 Ethical Permission

This is described in section 3.2.3.

5.2.4 Statistical Analyses

Data were analysed using SPSS 18.0 for Windows (IBM Inc, http://www-01.ibm.com/software/analytics/spss/) and MedCalc version 12.0 (MedCalc Software, www.medcalc.org). All data were assessed for normality, and appropriate summary statistics are presented.

In order to adjust the ACR and TPCR for creatinine excretion, a number of derived or predicted values were calculated as follows:

Estimated creatinine excretion:

Creatinine excretion was measured directly from a 24-hour urine collection (urine creatinine concentration per litre multiplied by volume) in those patients who performed one.

Estimated creatinine excretion (ECE) was calculated for all patients, using 5 prediction equations, as follows:

1. Ix (equation D) (41):

- 879.89+12.51 x weight (kg) – 6.19 x age + (34.51 if black) – (379.42 if female)

2. Cockcroft and Gault formula (32):

- 24-hr creatinine excretion (g) = (140-age) x weight (kg) x 0.0002 [x 0.85 if female]

3. Goldwasser formula(39):

- [23.6-(age/8.3)(+1.9 if black)] x weight (kg)

4. Walser formula (38):

- Male: (28.2-0.172 x age) x weight (kg)
- Female: (21.9-0.115 x age) x weight (kg)

5. Rule (Mayo Quadratic) Formula (40):

- {exp[7.26-0.26(if female) – (0.011 x (age – 55) if age>55)]} xBSA/1.73

Secondly, in addition to calculating ECE using actual body weight (ABW), ideal and lean body weight were also used in the equations. The rationale for this was that not all of the formulae are explicit regarding the most appropriate measure of body weight to use, and there are a number of issues related to body mass, as outlined in the introduction (chapter 1). Ideal body weight (IBW) and lean body weight (LBW) were calculated for each patient as follows (231-233):

Ideal body weight = 22 x Height $(m)^2$

Lean Body Weight (men) =

$(1.10 \times \text{Weight (kg)}) - 128 \left(\text{Weight}^2/(100 \times \text{Height(m)})^2 \right)$

Lean Body Weight (women) =

$(1.07 \times \text{Weight (kg)}) - 148 \left(\text{Weight}^2/(100 \times \text{Height(m)})^2 \right)$

Finally TPCR was adjusted for ECE, by multiplying the original value by the ECE using the following equation:

Adjusted TPCR = (P/V)/(C/V)*ECE = [P]/[C]*ECE = g/day

where:

[P] = urine protein concentration = protein/ volume = P/V

[C] = urine creatinine concentration = creatinine/ volume = C/V

PCR=protein/ creatinine ratio = [P]/[C]

ECE = estimate of 24-hour Creatinine

The equivalent equation was used to calculate adjusted ACR.

This adjustment to TPCR and ACR was performed using ECE calculated, in turn, using the 3 weight variables as described above. This produced eight variables (four variables for each of TPCR and ACR) as follows:

1. raw: $TPCR_{raw}$ and ACR_{raw}
2. adjusted for ECE using actual body weight: $TPCR_{ABW}$ and ACR_{ABW}
3. adjusted for ECE using ideal body weight: $TPCR_{IBW}$ and ACR_{IBW}
4. adjusted for ECE using lean body weight: $TPCR_{LBW}$ and ACR_{LBW}

ROC curves were constructed to compare the performance of $TPCR_{raw}$ and ACR_{raw} with the adjusted variables as predictors of 24-hr total proteinuria and patient outcomes of all-cause mortality, commencement of renal replacement therapy and doubling of serum creatinine. The AUC was compared using the method of Delong et al (234). Then, in addition to ROC curves, The Net Reclassification Index (NRI), a relatively new technique to compare the performance of tests as predictors of dichotomous outcomes, was also calculated for the patient outcomes described above (235). The NRI indicates the proportion of patients correctly reclassified into high or low risk groups using a new biomarker (in this case the adjusted TPCR and ACR compared to the raw value). The threshold for significant proteinuria of 1g/day equivalent was used to classify the groups into high and low risk for the purposes of the NRI.

5.3 Results

5.3.1 Demographics

A flowchart of the study population and exclusions in shown in figure 5-1. Baseline data for this cohort of 5586 patients with ACR and TPCR measured on the same day between 24th November 1999 and 28th May 2008 are described in chapter 3 (table 3-1). Twenty four hour urine collections were performed by 1808 patients (32% of the cohort) and the baseline demographics of this group are also described in the same table in chapter 3. Additional baseline measurements of the cohort, pertinent only to this analysis, are shown in table 5-1.

Figure 5-1 - Flowchart of study population and exclusions

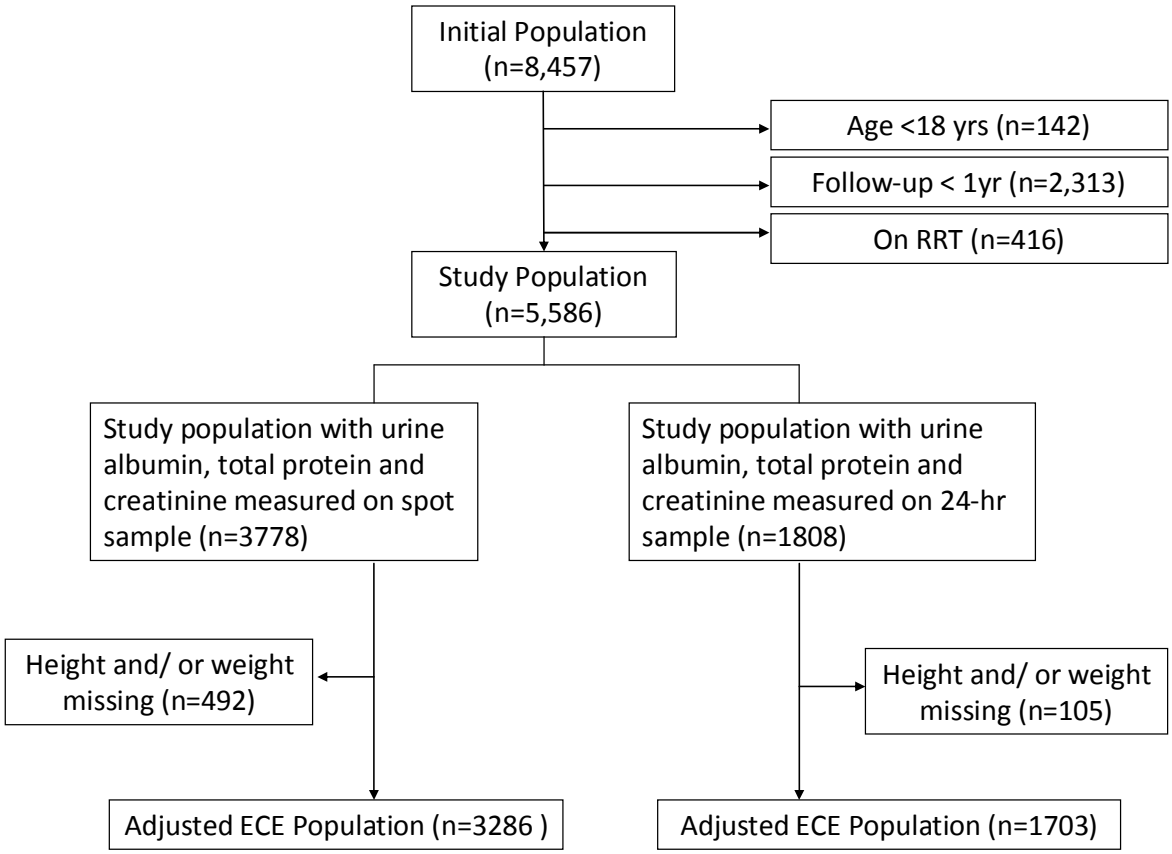


Table 5-1 - Selected demographic information for the cohort of 5586 patients attending a nephrology clinic

	Total cohort (n=5586)	% available	Cohort with 24-hr urine (n=1808)	% available
Height (m; ± SD)	1.65 ± 0.1	90.5	1.65 ± 0.1	95.9
Actual body weight (kg; ± SD)	77.8 ± 18.3	90.5	78.1 ± 18.4	94.8
Ideal body weight (kg; ± SD)	60.3 ± 7.3	91.2	60.8 ± 7.4	96.0
Lean body weight (kg; ± SD)	53.0 ± 10.3	89.3	53.6 ± 10.6	94.2
Measured urine creatinine (mmol/day; IQR)	-	-	9.3 (7.0 – 12.4)	100
Estimated creatinine excretion using ABW (mmol/day; IQR)	11.1 (8.9 – 14.2)	90.5	11.7 (9.3 – 14.7)	98.4
Estimated creatinine excretion using IBW (mmol/day; IQR)	8.8 (7.1 – 10.8)	91.2	9.3 (7.6 – 11.3)	95.9
Estimated creatinine excretion using LBW (mmol/day; IQR)	7.7 (6.0 – 9.8)	89.3	8.1 (6.3 – 10.2)	94.2

5.3.2 Outcomes

As described in chapter 3 (844 deaths at median of 3.0 years [IQR 1.8 – 4.7 years], 468 patients commencing RRT at 1.7 years [IQR 0.6 – 3.4 years], and serum creatinine doubling in 999 patients at 2.2 years [1.1 – 3.8 years]).

5.3.3 24-hour Creatinine Excretion

The median 24-hr excretion of creatinine (in those who had performed timed urine collections) was 9.3mmol/day (7.0 – 12.4). The estimated creatinine excretion was calculated using five predictive equations (and actual body weight) (32, 38-41), and correlated with the measured creatinine excretion: Ix 0.638 ($p<0.005$), Cockcroft and Gault 0.619 ($p<0.005$), Goldwasser 0.562 ($p<0.005$), Walser 0.562 ($p<0.005$), Rule 0.555 ($p<0.005$), using Pearson's correlation co-efficient. Both the Cockcroft & Gault formula and the Ix formula performed well with correlations >0.6 (figure 5-2 and 5-3). As Cockcroft and Gault is the established equation in most widespread use, we used it to calculate ECE for all subsequent analyses. A Bland-Altman plot of ECE (Cockcroft and Gault) and measured creatinine excretion is shown in figure 5-4. In addition, the scatterplots of the measured creatinine excretion and the estimated creatinine excretion using Cockcroft and Gault, but using the alternative weight measurements of ideal and lean body weight are show in figures 5-5 and 5-6; the correlation co-efficient was highest when lean body weight was used.

Figure 5-2 - Simple scatterplot of measured 24-hr creatinine excretion, versus predicted 24-hr creatinine excretion using the Cockcroft and Gault formula and actual body weight (Pearson's correlation coefficient 0.619 [p<0.005])

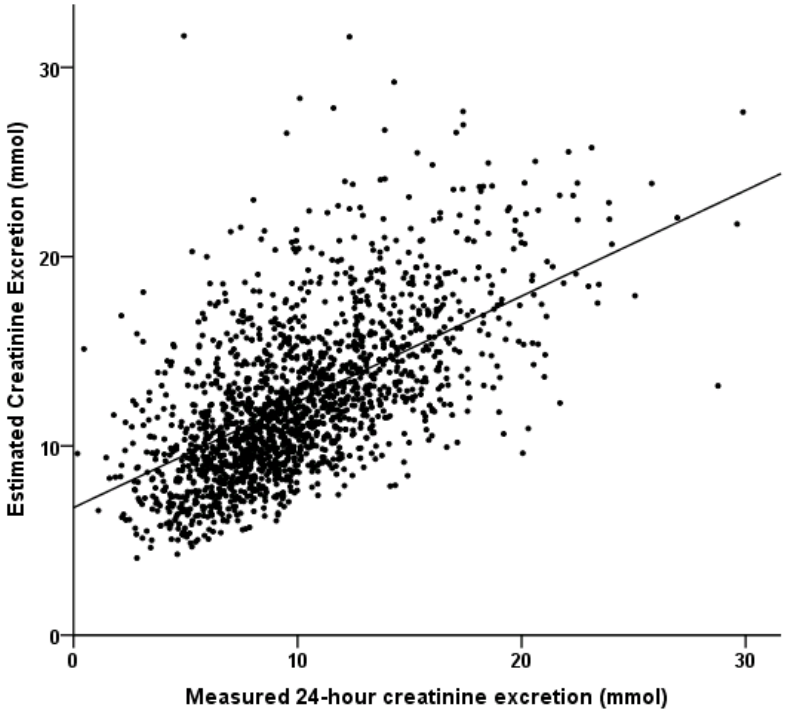


Figure 5-3 - Simple scatterplot of measured 24-hr creatinine excretion, versus predicted 24-hr creatinine excretion using the Ix Equation D formula (Pearson's correlation coefficient 0.638 [p<0.005])

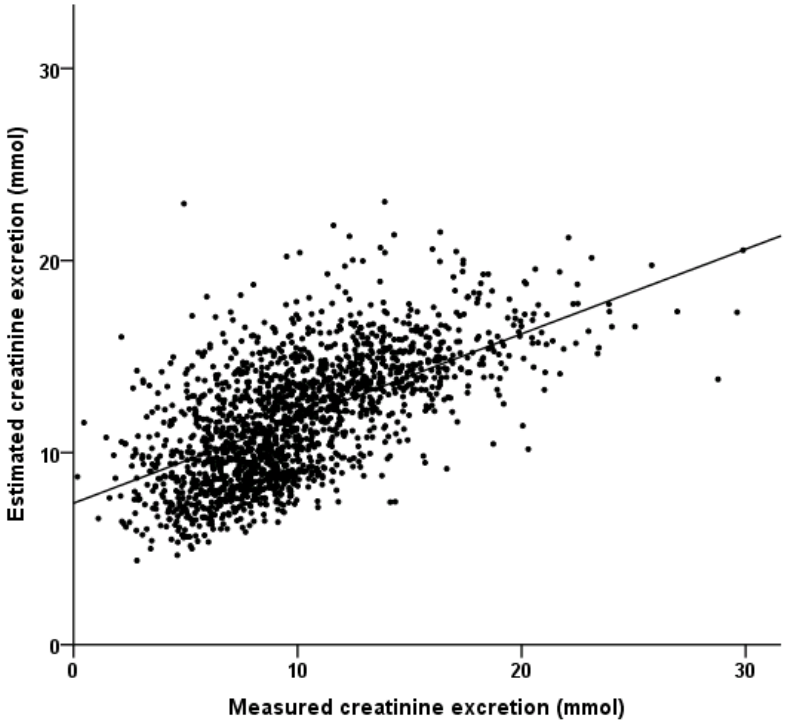


Figure 5-4 - Bland Altman plot of estimated creatinine excretion using Cockcroft and Gault formula (and actual body weight) versus measured creatinine excretion in timed urine collections

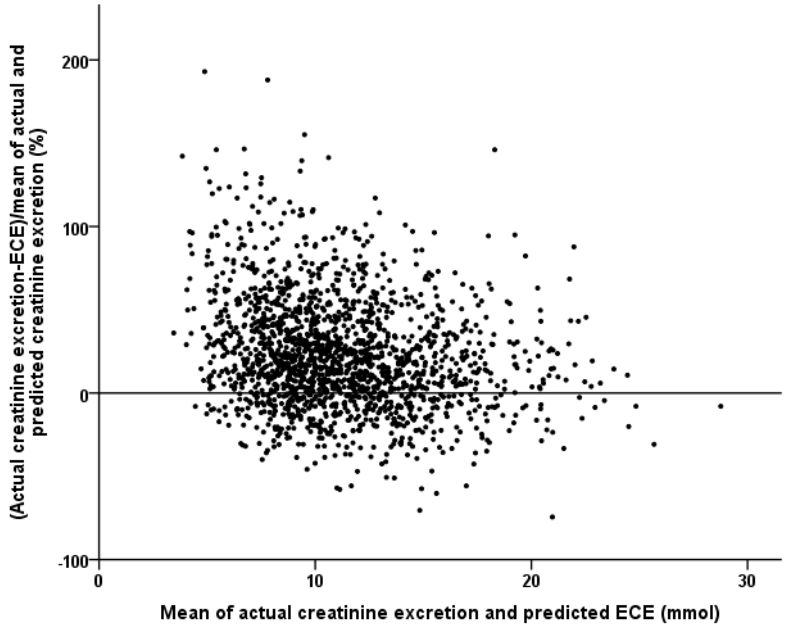


Figure 5-5 - Simple scatterplot of measured 24-hr creatinine excretion, versus predicted 24-hr creatinine excretion using the Cockcroft and Gault formula and ideal body weight (Pearson's correlation coefficient 0.566 [p<0.005])

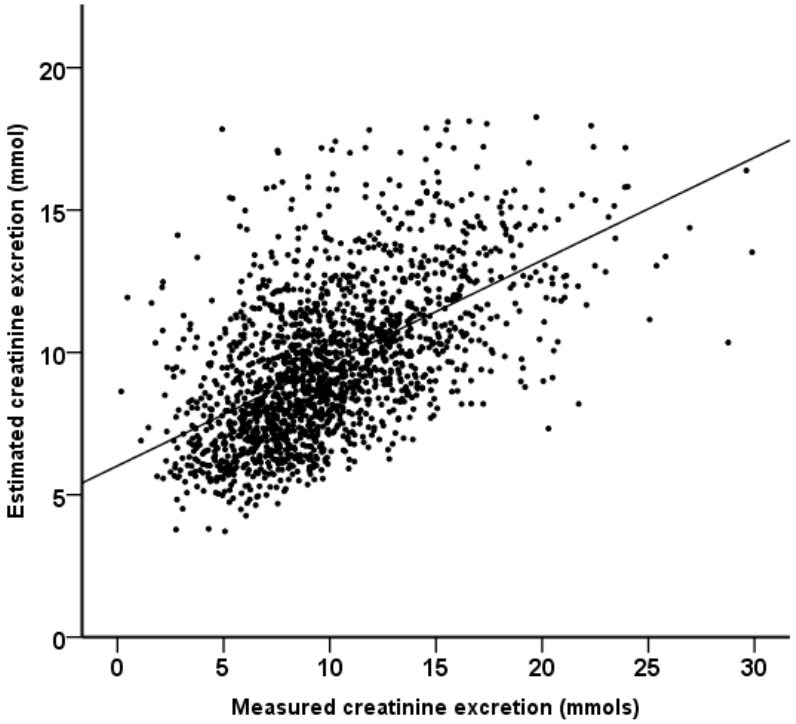
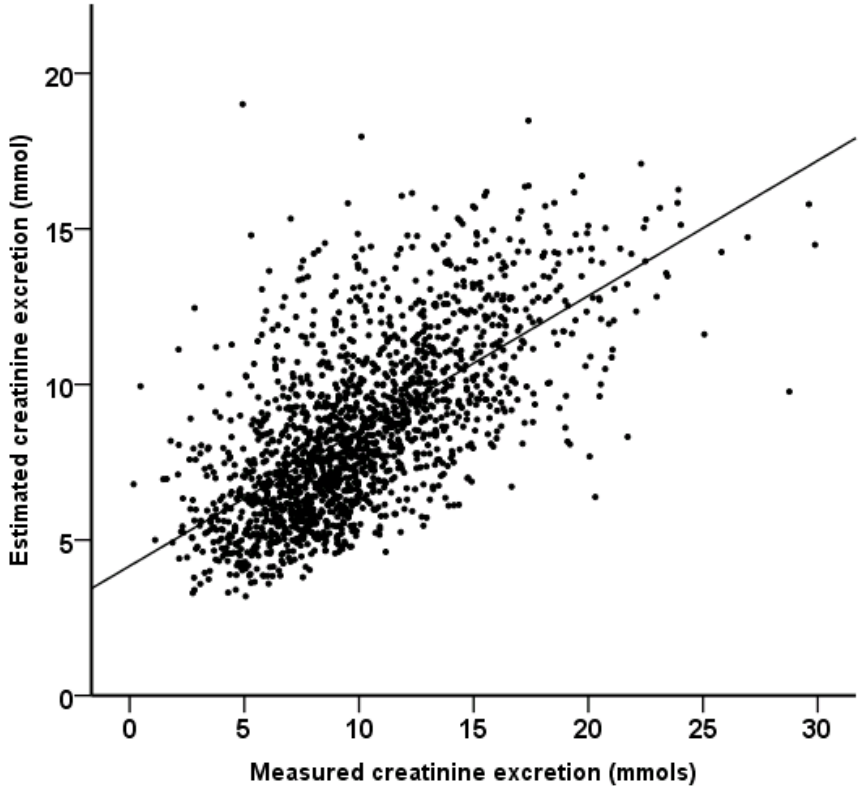


Figure 5-6 - Simple scatterplot of measured 24-hr creatinine excretion, versus predicted 24-hr creatinine excretion using the Cockcroft and Gault formula and lean body weight (Pearson's correlation coefficient 0.651 [p<0.005])



5.3.4 Prediction of 24-hr urine protein and albumin excretion: Impact of adjusting for estimated creatinine excretion

ROC curves were constructed for TPCR and ACR to predict 1g/day of urine total protein (as measured in the 24-hour urine collection), with no adjustment ("raw") or adjusted for estimated creatinine excretion (ECE). ECE was calculated using actual, ideal and lean body weight, as described in the methods. Areas under the curve were compared. Adjustment for ECE results in a statistically significant improvement in the performance of both TPCR ($p<0.001$ for all weight measurements) and ACR ($p<0.05$ except ACR adjusted for ideal body weight, $p=0.078$). The cohort was also divided according to age, gender and eGFR, and further ROC curves constructed (Table 5-2 and 5-3). The performance of raw TPCR and ACR to predict 1 g/day of urine protein varies according to age and kidney function, with an inferior test performance in the elderly and those with advanced renal impairment. Adjustment for ECE generally improved performance in the sub-group analysis similar to the overall cohort. Of the variables used for body weight, actual body weight produces the largest increment in AUC (i.e. improvement in test performance). The cohort was also divided according to body mass index and the test performance in those with a BMI<20kg/m^2 was markedly inferior to the overall cohort (AUC -0.020), but was improved by adjustment for ECE (AUC +0.013 for actual body weight).

Table 5-2 – Performance of urine TPCR to predict 1g/day of urine total protein, adjusted for estimated creatinine excretion using the Cockcroft and Gault prediction equation. Actual (ABW), lean (LBW) and ideal body weights (IBW) have each been used in the equation. The figures shown are the AUC of the ROC Curve

TPCR	N	Raw	ΔAll	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	1636	0.986 (0.98-0.99)	N/A	0.994 (0.99-1.00)	+0.008	0.993 (0.99-1.00)	+0.007	0.992 (0.99-1.00)	+0.006
Male	858	0.988 (0.98-0.99)	+0.002	0.993 (0.99-1.00)	+0.005	0.993 (0.99-1.00)	+0.005	0.991 (0.99-1.00)	+0.003
Female	778	0.991 (0.99-1.00)	+0.005	0.995 (0.99-1.00)	+0.004	0.994 (0.99-1.00)	+0.003	0.993 (0.99-1.00)	+0.002
Age ≤49	551	0.992 (0.99-1.00)	+0.006	0.996 (0.99-1.00)	+0.004	0.996 (0.99-1.00)	+0.004	0.995 (0.99-1.00)	+0.003
Age 49 – 64	469	0.985 (0.98-0.99)	-0.001	0.993 (0.99-1.00)	+0.008	0.992 (0.99-1.00)	+0.007	0.990 (0.98-1.00)	+0.005
Age 64 – 74	426	0.990 (0.98-1.00)	+0.004	0.995 (0.99-1.00)	+0.005	0.994 (0.99-1.00)	+0.004	0.993 (0.99-1.00)	+0.003
Age >74	190	0.973 (0.95-0.99)	-0.013	0.984 (0.97-1.00)	+0.011	0.989 (0.98-1.00)	+0.016	0.985 (0.97-1.00)	+0.012
BMI <20	68	0.966 (0.93-1.00)	-0.020	0.979 (0.95-1.01)	+0.013	0.976 (0.94-1.01)	+0.010	0.978 (0.95 – 1.01)	+0.012
BMI 20-25	464	0.994 (0.99-1.00)	+0.008	0.997 (0.99-1.00)	+0.003	0.997 (0.99-1.00)	+0.003	0.996 (0.99-1.00)	+0.002
BMI 25-30	608	0.988 (0.98-0.99)	+0.002	0.994 (0.99-1.00)	+0.006	0.995 (0.99-1.00)	+0.007	0.995 (0.99-1.00)	+0.007
BMI >30	563	0.986 (0.98-0.99)	-	0.992 (0.99-1.00)	+0.006	0.993 (0.99-1.00)	+0.007	0.993 (0.99-1.00)	+0.007
eGFR <15	85	0.936 (0.88-0.99)	-0.050	0.983 (0.96-1.00)	+0.047	0.981 (0.95-1.00)	+0.045	0.972 (0.94-1.00)	+0.036
eGFR 15-29	307	0.974 (0.96-0.99)	-0.012	0.988 (0.98-1.00)	+0.014	0.987 (0.98-1.00)	+0.013	0.981 (0.97-0.99)	+0.007
eGFR 30-59	721	0.993 (0.99-1.00)	+0.007	0.996 (0.99-1.00)	+0.003	0.996 (0.99-1.00)	+0.003	0.995 (0.99-1.00)	+0.002
eGFR ≥ 60	523	0.993 (0.99-1.00)	+0.007	0.996 (0.99-1.00)	+0.003	0.997 (0.99-1.00)	+0.004	0.995 (0.99-1.00)	+0.002

Table 5-3 – Performance of ACR to predict 1g/day of urine total protein, adjusted for estimated creatinine excretion using the Cockcroft and Gault prediction equation. Actual (ABW), lean (LBW) and ideal body weights (IBW) have each been used in the equation. The figures shown are the AUC of the ROC Curve

	n	Raw	ΔAll	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	1636	0.985 (0.98-0.99)	N/A	0.987 (0.98-0.99)	+0.002	0.987 (0.98-0.99)	+0.004	0.986 (0.98-0.99)	+0.003
Male	858	0.985 (0.98-0.99)	-	0.984 (0.98-0.99)	-0.001	0.985 (0.98-0.99)	-	0.984 (0.98-0.99)	-0.001
Female	778	0.988 (0.98-0.99)	+0.003	0.990 (0.98-1.00)	+0.002	0.989 (0.98-1.00)	+0.001	0.988 (0.98-0.99)	-
Age ≤49	551	0.989 (0.98-1.00)	+0.004	0.991 (0.99-1.00)	+0.002	0.991 (0.98-1.00)	+0.002	0.990 (0.98-1.00)	+0.001
Age 49 – 64	469	0.984 (0.97-0.99)	-0.003	0.987 (0.98-0.99)	+0.003	0.987 (0.98-0.99)	+0.003	0.986 (0.98-0.99)	+0.002
Age 64 – 74	426	0.989 (0.98-1.00)	+0.004	0.992 (0.99-1.00)	+0.003	0.991 (0.99-1.00)	+0.002	0.990 (0.98-1.00)	+0.001
Age >74	190	0.964 (0.94-0.99)	-0.021	0.969 (0.94-0.99)	+0.005	0.974 (0.95-1.00)	+0.010	0.972 (0.95-0.99)	+0.008
BMI <20	68	0.966 (0.93–1.00)	-0.019	0.975 (0.94–1.01)	+0.009	0.978 (0.95–1.01)	+0.012	0.969 (0.93–1.01)	+0.003
BMI 20-25	464	0.991 (0.98-1.00)	+0.006	0.991 (0.98–1.00)	+0.006	0.991 (0.98–1.00)	+0.006	0.990 (0.98–1.00)	+0.005
BMI 25-30	608	0.985 (0.98-0.99)	-	0.988 (0.98-0.99)	+0.003	0.988 (0.98-0.99)	+0.003	0.988 (0.98-0.99)	+0.003
BMI >30	563	0.985 (0.98-0.99)	-	0.984 (0.98-0.99)	-0.001	0.986 (0.98-0.99)	+0.001	0.986 (0.98-0.99)	+0.001
eGFR <15	85	0.924 (0.87-0.98)	-0.061	0.943 (0.90-0.99)	+0.019	0.946 (0.90-0.99)	+0.022	0.942 (0.90-0.99)	+0.018
eGFR 15-29	307	0.970 (0.95-0.98)	-0.015	0.980 (0.97-0.99)	+0.010	0.977 (0.96-0.99)	+0.007	0.973 (0.96-0.99)	+0.003
eGFR 30-59	721	0.991 (0.98-1.00)	+0.006	0.993 (0.99-1.00)	+0.002	0.994 (0.99-1.00)	+0.003	0.993 (0.99-1.00)	+0.002
eGFR ≥ 60	523	0.991 (0.98-1.00)	+0.006	0.991 (0.98-1.00)	-	0.991 (0.98-1.00)	-	0.991 (0.98-1.00)	-

5.3.5 Prediction of patient outcomes: Impact of adjusting TPCR and ACR for estimated creatinine excretion

ROC curves were then constructed for TPCR and ACR, for the total study population of 4989 patients who had measurements available, to predict the three major outcomes of all-cause mortality, commencement of RRT and doubling of serum creatinine, with no adjustment ("raw") or adjusted for estimated creatinine excretion, using ABW, IBW and LBW, as before. The AUCs were compared (Table 5-4) and the ROC curves for TPCR are shown in Figure 5-7 to 5-9. For all three outcome measures, the adjustments for ECE resulted in a statistically significant fall in test performance as represented by a smaller area under the ROC curve (except PCR_{IBW} to predict RRT p=0.08).

Table 5-4 – Performance of ACR and TPCR using ROC curve analysis to predict the outcomes of all-cause mortality, commencement of RRT and doubling of SCr, comparing the unadjusted values with those adjusted for estimated creatinine excretion using actual body weight (ABW), lean body weight (LBW) and ideal body weight (IBW). The area under the ROC curve is shown (with 95% confidence intervals). * denotes a statistically significant (p<0.005) difference from the raw measurement

	TPCR				**ACR**			
	Raw	**ABW**	**LBW**	**IBW**	**Raw**	**ABW**	**LBW**	**IBW**
Death	0.669 (0.65-0.69)	0.637* (0.62-0.66)	0.640* (0.62-0.66)	0.641* (0.62-0.66)	0.653 (0.63-0.67)	0.629* (0.61-0.65)	0.632* (0.61-0.65)	0.632* (0.61-0.65)
RRT	0.829 (0.81-0.85)	0.818* (0.80-0.84)	0.820* (0.80-0.84)	0.824 (0.80-0.84)	0.816 (0.80-0.83)	0.807* (0.79-0.83)	0.808* (0.79-0.83)	0.811* (0.79-0.83)
Doubled sCr	0.728 (0.71-0.75)	0.719* (0.70-0.74)	0.718* (0.70-0.74)	0.717* (0.70-0.74)	0.730 (0.71-0.75)	0.723* (0.70-0.74)	0.722* (0.70-0.74)	0.722* (0.70-0.74)

Figure 5-7 - ROC curves comparing the ability of unadjusted (raw) TPCR and TPCR adjusted for ideal body weight (IBW) to predict all-cause mortality. The curves for TPCR adjusted for actual body weight and lean body weight are coincident with that of TPCR adjusted for ideal body weight and are therefore not shown

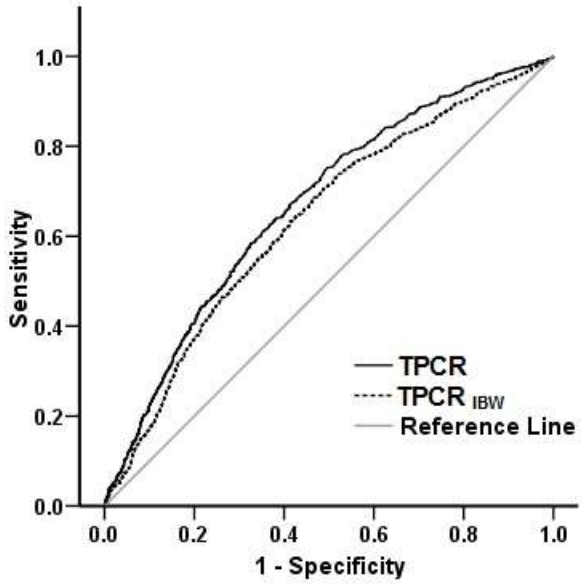


Figure 5-8 - ROC curves comparing the ability of unadjusted (raw) TPCR and TPCR adjusted for ideal body weight (IBW) to predict commencement of RRT. The curves for TPCR adjusted for actual body weight and lean body weight are coincident with that of PCR adjusted for ideal body weight and are therefore not shown

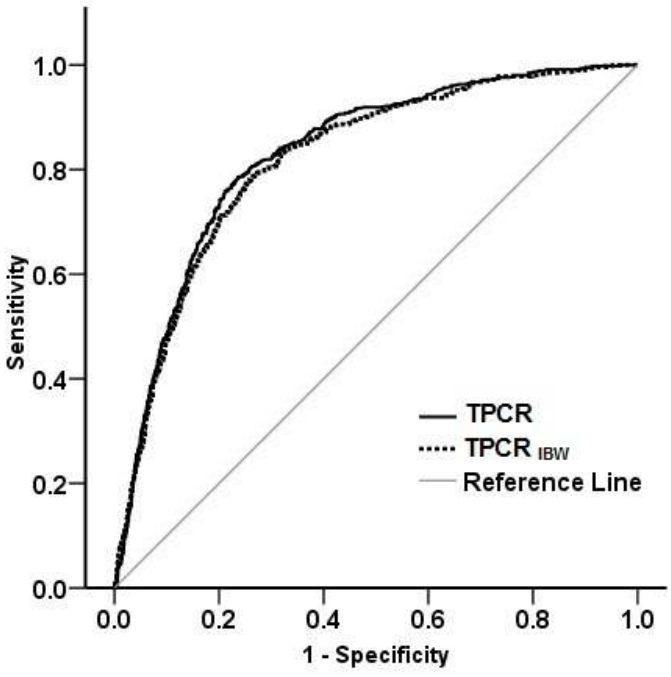
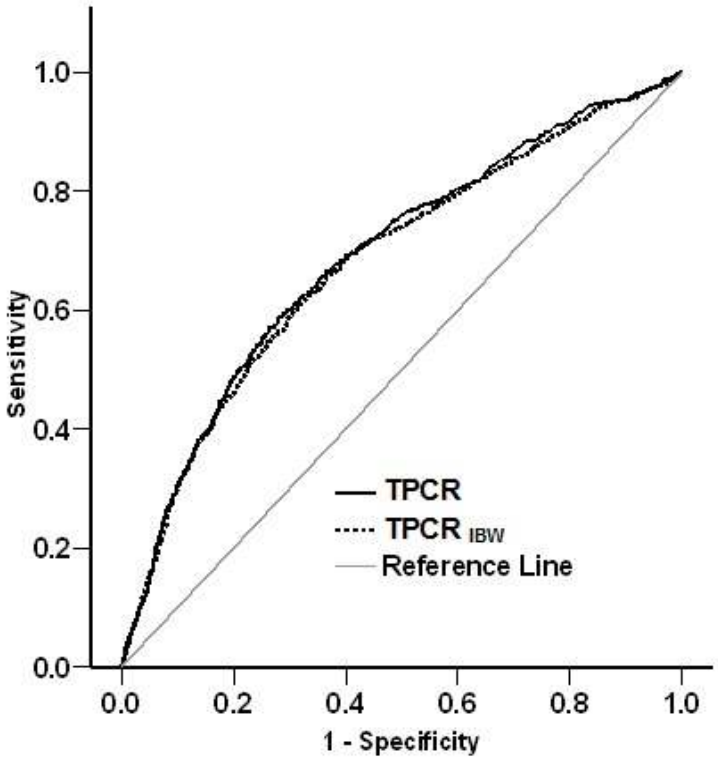


Figure 5-9 - ROC curves comparing the ability of unadjusted (raw) TPCR and TPCR adjusted for ideal body weight (IBW) to predict doubling of serum creatinine. The curves for TPCR adjusted for actual body weight and lean body weight are coincident with that of TPCR adjusted for ideal body weight and are therefore not shown



We went on to investigate this relationship further using the technique of net reclassification index, and the results are presented in table 5-5. They confirm that adjustment for ECE produces at best no difference in test performance and often a significantly inferior performance for prediction of renal and patient survival.

Table 5-5 – Net Reclassification Index (NRI) analysis of TPCR and ACR comparing the unadjusted values with those adjusted for estimated creatinine excretion using actual body weight (ABW), lean body weight (LBW) and ideal body weight (IBW) to predict the outcomes of all-cause mortality, commencement of RRT and doubling of SCr, The NRI is shown (with significance test) using the unadjusted TPCR and ACR as the reference value respectively

	NRI (%) for raw TPCR versus adjusted TPCR			**NRI (%) for raw ACR versus adjusted ACR**		
	ABW	**LBW**	**IBW**	**ABW**	**LBW**	**IBW**
Death	-2.3 (p=0.0017)	-4.47 (p<0.001)	-3.09 (p<0.001)	-3.52 (p<0.001)	-4.06 (p<0.001)	-3.36 (p<0.001)
RRT	-2.75 (p=0.0014)	-5.45 (p<0.001)	-2.40 (p=0.018)	-0.18 (p=0.560)	-3.61 (p=0.002)	-3.31 (p=0.001)
Doubled sCr	-1.59 (p=0.022)	-3.97 (p<0.001)	-2.58 (p<0.001)	-0.48 (p=0.261)	-2.84 (p<0.001)	-2.01 (p=0.002)

The cohort was again divided according to age, gender and eGFR, and further ROC curves constructed for the three patient outcomes and the AUCs for TPCR and ACR to predict all-cause mortality are shown in table 5-6 and 5-7 respectively, AUCs for TPCR and ACR to predict commencement of RRT are shown in table 5-8 and 5-9 respectively, and TPCR and ACR to predict doubling of serum creatinine in table 5-10 and 5-11.

On sub-group analysis, the performance of TPCR to predict all-cause mortality is superior in women, those <49 years and those with eGFR≥60ml/min/1.73m^2 compared with the total cohort. However adjustment for ECE results in inferior performance in all sub-groups except those >64 years, using lean or ideal body weight. For the prediction of commencement of RRT, the performance of raw TPCR also varies. The performance is superior in males, those <64 years and those with eGFR>30ml/min/1.73m^2, compared with

the overall cohort. When adjustment is made for ECE, the performance of the tests generally fell, except in the eGFR subgroups where there was a trend towards improvement (not statistically significant).The cohort was also divided according to body mass index, and within these subgroups, the pattern remained that adjustment for actual, lean or ideal body weight led to an inferior test performance.

Table 5-6 - Sub-group analysis of the impact of gender, age, body mass index and kidney function on the performance of TPCR to predict all-cause mortality comparing the unadjusted values with those adjusted for estimated creatinine excretion using actual body weight (ABW), lean body weight (LBW) and ideal body weight (IBW) in the Cockcroft and Gault prediction equation

	n	Raw	ΔAll	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	4989	0.669 (0.65-0.69)	N/A	0.637 (0.62-0.66)	-0.032	0.640 (0.62-0.66)	-0.029	0.641 (0.62-0.66)	-0.028
Male	2498	0.661 (0.63-0.69)	-0.008	0.625 (0.60-0.65)	-0.036	0.627 (0.60-0.65)	-0.034	0.630 (0.60-0.66)	-0.031
Female	2491	0.676 (0.64-0.71)	0.007	0.640 (0.61-0.67)	-0.036	0.642 (0.61-0.67)	-0.034	0.643 (0.61-0.67)	-0.033
Age ≤49	1454	0.755 (0.68-0.83)	0.106	0.748 (0.67-0.83)	-0.007	0.748 (0.67-0.82)	-0.007	0.749 (0.67-0.88)	-0.006
Age 49-64	1299	0.668 (0.62-0.71)	-0.001	0.666 (0.62-0.71)	-0.002	0.668 (0.62-0.71)	0	0.668 (0.62-0.71)	0
Age 64-74	1281	0.649 (0.61-0.68)	-0.020	0.647 (0.61-0.68)	-0.002	0.651 (0.62-0.69)	0.002	0.653 (0.62-0.69)	0.004
Age >74	955	0.665 (0.62-0.70)	-0.004	0.659 (0.62-0.70)	-0.006	0.666 (0.63-0.70)	0.001	0.668 (0.63-0.71)	0.003
BMI <20	224	0.697 (0.62-0.78)	0.028	0.656 (0.57-0.74)	-0.041	0.658 (0.57-03.74)	-0.039	0.656 (0.57-0.74)	-0.041
BMI 20-25	1246	0.662 (0.62-0.70)	-0.007	0.626 (0.59-0.67)	-0.036	0.628 (0.59-0.67)	-0.034	0.626 (0.58-0.67)	-0.036
BMI 25-30	1821	0.662 (0.63-0.70)	-0.007	0.638 (0.60-0.67)	-0.024	0.639 (0.60-0.68)	-0.023	0.638 (0.60-0.67)	-0.024
BMI >30	1698	0.674 (0.64-0.71)	0.005	0.652 (0.62-0.69)	-0.022	0.653 (0.62-0.69)	-0.021	0.651 (0.62-0.69)	-0.023
eGFR<15	311	0.545 (0.48-0.61)	-0.124	0.515 (0.45-0.58)	-0.030	0.517 (0.45-0.58)	-0.028	0.508 (0.44-0.57)	-0.037
eGFR 15-29	1100	0.587 (0.53-0.63)	-0.082	0.568 (0.53-0.61)	-0.019	0.576 (0.54-0.61)	-0.011	0.576 (0.54-0.61)	-0.011
eGFR 30-59	2100	0.610 (0.57-0.64)	-0.059	0.594 (0.56-0.63)	-0.016	0.599 (0.56-0.63)	-0.011	0.599 (0.56-0.63)	-0.011
eGFR≥60	1366	0.687 (0.61-0.77)	0.018	0.658 (0.58-0.74)	-0.029	0.661 (0.58-0.74)	-0.026	0.661 (0.58-0.74)	-0.026

Table 5-7 - Sub-group analysis of the impact of gender, age, body mass index and kidney function on the performance of ACR to predict all-cause mortality comparing the unadjusted values with those adjusted for ECE using actual body weight (ABW), lean body weight (LBW) and ideal body weight (IBW) in the Cockcroft and Gault prediction equation

	n	Raw	Δ All	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	4989	0.653 (0.63-0.67)	N/A	0.629 (0.61-0.65)	-0.024	0.632 (0.61-0.65)	-0.021	0.632 (0.61-0.65)	-0.021
Male	2498	0.635 (0.61-0.66)	-0.018	0.608 (0.58-0.64)	-0.027	0.610 (0.58-0.64)	-0.025	0.611 (0.58-0.64)	-0.024
Female	2491	0.669 (0.64-0.70)	0.016	0.643 (0.61-0.67)	-0.026	0.645 (0.61-0.68)	-0.024	0.646 (0.61-0.68)	-0.023
Age ≤49	1454	0.751 (0.67-0.83)	0.098	0.742 (0.66-0.82)	-0.09	0.741 (0.66-0.82)	-0.010	0.742 (0.66-0.82)	-0.09
Age 49-64	1299	0.661 (0.62-0.70)	0.008	0.659 (0.61-0.70)	-0.002	0.660 (0.62-0.70)	-0.001	0.661 (0.62-0.70)	0
Age 64-74	1281	0.650 (0.62-0.68)	-0.003	0.648 (0.61-0.68)	-0.002	0.651 (0.62-0.68)	0.001	0.653 (0.62-0.69)	0.003
Age >74	955	0.658 (0.62-0.70)	0.005	0.654 (0.61-0.69)	-0.004	0.657 (0.62-0.70)	-0.001	0.660 (0.62-0.70)	0.002
BMI <20	224	0.637 (0.55-0.72)	-0.016	0.608 (0.52-0.70)	-0.029	0.608 (0.52-0.70)	-0.029	0.607 (0.52-0.70)	-0.030
BMI 20-25	1246	0.646 (0.61-0.68)	-0.007	0.619 (0.58-0.66)	-0.027	0.620 (0.58-0.66)	-0.026	0.619 (0.58-0.66)	-0.027
BMI 25-30	1821	0.646 (0.61-0.68)	-0.007	0.628 (0.59-0.66)	-0.018	0.629 (0.59-0.66)	-0.017	0.628 (0.59-0.66)	-0.018
BMI >30	1698	0.670 (0.64-0.70)	0.017	0.652 (0.62-0.69)	-0.018	0.653 (0.62-0.69)	-0.017	0.653 (0.62-0.69)	-0.017
eGFR<15	311	0.513 (0.45-0.58)	-0.140	0.487 (0.42-0.55)	-0.026	0.491 (0.423-0.56)	-0.022	0.487 (0.42-0.55)	-0.026
eGFR15-29	1100	0.584 (0.54-0.62)	-0.069	0.572 (0.53-0.61)	-0.014	0.576 (0.54-0.61)	-0.008	0.576 (0.54-0.61)	-0.008
eGFR 30-59	2100	0.610 (0.58-0.64)	-0.043	0.598 (0.56-0.63)	-0.012	0.602 (0.57-0.63)	-0.008	0.602 (0.57-0.64)	-0.008
eGFR≥60	1366	0.693 (0.62-0.77)	0.040	0.666 (0.59-0.74)	-0.027	0.668 (0.59-0.75)	-0.025	0.668 (0.59-0.75)	-0.025

Table 5-8 - Sub-group analysis of the impact of gender, age, body mass index and kidney function on the performance of TPCR to predict commencement of RRT comparing the unadjusted values with those adjusted for ECE using actual body weight (ABW), lean body weight (LBW) and ideal body weight (IBW) in the Cockcroft and Gault prediction equation

	n	Raw	Δ All	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	4989	0.829 (0.81-0.85)	N/A	0.818 (0.80-0.84)	-0.011	0.820 (0.80-0.84)	-0.009	0.824 (0.80-0.84)	-0.005
Male	2498	0.841 (0.82-0.86)	0.012	0.836 (0.81-0.86)	-0.005	0.840 (0.81-0.86)	-0.001	0.842 (0.82-0.87)	0.001
Female	2491	0.818 (0.79-0.85)	-0.011	0.808 (0.78-0.84)	-0.010	0.814 (0.79-0.84)	-0.004	0.815 (0.79-0.84)	-0.003
Age ≤49	1454	0.856 (0.82-0.89)	0.027	0.847 (0.81-0.88)	-0.009	0.848 (0.81-0.88)	-0.008	0.850 (0.81-0.89)	-0.006
Age 49-64	1299	0.834 (0.80-0.86)	0.005	0.817 (0.78-0.85)	-0.017	0.824 (0.79-0.86)	-0.010	0.832 (0.80-0.86)	-0.002
Age 64-74	1281	0.803 (0.77-0.84)	-0.026	0.799 (0.76-0.84)	-0.004	0.795 (0.76-0.83)	-0.008	0.797 (0.76-0.83)	-0.006
Age >74	955	0.803 (0.75-0.86)	-0.026	0.792 (0.74-0.84)	-0.011	0.794 (0.74-0.85)	-0.009	0.798 (0.74-0.85)	-0.005
BMI <20	224	0.818 (0.75-0.89)	-0.011	0.823 (0.75-0.90)	0.005	0.823 (0.75-0.90)	0.005	0.821 (0.74-0.90)	0.003
BMI 20-25	1246	0.793 (0.75-0.83)	-0.036	0.792 (0.75-0.83)	-0.001	0.790 (0.75-0.83)	-0.003	0.791 (0.75-0.83)	-0.002
BMI 25-30	1821	0.835 (0.80-0.87)	0.006	0.831 (0.80-0.86)	-0.004	0.829 (0.80-0.86)	-0.007	0.832 (0.80-0.86)	-0.003
BMI >30	1698	0.859 (0.83-0.89)	0.030	0.847 (0.82-0.88)	-0.012	0.844 (0.81-0.87)	-0.015	0.847 (0.82-0.88)	-0.012
eGFR<15	311	0.608 (0.54-0.67)	-0.221	0.628 (0.56-0.69)	0.020	0.631 (0.57-0.69)	0.023	0.626 (0.563-0.690)	0.018
eGFR15-29	1100	0.749 (0.71-0.79)	-0.080	0.760 (0.72-0.78)	0.011	0.763 (0.73-0.80)	0.014	0.765 (0.73-0.80)	0.016
eGFR 30-59	2100	0.864 (0.82-0.91)	0.035	0.865 (0.82-0.91)	0.001	0.868 (0.82-0.91)	0.004	0.871 (0.82-0.92)	0.007
eGFR≥60	1366	0.835 (0.68-0.99)	0.006	0.870 (0.76-0.97)	0.035	0.880 (0.78-0.98)	0.045	0.881 (0.80-0.98)	0.046

Table 5-9 – Sub-group analysis of the impact of gender, age, body mass index and kidney function on the performance of ACR to predict commencement of RRT comparing the unadjusted values with those adjusted for ECE using actual body weight (ABW), lean body weight (LBW) and ideal body weight (IBW) in the Cockcroft and Gault prediction equation

	n	Raw	Δ All	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	4989	0.816 (0.80-0.83)	N/A	0.807 (0.79-0.83)	-0.009	0.808 (0.79-0.83)	-0.008	0.811 (0.79-0.83)	-0.005
Male	2498	0.824 (0.80-0.85)	0.008	0.819 (0.79-0.85)	-0.005	0.822 (0..-80-0.85)	-0.002	0.824 (0.80-0.85)	0
Female	2491	0.814 (0.79-0.84)	-0.002	0.808 (0.78-0.83)	-0.006	0.811 (0.78-0.84)	-0.003	0.811 (0.79-0.84)	-0.003
Age ≤49	1454	0.852 (0.82-0.89)	0.036	0.846 (0.81-0.88)	-0.006	0.847 (0.81-0.88)	-0.005	0.848 (0.81-0.88)	-0.004
Age 49-64	1299	0.806 (0.77-0.84)	-0.010	0.794 (0.76-0.83)	-0.012	0.798 (0.76-0.83)	-0.008	0.804 (0.77-0.84)	-0.002
Age 64-74	1281	0.799 (0.76-0.83)	-0.017	0.795 (0.76-0.83)	-0.004	0.793 (0.76-0.83)	-0.006	0.795 (0.76-0.83)	-0.004
Age >74	955	0.792 (0.74-0.84)	-0.024	0.783 (0.73-0.83)	-0.009	0.784 (0.73-0.83)	-0.008	0.789 (0.74-0.84)	-0.003
BMI <20	224	0.816 (0.74-0.90)	0	0.810 (0.72-0.88)	-0.006	0.806 (0.72-0.89)	-0.010	0.808 (0.72-0.89)	-0.008
BMI 20-25	1246	0.788 (0.75-0.83)	-0.028	0.786 (0.75-0.83)	-0.002	0.785 (0.74-0.82)	-0.003	0.785 (0.74-0.83)	-0.003
BMI 25-30	1821	0.822 (0.79-0.85)	0.006	0.819 (0.80-0.85)	-0.003	0.817 (0.79-0.85)	-0.005	0.819 (0.79-0.85)	-0.003
BMI >30	1698	0.845 (0.82-0.87)	0.029	0.834 (0.81-0.86)	-0.011	0.832 (0.80-0.86)	-0.013	0.835 (0.81-0.86)	-0.010
eGFR<15	311	0.652 (0.60-0.71)	-0.164	0.659 (0.60-0.72)	0.007	0.661 (0.60-0.72)	0.009	0.659 (0.60-0.72)	0.007
eGFR 15-29	1100	0.765 (0.73-0.80)	-0.051	0.772 (0.74-0.81)	0.007	0.774 (0.74-0.81)	0.009	0.776 (0.74-0.81)	0.011
eGFR 30-59	2100	0.865 (0.81-0.91)	0.049	0.866 (0.82-0.91)	0.001	0.867 (0.82-0.92)	0.002	0.869 (0.82-0.92)	0.004
eGFR≥60	1366	0.839 (0.67-1.00)	0.023	0.856 (0.72-0.99)	0.017	0.867 (0.74-0.99)	0.028	0.869 (0.74-1.00)	0.030

Table 5-10 - Sub-group analysis of the impact of gender, age, body mass index and kidney function on the performance of TPCR to predict doubling of serum creatinine comparing the unadjusted values with those adjusted for ECE using actual body weight, lean body weight and ideal body weight in the Cockcroft and Gault prediction equation

	n	Raw	ΔAll	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	4989	0.728 (0.71-0.75)	N/A	0.719 (0.70-0.74)	-0.009	0.718 (0.70-0.74)	-0.010	0.717 (0.70-0.74)	-0.011
Male	2498	0.715 (0.69-0.74)	-0.013	0.708 (0.68-0.73)	-0.007	0.706 (0.68-0.73)	-0.009	0.704 (0.68-0.73)	-0.011
Female	2491	0.741 (0.71-0.77)	0.013	0.729 (0.70-0.46)	-0.012	0.730 (0.70-0.76)	-0.011	0.730 (0.70-0.76)	-0.011
Age ≤49	1454	0.780 (0.74-0.82)	0.052	0.778 (0.74-0.81)	-0.002	0.776 (0.74-0.81)	-0.004	0.776 (0.74-0.81)	-0.004
Age 49-64	1299	0.744 (0.71-0.78)	0.016	0.741 (0.71-0.77)	-0.003	0.744 (0.71-0.78)	0	0.746 (0.71-0.78)	0.002
Age 64-74	1281	0.728 (0.69-0.76)	0	0.732 (0.70-0.77)	0.004	0.730 (0.70-0.76)	0.002	0.728 (0.69-0.76)	0
Age >74	955	0.606 (0.56-0.66)	-0.102	0.607 (0.56-0.66)	0.001	0.605 (0.56-0.65)	-0.001	0.602 (0.55-0.65)	-0.004
BMI<20	224	0.730 (0.65-0.80)	0.002	0.697 (0.61-0.78)	-0.033	0.700 (0.62-0.78)	-0.030	0.702 (0.62-0.78)	-0.028
BMI 20-25	1246	0.751 (0.71-0.79)	0.023	0.742 (0.70-0.78)	-0.009	0.740 (0.70-0.78)	-0.011	0.741 (0.70-0.78)	-0.010
BMI 25-30	1821	0.724 (0.69-0.76)	-0.004	0.713 (0.679-0.747)	-0.011	0.712 (0.68-0.74)	-0.012	0.713 (0.68-0.75)	-0.011
BMI>30	1698	0.714 (0.68-0.75)	-0.014	0.710 (0.68-0.74)	-0.004	0.710 (0.68-0.74)	-0.004	0.708 (0.68-0.74)	-0.006
eGFR<15	311	0.558 (0.49-0.6)	-0.170	0.572 (0.50-0.64)	0.014	0.573 (0.50-0.64)	0.015	0.572 (0.50-0.64)	0.014
eGFR 15-29	1100	0.703 (0.67-0.74)	-0.025	0.711 (0.68-0.74)	0.008	0.713 (0.68-0.75)	0.010	0.713 (0.68-0.75)	0.010
eGFR 30-59	2100	0.700 (0.67-0.73)	-0.028	0.698 (0.6-0.73)	-0.002	0.695 (0.66-0.73)	-0.005	0.694 (0.66-0.73)	-0.006
eGFR≥60	1366	0.725 (0.66-0.78)	-0.003	0.723 (0.66-0.78)	-0.002	0.726 (0.67-0.78)	0.001	0.723 (0.66-0.78)	-0.002

Table 5-11 - Sub-group analysis of the impact of gender, age, body mass index and kidney function on the performance of ACR to predict doubling of serum creatinine comparing the unadjusted values with those adjusted for ECE using actual body weight, lean body weight and ideal body weight in the Cockcroft and Gault prediction equation

	n	Raw	Δ All	ABW	ΔRaw	LBW	ΔRaw	IBW	ΔRaw
All	4989	0.730 (0.71-0.75)	N/A	0.723 (0.70-0.74)	-0.007	0.722 (0.70-0.74)	-0.008	0.722 (0.70-0.74)	-0.008
Male	2498	0.718 (0.69-0.74)	-0.012	0.711 (0.68-0.74)	-0.007	0.710 (0.68-0.74)	-0.008	0.709 (0.68-0.73)	-0.009
Female	2491	0.747 (0.72-0.77)	0.017	0.738 (0.71-0.76)	-0.009	0.739 (0.71-0.76)	-0.008	0.739 (0.71-0.76)	-0.008
Age ≤49	1454	0.796 (0.76-0.83)	0.066	0.794 (0.76-0.83)	-0.002	0.793 (0.76-0.83)	-0.003	0.793 (0.76-0.83)	-0.003
Age 49-64	1299	0.738 (0.70-0.77)	0.008	0.736 (0.70-0.77)	-0.002	0.737 (0.70-0.77)	-0.001	0.738 (0.70-0.77)	0
Age 64-74	1281	0.744 (0.71-0.78)	0.014	0.746 (0.71-0.78)	0.002	0.744 (0.71-0.78)	0	0.744 (0.71-0.78)	0
Age >74	955	0.621 (0.57-0.67)	-0.109	0.622 (0.58-0.67)	0.002	0.621 (0.57-0.67)	0	0.620 (0.57-0.67)	-0.001
BMI<20	224	0.694 (0.61-0.77)	-0.036	0.673 (0.59-0.76)	-0.021	0.672 (0.59-0.76)	-0.022	0.674 (0.59-0.76)	-0.020
BMI 20-25	1246	0.747 (0.71-0.78)	0.017	0.739 (0.70-0.78)	-0.008	0.738 (0.70-0.78)	-0.009	0.738 (0.70-0.78)	-0.009
BMI 25-30	1821	0.732 (0.70-0.76)	0.002	0.724 (0.69-0.76)	-0.008	0.723 (0.69-0.75)	-0.009	0.724 (0.69-0.76)	-0.008
BMI>30	1698	0.725 (0.69-0.76)	-0.005	0.721 (0.69-0.75)	-0.004	0.721 (0.69-0.75)	-0.005	0.720 (0.69-0.75)	-0.005
eGFR<15	311	0.576 (0.50-0.65)	-0.154	0.581 (0.509-0.653)	0.005	0.585 (0.51-0.66)	0.009	0.582 (0.51-0.65)	0.006
eGFR 15-29	1100	0.716 (0.68-0.75)	-0.014	0.721 (0.69-0.75)	0.005	0.721 (0.69-0.75)	0.005	0.722 (0.69-0.75)	0.006
eGFR 30-59	2100	0.706 (0.67-0.74)	-0.024	0.704 (0.67-0.73)	-0.002	0.702 (0.67-0.73)	-0.004	0.701 (0.67-0.73)	-0.005
eGFR≥60	1366	0.753 (0.70-0.81)	0.023	0.748 (0.69-0.80)	-0.005	0.748 (0.69-0.80)	-0.005	0.746 (0.69-0.80)	-0.007

5.3.6 Impact of adjusting TPCR or ACR for measured creatinine excretion

Given that adjusting for ECE gave inferior test performance in predicting patient outcomes, we repeated the analysis using actual measured creatinine excretion (MCE) in the subpopulation with 24h urine results available, to ensure the accuracy of the creatinine excretion prediction equation was not influencing the results itself. 24-hr creatinine excretion was available in the subgroup of 1808 patients who performed timed urine collections. ROC curves were constructed, as above, and the AUC calculated. For each outcome the pattern was the same: the AUC for TPCR adjusted for measured creatinine excretion was lower than that of TPCR adjusted for estimated creatinine excretion, which was lower in turn than the unadjusted (raw) TPCR. The ROC curves are shown in figures 5-10 – 5-12. The same relationships were demonstrated for ACR, for all 3 of the outcome measures. Excluding urine collections with biologically implausible 24-hr creatinine excretion (<3mmol/day or >30mmmol/day) did not alter the results.

Figure 5-10 - ROC curves comparing the ability of unadjusted (raw) TPCR, TPCR adjusted for ECE using actual body weight (ABW) and TPCR adjusted for measured creatinine excretion (MCE) to predict all cause mortality.

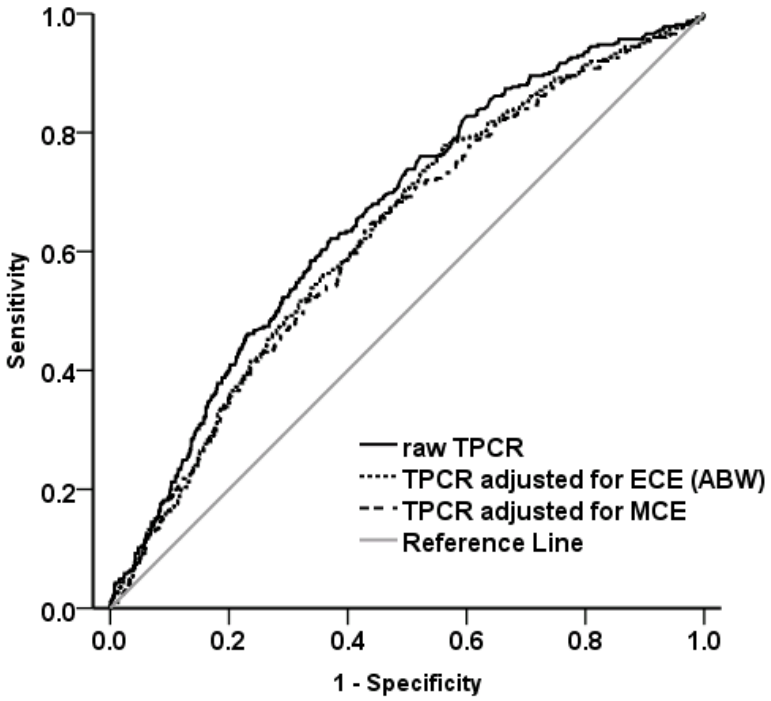


Figure 5-11 - ROC curves comparing the ability of unadjusted (raw) TPCR, TPCR adjusted for ECE using actual body weight (ABW) and TPCR adjusted for measured creatinine excretion (MCE) to predict commencement of renal replacement therapy.

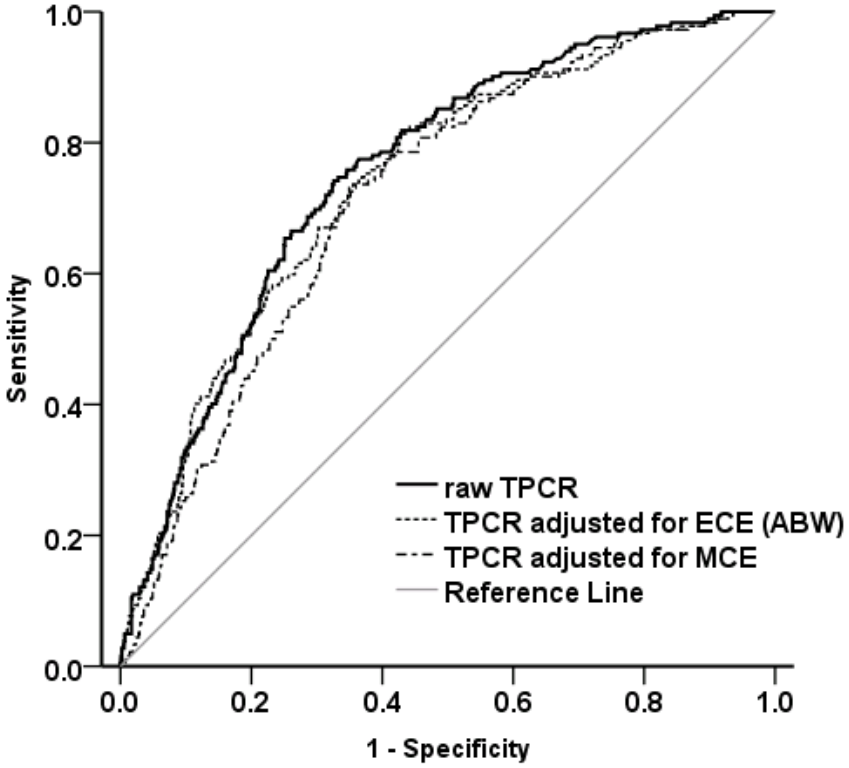
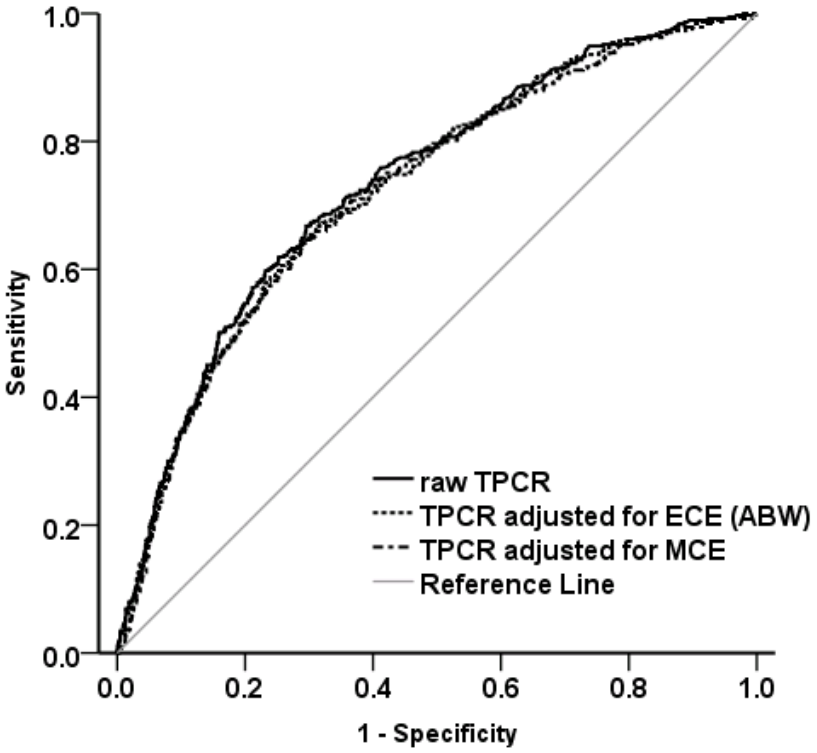


Figure 5-12 - ROC curves comparing the ability of unadjusted (raw) TPCR, TPCR adjusted for ECE using actual body weight (ABW) and TPCR adjusted for measured creatinine excretion (MCE) to predict doubling of serum creatinine.



Secondly, the predictive ability of the actual 24-hr total protein excretion versus the 24-hr total protein excretion corrected for body weight, were compared. There was no statistically significant difference in test performance between 24-hr total protein corrected for actual body weight and raw 24-hr total protein excretion, to predict all-cause mortality (AUC 0.626 (95%CI 0.592 – 0.659) v 0.623 (95%CI 0.590 -0.657)) and commencement of RRT (0.786 (95%CI 0.755 – 0.817) v 0.777 (95%CI 0.745 – 0.809). Using IBW or LBW did not afford any further improvement in performance.

5.4 Discussion

5.4.1 Findings of this study

In chapter 2 we showed that TPCR and ACR perform well as predictors of significant proteinuria (≥1 g/day) in a cohort of adults attending a nephrology clinic. However, performance is inferior in the elderly, females and those with advanced kidney disease. This was presumed to be due to the confounding influence of lower muscle mass on the TPCR/ACR result: patients with low muscle mass will have lower creatinine excretion and therefore higher TPCR/ACR for any given degree of protein excretion. In this analysis, adjustment of TPCR and ACR for estimated creatinine excretion (using the Cockcroft and Gault formula), improves the performance of these tests. This held true, whether estimated creatinine excretion was calculated using actual body weight, lean body weight or ideal body weight. The largest improvement in AUC was seen in the elderly and those with advanced kidney disease (eGFR <15ml/min/1.73m^2), i.e. the groups with the poorest baseline test performance. This effect was particularly pronounced, using lean body weight in place of actual body weight in the >74 years group, which may reflect the increase in fat as a proportion of actual body weight with increasing age (38).

However, what is potentially more important than the prediction of urine protein excretion is whether these adjustments improve the ability of TPCR and/or ACR to predict patient-relevant outcomes. Our results suggest that the converse is true, with performances of "raw" TPCR and ACR (i.e. unadjusted) significantly superior to the values adjusted for ECE, to predict the clinically important end-points of all-cause mortality, commencement of RRT and doubling of serum creatinine. This was confirmed using a net reclassification index analysis. Again, there were important differences within sub-group analysis, as for

prediction of 24-hr urine protein excretion, but the effects of adjusting for ECE were not consistent across the groups, and no subgroup gained statistically improved prediction of outcomes with adjustment for ECE.

As a confirmatory analysis in the subgroup with 24h urine samples available, we used the actual measured creatinine excretion to adjust the TPCR or ACR. This confirmed that adjusting TPCR or ACR for creatinine excretion gave an inferior performance compared to unadjusted TPCR or ACR. Furthermore, the performance of TPCR and ACR adjusted for measured 24-hour creatinine excretion was inferior to that of the spot samples adjusted for estimated creatinine excretion (calculated using the Cockcroft & Gault equation), which in turn was inferior to the "raw" TPCR/ACR.

It is conceivable that 1g/day of proteinuria may carry a higher prognostic risk in a 50 kg frail, elderly woman, than in a 120 kg young, muscular man. Thus, one explanation for the unadjusted TPCR/ACR predicting risk more effectively is that the denominator corrects to some extent for weight. To assess that further we examined the prognostic risk associated with 24-hour total protein excretion compared with that adjusted for actual, ideal or lean body weight (rather than adjusted for creatinine excretion). No difference was found between the two approaches in their ability to predict mortality, RRT or doubling of serum creatinine.

5.4.2 The role of urine creatinine

Low creatinine excretion as a result of low muscle mass will result in a low denominator in TPCR or ACR, leading to a higher result for any given protein excretion. The converse is also true: for example, in a young man with high creatinine excretion, the TPCR will underestimate 24-hr protein excretion (as demonstrated in chapter 2). Therefore adjusting for this, by taking account of estimated creatinine excretion in the derived formulae, will

reduce these important discrepancies between subgroups, and improve the overall predictive ability of the spot samples to accurately quantify 24-hr proteinuria, as we have demonstrated here. However the accurate prediction of 24-hour urine protein is not necessarily the ultimate goal, as many would argue that it is only a surrogate end-point used to identify those at risk of renal decline and increased risk of mortality. The goal is to identify the optimal measure of urine protein that accurately predicts renal and patient survival.

Why should it be the case that adjustment of spot urine samples for estimated creatinine excretion improves the prediction of 24-hr urine protein excretion, but not outcomes such as all-cause mortality or commencement of RRT? Timed urine collections have been considered the gold standard for the measurement of proteinuria, but have a number of acknowledged technical and practical drawbacks such as incomplete collection. It may be that a spot sample corrected for urine creatinine (e.g. TPCR) is a superior measure of 24-hour urine protein than the timed urine collection itself. The additional finding that adjusting the TPCR/ACR for *estimated* creatinine excretion had a superior predictive performance than adjusting TPCR/ACR for *measured* creatinine excretion, goes some way to supporting this hypothesis.

An alternative hypothesis is that urine creatinine excretion *per se* may have a role in predicting patient outcome, other than just correcting proteinuria for urine concentration. A *post hoc* analysis of the PREVEND study from the Netherlands demonstrated that urine creatinine excretion is an independent predictor of cardiovascular disease and mortality in a general population cohort (228). A study of patients with established cardiovascular disease also showed creatinine excretion to be an independent predictor of mortality (236).

The performance of "raw" TPCR/ACR is superior to the adjusted TPCR/ACR, but raw 24-hour total urine protein is not superior to 24-hour total urine protein adjusted for body weight. The notable difference between these analyses is the absence of urine creatinine in the latter analysis. Therefore, in our cohort, is the urine creatinine component of the TPCR and ACR acting as an independent predictor of outcome, rather than as a correction for urine flow rate alone? This might account for the marked fall in test performance following adjustment for ECE, for all-cause mortality when compared to renal outcomes? Therefore is TPCR/ACR the optimal measurement as it inadvertently includes two markers of outcome; the total protein/ albumin and the urine creatinine?

5.4.3 Implications of the study

Whether these are, or are not, the correct mechanisms to explain the phenomenon described here, the practical implications are clear. Adjustment for estimated creatinine excretion improves the identification of significant proteinuria in those with low muscle mass such as females and the elderly. However, it does not significantly improve the ability of TPCR or ACR to predict outcomes in this cohort. Therefore the utility of this technique is entirely dependant on the proposed application of the result; if the TPCR/ACR will be used to identify those above a proteinuria threshold who may benefit from the use of ACE inhibitors (where the evidence is derived from measurements in 24-hour collections and therefore the accurate quantification of urine protein is paramount) then adjustment of TPCR/ACR will be advantageous. However, if the TPCR/ACR is being utilised as a prognostic marker, then the unadjusted value, with the influence of the urine creatinine, will be more informative.

The optimal method of assessment of proteinuria is an ongoing challenge in nephrology. The proposed addition of proteinuria to the international CKD staging system underlines

the importance of this. Major national and international guidelines recommend screening for the presence of proteinuria using spot samples (either TPCR or ACR) (5, 7, 8, 214). Therefore clarification regarding the need for refinement of TPCR and ACR is urgently needed. The findings of this study contribute to that.

5.4.4 Limitations

This study has limitations. Those relating to the cohort have already been outlined in previous chapters. Of particular relevance to this analysis, only one third (approximately) of the cohort performed 24-hr urine collections, from which actual 24-hr creatinine excretion was measured. We do not have any additional measurements of muscle mass, in addition to actual body weight. A number of formulae have been used in this analysis, all of which are based on a number of assumptions that may be inaccurate. The cohort is a predominantly white population attending a hospital nephrology clinic and may not be representative of other populations. This is of particular importance given that the MDRD eGFR prediction equation using serum creatinine has a factor of 1.2 for black race. It will be important to examine these issues in other racial groups.

5.4.5 Direction of further research

Further research is required to clarify the role of urine creatinine in the prediction of outcomes in patients with chronic kidney disease. The results of this study should be confirmed prospectively, and in other populations.

5.4.6 Conclusion

Adjusting TPCR and ACR for estimates of muscle mass including estimated creatinine excretion improves the prediction of significant proteinuria in sub-groups with low muscle mass (such as the elderly and females). However, adjustment does not improve prediction of renal outcome or mortality in patients with chronic kidney disease.

6 Chapter 6: Estimated glomerular filtration rate: A retrospective study of the prevalence of CKD in the general population and secular trends: the impact of the MDRD and CKD-EPI Formulae

6.1 Introduction

The NKF-KDOQI classification of CKD was rapidly adopted internationally after its publication in 2002, and is primarily based on a reduced glomerular filtration rate, or in combination with other markers of kidney damage at GFR >60ml/min/1.73m^2 (5) (as described in detail in chapter 1). To allow the use of the NKF-KDOQI classification in the UK, it has been recommended that eGFR be reported routinely with serum creatinine measurements in adults (21) Most laboratories use the Modification of Diet in Renal Disease four variable formula, which estimates GFR from serum creatinine, age, sex and race (if available) (44, 215)

The MDRD4 formula was derived from a United States (US) CKD cohort with a mean GFR of 40 mL/min/1.73m^2, and underestimates higher GFRs (237, 238). Some have concerns that this formula may lead to overdiagnosis of CKD, particularly in the elderly and in women (239). The Chronic Kidney Disease Epidemiology Collaboration recently derived a series of new eGFR equations with improved accuracy at higher GFRs as described in detail in chapter 1 (47)

This study has two aims: the primary aim was to compare the impact of the MDRD4 and CKD-EPI formulae on estimates of CKD prevalence in a United Kingdom population, and the secondary aim was to examine the impact on secular trends in CKD prevalence over a five year period.

6.2 Methods

6.2.1 Laboratory Assays

Serum creatinine was measured using Roche Modular reagent Jaffe method, with a mean between batch coefficient of variation of 2.3% at a sCr concentration of 148 μmol/L and 1.7% at 326 μmol/L. In 2004, serum creatinine was measured by the O'Leary modifications of picrate method of Jaffe. The between-day CVs were <2.0% at concentrations of 100 and 485mmol/l. Both assays were performed using Roche Modular P Units. We used the adjustment factors produced by the UK National External Quality Assessment Service for each creatinine assay, to produce IDMS-traceable serum creatinine values (43).

6.2.2 Study Population

NHS Ayrshire & Arran provides healthcare for its geographically defined population in the West of Scotland, with only limited coverage by other providers at the boundaries. Biochemistry services are provided by a single laboratory in University Hospital Crosshouse, Kilmarnock. We downloaded all serum creatinine results reported between 1/4/2009 and 31/3/2010. Individual patients were identified using unique community health index (CHI) numbers, which are utilised in 98% of samples received by the laboratory. Samples with no CHI number, patients below 18 years of age and those receiving RRT were excluded. The lowest serum creatinine available for each individual over the year was selected, to minimise the potential effects of acute illness on kidney function. For analysis of secular trends, we performed the same data extraction for the period 1/1/2004 – 31/12/2004.

eGFR was calculated using the IDMS traceable versions of the MDRD4 and CKD-EPI formulae, (see chapter 1 for the formulae). No modification was made for race as our population is relatively homogeneous (99.35% white, 0.44% Indo-Asian, 0.04% black, and 0.20% other)(217). CKD stage was classified using the modified version of the NKF-KDOQI CKD classification (7, 8), with stage 3 subdivided into 3A (45-59 mL/min/1.73m^2) and 3B (30-44 mL/min/1.73m^2). Local population statistics were obtained from the General Register Office for Scotland (240).

6.2.3 Statistical Analyses

Data were analysed using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA). Appropriate summary statistics were obtained and comparison tables constructed. Agreement between the estimated GFR predicted by each formula was assessed using the Bland-Altman method.

We compared the prevalence in our study population with that of representative population studies (US National Health and Nutrition Examination Survey [NHANES] 1999-2006 data (47) and from the Health Survey for England [HSE]) in order to calculate the prevalence in the unbled proportion of our population that would be necessary to produce the same overall prevalence as NHANES/HSE. Specifically we used the CKD-EPI formulae with age-adjusted data from 2009 and estimated the expected prevalence of CKD in our population. We then used the difference between the CKD prevalence from our laboratory data and the expected prevalence from the NHANES and HSE data to calculate the necessary prevalence in the population with no blood sample taken in the study year.

6.3 Results

The adult population of Ayrshire and Arran in 2009 was 293,880 (240). Between April 2009 and March 2010, 438,872 serum samples were analysed for creatinine. Following removal of repeat samples on individuals, and application of the exclusion criteria, the study population was 123,121 (figure 6-1 and table 6-1). The population distribution and percentage with serum creatinine results by age band is shown in figure 6-2. Overall 42% of the adult population, and 71% of those over 65 years old had serum creatinine measured in the year.

Figure 6-1 - Flowchart of the study populations and exclusions

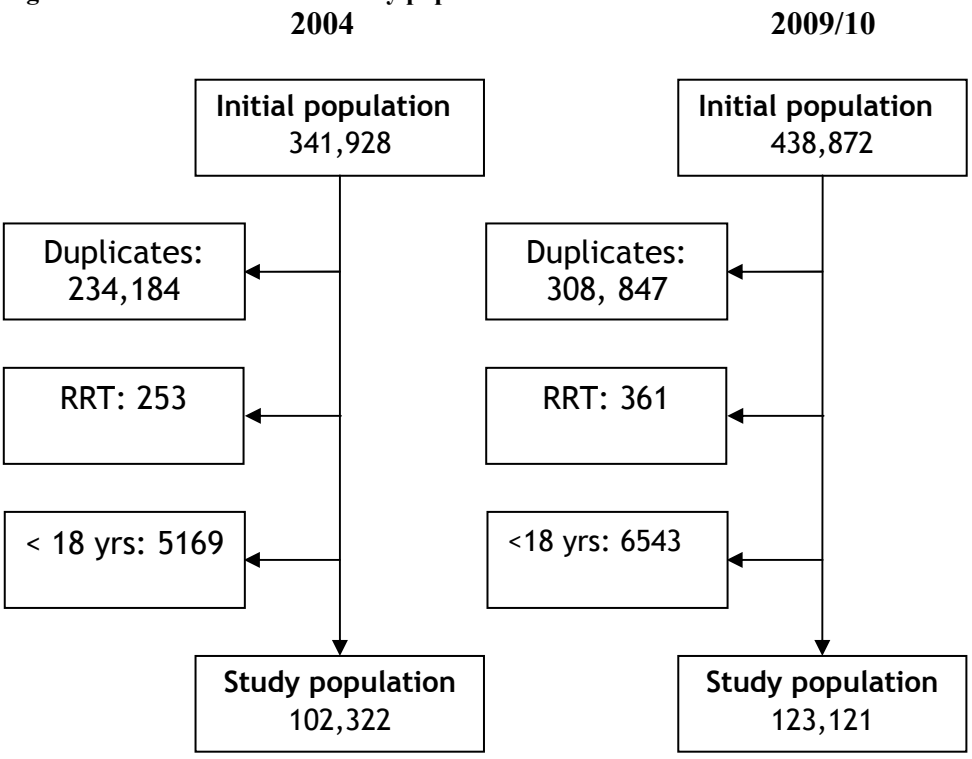
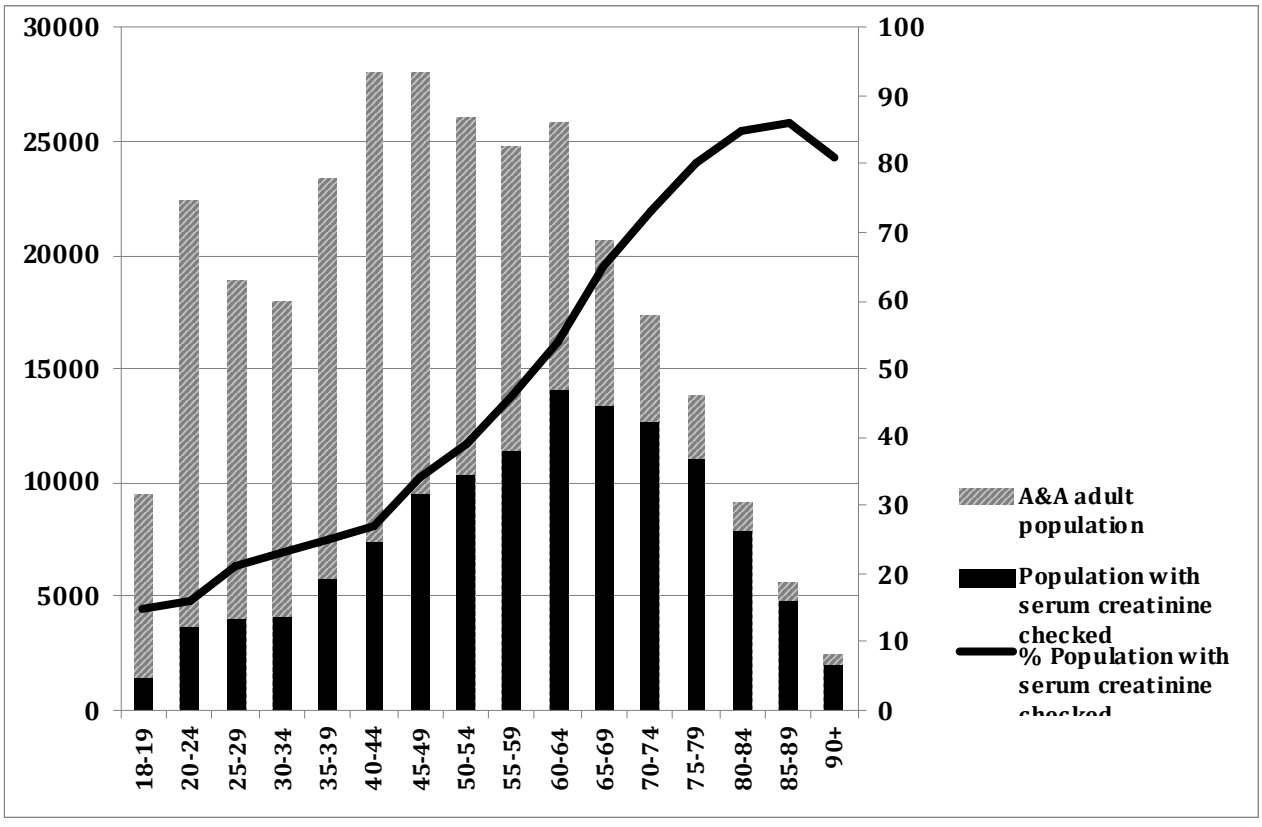


Table 6-1 –Population characteristics. The results are shown as mean ± standard deviation (SD) or median (interquartile range)

	2004	2009-2010
Adult population	289,386	293,880
Study population	102,322	123,121
Sex (% male)	43	44
Age (years) [range]	60±18 [18-105]	59±18 [18-109]
Serum creatinine (µmol/L)	88 (79-99)	75 (64-88)
eGFR $_{MDRD4}$ (mL/min/1.73m^2)	81 (67-95)	84 (69-100)
eGFR $_{CKD-EPI}$ (mL/min/1.73m^2)	86 (70-100)	89 (73-102)

Figure 6-2 - Population distribution by age in Ayrshire and Arran 2009-10 (240) **and the proportion with serum creatinine checked**



The relationship between eGFR as estimated by the MDRD4 formula and the CKD-EPI formulae are shown in figures 6-3 and 6-4. The prevalence of CKD stages 3-5 fell from 13.4% of our study population when using the MDRD4 formula, to 11.8% using the CKD-EPI formulae. The impact on prevalence by CKD stage, age and gender is shown in table 6-2 and 6-3. The difference between eGFR as estimated by the different formulae in relation to age is shown in Figure 6-5.

The CKD prevalence that would be required in the unbled population in order to match NHANES and HSE data is shown in table .6-4

Figure 6-3 - Comparison of eGFR calculated using the CKD-EPI formulae and the MDRD4 study formula in a population of 123,121 adults in Ayrshire. The line is the line of identity.

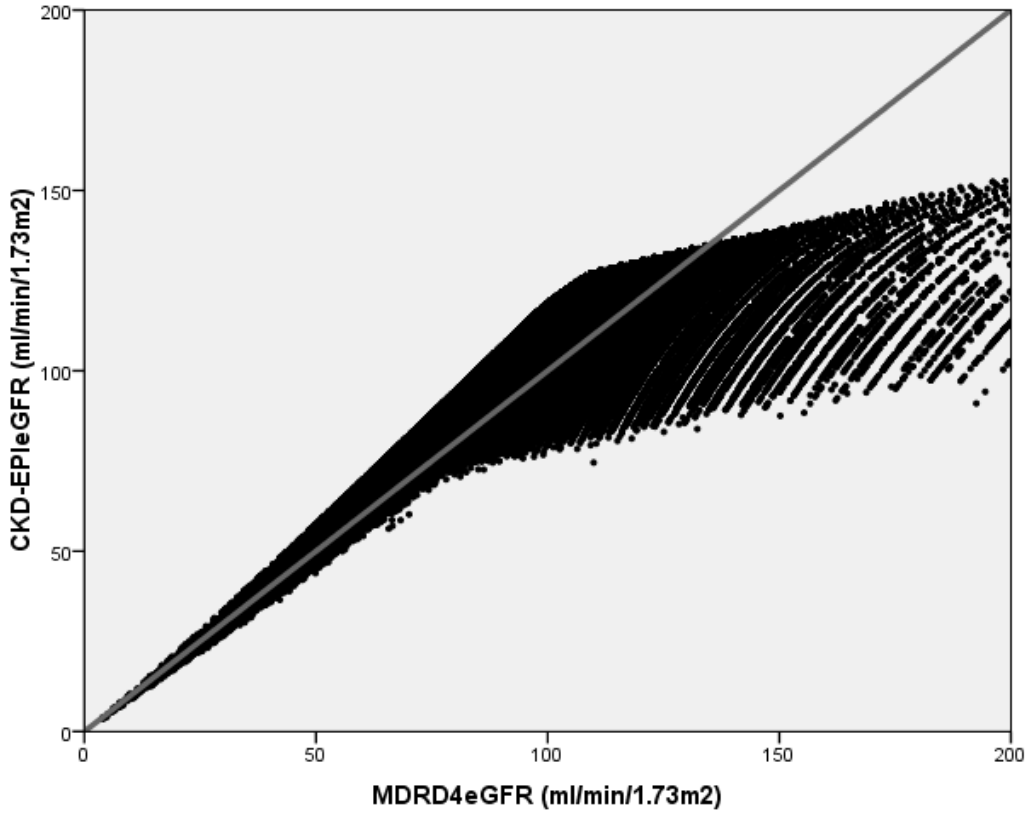


Figure 6-4 - Bland-Altman plot of eGFR calculated using the CKD-EPI formulae and the MDRD4 formula

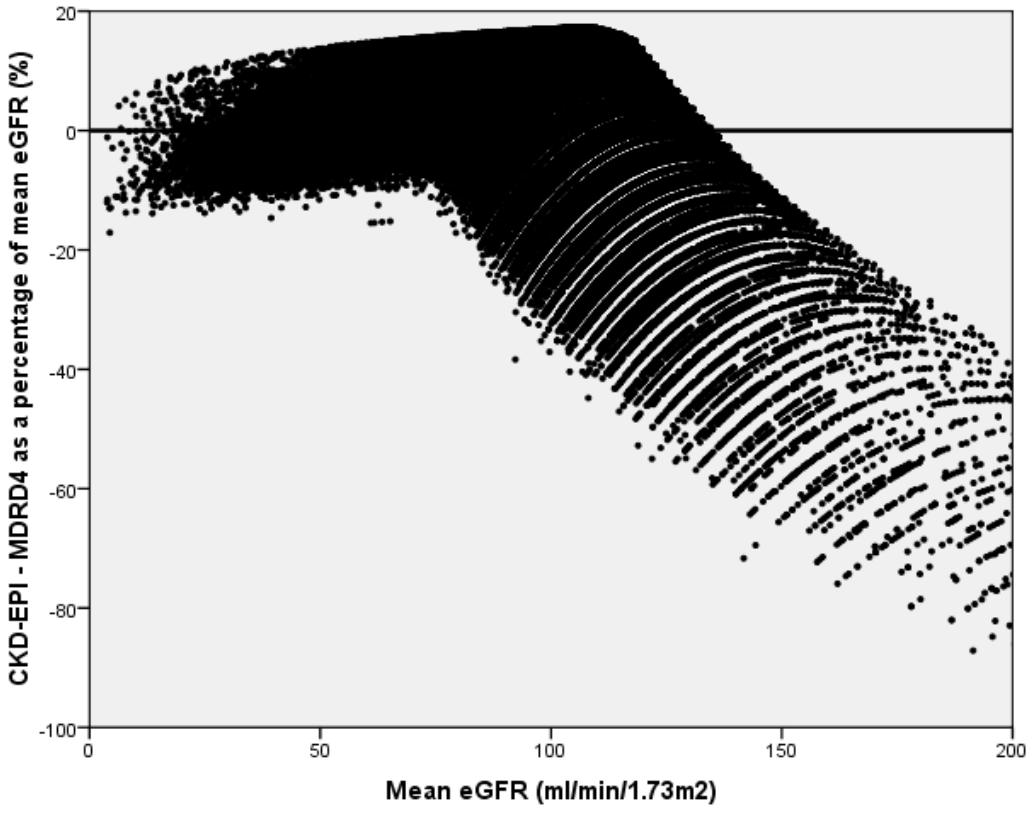


Table 6-2 - Observed frequencies of CKD stage, age, proportion of females and difference by eGFR formula. Bold figures are observed frequencies of agreement. Percentages of the study population (i.e. those patients with blood samples taken during the study year) are shown for each CKD stage by eGFR formula

	CKD stage (MDRD4)						
CKD stage (CKD-EPI)	**eGFR ≥90**	**eGFR 60-89**	**3A**	**3B**	**4**	**5**	**Total**
eGFR ≥90	**n=44965 F 52.4% Age 48.6±16.6**	n=14339 F 64.5% Age 48.4±11.6	--	--	--	--	*59304 (48.1%)*
eGFR 60-89	n=4155 F 64.8% Age 80.4±6.3	**n=42921 F 54.6% Age 65.8±12.9**	n=2307 F 81.1% Age 59.4±11.4	--	--	--	*49384 (40.1%)*
3A	--	n=481 F 32.0% Age 86.2±5.4	**n=8769 F 64.0% Age 74.6±10.6**	n=334 F 85.6% Age 60.1±10.7	--	--	*9584 (7.8%)*
3B	--	--	n=368 F 50.0% Age 86.4±5.6	**n=3447 F 67.5% Age 77.6±10.6**	n=28 F 75.0% Age 55.5±10.7	--	*3843 (3.1%)*
4	--	--	--	n=153 F 60.1% Age 86.6±5.5	**n=755 F 67.1% Age 75.8±13.0**	n=6 F 83.3% Age 46.5±10.3	*914 (0.7%)*
5	--	--	--	--	n=15 F 60.0% Age 83.9 ± 5.3	**n=87 F 57.3% Age 70.6±14.3**	*102 (0.1%)*
Total	*49120 (39.9%)*	*57741 (46.9%)*	*11444 (9.3%)*	*3934 (3.2%)*	*798 (0.7%)*	*94 (0.1%)*	

Table 6-3 – Change in the prevalence of CKD in the study population (i.e. those patients with blood samples taken during the study year) when using CKD-EPI formulae instead of MDRD4 eGFR formula

CKD stage	Overall	Sex		Age (years)				
		M	F	< 20	20-39	40-59	60-69	≥70
eGFR ≥90	8.1%	4.4%	11.2	7.3%	17.7%	21.4%	9.9%	-10.7%
eGFR 60-89	-6.6%	-4.1%	-8.6%	-7.1%	-16.9%	-19.1%	-6.5%	10.6%
3A	-1.6%	-0.4%	-2.5%	-0.2%	-0.7%	-1.9%	-2.9%	-0.8%
3B	-0.1%	0.2%	-0.3%	--	-0.1%	-0.2%	-0.5%	0.5%
4	0.1%	0.1%	0.1%	--	--	--	--	0.3%
5	--	-0.01%	0.01%	--	--	--	--	0.1%

Figure 6-5 - Relationship between age and the difference in eGFR calculated using the CKD-EPI formulae and the MDRD4 study formula

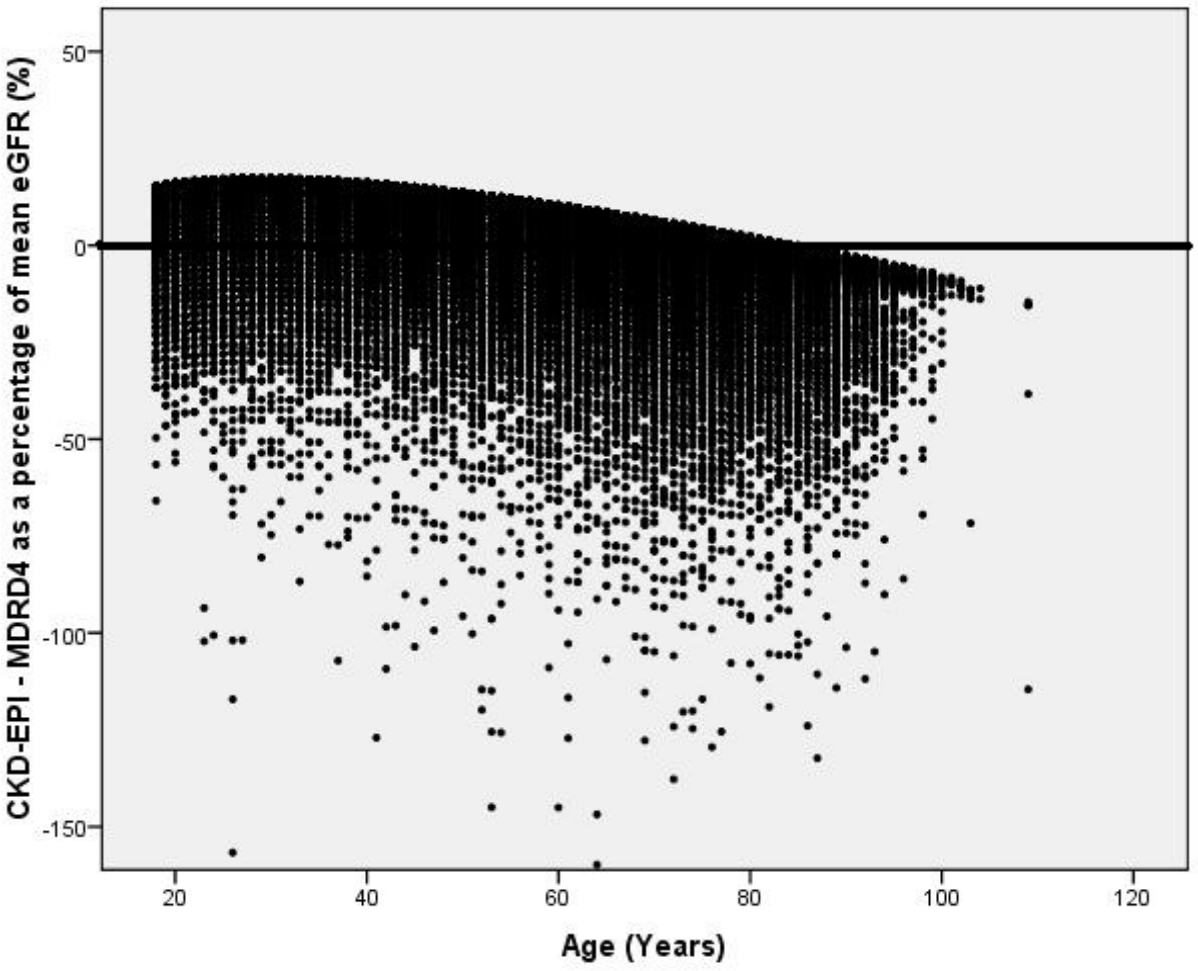


Table 6-4 - Measured and derived CKD prevalence in NHS Ayrshire & Arran (A&A) in 2009-10.

Calculations based on laboratory eGFR (using the CKD-EPI formulae), census population estimates, (240)**and the application of prevalence estimates from the NHANES** (47) **and the HSE to the NHS A&A population**

Age Band	***20-39 yrs***		***40-59 yrs***		***60-69 yrs***		***≥70 yrs***	
CKD stage	***3***	***4***	***3***	***4***	***3***	***4***	***3***	***4***
Prevalence in NHANES population (%)	0.17	0.01	2.04	0.05	10.06	0.72	35.33	2.44
Prevalence in HSE population (%)	0.10	-----	0.89	0.11	3.66	1.08	28.26	0.58
Identified prevalence in A&A population (%)	0.15	0.02	0.85	0.07	5.03	0.21	20.84	1.52
A&A unbled population (n)	64,967		68,311		18,994		10,103	
Predicted prevalence in A&A unbled population (NHANES data)	0.02	-----	1.86	-----	12.3	1.24	69.42	4.39
Predicted prevalence in A&A unbled population (HSE data)	-----	-----	0.06	0.07	-----	2.12	35.54	-----

6.3.1 Comparison of 2004 and 2009-2010 cohorts

The characteristics of the 2004 cohort are shown in table 6.1. The proportion bled by age group has been published previously (241). From 2004 to 2009-10, the number of serum samples analysed for creatinine in our laboratory has increased by 28% from 341,928 to 438,872. The total adult population has grown by 0.5%, whereas the number of individual adults having their serum creatinine measured increased by 20% from 102,322 to 123,121. The change in prevalence of CKD between the two time periods is shown in table 6-5 below, according to MDRD4 and CKD-EPI formulae.

Table 6-5 – Change in general adult population CKD prevalence between 2004 and 2009-10. eGFR was calculated using the MDRD4 formula or the CKD-EPI formulae

eGFR	MDRD 4		Prevalence Change	CKD EPI		Prevalence Change
	2004	2009/10		2004	2009/10	
≥ 90	11.7%	16.9%	5.2%	15.1%	20.4%	5.3%
60-89	18.2%	19.8%	1.6%	15.3%	17.0%	1.7%
3A	3.8%	3.9%	0.1%	3.3%	3.3%	--
3B	1.3%	1.4%	0.1%	1.3%	1.3%	--
4	0.3%	0.3%	--	0.3%	0.3%	--
5	0.04%	0.03%	-0.01%	0.04%	0.04%	--
Stages 3-5	5.44	5.63	+0.19	4.94	4.94	--

6.4 Discussion

6.4.1 Findings of this study

Our study shows that changing from the MDRD4 formula to the CKD-EPI formulae to measure eGFR in a UK population, would result in a small reduction in the overall prevalence of CKD stage 3A by 0.6% (from 5.6% to 4.9%), with most of these patients reclassified to eGFR >60 mL/min/1.73m^2. There is relatively little reclassification of CKD stage seen with more severe kidney disease. The 17014 (5.8% of the adult population) patients reclassified to milder stages of CKD are mainly female (67%) with a mean age of 50±12.2 years. Additionally, we found that the CKD-EPI formulae reclassified 5,172 (1.8%) of patients to more severe CKD stages, mostly affecting elderly females (mean age 81.5±6.6 years). Kidney function was assessed in a remarkably large proportion of the adult population with 42% assessed in 2009-10 (compared to 35% in 2004), rising to a peak of 86% in those aged 85-89 years. Of note, there was no rise in population CKD prevalence between 2004 and 2009-10 when eGFR was assessed by the CKD-EPI formulae, and only a small rise of 0.2% when using the MDRD4 formula, despite the increasing numbers being assessed.

6.4.2 Other studies of CKD prevalence

The CKD-EPI formulae were derived in 5504 subjects from 10 studies with formal GFR measures performed. The formulae were validated in 2750 additional subjects from the same studies, and also against an external set of 3896 subjects from 16 other studies. The mean GFR from the combined populations was 68 mL/min/1.73m^2, 44% were female, 71% were white and the mean age was 48 years. The CKD-EPI formulae in these studies estimated GFR as accurately as the MDRD4 formula in subjects with eGFR <60 mL/min/1.73m^2, but was more accurate at higher levels of eGFR. Precision remained relatively poor.

Levey and colleagues assessed the impact of the different formulae on CKD prevalence, using the NHANES 1999-2006 population (47). They found a rise in mean eGFR of 6.9 mL/min/1.73m^2, and a fall of 1.5% in prevalence of stage 3 CKD using the CKD-EPI formulae. Women and those aged 20-69 years had a disproportionate reduction in prevalence of CKD. An Italian study of 38,188 patients calculated eGFR using both formulae (242). The estimated prevalence of CKD fell by 1.6% using the CKD-EPI formulae, with a significant drop in the prevalence of stage 2 disease (15.3%). In a cohort of 14,427 Spanish patients, the mean eGFR was 0.6 mL/min/1.73m^2 higher with CKD-EPI than MDRD4, and CKD-EPI led to reclassification of patients to lower stages of CKD, particularly affecting stages 2, 3A and 3B, women and those <70 years old. In keeping with our study, they found some reclassification of older females to higher CKD stages with CKD-EPI (243). Similarly a Dutch population based cross-sectional survey of 6097 participants concluded that the CKD-EPI formulae provide higher estimates of GFR than the MDRD4 formula. However, women >75 and men >70 years had lower median eGFR values (244). A Belgian screening study of 1,992 volunteers aged between 45-84 years old showed mean eGFR to be 2 mL/min/1.73m^2 higher with CKD-EPI, and prevalence of CKD stage 3 fell from 11.04 to 7.98%, with greater impact in women(245). Compared to these studies, we found a relatively small change in CKD prevalence when using the CKD-EPI formulae. This is at least in part because our population is substantially older, and the reduction in CKD amongst adults under 70 years old is partially offset by the increased prevalence amongst the elderly, as could be predicted from Figure 5. Two other UK studies, in Oxfordshire and East Kent, have both reported similar findings regarding the relationship of lower CKD-EPI eGFR estimation with increasing age (246, 247). Of note, only 0.6% of the CKD-EPI population were >80 years old, and only 5.3% over 70 years old. A recent large UK primary care study found an overall reduction in CKD prevalence using CKD-EPI, but fluctuation in eGFR measurements accounted for a greater proportion of the change in prevalence, than changing formulae (248).

6.4.3 Limitations

This study has a number of limitations. There was no formal GFR measurement performed to allow direct comparison with the prediction formulae. The serum creatinine assay changed between the two study periods but both were converted to the IDMS-traceable serum creatinine value before the eGFR was calculated, thereby minimising bias. We defined patients as having CKD on the basis of a single eGFR, rather than two samples >90 days apart, but we minimised the impact of this by using the lowest serum creatinine available for each patient in the study year. Nevertheless, we may have over-diagnosed CKD in some patients. The strength of our study is its size, and that the population is unselected and clinically relevant.

Our study cohort comprised patients who have had kidney function assessed for a clinical indication, raising the possibility of some selection bias. To ascertain true population prevalence would require a population survey. In order to explore this we calculated the required prevalence in the unbled population to produce comparable prevalence to the NHANES population (adjusting for our older population). Using this method the prevalence in the unbled population is high. Whilst there will be some unidentified CKD, it seems unlikely to be the complete explanation. Compared to the NHANES population, our population has a slightly higher proportion of females, and a far higher proportion of whites, both of which should lead to higher rather than lower CKD prevalence. Furthermore, NHANES excluded adults living in institutions, who have a high prevalence of CKD (249), whereas our data includes such patients. It therefore seems likely that our population has a genuinely lower prevalence of CKD than in the USA. Previous estimates of the prevalence of kidney disease in the UK have varied substantially (250). Despite the different methodological approach of the Health Survey for England, and allowing for their relatively small sample size (n=2,171), our CKD prevalence rates are similar (251). This suggests that age-adjusted CKD prevalence is genuinely lower in the UK than in the USA.

A study with a similar design to this one, from the South of England, found similar prevalence at 4.4% with the CKD-EPI formulae and 4.9% using MDRD4 (247). Larger population-based surveys of CKD prevalence would be warranted in the UK to give more precise estimates of prevalence over time.

6.4.4 Outcomes and the CKD-EPI formulae

Three studies have assessed clinical outcomes in patients who have been reclassified by the CKD-EPI formulae. The AUSDIAB study compared outcomes in three categories of patients: those with CKD by both equations, those with CKD only by MDRD4 and those without CKD by either formula (252). In keeping with our study, they found that those reclassified were mainly women. They found no evidence of increased all-cause mortality in the reclassified group, suggesting that they are low-risk individuals. A similar *post hoc* analysis of the ARIC study (253) found reclassified individuals were more likely to be female, middle-aged and white. Moreover the reclassification of this sub-group was more appropriate with regard to their comparable risk of unfavourable outcomes such as end-stage renal disease, all cause mortality, coronary heart disease and stroke. An analysis of the participants of the kidney early evaluation programme (KEEP), a community based CKD screening programme in the Unites States, found the reclassified group to be younger, less likely to have chronic conditions and had a lower risk of mortality (254). There are no outcome studies from the UK. In our study population, 1.8% of subjects were reclassified to higher stages of CKD – no patients in ARIC and few in AUSDIAB were reclassified in this way. It will be of importance to examine outcomes in this group before the equation is implemented in clinical laboratories.

In practical terms, the implementation of the routine use of eGFR CKD-EPI formulae in place of the MDRD4 formula in the United Kingdom, would result in fewer people being identified as suffering from CKD, without apparent increased risk to those reclassified to

milder disease (though this should be confirmed in a UK population). The resultant fall in the number undergoing routine monitoring may result in reduced medicalisation of patients, and reduce the financial burden and workload in primary care. However, a large proportion of those on primary care CKD registers also suffer from hypertension, vascular disease and/or diabetes mellitus and would continue to receive similar monitoring as a result of these other conditions. The magnitude of any potential saving would have to be directly assessed. Implementation costs would be low, as it requires no change to instrumentation or assays.

6.5 Conclusion

Measurement of eGFR using the CKD-EPI equation reduced the overall prevalence of CKD, in particular stage 3A, in a predominantly Caucasian general population cohort. This was particularly prominent in females and those middle-aged. Using the MDRD4 formula, there is an apparent rise in CKD prevalence over the study time period, but this is not seen when using the CKD-EPI formulae. The CKD-EPI formulae may reduce overdiagnosis of CKD, but further assessment in the elderly is required before widespread implementation.

7 Chapter 7: Assessing Patient Outcomes In Hypertension: The Predictive Ability Of Proteinuria And Estimated Glomerular Filtration Rate

7.1 Introduction

Impaired excretory renal function and proteinuria are associated with an increased risk of cardiovascular morbidity and mortality as described in detail in the introduction. This has been shown in the general population (20) and in groups already at increased risk of vascular disease (255-257). In hypertensive patients, reduced renal function is associated with a greater likelihood of all cause and cardiovascular mortality, (258, 259). Furthermore, albuminuria predicts cardiovascular risk in this group (260), even at levels below the traditional threshold for microalbuminuria (5, 261). There is limited evidence of the effects of these markers in combination in a hypertensive population, with only 2 studies to date having evaluated the combination of these risk markers in a hypertensive population (262, 263) and this study extends the scope of those findings with the results from the Glasgow Blood Pressure Clinic (GBPC), a large secondary and tertiary care hypertensive cohort. Kidney function and urine protein have been recorded, in this large cohort of hypertensive patients in the West of Scotland since the 1960's.

In chapters 2-5 we have focused on proteinuria in patients with CKD and chapter 6 focused on how to measure eGFR in the general population. The aim of this study was to assess the utility of eGFR and proteinuria as individual and combined predictors of all-cause and cardiovascular mortality in a high risk hypertensive population.

7.2 Methods

7.2.1 Clinical Measurements and Laboratory Assays

Blood pressure was measured manually by specialist hypertension nurses. Patients were asked to rest for five minutes in the supine position before blood pressure was recorded, using standard mercury sphygmomanometers. It was measured three times and the mean of the last two measurements recorded. Blood pressure was recorded between 09:00 hours and 11:00 hours for all patients.

Proteinuria was measured by urine dipstick and considered to be positive if it was greater than or equal to "1+". This correlates to approximately greater than or equal to 0.3g/L of urinary protein.

Laboratory measurements were performed using standard operating procedures in the biochemistry and haematology laboratories of the Western Infirmary, Glasgow. The 4 variable MDRD formula was used to calculate eGFR (44, 215). It was not possible to calculate IDMS traceable values for serum creatinine. Data regarding race were not available, but the population of the West of Scotland is predominantly Caucasian with less than 0.25% being black. (217)

7.2.2 Participants and Setting

The Glasgow Blood Pressure Clinic is a secondary and tertiary referral clinic for patients with hypertension in the West of Scotland. It has a computerised database of all patients attending the clinic since November 1968, on which demographic and clinical data are prospectively recorded. The records of more than 11 000 patients are held. For the purposes of this analysis, the last new patient was added to the database on 17th September 2003, and followed up until February 2009.

Patients with at least one measure of serum creatinine and urine protein were included in the study. Patients were excluded from the analysis if they were less than 18 years old, or had a baseline eGFR of less than 15ml/min/1.73m^2. Baseline clinical and demographic data recorded included age, gender, blood pressure (BP) and serum creatinine, dipstick urinalysis, body mass index, haemoglobin, serum albumin, total cholesterol, diagnosis of diabetes or vascular disease. Subsequent measurements of blood pressure were also downloaded. Outcome data were obtained from the General Register Office for Scotland which records date and cause of death (according to the International Classification of Disease 9 and 10). All patient data were anonymised.

7.2.3 Ethical Permission

Ethical approval has been received for analysis of the GBPC cohort.

7.2.4 Statistical Analysis

Data were analysed using SPSS Version 15 (SPSS Inc, Chicago, Ill, USA). Renal dysfunction was classified according to the international CKD Staging System (5), using eGFR and other markers of kidney damage such as urinary or structural abnormalities.

Smoking was recorded as current smoker, ex-smoker or lifelong non-smoker. Diabetes was defined as random blood glucose ≥11.1mmol/L, receiving diabetic medication or patient reported diagnosis. Vascular disease was defined as a definitive vascular event or angiographically proven peripheral vascular disease. Cardiovascular mortality was defined as a cardiovascular cause listed in Part I of the death certificate (according to ICD 9 and 10 codes). The cohort was divided into two groups according to eGFR (≥60ml/min/1.73m^2 or <60ml/min/1.73m^2 as this is the important clinical threshold) and the presence or absence of dipstick proteinuria, producing four groups. Summary statistics are presented as mean ±

standard deviation or median (interquartile range). Significance testing was performed using one way analysis of variance (ANOVA) with post-hoc tests (Tukey), the Chi squared test, Fisher's exact test and paired t-test as appropriate. Survival analysis was performed using Cox proportional hazards regression, using a forward stepwise conditional model. The assumptions of proportionality were assessed using log minus log plots and Schoenfeld residuals. There was no violation of these assumptions. To take account of variation between time periods, a variable was constructed which divided the cohort into quintiles according to the date of baseline measurements with the first quintile being the earliest, and this was included in the survival model (quintile 1; 1968 – 1975, quintile 2; 1976 – 1983, quintile 3; 1984 – 1989, quintile 4; 1990 – 1995, quintile 5; 1996 – 2003) . The analyses were repeated, excluding those with follow-up <2 years, to ensure the findings were robust.

7.3 Results

7.3.1 Demographics of the cohort

A total of 11397 patients who attended the Glasgow Blood Pressure Clinic between 6th November 1968 and 17th September 2003 were identified. One thousand four hundred and sixteen patients were excluded as they had no recorded measure of kidney function, and a further 1445 patients were excluded as they did not have a documented measure of urinary protein. Those under 18 years of age (n=29) and those with an eGFR <15ml/min/1.73m^2 at baseline (n=31) were also excluded. A flowchart of the exclusions is shown in Figure 7-1. The baseline demographics of the remaining 8476 participants are presented in Table 7-1. One thousand nine hundred and fifty three (23.0%) had a baseline eGFR of <60ml/min/1.73m^2, 1827 (21.6%) had proteinuria detected by dipstick and 545 (6.4%) had both. Median follow-up was 15.2 years (interquartile range 9.4, 22.9). Those with a reduced eGFR (<60ml/min/1.73m^2) were older, more hypertensive, and had a greater burden of diabetes and vascular disease at baseline (p<0.001).

Figure 7-1 - Flowchart of the population and exclusions

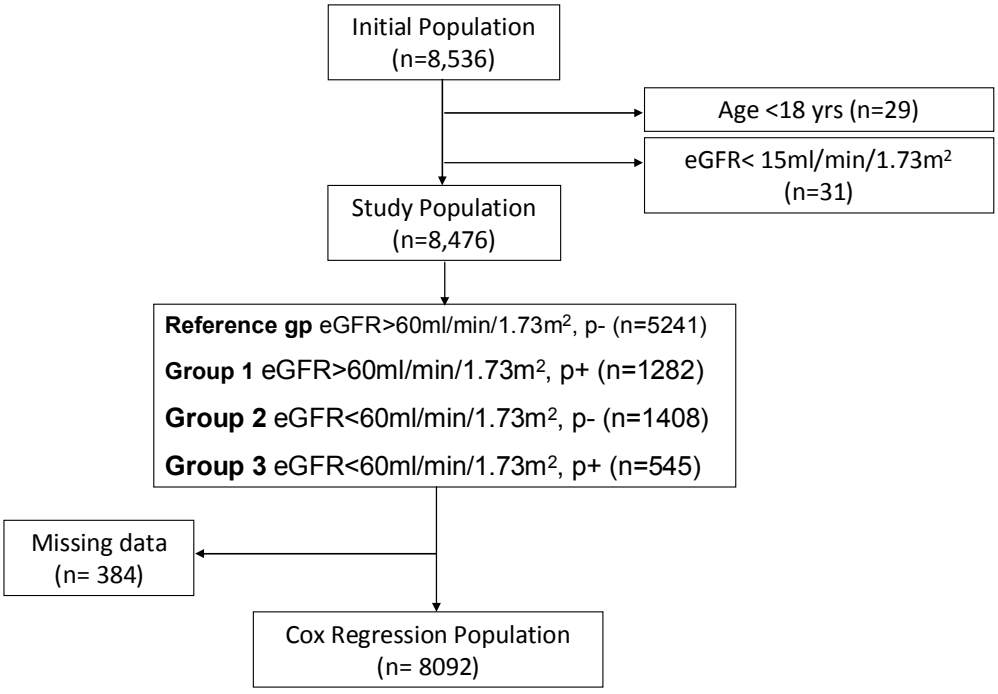


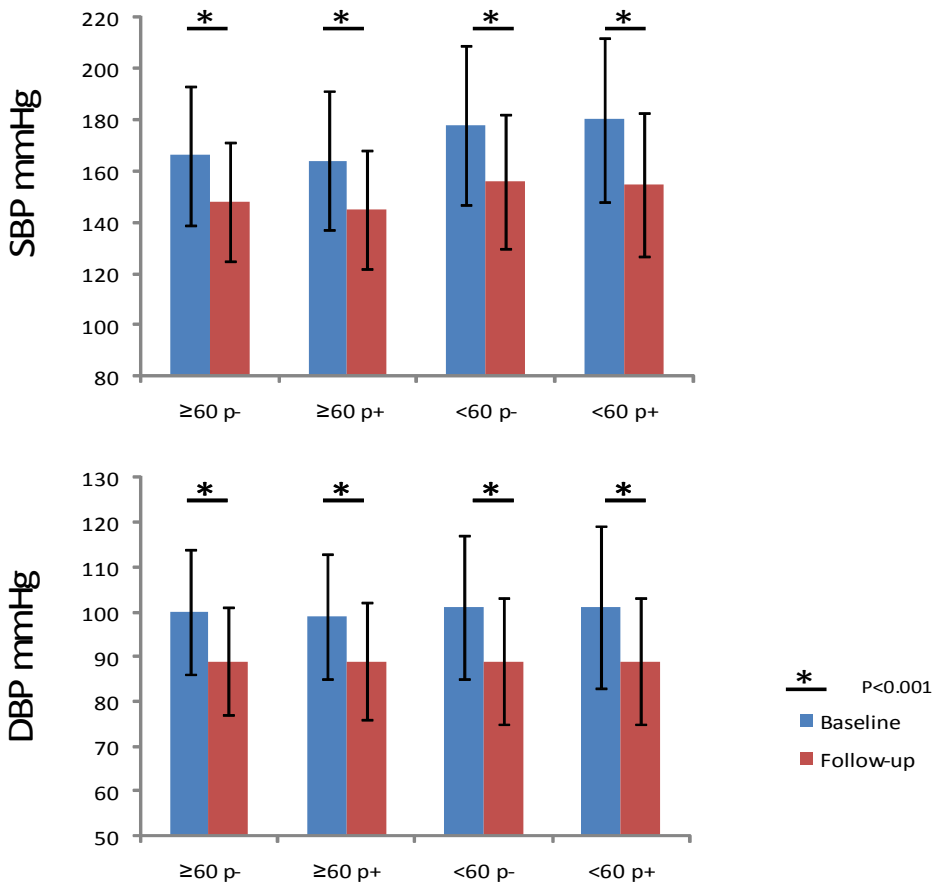
Table 7-1 – Baseline demographics for the 8476 participants. Results are presented as mean ±standard deviation, median (interquartile range) or percentage of cohort with the described characteristic

Variable	All	eGFR >60, p-	eGFR >60, p+	eGFR <60, p-	eGFR <60, p+	Sig	% available
n	8476	5242	1282	1407	545	-	-
Age (years)	50.3 ± 13.3	47.9 ± 12.5	46.9 ± 13.4	59.3 ± 10.3	58.6± 13.1	P<0.001	100
Gender (% male)	48	49	56	34	50	P<0.001	100
Serum creatinine (µmol/l)	89 (77 – 104)	82 (73 – 94)	86 (78 – 98)	114 (97 – 130)	128 (110 – 159)	P<0.001	100
eGFR (ml/min/1.73m^2)	73 (61-85)	78 (69 – 90)	77 (68 – 88)	52 (46 – 57)	47 (37 – 55)	P<0.001	100
Diabetes (%)	7	6	9	8	12	P<0.001	100
SBP (mmHg)	169 ± 29	167 ± 27	164 ± 27	179 ± 31	181 ± 32	P<0.001	100
DBP (mmHg)	100 ± 15	100 ± 14	100 ± 14	101 ± 16	101 ± 18	P=0.021	100
Pulse pressure (mmHg)	69 ± 23	67 ± 21	64 ± 21	78 ± 25	80 ± 27	P<0.001	100
BMI (kg/m^2)	27.6 ±5.3	27.4 ±5.2	28.7± 6.1	27.4 ± 5.0	27.9 ± 5.2	P<0.001	98.4
Vascular disease at baseline (%)	10	8	7	16	17	P<0.001	100
Current smoker (%)	29	30	26	26	28	P<0.001	95.5
Cholesterol (mmol/L)	6.1 ± 1.3	6.0 ± 1.2	5.9 ± 1.2	6.4 ± 1.3	6.3 ± 1.5	P<0.001	80.7
Haemoglobin (g/dL)	14.4 ± 1.5	14.6 ± 1.4	14.5 ± 1.5	14.1 ± 1.6	13.8 ± 1.8	P<0.001	93.5
Serum Albumin (g/L)	44 ± 4	44 ± 3	45 ± 3	43 ± 4	43 ± 4	NS	64.8

7.3.2 Hypertension

The baseline and last follow-up blood pressure recordings are presented, divided into four groups according to eGFR (≥60 or <60ml/min/1.73m^2 and presence (+) or absence (-) of proteinuria) in figure 7-2. There were significant differences in the baseline systolic BP between the four groups using an ANOVA with post-hoc analysis (except between eGFR<60 p- and eGFR<60 p+ groups). On follow-up, mean systolic and diastolic BP was significantly lower than baseline in all groups (paired t-test). However the differences in follow-up systolic BP between the groups remained statistically significant ($p<0.005$) except between eGFR<60 p- and eGFR<60 p+, as before. When the analysis was limited to the modern era of blood pressure targets (baseline measurements from 1990 onwards, n=3333), the initial and follow-up blood pressures were lower but the relationship of higher baseline and follow-up blood pressure, depending on severity of renal disease, remained.

Figure 7-2 - Baseline and follow up blood pressures divided according to eGFR (threshold 60ml/min/1.73m^2) and presence of proteinuria



7.3.3 Outcomes

A total of 3562 participants died during the follow-up period. Of these, 2522 (70.8%) died as a result of vascular disease. Overall median time to death from baseline blood pressure measurement was 11.7 years (interquartile range 6.2, 18.5). Increasing age, male gender, reduced GFR and /or urinary protein, diabetes, smoking, higher baseline systolic BP, higher baseline diastolic BP, higher cholesterol at baseline and the presence of vascular disease at baseline (Table 7-2) were individually associated with increased all cause and cardiovascular mortality. Joining the cohort in an earlier era was also associated with increased all-cause and cardiovascular mortality, and this was subsequently included in the multivariate model to take account of this influence. These variables were then entered into a multivariate Cox regression (Table 7-3). For all-cause mortality diastolic BP and cholesterol were not included as independent predictors of outcome while for cardiovascular mortality diastolic BP was no longer included. Both low eGFR and presence of proteinuria were associated with a greater likelihood of both outcomes. Survival curves from the multivariate Cox regression for all cause mortality and cardiovascular mortality, according to these markers of kidney disease, are shown in figure 7-2 and 7-3 (respectively). Exclusion of those with < 2 years follow-up did not influence the model.

Table 7-2 – Univariate analyses derived from a Cox regression model for all-cause mortality and cardiovascular (CV) mortality, with hazard ratios and 95% confidence intervals shown in brackets

	All-cause mortality	Significance	CV mortality	Significance
Age (per 10 years)	1.95 (1.089 – 2.00)	P<0.001	1.93 (1.87 – 2.00)	P<0.001
Male Sex	1.41 (1.32 - 1.50)	P<0.001	1.52 (1.41 - 1.65)	P<0.001
eGFR< 60 (ml/min/1.73m^2)	2.17 (2.02 – 2.32)	P<0.001	2.27 (2.09 – 2.46)	P<0.001
Presence of urinary protein	1.25 (1.15 - 1.36)	P<0.001	1.25 (1.13 - 1.38)	P<0.001
Diabetes	1.47 (1.31 - 1.65)	P<0.001	1.60 (1.40 - 1.83)	P<0.001
SBP (per 10 mmHg)	1.14 (1.13 - 1.16)	P<0.001	1.16 (1.14 - 1.17)	P<0.001
DBP (per 10 mmHg)	1.13 (1.11 - 1.15)	P<0.001	1.17 (1.14 - 1.20)	P<0.001
Current smoker	1.85 (1.72 – 2.00)	P<0.001	1.96 (1.79 – 2.14)	P<0.001
Cholesterol (per mmol/L)	1.11 (1.08 – 1.15)	P<0.001	1.15 (1.11 – 1.19)	P<0.001
Presence of vascular disease at baseline	2.51 (2.30 - 2.75)	P<0.001	2.95 (2.67 – 3.26)	P<0.001

Table 7-3 – Multivariate analyses derived from a Cox regression model for all-cause mortality and cardiovascular (CV) mortality, with hazard ratios and 95% confidence intervals shown in brackets

	All-cause mortality	Significance	CV mortality	Significance
Age (per 10 years)	2.01 (1.94 – 2.09)	P<0.001	2.06 (1.96 – 2.17)	P<0.001
Male Sex	1.60 (1.49 – 1.72)	P<0.001	1.78 (1.61 – 1.96)	P<0.001
eGFR< 60 (ml/min/1.73m^2)	1.25 (1.16 – 1.35)	P<0.001	1.32 (1.19 – 1.46)	P<0.001
Presence of urinary protein	1.37 (1.26 – 1.50)	P<0.001	1.47 (1.30 – 1.65)	P<0.001
Diabetes	1.25 (1.11 – 1.41)	P<0.001	1.35 (1.16 – 1.57)	P<0.001
SBP (per 10 mmHg)	1.04 (1.03 – 1.05)	P<0.001	1.05 (1.03 – 1.07)	P<0.001
Current smoker	1.84 (1.70 – 1.98)	P<0.001	1.88 (1.69 – 2.08)	P<0.001
Cholesterol (per mmol/L)	-	-	1.08 (1.04 – 1.12)	P<0.001
Presence of vascular disease at baseline	1.46 (1.33 – 1.60)	P<0.001	1.84 (1.63 – 2.08)	P<0.001
Quintile 1 (earliest era)	1 (Ref gp)	-	1 (Ref gp)	-
Quintile 2	0.80 (0.74 – 0.88)	P<0.001	0.78 (0.70 – 0.88)	P<0.001
Quintile 3	0.58 (0.53 – 0.65)	P<0.001	0.54 (0.47 – 0.63)	P<0.001
Quintile 4	0.51 (0.45 – 0.57)	P<0.001	0.39 (0.33 – 0.46)	P<0.001
Quintile 5	0.38 (0.32 – 0.45)	P<0.001	0.32 (0.26 – 0.40)	P<0.001

Figure 7-3 - Survival curve for all cause mortality according to the presence of proteinuria and reduced eGFR

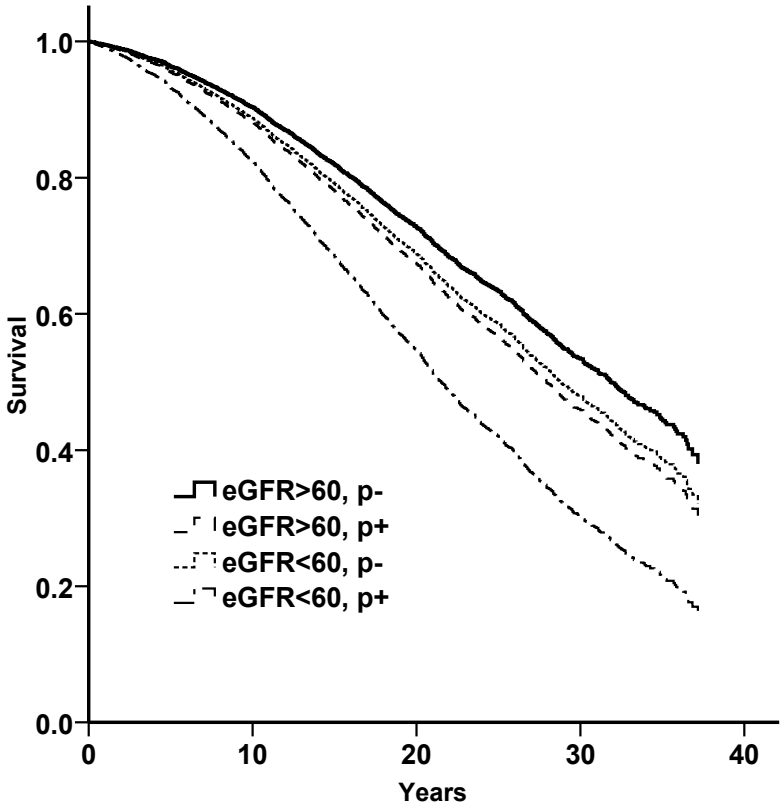
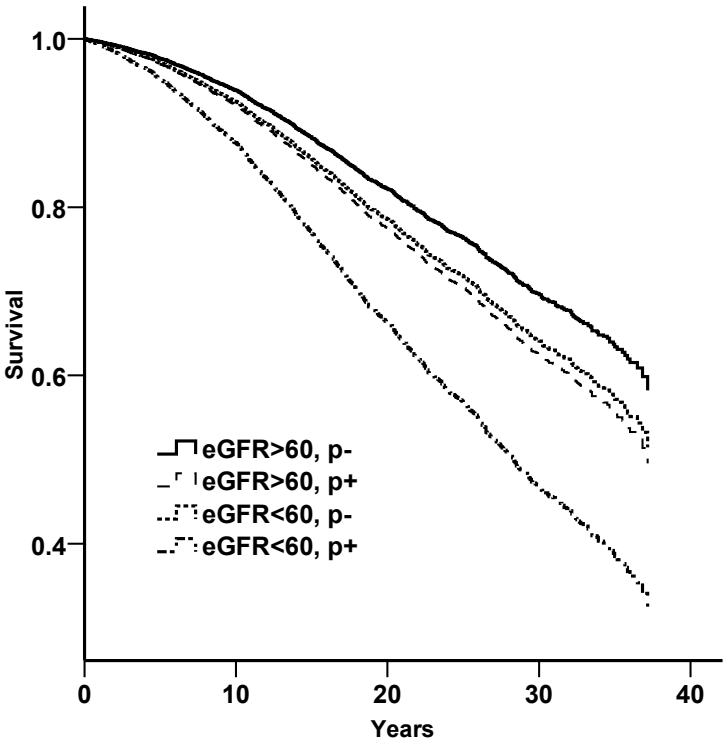


Figure 7-4 - Survival curve for cardiovascular mortality according to the presence of proteinuria and reduced eGFR



p- - no proteinuria detected on dipstick; p+ - proteinuria detected on dipstick

In order to combine urinary protein and eGFR as predictors of outcome, the cohort was divided into 4 groups using eGFR (greater or less than 60ml/min/1.73m^2) and presence or absence of proteinuria, and then entered into the model as a categorical variable, with age, sex, smoking, cholesterol, SBP, history of diabetes, history of vascular disease and the time quintiles as covariates. The group with eGFR>60ml/min/1.73m^2 and no detectable proteinuria was the reference group, and the results are shown in table 7-4.

Table 7-4 – Adjusted hazard ratios derived from a multivariate Cox regression model, using eGFR and proteinuria as combined categorical variables for all-cause and cardiovascular (CV) mortality. 95% confidence intervals shown in brackets

	All-cause mortality	CV mortality
eGFR≥60ml/min, p-	1 (Reference)	1 (Reference)
eGFR≥60ml/min, p+	1.24 (1.11 – 1.39)	1.29 (1.12 – 1.47)
eGFR<60ml/min, p-	1.18 (1.08 – 1.29)	1.25 (1.12 – 1.38)
eGFR<60ml/min, p+	1.88 (1.66 – 2.14)	2.07 (1.79 – 2.40)

p+ - detectable proteinuria; p- - no detectable proteinuria

7.4 Discussion

7.4.1 Findings of this study

In this study we show that eGFR and dipstick proteinuria measurements at first referral to a specialist hypertension clinic are strong independent predictors of long-term mortality, independently and in combination. The patients referred to the GBPC are those who are difficult to treat in primary care; they have been treated and followed up in primary practice before referral to the blood pressure clinic for treatment escalation, extensive investigation and specialist follow-up. In this setting, despite specialist follow-up, eGFR and dipstick proteinuria at presentation are powerful predictors of long-term outcomes.

There was a significant burden of kidney dysfunction in this study cohort; 23% had reduced excretory renal function (eGFR<60ml/min/1.73m^2) and 22% had proteinuria detectable on urinary dipstick. Over one third (38.2%) had at least one of these manifestations of kidney disease, but only 6% had both. This is considerably higher than prevalence estimates of the general population, such as NHANES 1999 – 2004, when 8% had an eGFR<60ml/min/1.73m^2, and 1.3% had frank proteinuria (24). However, it is in-keeping with the prevalence of a reduced eGFR in a recent Italian study -of a primary care hypertensive population; proteinuria data were not available in that cohort (264).

This finding highlights the importance of evaluating both eGFR and proteinuria in hypertensive patients as the tests identify two high risk groups and refines risk for the small number with both abnormalities. It is not necessarily surprising that these markers identify different sub-groups, as the primary underlying pathophysiological mechanisms may be different; loss of nephron mass secondary to glomerulosclerosis leading to reduced excretory capacity versus widespread endothelial dysfunction leading to proteinuria. For a minority with ongoing significant proteinuria, this will lead to renal interstitial fibrosis,

further glomerulosclerosis, progressive kidney disease and cardiovascular disease. Thus the highest risk group is those with both abnormal findings (reduced eGFR and proteinuria), as shown in this study. All cause and cardiovascular mortality increased with falling eGFR. This effect persisted after adjustment for other major cardiovascular risk factors including age, sex, blood pressure and smoking. The presence of urinary protein was also a strong predictor of mortality in this cohort. When reduced eGFR and proteinuria are combined in the survival model, the adjusted hazard ratio is significantly higher than for either parameter alone. The combination of a preserved eGFR but the presence of proteinuria was associated with a higher risk of all-cause and cardiovascular mortality than the converse, a reduced eGFR but no proteinuria, though the confidence intervals overlap.

7.4.2 Role of reduced eGFR and proteinuria in patients at high cardiovascular risk

There is extensive evidence supporting eGFR and proteinuria as predictors of cardiovascular disease and death in the general population, in the elderly, in high CV risk cohorts (1-4, 20-22). The CKD Prognosis consortium meta-analysis of these predictors in the general population showed that an eGFR<60ml/min/1.73m^2 and an ACR of ≥1.1.mg/mmol (or a trace or more of urinary protein on dipstick) are strong independent predictors of cardiovascular and all-cause mortality (20). These factors are more powerful predictors of risk when combined in the general population (198), and stratified by age (265). Various high risk groups have also been studied; for instance in the HOPE (Heart Outcomes and Prevention Evaluation) study of participants with a history of cardiovascular disease or diabetes mellitus and at least one cardiovascular risk factor, but serum creatinine <200μmol/L, both renal insufficiency and albuminuria were predictors of subsequent cardiovascular events (255, 256). This relationship was confirmed in the elderly by the Cardiovascular Health Study, which showed microalbuminuria and elevated cystatin C to be predictors of cardiovascular events and mortality (266). Most recently, a meta-analysis

of albuminuria and reduced GFR in high risk populations, identified by diabetes, cardiovascular disease or hypertension, has confirmed this strong relationship (257).

The combined role of eGFR and proteinuria in hypertensive cohorts is evident from a post-hoc analysis of the Nordic Diltiazem study which showed eGFR and ACR to be predictors of cardiovascular disease in the trial cohort of 10 881 participants, and a small Italian study of 837 hypertensive patients showed the combination of reduced eGFR and proteinuria (measured by ACR) to be a risk factor for cardiovascular events and all-cause mortality. We have recapitulated and extended this relationship, in our large unselected cohort with long follow-up and more than 4000 deaths including a wider range of kidney function and using a cheaper and easier means of detecting proteinuria. Our study has shown a reduction in excretory renal function (eGFR<60ml/min/1.73m^2), accompanied by dipstick proteinuria is associated with approximately doubling of all-cause and cardiovascular mortality. All the patients were managed according to the contemporaneous British Hypertension Society guidelines and show significant reduction in BP during follow-up at the clinic, but the increased risk, associated with markers of kidney disease, remained.

7.4.3 Limitations

The current study has several limitations. Firstly, it is retrospective. Urinary protein was measured by dipstick, which is reliant on the urine concentration and flow rate, though all samples were taken at the same time of day. We may have overestimated the prevalence in our cohort, by using single samples of blood and urine; however, even taking this into account, the prevalence is high. Generally prevalence estimates from single samples may overestimate because of intercurrent illness leading to acute renal dysfunction. As our cohort was attending a blood pressure clinic as asymptomatic out-patients, the impact is likely to be marginal. The inclusion period is long, but the data were collected consistently and recorded reliably and prospectively over the whole time period, and an era covariate

was included in the survival model to take account of secular trends. The use of renin-angiotensin system inhibitors was not included in the multi-variate analysis, since such drugs were not in use at the inception of the cohort, and while these reduce proteinuria, the residual level of proteinuria is strongly predictive of outcome, and this is still captured in this analysis. Furthermore, while renin angiotensin system blockers may slow the progression to renal disease, there is little evidence for specific reduction in cardiovascular outcomes or mortality in hypertension. An index of deprivation was not included in the multi-variate analysis as this was not available. The strength of the study lies in its representative nature: a large unselected population attending a hypertension clinic. The use of urinary dipstick to assess proteinuria does have some strengths; dipsticks are cheap, easy to use and allow an assessment of proteinuria at the point of care, which is particularly important in resource scarce healthcare environments.

7.4.4 Conclusion

In conclusion, in this large cohort of patients with severe and difficult to treat hypertension, simple measures of dipstick urinary protein and eGFR at presentation are powerful predictors of future risk of all-cause and cardiovascular mortality. These data illustrate the utility of the combined measurement of eGFR and proteinuria. They are essential to aid accurate risk stratification across a spectrum of patients with hypertension, in order to allow intensive treatment to be targeted at those at greatest risk, and should be included in the assessment of cardiovascular risk in primary care and specialist practice.

8 Chapter 8: Assessing the Impact of CKD in the community: the Triple A Kidney Project

8.1 Introduction

Chronic kidney disease (CKD) affects 6 - 13% of the population as outlined in detail in the introduction (250). The vast majority of people with CKD do not develop advanced kidney disease requiring dialysis. However it is thought to be important to recognise patients with early CKD because it is associated with an increased risk of vascular disease (179), and to minimise risk of progression to advanced kidney disease requiring renal replacement therapy. The mechanisms governing the excess burden of cardiovascular disease are poorly understood and it is not known if traditional cardiovascular risk factors are relevant in patients with CKD. It is also not clear whether cardiovascular risk assessment tools used in the general population should also be utilised in patients with CKD. As we have shown in earlier chapters, there is growing evidence that other non-traditional risk factors, such as persistent proteinuria are associated with cardiovascular disease.

In the UK, the majority of patients with CKD are looked after by their General Practitioner. This arrangement was formalised by the Quality and Outcomes Framework, as described in the introduction. However the majority of evidence available to guide the management of patients with CKD comes from studies of patients attending hospital clinics. Assuming that the patients in primary care can be treated in the same way may be unwise, as the patients under hospital follow-up are a selected population with more severe disease and a higher risk of developing complications.

In this study I recruited patients with CKD stage 3 in the community in Ayrshire and Arran ("Triple A"), performed a detailed baseline assessment, and plan to collect subsequent data and outcomes, including progression of kidney disease, development of vascular disease and death, with the aim of identifying predictors of renal and patient outcome.

8.2 Methods

8.2.1 Laboratory Assays

All samples (except parathyroid hormone and vitamin D) were analysed at the biochemistry and haematology laboratories of University Hospital Crosshouse, Kilmarnock Ayrshire. Serum creatinine was measured using Roche Modular reagent Jaffe method, with a mean between batch coefficient of variation of 2.3% at a serum creatinine concentration of 148μmol/L and 1.7% at 326μmol/L. The adjustment factors produced by the UK National External Quality Assessment Service were used for the creatinine assay, to produce IDMS-traceable serum creatinine values and then the eGFR was calculated using the MDRD formula and CKD-EPI formulae (47, 215). Serum albumin was measured using bromocresol green, with CV of 2% between batches. Sodium, potassium and chloride were measured using indirect ion selective electrodes (between batch CV <1%). Bicarbonate and urea were measured using coupled enzyme reactions. Parathyroid hormone and vitamin D were measured at Glasgow Royal Infirmary on an Abbott Architect analyser using automated immunoassays. Total protein was measured using a turbidimetric method with benzethonium precipitation, with a mean between batch CV of 3.9%. Urinary albumin was measured using an immunoturbidimetric method with a mean between batch CV of 4.3%. The urine creatinine was assayed by a reaction rate Jaffe method with a mean between batch CV is 3.6%.

8.2.2 Study Population

General Practices (GP) in Ayrshire, Scotland were invited to take part in the study, with a mix of urban and rural populations, and diverse geographical locations to reflect the overall catchment area. General practices in the United Kingdom are remunerated to maintain an electronic database of patients with chronic kidney disease stage 3 – 5 (known as the CKD register). In each participating practice a member of administrative staff interrogated the

database to identify potential participants with Stage 3 CKD who met the inclusion and exclusion criteria. All eligible patients within each practice were approached by letter inviting them to participate (see appendix for a sample invite letter). This was accompanied by a participant information sheet (see appendix). They were asked to contact their general practice to make an appointment to meet the study investigator. If they did not respond within two weeks a follow-up invite letter was sent. Thereafter no further contact was made.

8.2.3 Determination of Sample Size

This study was intended as a pilot study for a large cohort study. Pilot phase aimed for 500 patients (3:2 stage 3A: stage 3B), recruited over 12 months. The pilot phase would allow refinement of power calculations for the main study, as there is little current data available regarding the primary outcome measure. The population of NHS Ayrshire & Arran is 367,000. With an estimated prevalence of stage 3 CKD of 4.5% (low prevalence estimate), there are likely to be 16,500 patients with CKD stage 3. Based on other studies there is a 1:4 split of stage 3B:3A, equating to 3,300 stage 3B and 13,200 stage 3A. There are 54 general practices in Ayrshire. Thus we aim to recruit 2.3% of the stage 3A and 6.1% of the stage 3B patients. This was anticipated to be achievable from 6 - 10 practices, which required us to recruit 12.3% of their stage 3A and 32.7% of their stage 3B patients.

8.2.4 Inclusion Criteria

- Age 18 years or older
- Chronic Kidney Disease stage 3 as defined by the NKF-KDOQI classification (eGFR 30 – 59ml/min/1.73m^2 on 2 occasions at least 3 months apart) (patients are added to the primary care CKD Register if eGFR<60 ml/min/1.73m^2).

8.2.5 Exclusion Criteria

- Unable or unwilling to consent to take part in a study
- Unable to provide a spot urine sample
- Pregnant or lactating women
- Aged less than 18 years (the eGFR formulae are not validated in those under 18 years)
- Renal transplant recipient

8.2.6 Incorrectly enrolled participants

It was anticipated that a number of participants would have an eGFR> 60ml/min/1.73m^2 on the meat fasted study sample. However the primary basis of the selection criteria for the study was that the participant was included in the primary care CKD register, and was not re-assessed by the study investigators for the presence of CKD. It was hypothesised that patients may have been added to the register on the basis of a single serum creatinine measurement, or a sample after consuming cooked meat, or taken during an intercurrent illness reflecting a transient low eGFR that is not found on subsequent samples. These participants may represent a high risk group for developing subsequent CKD. Therefore the study was designed pragmatically to most accurately reflect current clinical practice, acknowledging the fact that a proportion of those on the CKD register will not fully fit the criteria. A decision was made, a priori, that participants with eGFR>60 ml/min/1.73m^2

would be retained in the study, in order to observe any renal progression. The participant's GP was informed of this.

8.2.7 Ethical Permission

Ethical approval was obtained from the West of Scotland Research Ethics Service and organisational approval was obtained from the Research and Development committee of NHS Ayrshire and Arran.

8.2.8 Recruitment

All participants were recruited and assessed by a single investigator (SM). They were assessed in the local primary care practice to minimise travel and maximise participation. Written informed consent was obtained (see appendix for consent form). Participants were installed on the Scottish Electronic Renal Patient Record (SERPR) to allow automated collection of all blood and urine results regardless of source (using the unique Scottish community health index number), and via an electronic link with the Scottish Renal Registry subsequent outcome data were collected (including date and cause of death, cardiovascular events etc). Participants gave written consent for remote follow-up for ten years.

8.2.9 Primary endpoint

- Incidence of cardiovascular disease

This is defined as:

- Myocardial infarction (Fatal or non-fatal ST elevation myocardial infarction and non ST elevation myocardial infarction).

  Myocardial infarction defined as hospital discharge diagnosis code or cause of death
- Need for coronary revascularisation
- Transient ischaemic attack
- Fatal or non-fatal ischaemic stroke
- Fatal or non-fatal haemorrhagic stroke
- Need for peripheral revascularisation
- Amputation for peripheral vascular disease
- Death due to peripheral vascular disease
- Sudden cardiac death

8.2.10 Secondary Endpoints

- All-cause mortality
- Commencement of renal replacement therapy
- Decline in renal function as measured by;

  Doubling of serum creatinine

  Decline in eGFR

8.2.11 Clinical assessment

Participants were requested to fast for 12 hours prior to the assessment. If they were unable to do this (e.g. diabetic, on medication) they were asked to refrain from eating meat for 12 hours to avoid the interference of cooked meat on the creatinine measurement (267).

8.2.12 Questionnaire

Participants completed a questionnaire regarding their medical history, current medication, lifestyle, family and occupational history (see appendix for a sample questionnaire). During the baseline assessment, this was checked by the researcher (SM) for completeness and cross-checked with the electronic GP records for medication and diagnoses (recorded on the GP electronic records using Read codes).

All participants completed the International Consultation on Incontinence Questionnaire urinary incontinence form (ICIQ-UI). The ICIQ-UI scores urinary incontinence across 3 domains (frequency, amount and impact on quality of life) and awards a score from zero (never) to 21 (frequent, large volume leakage with significant impact of lifestyle). In addition male participants completed the International Prostate Symptom Score (IPSS). The IPSS assesses lower urinary tract symptoms in men across seven domains, awarding a score from zero (no symptoms) to five (severe symptoms), with a total score of 8 – 19 reflecting moderate symptoms and ≥20 suggesting severe symptoms. These were completed independently by participants (see appendix for sample questionnaires).

Participants also completed a short quality of life questionnaire (the European Quality of Life- 5 Dimensions [EQ-5D] questionnaire) which assesses five domains of mobility, self care, usual activities, pain and anxiety and depression, with a choice of three possible answers; no problems, some problems or unable to perform for the former three domains

and no symptoms, moderate or severe symptoms for the latter two domains (see appendix). This generates a score such as 11121, where there are no problems in four of five domains, except the penultimate. The developers of the EQ-5D questionnaire have then created country specific indexes where 0.0 is death, and 1.0 is full health, to allow comparisons between groups and internationally (a negative score is possible where the quality of life is considered to be worse than death). A global assessment using a visual analogue scale is also included (see appendix for a sample questionnaire). The EQ-5D index was calculated for the cohort (268).

Permission was obtained for the use of the IPSS, ICIQ and EQ5D questionnaires.

8.2.13 Scottish Index of Multiple Deprivation

The Scottish Index of Multiple Deprivation (SIMD) score was recorded. Scotland is divided into 6,505 small areas, known as datazones, based on postcode, and a rank is assigned for each datazone from 1 to 6,505 (from most to least deprived), by assessing deprivation across seven domains – income, employment, health, education, access to services, crime and housing. The overall index is a weighed sum of the seven domain scores and is designed to give a relative measure of deprivation. It is obtained from the Scottish Government website, by entering the postcode into the appropriate spreadsheet for each local authority (269). The commonly applied cut-point to compare groups is the most deprived 15%.

8.2.14 Physical Assessment

The physical assessment comprised of the following:

- anthropometric measurements:
- height (measured barefoot, using wall mounted measure)
- weight, (measured barefoot, outdoor clothing removed, pockets emptied using calibrated scales)
- body mass index, (weight (kg)/ height $(m)^2$)
- waist to hip ratio, calculated as follows:
  - *waist girth*: taken at the narrowest waist level. If this is not apparent, at the midpoint between the lowest rib and the top of the hip bone (iliac crest).
  - *hip girth*: taken over minimal clothing and empty pockets at the widest point of the hips and greatest protrusion of the gluteal muscles. Participant stands straight with weight evenly distributed on both legs and feet.
- blood pressure, measured as follows:
  - 3 readings, using a portable automated machine (A&D Medical UA-767 Plus 30) measured in the seated position after 5 minutes rest
- electrocardiogram (portable device)

8.2.15 Biochemical Assessment

A blood sample for:

- urea and electrolytes
    - sodium, potassium, chloride, bicarbonate, urea and creatinine
- bone profile
    - calcium, phosphate, albumin, alkaline phosphatase, total protein, adjusted calcium calculated = Ca + 0.02(40 – Alb))
- full blood count
    - haemoglobin, platelets, total white cell count and differential count
- lipids
    - total cholesterol, high density lipoprotein, triglycerides, and low density lipoprotein calculated using Friedewald equation ;
    - LDL=Total Chol – HDL – (Trig/2.2)
- glucose
- urate
- Parathyroid hormone
- 25-hydroxy vitamin D
- serum, plasma and whole blood (for DNA analysis) for storage

serum and plasma were centrifuged at 3500rpm for 5 minutes and supernatant frozen at -80°C, whole blood was frozen unchanged at -80°C

Participants provided 5-10mL first morning urine sample for:

- dipstick urinalysis (Siemens 10 SG multistix)
- laboratory quantification of:
  - total protein
  - albumin
  - creatinine
  - sodium
  - aliquot for storage at -80°C (not centrifuged)

A small sub-set of participants performed a 24-hour urine collection, in addition to the spot sample, (if the urine dipstick ≥1+ for protein) for:

- total protein
- albumin
- creatinine
- sodium
- aliquot for storage

8.2.16 Statistical analysis

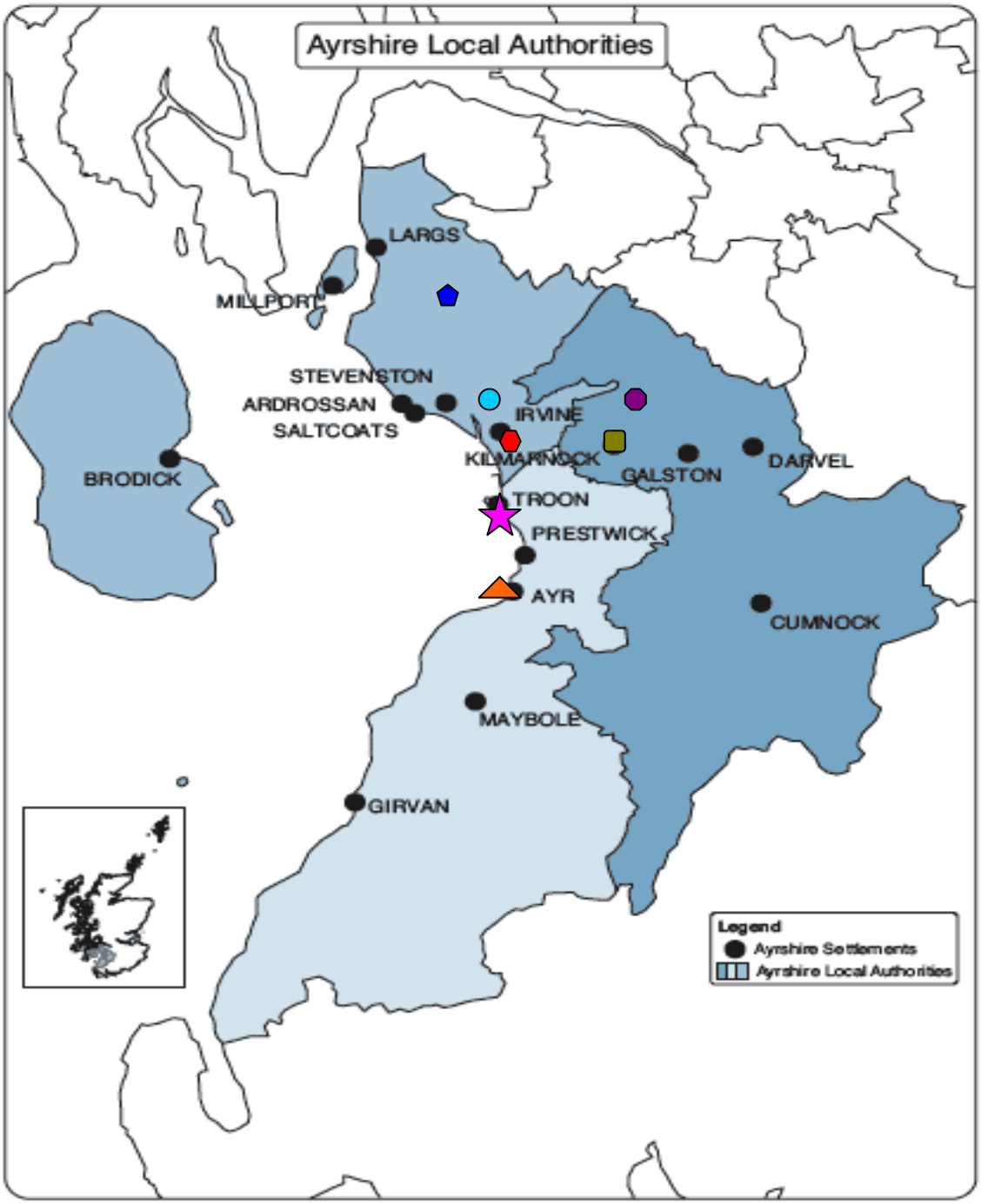
Data were downloaded and patient identifiers removed prior to further analysis. Analysis was performed using SPSS version 18.0 for Windows (IBM Inc, http://www-01.ibm.com/software/analytics/spss/). All data were assessed for normality, and appropriate summary statistics are presented. Significance testing was performed was student's T test, Mann-Whitney U test, chi squared test and analysis of variance as appropriate.

8.3 Results

8.3.1 Participating General Practices

Participants were recruited from seven general practices around Ayrshire as shown in Figure 8-1. The number of participants, grouped according to GP and response rate are shown in table 8-1.

Figure 8-1 - Map of Ayrshire with Recruitment Locations



Legend

- Beith Health Centre, Beith
- Stewarton Health Centre, Stewarton
- Kilwinning Medical Practice, Kilwinning
- Townhead Surgery, Irvine
- Old Irvine Road Surgery, Kilmarnock
- Portland Surgery, Troon
- Racecourse Road Medical Group, Ayr

Table 8-1 - Breakdown of Participants by General Practice

	Number of participants	Proportion of Total Cohort (%)	Response Rate (% of invites sent)
Beith	29	7.1	20
Stewarton	48	11.7	20
Kilwinning	107	26.0	13
Irvine	125	30.4	11
Kilmarnock	34	8.3	17
Troon	36	8.8	29
Ayr	32	7.8	7
Total	411	100.0	13

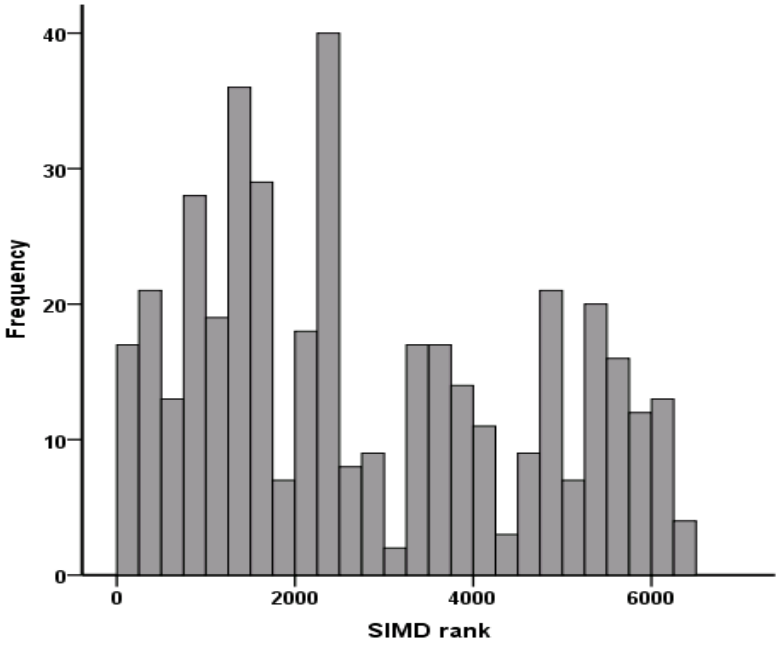
8.3.2 Baseline demographics

The demographic information of the total cohort, and divided according to renal function is shown in Table 8-2. The cohort includes 17% from the most deprived 15% of datazones in the Scottish Index of Multiple Deprivation, which is a reflection of the overall deprivation of the population of Ayrshire and Arran. This is demonstrated in the histogram of SIMD rank of the cohort (Figure 8-2) and the corresponding histograms of East Ayrshire and South Ayrshire Council areas, for comparison (Figure 8-3 and 8-4).

Table 8-2 – Demographic information of the cohort, divided according to renal function.

	Total Cohort (n=411)	eGFR>60 ml/min/1.73m^2 (n=109)	CKD Stage 3A (n=187)	CKD Stage 3B (n=94)	Sig
Age (yrs; ± SD) [range]	70.6 ±9.6 [30 – 90]	69.0 ± 10.3 [31 – 90]	70.5 ± 8.7 [42 – 89]	73.2 ± 9.6 [30 – 90]	0.014
Female (%)	59	64	54	66	0.079
Ethnicity White (%)	99.5	99.1	100	100	0.855
Mixed (%)	0.5	0.9	0	0	-
Serum creatinine (µmol/L; IQR)	107 (89 – 123)	83 (73 – 97)	107 (94 – 118)	128 (116 – 148)	<0.005
eGFR (MDRD) (ml/min/1.73m^2; IQR)	54 (44 – 61)	65 (62 – 70)	54 (49 – 57)	40 (36 – 43)	<0.005
SIMD rank (median; IQR)	2315 (1273 – 4324)	2351 (926 – 3775)	2754 (962 – 4546)	1954 (769 – 3138)	0.023
Lowest 15% SIMD rank (%)	17.0	17.4	17.0	16.7	0.999

Figure 8-2 - Histogram of Scottish Index of Multiple Deprivation (SIMD) rank of t-he cohort



Rank 1 represents most deprived

Figure 8-3 - SIMD deciles for the general population of East Ayrshire

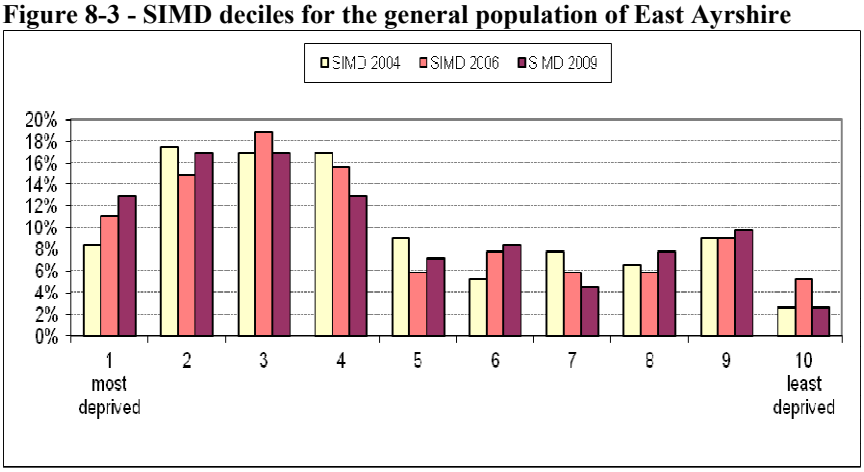
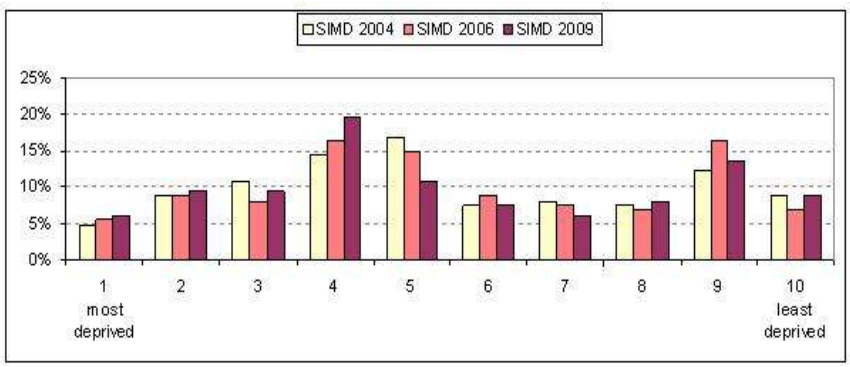


Figure 8-4 - SIMD deciles for the general population of South Ayrshire



8.3.3 Baseline renal function

Participants were recruited on the basis that they were included in their general practice's CKD register. The study samples were taken after a 12-hour meat free period. The estimated GFR and corresponding CKD stage of the cohort are shown in table 8-3 and figure 8-5, according to the MDRD formula (currently used in UK biochemistry laboratories,) and the CKD-EPI equation using IDMS traceable values of serum creatinine. The equations identify different numbers of patients as having CKD, especially Stage 3A CKD, and a proportion as having no kidney disease. Figure 8-6 shows the relationship between the eGFR derived from the formulae.

Table 8-3 – Prevalence of CKD Stages, according to the MDRD and CKD-EPI equations for estimating glomerular filtration rate

	MDRD Formula	CKD-EPI Formulae
Median eGFR (ml/min/1.73m^2; IQR)	54 (44 – 61)	55 (44 – 63)
No kidney disease (number [%])	83 (20.2)	104 (25.3)
CKD Stage 1/ 2 (number [%])	26 (6.3)	35 (8.5)
CKD Stage 3A (number [%])	194 (47.2)	164 (39.9)
CKD Stage 3B (number [%])	96 (23.4)	93 (22.6)
CKD Stage 4 (number [%])	12 (2.9)	15 (3.6)
CKD Stage 5 (number [%])	0 (0.0)	0 (0.0)

CKD Stage 1/2: defined as eGFR>60ml/min/1.73m^2 plus evidence of kidney damage such as TPCR>15mg/mmol or ACR>3mg/mmol.

Figure 8-5 - Prevalence of CKD Stages in the cohort according to the MDRD and CKD-EPI formulae

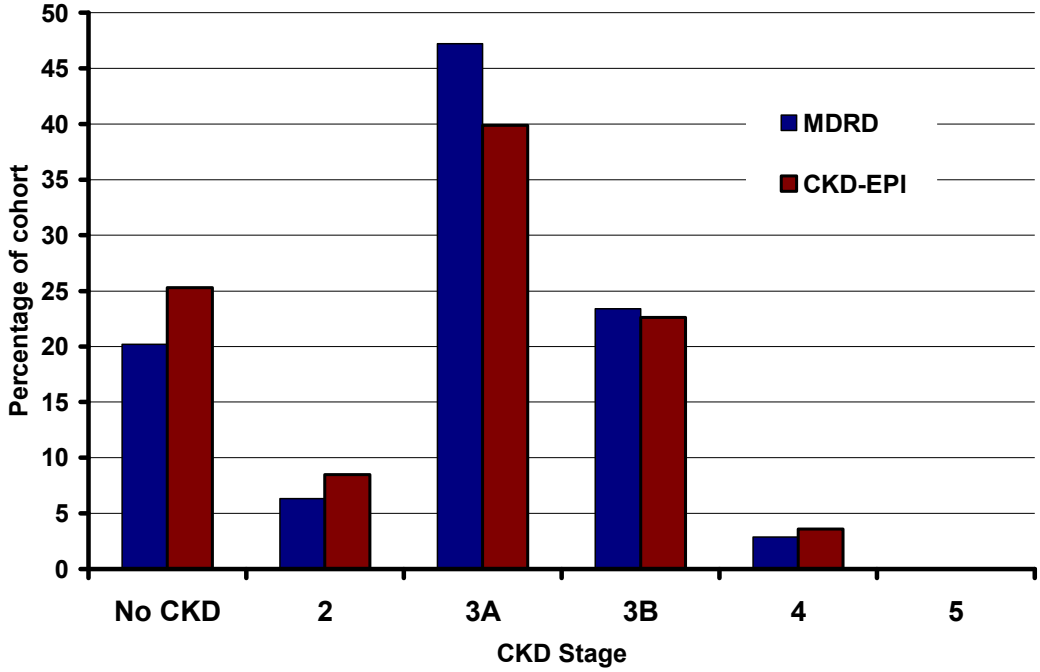
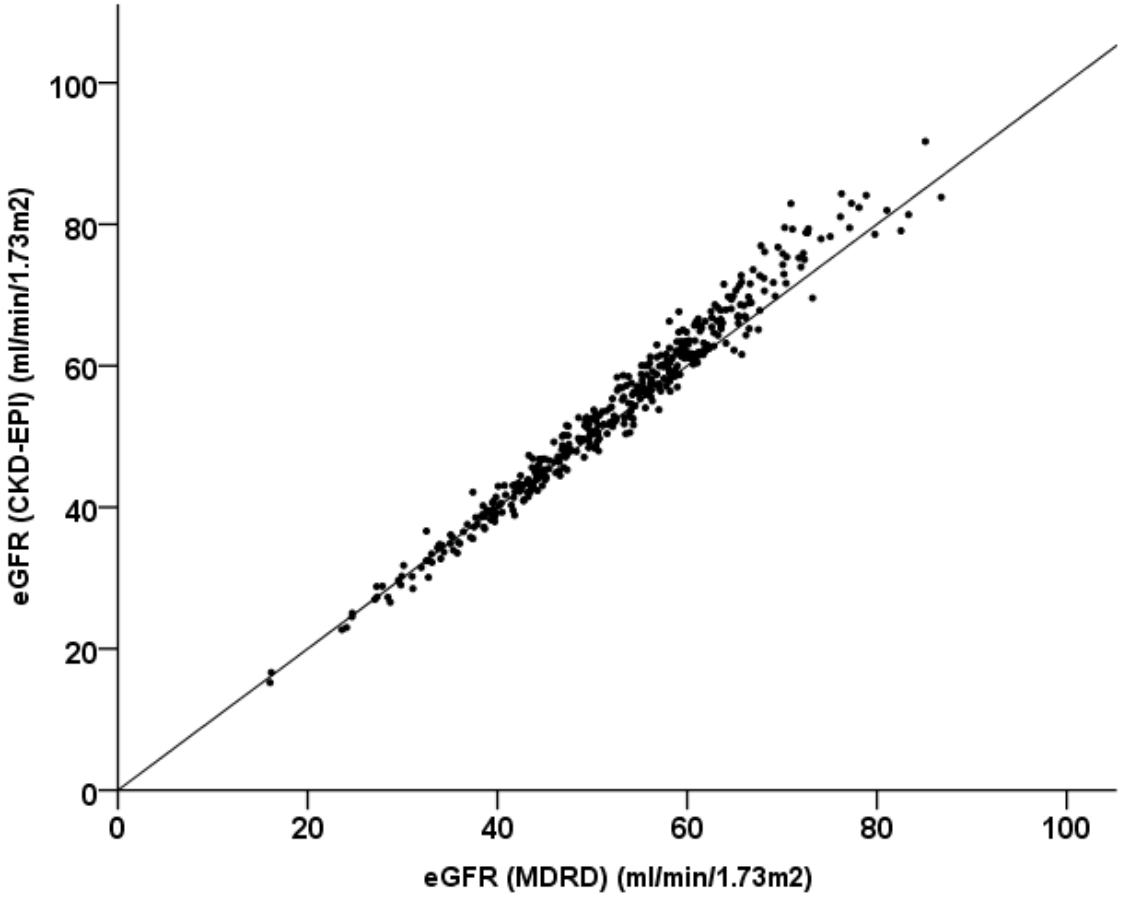


Figure 8-6 - Scatterplot of eGFR according to the MDRD and CKD-EPI formulae demonstrating the changing level of agreement between the results according to level of kidney function



8.3.4 Measurements of renal function prior to the study

Over a quarter (26.5%) of the cohort have an eGFR >60ml/min/1.73m^2 (according to the MDRD formula) following a 12-hour meat free period. In order to investigate the impact of the participants being meat free at the time of sampling on the prevalence of CKD in the cohort, prior measurements of creatinine and eGFR (performed during routine clinical care and measured in the same laboratory in the preceding year) were obtained for the group of participants found to have an eGFR>60ml/min/1.73m^2 (MDRD formula) at the baseline study measurement.

63 (58%) of those had an eGFR measurement of <60ml/min/1.73m^2 in the year preceding the baseline study visit, and 22 of those had 2 recorded measurements of eGFR <60ml/min/1.73m^2, greater then 90 days apart.

8.3.5 Characterisation of renal disease

The cohort have predominantly early renal disease, receiving care from their general practice multidisciplinary team, with less than 10% under current hospital review and less than 5% having undergone a diagnostic percutaneous renal biopsy (table 8-4).

Table 8-4 - Characterisation of renal disease in the cohort

	Total Cohort (n=411)	eGFR>60ml/min/1.73m^2 (n=109)	CKD Stage 3A (n=187)	CKD Stage 3B (n=94)
Nephrology clinic follow-up				
Never (number [%])	341 (83)	101 (92.7)	163 (84)	75 (78.1)
Current (number [%])	32 (7.8)	3 (2.8)	8 (4.1)	12 (12.5)
Discharged (number [%])	38 (9.2)	5 (4.6)	23 (11.9)	9 (9.4)
Renal biopsy performed (number [%])	11 (2.7)	2 (1.8)	4 (2.1)	4 (4.2)
Primary Renal Disease (%)				
Primary glomerulonephritis	2.7	2.8	2.6	2.1
Interstitial disease	8.5	5.5	8.2	12.5
Multisystem diseases	3.6	1.8	4.1	4.2
Diabetic nephropathy	1.0	0.0	0.0	0.0
Other	0.2	0.0	0.5	0.0
CKD; aetiology unknown	83.9	89.9	84.5	81.3
Renal tract malignancy (inc prostate) (%)	4.4	0.9	5.7	6.3
IPSS (score; IQR)	6 (2 – 10)	5.5 (2 – 12.5)	6 (3 – 10)	3.5 (2 – 8)
Moderate to severe LUTS (IPSS ≥8) (%)	38.3	33.3	43.3	23.3
Gynaecological malignancy (% of females)	2.9	3.6	2.9	3.2
ICIQ-UI >10 (%)	8.6	13.3	7.3	8.4
Self-reported recurrent UTI (%)	11.4	10.1	11.9	12.5
Family history of renal disease (%)	10.5	9.2	4.9	2.9
Occupational exposure to nephrotoxins (%)	8.5	5.5	11.3	7.3

Primary renal disease; reported as recorded in the GP records

Occupational exposure to nephrotoxins includes; lead, cadmium, mercury, silica, beryllium, uranium, chromium, ethylene glycol

8.3.6 Urinary abnormalities at baseline

The prevalence of dipstick proteinuria (≥1+) in the cohort was low (8.1%) and this was reflected in the median TPCR of 10mg/mmol and ACR of 0.9mg/mmol. The prevalence of proteinuria was significantly higher in participants with diabetes ($p<0.001$) (shown in table 8-5).

Overall urinary sodium excretion was high with a median of 85mmol/L in the cohort, with differences noted between the diabetic and non-diabetic subgroups. Diuretic usage was not significantly different between the groups ($p=0.113$), but urine sodium was significantly higher in the diuretic users ($p<0.001$) as expected. To correct for urine flow rate sodium: creatinine ratios were calculated and these were significantly higher in the non-diabetic group.

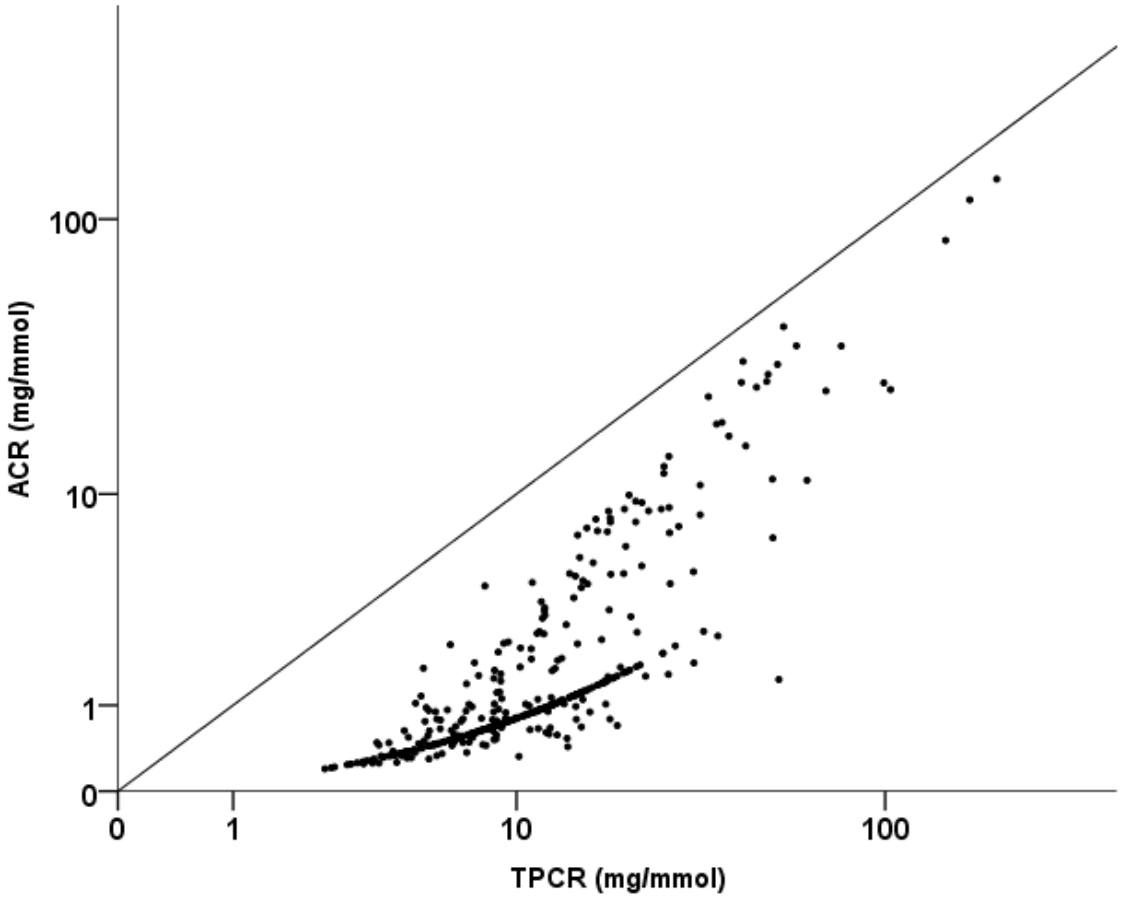
Table 8-5 - Urinary abnormalities of the cohort divided according to diabetic status

	Total cohort (n=407 with dipstick)	Non-diabetics (n=324)	Diabetics (n=83)	Significance
Dipstick proteinuria Negative (number [%])	275 (66.9)	230 (71.0)	45 (54.2)	0.03
Trace (number [%])	99 (24.1)	73 (22.5)	26 (31.3)	
≥1+ (number [%])	33 (8.1)	21 (6.5)	12 (14.4)	
Median TPCR (mg/mmol; IQR)	10 (7-15)	9 (7 – 15)	12 (7 – 18)	0.03
TPCR 15 - 50mg/mmol (number [%])	91 (22.4)	68 (21.0)	23 (28.0)	0.171
TPCR> 50mg/mmol (number [%])	15 (3.7)	8 (2.5)	7 (8.5)	0.009
Median ACR (mg/mmol; IQR)	0.9 (0.5-1.7)	0.9 (0.6 – 1.5)	1.0 (0.6 – 5.3)	0.015
Microalbuminuria (number [%])	62 (15.1)	45 (13.8)	17 (20.5)	0.127
Macroalbuminuria (number [%])	9 (2.2)	3 (0.9)	6 (7.2)	<0.001
Dipstick non-visible haematuria ≥ 1+ (number [%])	28 (6.9)	22 (6.8)	6 (7.2)	0.524
Urine sodium (mmol/L; IQR)	85 (59 – 113)	87 (59 – 115)	76 (54 – 106)	0.038
Urine sodium/ creatinine ratio (mmol/mmol; IQR)	13 (8 – 18)	13 (8 – 18)	11 (7 – 18)	0.015

8.3.7 Relationship between TPCR and ACR in the cohort

In earlier chapters the relationship between TPCR and ACR was explored in a cohort of patients attending a hospital renal clinic, with a high prevalence of proteinuria. In figure 8-7, the relationship is shown in this low prevalence community cohort.

Figure 8-7 - Scatterplot of the relationship between TPCR and ACR in the cohort



Of those with significant proteinuria (TPCR>50mg/mmol, n=15) seven participants had discordant levels of TPCR and ACR (i.e. high levels of non-albumin proteinuria), using the thresholds of TPCR 50mg/mmol and ACR 30mg/mmol (as described in previous chapters). One participant had a TPCR>100mg/mmol, with ACR <70mg/mmol. One participant had a high ACR (>30mg/mmol) and a low TPCR (<50mg/mmol). The characteristics of those with significant proteinuria are shown in table 8-6, divided according to the level of non-albumin proteinuria. No one in the group had a diagnosis of multiple myeloma.

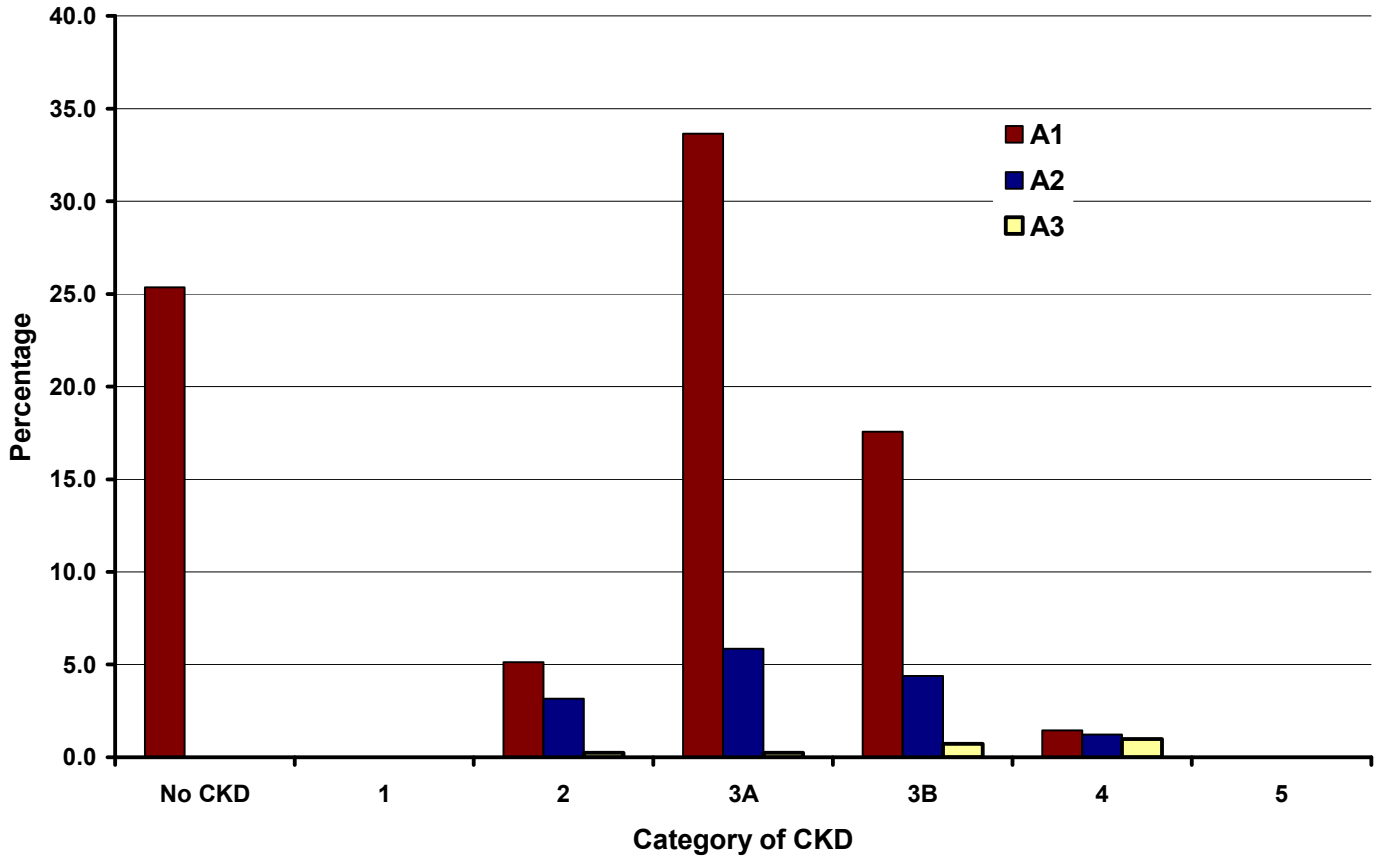
Table 8-6 - Characteristics of those with significant proteinuria (TPCR>50mg/mmol) divided according to level of non-albumin proteinuria (NAP)

	High NAP ACR <30, PCR >50	Low NAP ACR>30 PCR>50	Significance
N	7	8	-
Age (year; ± SD s)	76.0 (± 4.6)	67.0 (±8.8)	0.031
Female (%)	86	38	0.166
On RAASi (%)	43	87	0.336
SBP (mmHg;± SD)	135 (±39)	146 (± 20)	0.504
DBP(mmHg± SD)	72 (±14)	75 (± 13)	0.673
sCr (micromols/l; IQR)	100 (75 – 142)	171 (117 - 305)	0.002
eGFR (ml/min/1.73m^2; IQR)	50 (36 – 68)	31 (18 43)	0.002
CKD; aetiology unknown (%)	86	37	<0.001
ACR (mg/mmol; IQR)	11 (7 – 24)	63 (35 – 133)	<0.001
TPCR(mg/mmol; IQR)	62 (50 – 99)	110 (55 – 189)	<0.001

8.3.8 Combining proteinuria and eGFR in the classification of CKD

It has been proposed to add proteinuria to the eGFR stage in the classification of CKD (11). The modified classification describes the cause, eGFR, and albuminuria using ACR; called the CGA classification. In order to investigate the impact of applying this classification to this cohort they were divided according to the level of CKD-EPI eGFR (using Stages 1 – 5, including 3A and 3B) and ACR (using 3 categories, ACR <3mg/mmol; A1, ACR 3-30mg/mmol; A2 and ACR>30mg/mmol; A3). Then prevalence of CKD using this approach is shown in figure 8-8.

Figure 8-8 - Bar chart of the prevalence of CKD using the CKD-EPI eGFR equations and ACR divided into three categories (A1; ACR<3mg/mmol, A2; ACR 3 - 30mg/mmol and A3; ACR>30mg/mmol)



8.3.9 Burden of cardiovascular disease

Three quarters of the cohort have been given a diagnosis of hypertension (self-reported or according to GP records), but blood pressure is well controlled and only 7.4% of electrocardiograms met the criteria (by voltage) for left ventricular hypertrophy (270). In excess of 40% are known to suffer from vascular disease, and 20% are diabetic. Overall there is a large burden of cardiovascular disease in this cohort, as shown in detail in Table 8-7 and 8-8.

Participants were receiving a median of two anti-hypertensive agents, and the majority were receiving an ACE inhibitor or angiotensin receptor blocker, (figure 8-9). The prevalent use of renin angiotensin system blockade rose with severity of CKD.

Table 8-7 - Cardiovascular parameters of the cohort divided according to severity of renal disease

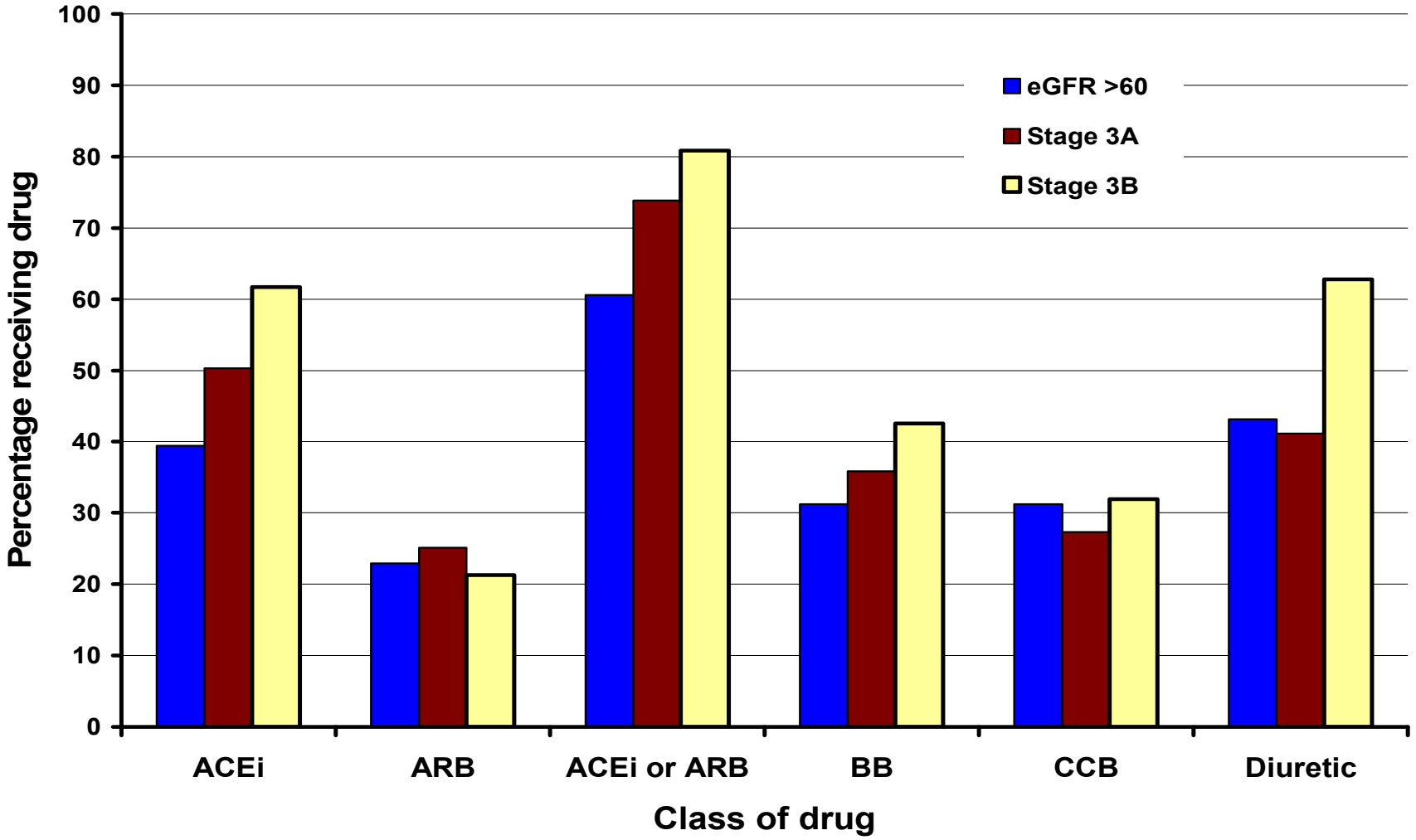
	Total cohort (n=411)	eGFR>60ml/min/1.73m^2 (n=109)	CKD Stage 3A (n=187)	CKD Stage 3B (n=94)	Significance
Mean systolic BP (mmHg; ± SD)	141 (±22)	143(±23)	142(±21)	138(±23)	0.236
Mean diastolic BP (mmHg; ± SD)	79 (±12)	82(±11)	79(±12)	75(±12)	<0.001
Lowest systolic BP (mmHg; ± SD)	135(±22)	138(±22)	136(±21)	132(±21)	0.200
Lowest diastolic BP (mmHg; ± SD)	76(±12)	79(±11)	76(±12)	72(±12)	<0.001
Self-reported or GP diagnosed hypertension (%)	75.0	70.6	77.5	78.7	0.150
Antihypertensive drugs (number)	2 (1 – 3)	2 (1 – 3)	2 (1 – 2)	2 (1.25 – 3)	0.002
ECG findings: Normal	56.4	61.7	60.9	41.1	0.008
Voltage criteria LVH	7.4	4.7	6.8	11.7	0.286
Ischaemia	20.3	15.9	18.5	28.3	0.143
Arrythmia	8.6	5.6	9.8	11.6	0.659
Any vascular disease (%)	42.6	37.6	43.3	45.8	0.609
Ischaemic Heart Disease (%)	31.1	28.4	29.4	38.5	0.472
Cerebrovascular disease (%)	12.4	10.1	14.4	11.7	0.906
Peripheral Vascular Disease (%)	12.2	11.9	15.0	8.5	0.577
Family History of vascular disease (%)	51.8	55.0	52.6	47.9	0.664

Arrhythmia; paced rhythm or arrhythmia seen on ECG

Table 8-8 – Cardiovascular parameters of the cohort (2)

	Total cohort (n=411)	eGFR>60ml/min/1.73m^2 (n=109)	CKD Stage 3A (n=187)	CKD Stage 3B (n=94)	Significance
Type 1 diabetes (%)	0.24	0	0	0	-
Type 2 diabetes (%)	20.0	18.3	18.2	25.5	0.359
Diet controlled (% of DM)	30.5	35.0	11.8	16.7	-
OHA (% of DM)	48.8	55.0	70.6	58.3	-
Insulin requiring (% of DM)	20.7	10.0	17.6	25.0	-
Retinopathy/ neuropathy (%)	8.5	8.3	4.8	13.8	0.004
Impaired glucose tolerance (%)	3.2	2.7	3.7	3.2	0.648
Cholesterol (mmol/L; ± SD)	4.9 (± 1.2)	5.0 (±1.1)	4.8 (±1.2)	4.9 (±1.3)	0.298
HDL (mmol/L; ± SD)	1.5 (±0.5)	1.5(±0.4)	1.4 (±0.5)	1.4(±0.5)	0.110
LDL (mmol/L; ± SD)	2.7 (±1.0)	2.8(±0.9)	2.7(±1.0)	2.7(±1.1)	0.382
Trigs (mmol/L; ± SD)	1.7(±1.0)	1.6(±1.0)	1.6(±0.8)	1.9(±1.1)	0.060
TC:HDL ratio	3.6 (±1.2)	3.5 (±1.1)	3.7 (±1.1)	3.7 (±1.1)	0.240
Statin usage (%)	61.3	57.8	62.4	60.4	0.370
Ezetimibe usage (%)	1.2	1.2	1.0	2.0	0.873
Uric acid (mmol/L; mean ± SD)	0.38 (± 0.10)	0.34 (± 0.08)	0.38(± 0.10)	0.43(± 0.10)	<0.001
Previous clinical episode of gout (%)	9.5	2.8	10.8	13.5	0.033

Figure 8-9 - Use of antihypertensive drugs according to severity of CKD



8.3.10 Complications of renal disease

The prevalence of biochemical complications of renal disease, such as hyperkalaemia and metabolic acidosis, are low (table 8-9). However the prevalence of abnormalities of vitamin D metabolism is high in the cohort, independent of renal function, with only a quarter (25.8%) of the cohort having adequate levels of 25-hydroxy-vitamin D (table 8-10). Mild hyperparathyroidism is also common, independent of renal function, but more severe hyperparathyroidism was related to severity of renal disease ($p<0.001$) and mean PTH levels were higher in the participants with CKD Stage 3B (10.9pmol/L) versus 7.7pmol/L in CKD Stage 3A, ($p<0.001$).

Table 8-9 - Complications of renal disease, divided according to severity of renal disease

	Total cohort (n=411)	eGFR>60ml/min/1.73m^2 (n=109)	CKD Stage 3A (n=187)	CKD Stage 3B (n=94)	Significance
Potassium (mmol/L; ± SD)	4.2 (±0.5)	4.0 (± 0.4)	4.2 (± 0.4)	4.3(±0.5)	0.025
Hyperkalaemia (K>5.0mmol/L) (%)	4.7	0.0	2.7	7.4	<0.001
Serum bicarbonate (mmol/L; ± SD)	26 (±3)	26(±3)	26(±3)	25(±3)	0.096
Metabolic acidosis (serum bicarb <22mmol/L) (%)	5.4	0.9	4.6	9.4	0.002
Haemoglobin (g/dL; ± SD)	13.6 (±1.5)	13.8(±1.5)	13.7(±1.5)	13.1(±1.5)	0.002
Anaemia (Hb< 11g/dL) (%)	4.9	2.8	4.6	5.3	0.02
Albumin (g/L; ± SD)	45 (±3)	45(±3)	45(±3)	45(±3)	0.321
Hypoalbuminaemia (serum albumin< 35g/L) (%)	0	-	-	-	-

Table 8-10 – Features of CKD-MBD, divided according to severity of renal disease

	Total cohort (n=411)	eGFR>60ml/min/1.73m^2 (n=109)	CKD Stage 3A (n=187)	CKD Stage 3B (n=94)	Significance
Adjusted calcium (mmol/L; ± SD)	2.2 (± 0.1)	2.3(±0.1)	2.2(±0.1)	2.3(± 0.1)	0.043
Hypocalcaemia (Adj Ca <2.1mmol/L) (%)	3.9	0.9	5.2	5.2	0.353
Phosphate (mmol/L; ± SD)	0.9 (± 0.2)	0.9(±0.2)	0.9(±0.2)	1.0(±0.2)	0.055
Hyperphosphataemia (PO_4 >1.3mmol/L) (%)	3.2	3.7	2.1	4.3	0.002
Alkaline Phosphatase (µg/L; ± SD)	78 (±29)	75(±23)	77(±29)	80(±37)	0.461
PTH (pmol/L; ± SD)	8.7 (± 4.9)	7.8(±4.8)	7.7(±3.8)	10.9(±5.5)	<0.001
Hyperparathyroidism					
> ULN (7.6 – 15.0pmol/L)	35.7	41.7	34.6	42.9	<0.332
>2X ULN (15.1 – 22.5pmol/L)	10.3	5.9	5.9	22.0	<0.001
>3X ULN (>22.5pmol/L)	1.2	1.0	0.0	2.2	<0.001
25-OH Vitamin D (nmol/L; ± SD)	38 (± 22)	37(±23)	40(±21)	36(±21)	0.193
Insufficiency (25-49nmol/L(%)	40.3	36.5	42.7	39.8	0.795
Deficiency (14 – 24nmol/L) (%)	20.2	21.9	17.4	22.0	0.579
Undetectable (<14nmol/L) (%)	13.7	15.6	11.2	14.8	0.315

8.3.11 Factors related to lifestyle

The cohort has a high prevalence of obesity with 45% having a body mass index >30kg/m^2. There was no correlation between eGFR and BMI nor waist: hip ratio in this cohort. However less than 10% of the cohort are current smokers, with 44% reporting a past history of tobacco use. Alcohol consumption is notably low (table 8-11).

Table 8-11 - Factors related to lifestyle in the total cohort and divided according to gender

	Total Cohort (n=411)	Males (n=169)	Females (n=242)
Weight (kg) (±SD)	81 (±18)	88 (±17)	76 (±16)
Height (cm) (±SD)	164 (±11)	171 (±11)	158 (±7)
BMI (kg/m^2) (±SD)	30 (±6)	30 (±5)	30 (±6)
BMI 25.1 – 30kg/m^2 (overweight) (%)	36	43	31
BMI > 30kg/m^2 (obese) (%)	45	40	48
Waist (cm) (±SD)	96 (±14)	102 (±12)	92 (±13)
Hips (cm) (±SD)	106 (±11)	103 (±9)	107 (±11)
Waist :Hip ratio	0.9 (±0.1)	1.0 (±0.1)	0.8 (±0.1)
Central fat distribution (%)	82	88	77
Waist: Height ratio	0.59 (±0.08)	0.59 (±0.007)	0.58 (±0.08)
Current smoker (%)	9.5	7.1	11.1
Pack years	25 (11 – 45)	15 (5 – 50)	25 (19 – 41)
Ex-smoker (%)	44.0	55.0	36.4
Pack years	22 (10 – 36)	25 (10 – 40)	20 (5 – 31)
Non-smoker (%)	46.5	37.9	52.5
Alcohol (units/week)	1 (0 – 8)	4 (0 – 18)	0.5 (0 - 4)

Central fat distribution defined as waist: hip ratio >0.8 in females or >0.9 in males.

8.3.12 Co morbidity and Quality of Life

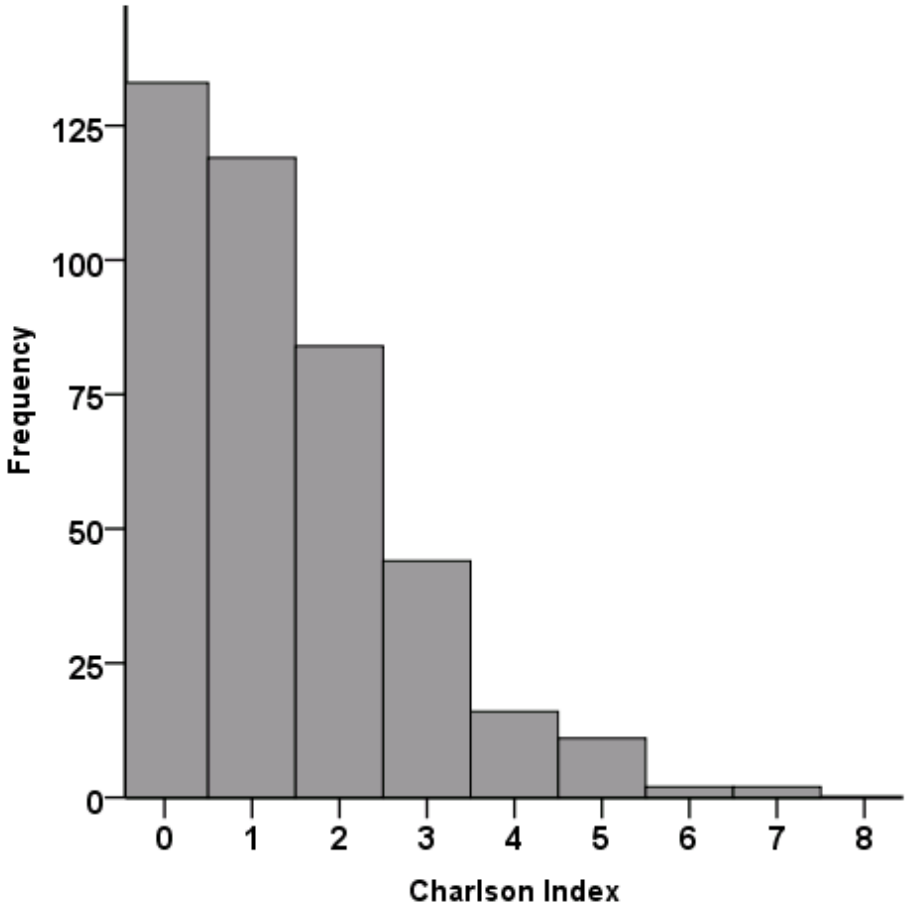
The burden of co morbidity in the cohort was significant, as measured by the Charlson index, shown in table 8-12 and figure 8-10. Quality of life was measured using the EQ-5D questionnaire and the findings for each domain are shown in table 8-12. Median score was 7 (minimum possible is 5 and maximum 15) and the median EQ-5D index for the whole cohort was 0.81 (0.76 – 0.85). A visual analogue scale (VAS) was used for participants' global assessment of wellbeing and the median value was 75% for the whole cohort.

Table 8-12 – Co morbidity, as measured by the Charlson Score and Quality of Life measurements for the cohort, and divided according to severity of renal disease

	Total cohort (n=411)	eGFR>60 ml/min/1.73 m^2 (n=109)	CKD Stage 3A (n=187)	CKD Stage 3B (n=94)	P value
Charlson Index (median; IQR)	1 (0 – 2)	1 (0 – 2)	1 (0 – 2)	1 (0 – 2)	0.063
Charlson Index >2 (%)	18.0	18.3	16.6	20.2	-
EQ-5D scores (5)					
Mobility (%)					
Level 1	51.6	51.4	57.2	38.5	
Level 2	48.4	48.6	42.8	61.5	0.018
Level 3	0.0	0.0	0.0	0.0	
Self care (%)					
Level 1	86.4	85.3	87.6	84.4	
Level 2	13.6	14.7	12.4	15.6	0.808
Level 3	0.0	0.0	0.0	0.0	
Usual activity (%)					
Level 1	63.0	65.1	64.4	57.3	
Level 2	33.8	30.3	34.0	37.5	
Level 3	3.2	4.6	1.5	5.2	0.493
Pain (%)					
Level 1	35.3	31.2	38.7	30.2	
Level 2	56.0	58.7	54.1	58.3	
Level 3	8.8	10.1	7.2	11.5	0.316
Anxiety & Depression (%)					
Level 1	71.8	73.4	73.2	65.6	
Level 2	26.5	24.8	25.8	32.3	
Level 3	1.7	1.8	1.0	2.1	0.286
EQ-5D Total (median; IQR)	7 (6 – 8)	7 (6 – 8)	6 (5 – 8)	7 (6 – 9)	0.833
EQ-5D Index	0.81 (0.76–0.85)	0.79 (0.59-0.83)	0.83 (0.78–1.00)	0.81 (0.71-0.84)	0.024
EQ-5D VAS (median; IQR)	75 (55 - 90)	80 (60 – 90)	75 (60 – 90)	70 (50 – 84)	0.278

Level 1; no problems, Level 2; some problems, Level 3; severe problems

Figure 8-10 - A histogram of Charlson Index Scores in the cohort (n=411)



8.3.13 Co morbidity and the Quality Outcomes Framework

The cohort was identified using the GP CKD register, maintained as part of the Quality and Outcomes Framework. The participants are offered regular monitoring of blood and urine parameters and blood pressure measurements. In addition to CKD there a number of other chronic conditions that are included in the QOF such as diabetes and ischaemic heart disease. Only 14% of the cohort (n=56) do not have another condition included in the QOF that would already result in regular monitoring of these parameters. Of those with an eGFR>60ml/min/1.73m^2 (i.e. those who do not currently meet the criteria to be included on the register) only 23 (20.4% of those with eGFR>60ml/min/1.73m^2) or 5.6% of the total cohort did not have another condition included in the QOF monitoring.

8.3.14 The impact of age in the cohort

The median age of the cohort was 70.6 years. Therefore the cohort was divided into 2 groups using 70 years as the cut-point to produce two approximately equal groups. The comparison of key features between the groups is shown in table 8-13.

Table 8-13 - Demographic information divided according to age

	Age ≤ 70 years	Age >70 years	Significance
Female (%)	58	59	0.851
SIMD rank (median; IQR)	2365 (1197 – 4323)	2306 (1273 -4759)	0.829
sCr (micromol/L; IQR)	103 (88 - 121)	110 (91 – 124)	0.215
eGFR (ml/min/1.73m^2; IQR)	56 (47 - 63)	51 (43 - 59)	0.002
eGFR<45ml/min/1.73m^2 (%)	21.1	33.6	0.005
TPCR (mg/mmol; IQR)	9.4 (6.4 – 14.1)	10.6 (6.9 – 17.3)	0.077
ACR (mg/mmol:IQR)	0.84 (0.52 – 1.7)	0.9 (0.6 – 1.7)	0.106
BMI (kg/m^2; ±SD)	31.1 (±6.0)	29.0 (±5.1)	<0.001
SBP (mmHg; ±SD)	131 (±20)	139 (±23)	<0.001
DBP (mmHg; ±SD)	80 (±11)	73 (±12)	<0.001
Pulse pressure (mmHg; ±SD)	52 (±17)	66 (±20)	<0.001
Hypertension (%)	72.4	77.0	0.289
Diabetes (%)	21.1	19.5	0.685
Vascular disease (%)	33.0	50.4	<0.001
ACEi usage (%)	66.5	73.9	0.101
Charlson score (median; IQR)	1 (0 – 2)	1 (0 – 2)	0.082
EQ5D score (median;IQR)	7 (5 – 8)	7 (6 – 8)	0.384
Haemoglobin (g/dL; ±SD)	13.8 (±1.5)	13.3 (±1.6)	0.001
Adjusted calcium (mmol/L; ±SD)	2.2 (±0.1)	2.2 (±0.1)	0.095
PO_4 (mmol/L; SD)	0.9 (±0.2)	0.9 (±0.2)	0.654
PTH (pmol/L; ±SD)	8.1 (±4.6)	9.1 (±5.1)	0.039
Vitamin D (nmol/L; ±SD)	39 (±21)	37 (±22)	0.295
Urate (mmol/L; ±SD)	0.38 (±0.10)	0.38 (±0.1)	0.742
Total cholesterol (mmol/L; ±SD)	5.0 (±1.2)	4.7 (±1.2)	0.006

8.3.15 Outcomes

The cohort has been followed for a median of 13.2 months (11.1 – 15.7) to date. During this time 8 participants (1.9%) died, one has commenced renal replacement therapy, and no participant sustained a doubling of serum creatinine.

Three hundred and fifty eight participants (87%) have had subsequent serum creatinine and eGFR measurements. Of those with baseline eGFR >60ml/min/1.73m^2 (n=109), 88 have had follow-up measurements recorded and 51% of those now have eGFR <60ml/min/1.73m^2. The median change in eGFR from baseline measurement is -0.2ml/min/1.73m^2 (-0.73 – 0.20).

Data regarding cardiovascular events is not yet available.

8.4 Discussion

8.4.1 Findings of the Study

In this chapter we have presented the baseline results of an observational longitudinal cohort study of patients with a diagnosis of chronic kidney disease in primary care, recruited from varied rural and urban settings and differing levels of deprivation in order to obtain a representative sample of the Ayrshire population. The participants are predominantly female, white and elderly with a mean age of 70.6 years. The socioeconomic and ethnic make-up of the cohort is representative of Ayrshire (217, 269). Overall the cohort has early CKD with a median eGFR of 54ml/min/1.73m^2. A significant proportion of the cohort had an eGFR >60ml/min/1.73m^2 (26.5% using the MDRD formula) which rose to 23.8% when eGFR was calculated using the CKD-EPI formulae. The design of the study was pragmatic; all patients with a coded diagnosis of CKD were invited to participate, rather than those who we identified as having CKD stages 3-5 using the KDIGO definition (6). There are strengths and weaknesses to this approach. The strength is to obtain a truly representative sample of those patients who are being *treated* as having CKD by their GPs rather than those who strictly fit the criteria, and allows comparison over time with those with consistent eGFR<60ml/min/1.73m^2. The weakness is that the absolute number of those with eGFR< 60ml/min/1.73m^2 in the study is lower and therefore some statistical power is lost. However the natural history of the group with eGFR> 60ml/min/1.73m^2 will be of interest during the follow-up period. Some of this group may have borderline function and eGFR dips below the threshold on random samples, but is >60ml/min/1.73m^2 when a meat fasted sample is taken (as the baseline study sample was). Alternatively they may have highly variable renal function as a result of altered haemodynamics and renal perfusion and therefore still represent a high risk group. Or some patients may have simply been misclassified; perhaps as a result of creatinine being checked during an intercurrent illness (over 60% are taking renin

angiotensin system blockade which puts them at increased risk of AKI (134)). This will become clear during follow-up. Reviewing the previous results gave some insight into this with 58% of the eGFR> 60 ml/min/1.73m^2 group having had a single low eGFR result in the preceding year, and 20% having had 2 low results greater than 90 days apart (the NKF-KDIGO definition of CKD stage 3 - 5). The vast majority have never seen a nephrologist and 84% did not have a cause of their renal disease recorded in their GP records. The prevalence of proteinuria in the cohort was notably low with 67% having no urine protein detectable on dipstick and 74% having a normal TCPR (<15mg/mmol) and 83% having a normal ACR (<3mg/mmol). The prevalence of macroalbuminuria was higher in the group with diabetes, but overall prevalence was still low. Only 15 participants had a TPCR >50mg/mmol, and 7 of those had discordant results with a high proportion of non-albumin proteinuria (high TPCR but low ACR). The discordant group were older, with better renal function and significantly more CKD of unknown aetiology. The urine sodium levels of the participants were high in keeping with a high salt diet (as measured by urine sodium in mmol/ L and the sodium: creatinine ratio [mmol/mmol]). The long term significance of sodium: creatinine ratios derived from spot samples is not known currently, and this will be of interest in the future. There is a large burden of pre-existent cardiovascular disease and cardiovascular risk factors in the cohort. Seventy five per cent have been diagnosed with hypertension, which is generally well controlled with a median of two antihypertensive agents. Forty three per cent have a history of vascular disease (ischaemic heart disease, peripheral vascular disease or cerebrovascular disease). Over 60% are currently receiving a statin, though very few are receiving combination therapy with ezetimibe as the recent SHARP study would suggest they should (161), over 50% have a family history of vascular disease and over 50% are either current or former smokers. There is also a very high prevalence of obesity with 45% of the overall cohort having a BMI of >30kg/m^2 (approximately 30% of age matched men and women have BMI 30kg/m^2 in the Scottish Health Survey) (271). Alcohol consumption was generally very

low. The participants have a number of co-morbid conditions (as measured by the Charlson score) with 18% having a score >2. Quality of life was measured using the EQ-5D questionnaire and the median value for global health state using a visual analogous scale was 75%, comparable to that of patients with many cancers (272) and much lower than the UK mean of 83% (273). The median calculated EQ-5D index was 0.81 (0.76 – 0.85) which is very similar to other studies of patients with conditions such as type 2 diabetes (274) but lower than a Japanese CKD cohort (275).

Comparison was made between those over 70 years of age and those 70 years or less. Kidney function was significantly worse in the older group as measured by eGFR ($p=0.002$). Systolic blood pressure and pulse pressure were significantly higher in the older group, as was the prevalence of vascular disease.

Overall the cohort has an apparently low risk of progressive renal decline and the need for renal replacement therapy, with low prevalence of proteinuria, older age and relatively preserved excretory renal function. However the risk of future cardiovascular events is high with a significant proportion having pre-existing vascular disease and a large burden of cardiovascular risk factors. These outcomes will be followed over the next 10 years.

8.4.2 Comparison with Other Cohorts

The demographics of this cohort are similar to those of the patients with equivalent severity of CKD in the overall population, as presented in the laboratory database analysis in chapter 6, demonstrating that we have recruited a broadly representative sample of the Ayrshire population. Compared to the general population of Ayrshire (and Scotland as a whole), the cohort is older, with a higher proportion of women. The proportion of study participants from minority ethnic groups is very low compared to the general population of Scotland, but is more representative of Ayrshire which is not an ethnically diverse area (217). The demographics are strikingly similar to those of the CKD cohort from the Renal Research in Derby (RRID) study and a longer term comparison of the outcomes in these similar CKD cohorts from different parts of the UK should be informative (276). It is notable that the demographics of the UK studies are quite different to those of the large American Chronic Renal Insufficiency Cohort (CRIC) which has a younger cohort with more severe renal disease and a large proportion of black and hispanic participants (277). The selection criteria for this cohort and RRID were very similar and designed to reflect real life practice in the UK, while CRIC purposefully limited the number of older individuals recruited by using age-based eGFR entry criteria and an upper age limit of 74 years.

We can also compare the community cohort to that of the patients under follow-up in the Glasgow nephrology clinic, the cohort described in chapters 2 – 5, with the community cohort being older, with a higher proportion of women, better renal function and less proteinuria. A table comparing the key demographics of these cohorts is shown below (table 8-12). Patients receiving RRT in Scotland are also younger with a higher proportion of men (12). These important differences should be taken into account when providing advice to primary care, and cohorts such as this one will provide evidence to guide such recommendations in the future.

Table 8-14 - Comparison of key demographics of the study cohort with other CKD cohorts

	Triple A	RRID	Glasgow Nephrology Clinic	CRIC
Age (years; ±SD)	71 ± 10	73 ±9	59 ± 16	58 ±11
Sex (% female)	59	60	50	46
Ethnicity (% white)	99.5	97.5	-	45
Serum creatinine (adjusted) (µmol/L; IQR)	103 (86 – 119)	-	140 (100 – 190)	153 ±50
MDRD eGFR (ml/min/1.73m^2)	54 (44 – 61)	52±10	42 (28 – 63)	43±13
24-hour urine protein (g/day)	-	-	0.34 (0.15 – 0.92)	0.17 (0.07 – 0.81)
ACR (mg/mmol; IQR))	0.9 (0.5 – 1.7)	0.33 (0 – 1.5)	10 (2 – 48)	-
TPCR (mg/mmol; IQR))	10 (7 – 15)		35 (17 – 106)	-
Blood pressure (mmHg; ±SD)	141/79 ± 22/12	134/73 ±18/11	144/78 ±27/14	128/71 ± 22/13
ACEi/ARB therapy (%)	69	65	22	68

8.4.3 The added value of the Primary Care CKD Register?

This cohort was recruited by identifying potential participants from primary care CKD registers. These registers allow automatic recall notices to be sent to patients to facilitate regular monitoring of kidney function (blood and urine) and blood pressure control. However the observation that only 14% of the cohort do not have another condition that would lead to regular monitoring calls in to question the value of the CKD register, in addition to those for conditions such as diabetes and ischaemic heart disease.

Additionally over 26% of the cohort had an eGFR>60ml/min/1.73m^2 and may not require specific renal monitoring. Twenty per cent of those did not have another condition that

would lead to regular monitoring and therefore may be receiving this monitoring spuriously as they do not meet the criteria for CKD in the first instance. Potentially this may lead to anxiety and definitely adds to costs with little current evidence of benefit. According to the QOF data, the prevalence of CKD in Ayrshire is around 4.3%. The prevalence in the laboratory database study in chapter 6 was 5.63% using the MDRD formula, though this was based on single creatinine measurements which can lead to a degree of overestimation. The QOF prevalence in Ayrshire is the highest in Scotland. However this study would suggest that a significant proportion (26%) of those on the CKD registers do not have an eGFR<60ml/min/1.73m^2, and if we extrapolate these findings to the whole Scottish and UK population it may be that thousands of patients are included in registers of CKD who are ineligible, and thousands of patients who do meet the criteria have not yet been registered, five years after the introduction of CKD to the QOF.

8.4.4 Limitations

Over one fifth of the cohort had eGFR>60ml/min/1.73m^2 on the meat fast sample and the implications of this are discussed in section 8.4.1. Further detailed assessment of vascular function would have been beneficial but this could not be performed in the primary care setting. A number of the primary care premises where participants were recruited were a significant distance from hospital. The requirement of an additional compulsory hospital visit may have deterred frail participants or resulted in selection bias. The primary care setting was one of the key aspects of the study design to maximise the recruitment of a representative cohort. The data regarding outcomes will be gathered via remote follow-up, using the Scottish electronic renal patient record and data from the information and statistics division of NHS Scotland. This limits the availability of further clinical parameters such as blood pressure measurements. However it is an extremely robust method, as by using the unique community health index identifier, all laboratory blood and urine parameters will be electronically downloaded and this will limit the number of

participants who are lost to follow-up. Likewise, the data on cardiovascular events and date and cause of death will be electronically downloaded from a central source allowing more complete data collection.

The strengths of this study are the detailed baseline assessment, performed by a single investigator (SM) which eliminates inter-observer variation, and the representative nature of the cohort.

8.4.5 Conclusion

This study presents a representative cohort of patients with CKD in primary care in Ayrshire, who are predominantly female, elderly, overweight or obese, with early CKD. The prevalence of proteinuria is notably low and the prevalence of pre-existent cardiovascular disease and cardiovascular risk factors is high. They represent a cohort at high risk of subsequent cardiovascular events and death and low risk of requiring renal replacement therapy. They have a different risk profile from those attending hospital nephrology clinics and the outcomes of this cohort will provide evidence for appropriate guidance on the investigation, monitoring and management of patients with CKD in primary care in the future.

9 Chapter 9: Discussion

9.1 Summary of findings

The aim of this thesis was to explore the optimal measurements of renal function and the optimal predictors of renal and patient outcome in those with chronic kidney disease in a variety of settings including a hospital nephrology clinic, a laboratory database of the general population, a specialist hypertension clinic and a primary care setting.

The main findings were:

- TPCR is a more sensitive predictor of 24-hour total proteinuria than ACR in a cohort of patients with CKD attending a hospital nephrology clinic, though the performance of both varies significantly with age and gender.

- TPCR and ACR are both strong independent predictors of renal and patient outcome in a cohort of patients with CKD attending a hospital nephrology clinic. They were as powerful as 24-hour total proteinuria and albuminuria to predict outcomes. TPCR performed well at low levels (15-50 mg/mmol), where albuminuria has traditionally been considered to be the superior marker.

- Using TPCR as a screening test identifies a group of patients with a high proportion of non-albumin proteinuria, who are not identified using ACR alone. This group is at higher risk of adverse outcomes than those with equivalent levels of albuminuria.

- TPCR and ACR vary between individuals as a result of differences in creatinine excretion in addition to changes in protein excretion. Adjusting TPCR and ACR for creatinine excretion (measured or estimated) improves the performance of TCPR/ACR to predict 24-hour proteinuria. However, this adjustment does not improve the prediction of renal and patient outcomes.

- The prevalence of CKD stages 3 – 5 falls when MDRD eGFR is replaced by the CKD-EPI formula in a laboratory database of all creatinine samples measured during two 12-month periods in Ayrshire. The population prevalence of CKD stages 3 – 5 remained stable over a 5-year period, when the CKD-EPI equation was used to calculate eGFR, despite a rise in the number of patients having their creatinine measured.

- Baseline reduced eGFR and dipstick proteinuria independently predict poor outcome in patients attending a specialist hypertension clinic, despite subsequent intensive management of blood pressure.

- Patients in the community with CKD are predominantly overweight or obese, elderly white women with early CKD. They have a large burden of pre-existent cardiovascular disease and cardiovascular risk factors and a low risk of requiring renal replacement therapy as a result of an extremely low prevalence of proteinuria and relatively preserved excretory renal function.

- The community cohort was recruited by interrogating the primary care CKD registers for those identified as having a diagnosis of CKD stages 3-5. However, over one quarter of the community CKD cohort did not meet the diagnostic criteria for CKD stage 3-5 when creatinine analysis was performed on a fasted sample.

9.2 Strengths of the studies

The nephrology clinic cohort (chapters 2-5) is large and unique in the fact that both TPCR and ACR were measured prospectively in all those attending the clinic, allowing the comparisons presented here. The geography of Ayrshire means that virtually all blood samples taken within the health board area are analysed at a single laboratory, meaning that the prevalence estimates in chapter 6 are reliable (allowing for the inherent weaknesses of any laboratory database analysis). The community cohort was extremely well phenotyped by a single investigator (SM) and robust arrangements are in place for follow-up for the next 10 years. The hypertension database is also relatively unique as a result of its size, completeness, length of follow-up and large number of events.

9.3 Prediction of 24-hour Total Proteinuria

Given that proteinuria is the single strongest predictor of renal progression, the accurate quantification of urine protein is paramount. Total proteinuria was traditionally measured until relatively recently when albumin came to the fore. The theory was that albuminuria is pathological, in contrast to physiological non-albumin proteins such as uromodulin. Therefore measuring only the single entity of albumin would improve the signal: noise ratio (with albumin the signal and NAP the noise) and subsequently improve the diagnosis of renal disease. However the evidence for clinical interventions in non-diabetic CKD such as target blood pressure and renin-angiotensin system blockade is based on the measurement of 24-hour total proteinuria (138) and there have been few studies comparing the performance of total proteinuria and albuminuria as diagnostic tests.

Twenty-four-hour urine collections have traditionally been considered the gold standard, however there is increasing evidence of the utility of spot samples and they are in widespread use. Therefore the spot measurement (whether that be total protein or albumin)

needs to have a good overall correlation with 24-hour total proteinuria. Additionally, it should correctly identify patients excreting >0.5 g/day or >1 g/day of proteinuria as these are important diagnostic thresholds. In chapter 2 we studied a secondary care cohort of 6842 patients with CKD who had undergone simultaneous measurements of total protein, albumin and creatinine on spot urine samples. TPCR had a superior test performance to ACR to predict 24-hour proteinuria. {{}} ACR had a sensitivity of only 79.0%, compared to TPCR's sensitivity of 93.9% to predict 1 g/day of total proteinuria. To improve the sensitivity of ACR to a comparable level with TPCR, the cut point fell to an ACR of 17.5 mg/mmol, with resultant fall in specificity to 69.8% (cf. TPCR 88.5%). Given that spot urine tests are primarily used by non-nephrologists to identify high risk patients for onward referral, investigation and treatment, sensitivity is of prime importance.

9.4 Prediction of Outcomes Relevant to Patients

It is perhaps unsurprising that TPCR is superior to ACR at predicting 24-hour urine total protein. Far more important than the ability to predict 24-hour total proteinuria, is the prediction of patient-relevant outcomes in CKD. There is increasingly strong evidence demonstrating the importance of proteinuria as a predictor of patient outcomes, whether measured by dipstick, ACR or TPCR (20, 69, 197). In chapter 3, we demonstrated that TPCR and ACR had comparable performance to predict doubling of serum creatinine, commencing RRT and all-cause mortality in a retrospective study of 5586 patients with CKD attending hospital renal clinics. This has not been shown previously. We also showed that the performance of TPCR and ACR was comparable to that of 24-hour collections to predict outcomes in this cohort.

9.5 The Issue of Non-Albumin Proteinuria

TPCR takes account of non-albumin proteins while ACR only measures albumin.

In chapter 4 using the same retrospective secondary care cohort, we examined the outcomes of patients with high levels of non-albumin proteinuria who would not have been identified as having significant proteinuria using ACR alone. Those with high levels of NAP were older, with poorer kidney function and had worse outcomes than those with heavy albuminuria (in terms of all-cause mortality and progressive renal decline measured by doubling of serum creatinine and commencement of RRT). When these factors were entered into a multi-variate model some of the excess risk was truncated, but they still remained a high risk group that would be identified using TPCR, but not ACR which failed to identify 16% of patients with proteinuria >1 g/day. The AusDIAB study in a general population sample, and using a lower threshold for proteinuria (≤0.2 mg/mg equating to 0.2 g/day) also found that ACR failed to detect 8% of patients with proteinuria (95). Non-albumin proteinuria covers a diverse spectrum from small proteins such as beta-2-microglobulin, representing tubular dysfunction, to large proteins such as transferrin, whose presence in the urine suggests loss of glomerular size selectivity. Despite this diversity the presence of a high proportion of non-albumin proteins in the urine would appear to offer additional prognostic information.

9.6 The Issue of Microalbuminuria

One of the arguments often given in favour of ACR is that it has superior sensitivity at low levels (around 3 – 30 mg/mmol, known as microalbuminuria) because of the signal: noise ratio described above. This assumes that the quantity of non-albumin proteinuria adds no additional information to albumin, and that the quantity of physiological proteinuria is irrelevant to risk.

However, our study showed that TPCR had comparable performance to ACR at microalbuminuria levels. Risk associated with albuminuria is raised even at levels lower than microalbuminuria (20). However, even when our reference group was divided in two, and the lower half used as the reference group in survival modelling, the utility of TPCR persisted showing that low levels of TPCR are clinically useful. These unexpected findings need to be confirmed by others, but challenge the paradigm.

9.7 The Issue of Muscle Mass

In chapter 2 the sensitivity and specificity of TPCR and ACR were found to vary according to age and sex, with a three-fold difference in the diagnostic threshold for an elderly woman versus a young man. We hypothesised that this was as a result of differing muscle mass, and therefore differing urine creatinine excretion (used as the denominator in TPCR and ACR). This has been acknowledged in some guidelines in the past where a different ACR reference range was recommended for males versus females (usually <2.5mg/mmol for males and <3.5mg/mmol for females) however a differential threshold has not previously been recommended for higher thresholds (such as 30 or 70mg/mmol) nor for TPCR. Therefore in chapter 5 we adjusted TPCR and ACR for predicted creatinine excretion and found that this approach was successful in improving the prediction of 24-hour proteinuria, especially in those with low muscle mass such as the elderly and females, with the result that differences in performance between sub-groups were eliminated. However the adjustment led to an inferior test performance for the prediction of renal and patient outcomes. This may reflect that urine creatinine was acting as an independent predictor of outcome. Therefore, whether TPCR and ACR should be adjusted for creatinine excretion depends on the intended purpose of the test: if TPCR is being measured to accurately quantify urine protein excretion (for instance to guide the decision to perform a renal biopsy) then adjusting for urine creatinine will afford an advantage in certain groups

of patients. However if the TPCR or ACR is being measured as a prognostic marker (i.e. to identify those at risk from subsequent adverse outcomes such as cardiovascular disease) then the unadjusted value, including the influence of urine creatinine, will provide more information that the proteinuria quantification alone. This issue is of particular importance given the ongoing development of renal and cardiovascular risk scores: the TPCR or ACR consist of 2 distinct biomarkers.

9.8 eGFR estimation

In chapter 6 we assessed the impact on CKD prevalence of changing from the MDRD formula to the CKD-EPI formulae for the routine estimation of eGFR in the Ayrshire population. The prevalence fell using the CKD-EPI formula predominantly as a result of reclassification from Stage 3A CKD to eGFR >60ml/min/1.73m^2 in middle aged women. The formulae were applied longitudinally in a 12 month period in 2006 and then in 2010-11; prevalence rose slightly between the study periods using the MDRD formulae but remained stable when the CKD-EPI formulae were used. The group that were re-classified as having better renal function have been shown to be low risk in other cohorts(254), however there is also a group (of predominantly elderly women) who were re-classified downwards to a more advanced stage of CKD and their outcomes remain unclear at present. Therefore, it may be premature to replace the MDRD formula with the CKD-EPI formulae in the UK at present.

9.9 Have 24-hour urine collections had their day?

In the studies described in this thesis we have shown that 24-hour urine collections are not needed to identify significant proteinuria, nor are they necessary as a prognostic indicator. Formulae to calculate eGFR are becoming ever more precise and accurate and so the role of the 24-hour collection to measure creatinine clearance is becoming more limited. Based

on these findings some might conclude that the 24-hour urine collection for total protein, albumin and creatinine(92) is no longer indicated except for very specific circumstances (such as cytotoxic drug dosing), though there are still some who disagree (92).

9.10 CKD in a community cohort

In chapter 7 the baseline characteristics of a prospective community cohort of patients with CKD are described. They differ markedly from the demographics of those attending hospital clinics, and those receiving RRT. They are predominantly elderly women with a large burden of hypertension and cardiovascular disease. Proteinuria is of notably low prevalence, however the use of renin angiotensin system blockade is high. Primary vitamin D deficiency is extremely common, but renal bone disease is not. The incidence of other complications of kidney disease such as anaemia, metabolic acidosis and hypoalbuminaemia is low. They represent a cohort at high risk of cardiovascular death, and a low risk of progressive renal decline. The cohort will be followed-up for 10 years and should provide valuable insights to guide recommendations for the management of CKD in the community.

9.11 The issue of overdiagnosis

The community cohort was recruited from 7 general practices by inviting all patients included in the CKD register. Over one quarter did not have an eGFR<60ml/min/1.73m^2 according to the meat fasted sample taken at the study visit (the criterion by which patients should be added to the register) and over one fifth did not have any sign of kidney disease at all. Estimates of CKD prevalence differ around the world, but are generally high. There has been considerable debate in the nephrology community regarding the possible overdiagnosis of CKD (278). Areas of disagreement include issues such as medicalisation of old age, over ascertainment of CKD because of the imprecision of eGFR formulae or

incorrect measurement of non meat-fasted samples (239, 267). The inclusion of samples taken during intercurrent illness which are not repeated during the convalescent phase to confirm CKD (rather than AKI) may exacerbate the problem. These issues are not confined to nephrology with other conditions including prostate cancer and gestational diabetes being the subject of similar debate (279).

9.12 CKD in a hypertension cohort

In chapter 8 baseline measurements of eGFR and dipstick proteinuria are shown to be independent predictors of cardiovascular and all-cause mortality in a cohort of patients attending a specialist hypertension clinic. A reduced eGFR and dipstick proteinuria identify almost mutually exclusive high risk groups, and in the minority with both abnormalities the risk is even greater. It is notable that these simple baseline measures remain such powerful predictors despite subsequent aggressive modification of hypertension and could be utilised as powerful risk markers at baseline.

9.13 Limitations of these studies

Chapters 2 – 5 are based on retrospective data gathered in the course of routine clinical care and therefore have limitations of some missing data and potential bias. For instance, everyone attending the clinics had their urine sent for spot quantification but 24-hour collections were requested at the discretion of the individual clinician.

The study of CKD prevalence in chapter 6 was a laboratory database study which has inherent limitations; only those individuals with a serum creatinine measurement in the course of their clinical care were included, and no clinical data were available to correlate with biochemical parameters. However, reassuringly, between 2006 and 2011 (the first and second study periods) the number of individuals undergoing creatinine measurement rose

dramatically but the prevalence of CKD did not, implying that ascertainment is already high.

In the community cohort study a significant number of the participants had an eGFR >60ml/min/1.73m^2 on the meat fasted study sample. This was anticipated (though the scale was not expected) but gives an extremely useful insight into the limitations of the CKD classification, and the realities of the identification of CKD in primary care. Interestingly the proportion with eGFR >60ml/min/1.73m^2 in the cohort on the follow up measurements (after median 13 months follow-up) was much lower.

In the hypertension cohort the urine protein was measured by dipstick which is less sensitive and specific than laboratory quantification, however the prognostic importance of proteinuria was still demonstrated.

In both chapters 6 and 7 eGFR was based on a single creatinine measurement, which has limitations as described above, though measures were taken to minimise the impact of this. The samples were not fasted.

9.14 Future studies

A prospective study comparing TPCR and ACR as predictors of outcome is needed. Comparison with other cohorts would be valuable. Both total protein and albumin have been measured in our community cohort study but this population has a low prevalence of proteinuria and therefore may not be able to answer the question. The importance of specific non-albumin proteins in the urine should be examined, both for prognostication and to shed light on underlying pathophysiology. The expanding field of urine proteomics may allow the identification of novel prognostic urine markers. Clinical assessment of

muscle mass (for instance using bio-impedance techniques) would be worthwhile in any future study of urine creatinine excretion. An evaluation of outcomes in a large cohort of people who are re-classified from CKD Stage 3A to eGFR>60ml/min/1.73m^2 and those re-classified to a more advanced stage of kidney disease is necessary before the new CKD-EPI formulae are adopted into clinical practice in the UK.

Samples from the community cohort have been stored to allow future study of novel biomarkers in the urine and blood, and the 10-year follow-up data will provide data on renal and patient outcomes. These data, when combined with other cohorts, will hopefully provide further insights into the predictors of outcome in early CKD and allow the development of robust renal risk scores. This is essential for the future, given the high and possibly rising prevalence of CKD, in order to allow the limited healthcare resources to be targeted at those at highest risk of adverse renal and cardiovascular outcomes in the future. Whole blood has also been stored from the cohort to allow DNA analysis in the future, perhaps to look for genetic promoters or inhibitors of renal progression.

9.15 Conclusions

This thesis investigated optimal measurements of renal function and the optimal predictors of renal and patient outcome in patients with CKD. Firstly, the role of TPCR and ACR in the risk stratification of patients with CKD was examined. TPCR is the superior predictor of 24-hour total proteinuria on which the evidence for our current interventions is based. It performs equally well as ACR to predict clinically relevant outcomes including renal and patient survival and performs well at low levels where albumin has traditionally been seen to be the superior marker. Only TPCR takes account of non-albumin proteins that may play an important pathological role in the progression of renal disease. Furthermore, adjusting TPCR and ACR for creatinine excretion improves the accuracy of urine protein quantification, but does not improve prediction of patient relevant outcomes. More research is needed to compare these tests prospectively before a definitive answer can be found but in the meantime TPCR remains a cheap, powerful test to identify patients at high risk of adverse outcomes.

Secondly the role of eGFR was examined. The prevalence of CKD Stages 3 – 5 falls if the CKD-EPI formulae are used in place of the MDRD formula for the estimation of eGFR in the Ayrshire population. The predictive role of eGFR was also examined. Baseline eGFR is a powerful independent predictor of adverse outcomes in a specialist hypertension cohort despite subsequent specialist intervention. Dipstick proteinuria also identifies a high risk group in this cohort and the two combine to provide important prognostic information and should be used for risk stratification in patients with hypertension.

Lastly in order to examine the prognostic role of eGFR and proteinuria prospectively, along with other predictors of outcome, a community cohort of patients with CKD was recruited. They differ significantly from those under hospital follow-up with a low risk of

renal progression versus a large burden of cardiovascular disease. A considerable proportion of the cohort did not meet the eGFR criteria for CKD stages 3-5 on the serum creatinine measurement of the meat fasted study sample, suggesting that overdiagnosis of CKD may be an issue in the community in the UK.

Proteinuria and eGFR are two of the key aspects of the diagnosis and monitoring of chronic kidney disease. Identification of the optimal measures of both is therefore essential, and the findings presented in this thesis contribute to that. There is an urgent need to refine our risk stratification abilities in CKD, in order to identify those who require intensive intervention, and to reassure the others. The findings of this thesis also contribute to that. Further study is required to refine these core aspects of the diagnosis, investigation and management of CKD.

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11 Appendices

Paperwork related to the community cohort study

11.1.1 Letter of invitation to potential participants

11.1.2 Participant Information Sheet

11.1.3 Consent Form

11.1.4 Baseline Information Questionnaire

11.1.5 ICIQ-UI Short Form

11.1.6 IPSS Questionnaire

11.1.7 EQ-5D Questionnaire

GP Headed Notepaper
Date as postmark

Dear

A study of the factors influencing outcomes in patients with chronic kidney disease

We would like to invite you to take part in the above research study that is happening in your GP practice. The study aims to look into factors that cause worsening kidney disease and to look at factors that may contribute to heart disease in patients with kidney disease. Our records indicate that you have a mild to moderate reduction in your kidney function and therefore qualify to be included in the study. Please take a few moments to read the following information regarding the term Chronic Kidney Disease.

- Chronic Kidney Disease is the international term used to describe deterioration in kidney function over a long period of time. However, it may not necessarily mean there is a disease as such, but more that the kidneys aren't functioning at the level they once were.
- The level of kidney function that you may have affects about 5% (1 in 20) of the UK population.
- For the vast majority of people nothing else will happen. However there will be a small minority of people who will develop more advanced kidney problem, and will need specialist kidney care.
- This research is to try to work out who will and who won't develop more severe kidney disease and to identify what it is that makes most people stable, but for some makes their condition progress.

The researchers are kidney doctors based in Crosshouse Hospital Renal Unit and your GP practice is helping them with the study. In the envelope, along with this letter you will find a participant information sheet, which explains in some detail what the study will involve. Please read this carefully. You will not be paid to take part in the study, but we can refund the cost of your travel (bus fare, petrol etc) so please keep your ticket.

If you would like to discuss the study further or have questions, please contact the researchers (contact details on the Information sheet) or me. Once you have decided whether or not to participate please telephone **01563 825132** to arrange the first study visit (at the GP practice) or to inform the researchers that you do not wish to participate. If there is no answer, please leave your name and telephone number and somebody will get back to you to make arrangements. Due to the high volume of calls, please do not worry if your call is not returned on the same day.

You can also contact us by post or email:

By post: Dr Shona Methven, Renal Office, Level 2 East, Crosshouse Hospital, Kilmarnock KA2 0BE
By email: shona.methven@nhs.net

If we do not hear from you in the next 2 weeks, we will write again to ask you to take part. If we still do not hear from you, we will not get back in touch.

We look forward to hearing from you.

Yours sincerely

General Practitioner

Participant Information Sheet
A study of the factors influencing outcomes in patients with chronic kidney disease

You are being invited to take part in a research study. Before you decide if you wish to take part, it is important for you to understand why the research is being done and what it involves. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?
Chronic kidney disease (CKD) is common. It affects 8 - 13% of the population. It is important because it is associated with an increased risk of disease of the blood vessels (vascular disease). Most of the research on this condition has been performed with people who were attending hospital clinics. We do not know if people with CKD, who are looked after by their General Practitioner (GP) should be looked after the same way. The aim of this project is to study people with CKD in the community for the next 10 years, to find out what happens to them.

Why have I been chosen?
You have been invited to take part because you have chronic kidney disease, and your GP looks after you. We plan to study 500 people with chronic kidney disease.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. You will be given this information sheet and a copy of the signed consent form, to keep. If you decide to take part, you are free to withdraw at any time and without giving a reason. Your usual treatment will not be affected in any way if you do not wish to take part or if you withdraw.

What will happen to me if I take part?
Most people will be asked to come for one visit to their GP practice. Some people may be asked to come back for a second visit to their GP practice or the hospital. There will be no further visits for the study. We will contact you by letter after a year to ask you to fill in a questionnaire.

The doctors who are running the research project will look at the computer records that your GP practice keep about you (not the paper records). We will enter your personal details onto a confidential NHS database called the Scottish Electronic Renal Patient Record. This is linked to other NHS computers, which contain records of laboratory results, hospital admissions, diagnoses, and operations. This will allow us to see what happens to your health over the next 10 years, without having to contact you regularly.

If something happens to you, and you are no longer able to make decisions about being in the study, we will not ask for any more blood or urine samples. If you have consented to be in the study, we will assume that we can continue to gather information about your health as described above. If you change your mind, and ask us to stop collecting information, we will of course, stop immediately. You would not have to explain why.

You will not be given any specific treatment as part of the study, you will receive all the usual treatments from you own doctor, and we will keep a record of those.

If you take part in the study, you will be asked to visit your GP practice to meet the research doctor. You will be able to ask questions about the study and discuss anything you are not sure about, or do not understand. If you are happy to take part in the study you will be asked to sign the consent form. You will be given a copy of the form to keep (along with this information sheet). Before the visit you will be sent a questionnaire to fill out, with questions about your health and treatment. You will be asked to bring the completed questionnaire to the GP practice when you come. On the

morning of the visit, you should not have anything to eat or drink, except water. When you arrive, you will have your height, weight, waist and hips measured and have your blood pressure checked. You will be asked some questions about how you feel, and how your health affects your life. You will be asked to provide a urine sample (around 30mls or 6 teaspoons). An electrocardiogram (ECG) will be performed. This is a trace of the electrical activity of the heart, and is measured by applying sticky pads to the chest, and attaching wires to the pads which are connected to the ECG machine. You lie on the examination couch for this test, and stay still while this is done. You do not feel anything when the recording is being taken. You will also have a blood sample taken, of around 30mls (6 teaspoons) of blood. If you would like to lie down while the sample is taken, please let the researcher know. This visit will last approximately 50 minutes.

Some people may also be asked to perform a 24-hour urine collection. This involves collecting all the urine that you pass for 24 hours, and storing it in a plastic container (that we will provide). This can be performed when it suits you, and handed in. On the first day of the test, when you get up in the morning and pass urine, discard it in the toilet, then collect all the urine you pass that day, and overnight, and the first time you pass the following morning, then stop the collection.

Small amounts of blood and urine from the samples you give will be stored in a freezer. Your details will be removed from the samples. If this study gives new information about kidney disease, we may do extra tests on the frozen samples, and may analyse the samples for DNA to look for which inherited traits are associated with kidney disease. If your samples were analysed for DNA in the future, this would be done anonymously and you would not be identified. We would also ask permission from the West of Scotland Research Ethics Service before doing any further tests, but we would not get in touch with you again to ask further permission. We will not give or sell your samples to insurance companies or other similar organisations.

What do I have to do?
You should not eat or drink anything apart from water on the morning of your study visit. You may be asked to perform a 24-hour urine collection (described above) or attend the hospital for a scan. There are no other restrictions during the study, you can eat and drink normally, and go about your usual business.

What are the possible disadvantages and risks of taking part?
The visit to the GP practice or hospital or performing a 24-hour urine collection may be inconvenient. Giving a blood sample is uncomfortable, and can leave a bruise at the site of the needle. Some people feel faint when having blood taken. It can be uncomfortable when the sticky pads are removed from the chest, after the ECG recording, particularly if you have hairs on your chest. The ultrasound of the neck, kidneys or heart can be slightly uncomfortable when the probe is pressed on the skin.

What are the possible benefits of taking part?
If we find any health problem that has not been noted before, for instance protein in the urine, we will inform your GP, and recommend the best treatment for this. Otherwise you will receive no direct benefit from taking part in this study. The information that is collected during this study will give us a better understanding of chronic kidney disease and how that affects people's lives in the community.

Will my taking part in this study be kept confidential?
All information, which is collected, about you during the course of the research will be kept strictly confidential. You will be identified by an ID number and any information about you will have your name and address removed so that you cannot be recognised.

What will happen to the results of the research study?
The results will be presented at conferences, and published in medical journals. The first set of results is likely to be published around 12 – 18 months after your study visit. You can contact us, if you would like a copy of the published results. The results will be anonymous and no individual person will be identified in the publications.

Who is organising and funding the research?
The research is funded by NHS Ayrshire and Arran, and an unrestricted educational grant from Bristol Myers Squibb.

Who has reviewed the study?
This study has been reviewed by the West of Scotland Research Ethics Service.

Will my General Practitioner (GP) know?
Yes. We will tell your GP that you are taking part in the study.

Contact for Further Information
1. Dr Shona Methven, Renal Office, Level 2 East, Crosshouse Hospital, Kilmarnock, KA2 0BE
Telephone: 01563 825177 Email: shona.methven@nhs.net
2. Your General Practitioner

If you wish to make a complaint
A leaflet called "Information for Patients and Carers – Complaints Procedure" is available from your GP practice with details of how to make a complaint.

Study Identification Number:

CONSENT FORM

A study of the factors influencing outcomes in patients with chronic kidney disease

Name of Researcher: Dr Shona Methven

Please initial box

1. I confirm that I have read and understand the information sheet dated 19/4/2010 (version 2) for the above study and have had the opportunity to ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I agree to my GP being informed of my participation in this study ☐

4. I understand that my details will be added to the Scottish Electronic Renal Patient Record, and these records will be used to follow up my health status for the next 10 years ☐

5. I agree that a small sample of my blood and urine can be stored for possible further testing in the future, including DNA testing ☐

6. I agree to take part in the above study. ☐

Name of participant	**Date**	**Signature**
Dr Shona Methven		
Researcher	**Date**	**Signature**

1 for participant; 1 for researcher; 1 (original) to be kept in medical notes

Background Information Questionnaire

Please complete this questionnaire and bring it with you to your study visit

Date completed ……
Study ID Number …….. (the researcher will fill this in)

1. PERSONAL MEDICAL HISTORY

1. Have you ever attended a hospital kidney clinic?

Yes currently **Yes previously** **No** (Please circle)

2. Have you ever had a kidney biopsy?
(a small sample of the kidney is taken to be examined in detail)

Yes **No** **(**Please circle)

3. If yes to either question 1 or 2, what is the cause of your kidney problem (if known)?

…….....

4. Do you suffer from or are you receiving treatment for any of the following conditions?

If yes, please circle all that apply:

High Blood Pressure	Heart attack
Angina	Heart Failure
Stroke	Diabetes
Emphysema/chronic bronchitis (COPD)	Kidney Stones
Frequent urine infections	Enlarged prostate
Rheumatoid arthritis	Asthma
Narrowed blood vessels in your legs	Liver disease

5. Have you had an operation performed on your kidneys or bladder?

Yes **No** (Please circle)

If yes please give details…….

6. Do you have any problems passing urine? **Yes** **No** (Please circle)

If yes please circle all that apply:

Passing urine more than once during the night	Pain passing urine
Difficulty starting to urinate	Poor flow
Difficulty stopping urination	Blood in the urine

2. MEDICAL SUMMARY

Please list any serious illnesses, hospital admissions or operations. Continue overleaf if necessary.

DATE	PROBLEM

3. ALCOHOL INTAKE

Do you drink alcohol? **Yes** **No** **(please circle)**

If yes how many of the following do you have a week?

Pints of beer/ cider ……..

Glasses of wine ……..

Measures of spirits ……..

Cocktails or other drinks ……..

4. SMOKING STATUS

Do you smoke? **Yes** **Ex-Smoker** **Never (please circle)**

If Yes or Ex-smoker – how many per day? …….....for how long?……..

5.MEDICATION

Please include any medicine or supplement you take regularly or as needed, not just prescribed medication. Continue overleaf if needed.

Name of medication	*Dose*	*Number per day*	*What is it for?*

Please list any allergies to medication: ……...……

Do you regularly take any of the following painkillers? **(please circle)**

Paracetamol Co-codamol

Ibuprofen (Brufen/ Nurofen) Diclofenac (Voltarol)

If yes, for how long have you taken them? ……..

6. *FAMILY HISTORY*

Have your parents, brothers or sisters had any of the following conditions? **(please circle)**

Kidney Disease Diabetes

Heart Disease Stroke

High blood pressure

Any other important family illness ……

7. OCCUPATION

Have you ever worked with any of the following? Please circle

Lead Cadmium

Mercury Silica

Beryllium Uranium

Chromium Ethylene glycol

8. ETHNICITY

What is your ethnic group? Choose ONE section A to E, and then circle to indicate your ethnic group.

A: White

Scottish
English
Welsh
Northern Irish
British
Irish
Gypsy/ Traveller
Any other White ethnic group, please state ……...

B: Mixed or multiple ethnic groups

Please state ……..

C: Asian, Asian Scottish or Asian British

Indian
Pakistani
Bangladeshi
Sikh
Chinese
Any other Asian background, please state ……..

D: Black, Black Scottish or Black British

Caribbean
African
Any other Black background, please state ……..

E: Other ethnic group

Arab
Jewish
Any other, please state …….

Not stated

Thank you very much for answering these questions, please turn over to answer a few more questions.

Initial number

ICIQ-UI Short Form

CONFIDENTIAL

DAY MONTH YEAR

Today's date

Many people leak urine some of the time. We are trying to find out how many people leak urine, and how much this bothers them. We would be grateful if you could answer the following questions, thinking about how you have been, on average, over the PAST FOUR WEEKS.

1 Please write in your date of birth: ☐☐ ☐☐ ☐☐

DAY MONTH YEAR

2 Are you *(tick one)*: Female ☐ Male ☐

3 How often do you leak urine? *(Tick one box)*

never ☐	0
about once a week or less often ☐	1
two or three times a week ☐	2
about once a day ☐	3
several times a day ☐	4
all the time ☐	5

4 We would like to know how much urine <u>you think</u> leaks.
How much urine do you <u>usually</u> leak (whether you wear protection or not)?
(Tick one box)

none ☐	0
a small amount ☐	2
a moderate amount ☐	4
a large amount ☐	6

5 Overall, how much does leaking urine interfere with your everyday life?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all a great deal

ICIQ score: sum scores 3+4+5 ☐☐

6 When does urine leak? *(Please tick all that apply to you)*

never – urine does not leak	☐
leaks before you can get to the toilet	☐
leaks when you cough or sneeze	☐
leaks when you are asleep	☐
leaks when you are physically active/exercising	☐
leaks when you have finished urinating and are dressed	☐
leaks for no obvious reason	☐
leaks all the time	☐

Thank you very much for answering these questions.

International prostate symptom score (IPSS)

Name: Date:

	Not at all	Less than 1	Less than half	About half the	More than half	Almost always	**Your score**
Incomplete emptying Over the past month, how often have you had a sensation of not emptying your bladder completely after you finish urinating?	0	1	2	3	4	5	
11.1.8 Frequency Over the past month, how often have you had to urinate again less than two hours after you finished urinating?	0	1	2	3	4	5	
11.1.9 Intermittency Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
11.1.10 Urgency Over the last month, how difficult have you found it to postpone urination?	0	1	2	3	4	5	
11.1.11 Weak stream Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5	
11.1.12 Straining Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5	
	None	1 time	2 times	3 times	4 times	5 times or more	**Your score**
Nocturia Over the past month, many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?	0	1	2	3	4	5	
11.1.13 Total IPSS score							

Quality of life due to urinary symptoms	Delighted	Pleased	Mostly satisfied	Mixed – about equally	Mostly dissatisfied	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition the way it is now, how would you feel about that?	0	1	2	3	4	5	6

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.