# Risk Prediction and Outcomes in Chronic Kidney Disease Stage 3: a Prospective Cohort Study in Primary Care

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For Annie and Naomi

# Abstract


## Introduction

Chronic Kidney Disease (CKD) is common, and affects many people who are predominantly managed in primary care. Guidelines for management of CKD are often based around evidence from referred secondary care populations. Many risk factors for progressive CKD have been described, but not examined in a primary care population. Outcomes in CKD are variable, and concern long-term changes. Therefore, risk stratification is key to guide clinical management. This thesis will examine outcomes over 5 years in a cohort of people with CKD stage 3 recruited from primary care. It will evaluate cystatin C, Fibroblast Growth Hormone (FGF23), vitamin D and parathyroid hormone (PTH) as biochemical markers of risk in this population, and additionally will assess Skin autofluorescence (SAF) as a non-invasive measure of tissue advanced glycation endproducts (AGEs) and another potential strategy for risk stratification.

## Methods

The Renal Risk in Derby (RRID) study is a prospective cohort of 1,741 people with CKD stage 3, defined by two GFR measurements more than 90 days apart, prior to study entry.

Participants were individually assessed at baseline, 1 year, and 5 year follow-up visits. Blood and urine were sampled for biochemistry, and anthropometric data were additionally collected. SAF was measured at each study visit using an AGE reader (Diagnoptics).

**Results**

Five-year follow-up demonstrated low rates of CKD progression. The largest group of participants (593 of 1,741, 34.1%) evidenced stable CKD over the 5 year study period. End Stage Kidney Disease (ESKD) was rare, and was seen in 0.2% (4 of 1,741) of participants. Additionally, a significant minority of participants (19.3%, 336 of 1,741) demonstrated no evidence of CKD at their year 5 visit. The risk of CKD progression can be predicted with moderate accuracy using common clinical variables including age, gender, baseline eGFR, urine albumin to creatinine ratio (uACR), haemoglobin, bicarbonate, and diabetes.

Cystatin C was evaluated in the context of national and international guidance suggesting it be used to confirm diagnosis of CKD in those with CKD 3a A1 on creatinine based estimates of GFR. In this cohort, mean cystatin C based eGFR

values were significantly lower than those derived from creatinine (45.1 ml/min/1.73m$^2$, 95% Confidence Interval (CI) 44.4 to 45.9 versus 53.6 ml/min/1.73m$^2$, 95% CI 53.0 to 54.1). Use of cystatin C based eGFR in this population would therefore reclassify many participants as having more severe disease.

Markers of mineral bone disease, including FGF23, vitamin D, and parathyroid hormone (PTH) have been of interest in CKD risk prediction. In the RRID study, vitamin D deficiency (Hazard Ratio 1.62, 95% CI 1.01 to 2.58) and raised PTH (HR 1.42, 95% CI 1.09 to 1.84) were independently associated with increased risk of all-cause mortality, but FGF23 was not.

Skin Autofluorescence (SAF) was examined as a risk marker of all-cause mortality and CKD progression. In fully adjusted multivariable analysis, both baseline SAF (HR 1.16, 95% CI 1.02 to 1.32), and the change in SAF over the first year of the study (HR 1.16, 95% CI 1.00 to 1.34) were associated with all-cause mortality but not CKD progression.

**Conclusions**

The results presented in this thesis show that, in a primary care cohort of predominantly older adults, CKD progression is uncommon and can be predicted using common clinical variables. Cystatin C did not improve diagnosis or risk prediction in CKD in the way that it was intended in recent national and international guidance. Some novel risk markers evaluated in this study did predict risk in this population, including SAF, vitamin D, and PTH; however, these do not necessarily contribute additionally to common clinical variables. Risk prediction models in primary care should therefore focus on these. Thus, these results suggest that people with CKD stage 3 can be adequately managed and risk stratified in primary care without the need for additional, potentially expensive, investigations.

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# List of Abbreviations

| | |
|---|---|
| Acute Kidney Injury | AKI |
| Advanced Glycation Endproducts | AGE |
| Angiotensin Converting Enzyme | ACE |
| Angiotensin Receptor Antagonist | ARB |
| Analysis of Variation | ANOVA |
| Arbitrary Unit | AU |
| Berlin Initiative Study | BIS |
| Cardiovascular Events | CVE |
| Case Report Form | CRF |
| Chronic Kidney Disease | CKD |
| Chronic Renal Insufficiency Cohort | CRIC |
| Confidence Interval | CI |
| Deoxyribonucleic acid | DNA |
| Diastolic Blood Pressure | DBP |
| End Stage Kidney Disease | ESKD |
| Estimated Glomerular Filtration Rate | eGFR |
| Fibroblast Growth Factor 23 | FGF23 |
| Focal Segmental Glomerulosclerosis | FSGS |

| | |
|---|---|
| Free Light Chains | FLC |
| Glomerular Filtration Rate | GFR |
| Health and Social Care Information Centre | HSCIC |
| High-density lipoprotein | HDL |
| Hospital Episode Statistics | HES |
| Interquartile range | IQR |
| Isotope Dilution Mass Spectrometry | IDMS |
| Kidney Failure Risk Equation | KFRE |
| Left Ventricular Hypertrophy | LVH |
| Low-density lipoprotein | LDL |
| Lund-Malmö revised (estimating equation) | LMR |
| Mean Arterial Pressure | MAP |
| National Health and Nutrition Examination Surveys | NHANES |
| National Institute for Health and Care Excellence | NICE |
| Parathyroid Hormone | PTH |
| Patient Information Sheet | PIS |
| Quality Improvement in Chronic Kidney Disease | QICKD |
| Receptor for Advanced Glycation Endproducts | RAGE |

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|---|---|
| Reduction in Endpoints in Non-Insulin Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan | RENAAL |
| Renal Association | RA |
| Renal Risk in Derby | RRID |
| Research Ethics Committee | REC |
| Skin Autofluorescence | SAF |
| Systolic Blood Pressure | SBP |
| Urine Albumin to Creatinine Ratio | uACR |
| Urine Protein to Creatinine Ratio | uPCR |
| Soluble Urokinase-type Plasminogen Activator Receptor | suPAR |
| Systolic Blood Pressure Intervention Trial | SPRINT |

# Acknowledgements

Firstly, I'm tremendously grateful to Prof. Maarten Taal, for the opportunity to work on this project, and for his constant support, encouragement and mentorship, both academically and clinically. I hope I've done justice to it.

The Renal Risk in Derby (RRID) study represents the results of a monumental effort from a few very dedicated people. Alongside Prof. Taal, the study would not have happened without the dedication of Dr. Natasha McIntyre, who personally recruited all the participants and completed the baseline visits and the majority of the first year follow-up. Rebecca Packington has worked on RRID almost since its inception, and I'm fairly sure that no 5 year follow-up would have happened at all without her knowledge and hard work.

No study would be possible without its participants, and I am therefore indebted to the people who gave up their own time (and the minor inconvenience of storing urine samples in their fridges) to participate in this work. Seeing over a thousand people was not nearly as arduous as I first thought, as everyone was interested and so willing to help. The many GP practices involved in the study were also invaluable, providing

a place to see participants close to home. I'm sure that this increased participation rates tremendously.

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My family and friends have been a constant source of support, throughout my education and training. My parents and sisters particularly have always been there for me at difficult times. My grandparents were always interested in my academic progress. I'm sorry they didn't see me finish this.

Naomi, I can't believe how much you've grown over the time I've been writing this. Probably, meaning I've taken too long to finish it. Watching you grow has been more amazing than I thought possible. I hope that you gain as much enjoyment in finding out about the world as I have over the years and wish you every happiness possible for the future.

Finally, Annie, thank you so much for all your patience and support. I wouldn't have finished this without you. I'm sorry

that this thesis has taken so much of my time over the past few years. You’re the best partner I could have found and I love you very much.

# Contributions to the Renal Risk in Derby Study

The Renal Risk in Derby (RRID) study is a prospective cohort study of people with CKD stage 3 in primary care. It commenced recruitment in June 2008 and as such many people have been involved in its design and implementation. It was initially designed and developed by Prof. Maarten Taal and Dr. Natasha McIntyre. Consultant colleagues at the Renal unit, Royal Derby Hospital, Dr. Richard Fluck and Prof. Chris McIntyre, were also involved in the design of the study.

Participants were recruited by Dr. N McIntyre, who conducted all baseline study visits. First year follow-up visits were conducted by Dr. Natasha McIntyre and Research Nurse Paula Welch. Administrative support throughout baseline visits and year 1 follow-up was provided by Rebecca Packington.

My contributions to the study include design of the year 5 study visits and the writing and submission of the major protocol amendments to the Research Ethics Committee (REC). For this amendment, I designed the functional testing elements of the study visit, updated the Patient Information Sheet (PIS), Case report from (CRF) and consent form.

Additionally, I completed all Year 5 Follow-up visits personally, studying over 1,000 participants, and was involved in all stages of the preparation for these visits alongside Rebecca Packington. This included correspondence with participants by letter inviting them to continue their study participation, arranging study visits with participants by telephone, and preparing packs of materials sent out prior to each study visit.

I managed samples of blood and urine taken from participants at each visit, and organised the storage of these in -80C freezers. I was involved with the arrangement of transport and testing of blood and urine samples

I entered a large majority of the data collected into a database, and was responsible for data 'cleaning' and accuracy. This data was subsequently exported into SPSS for statistical analysis.

Through regular meetings with Prof. Taal, I developed the design and direction of study analysis. I carried out all statistical work described in this thesis. I was lead author for three papers that involve work presented in this manuscript.

In this capacity, I wrote and edited the manuscripts with the input of co-authors. I prepared all figures presented both in these papers and in this thesis.

I was also involved in the arrangement and organisation of two participant feedback meetings, allowing study results to be fed back to participants, and allowing them to ask questions, and give their thoughts on the direction of this research and on future areas of focus for the department.

Results from this thesis have been published in three papers in peer-reviewed journals as follows:

1. Shardlow A, McIntyre NJ, Fraser SDS, Roderick P, Raftery J, Fluck RJ, McIntyre CW, Taal MW. The clinical utility and cost impact of cystatin C measurement in the diagnosis and management of chronic kidney disease: A primary care cohort study. PLoS Med. 2017 Oct 10;14(10): e1002400.

2. Shardlow A, McIntyre NJ, Fluck RJ, McIntyre CW, Taal MW. Associations of fibroblast growth factor 23, vitamin D and parathyroid hormone with 5-year outcomes in a prospective primary care cohort of people with chronic kidney disease stage 3. BMJ Open. 2017 Aug 23;7(8): e016528.

3. Shardlow A, McIntyre NJ, Fluck RJ, McIntyre CW, Taal MW. Chronic Kidney Disease in Primary Care: Outcomes after Five Years in a Prospective Cohort Study. PLoS Med. 2016 Sep 20;13(9):e1002128.

# 1. Introduction and Background

## 1.1 Chronic Kidney Disease

Chronic kidney disease (CKD) is a clinical syndrome that encompasses a wide range of disease severity and aetiologies. Universally, CKD is defined though the demonstration of renal damage that persists for at least three months. This includes demonstration of loss of renal filtration function, the detection of abnormal levels of blood or protein in the urine, or structural abnormalities of the kidney at a macroscopic level. Unsurprisingly, as a heterogeneous spectrum of clinical conditions fall under the diagnosis of CKD, outcomes in people with CKD are variable. Therefore, the assessment of risk of adverse outcomes, and the translation of this to clinical practice is of vital importance for management of patients with CKD.

Essential to the diagnosis and management of CKD is glomerular filtration rate (GFR), which is a measure of the volume of filtrate produced in a given unit of time. This can be measured directly, using compounds such as inulin or iohexol, which are freely filtered at the glomerulus and not secreted or absorbed in the renal tubule. However, in clinical practice GFR is more commonly estimated using equations based upon

endogenous filtration markers and patient characteristics. Estimated GFR (eGFR) is crucial for the diagnosis of CKD, and its clinical management.

## 1.2 Chronic Kidney Disease Diagnostic Criteria

CKD was first defined in 2002, following the development of the Modification of Diet in Renal Disease (MDRD) estimating equation for eGFR.[1] The initial classification system described CKD stage 1 to 5 based upon GFR. CKD stage 3-5 can be defined on the basis of a GFR reduced to <60 ml/min/1.73m$^2$ alone, which persists for at least three months. Diagnosis of CKD stages 1 and 2 requires further evidence of chronic renal damage, principally proteinuria. Prior to this, there was uncertainty in the description and management of people with renal disease,[2] and no agreed standards for research. Classification by GFR allows comparison easily between people of different ages, in whom creatinine values may be disparate for the same GFR. Subsequent classification modifications included the division of stage 3 CKD into CKD 3A and 3B.[3] The most recent KDIGO classification was described in 2012.[4]

### 1.2.1 Kidney Disease: Improving Global Outcomes 2012 Classification

Based on extensive epidemiological research conducted in the years since the first CKD classification system, the 2012 KDIGO classification sought to provide categories that reflect the risk status of individual patients. CKD is classified in using 3 dimensions.[4] These include estimated GFR categories, definitions of levels of albuminuria (A1, A2, and A3, defined as urine albumin to creatinine ratio (uACR) <3 mg/mmol, 3 to 30 mg/mmol, and >30 mg/mmol respectively), and also consideration of the aetiology of kidney disease, which is significant when considering risk of progression. This classification system is represented in Figure 1.1 The heat map reflects the risk status of each category for multiple adverse outcomes including end-stage kidney disease (ESKD), cardiovascular events, acute kidney injury and all-cause mortality. At each stage in the evolution of the CKD classification system, guidelines for the management of people with CKD have been produced, and this has had definite benefit in the management of people with kidney disease. Similarly, in research, comparison between projects can be drawn more easily as the level of kidney function, and the methods used to estimate this from common clinical variables is now standardised.

**Figure 1.1 2012 KDIGO CKD classification and heat map. Colours demonstrate levels of risk from green (low risk) to red (high risk).**

**Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012**

| GFR categories (ml/min/ 1.73m²) Description and range | | | | Persistent albuminuria categories Description and range | | |
|---|---|---|---|---|---|---|
| | | | | A1 | A2 | A3 |
| | | | | Normal to mildly increased | Moderately increased | Severely increased |
| | | | | <30 mg/g <3 mg/mmol | 30-300 mg/g 3-30 mg/mmol | >300 mg/g >30 mg/mmol |
| | G1 | Normal or high | ≥90 | | | |
| | G2 | Mildly decreased | 60-89 | | | |
| | G3a | Mildly to moderately decreased | 45-59 | | | |
| | G3b | Moderately to severely decreased | 30-44 | | | |
| | G4 | Severely decreased | 15-29 | | | |
| | G5 | Kidney failure | <15 | | | |

Whilst the concept and definitions of CKD are well established, the utility and applicability of these in all populations is controversial. In the elderly, there is debate as to whether the same classification system should be used as in younger people.[5, 6] Additionally, the definitions provide no clear guidance on when progression from one stage to another has occurred (i.e. single GFR value in next category or two required), or how to define when CKD is no longer present. These are of importance for the development of clinical trials, as well as clinical practice.

Finally, in recognition of the limitations associated with using serum creatinine to estimate GFR, the 2012 KDIGO guidelines,[4] and subsequent 2014 National Institute of Health and Care Excellence (NICE) guidelines[7] have suggested that cystatin C based estimates of GFR be used in addition to creatinine. This will be discussed further in section 1.9.10.

## 1.3 Epidemiology of Chronic Kidney Disease

Epidemiological studies in different populations across the world have shown CKD to be a common phenomenon.[8] In a recent meta-analysis, Hill et al. estimated global CKD prevalence to be 13·4% (95% Confidence Interval (CI) 11·7-15·1%). The largest proportion of these had CKD stage 3 (7·6%, 95% CI 6·4-8·9%).[8] A recent analysis of data from general population cohorts across Europe has found differences in prevalence of CKD across different regions. Adjusted CKD stage 1 to 5 prevalence varied from 3.3% in Norway, to 17.3% in North East Germany.[9] Prevalence increased in all groups with increasing age. United States data from the National Health and Nutrition Examination Surveys (NHANES) showed similar prevalence of CKD stage 3 in 1999

to 2004 (7.7%, 95% CI 7.0%-8.4%), and an increase from (5.4%, 95% CI, 4.9%-6.0%) in 1988-1994.[10]

Prevalence estimates depend upon the diagnostic criteria for CKD applied. In most epidemiological studies, including in the UK, a single eGFR less than 60 ml/min/1.73m$^2$ has been used to define CKD. One study based on data from GP databases reported a prevalence of about 8% for CKD stages 3 to 5.[11] A more recent, population-based study found a similar prevalence of 5.2%.[12] Strictly, CKD diagnosis requires 2 eGFR readings below 60 ml/min/1.73m$^2$, at least 90 days apart. Applying this criteria, the Quality Improvement in Chronic Kidney Disease (QICKD) study, based on data from GP databases, quotes a prevalence in the UK adult population of 6.76% for CKD stages 3 to 5.[13, 14] The national CKD Audit in England and Wales reported a similar prevalence of 5.5%, based upon primary care coding records and biochemical data.[15]

CKD is a significant global health burden, and is of particular relevance in ageing populations. Understanding the outcomes and risks associated with CKD is therefore of importance both in identifying those at high risk, and in targeting interventions to reduce these risks. Similarly, in a resource limited

environment, sparing those at low risk from unnecessary interventions has benefits on both the individual and societal levels.

## 1.4 Causes of Chronic Kidney Disease

CKD should be regarded as a clinical syndrome that may result from many distinct pathological processes. The underlying cause of CKD varies depending on the population studied, but the most common cause in the UK and other developed countries is diabetic kidney disease.[16] Other common causes of CKD include renovascular disease, obstructive uropathy and intrinsic renal diseases, including glomerulonephritis and tubulointerstitial nephritis. Renal biopsy results and pathological diagnosis may separate different categories of glomerulonephritis. However, even within a single pathological diagnosis, there may be significant variation in outcomes. This is well characterised in IgA nephropathy with a range of pathological and clinical features associated with adverse outcomes.[17-19] Furthermore, the cause of CKD is not identified in a substantial proportion of patients.

## 1.5 Chronic Kidney Disease in Primary Care

Owing to its high prevalence, particularly in older people, CKD is a significant problem in primary care. In the UK, national guidance suggests that most people could be managed in primary care until they develop CKD stage 4, or unless their eGFR falls rapidly over time.[7] This implies that the majority of people with CKD are managed primarily by non-specialists. On the other hand, much research on CKD has been undertaken in secondary care cohorts, and focuses on risks of End Stage Kidney Disease (ESKD), which may be less important than other adverse outcomes in lower risk, primary care populations. There is therefore a lack of data on risk factors and outcomes associated with CKD, specifically relevant to the population managed in primary care.

Previous analysis from questionnaire data from the Renal Risk in Derby Cohort has shown that many people managed in primary care are unaware of being diagnosed with CKD.[20] Improving patient engagement and awareness may help outcomes and engagement with self-management in this group. People with CKD in primary care have many other health conditions and may be on multiple medications,[21, 22] which complicates their clinical management.

## 1.6 Outcomes in People with Chronic Kidney Disease

### 1.6.1 CKD Progression and End Stage Kidney Disease

Much research in nephrology has focussed on the development of End Stage Kidney Disease (ESKD). Clinical practice in nephrology clinics often focuses on the identification, education and preparation of individuals with high risk CKD for renal replacement therapy or renal transplantation. Often, these people have a clear diagnosis underlying their CKD, commonly including glomerulonephritis, diabetic nephropathy, or inherited conditions such as autosomal dominant polycystic kidney disease. Large epidemiological studies based upon meta-analysis of many CKD and general population cohorts from the CKD Prognosis Consortium have demonstrated significant associations between reduced eGFR and raised uACR and a range of adverse outcomes, including progression to ESKD, acute kidney injury (AKI), cardiovascular events, and all-cause mortality.[23-27] These analyses include individuals with a range of different underlying diagnoses, suggesting that there are common factors that contribute to CKD progression and other adverse outcomes regardless of diagnosis.

### 1.6.2 Definition of CKD progression

For many with CKD, renal function may decline slowly over many years. In order to evaluate risk it is necessary either to follow study participants for significant lengths of time, or to use surrogate endpoints, which associate with future adverse outcomes. For studies of CKD progression, development of ESKD, requiring renal replacement therapy, is the outcome most commonly used. Additionally, many studies have coupled this with a doubling of serum creatinine (corresponding change in eGFR is a 57% decline, using CKD EPI eGFR). Alternatively, some studies, including the MDRD study have evaluated the rate of GFR change over time.[28] Recent work from the CKD prognosis consortium has shown that a 30% decline in GFR over 2 years associates strongly (10 year risk of ESKD with 30% decline in eGFR over 2 years was 64%) with future development of ESKD and all-cause mortality and are more sensitive than more marked declines (57% decline) in detecting those at risk.[29] This would support the view that a moderate decline in GFR over a two-year follow-up period would identify individuals at higher risk of adverse outcomes. This is of use both for observational studies and clinical trials, as follow-up required to detect these changes will be shorter than requiring follow-up to ESKD.

Current KDIGO guidelines define CKD progression in terms of changes in both albuminuria and eGFR. A suggested definition of GFR progression is stated, (25% decline in renal function coupled with a worsening of GFR category) but there is no definition based upon change in albuminuria.[4] Whilst it is well known that higher levels of albuminuria associate with adverse renal outcomes, change in albuminuria has not been intensively studied as a risk factor for adverse outcomes. It would be possible to define CKD progression with respect to albuminuria in several different ways including development of clinically significant levels (e.g. urine protein to creatinine ratio (uPCR) >100 mg/mmol, uACR >50 or 70 mg/mmol), or increasing albuminuria category. Increasing albuminuria has been associated with increasing risk of incident CKD and rapidly progressive CKD in a cohort of predominantly (91%) diabetic US veterans.[30] Additionally, measurements from a Swedish cohort have demonstrated associations with a 4-fold increase of albuminuria and ESKD and all-cause mortality.[31]

### 1.6.3 Cardiovascular Disease and CKD

Chronic Kidney Disease has been well established as a risk factor for cardiovascular disease. In 2004, Go et al showed that reduced GFR independently associated with cardiovascular

events.[32] More recent work from the CKD Prognosis Consortium has shown similar increasing risk of cardiovascular events and cardiovascular mortality with lower baseline eGFR. Additionally this study showed increasing cardiovascular mortality with albuminuria as an independent risk factor.[33] The Chronic Renal Insufficiency Cohort (CRIC) study has also reported an association between heart failure and progression of CKD, defined as incident ESKD or a 50% decline in eGFR.[34]

Recent global epidemiological work has quantified the impact of CKD on cardiovascular outcomes. Thomas et al. found that 1.2 million deaths due to cardiovascular causes in 2013 were attributable to CKD.[35] In this study, cardiovascular outcomes were found to be more prevalent than progression to ESKD, perhaps due to greater numbers of people with mildly reduced GFR and low risk of progression. In populations with less marked reduction in GFR, cardiovascular outcomes are more frequent than progression to ESKD. In a study following a large Norwegian general population cohort, Hallan et al. demonstrated minimal progression to ESKD in those participants with a baseline GFR above 30 ml/min/1.73m$^2$. In comparison, mortality from cardiovascular disease was much

more common in those with a GFR <60 ml/min/1.73m$^2$ compared to those without CKD at baseline (3.5 versus 0.4 deaths per 100 person-years).[36] This demonstrates the importance of competing risks when considering prognosis in people with CKD.

### 1.6.4 Acute Kidney Injury

Acute kidney injury (AKI) represents a rapid decline in renal function, often associated with an acute illness and hospital admission. AKI is currently defined using change in creatinine levels or urine output criteria.[37] These parameters also allow the severity of renal injury to be measured. AKI is known to contribute to the development of chronic kidney disease. A meta-analysis of cohort studies showed a hazard ratio of 8.8 for development of CKD after AKI. Additionally, this depended upon the severity of AKI.[38] Mechanistically, this is likely to relate to the development of fibrosis after an acute injury. A recent prospective case-control study has demonstrated that, as well as contributing to reduction in eGFR, AKI may also contribute to development, and worsening of proteinuria.[39] Additionally, work from the CKD Prognosis Consortium has shown that both reduced eGFR and increasing albuminuria are independent risk factors for AKI.[25, 26] Current evidence

therefore suggests an interacting relationship between CKD and AKI, with each potentiating development of the other.[40]

## 1.7 Chronic Kidney Disease in Older People

The prevalence of CKD has been shown to increase with advancing age.[10, 41] A multicentre study in primary care reported the mean age of over 23,000 individuals with CKD at 75 years.[42] Age has been shown to be a modulating factor in terms of an individual's outcome in CKD. In a large cohort of American Veterans, O'Hare et al. showed that the eGFR at which the risk of ESKD drops below the risk of all-cause mortality declines with increasing age such that in those aged 65 to 74 years, the risk of ESKD exceeds the risk of death only when GFR falls below 15 ml/min/1.73m$^2$.[43] Renal structure and function change with increasing age, and these changes over time tend to reduce glomerular filtration rate. For example, increased numbers of sclerosed glomeruli were found in donor kidneys from individuals aged over 55 years compared to younger donors.[44] A recent, more comprehensive study of kidneys from living donors has reported a 7.3% reduction in the number of glomeruli for each decade increase in age.[45] There is ongoing debate regarding the diagnosis of CKD in older people. Some argue that the

application of KDIGO diagnostic criteria in older adults leads to diagnosing disease in those demonstrating normal physiological ageing.[5] Others, however, have argued that the reduction of GFR into CKD stage 3 ranges always carries increased risk, regardless of age.[6] The development of sarcopaenia in older people and its impact on creatinine levels may result in inaccuracy of the creatinine based estimating equations generated in younger populations. Equations have been developed, specifically for use in older people, to try and reduce this problem. These will be discussed in the next section.

## 1.8 Equations for Estimating Glomerular Filtration Rate

Diagnosis, monitoring and management of CKD require accurate measurement of renal function, and its change over time. The gold standard measurement of GFR requires intravenous injection of exogenous substances, such as inulin or iohexol. This is time-consuming and not suitable for routine clinical practice, and as such is rarely undertaken outside of research. As an alternative, the serum concentration of an endogenous marker of glomerular filtration can be used to estimate GFR. To be useful as a filtration marker, a substance should ideally be filtered freely at the glomerulus and neither

be absorbed or secreted by the nephron.[46] The most commonly used endogenous filtration marker is creatinine, but several others including cystatin C are also being investigated. Multiple equations to estimate GFR have been developed.

### 1.8.1 Creatinine-based Estimating Equations

Creatinine has been used in a range of methods to estimate renal function (creatinine clearance and glomerular filtration rate). Creatinine is a metabolite of creatine, and is formed at a constant rate in muscle tissue through a non-enzymatic reaction. Serum levels are dependent upon muscle mass, and hence can be dependent upon body habitus as well as GFR. In addition, creatinine is secreted by the renal tubule, and is not therefore a perfect glomerular filtration marker. Nevertheless, creatinine is simple and inexpensive to measure and therefore has become the most commonly used marker of GFR.

#### *1.8.1.1 MDRD Equation*

The MDRD equation was developed from the MDRD study population and includes age, gender, and ethnicity alongside serum creatinine.[47] This equation was subsequently modified for use with isotope dilution mass spectrometry (IDMS) standardised creatinine measurements.[48] The

original definition of CKD followed the development of this equation, as it allowed standardisation of measurement of renal function between individuals. The MDRD equation also made possible the automated reporting of eGFR values by laboratories, which contributed to a greater awareness of CKD worldwide. Nevertheless, the MDRD equation is known to over-estimate renal function in those with eGFR above 60 ml/min/1.73.$m^2$ and was not well validated in older people.[47, 49] The MDRD equation has subsequently been replaced by the CKD Epidemiology Consortium estimating equation (CKD EPI) in the KDIGO and NICE guidelines.[4, 7]

#### *1.8.1.2 CKD EPI Equation*

The CKD EPI estimating equation was described in 2009, and was derived from a series of cohort studies with participants more representative of the general population than the MDRD study. 5,504 participants were included in the development cohort and 2,750 in the internal validation cohort.[50] The CKD EPI equation includes the same variables as MDRD but large number of participants, and diverse clinical groups included resulted in an equation that was more accurate and evidenced less bias than MDRD at GFR ranges both above and below 60 ml/min/1.73$m^2$.

#### *1.8.1.3 Newer Creatinine-based Equations*

Several other groups have published attempts to improve accuracy of creatinine-based estimates of GFR. The Lund-Malmo revised (LMR) estimating equation was developed in a Swedish cohort, and is based upon serum creatinine, age and gender. In the development cohort, it was more accurate than CKD EPI.[51] More recently, the Full age spectrum (FAS) equation has been described. This uses a different theoretical approach than the CKD EPI equation, and describes standardised creatinine values for people of different ages.[52, 53] These equations have not yet found widespread use in clinical practice, and studies in general populations have not shown significant improvements compared to the CKD EPI equations.[54]

### 1.8.2 Cystatin C and Combined Creatinine-Cystatin C estimates

Whilst creatinine has been used for the assessment of renal function for many years, it is limited by its association with muscle mass. Cystatin C has been evaluated as a potential alternative endogenous filtration marker. Cystatin C is a 13.3 kDa protein, produced by all nucleated cells, and is therefore less dependent on muscle mass than creatinine. Grubb et al.

first described an equation using cystatin C as an endogenous filtration marker to estimate GFR in 2005.[55] This equation was reported to outperform the MDRD and the Schwartz equations across the full age range, including paediatric populations.[56] Cystatin C has been incorporated into new CKD-EPI equations, both independently and in conjunction with creatinine. The CKD EPI combined creatinine and cystatin C equation evidenced greater accuracy and precision than equations that included creatinine or cystatin C alone.[57]

More recent estimating equations involving cystatin C have been published. The Berlin Initiative Study (BIS) equations, including a combined creatinine and cystatin equation, and a creatinine only version were developed in a cohort of older adults (over 70 years), and has been externally validated.[58, 59] The authors describe improved classification using the combined creatinine-cystatin equation (BIS2), with respect to measured GFR when compared to the combined CKD EPI equation. Additionally, The FAS equation has also been extended to include cystatin C and combined creatinine-cystatin C versions.[60]

*1.8.2.1 Non-GFR Determinants of Cystatin C*

Cystatin C has been suggested as an alternative filtration marker because it is less dependent on muscle mass than creatinine.[61] Nevertheless, it has emerged that cystatin C concentration also has non-GFR determinants. Obesity, smoking, diabetes and inflammatory states have all been associated with serum cystatin C concentrations.[62-65] Cystatin C has also been considered as a risk marker in populations with and without kidney disease. This will be discussed in more detail below (Chapter 1.9.10).

### 1.8.3 Other Filtration Markers

Other potential endogenous filtration markers, including β2 microglobulin and beta-trace protein have been included in estimating equations in an attempt to find more accurate ways of estimating GFR. However, a combined estimating equation generated from these filtration markers was not more accurate than the CKD EPI combined creatinine and cystatin equation.[66]

### 1.8.4 Comparison of Estimating Equations

Comparison of estimating equations depends upon the population studied. Comparing LMR, FAS to CKD EPI

equations, in the CKD EPI validation cohort, Levey et al. found that none of these newer equations was more accurate in an diverse, American general adult cohort.[54] Additionally, the combined creatinine and cystatin CKD EPI equation did not perform worse to the BIS equations in a study of older people in Iceland.[67] Whilst there may be situations and populations in which alternative estimating equations for GFR may be useful, current evidence would support using CKD EPI estimating equations for the diagnosis and management of CKD.

## 1.9 Risk Factors in Chronic Kidney Disease

People with CKD are at risk of multiple adverse outcomes including death, progressive CKD, development of ESKD, AKI, and cardiovascular events. An understanding of risk factors that promote both development of CKD and its progression is key in understanding individual risk of adverse outcomes, and may additionally provide potential targets for disease modifying treatment. This section examines the range of factors that have been studied as markers of the different risks associated with CKD.

Many factors have been shown to be associated with the development and natural history of CKD. These can be considered as predisposing, initiating, and perpetuating factors.[68, 69] Predisposing factors include lower nephron number and older age. For example, low birth weight has been proposed to associate with lower nephron number, and therefore represents a predisposing factor for kidney disease. Birth weight as a measure of nephron endowment has been assessed recently in the Helsinki Birth Cohort Study, which found that both smaller size at birth and prematurity were associated with the development of CKD in adulthood.[70] Initiating factors include the development of a specific renal or urological condition (for example glomerulonephritis, or urinary obstruction). Factors perpetuating progression include albuminuria and hypertension. Many risk factors may fall under several of these categories, including AKI, which could be thought of as both an initiating and perpetuating risk.

### 1.9.1 Proteinuria and Albuminuria

Under normal conditions a small amount of albumin is filtered into the urine at the glomerulus but is subsequently reabsorbed in the proximal tubule.[71] The loss of protein in the urine is therefore an indicator of renal damage due to loss

of glomerular filtration barrier integrity or failure of tubular reabsorption. Albuminuria, typically measured as uACR, therefore forms part of the CKD diagnostic criteria. Though proteinuria has long been recognised as a marker of kidney damage, a large body of evidence has accumulated over the past two decades to support the hypothesis that proteinuria also contributes directly to CKD progression by provoking a proinflammatory and profibrotic phenotype in tubule cells that contributes to the development of progressive interstitial fibrosis. Proteinuria has been identified in multiple studies as a key risk factor for progressive renal disease.[72] Moreover, albuminuria has been identified in large meta-analyses as a powerful independent risk factor for AKI and cardiovascular disease.[26, 73]

Proteinuria has been well established as a risk factor for the progression of CKD. Results from the CKD Prognosis Consortium have shown strong associations between proteinuria and adverse outcomes including ESKD, all-cause mortality cardiovascular events (CVEs), and AKI.[24, 25, 73] Reduction of proteinuria through inhibition of the renin-angiotensin system, most commonly using angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor

blockers (ARBs) remains a key therapeutic strategy, and many randomised controlled trials have shown significant benefit in slowing progression of CKD in both diabetic and non-diabetic groups.[74-77]

Clinically, proteinuria can be quickly detected using urine dipsticks but it should be remembered that this provides a qualitative, not a quantitative result and the use of dipsticks to detect proteinuria is specifically discouraged in the NICE guideline.[7] Nevertheless, proteinuria detected by urine dipstick has been shown to predict the development of ESKD.[78] By contrast, in the same study, the detection of haematuria by dipstick was associated with little increased risk for development of ESKD.

### 1.9.2 Hypertension

Hypertension is both a cause and a consequence of renal disease. In the MDRD study, treatment of blood pressure to a low blood pressure target (mean arterial pressure (MAP) <92 mmHg) versus a higher target (MAP <107 mmHg) was not associated with any initial benefit in renal outcomes except in a subgroup with proteinuria of greater than 1 g/day at baseline,[72] but after long-term post-trial follow-up the low

blood pressure target was associated with improved renal survival.[79] Earlier analyses of the MDRD study suggested that tight blood pressure control can delay increases in proteinuria seen in those with less stringent control.[80] More recently, the Systolic Blood Pressure Intervention Trial (SPRINT) showed, in people aged over 50 years with and without CKD, that lower blood pressure targets in older people (mean age 68 years) lead to fewer cardiovascular events in the trial population. Renal endpoints, including progression to ESKD were studied, but a difference between those randomised to lower (Systolic blood pressure (SBP) <120 mmHg) versus standard (SBP <140 mmHg) blood pressure targets was not seen.[81] In a recent subgroup analysis of SPRINT that included only participants with CKD confirmed that the lower systolic blood pressure target was associated with lower all-cause mortality (HR, 0.72; 95% CI, 0.53 to 0.99) and risk of CVE (HR, 0.81; 95% CI, 0.63 to 1.05). No increase in serious adverse events was observed, though the lower SBP target was associated with a greater decrease in GFR after 6 months (-0.47 versus -0.32 ml/min/1.73 $m^2$ per year) and the incidence of AKI, hypokalaemia and hyperkalaemia were increased.[82]

ACE inhibitors and ARBs have been previously discussed in the context of proteinuria, in that they provide additional renoprotective and antiproteinuric effect above what would be expected for their effect on blood pressure. These drugs are therefore regarded as first line treatment for hypertension in the context of proteinuria.

### 1.9.3 Serum Bicarbonate and Acidosis

Serum bicarbonate is a marker of metabolic acidosis and is commonly measured in people with CKD. This is of interest both from a clinical and a research perspective as acidosis is potentially modifiable through administration of oral sodium bicarbonate. Bicarbonate has also been associated with progression of CKD, and potentially reflects tubular dysfunction and tubulointerstitial atrophy. Several studies have shown benefit of bicarbonate supplementation, suggesting that acidosis does promote CKD progression.[83-85] Additionally, bicarbonate was found to be a predictor of ESKD in the Kidney Failure Risk Equations, which will be further described below.[86]

Abnormalities of serum bicarbonate have also been shown to associate with all-cause mortality. Kovesdy et al. found a U-

shaped association of with mortality, with the lowest risk of death occurring in those with serum bicarbonate between 26 and 29 mmol/l.[87] In the CRIC study, lower serum bicarbonate was associated with progression of renal disease, but no association with mortality was seen.[85] In further work, looking at bicarbonate levels throughout the study follow-up period, the same group did show associations between serum bicarbonate persistently above 26 mmol/l, all-cause mortality, and episodes of heart failure.[88]

### 1.9.4 Haemoglobin

Through the production of erythropoietin in response to tissue hypoxia, the kidney is directly involved in erythropoiesis. It is therefore not surprising that lower haemoglobin concentration has been linked to progressive renal disease. Analysis of the Reduction in Endpoints in Non-Insulin Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) study cohort showed increased risk of progression to ESKD in type 2 diabetics with CKD even with mild levels of anaemia [89]. Similarly to bicarbonate, haemoglobin reflects a function of the kidney not directly incorporated in measurements of glomerular filtration. Physiologically, this reflects the ability of the kidney to produce erythropoietin, a hormone that

stimulates production of erythrocytes. Lower haemoglobin could therefore be seen as a marker of increased tubulointerstitial injury. Alternatively, lower haemoglobin may also reflect other health conditions, which could also impact upon risk of renal progression.

### 1.9.5 Diabetes Mellitus

Diabetes mellitus is the commonest cause of incident ESKD in the UK [90] and other developed countries. Its role as a key risk factor and cause of CKD is well established. Diabetes is typically associated with specific histological features termed diabetic nephropathy but it has also become clear that kidney disease due to a variety of other causes may be present in those with diabetes.[91] Glycaemic control has been shown in randomised controlled trials to be important in reducing the incidence of diabetic nephropathy in both type 1 and type 2 diabetics.[92, 93]

### 1.9.6 Dyslipidaemia

Dyslipidaemias are well known to increase the risk of cardiovascular disease [94]. In some studies, higher triglyceride levels and lower high density lipoprotein (HDL) cholesterol levels are found to be a risk factor for CKD

progression.[95, 96] These findings have not been universally confirmed, however.[97] Additionally, more recent results from the CRIC study have suggested no association between HDL, low-density lipoprotein (LDL) and triglyceride levels and progression of renal disease at 4 years median follow-up (CKD progression defined as development of ESKD or a 50% loss of GFR). However, in a subgroup analysis of people with low levels of proteinuria, (<0.2 g/day) LDL cholesterol was inversely associated with progression (higher levels found to be beneficial).[98]

There is some evidence for the role of treatment of dyslipidaemia in reduction of risk of cardiovascular events in people with CKD. The Study of Heart and Renal Protection (SHARP) compared the use of simvastatin and ezetimibe versus placebo as lipid-lowering agents in people with CKD (mean eGFR = 27 ml/min/1.73m$^2$). The SHARP trial did not show a benefit in terms of reducing progression to ESKD, but the treatment arm did evidence reduced incidence of atherosclerotic CVE.[99]

### 1.9.7 Smoking

Whilst smoking is well established as a risk factor for cardiovascular disease, there is mixed evidence for the role of smoking as a potentially modifiable risk factor for progression in CKD. In a recently published analysis of data collected as part of the SHARP study, smoking was associated with an increased risk of cardiovascular disease, cancer and all-cause mortality, but did not associate with ESKD. Additionally, rates of change of eGFR were similar in current, previous and never smokers across different groups split by baseline eGFR.[100] This is in contrast with results from the CRIC study, in which non-smokers evidenced a reduced rate of progressive renal disease.[101]

### 1.9.8 Obesity

Obesity, defined using body mass index (BMI), is well known to be associated with cardiovascular risk and mortality. Obesity has also been associated with the development of CKD,[102] and its progression to ESKD.[103] Waist to hip ratio has been used alternatively to define central obesity and avoid problems associated with BMI due to different body compositions. For example, a lean individual with a high muscle mass may be characterised as obese through BMI

based-definitions. In a previous analysis of the RRID cohort, Evans et al. showed a greater association between WHR and other CKD risk factors than direct BMI measurement.[104]

### 1.9.9 Uric Acid

Uric acid is the end result of purine metabolism, and has been associated with cardiovascular and renal risk. Small trials have shown that lowering of uric acid with allopurinol[105] or febuxostat[106] is associated with reduced risk of progressive renal disease. However, both trials were small (113 and 93 participants respectively), and results must therefore be interpreted with caution. Recent work from the CRIC cohort suggests that uric acid is a risk marker for progressive CKD in people with CKD stage 3.[107]

### 1.9.10 Cystatin C

Cystatin C is a low molecular weight protease inhibitor that is produced by all nucleated cells. Cystatin C has previously been considered as an endogenous filtration marker in estimating equations of GFR in section 1.8.2. Additionally, cystatin C has been identified as a marker of risk, which has underpinned the recommendations in national and international guidance that it be used in the diagnosis of CKD stage 3a in those with no

proteinuria.[108] Cystatin C has been described as a marker of risk of all-cause mortality in healthy older adults (aged 70 to 79 years at study recruitment),[109] and in those with heart failure.[110] Shlipak et al. analysed cystatin C independently and in conjunction with creatinine, using the CKD EPI equations in a meta-analysis of cohort studies within the CKD prognosis consortium. They reported improvements in association between GFR and both all-cause and cardiovascular mortality. Cystatin C did not, however, benefit reclassification in terms of risk prediction for ESKD.[111] It should be noted that many of the non GFR determinants of cystatin C that were considered previously are also cardiovascular risk factors, which may explain its closer association with mortality and CVE compared to creatinine.

### 1.9.11 Fibroblast Growth Factor 23 and Markers of Mineral Bone Disease

Fibroblast Growth Factor 23 (FGF23) is a protein hormone secreted by osteoblasts. It promotes phosphate excretion in the urine, and becomes elevated with declining renal function, possibly as a homeostatic response to maintain phosphate balance. FGF23 was initially identified in rare hypophosphataemic syndromes[112, 113] and was

subsequently found to be independently associated with increased mortality in patients undergoing haemodialysis.[114] Analysis of data from the CRIC study showed an independent association between FGF23 levels and all-cause mortality.[115] Increased levels of FGF23 have also been shown to be associated with mortality in those with coronary artery disease[116] and in kidney transplant recipients.[117] The CRIC study team have also described an association between FGF23 and left ventricular hypertrophy (LVH), with higher rates of LVH seen in those with higher levels of FGF23. Additionally, high levels of FGF23 were associated with an increased risk of developing LVH at 2.9 years of follow-up.[118] This paper also reports results of animal experiments showing LVH in rats injected with FGF23, coupled with an increase in cell size and actin expression. This was demonstrated to be independent of klotho, a previously described co-receptor for FGF23.[119]

In some cohorts, FGF23 has been shown to be associated with CKD progression. In a small cohort of people with non-diabetic CKD, Fliser et al. described an association between higher levels of FGF23 and progression to ESKD or doubling of serum creatinine.[120] In a larger analysis from the Homocysteine in

Kidney and End Stage Renal Disease study, a population of 1,099 with advanced CKD (median eGFR = 18ml/min/1.73m$^2$) also evidenced an association between higher levels of FGF23 and CKD progression.[121]

Parathyroid Hormone (PTH) is, like FGF23, a phosphaturic hormone that is elevated in people with CKD. In early CKD, potentially both PTH and FGF23 play a role in maintaining phosphate balance. Earlier analysis from the RRID study suggests that vitamin D status determines whether PTH or FGF23 was elevated primarily. Vitamin D deficiency was found to be associated with PTH elevation, whereas those who were vitamin D replete tended to have elevated FGF23 levels.[122]

### 1.9.12 Advanced Glycation End Products

Advanced glycation end-products (AGE) are the end result of series of non-enzymatic reactions, collectively termed the Maillard reaction.[123, 124] Factors promoting tissue AGE accumulation include hyperglycaemia, oxidative stress,[124] tobacco smoking and reduced renal clearance of AGE precursors. AGE precursors are present in the diet at varying levels depending on food types and cooking methods. Cooking at high temperatures leads to AGE formation in food. The mechanisms by which AGE may affect physiological systems

include both intracellular and extracellular mechanisms. A cell surface receptor for AGE (RAGE) has been identified, which promotes production of inflammatory cytokines.[125] Extracellular effects are medicated through the structural modification of proteins in the extracellular matrix, including collagen. This reduces elasticity, and therefore is of particular importance in the walls of elastic arteries.

In people with diabetes mellitus, AGE accumulation has been shown to be a marker of risk for diabetic complications. In a subgroup from the Diabetes Control and Complications Trial, AGE measured from skin biopsy samples was strongly associated with complications of diabetes, including nephropathy, defined by albuminuria.[126]

AGE can be measured directly on skin biopsy but skin autofluorescence (SAF) has been proposed as a non-invasive method of measuring tissue AGE. It has been validated and found to be reliable compared to direct measurement of tissue AGE.[127] SAF measurements can be taken quickly in a clinical setting, and could be used within a consultation. Higher SAF has been shown to predict mortality in patients on haemodialysis.[128] Higher levels have been found in people

on haemodialysis, compared to those with CKD who have not progressed to ESKD and those who have received a transplant. As many transplant patients in this study were previously exposed to haemodialysis, these data also suggest that AGE levels may be modifiable.[129] The RRID study has previously shown that whilst a single SAF reading is associated with all-cause mortality in people with CKD stage 3 after a median of 3.6 years of follow-up, the association became non-significant when adjusted for known markers of risk, including eGFR and uACR.[130]

It is proposed that vascular disease and in particular, arterial stiffness, plays an important part in the progression of renal disease.[131] Therefore, as AGE accumulation is associated with worsening several vascular parameters, SAF may have a role in risk prediction in CKD. However, as well as showing that it is of use in identifying those at higher risk of negative outcomes (cardiovascular events, progressive renal disease and mortality), it would be important to investigate whether interventions are able to modify SAF and, in the longer term, have a beneficial effect on these outcomes.

Tanaka et al. showed that, in a Japanese cohort, SAF was associated with progression of chronic kidney disease. This study included people with a wide range of baseline renal function.[132] These results have not been confirmed in other populations, however. Single SAF readings have been associated with progressive renal disease and cardiovascular outcomes but it is not yet known whether changes in SAF over time may also contribute to risk prediction. SAF may also provide a future outcome measure for interventional studies. However more understanding of the relationship between SAF and outcomes in CKD is required before SAF could be considered as an outcome measure.

### 1.9.13 Free Light Chains

Light chains are a normal component of immunoglobulins and are produced by leucocytes of the B-cell lineage. Each B cell produces either a kappa ($\kappa$) or lambda ($\lambda$) light chain isotype. Whilst the majority of light chains are incorporated into immunoglobulins, a proportion are detectable in the serum independently as free light chains (FLC). These are largely excreted via the kidneys. Combined ($\kappa$ and $\lambda$) serum FLC concentration is increased in conditions associated with inflammation. Previous studies, including an analysis of data

from the RRID study, have shown associations between λ FLC[133] or total FLC[134, 135] and mortality. Associations between FLC and both mortality and progression to ESKD were confirmed in a recent meta-analysis.[136]

### 1.9.14 Soluble Urokinase Plasminogen Activator Receptor

Recent work has shown that soluble urokinase-type plasminogen activator receptor (suPAR) has been associated with increased renal function decline and development of incident CKD.[137] suPAR is the soluble form of a receptor commonly found on immune cells, endothelial cells, and podocytes. This receptor has been shown to be involved in the adhesion and migration of leucocytes across the endothelial barrier, suggesting a potential role in glomerular pathology. In mouse models, suPAR has been shown to activate podocyte $\beta_3$ integrins, resulting in a focal segmental glomerulosclerosis (FSGS)-like glomerulopathy.[138] Schulz et al. found that higher suPAR levels at baseline were associated with increased rates of progression and incident CKD in a large cohort of healthy individuals.[139] This has also been demonstrated in a paediatric cohort.[140]

### 1.9.15 Arterial Stiffness

Many factors may affect renal function through their effects on the cardiovascular system. Arterial stiffness is a measurement of the elasticity of the large arteries in the body. This is closely associated with atherosclerosis and vascular disease. Arterial Stiffness is known to associate with mortality in patients with ESKD.[141] In the CKD population, arterial stiffness correlates closely with age.[142] Increasing arterial stiffness may also exacerbate renal function decline as it reduces damping of the pulse wave form throughout the arterial tree, exposing the renal vasculature to an increased pulse pressure. Some studies have shown a relationship between arterial stiffness and progression of CKD.[131, 143] The methods used to measure arterial stiffness are important and varied. We have used pulse wave velocity (PWV) as a method of assessing arterial stiffness. This is non-invasive and can be carried out quickly and reliably with minimal equipment. In a European expert consensus document, PWV was considered as the gold standard for measurement of arterial stiffness.[144]

### 1.9.16 Genetic factors

Epidemiological studies have shown that CKD progression occurs with different frequencies in people of different

ethnicities. Technological advances, including whole genome sequencing, have led to an increasing range of genes that have association with the development and progression of CKD.[145] For example, the APOL1 gene has been shown to have two high-risk variants that associate with an increased risk of progressive CKD in the African American population.[146] Recently, an interaction between suPAR and the high-risk alleles, promoting activation of $\alpha_v\beta_3$ integrin signalling, has been described.[147] This provides both a potential mechanism by which APOL1 gene variants promote CKD, and a demonstration of the interaction between genetic and other risk factors. Other single gene polymorphisms have been identified to associate with CKD progression and include GLUT9, a gene encoding a uric acid transporter,[148] and RAGE, the receptor for AGEs.[149] Both of these genetic factors may influence CKD progression in relation to factors and mechanisms discussed elsewhere in this chapter.

There has been some attempt to incorporate genetic testing into risk prediction for incident CKD. Ma et al. developed a genetic risk score based upon 53 single nucleotide polymorphisms known to associate with CKD in the

Framingham original and Framingham offspring cohorts. However, this did not improve on clinical risk prediction.[150]

Epigenetics describes changes in gene expression linked to environmental factors, rather than the direct alteration of the nucleotide code. Deoxyribonucleic acid (DNA) methylation is one example of an epigenetic factor that affects gene expression.[151] Methylation typically reduces gene expression, whereas active genes are typically hypo- or un-methylated. DNA methylation has been evaluated in CKD by the CRIC study investigators, who found differences in the patterns of DNA methylation between individuals with rapidly progressive CKD compared to those with more stable disease.[152]

There is good evidence that genetic factors play an important part in predisposing individuals towards the development of CKD. Additionally, genetic analysis has huge potential to improve knowledge of the mechanisms of CKD, the identification of individuals at risk, and potential therapeutic targets. However, currently this is not developed to a level where it is useful in clinical practice.

### 1.9.17 Newer Strategies for Assessing Risk

Metabolomics (studying the whole range of metabolites present in a biological sample) and proteomics (studying the multiple proteins present in a biological sample) have both been investigated in the context of CKD progression. Additionally, multiple panels of biomarkers have been used in conjunction to identify high-risk individuals in large populations. Good et. al identified a panel of 273 urinary proteins that predict the presence of CKD with 85.5% sensitivity and 100% specificity in a test cohort.[153] Proteomic strategies have also been used to identify those at high risk of progressive CKD. Looker et al. identified 62 biomarkers associated with rapid decline of renal function in people with type 2 diabetes and CKD stage 3, and developed a panel of 14 biomarkers associated with an increase in AUROC of predictive models from 0.706 based on clinical characteristics to 0.868.[154]

## 1.10 Risk Prediction in Chronic Kidney Disease

Risk factors become clinically useful through the identification of individuals of high risk who may benefit from early intervention in the course of their disease. Conversely, accurate risk prediction allows for reassurance of those at low

risk of harm. There have been several attempts at generating risk prediction equations for patients with CKD, based upon the results of multivariable regression analysis in trial cohorts, which are subsequently extrapolated to other groups. Most commonly used at present is the Kidney Failure Risk Equation (KFRE), developed by Tangri et al.[86] This paper presents multiple models for prediction of risk of developing End Stage Kidney Disease; the most simple of these includes eGFR, gender, age, and albuminuria as predictors. Addition of risk factors, including phosphate, albumin, bicarbonate, and calcium, increased slightly the accuracy of this equation. Additionally, the KFRE has been shown to predict progression in a paediatric cohort.[155] Similar predictors were used in risk scores from the CKD risk in Birmingham (CRIB) study although this study derived risk equations in a much smaller cohort.[156]

The extrapolation of risk scores beyond the population in which they were developed is often problematic, as different groups may display different levels of risk compared to the population in which they were developed. The KFRE risk equation was developed using data from two Canadian cohorts of people with CKD stages 3-5, who had been referred to

nephrologists. Whilst this equation has subsequently been validated in a large meta-analysis of data derived from multiple international cohorts in the CKD Prognosis Consortium,[157] it may be less useful in discriminating risk early in the course of CKD, when 2- and 5 year risk of requiring dialysis is low, but some patients are at risk of developing ESKD in the longer term. This would be typical for many patients with CKD stage 3 who are managed in primary care. Additionally, the KFRE predicts only a single adverse outcome, and may overestimate risk of requiring dialysis in those who have high mortality risk. The KFRE has also recently been published in a format that incorporates multiple creatinine readings to account for the changing pattern of risk over time.[158]

Other risk scores have been generated in a range of different settings. One group have developed a risk prediction tool for progressive chronic kidney disease in primary care, and reported a risk prediction equation for loss of 7.5 ml/min/1.73m$^2$ of eGFR over 3 years.[159] This study was based upon a retrospective analysis of data, and included both those with established CKD and those at high risk of developing kidney disease. AKI has been shown to link with

progression of CKD. A risk score has been developed to identify those at high risk of developing advanced kidney disease (defined as two measurements of eGFR below 30 ml/min/1.73m$^2$ more than 30 days apart) in the year after discharge. Older age, female sex, lower baseline eGFR, higher levels of albuminuria, severity of AKI and higher serum creatinine levels at discharge were found to associate with a greater chance of advanced CKD after AKI.[160] This risk prediction equation was developed in a large Canadian population (9,973 participants were included in the development cohort), however the period of time participants were followed was short at 1 year. This may be useful, as the authors state, in identifying individuals at risk of advanced CKD in the short term. It did not, however, assess the long-term risk of CKD progression or ESKD risk. Both of these factors may be affected by episodes of AKI.

Risk prediction in CKD is developing, with many potential risk markers identified and several risk prediction equations published. However, risk prediction has yet to be used widely in the clinical setting, or to be incorporated into national and international guidance. Furthermore, it is not widely utilised in primary care, and may potentially be a valuable tool in

decisions regarding referral, or to explain future risks to patients.

## 1.11 Summary

Chronic Kidney disease is a common condition that covers a wide range of clinical diagnoses and severity. In the UK, the majority of people are managed in primary care but much of the evidence underlying our understanding of CKD comes from secondary care referred populations. This is important because outcomes in CKD are distinct according to the population studied. The Renal Risk in Derby study has sought to address the knowledge gap regarding CKD in primary care to provide data on outcomes over 5 years to better inform patient care.

# 2. Research Questions and Aims of this Thesis

This thesis will address the following research questions:

**What are the 5 year Outcomes of CKD stage 3 in a primary care cohort?**

Much research on CKD has focussed on populations referred to secondary care, however the majority of people with CKD have CKD stage 3 and, in the UK are managed in primary care. Results chapter 1 will examine outcomes in a primary care cohort over 5 years of follow-up as well as risk factors associated with these outcomes.

**Is the use of cystatin C in primary care, according to national and international guidelines, beneficial in the diagnosis and risk assessment of people with CKD stage 3?**

NICE and KDIGO guidance has suggested using cystatin C to confirm a diagnosis of CKD in people with CKD stage 3a disease with no proteinuria. This strategy has not been previously evaluated in a primary care population. Results chapter 2 will evaluate the use of cystatin C both as a filtration marker in the diagnosis of CKD, and as a marker of risk in a primary care population.

**Can markers of mineral bone disease be used to assess risk in people with CKD stage 3 in primary care?**

Much recent work has focussed on FGF23 as a marker of cardiovascular and renal risk in populations with CKD. This chapter will examine FGF23, vitamin D and PTH as markers of risk in a primary care cohort.

**Can skin autofluorescence be used to non-invasively assess risk in people with CKD stage 3 in primary care?**

Advanced glycation end-products (AGEs) have been examined as a risk marker in different populations, and have been shown to predict adverse outcomes in people receiving maintenance haemodialysis. AGEs can be measured non-invasively using skin autofluorescence that could be quickly assessed in a primary care consultation. Results chapter 4 will investigate the additional benefit this measurement provides in a primary care population.

# 3. Methods

## 3.1 Study Design

The Renal Risk in Derby (RRID) study is a prospective cohort study of people with CKD stage 3 recruited from primary care across Derbyshire. It was designed to measure outcomes and investigate risk prediction strategies specifically in this population.

## 3.2 Recruitment of Participants

Study participants were individually recruited from 32 general practices across Derbyshire between 2008 and 2010. Participating general practices were asked to invite people over 18 years of age with CKD stage 3 from CKD registers. 8,280 people were invited to take part in the study by post. 1,822 people attended for baseline recruitment visits. Participants were selected using eGFR values calculated using the 4-variable MDRD equation modified for use with isotope dilution mass spectrometry (IDMS)-standardised creatinine measurement. Two eGFR readings more than 90 days apart in the range 30 to 59 ml/min/1.73m$^2$ were required to be eligible. Of the 1,822 people who attended baseline visits, 1,741 were suitable from recruitment into the study cohort. People with a previous renal or other solid organ transplant,

those with an expected life-expectancy of less than one year and those who were unable to attend the baseline visit at their general practice surgery were excluded from the study.

## 3.3 Study Visits

Study visits were scheduled to take place at baseline, year 1 and year 5. Study visits took place predominantly at the participants' general practice surgery, and were planned to last 30 minutes. Before the 1 and 5 year follow-up periods, participants received a written letter reminding them of the study, and asking them to telephone a central office to make an appointment for their study visit. Participants who did not respond received a telephone call, again inviting them to make an appointment. Prior to each visit, participants completed a background questionnaire covering demographics, medical history, smoking history, and medication history. Participants' responses were reviewed during the study visit and clarified as appropriate.

Each participant was asked to bring 3 consecutive days' early morning urine samples, having stored these in a refrigerator prior to coming to the study visit. The most recent sample was tested by urine dipstick (Combur test, Roche) and urine

culture was performed for any participants with evidence of haematuria on urine dipstick. All three urine samples were analysed for total protein, albumin, electrolytes, uPCR and uACR. Blood samples for biochemistry and haematology were taken from each participant. Additional serum and plasma samples were collected and stored in -80°C freezers for future analysis. Participants were asked to avoid eating meat for 12 hours prior to their study visit to avoid confounding their serum creatinine results.[161]

At each study visit, participants' height (measured without shoes) weight, waist- and hip circumference were measured. Waist circumference was measured at the narrowest waist level, or at the mid point between the lower border of the ribs and the anterior superior iliac spine. Hip circumference was measured at the widest point around the gluteal muscles and hips. Three blood pressure measurements were taken at each study visit using an automatic oscillometric device (UA-767 Plus 30, A&D Medical). Readings were taken after at least 5 minutes rest, and were repeated so that values were different by no more than 10%.

SAF was measured at each study visit using an AGE reader (Diagnoptics Technologies B.V. Groningen, the Netherlands). 3 readings were taken from the skin of the forearm at each visit, and a mean calculated. Areas of tattooed or heavily pigmented skin were avoided.

Additionally, although not analysed in this thesis, Pulse Wave Velocity (PWV) was measured using a Vicorder (Smart medical) At the year 5 visits, additional data was collected regarding the functional status of each participant. Each participant was scored on the Karnofsky scale,[162] based upon the information provided in the background questionnaire and on direct questioning. Participants completed the 5 level EQ-5D questionnaire[163] and completed a timed sit to stand 5 test (time taken to stand 5 times from a chair of standard height with arms crossed across the chest).[164] Mid upper arm circumference was measured around the widest point of the upper arm. Gait speed was assessed using the time taken to cover a distance between two points measured to be 4 metres apart. Participants were instructed to walk between the two points at their normal walking speed. The four-metre walk was repeated twice, and

the mean time used to calculate gait speed.[165] These data will be analysed by other investigators.

## 3.4 Laboratory Methods

Study blood and urine samples were analysed at a single clinical laboratory at the Royal Derby Hospital. Creatinine was measured using the Jaffe reaction and was standardised to isotope dilution mass spectrometry (IDMS) methods. Additionally, serum was analysed for standard electrolytes and bone profile. A full blood count was undertaken for all participants. uACR and uPCR were measured from all three urine samples from each participant, and a mean value was used for analysis.

## 3.5 Review of Results

Following the study visit, all results were reviewed by the study principal investigator. Advice letters, including specific actions to be taken based on NICE guidance for the management of CKD, were written to general practitioners for all participants. Additionally, the blood results of each participant were screened to identify any episode of AKI, defined by KDIGO eGFR criteria.[37]

## 3.6 Endpoint Definitions

### 3.6.1 CKD Progression

The RRID study pre-specified endpoint for progressive renal disease was defined as development of ESKD or doubling of serum creatinine. This was met in very few participants by the end of the 5 year follow-up period. Therefore, these analyses use a definition for CKD progression based upon the KDIGO guidelines.[4] These state that progression should be based upon worsening of GFR or increasing uACR. CKD progression was therefore defined as a 25% decline in GFR coupled with a worsening of GFR category, or an increase in albuminuria category.

## 3.7 Clinical Definitions

Hypertension was defined as systolic blood pressure greater than 140 mmHg, or diastolic blood pressure greater than 90 mmHg, or treatment with antihypertensive medication. Previous cardiovascular disease included those who had previously suffered from a stroke, transient ischaemic attack, myocardial infarction or required a coronary artery bypass or percutaneous coronary intervention.

## 3.8 Statistical Methods

Detailed statistical methods used are presented in each results chapter. Results were recorded onto case report forms (CRFs) at baseline, year 1 and year 5 visits (Year 5 CRF is given in Appendix 1). Subsequently, these data were entered into a Microsoft access database, and then transferred into SPSS version 22 (IBM corporation, NY, USA) for analysis. Prior to analysis, the data were thoroughly checked for inaccuracies.

Continuous variables were assessed graphically for their approximation to a normal distribution. Several continuous variables, including uACR were positively skewed, and were therefore logarithmically transformed prior to multivariable analysis. Normally distributed variables were compared at different time points using paired t-tests. Non-parametric variables instead used Mann-Witney U tests.

### 3.8.1 Multivariable analysis

Binomial logistic regression was used to model outcomes such as CKD progression where no time to event data was available. Survival analysis was undertaken using Cox proportional Hazards models. Categorical variables were used

to plot survival curves to ensure the proportional hazards assumption was met.

## 3.9 Ethical Approval

Prior to study commencement, ethical approval was obtained from the Nottingham Research Ethics Committee (REC) 1. This study is included on the National Institute for Health Research (NIHR) clinical research portfolio (NIHR Study ID 6632). All participants gave written informed written consent at recruitment. All participants attending year 5 visits again gave written consent, following protocol amendments to include measures of functional status. The Nottingham REC 1 approved all protocol amendments. The study follows the principles of Good Clinical Practice and is in accordance with the Declaration of Helsinki.

## 3.10 Outcome Data

The RRID study cohort is currently linked to NHS Digital (Previously the Health and Social Care Information Centre). NHS Digital provides details on date and cause of death (as given on death certificates) for all study participants. Additionally, application was made to NHS digital for Hospital Episode Statistics (HES) data. Data has been requested with

the intention of studying episodes of AKI within the study population, and additionally to provide detail of cardiovascular events sustained by the study participants, which will be examined in subsequent analyses.

## 3.11 Socio-economic Status

All participants' socioeconomic status was measured using postcode and the Index of Multiple Deprivation (IMD) 2007 measure. The IMD covers several domains including income, employment, housing, health and disability, and assigns a numeric score to an area based on postcode. This score is derived from national statistics, local government data and census data. Postcodes were collected at baseline from general practice records. Addresses were confirmed with study participants at time of arranging a baseline visit.

## 3.12 Study Funding

Funding for the Year-5 visits and cystatin-C analysis was obtained from the Dunhill Medical Trust. Previous funding includes a joint Kidney Research UK/British Renal Society Research Fellowship (To Natasha McIntyre) and an unrestricted educational grant from Roche products. No

external organisation had input into study design or data analysis.

# 4. Results Chapter 1

# Five Year Outcomes from a Prospective Cohort Study of People with CKD Stage 3 in Primary Care

## 4.1 Abstract

### 4.1.1 Introduction

Chronic kidney disease (CKD) is commonly managed in primary care but most guidelines have a secondary care perspective emphasizing the risk of end-stage kidney disease (ESKD) and need for renal replacement therapy. This chapter aims to describe outcomes in the Renal Risk in Derby (RRID) cohort over 5 years, and to evaluate common baseline factors that predict these outcomes with a view to evaluating risk.

### 4.1.2 Methods

1,741 people with CKD stage 3 were individually recruited from 32 local primary care practices. Study visits were undertaken at baseline, year 1 and year 5. Binomial logistic regression and Cox proportional hazards models were used to model progression, CKD remission, and all-cause mortality. CKD progression was defined based on KDIGO criteria. CKD remission was defined as absence of diagnostic criteria (eGFR >60 ml/min/1.73m$^2$ and uACR <3 mg/mmol) at any study visit.

### 4.1.3 Results

Participants were predominantly elderly (mean age 72.9 ± 9.0 years) with relatively mild reduction in GFR (mean eGFR 53.5 ± 11.8 mL/min/1,73m$^2$) and a low prevalence of albuminuria (16.9%). After five years, 247 participants (14.2%) had died, most of cardiovascular causes. Only four (0.2%) developed ESKD but 308 (17.7%) evidenced CKD progression by KDIGO criteria. Stable CKD was observed in 593 participants (34.1%) and 336 (19.3%) met the criteria for remission. Remission at baseline and year one was associated with a high likelihood of remission at year 5 (OR = 23.6, 95% CI 16.5-33.9 relative to participants with no remission at baseline and year 1 study visits). Multivariable analyses confirmed estimated glomerular filtration rate (eGFR) and albuminuria as key risk factors for predicting adverse as well as positive outcomes. Limitations of this study include reliance on GFR estimated using the MDRD equation for recruitment (but not subsequent analysis) and a study population that was predominantly Caucasian and elderly, implying that the results may not be directly applicable to younger populations, or populations of more diverse ethnicity.

### 4.1.4 Conclusions

Management of CKD in primary care should focus principally on identifying the minority of people at high risk, to allow

intervention to slow CKD progression and reduce cardiovascular events. Efforts should also be made to identify and reassure the majority who are at low risk of progression to ESKD. Consideration should be given to adopting an age-calibrated definition of CKD to avoid labelling a large group of people with age-related decline in GFR and low associated risk as having CKD.

Results presented in this chapter have been published previously in the article *'Chronic Kidney Disease in Primary Care: Outcomes after Five Years in a Prospective Cohort Study' PLoS Medicine 13(9):e1002128.* A Shardlow, NJ McIntyre, RJ Fluck, CW McIntyre and MW Taal (2016)[166]. These results were also presented in oral abstract form at EDTA-ERA and UK Kidney Week Conferences 2016.

## 4.2 Introduction

In the United Kingdom and many other countries, the majority of people with chronic kidney disease (CKD) are diagnosed and managed in primary care clinics without ever being referred to a nephrologist. In contrast, most detailed studies investigating the risks associated with CKD have been led by nephrologists, resulting in a predominantly secondary care perspective. Consequently, guidelines based on these studies tend to emphasize the risk of end-stage kidney disease (ESKD) and the need for timely referral to facilitate preparation for renal replacement therapy. Landmark epidemiological studies have highlighted the importance of diagnosing CKD because the abnormalities that define CKD (reduced glomerular filtration rate (GFR) and albuminuria) are powerful independent risk factors for multiple adverse outcomes including progression of CKD, development of ESKD,[29, 86] acute kidney injury (AKI),[25] excess cardiovascular events (CVE),[73] and increased mortality.[32] Nevertheless, the prognosis associated with CKD is extremely heterogeneous and the risk of adverse outcomes varies widely according to the population studied. For example, population-based studies have reported that the majority of people with CKD are at low risk of developing ESKD,[36] whereas studies of people known to

have CKD and managed in secondary care report ESKD as a common outcome.[167, 168]

Many previous studies have understandably focussed on risks associated with CKD.[25, 29, 32, 73, 86] However, it is arguably as important to study positive outcomes such as stable CKD or remission of CKD so that people at low risk for adverse outcomes can be spared unnecessary intervention and referral to Nephrology clinics. Interestingly, there is currently no consensus on when CKD should be considered no longer present (in remission) and there is therefore a lack of readily comparable data to indicate how frequently remission occurs or the factors that contribute to it.

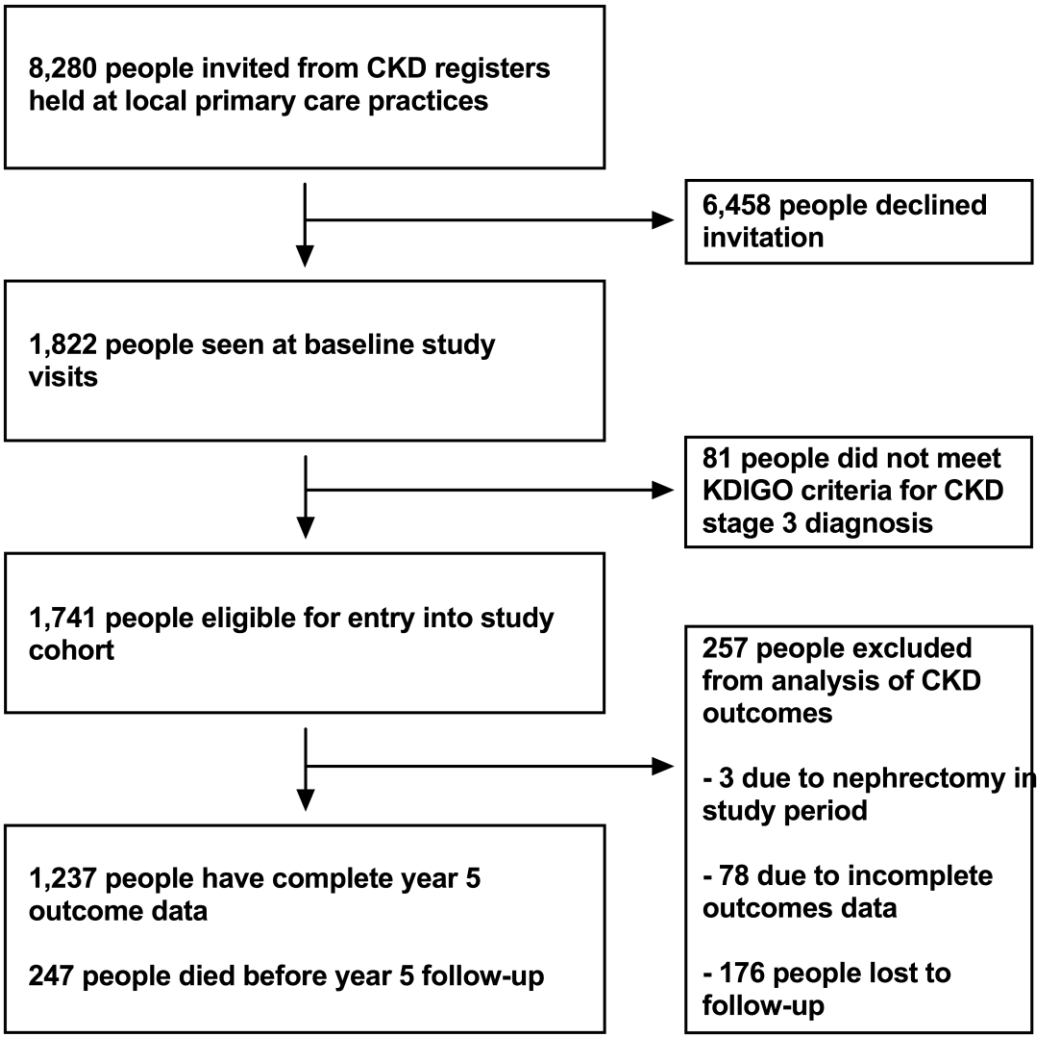
The majority of people with CKD in primary care are elderly and albuminuria is present in only a minority,[169] suggesting that overall this population is at low risk for progression to ESKD. This chapter aims to describe to the natural history of CKD in a primary care to better inform the appropriate emphasis for future guidance on caring for people with CKD in a primary care setting.

## 4.3 Methods

### 4.3.1 Participants

Detailed methods for the Renal Risk in Derby (RRID) study are described in chapter 3, and have been published previously.[169] In brief, participants were individually recruited from 32 local primary care clinics between 2008 and 2010 and prospectively studied. In total, 8,280 people were invited from registers of people with CKD stage 3, 1,822 attended baseline visits and 1,741 were eligible to participate (Figure 4.1). Participants were aged >18 years and at least two eGFR results (derived from the Modification of Diet in Renal Disease study (MDRD) equation) of 30 to 59 ml/min/1.73m$^2$, more than 90 days apart, were required to be eligible.[4] People judged to have a life expectancy of less than one year, unable to attend study visits or with a solid organ transplant were excluded.

**Fig 4.1 : Flowchart showing study participant outcomes and follow-up**




### 4.3.2 Study Visits

Study visits were conducted as described in Methods (Section 3.3). Visits were conducted at baseline and repeated at 1 and 5 years. Prior to each visit, participants completed a background questionnaire covering demographic variables, social, medical, and medication history. Questionnaires were reviewed at study visits and clarified as required. Height, weight, waist- and hip circumference were measured. Three

blood pressure measurements that differed by <10% were taken using an oscillometric device (UA-767 Plus 30, A&D Medical) after at least five minutes rest.

### 4.3.3 Laboratory Methods

Participants were asked to abstain from eating meat for 12 hours prior to study visits to avoid confounding the serum creatinine assay. Blood and urine samples were analysed in a single clinical laboratory at the Royal Derby Hospital for standard haematological and biochemical variables. Creatinine was measured using the Jaffe method, standardized against an isotope dilution mass spectrometry (IDMS) method. GFR was estimated using the CKD-EPI equation in the primary analysis, with an additional sensitivity analysis performed using the MDRD equation. The average urine albumin to creatinine ratio (uACR) from three consecutive early morning specimens was used for analysis.

Thirty-one participants who were unable to attend for year 5 study visits were able to submit blood and urine samples specifically for the study. For 176 participants, unable to either attend for a study visit or to submit study samples, blood and uACR results taken for clinical purposes were incorporated into

the data set. These were selected to be less than 6 months from the year 5 visit due date. Samples taken during hospital admissions were not used.

### 4.3.4 Mortality Data

Date and cause of death as stated on death certificates was obtained from the Office of National Statistics via the Health and Social Care Information Centre (HSCIC). Independent of each other and without knowledge of other participant data, three RRID study investigators (AS, RJF and MWT) assessed the information on death certificates and classified cause of death into four categories (cardiovascular, malignancy, infection and other). Differences in classification were subsequently resolved by discussion.

### 4.3.5 Endpoint Definitions

This analysis used Kidney Disease Improving Global Outcomes (KDIGO) definitions for CKD and CKD progression.[4] Progression of CKD was defined as a 25% decline in GFR, coupled with a worsening of GFR category or an increase in albuminuria category. Our pre-specified endpoint from study design (development of ESKD or doubling of serum creatinine) was not used, due to a very small number of events. We

defined CKD remission as the presence of both eGFR >60 ml/min/1.73m$^2$ and uACR <3 mg/mmol at any study visit in an individual who had previously met KDIGO diagnostic criteria for CKD.

### 4.3.6 Statistical Analysis

Analysis was performed using IBM SPSS version 22. Urine ACR was logarithmically transformed prior to multivariable analysis. Outcomes were analysed using binomial logistic regression models. For analyses of progression and remission, participants with a complete year 5 outcome (eGFR and uACR values, or a date of death before the year 5 visit) were included. Basic models were initially produced including baseline age, eGFR log uACR and gender. Variables that were significantly associated with the outcome on univariable analysis were then added in groups, according to biological and clinical relevance. The final models included all variables that were significantly associated with the outcome in previous models and gave the best discrimination. For each model, predicted probabilities were used to draw receiver operating characteristics (ROC) curves, and area under the ROC curve (AUROC) was used to compare models. Survival analysis was performed using Cox proportional hazards models. All 1,741

participants were included in the survival analysis. Death prior to a year 5 visit was considered an event. Participants were censored at date of year 5 visit, date of first dialysis or 5 years from the time of baseline visit if neither of these events occurred. Three participants underwent unilateral nephrectomy during the study follow-up period and therefore were excluded from analysis of CKD progression and remission.

## 4.4 Results

### 4.4.1 Cohort Description and Outcomes

Description of the cohort at baseline has previously been published.[169] Baseline, Year 1 and Year 5 results are given in table 4.1.

**Table 4.1 Demographic, anthropometric, and laboratory data from the baseline, year 1, and year 5 study visits**

| Variable | Baseline | | Year 1 | | Year 5 | |
|---|---|---|---|---|---|---|
| | | N | | N | | N |
| CKD EPI eGFR | 53.5 ± 11.8 | 1741 | 53.1 ± 12.7 | 1619 | 53.1 ± 14.8 | 1311 |
| Age (years) | 72.9 ± 9.0 | 1741 | 73.6 ± 9.0 | 1619 | 76.8 ± 8.9 | 1311 |
| Female Sex | 1052 (60.4) | 1741 | 981 (60.6) | 1619 | 819 (62.5) | 1311 |
| Caucasian | 1698 (97.5) | 1741 | 1579 (97.5) | 1619 | 1281 (97.7) | 1311 |
| Diabetes at baseline | 294 (16.9) | 1741 | 266 (16.4) | 1619 | 211 (16.1) | 1311 |
| Previous CVD | 387 (22.2) | 1741 | 339 (20.9) | 1610 | 244 (18.6) | 1311 |
| Haemoglobin (g/dl) | 13.2 ± 1.4 | 1736 | 13.2 ± 1.4 | 1613 | 13.1 ±1.5 | 1050 |
| Corrected Calcium (mmol/l) | 2.38 ± 0.10 | 1730 | 2.38 ± 0.10 | 1619 | 2.44 ± 0.09 | 1062 |
| Phosphate | 1.1 ± 0.2 | 1709 | 1.1 ± 0.2 | 1610 | 1.1 ± 0.2 | 1058 |
| Albumin | 40.6 ± 3.2 | 1738 | 39.7 ± 3.2 | 1619 | 36.8 ± 2.9 | 1065 |
| Bicarbonate | 25.5 ± 2.7 | 1722 | 25.3 ± 2.7 | 1616 | 25.2 ± 2.6 | 1055 |
| Urate | 384 ± 91 | 1731 | 384 ± 92 | 1614 | 371 ± 88 | 1051 |
| Total Cholesterol | 4.8 ± 1.2 | 1732 | 4.6 ± 1.1 | 1616 | 4.6 ± 1.2 | 1062 |
| uACR (mg/mmol) | 0.3 (0.0 to 1.5) | 1738 | 0.6 (0.2 to 2.0) | 1618 | 0.8 (0.0 to 3.4) | 1236 |
| Height (m) | 1.64 ± 0.09 | 1740 | 1.63 ± 0.09 | 1615 | 1.63 ± 0.09 | 1019 |
| Weight (kg) | 78.2 ± 15.5 | 1740 | 78.0 ± 15.5 | 1616 | 77.4 ± 15.5 | 1017 |
| BMI | 29.0 ± 5.1 | 1740 | 29.2 ± 5.2 | 1615 | 29.2 ± 5.3 | 1015 |
| Waist Hip ratio | 0.91 ± 0.09 | 1740 | 0.91 ± 0.09 | 1615 | 0.90 ± 0.07 | 1019 |
| SBP (mmHg) | 133 ± 18 | 1741 | 131 ± 17 | 1619 | 139 ± 20 | 1030 |
| DBP (mmHg) | 73 ± 11 | 1741 | 70 ± 10 | 1619 | 75 ± 11 | 1030 |
| Mean SAF (AU) | 2.7 ± 0.6 | 1707 | 2.6 ± 0.6 | 1567 | 2.7 ± 0.6 | 953 |
| PWV (m/s) | 9.9 ± 2.0 | 1717 | 9.7 ± 1.8 | 1596 | 10.8 ± 2.1 | 978 |

All data are mean ± standard deviation, median (Interquartile range) or number (percentage of total)
N = number of participants
Cardiovascular disease (CVD), urine albumin to creatinine ratio (uACR), Body mass index (BMI), Systolic blood pressure (SBP), Diastolic blood pressure DBP, Skin autofluorescence (SAF), Pulse wave velocity (PWV)

Mean eGFR changed little in absolute terms over the course of the study (53.5 ml/min/1.73m$^2$ at baseline versus 53.1 ml/min/1.73m$^2$).

Data on renal outcomes at 5 years were obtained in 1,484 participants (85.2%) and survival data were available in all participants (from the Office of National Statistics). Two

hundred and forty seven of 1,741 people (14.2%) died prior to their year 5 visit, representing an age standardised mortality rate per year of 4.2% in males and 2.2% in females. This compares to a UK age standardised mortality rate of 3.9% and 2.9% in males and females respectively.[170] Four participants (0.2%) reached ESKD, and 308 (17.7%) evidenced progression of CKD by KDIGO criteria. However, 593 participants (34.1%) had stable CKD and 336 (19.3%) met our criteria for CKD remission. Table 4.2 summarises baseline results according to year 5 outcome. Outcomes are summarised in Figure 4.2. Outcomes are further subdivided by baseline CKD category in Figure 4.3 and table 4.3.

**Table 4.2 : Baseline Cohort Descriptive Statistics and Breakdown by Year 5 Outcome**

| Variable (n) | Total (1741) | CKD Remission (336) | Stable CKD (593) | CKD Progression (308) | Died (247) | Lost to Follow-up (257) |
|---|---|---|---|---|---|---|
| Female Gender (%) | 1052 (60.4) | 252 (75.0) | 350 (59.0) | 160 (51.9) | 104 (42.1) | 186 (72.4) |
| Age (years) | 72.9 ± 9.0 | 67.1 ± 8.5 | 72.6 ± 8.1 | 73.3 ± 8.7 | 78.7 ± 6.9 | 74.9 ± 9.3 |
| eGFR–CKD-EPI (ml/min/1.73m$^2$) | 53.5 ± 11.8 | 64.1 ± 9.0 | 51.6 ± 9.6 | 50.3 ± 11.8 | 46.3 ± 10.0 | 55.1 ± 12.0 |
| eGFR–MDRD (ml/min/1.73m$^2$) | 52.5 ± 10.4 | 60.9 ± 7.8 | 50.7 + 8.7 | 49.7 ± 10.9 | 47.1 ± 95 | 54.2 ± 10.4 |
| uACR (mg/mmol) | 0.3 (0.0–1.5) | 0.1 (0.0–0.4) | 0.3 (0.0–1.4) | 0.9 (0.2–2.1) | 1.1 (0.1–3.9) | 0.3 (0.0–1.3) |
| Diabetes (%) | 294 (16.9) | 30 (8.9) | 93 (15.7) | 78 (25.3) | 57 (23.1) | 36 (14.0) |
| CVD (%) | 387 (22.2) | 49 (14.6) | 107 (18.0) | 70 (22.7) | 99 (40.1) | 62 (24.1) |
| Current or Previous Smoker (%) | 947 (54.4) | 159(47.3) | 307 (51.8) | 173 (56.2) | 165(66.8) | 143 (55.6) |
| ACE/ARB use (%) | 1123 (64.5) | 186 (55.4) | 374 (63.1) | 222 (72.1) | 173 (70.0) | 168 (65.4) |
| Weight (kg) | 78.2 ± 15.5 | 77.6 ± 15.5 | 79.5 ± 14.3 | 79.2 ± 15.8 | 775 ± 175 | 75.8 ± 15.6 |
| BMI (kg/m$^2$) | 29.0 ± 5.1 | 29.0 ± 5.2 | 29.5 ± 4.9 | 28.9 ± 4.9 | 28.3 ± 5.4 | 29.0 ± 5.4 |
| Waist:Hip ratio | 0.91 ± 0.09 | 0.88 ± 0.09 | 0.91 ± 0.08 | 0.92 ± 0.09 | 0.94 ± 0.09 | 0.90 ± 0.09 |
| SBP (mmHg) | 134.0 ± 18.3 | 130.0 ± 16.8 | 133.7 ± 17.1 | 136.9 ± 17.4 | 135.7 ± 22.0 | 134.8 ± 195 |
| DBP (mmHg) | 72.8 ± 11.0 | 74.7 ± 10.7 | 73.2 ± 11.0 | 72.2 ± 10.6 | 70.2 ± 11.6 | 72.8 ± 11.0 |
| Haemoglobin (g/dl) | 13.2 ± 1.4 | 13.6 ± 1.2 | 13.3 ± 1.3 | 12.9 ± 15 | 12.8 ± 15 | 13.2 ± 15 |
| Corrected Calcium (mmol/l) | 2.38 ± 0.10 | 2.38 ± 0.09 | 2.38 ± 0.10 | 2.37 ± 0.10 | 2.36 ± 0.10 | 2.39 ± 0.12 |
| Phosphate (mmol/l) | 1.11 ± 0.18 | 1.11 ± 0.18 | 1.10 ± 0.18 | 1.12 ± 0.17 | 1.11 ± 0.18 | 1.11 ± 0.18 |
| Albumin (g/l) | 40.7 ± 3.2 | 41.4 ± 2.9 | 40.8 ± 2.9 | 40.4 ± 3.6 | 39.6 ± 35 | 40.6 ± 3.0 |
| Bicarbonate (mmol/l) | 25.5 ± 2.7 | 26.0 ± 2.5 | 25.6 ± 25 | 24.9 ± 2.7 | 25.4 ± 3.1 | 255 ± 2.7 |
| Total Cholesterol (mmol/l) | 4.8 ± 1.19 | 5.0 ± 1.2 | 4.8 ± 1.2 | 4.7 ± 1.3 | 4.4 ± 1.1 | 4.8 ± 1.1 |
| Urate (μmol/l) | 384 ± 91 | 334 ± 79 | 391 ± 89 | 402 ± 85 | 409 ± 100 | 374 ± 92.8 |
| **Change in given variables at different time points** | | | | | | |
| Change in CKD-EPI eGFR at Y1 | -0.74 ± 7.8 | 0.40 ± 8.4 | -0.35 ± 7.4 | -2.7 ± 7.7 | -0.81 ± 8.1 | -0.73 ± 7.6 |
| Change in SBP at Y1 (mmHg) | -3.2 ± 16.0 | -1.8 ± 8.6 | -3.4 ± 14.6 | -4.1 ± 17.1 | -5.0 ± 18.8 | -2.1 ± 18.3 |
| Change in DBP at Y1 (mmHg) | -2.4 ± 9.3 | -1.7 ± 8.6 | -2.6 ± 9.1 | -2.7 ± 9.3 | -2.8 ± 10.0 | -2.2 ± 105 |
| Change in uACR at Y1 | 0.17 (-0.1–0.6) | 0.17 (0.0–0.4) | 0.15 (-0.1–0.4) | 0.4 (-0.0–1.6) | 0.3 (-1.1–1.3) | 0.2 (-0.1–0.7) |
| Change in weight at Y5 (kg) | -1.6 ± 5.9 | -1.1 ± 5.4 | -1.7 ± 6.2 | -1.9 ± 5.9 | N/A | N/A |

**Fig 4.2 : Breakdown of study cohort by outcome. Independent predictors for CKD remission, CKD progression and Mortality before year 5 are given in boxes.**

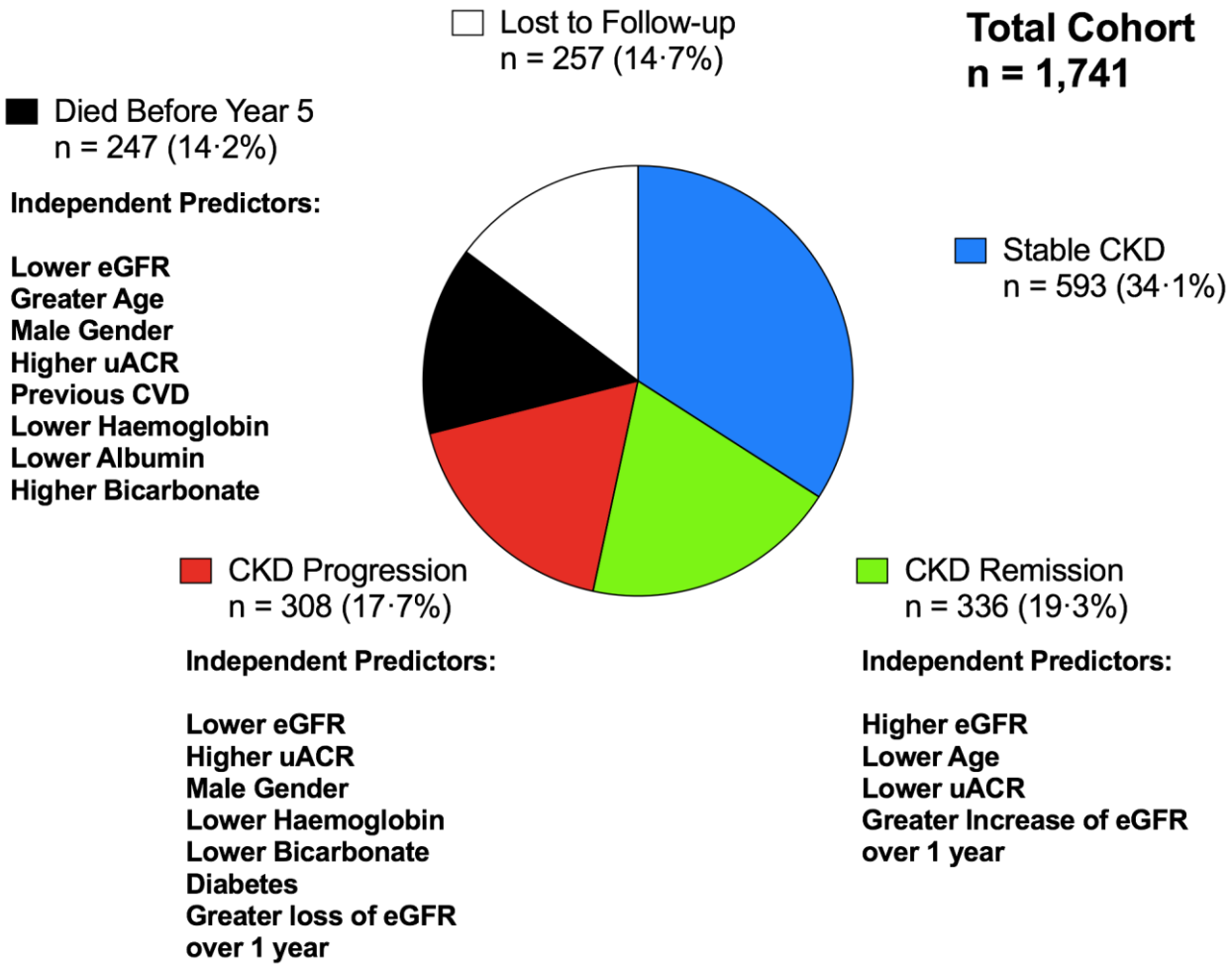


**Figure 4.3 Proportion of participants with each outcome by baseline CKD stage (G1 to 4 refer to KDIGO GFR categories, A1 to 3 refer to KDIGO albuminuria categories)**

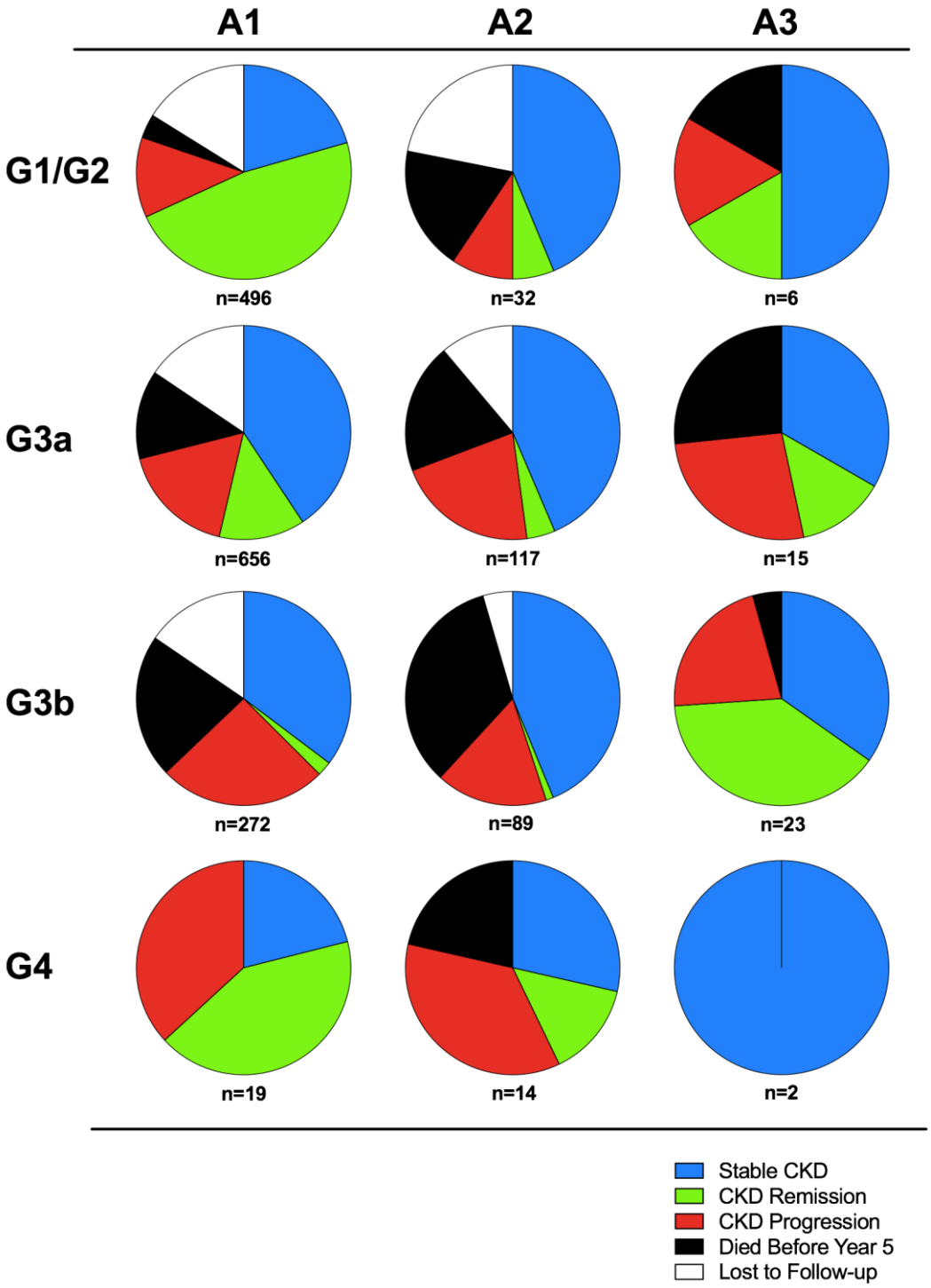


**Table 4.3 Frequency of Outcomes at year 5 by baseline KDIGO CKD category**

| | CKD Remission | | | | Stable CKD | | |
|---|---|---|---|---|---|---|---|
| | **A1** | **A2** | **A3** | | **A1** | **A2** | **A3** |
| **G1/G2** | 236 (47.5%) | 2 (6.3%) | 1 (16.7%) | **G1/G2** | 102 (20.8%) | 14 (43.8%) | 3 (50%) |
| **G3a** | 85 (13.0%) | 5 (4.3%) | 0 (0%) | **G3a** | 268 (40.9) | 51 (43.6%) | 5 (33.3%) |
| **G3b** | 6 (2.2%) | 1 (1.1%) | 0 (0%) | **G3b** | 96 (35.3%) | 39 (43.8%) | 8 (34.8%) |
| **G4** | 0 (0%) | 0 (0%) | 0 (0%) | **G4** | 4 (21.1%) | 4 (28.6%) | 0 (0%) |
| | **CKD Progression** | | | | **All-cause Mortality** | | |
| | **A1** | **A2** | **A3** | | **A1** | **A2** | **A3** |
| **G1/G2** | 60 (12.1%) | 3 (9.4%) | 1 (16.7%) | **G1/G2** | 18 (3.6%) | 6 (18.8%) | 0 (0%) |
| **G3a** | 114 (17.4%) | 25 (21.4%) | 2 (13.3%) | **G3a** | 88 (13.4%) | 23 (19.7%) | 4 (26.7%) |
| **G3b** | 69 (25.7%) | 15 (16.9%) | 9 (39.1%) | **G3b** | 59 (21.7%) | 30 (33.7%) | 5 (21.7%) |
| **G4** | 8 (42.1%) | 2 (14.3%) | 0 (0%) | **G4** | 7 (36.8%) | 5 (35.7%) | 2 (100%) |

All data presented as number (percentage of cohort with the given stage of CKD at baseline)

### 4.4.2 Associations with Progression of Chronic Kidney Disease

A basic multivariable model that included baseline eGFR, gender, uACR and age as predictors of CKD progression was associated with an AUROC of 0.69. Addition of baseline haemoglobin, bicarbonate, diabetes status and systolic blood pressure (SBP) produced a model with an AUROC of 0.73. In this model, baseline age was not statistically significant. The impact of the change at 1 year in SBP, diastolic blood pressure (DBP) and eGFR was also assessed. Change in eGFR at year 1 was a significant determinant of CKD progression but change in SBP and DBP at 1 year did not enter the model (Table 4.4).

When the recently validated 4-variable Kidney Failure Risk Equation (KFRE)[86, 157] was applied to our study population at baseline, the majority were assessed to be at extremely low risk (median 5 year probability of ESKD = 0.08%). In the 4 participants who did progress to ESKD, predicted 5 year risks at baseline were 54.0%, 33.1%, 14.9%, and 9.0% respectively.

**Table 4.4 Univariable and multivariable associations of CKD progression at year 5 study visit**

| Variable | Univariable Odds Ratio (95% CI) | Multivariable Odds Ratio (95% CI) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Model 1 (Basic Model) | Model 2 | Model 3 | Model 4 | Model 5 | Model 6 (Best Model) |
| eGFR | 0.59 (0.51–0.68)* | 0.72 (0.62–0.84)* | 0.75 (0.64–0.87)* | 0.85 (0.71–1.02) | 0.74 (0.63–0.86)* | 0.82 (0.70–0.97)* | 0.71 (0.60–0.85)* |
| Age | 1.41 (1.22–1.62)* | 1.25 (1.07–1.45)* | 1.25 (1.07–1.45)* | 1.22 (1.04–1.44)* | 1.12 (0.95–1.32) | 1.17 (0.98–1.38) | 1.12 (0.94–1.34) |
| Male Gender | 1.70 (1.31–2.21)* | 1.24 (0.94–1.64) | 1.23 (0.92–1.64) | 1.61 (1.15–2.25)* | 1.25 (0.94–1.66) | 1.50 (1.10–2.03)* | 1.48 (1.08–2.02)* |
| Log uACR | 1.76 (1.53–2.04)* | 1.59 (1.37–1.85)* | 1.56 (1.34–1.81)* | 1.59 (1.36–1.86)* | 1.56 (1.35–1.82)* | 1.53 (1.31–1.79)* | 1.50 (1.28–1.75)* |
| Haemoglobin | 0.68 (0.60–0.79)* | | | 0.71 (0.60–0.85)* | | 0.73 (0.62–0.86)* | 0.75 (0.64–0.89)* |
| Phosphate | 1.11 (0.97–1.26) | | | 1.04 (0.89–1.13) | | | |
| Corrected Calcium | 0.78 (0.76–1.00) | | | 0.97 (0.83–0.96) | | | |
| Bicarbonate | 0.71 (0.62–0.82)* | | | 0.83 (0.71–0.96)* | | 0.83 (0.72–0.96)* | 0.83 (0.71–0.96)* |
| Albumin | 0.83 (0.73–0.95)* | | | 0.98 (0.84–1.14) | | | |
| Total Cholesterol | 0.89 (0.78–1.02) | | | 1.10 (0.95–1.27) | | | |
| Urate (µmol/l) | 1.37 (1.20–1.56)* | | | 1.09 (0.92 -1.29) | | | |
| Diabetes | 2.22 (1.62–3.06)* | | 1.71 (1.22–2.40)* | | | 1.53 (1.07–2.19)* | 1.52 (1.06–2.20)* |
| Previous CVD | 1.46 (1.06–2.00)* | | 1.14 (0.82–1.60) | | | | |
| Current or ex-smoker | 1.27 (0.98–1.65) | | 1.05 (0.79–1.39) | | | | |
| SBP | 1.32 (1.15–1.51)* | | | | 1.30 (1.09–1.55)* | 1.22 (1.02–1.46)* | 1.17 (0.98–1.41) |
| DBP | 0.86 (0.76–0.99)* | | | | 0.98 (0.68–0.96)* | 0.96 (0.80–1.15) | 0.97 (0.80–1.16) |
| BMI | 0.92 (0.80–1.05) | | | | | | |
| Waist:Hip Ratio | 1.33 (1.16–1.51)* | | | | | | |
| Y1 Change eGFR | 0.70 (0.61–0.81)* | | | | | | 0.63 (0.54–0.75)* |
| Y1 Change SBP | 0.92 (0.80–1.05) | | | | | | |
| Y1 Change DBP | 0.96 (0.84–1.10) | | | | | | |
| AUROC | | 0.69 | 0.70 | 0.72 | 0.70 | 0.73 | 0.74 |

p value <0.05*

eGFR calculated using CKD EPI formula, all variables measured at baseline unless stated

Odds ratios are expressed per 1 standard deviation increase in the independent variable

Estimated Glomerular filtration rate (eGFR), Urine Albumin to creatinine ratio (uACR), Cardiovascular disease (CVD), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Body mass index (BMI), Area under receiver operator characteristics curve (AUROC)

### 4.4.5 Associations with All-cause Mortality

Cause of death was classified as cardiovascular in 94 cases (38.1%), malignancy in 63 (25.5%), infection in 50 (20.2%), "other" in 34 (13.8%), and "no data available" in six (2.4%). Two people died before year 5 follow-up, but after the development of ESKD. Participants who died tended to be older than other outcome groups, had lower eGFR and higher uACR on average at baseline (Table 4.2). Cox proportional hazards models identified age, male gender, baseline eGFR, log uACR, haemoglobin, albumin and bicarbonate as independent predictors of death (Table 4.5). Change in eGFR at year 1 did not enter the model (HR = 1.00, p = 0.67)

## Table 4.5 Cox Proportional Hazards Models for survival at 5 years

| Variable | Univariable Relative Hazard (95% CI) | Multivariable Relative Hazard (95% CI) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Model 1 | Model 2 | Model 3 | Model 4 | Model 5 | Model 6 |
| eGFR | 0.50 (0.44–0.57)* | 0.69 (0.60–0.80)* | 0.70 (0.60–0.81)* | 0.73 (0.61–0.87)* | 0.71 (0.61–0.82)* | 0.68 (0.57–0.82)* | 0.62 (0.62–0.84)* |
| Age | 2.47 (2.10–2.89)* | 2.01 (1.70–2.38)* | 1.96 (1.66–2.22)* | 1.93 (1.62–2.30)* | 1.99 (1.68–2.37)* | 2.14 (1.76–2.61)* | 1.90 (1.60–2.25)* |
| Male Gender | 2.20 (1.71–2.84)* | 1.62 (1.25–2.10)* | 1.46 (1.12–1.91)* | 1.87 (1.38–2.54)* | 1.67 (1.29–2.17)* | 1.81 (1.34–2.45)* | 1.89 (1.44–2.49)* |
| Log uACR | 1.57 (1.37–1.81)* | 1.30 (1.12–1.50)* | 1.24 (1.07–1.44)* | 1.22 (1.05–1.41)* | 1.31 (1.13–1.52)* | 1.26 (1.07–1.49)* | 1.22 (1.06–1.41)* |
| Haemoglobin | 0.70 (0.62–0.80)* | | | 0.83 (0.72–0.97)* | | | 0.84 (0.74–0.96)* |
| Phosphate | 1.00 (0.88–1.13) | | | 1.00 (0.87–1.16) | | | |
| Corrected Calcium | 0.86 (0.75–0.98)* | | | 0.97 (0.85–1.11) | | | |
| Bicarbonate | 0.97 (0.85–1.10) | | | 1.16 (1.02–1.32)* | | | 1.17 (1.03–1.32)* |
| Albumin | 0.73 (0.65 -0.81)* | | | 0.79 (0.70–0.91)* | | | 0.82 (0.72–0.93)* |
| Total Cholesterol | 0.68 (0.59–0.78)* | | | 0.90 (0.77–1.05) | | | |
| Urate | 1.33 (1.18–1.50)* | | | 1.02 (0.88–1.17) | | | |
| Diabetes | 1.56 (1.16–2.09)* | | 1.25 (0.92–1.68) | | | | 1.20 (0.88–1.63) |
| Previous CVD | 2.62 (2.03–3.38)* | | 1.84 (1.42–2.38)* | | | | 1.81 (1.39–2.35)* |
| Ever smoker | 1.76 (1.35–2.29)* | | 1.27 (0.97–1.68) | | | | |
| SBP | 1.10 (0.97–1.25) | | | | 0.97 (0.84–1.12) | | |
| DBP | 0.76 (0.67–0.86)* | | | | 0.90 (0.77–1.05) | | |
| BMI | 0.84 (0.74–0.96)* | | | | | | |
| Waist:Hip Ratio | 1.39 (1.23–1.57)* | | | | | | |
| Y1 Change eGFR | 1.00 (0.86–1.16) | | | | | 0.94 (0.79–1.11) | |
| Y1 Change SBP | 0.89 (0.77–1.04) | | | | | | |
| Y1 Change DBP | 0.96 (0.83–1.12) | | | | | | |

p value <0.05*

eGFR calculated using CKD EPI equation All variables measured at baseline unless stated

Relative hazards are expressed per 1 standard deviation increase in the independent variable

Estimated Glomerular filtration rate (eGFR), Urine Albumin to creatinine ratio (uACR), Cardiovascular disease (CVD), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Body mass index (BMI)

### 4.4.6 Associations with CKD Remission

At baseline, participants who evidenced remission at year 5 had a higher mean eGFR and a lower median uACR, compared to the rest of the study cohort (Table 4.2). In addition participants with remission were younger and evidenced a higher proportion of females (Table 4.4). A basic multivariable model, including age, gender, eGFR and log uACR as independent variables, was associated with an AUROC of 0.85 (Table 4.6). This was improved to 0.86 by addition of the change in eGFR seen at 1 year and baseline haemoglobin. There was no significant difference in weight change between renal outcome groups (Table 4.2), nor did weight change enter multivariable models as a predictor of remission.

## Table 4.6 Univariable and multivariable associations of CKD remission at 5 years

| Variable | Univariable Odds Ratio (95% CI) | Multivariable Odds Ratio (95% CI) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Model 1 (Basic Model) | Model 2 | Model 3 | Model 4 | Model 5 | Model 6 (Best Model) |
| eGFR | 4.92 (3.99–6.06)* | 4.07 (3.27–5.07)* | 4.01 (3.21–5.00)* | 3.80 (2.99–4.83)* | 4.18 (3.34–5.24)* | 5.25 (4.09–6.73)* | 5.10 (3.97–6.55)* |
| Age | 0.50 (0.44–0.58)* | 0.67 (0.56–0.79)* | 0.66 (0.56–0.79)* | 0.70 (0.59–0.84)* | 0.69 (0.57–0.83)* | 0.75 (0.63–0.90)* | 0.76 (0.64–0.91)* |
| Female Gender | 2.30 (1.74–3.04)* | 1.21 (0.87–1.69) | 1.22 (0.87–1.71) | 1.44 (0.96–2.18) | 1.16 (0.83–1.63) | 1.16 (0.83–1.63) | 1.34 (0.92–1.94) |
| Log uACR | 0.60 (0.52–0.68)* | 0.67 (0.57–0.79)* | 0.67 (0.57–0.79)* | 0.68 (0.57–0.80)* | 0.67 (0.57–0.79)* | 0.69 (0.58–0.81)* | 0.69 (0.58–0.81)* |
| Haemoglobin | 1.33 (1.17–1.53)* | | | 1.22 (1.00–1.49)* | | | 1.20 (0.99–1.45) |
| Phosphate | 1.01 (0.89–1.15) | | | 1.04 (0.88–1.23) | | | |
| Corrected Calcium | 1.08 (0.95–1.23) | | | 0.98 (0.83–1.15) | | | |
| Bicarbonate | 1.32 (1.16–1.51)* | | | 1.17 (0.98–1.38) | | | |
| Albumin | 1.30 (1.14–1.49)* | | | 1.16 (0.97–1.38) | | | |
| Total Cholesterol | 1.23 (1.09–1.39)* | | | 0.94 (0.80–1.11) | | | |
| Urate | 0.52 (0.45–0.61)* | | | 0.96 (0.79–1.17) | | | |
| Diabetes | 0.42 (0.28–0.63)* | | 0.79 (0.49–1.28) | | | | |
| Previous CVD | 0.70 (0.50–0.99)* | | 1.12 (0.73–1.70) | | | | |
| Current or ex-smoker | 0.79 (0.61–1.01) | | 0.97 (0.71–1.32) | | | | |
| SBP | 0.74 (0.64–0.85)* | | | | 0.87 (0.70–1.08) | | |
| DBP | 1.18 (1.04–1.35)* | | | | 0.95 (0.78–1.16) | | |
| BMI | 0.95 (0.83–1.08) | | | | | | |
| Waist:Hip Ratio | 0.68 (0.60–0.78)* | | | | | | |
| Y1 Change eGFR | 1.22 (1.07–1.38)* | | | | | 1.71 (1.45–2.01)* | 1.70 (1.44–2.00)* |
| Y1 Change SBP | 1.13 (0.99–1.30) | | | | | | |
| Y1 Change DBP | 1.11 (0.97–1.26) | | | | | | |
| AUROC | | 0.85 | 0.85 | 0.85 | 0.85 | 0.86 | 0.86 |

p value <0.05*

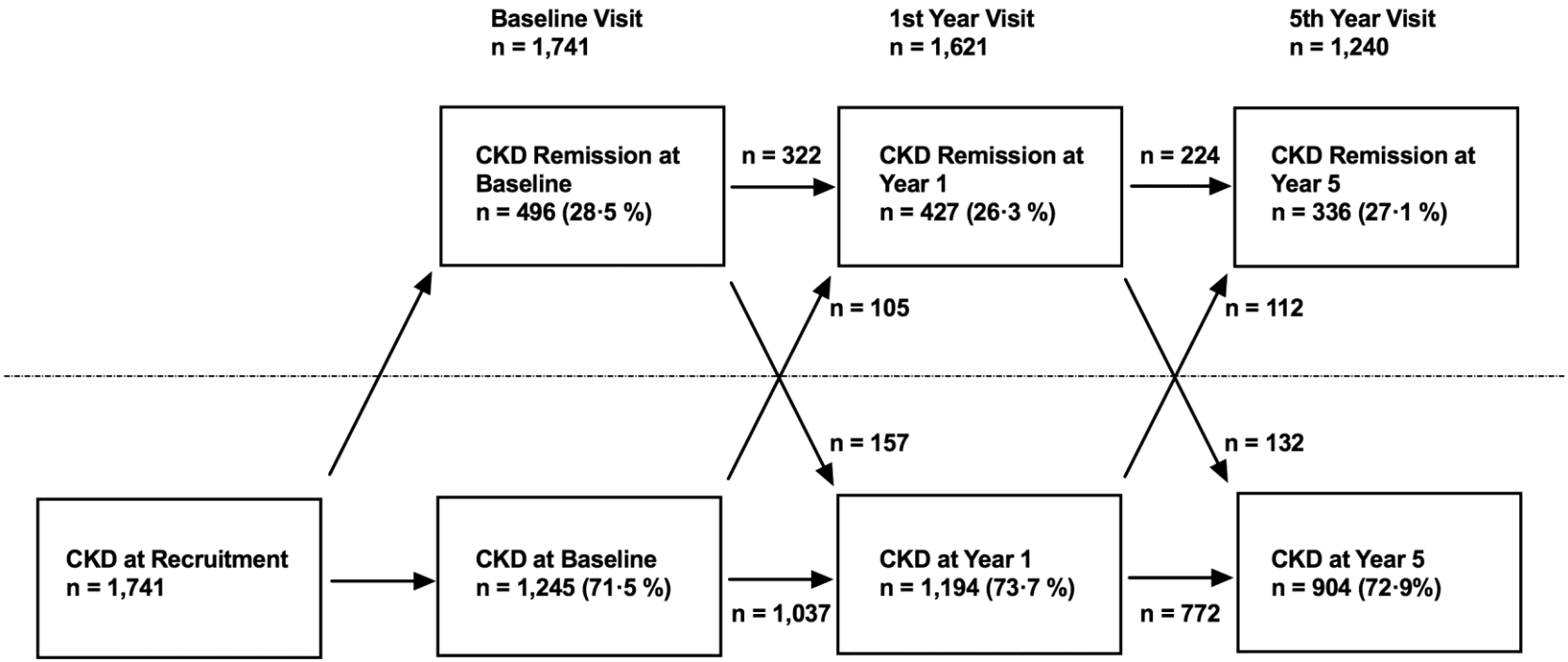
eGFR calculating using CKD EPI equation, all variables measured at baseline unless stated

Odds ratios are expressed per 1 standard deviation increase in the independent variable

Estimated Glomerular filtration rate (eGFR), Urine Albumin to creatinine ratio (uACR), Cardiovascular disease (CVD), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Body mass index (BMI), Area under receiver operator characteristics curve (AUROC)

The number of participants at each time point who demonstrated CKD remission is illustrated in Figure 4.4. Despite meeting KDIGO diagnostic criteria for CKD stage 3 prior to study entry, 496 participants (28.5%) no longer met the criteria for a diagnosis of CKD at the baseline study visit. Of this group, 224 (45.2%) remained in remission at year 5. Remission at 5 years was most likely in the group with remission at both baseline and year 1 (Odds ratio relative to those with no remission at baseline and year 1 = 23.6, 95% CI 16.5 to 33.9, p <0.001). Those with remission at baseline only (OR = 5.9, 95%, CI 3.8 to 9.2, p <0.001) and year 1 only (OR = 7.1, 95% CI 4.3 to 11.8, p <0.001) showed intermediate likelihood of remission at 5 years. The group with remission at baseline and year 1 also evidenced lower mortality (3.2%) over 5 years compared with those with remission at baseline only (5.0%) and those with remission at year 1 only (5.3%). Mortality was significantly lower in all groups demonstrating remission at any time point compared to the group who met criteria for CKD at both baseline and year 1 (15.7%; log-rank test p <0.001).

**Figure 4.4 Flowchart showing numbers of participants demonstrating CKD and CKD remission at each study visit. Arrows indicate numbers of participants transitioning from one condition to the other.**



### 4.4.7 Sensitivity Analysis

Repeat analyses using the MDRD formula to estimate GFR revealed similar numbers of participants reaching each outcome (Table 4.7). Predictors of different outcomes were similar using MDRD eGFR to those presented above using CKD-EPI eGFR.

**Table 4.7 Frequency of outcomes at the 5 year study visits, using the MDRD equation to calculate eGFR**

| Outcome | Stable CKD | CKD Remission | CKD Progression | Died before Year 5 | Lost to Follow-up |
|---|---|---|---|---|---|
| **Number** | 649 | 297 | 291 | 247 | 257 |
| **(%)** | (37.3) | (17.1) | (16.7) | (14.2) | (14.8) |

## 4.5 Discussion

In this population with CKD stage 3 in primary care, the majority of whom did not meet NICE criteria for referral to a nephrologist,[20] we observed a very low incidence of ESKD over five years (0.2%), and only a minority evidenced progression of CKD as defined by KDIGO (17.7%). Similar to population-based studies, the risk of all-cause (14.2%) and cardiovascular mortality (5.4%) greatly exceeded the risk of ESKD.[29, 43] Stable CKD (34.1%) was more common than progression and a substantial minority evidenced remission of CKD (19.3%).

These data confirm in a primary care population the previous findings that reduced GFR and albuminuria are important independent risk factors for adverse outcomes.[25, 29, 73, 86] Other risk factors were similar to those reported in previous studies.[68] Diabetes was a significant predictor of CKD progression, but not of mortality. Lower haemoglobin predicted both CKD progression and all-cause mortality.[89] As reported recently by the CRIC study investigators lower serum bicarbonate was associated with CKD progression.[85] Serum albumin was a predictor of mortality, but not CKD progression. Change in GFR at 1 year was an independent determinant of CKD progression at 5 years. This observation confirms that annual assessment of GFR is useful for monitoring and as a marker of prognosis even in people with relatively mild CKD. Overall the risk of progression to ESKD was extremely low over 5 years and less severe CKD progression was observed in only a minority. The KFRE successfully identified four participants who developed ESKD as moderate or high risk but risk prediction tools that predict risk over longer periods or give an estimate of "time to ESKD" may be more useful in primary care.

Despite globally applied criteria for the diagnosis of CKD, there is no consensus on when CKD should be considered no longer present (in remission) and therefore there is a lack of readily comparable data to indicate how frequently remission occurs or the factors that contribute to it. Using a definition based on lack of any diagnostic criteria for CKD, remission was observed in a substantial proportion of people who met the KDIGO criteria for CKD prior to the baseline study visit. This observation was, in part, due to diagnostic considerations. Study participants were recruited based on previous routine GFR values estimated using the MDRD equation. Prior to study visits, unlike standard clinical practice, participants were asked not to eat meat for 12 hours and for analysis. This may have confounded some results classifying people as having CKD. Additionally, the more accurate CKD-EPI formula (published in 2009, after initiation of the RRID study) was used to estimate GFR at study visits. Thus, these data illustrate the importance of applying the most accurate equation to estimate GFR and avoidance of meat prior to testing (previous studies have reported that meat ingestion can increase the serum creatinine by as much as 20 μmol/L).[161] Nevertheless, there were participants who met the diagnostic criteria for CKD at the baseline visit who evidenced remission at 1 and 5 years (Figure 4.4). Alternatively, changes in medication or hydration

status and healing of mild kidney damage may have contributed to this remission. A further possibility is that loss of muscle mass with increasing age may result in a decrease in serum creatinine and rise in eGFR but this does not seem to have been a contributory factor since weight change was not associated with remission in this cohort. Improvement in eGFR over time has been reported previously in 41% of participants in a large database study (median 7 ml/min/1.73m$^2$ improvement over two years).[171] This cohort had a similar mean age (76.1 years) to our study but these participants did not necessarily meet formal definitions for CKD and included some people with an eGFR >60 ml/min/1.73m$^2$.

Together, these data make a strong case for an internationally agreed definition of CKD remission so that it can be studied in other populations. Furthermore, a definition of remission will allow some patients to be removed from CKD registers which may have implications for their health and life insurance. These data support the definition of CKD remission (in persons previously diagnosed with CKD) as: absence of diagnostic criteria for CKD that persists for at least 1 year, since this was associated with a substantially higher likelihood of long-term remission than a single set of normal values. Remission at any

time point was also associated with lower mortality risk. This analysis has identified lower age, higher eGFR and lower uACR as predictors of remission with good discrimination and these simple variables may therefore be used to identify patients who should be re-assessed for remission or who should perhaps be given a provisional rather than a firm diagnosis of CKD. It remains to be shown, however, whether those meeting CKD criteria for a short period prior to improvement carry any residual excess risk.

The benign prognosis observed in participants in CKD category G3a A1 raises the question of whether older people with mildly reduced GFR but no albuminuria should be labelled as having CKD at all. This is important because our data confirm that G3a A1 represents the largest group of those diagnosed with CKD in primary care (Figure 4.3). It has been suggested that an age-calibrated definition of CKD should be applied to avoid labelling people with age-related GFR decline and low risk of adverse outcomes as having CKD. Based on an analysis of values in normal populations across the age spectrum and the risks associated with reduced GFR, it has been proposed that the GFR threshold for diagnosis of CKD in the absence of albuminuria in those over 65 years should be lowered from 60

to 45 ml/min/1.73m$^2$.[172] These data support this recommendation though it should be noted that progression of CKD over 5 years was observed in 17.4% of those with G3a A1 at baseline, suggesting that monitoring of GFR and uACR in this group should continue. Alternatively, an increasing role for risk prediction (progressive CKD risk, AKI risk and all-cause mortality risk) may help clinicians to provide information about a diagnosis of CKD 3A in an appropriate context.

### 4.5.1 Strengths and Limitations

Notable strengths of this study include use of strict KDIGO criteria for diagnosis of CKD stage 3 prior to study entry and detailed clinical characterisation of participants at each study visit. This analysis has focussed on basic risk factors that would be easily measurable in clinical settings, but the use of novel biomarkers may improve risk prediction in the future.

The RRID study cohort included predominantly Caucasian people, reflecting the demographic composition of the population of Derbyshire. In addition the study population was elderly (mean age 72.9 ± 9.0 years), reflecting the higher prevalence of CKD in older people. Although this population was generally representative of people with CKD cared for in

Primary Care in the UK,[42] the lack of ethnic diversity and relatively high age may limit application of our findings to populations with different ethnic composition or younger age. Additionally, CKD in younger groups may tend to have different aetiologies and natural histories, making the results from this study difficult to extend to other populations. Recruitment commenced in 2008, and therefore the MDRD equation was used to diagnose CKD. The MDRD equation is known to under-estimate GFR at higher levels of ≥60 ml/min/1.73m$^2$ and is less accurate than the CKD-EPI equation.[50] It is possible therefore, that some participants were included who would not have been diagnosed with CKD, were the CKD-EPI equation to have been used. Nevertheless, this study reflects a "real-world" situation with some people still having a diagnosis of CKD based on the MDRD equation. Sensitivity analyses using GFR estimated using the MDRD equation produced similar results to the primary analysis.

This analysis used definitions for CKD progression based upon KDIGO recommendations.[4] The protocol specified a definition of ESKD or doubling of serum creatinine but these endpoints were met too infrequently to be useful for analysis of our cohort. Nevertheless, analysis using less severe CKD

progression as an endpoint still remains relevant because it has been shown that those with greater declines in eGFR are at higher future risk of ESKD and all-cause mortality[29].

## 4.6 Conclusions

These results show, in a primary care setting, that the most common outcome associated with CKD stage 3 over 5 years was stable kidney function. Moreover, a significant minority of people evidenced CKD remission. These data therefore suggest that management of CKD in primary care should focus principally on identifying the minority of people who are at high risk for intervention to slow CKD progression and reduce cardiovascular events but efforts should also be made to identify and reassure the majority who are at low risk of progression to ESKD. Consideration should be given to adopting an age-calibrated definition of CKD to avoid labelling a large group of people with age-related decline in GFR and low associated risk as having CKD. Nevertheless, robust mechanisms should be in place to identify the minority at high risk for developing ESKD to facilitate timely referral.

# 5. Results Chapter 2

# Clinical Utility of Cystatin C in the diagnosis and management of CKD in primary care

## 5.1 Abstract

### 5.1.1 Introduction

To reduce over-diagnosis of chronic kidney disease (CKD) resulting from the inaccuracy of creatinine based estimates of glomerular filtration rate (GFR), national and international guidelines recommend that cystatin C based estimates of GFR be used to confirm or exclude the diagnosis in people with GFR 59 to 45 ml/min/1.73m$^2$ and no albuminuria (CKD G3a A1). Whilst there is good evidence for cystatin C being a marker of GFR and risk in people with CKD, its use to define CKD in this manner has not been evaluated in primary care, the setting in which most people with GFR in this range are managed.

### 5.1.2 Methods

1,741 people with CKD G3a or G3b defined by two eGFR values more than 90 days apart were recruited to the Renal Risk in Derby study between 2008 and 2010. Using CKD Epidemiology Collaboration (CKD EPI) equations, we compared GFR estimated from creatinine ($eGFR_{creat}$), cystatin ($eGFR_{cys}$) and both ($eGFR_{creat\text{-}cys}$) at baseline and over five years of follow-up. This chapter describes the proportion of participants

with CKD G3a A1 reclassified to "no CKD" or more advanced CKD with each equation. Additionally, this analysis examines the impact of using cystatin C based eGFR in risk prediction equations for CKD progression and all-cause mortality and investigated non-GFR determinants of $eGFR_{cys}$.

### 5.1.3 Results

Mean $eGFR_{cys}$ was significantly lower than $eGFR_{creat}$ (45.1 ml/min/1.73m$^2$, 95% CI 44.4 to 45.9 versus 53.6 ml/min/1.73m$^2$, 95% CI 53.0 to 54.1, P <0.001). $eGFR_{cys}$ reclassified 7.7% (50 of 653) of those with CKD G3a A1 by $eGFR_{creat}$ to eGFR >60 ml/min/1.73m$^2$. However, a much greater proportion (59.0%, 385 of 653) were classified to a lower eGFR category. A similar pattern was seen using $eGFR_{creat-cys}$ but lower proportions were reclassified. Change in $eGFR_{creat}$ and $eGFR_{cys}$ over five years were weakly correlated (r = 0.33, P <0.001) but $eGFR_{cys}$ identified more people as having CKD progression (18.2% versus 10.5%). Multivariable analysis using $eGFR_{creat}$ as an independent variable identified age, smoking status, body mass index (BMI), haemoglobin, serum urate, serum albumin, albuminuria and C reactive protein as non-GFR determinants of $eGFR_{cys}$. Use of $eGFR_{cys}$ or $eGFR_{creat-cys}$ did not improve discrimination in risk prediction models for CKD progression and all-cause mortality when compared to similar models with $eGFR_{creat}$. Limitations of this

study include the lack of a measured GFR and the potential lack of ethnic diversity in the study cohort.

### 5.1.4 Conclusions

Implementation of current guidelines on $eGFR_{cys}$ in our study population of older people in primary care resulted in only a small reduction in diagnosed CKD but reclassified a greater proportion as having more advanced CKD than $eGFR_{creat}$. Use of $eGFR_{cys}$ did not improve risk prediction in this population and was associated with increased cost. Our data therefore do not support implementation of these recommendations in primary care. Further studies are warranted to define the most appropriate clinical application of $eGFR_{cys}$ and $eGFR_{creat\text{-}cys}$.

Results presented in this chapter have been published in the article "*The clinical utility and cost impact of cystatin C measurement in the diagnosis and management of chronic kidney disease: A primary care cohort study.*" Shardlow A, McIntyre NJ, Fraser SDS, Roderick P, Raftery J, Fluck RJ, McIntyre CW, Taal MW. PLoS Med. 2017 Oct 10;14(10): e1002400 [173] and additionally have been presented in oral abstract form at Renal Association (best clinical abstracts session) and British Renal Society conferences 2017.

## 5.2 Introduction

The use of serum creatinine concentration to estimate glomerular filtration rate (GFR) has become widely adopted as the principal test for the diagnosis of chronic kidney disease (CKD). However, the dependence of serum creatinine on muscle mass and the tendency of creatinine based equations to under-estimate GFR at values close to the diagnostic threshold of 60 ml/min/1.73m$^2$, has raised concerns about the risk of over-diagnosis in otherwise healthy older populations when relying on this method and has prompted calls to identify more reliable endogenous filtration markers for the estimation of GFR.[174] On the other hand, concern has been expressed that the use of GFR estimated from creatinine but not corrected for age may result in under-diagnosis of CKD in younger people.[172] Cystatin C, a protein that normally crosses the glomerular filtration barrier, has been proposed as such an alternative endogenous marker. Cystatin C is produced by all nucleated cells, and is therefore less influenced by muscle mass than creatinine. [174-176] Though estimation of GFR from cystatin C alone was no more accurate than creatinine, estimated GFR (eGFR) derived from a combined creatinine and cystatin C equation was more accurate and showed greater precision than eGFR derived from creatinine or cystatin C alone.[57]

The National Institute for Health and Care Excellence (NICE) and Kidney Disease Improving Global Outcomes (KDIGO) guidance for the diagnosis of CKD stage 3 has recommended use of cystatin C-based eGFR to confirm or exclude a diagnosis in those found to have a creatinine-based eGFR between 45 and 59 ml/min/1.73m$^2$ and no albuminuria (CKD category G3a A1).[4, 7] However, the clinical impact of implementing this recommendation has not been adequately evaluated in the population in which it will be applied; those with mildly reduced eGFR, managed predominantly in primary care. This is important because this group represents the majority of people defined as having CKD. Population based studies have reported that 3.6% of adults in the US [177] and 3.2% of adults in the UK are in CKD category G3a A1.[12] Additionally, whilst cystatin C is not dependent on muscle mass, it has been reported to have other non-GFR determinants including sex, inflammation, obesity, diabetes, smoking and thyroid dysfunction that may adversely affect GFR estimation in some populations.[62-65, 178]

Cystatin C has also been shown to improve discrimination in equations to predict adverse outcomes in CKD stage 3 including end-stage kidney disease (ESKD),[179] all-cause

mortality[179] and cardiovascular mortality.[180] Potentially therefore, its use in the diagnosis and continuing evaluation of people with CKD in primary care may improve our ability to detect individuals at high risk of adverse outcomes to facilitate targeted monitoring and intervention including early referral to a nephrology service.[181] However, as yet there is little published evidence regarding the use of cystatin C based estimates of GFR for risk assessment in primary care.

This analysis, aims to assess the impact of use of cystatin C-based and combined creatinine and cystatin eGFR compared to standard creatinine-based estimates in a primary care population with baseline CKD stage 3, defined by two measures of GFR more than 90 days apart, and evaluate the non-GFR determinants of cystatin C-based eGFR. Additionally, this chapter compares creatinine- and cystatin-based estimates of GFR over five years of follow-up and evaluates the prognostic accuracy of cystatin C in risk prediction.

## 5.3 Methods

### 5.3.1 Participants

Detailed methods for the RRID study have been published previously.[169] 1,741 participants were individually recruited

and prospectively studied from 32 Derbyshire primary care practices between 2008 and 2010. In total, 8,280 people were invited from practice registers of people with CKD stage 3. Of these, 1,822 people attended baseline visits. All participants were aged over 18 years. Participants were selected using the 4-variable Modification of Diet in Renal Disease study (MDRD) equation modified for use with isotope dilution mass spectrometry (IDMS)-standardised creatinine measurement. Two MDRD eGFR results consistent with CKD stage 3 (30 to 59 ml/min/1.73m$^2$) more than 90 days apart were required to be eligible. People who were judged to have a life expectancy of less than one year, were unable to attend study visits at their primary care surgery or had previously received a solid organ transplant were excluded from the study. Of the 1,822 people who attended baseline visits, 1,741 were eligible, and therefore included in the study cohort. (Figure 5.1)

**Figure 5.1 Flow chart for participants invited and recruited into the study, and numbers involved at key time points**


### 5.3.2 Study visits

Study visits were conducted at baseline and repeated at one and five years. Prior to each visit, participants completed a background questionnaire covering demographic details, medical history, smoking history and medication history. Participants' responses to questions were reviewed at the

study visit and clarified as required. At each clinical visit the participant's height, weight, waist- and hip circumference were measured. Three blood pressure measurements were taken using an oscillometric device (UA-767 Plus 30, A&D Medical) after at least five minutes rest. Readings were repeated so that values differed by no more than 10%.

### 5.3.3 Laboratory Methods

Participants collected three consecutive days' early morning urine samples and stored these in a refrigerator prior to their study visit for subsequent albumin and creatinine analysis. The mean urine albumin to creatinine ratio (uACR) from three specimens was used for analysis. Blood samples were taken at each study visit. Participants were asked to abstain from eating meat for 12 hours prior to the study visit to avoid confounding the serum creatinine assay.[161] Blood and urine samples were analysed in a single clinical laboratory at the Royal Derby Hospital for standard haematological and biochemical variables. Creatinine was measured using a compensated Jaffe method, standardized against an isotope dilution mass spectrometry method, with an inter-assay coefficient of variance of 2.3% at 96 mmol/l (Roche P-analyser, Roche Diagnostics, W. Sussex, UK). Cystatin C was

measured from serum samples taken at baseline, year one and year five study visits, stored at -80°C. Measurement was undertaken at the biochemistry laboratory at the John Radcliffe Hospital, Oxford, UK, using a particle enhanced tubidimetric immunoassay assay (Abbott c16000 Analyser, Abbott Diagnostics, Maidenhead, UK) calibrated against the international reference material ERM-DA471/IFCC.63.[182] The assay used has a coefficient of variation of 1.5% at 0.89 mg/l and 1.1% at 4.06 mg/l.

### 5.3.4 Estimating Equations

This analysis compared GFR estimated using the creatinine-based, cystatin C-based and combined equations developed by the CKD Epidemiology Collaboration (CKD EPI), designated $eGFR_{creat}$, $eGFR_{cys}$ and $eGFR_{creat\text{-}cys}$, respectively.[50, 57]

### 5.3.5 Outcome Definitions

We used KDIGO definitions to classify participants' CKD stage according to $eGFR_{creat}$, $eGFR_{cys}$ and $eGFR_{creat\text{-}cys}$. The study pre-specified endpoint for CKD progression was the development of ESKD or doubling of serum creatinine. However this endpoint was observed in only four participants (0.2%) after five years[166] and for this analysis, CKD progression was

defined as a 25% or more loss of GFR coupled with a worsening of GFR category, or a worsening of albuminuria category. This is based upon progression definitions given in KDIGO diagnostic criteria.[4] Date and cause of death as stated on death certificates was obtained from the Office of National Statistics via the Health and Social Care Information Centre (HSCIC).

### 5.3.6 Statistical Analysis

Baseline variables were compared according to quartiles of cystatin C, using analysis of variation (ANOVA), Kruskal-Wallis or Chi-squared tests as appropriate. Participants were classified according to KDIGO GFR category initially using $eGFR_{creat}$. Reclassification was undertaken using both $eGFR_{cys}$ and $eGFR_{creat\text{-}cys}$. Bland-Altman plots were produced to compare the difference between $eGFR_{creat}$ and both $eGFR_{cys}$ and $eGFR_{creat\text{-}cys}$ across the range of eGFR. Multivariable linear regression models were constructed using $eGFR_{cys}$ as the dependent variable and $eGFR_{creat}$ as well as clinical variables previously reported as non-GFR determinants of cystatin C as covariates. Non-normally distributed variables (uACR, high sensitivity C reactive protein, (hsCRP)) were logarithmically transformed prior to multivariable analysis.

Results Chapter 1 has already described multivariable models predicting risk of CKD progression and all-cause mortality developed in this cohort.[166] Comparison of these models was undertaken using $eGFR_{cys}$ and $eGFR_{creat-cys}$ in place of $eGFR_{creat}$. Binomial logistic regression models were compared using area under the receiver operator characteristic curve (AUROC) based upon predicted probability of progression.

## 5.4 Results

### 5.4.1 Baseline data

Cystatin C was measured from stored samples in 1,732 participants at baseline. Baseline values for key variables for this cohort are given in Table 5.1. Mean ± standard deviation values for $eGFR_{creat}$, $eGFR_{cys}$, and $eGFR_{creat-cys}$ were 53.6 ± 11.8, 45.1 ± 16.0 and 48.3 ± 12.9 ml/min/1.73m$^2$, respectively ($p < 0.001$ for $eGFR_{cys}$ and $eGFR_{creat-cys}$ versus $eGFR_{creat}$). Higher cystatin C associated with male sex, higher prevalence of previous cardiovascular disease and diabetes mellitus, greater BMI, greater waist to hip ratio, higher SBP and lower DBP. Haemoglobin, total cholesterol and serum bicarbonate concentration were lower across quartiles of increasing cystatin C concentration. Serum uric acid and uACR were higher in quartiles with higher cystatin C. (Table 5.1)

**Table 5.1 Baseline variables by quartile of baseline serum cystatin C concentration (quartiles numbered 1 to 4 from lowest to highest cystatin C concentration)**

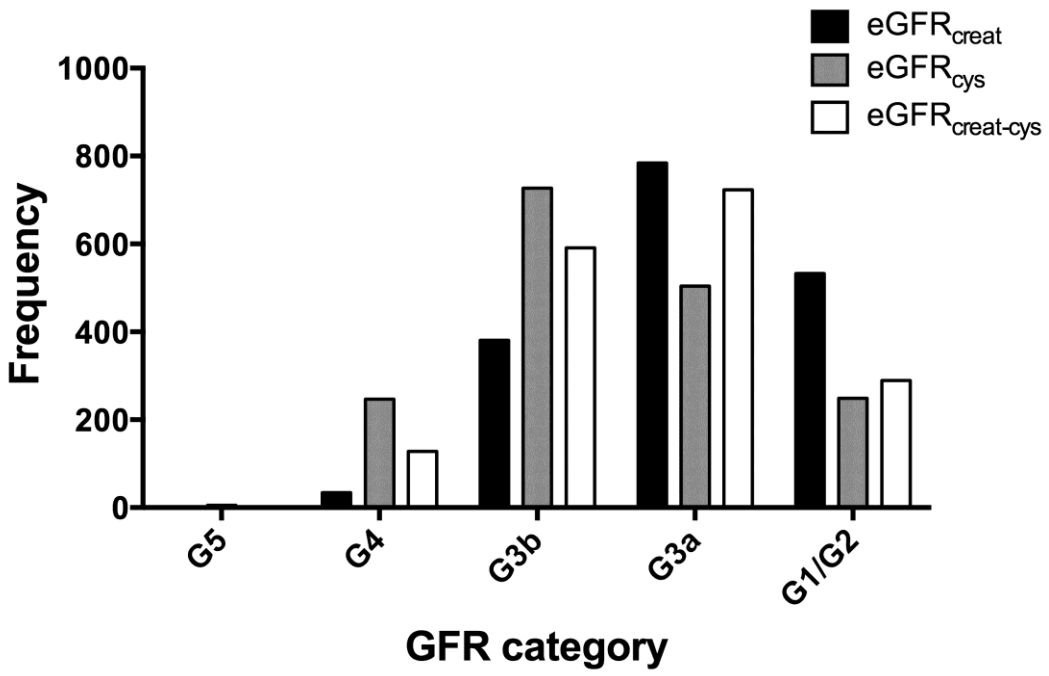
| Variable | Total (n = 1732) | Quartile 1 (n = 444) | Quartile 2 (n = 432) | Quartile 3 (n = 431) | Quartile 4 (n = 425) | p value for trend |
|---|---|---|---|---|---|---|
| $eGFR_{creat}$ (ml/min/1.73m$^2$) | 53.6 ± 11.8 | 63.6 ± 9.7 | 57.4 ± 7.4 | 51.1 ± 8.1 | 41.7 ± 8.9 | <0.001 |
| $eGFR_{cys}$ (ml/min/1.73m$^2$) | 45.1 ± 16.0 | 65.5 ± 14.4 | 47.7 ± 3.8 | 38.6 ± 3.0 | 27.9 ± 4.9 | <0.001 |
| $eGFR_{creat-cys}$ (ml/min/1.73m$^2$) | 48.3 ± 12.9 | 64.0 ± 9.2 | 51.7 ± 4.3 | 43.7 ± 4.3 | 33.2 ± 5.7 | <0.001 |
| Age (years) | 72.9 + 9.0 | 68.6 ± 9.0 | 72.2 ± 8.3 | 74.4 ± 8.1 | 76.4 ± 8.8 | 0.19 |
| Female Sex | 1047 (60.4%) | 350 (78.8%) | 270 (62.5%) | 230 (53.4%) | 197 (46.4%) | <0.001 |
| Diabetes | 292 (16.9%) | 43 (9.7%) | 60 (13.9%) | 92 (21.3%) | 97 (22.8%) | <0.001 |
| Current Smoker | 81 (4.7%) | 17 (3.8%) | 18 (4.2%) | 18 (4.2%) | 28 (6.6%) | 0.20 |
| Previous CVD | 385 (22.2%) | 67 (15.1%) | 84 (19.4%) | 111 (25.8%) | 123 (28.9%) | <0.001 |
| Thyroid Disorder | 217 (12.5%) | 61 (13.7%) | 58 (13.4%) | 44 (10.2%) | 54 (12.7%) | 0.39 |
| Haemoglobin (g/dl) | 13.2 ± 1.4 | 13.5 ± 1.3 | 13.4 ± 1.3 | 13.3 ± 1.4 | 12.8 ± 1.6 | <0.001 |
| Corrected Calcium (mmol/l) | 2.38 ± 0.10 | 2.38 ± 0.10 | 2.38 ± 0.09 | 2.38 ± 0.10 | 2.37 ± 0.10 | 0.37 |
| Phosphate (mmol/l) | 1.11 ± 0.18 | 1.11 ± 0.18 | 1.10 ± 0.19 | 1.09 ± 0.16 | 1.12 ± 0.18 | 0.07 |
| Albumin (g/l) | 40.7 ± 3.2 | 41.3 ± 3.0 | 41.0 ± 3.0 | 40.4 ± 3.1 | 40.0 ± 3.5 | 0.28 |
| Bicarbonate (mmol/l) | 25.5 ± 2.7 | 26.1 ± 2.4 | 25.7 ± 2.5 | 25.4 ± 2.7 | 24.9 ± 3.0 | <0.001 |
| Total Cholesterol (mmol/l) | 4.8 ± 1.2 | 5.1 ± 1.1 | 4.8 ± 1.1 | 4.7 ± 1.2 | 4.5 ± 1.2 | 0.04 |
| Uric Acid (□mol/l) | 384 ± 91 | 334 ± 75 | 364 ± 76 | 398 ± 78 | 443 ± 96 | <0.001 |
| BMI (kg/m$^2$) | 29.0 ± 5.1 | 28.4 ± 4.9 | 28.7 ± 4.7 | 29.3 ± 4.8 | 29.7 ± 5.9 | 0.003 |
| WHR | 0.91 ± 0.09 | 0.87 ± 0.08 | 0.90 ± 0.09 | 0.92 ± 0.08 | 0.94 ± 0.09 | 0.001 |
| SBP (mmHg) | 134 ± 18 | 133 ± 18 | 134 ± 17 | 135 ± 18 | 134 ± 21 | 0.001 |
| DBP (mmHg) | 73 ± 11 | 76 ± 11 | 73 ± 9.9 | 73 ± 11 | 70 ± 11.4 | 0.008 |
| uACR (mg/mmol) | 0.33 (0.00 – 1.50) | 0.13 (0.00 – 0.58 | 0.16 (0.00 – 0.97) | 0.50 (0.00 – 2.07) | 1.17 (0.15 – 4.2) | <0.001 |
| hs CRP (mg/l) | 2.23 (1.14 – 4.59) | 1.7 (0.8 – 3.4) | 2.0 (1.1 – 3.6) | 2.5 (1.3 – 5.5) | 3.3 (1.7 – 6.2) | <0.001 |

All data presented as mean ± standard deviation, median (interquartile range) or number (percentage)

Body mass index (BMI), Creatinine (creat), Cystatin C (cys) Creatinine and Cystatin C (creat-cys), Cardiovascular disease (CVD), Diastolic blood pressure (DBP), Estimated glomerular filtration rate (eGFR), High sensitivity C-reactive protein (hs CRP), Systolic Blood Pressure (SBP), Urine albumin to creatinine ratio (uACR), Waist to hip ratio (WHR)

A comparison of the frequency of people in each eGFR category using the different equations is shown in Figure 5.2. Fewer participants had a baseline eGFR >60 ml/min/1.73m$^2$ using either $eGFR_{cys}$ or $eGFR_{creat-cys}$ compared to $eGFR_{creat}$. Similarly, both $eGFR_{cys}$ and $eGFR_{creat-cys}$ classified more participants as having either CKD G3b or G4 compared to $eGFR_{creat}$.

**Figure 5.2 Histogram showing frequency of people in each eGFR category at baseline using different estimating equations.**




The use of $eGFR_{cys}$ in the 653 people with $eGFR_{creat}$ CKD G3a A1 at baseline reclassified 50 (7.7%) to eGFR >60 ml/min/1.73m$^2$(i.e. no CKD), 356 (54.5%) to G3b and 29

(4.5%) to G4 or 5. Similarly, using $eGFR_{creat-cys}$ reclassified 36 (5.5%) to no CKD, 239 (36.6%) to G3b and 2 (0.3%) to G4 or 5 (Table 5.3). Application of $eGFR_{cys}$ to the whole study population reclassified 57 of 784 (7.3%) with $eGFR_{creat}$ CKD G3a to eGFR >60 ml/min/1.73m$^2$and 488 (62.2%) to CKD G3b or worse. Similarly in the whole study population $eGFR_{creat-cys}$ reclassified 4.7% of participants (37 of 784) with $eGFR_{creat}$ CKD G3a to eGFR >60 ml/min/1.73m$^2$ and 311 (39.7%) to CKD G3b or G4 (Tables 5.4 and 5.5).

**Table 5.3 Reclassification in 653 participants classified as CKD G3a A1 by $eGFR_{creat}$ at baseline using $eGFR_{cys}$ and $eGFR_{creat-cys}$. Data shown are number (percent).**

| | eGFR >60 | CKD G3a | CKD G3b | CKD 4 | CKD 5 |
|---|---|---|---|---|---|
| **$eGFR_{cys}$** | 50 (7.7) | 218 (33.4) | 356 (54.5) | 28 (4.3) | 1 (0.2) |
| **$eGFR_{creat-cys}$** | 36 (5.5) | 376 (57.6) | 239 (36.6) | 2 (6.3) | 0 |

**Table 5.4 Reclassification in all study participants of baseline $eGFR_{creat}$ category using $eGFR_{cys}$. Data shown are number (percent).**

| | | $eGFR_{cys}$ Category | | | | | Total |
|---|---|---|---|---|---|---|---|
| | | G1/G2 | G3a | G3b | G4 | G5 | |
| **Baseline $eGFR_{creat}$ Category** | **G1/G2** | 182 (34.1) | 251 (47.1) | 96 (18.0) | 4 (0.8) | 0 | 533 (30.8) |
| | **G3a** | 57 (7.3) | 239 (30.5) | 446 (56.9) | 41 (5.2) | 1 (0.1) | 784 (45.2) |
| | **G3b** | 10 (2.6) | 12 (3.1) | 183 (48.0) | 174 (45.7) | 2 (0.5) | 381 (22.0) |
| | **G4** | 0 | 2 (5.9) | 2 (5.9) | 28 (82.4) | 2 (5.9) | 34 (2.0) |
| Cohen's Kappa for agreement between $eGFR_{creat}$ and $eGFR_{creat-cys}$ = 0.13 | | | | | | | |

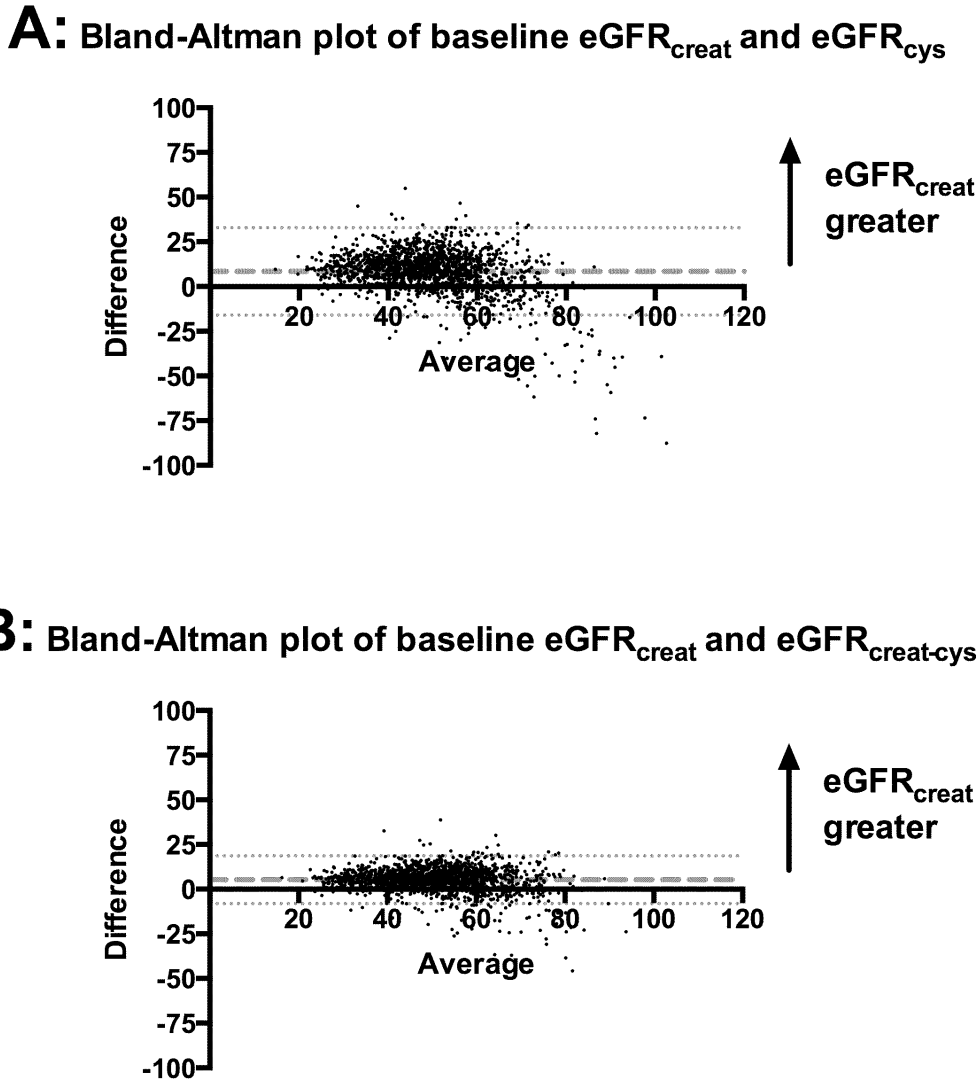
**Table 5.5 Reclassification in all study participants of baseline $eGFR_{creat}$ category using $eGFR_{creat-cys}$. Data shown are number (percent).**

| | | $eGFR_{creat-cys}$ Category | | | | | Total |
|---|---|---|---|---|---|---|---|
| | | G1/G2 | G3a | G3b | G4 | G5 | |
| Baseline $eGFR_{creat}$ Category | G1/G2 | 249 (46.7) | 274 (51.4) | 10 (1.9) | 0 | 0 | 533 (30.8) |
| | G3a | 37 (4.7) | 436 (55.6) | 309 (39.4) | 2 (0.3) | 0 | 784 (45.2) |
| | G3b | 3 (0.8) | 13 (3.4) | 270 (70.9) | 95 (24.9) | 0 | 381 (22.0) |
| | G4 | 0 | 0 | 2 (5.9) | 31 (91.2) | 1 (2.9) | 34 (2.0) |
| Cohen's Kappa for agreement between $eGFR_{creat}$ and $eGFR_{creat-cys}$ = 0.37 | | | | | | | |

### 5.4.2 Bland-Altman Plots

Bland-Altman plots of the whole cohort showed that, for the majority of participants, $eGFR_{creat}$ was greater than $eGFR_{cys}$ and $eGFR_{creat-cys}$. (Figure 5.3) Mean difference was (+) 8.4 ml/min/1.73m$^2$ between $eGFR_{creat}$ and $eGFR_{cys}$, and (+) 5.3 ml/min/1.73m$^2$ between $eGFR_{creat}$ and $eGFR_{creat-cys}$. Both plots showed a small minority of cases, at higher mean eGFR, where $eGFR_{cys}$ was greater than $eGFR_{creat}$. (Figure 5.3)

**Figure 5.3 Bland-Altman plots comparing $eGFR_{creat}$ to $eGFR_{cys}$ (A) and $eGFR_{creat-cys}$ (B). Dashed lines show mean difference between the two estimates of GFR. Dotted lines show 95% CI for mean difference between the two estimates.**




### 5.4.3 Non-GFR determinants of $eGFR_{cys}$

Non-GFR determinants of $eGFR_{cys}$ at baseline were assessed using linear regression, and correction for baseline $eGFR_{creat}$. In fully adjusted models, a range of factors remained significant including age, smoking status and BMI. Other significant determinants included markers of inflammation and

non-traditional cardiovascular risk factors (haemoglobin, urate, albumin, uACR and hsCRP) (Table 5.6).

**Table 5.6 Univariable and Multivariable associations of $eGFR_{cys}$.**

| Variable | Univariable B (95% CI) | Adjusted for $eGFR_{creat}$ B (95% CI) | Multivariable Model B (95% CI) |
|---|---|---|---|
| Male Sex | -4.58 (-6.10 to -3.05)* | 0.01 (-1.21 to 1.23) | |
| Age | -6.30 (-6.99 to -5.60)* | -2.76 (-3.38 to -2.14)* | -3.21 (-3.84 to -2.58)* |
| Current Smoker | -2.45 (-6.02 to 1.13) | -4.56 (-7.32 to -1.81)* | -7.80 (-11.14 to -4.46)* |
| Diabetes | -5.88 (-7.88 to -3.89)* | -2.06 (-3.63 to -0.49)* | -1.56 (-4.89 to 1.77) |
| Haemoglobin | 3.26 (2.52 to 4.00)* | 0.98 (0.39 to 1.58)* | 0.65 (0.01 to 1.29)* |
| Corrected Calcium | 0.38 (-0.38 to 1.14) | -0.12 (-0.70 to 0.47) | |
| Phosphate | -0.65 (-1.41 to 0.16) | -0.16 (-0.75 to 0.43) | |
| Albumin | 3.06 (2.32 to 3.80)* | 1.85 (1.27 to 2.44)* | 1.05 (0.45 to 1.65)* |
| Bicarbonate | 2.27 (1.52 to 3.03)* | 0.55 (-0.05 to 1.15) | |
| Total Cholesterol | 2.06 (1.31 to 2.81)* | 0.34 (-0.26 to 0.93) | |
| Urate | -6.22 (-6.92 to -5.52)* | -1.90 (-2.56 to -1.25)* | -1.92 (-2.58 to -1.26)* |
| BMI | -1.66 (-2.41 to -0.90)* | -1.61 (-2.18 to -1.03)* | -1.50 (-2.11 to -0.90)* |
| Waist Hip Ratio | -3.31 (-4.05 to -2.57)* | -1.03 (-1.63 to -0.44)* | -0.20 (-0.82 to -0.42) |
| Previous CVD | -4.14 (-5.94 to -2.33)* | -1.04 (-2.45 to 0.38) | |
| Ever Smoked | -3.39 (-4.90 to -1.88)* | -1.35 (-2.52 to -0.17)* | |
| SBP | -0.63 (-1.39 to 0.12) | -0.12 (-0.71 to 0.46) | |
| DBP | 3.31 (2.57 to 4.05)* | 1.27 (0.68 to 1.87)* | 0.57 (-0.04 to 1.17) |
| Log(uACR) | -3.76 (-4.49 to -3.03)* | -1.21 (-1.81 to -0.65)* | -0.81 (-1.41 to -0.22)* |
| Thyroid disorder | 0.33 (-1.95 to 2.61) | -1.02 (-2.78 to 0.75) | |
| Log(hsCRP) | -3.22 (-3.96 to -2.49)* | -1.82 (-2.41 to -1.24)* | -0.73 (-1.34 to -0.22)* |

All parameter estimates given per standard deviation for continuous variables

Multivariable Model 1 also adjusted for $eGFR_{creat}$

Body Mass Index (BMI), Cardiovascular Disease (CVD), Diastolic Blood Pressure (DBP), Creatinine-based estimated glomerular filtration rate ($eGFR_{creat}$), Cystatin C-based estimated glomerular filtration rate, High sensitivity C Reactive Protein, Systolic blood pressure (SBP), Urine Albumin to Creatinine ration (uACR)

### 5.4.4 Change in eGFR over five years

Nine hundred and ninety-nine participants had cystatin C measured at both baseline and year five (Figure 5.1). There was a weak correlation between five-year change in $eGFR_{cys}$ and $eGFR_{creat}$ (Pearson's correlation coefficient, r = 0.33, p <0.001), and a moderate correlation between five-year change in $eGFR_{creat-cys}$ and $eGFR_{creat}$ (r = 0.76, p <0.001).

Over five years, the KDIGO definition for CKD progression based on 25% loss of eGFR was met in 105 of 999 participants (10.5%) using $eGFR_{creat}$, 182 (18.2%) using $eGFR_{cys}$ and 135 (13.5%) using $eGFR_{creat-cys}$.

### 5.4.5 Risk Prediction

Overall, 306 participants (17.7%) met the KDIGO criteria for CKD progression at 5 years and 247 (14.2%) died. Replacing baseline $eGFR_{creat}$ with $eGFR_{cys}$ or $eGFR_{creat-cys}$ in previously developed multivariable prediction models for CKD progression[166] did not improve discrimination. The area under the receiver operator characteristics curve (AUROC) was comparable for all three models (Table 5.6). Similarly, in multivariable Cox proportional hazards models for all-cause

mortality over five years similar hazard ratios were obtained for eGFR with each estimating equation. (Table 5.7)

**Table 5.7 Risk prediction models for CKD progression in 999 participants and all-cause mortality in 1,732 participants using different estimating equations for eGFR.**

| | Models for KDIGO CKD Progression | | |
|---|---|---|---|
| **Estimating equation** | **Odds Ratio (95% CI)** | **P value** | **AUROC** |
| $eGFR_{creat}$ | 0.984 (0.971 – 0.998) | 0.023 | 0.722 |
| $eGFR_{cys}$ | 0.982 (0.971 – 0.993) | 0.001 | 0.726 |
| $eGFR_{creat-cyc}$ | 0.978 (0.965 – 0.991) | 0.001 | 0.726 |
| All progression models are adjusted for age, sex, urine Albumin to Creatinine ratio, haemoglobin, bicarbonate and diabetes<br>All Odds Ratios given per ml/min/1.73m$^2$ | | | |
| | **Models for All-cause mortality** | | |
| **Estimating Equation** | **Hazard Ratio (95% CI)** | **P value** | |
| $eGFR_{creat}$ | 0.973 (0.960 – 0.986) | < 0.001 | |
| $eGFR_{cys}$ | 0.975 (0.963 – 0.987) | < 0.001 | |
| $eGFR_{creat-cyc}$ | 0.967 (0.954 – 0.981) | < 0.001 | |
| All survival models are adjusted for age, sex, urine Albumin to Creatinine ratio, haemoglobin, albumin, bicarbonate diabetes and previous cardiovascular disease<br>All Hazard ratios are given per ml/min/1.73m$^2$ | | | |

## 5.5 Discussion

These results indicate that for the majority with CKD stage 3 (confirmed by two $eGFR_{creat}$ values) in primary care, use of $eGFR_{cys}$ or $eGFR_{creat-cys}$ results in lower estimates of GFR than $eGFR_{creat}$. The use of $eGFR_{cys}$ as recommended by NICE to

confirm a $eGFR_{creat}$ based diagnosis of CKD G3a A1 resulted in reclassification of 7.7% as not having CKD but a far greater proportion (59.0%) were reclassified as having more advanced CKD (G3b-5). Thus, in a primary care setting, the potential benefit of reducing over-diagnosis of CKD with $eGFR_{cys}$ would be eliminated by the unintended consequence of greater reclassification to more advanced CKD requiring more frequent monitoring and increased referrals to secondary care. Additionally, the use of $eGFR_{cys}$ did not improve discrimination in risk prediction models in a primary care population.

These results differ in many respects from those of a large meta-analysis to evaluate the clinical impact of using cystatin C versus creatinine to estimate GFR in 11 general population cohorts and five CKD cohorts. Though no difference was observed in mean eGFR calculated by the different methods, use of $eGFR_{cys}$ resulted in a higher prevalence of eGFR <60 ml/min/1,73$m^2$ than either $eGFR_{creat}$ or $eGFR_{creat\text{-}cys}$ (13.7%, 9.7% and 10.0%, respectively). On the other hand, use of $eGFR_{cys}$ resulted in reclassification of 35-47% of participants with CKD G3a to GFR >60 ml/min/1.73$m^2$ whereas a lower proportion (21-27%) were reclassified to a CKD G3b or worse. Moreover, reclassification to a less severe category was

associated with lower risks of all-cause mortality, cardiovascular mortality and ESRD.[111] One reason for the differences between these observations and this analysis is important differences in the cohorts studied. The mean age of 60 and 55 years for the general population and CKD cohorts, respectively, was substantially lower than the mean age of our cohort (73 years). This is an important distinction because our cohort is more representative of the majority of people affected by CKD in developed countries with predominantly Caucasian populations. A second important difference is that the studies included in the meta-analysis relied on only one abnormal $eGFR_{creat}$ for the diagnosis of CKD whereas the RRID study required confirmation with two abnormal $eGFR_{creat}$ values at least 90 days apart for study eligibility. Therefore, in the RRID cohort, those classified as CKD G3a at baseline had a minimum of three abnormal $eGFR_{creat}$ values. It is likely that simply retesting $eGFR_{creat}$ would have reclassified a proportion of those included in the meta-analysis as not having CKD and this would reduce the impact of reclassification by $eGFR_{cys}$. In a population-based study from England, use of $eGFR_{cys}$ resulted in a higher prevalence of CKD G3-5 than $eGFR_{creat}$ (7.7%, versus 5.2%, respectively).[181] Though similar to the above meta-analysis, $eGFR_{cys}$ reclassified 37% of those with CKD G3a A1 defined by $eGFR_{creat}$ as not having CKD, but the

proportion reclassified to a lower GFR category was not reported. Like the meta-analysis, the participants in this study were much younger than our cohort (median age 50 years) and only a single creatinine measurement was used to define CKD. In addition, the cystatin C assay used was not standardised to international reference material and the CKD-EPI equation could not be used.[181] In contrast, an analysis of National Health and Nutrition Examination Survey data revealed higher prevalence of reduced GFR by $eGFR_{cys}$ than $eGFR_{creat}$ in both diabetic and non-diabetic participants.[183]

In the RRID study, $eGFR_{cys}$ identified a higher proportion of participants as having progressive CKD (18.2%) than $eGFR_{creat}$ (10.5%) or $eGFR_{creat\text{-}cys}$ (13.5%) Thus, in addition to impact of lower baseline eGFR values seen with $eGFR_{cys}$, higher apparent progression rates would further promote the referral of patients from primary to secondary care. One could argue that increased referral would be appropriate if patients were at increased risk, but the very low rate of progression to ESKD observed in this study population after 5 years (0.2%)[166] implies that use of $eGFR_{cys}$ in this primary care population would tend to increase referrals and frequency of testing of people with low risk disease, who would be unlikely to benefit.

There is ongoing debate concerning the appropriateness of diagnosing CKD in older people with category G3a eGFR and no proteinuria.[184] In this analysis, we have applied current guidelines to diagnosis of CKD to our cohort. Results chapter 1 described the low rates of CKD progression and relative high rates of "remission" in this population.[166] Additionally, recent results from the Berlin Initiative Study (BIS) have shown that eGFR in older adults strongly depends upon the estimating equation used.[185] The BIS equations (creatinine only and combined creatinine cystatin) were developed in a cohort of people over the age of 70 years. These equations tend to produce lower eGFRs than the corresponding CKD EPI equations, and were more accurate in predicting measured GFR.[58] Comparable results have been shown using the full age spectrum (FAS) equation.[60] This analysis has focussed on the CKD EPI equations as these have been incorporated into KDIGO and NICE guidance, and are in widespread use clinically.

Several studies have reported that, like all endogenous markers of GFR, serum cystatin C concentration is independently associated with several non-GFR determinants including age, sex, diabetes, markers of obesity, inflammation

and smoking.[62-65] Though this analysis did not incorporate measured GFR data, multivariable analysis corrected for $eGFR_{creat}$ confirmed independent associations of $eGFR_{cys}$ with age, serum albumin, serum uric acid, haemoglobin, BMI, uACR, hsCRP and current smoking (Table 5.6).[186] These observations are important because several of these non-GFR determinants are also risk factors for cardiovascular disease and this may in part explain the better performance of $eGFR_{cys}$ as a risk factor for adverse outcomes in CKD cohorts and populations without CKD. Indeed, some have suggested that the ability of cystatin C concentration to predict mortality may have little to do with its association with GFR but instead is largely attributable to the non-GFR determinants.[187, 188] Alternatively, other investigators have proposed that in states of inflammation, filtration of cystatin C at the glomerulus is impaired, producing underestimates of GFR.[189] In addition understanding the non-GFR determinants of cystatin C is important for identifying patient groups in whom $eGFR_{cys}$ will be unreliable. Our data, though limited by lack of measured GFR, confirm previous reports suggesting that $eGFR_{cys}$ is likely to be less accurate for estimating GFR in elderly and obese patients as well as those with albuminuria or evidence of inflammation and in current smokers. However, there may be situations where measurement of $eGFR_{cys}$ may be preferred to

$eGFR_{creat}$, for example the assessment of renal function in younger people with extremes of body habitus and muscle mass.

Reduced GFR is widely recognised as an independent risk factor for multiple adverse outcomes including acute kidney injury (AKI), ESKD, cardiovascular mortality and all-cause mortality.[25, 73, 190] Several papers have reported improved discrimination if $eGFR_{cys}$ is used in risk prediction analyses instead of $eGFR_{creat}$, though it is unclear whether this is due to improved GFR estimation or associations with the non-GFR determinants of cystatin C. In the RRID cohort, cystatin C did not improve discrimination in risk prediction analyses for CKD progression or all-cause mortality suggesting that widespread use in primary care will not improve risk prediction. [179, 180]

### 5.5.1 Strengths and Limitations

Important strengths of this study are individual recruitment and clinical assessment at baseline, prospective protocol-driven follow-up and a requirement for two eGFR readings of <60 ml/min/1.73m$^2$ prior to inclusion in the study cohort.[191, 192] This latter aspect is of particular significance

because the majority of published studies have adopted the epidemiological study approach of requiring only one abnormal eGFR for CKD diagnosis. Moreover, our study population was predominantly elderly and most participants had only mildly reduced GFR. This is typical of the majority affected by CKD in developed countries[177] and is representative of populations in which NICE and KDIGO anticipated that use of $eGFR_{cys}$ would reduce over-diagnosis of CKD. We must, however, concede several important limitations. In this analysis, we were limited by the lack of a measured GFR at baseline in order to compare estimating equations to a "gold standard". However, the aim of this study was primarily to assess the clinical impact of introducing $eGFR_{cys}$ in primary care, where few people have a measured GFR, rather than the accuracy of the estimating equations. Few people with CKD have a measured GFR and our study therefore reflects the situation in clinical practice. The lack of a measured GFR also impacts upon our assessment of non-GFR determinants of $eGFR_{cys}$ due to potential confounding by non-GFR determinants of $eGFR_{creat}$ (used instead of measured GFR). Nevertheless, our results are consistent with previous published studies and strengthen the evidence by showing that non-GFR determinants of cystatin C are an important consideration in the primary care setting. The risk prediction models described in this paper are intended

to show that the use of $eGFR_{cys}$ did not improve discrimination when compared to $eGFR_{creat}$ in this cohort. It was not our intention to develop risk prediction models for general application and we concede that external validation would be required before this could be recommended. Our study population is predominantly Caucasian, elderly (mean age 73 years) and most had only mild reductions in GFR (mean $eGFR_{creat}$ 53.6 ml/min/1.73m$^2$). As discussed above, this is in some respects, a strength, but we concede that our results may not be applicable to younger or more ethnically diverse populations or to those in secondary care with more advanced CKD. The number of events of death and CKD progression was also relatively low and we may therefore have lacked statistical power to detect minor improvement is risk prediction with $eGFR_{cys}$.

## 5.6 Conclusions

This analysis has shown that, in a predominantly elderly population in primary care, application of NICE and KDIGO recommendations to use $eGFR_{cys}$ to confirm a diagnosis of CKD in those classified as CKD G3a A1 by $eGFR_{creat}$ results in reclassification to lower GFR category in a greater proportion than reclassification to a higher GFR category (no CKD).

Additionally, $eGFR_{cys}$ cannot be recommended to improve risk prediction in this population because it did not improve discrimination in risk prediction models for adverse outcomes when compared to $eGFR_{creat}$. These data therefore do not support implementation of these recommendations in primary care. Nevertheless it is likely that $eGFR_{cys}$ will be helpful in obtaining a more accurate estimate of GFR in people at extremes of muscle mass, in whom $eGFR_{creat}$ is known to be inaccurate, but account should also be taken of the non-GFR determinants of cystatin C. Further studies are warranted to define the most appropriate clinical application of $eGFR_{cys}$ and $eGFR_{creat\text{-}cys}$.

# 6. Results Chapter 3

# Associations of Fibroblast Growth Factor 23, Vitamin D, and Parathyroid Hormone with 5 Year Outcomes in People with Chronic Kidney Disease Stage 3

## 6.1 Abstract

### 6.1.1 Introduction

Vitamin D deficiency, elevated fibroblast growth factor 23 (FGF23) and elevated parathyroid hormone (PTH) have each been associated with increased mortality in people with chronic kidney disease (CKD). Recent studies have focussed on the effects of FGF23 in relatively advanced CKD. It is not known, however, whether FGF23 is similarly a risk factor in people with early CKD and how this risk compares to that associated with vitamin D deficiency or elevated PTH. We aimed to evaluate the relative importance of these risk factors in a prospective cohort of people with CKD stage 3 recruited from primary care, the Renal Risk in Derby study.

### 6.1.2 Methods

1,664 participants with CKD stage 3 were evaluated clinically and had vitamin D, PTH and FGF23 levels measured at baseline. All participants had CKD stage 3 prior to recruitment, defined as two eGFR measurements in the range 30 to 59

ml/min/1.73m$^2$ at least 90 days apart. Participants were followed for at least 5 years.

### 6.1.3 Results

289 participants died during the follow-up period. Vitamin D deficiency (Hazard Ratio 1.62, 95% Confidence Interval 1.01 to 2.58) and elevated PTH (HR 1.42, 95% CI 1.09 to 1.84) were independently associated with all-cause mortality. FGF23 was associated with all-cause mortality in univariable but not multivariable analysis. Fully adjusted multivariable models of CKD progression showed no association with FGF23, vitamin D status or PTH.

### 6.1.4 Conclusions

In this cohort of predominantly older people with CKD stage 3, vitamin D deficiency and elevated PTH were independent risk factors for all-cause mortality but elevated FGF23 was not. Whilst FGF23 may have a role as a risk marker in more advanced CKD, our data suggest that it may not be as important in early CKD.

Results presented in this chapter have been published previously in the article *'Associations of fibroblast growth factor 23, vitamin D and parathyroid hormone with 5 year outcomes in prospective primary care cohort of people with*

*chronic kidney disease stage 3' BMJ open* 2017;7:e016528. A Shardlow, NJ McIntyre, RJ Fluck, CW McIntyre and MW Taal (2017).[193] These results were also presented in poster abstract form at EDTA-ERA and UK Kidney Week Conferences 2016.

## 6.2 Introduction

Fibroblast growth factor 23 (FGF23), vitamin D and parathyroid hormone (PTH) interact in the control of phosphate, calcium and bone metabolism. These factors are all affected by the loss of glomerular filtration rate (GFR) that characterises chronic kidney disease (CKD). Since the observation that elevated FGF23 is linked to mortality in haemodialysis patients, there has been increasing interest in FGF23 as a risk factor in CKD.[114] FGF23 has subsequently been associated with left ventricular hypertrophy,[118] mortality,[115, 121] cardiovascular events[121, 194, 195] and CKD progression[115, 121] in populations with and without CKD. Reports from cross-sectional studies suggest that elevated FGF23 is the earliest alteration in mineral metabolism in CKD,[196] and have prompted calls for studies to explore the benefit of interventions to prevent increases FGF23 early in the course of CKD, including public health initiatives to reduce dietary phosphate intake.[197] However, the interaction between FGF23, vitamin D and PTH with respect to adverse outcomes in early CKD is not completely understood.

Like FGF23, PTH is a phosphaturic hormone that is commonly elevated in people with CKD. Previous studies have reported

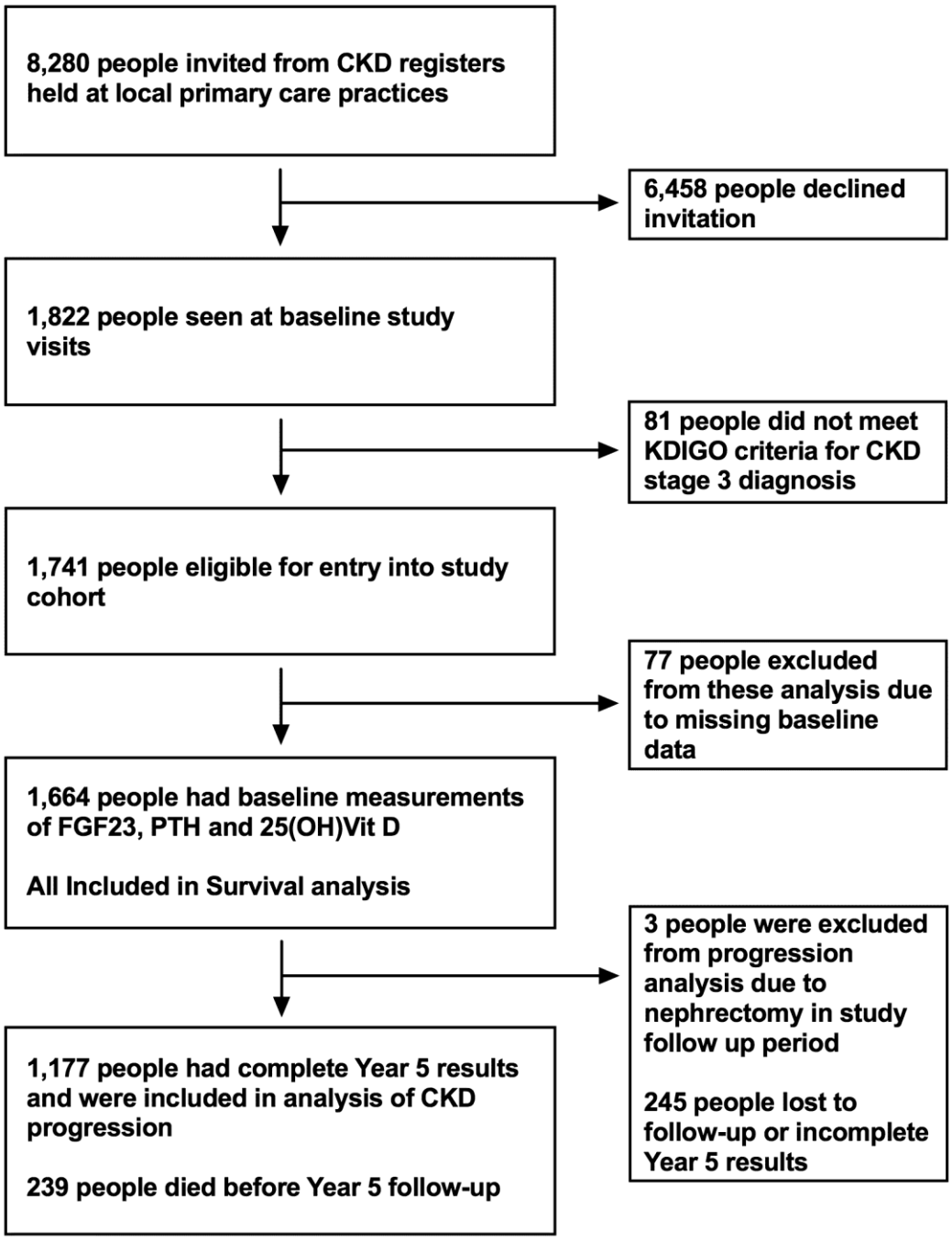
that PTH may become elevated with declining GFR before FGF23 in some populations [196, 198], including those without CKD.[199] Vitamin D status appears to be an important determinant of this difference. A previous analysis from the Renal Risk in Derby (RRID) study has shown that Vitamin D status affects the relative elevation of FGF23 and PTH in people with CKD stage 3. Those with vitamin D insufficiency were found to be more likely to have high PTH than FGF23. In contrast, FGF23 levels were elevated preferentially in those who were vitamin D replete.[122] Vitamin D deficiency has also been associated with an increased risk of all-cause mortality in the general population.[200] There is much mechanistic work to suggest significant interaction between FGF23, PTH and vitamin D,[201, 202] however the association between relative levels of these hormones and outcomes in people with early CKD is not well understood.[122] This chapter aims to address the question of whether those with elevated FGF23, or those with elevated PTH and vitamin D deficiency have worse outcomes in the context of early CKD.

## 6.3 Methods

Detailed methods for the Renal Risk in Derby (RRID) study have been published previously[122, 169]. 1,741 participants

were individually recruited and prospectively studied from 32 Derbyshire primary care practices between 2008 and 2010. In total, 8,280 people were invited from practice registers of people with CKD stage 3. 1,822 people attended baseline visits. All participants were aged over 18 years. Participants were selected using the 4-variable MDRD equation modified for use with isotope dilution mass spectrometry (IDMS)-standardised creatinine measurement. Two MDRD eGFR results consistent with CKD stage 3 (30 to 59 ml/min/1.73m$^2$) more than 90 days apart were required to be eligible. People who were judged to have a life expectancy of less than one year, were unable to attend study visits at their primary care surgery or had previously received a solid organ transplant were excluded from the study. Of the 1,822 people who attended baseline visits, 1,741 were eligible, and were included in the study cohort. (Figure 6.1)

**Figure 6.1 Flow chart showing numbers of participants at different time points of the study**




### 6.3.1 Study visits

Study visits were conducted at baseline, 1 and 5 years. Prior to each visit, participants completed a background questionnaire covering demographic details, medical history, smoking history and medication history. Participants'

responses to questions were reviewed at the study visit and clarified as required. At each study visit, the participant's height, weight, waist- and hip circumference were measured. Three blood pressure measurements were taken using an oscillometric device (UA-767 Plus 30, A&D Medical) after at least five minutes rest. Readings were repeated so that values differed by no more than 10%.

### 6.3.2 Laboratory Methods and GFR estimation

Participants collected three consecutive days' early morning urine samples and stored these in a refrigerator prior to their study visit for subsequent albumin and creatinine analysis. The mean urine albumin to creatinine ratio (uACR) from 3 specimens was used for analysis. Blood samples were taken at each study visit. Participants were asked to abstain from eating meat for 12 hours prior to the study visit to avoid confounding the creatinine assay.[161] Blood and urine samples were analysed in a single clinical laboratory at the Royal Derby Hospital for standard haematological and biochemical variables. Creatinine was measured using the Jaffe method, standardized against an isotope dilution mass spectrometry method. For these analyses, GFR was estimated using the creatinine based CKD-EPI equation. [50]

FGF23, 25-hydroxy vitamin D3 (25(OH)vit D) and PTH measurement was undertaken from frozen serum samples stored at -80°C following the baseline study visit. Serum FGF23 was measured using an intact two-site enzyme-linked immunosorbent assay (Kainos Laboratories, Tokyo, Japan). The upper limit of normal (51 pg/ml) was defined as 2 standard deviations above the mean from a previous study of people without disease.[203] 25(OH)vit D levels were measured with an API 4000TM quadrupole mass spectrometer/MS system (ABSciex, Warrington, UK), which was coupled to a Prominence ultrafast liquid chromatography system (Shimadzu Scientific Instruments, Columbia, MD). Vitamin D deficiency was defined as <25 nmol/l, Vitamin D insufficiency as 25 to 50 nmol/l and optimal vitamin D levels were defined as >75 nmol/l.[204] PTH was measured using an intact immunometric sandwich assay (Roche Diagnostics, Burgess Hill, UK, Modular Analytics E170). This assay has an upper limit of normal of 65 pg/ml.

### 6.3.3 Mortality Data

Date and cause of death as stated on death certificates was obtained from the Office of National Statistics via the Health and Social Care Information Centre (HSCIC). Three

investigators (AS, RJF and MWT) independently classified cause of death into 4 categories (cardiovascular, malignancy, infection and other). Differences were resolved by discussion.

### 6.3.4 Statistical Analysis

Independent samples T tests and Mann Whitney U tests were used to compare normally distributed and non-normally distributed variables respectively. FGF23 and PTH were assessed using strata based upon the upper limit of normal for the respective assays. Other cut points were chosen to provide four groups of reasonable size. Vitamin D strata were based upon existing definitions for vitamin D deficiency (<25 nmol/l) and insufficiency (25 to 50 nmol/l). Survival analysis was performed using Cox proportional hazards models. Participants surviving without reaching an endpoint were censored at the end of the year 5 follow-up period (1$^{st}$ May 2015). CKD Progression was assessed using binomial logistic regression. uACR, FGF23, 25(OH)vit D, and PTH were not normally distributed, and were therefore logarithmically transformed (base 10) before use in multivariable analysis as continuous variables. Three participants were excluded from progression analysis, as they underwent nephrectomy during the study period.

Independent predictors of outcomes from statistical models developed for a full analysis of year 5 outcomes for this cohort were used to adjust hazard ratios for FGF23, 25(OH)vit D and PTH in this analysis.[166]

For sensitivity analyses, the cohort was subdivided according to baseline eGFR (≥45 ml/min/1.73m$^2$, and <45 ml/min/1.73m$^2$) and baseline age (<75 years and ≥75 years).

### 6.3.5 Endpoint definitions

At year 5 of follow-up, we observed that few study participants reached End Stage Kidney Disease (ESKD). Therefore, in place of the RRID study pre-specified endpoint of development of ESKD, CKD progression was defined using the KDIGO guideline, which states that assessment of both GFR and albuminuria should be undertaken to evaluate progression.[4], We therefore defined CKD progression as a 25% decline in GFR, accompanied by a worsening of GFR category, or a worsening of albuminuria category.

## 6.4 Results

### 6.4.1 Baseline Data

Baseline data [169] as well as FGF23 and PTH levels in relation to vitamin D status have previously published.[122] Table 6.1 shows baseline data for the 1,664 participants and subgroups according to survival after 5 years. Participants were predominantly elderly with relatively well preserved GFR. Vitamin D deficiency was observed in 104 (6.3%) and vitamin D insufficiency in an additional 648 (38.9%). FGF23 was elevated (>51 pg/mL) in 475 (29%) and PTH was elevated (>65 pg/mL) in 422 (25%).

**Table 6.1 Baseline variables for participants included in this analysis (n = 1,664) by year 5 survival status.**

| Variable | Total cohort (n= 1,664) | Year 5 Outcome | |
|---|---|---|---|
| | | **Survived** (n= 1,375) | **Died** (n=289) |
| Female Gender (%) | 1010 (60.7) | 880 (64.0) | 130 (45.0)* |
| Age (years) | 73.0 ± 8.9 | 71.8 ± 8.8 | 78.5 ± 7.2* |
| CKD-EPI eGFR (ml/min/1.73m$^2$) | 53.7 ± 11.9 | 55.1 ± 11.7 | 46.7 ± 9.9* |
| uACR (mg/mmol) | 0.3 (0.0-1.5) | 0.3 (0.0-1.2) | 0.9 (0.1-3.6)* |
| Diabetes (%) | 275 (16.5) | 213 (15.5) | 62 (21.5)* |
| CVD (%) | 374 (22.5) | 263 (19.1) | 111 (38.4)* |
| Current or Previous Smoker (%) | 903 (54.3) | 713 (51.9) | 190 (65.7)* |
| ACE/ARB use (%) | 1074 (64.5) | 875 (63.6) | 199 (68.9) |
| Weight (kg) | 78.1 ± 15.4 | 78.3 ± 15.0 | 77.6 ± 17.3 |
| BMI (kg/m$^2$) | 29.0 ± 5.1 | 29.1 ± 5.0 | 28.4 ± 5.4* |
| Waist:Hip ratio | 0.91 ± 0.09 | 0.90 ± 0.09 | 0.93 ± 0.09* |
| SBP (mmHg) | 134.0 ± 18.4 | 133.8 ± 17.7 | 135.6 ± 21.3 |
| DBP (mmHg) | 72.8 ± 11.0 | 73.3 ± 10.9 | 70.3 ± 11.2* |
| Haemoglobin (g/dl) | 13.2 ± 1.4 | 13.3 ± 1.4 | 12.8 ± 1.6* |
| Corrected Calcium (mmol/l) | 2.38 ± 0.10 | 2.38 ± 0.10 | 2.37 ± 0.10 |
| Phosphate (mmol/l) | 1.11 ± 0.18 | 1.11 ± 0.17 | 1.11 ± 0.19 |
| Albumin (g/l) | 40.7 ± 3.2 | 40.8 ± 3.1 | 39.7 ± 3.4* |
| Bicarbonate (mmol/l) | 25.5 ± 2.7 | 25.6 ± 2.5 | 25.5 ± 3.1 |
| Total Cholesterol (mmol/l) | 4.8 ± 1.19 | 4.8 ± 1.2 | 4.5 ± 1.1* |
| Urate (µmol/l) | 384 ± 91 | 379 ± 88 | 408 ± 99* |
| FGF23 (pg/ml) | 42 (33-53) | 42 (33-52) | 45 (34-59)* |
| PTH (pg/ml) | 46 (34-66) | 45 (33-62) | 56 (39-86)* |
| 25(OH) Vitamin D (nmol/l) | 53 (38-71) | 54 (39-72) | 48 (31-67)* |
| On vitamin D supplementation (%) | 67 (4.0) | 49 (3.6) | 18 (6.2)* |

Data are presented as number (percentage), mean ± standard deviation or median (Interquartile range)

* p value < 0.05, versus group who survived to end of year 5 follow-up

End of year 5 follow-up period defined as 31$^{st}$ April 2015

Estimated glomerular filtration rate (eGFR), Urine albumin to creatinine ratio (uACR), Cardiovascular disease (CVD), Angiotensin converting enzyme inhibitor (ACE), Angiotensin 2 receptor blocker (ARB), Body mass index (BMI), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Fibroblast growth factor 23 (FGF23), Parathyroid hormone (PTH), 25 hydroxy-vitamin D (25(OH)vitD)

### 6.4.2 All-cause Mortality

Two hundred and eighty nine participants (18.4%) died prior to the end of the year 5 follow-up period: 101 (34.9%) from cardiovascular causes, 75 (26.0%) from malignancy, 61 (21.1%) from infection, 41 (14.2%) from other causes and no cause of death data was available for 11 (3.8%). On average, participants who died were older (78.5 ± 7.2 versus 71.8 ± 8.8 years) and had lower eGFR (46.7 ± 9.9 versus 55.1 ± 11.7 ml/min/1.73m$^2$) compared to those who survived to the end of year 5 follow-up (Table 1). Median FGF23 (45, interquartile range (IQR) 34 to 59, versus 42, IQR 33 to 52 pg/ml) and PTH levels (56, IQR 39 to 86, versus 45, IQR 33 to 62 pg/ml) were higher in those who died, and median vitamin D levels were lower (48, IQR 31 to 67 versus 54, IQR 39 to 72 nmol/l). Only 67 participants (4.0%) were taking vitamin D supplementation at study baseline. Four participants (0.2%) progressed to ESKD by the end of the year 5 follow-up period. Two of those who progressed to ESKD subsequently died prior to the year 5 follow-up visits, and were included as deaths in survival analysis.

Table 6.2 shows mortality outcomes at the end of the year 5 follow-up period by strata of FGF23, PTH and 25(OH)vit D. All-

cause mortality was more common at higher baseline levels of FGF23 and PTH, and in the vitamin D deficiency and insufficiency groups.

**Table 6.2 All-cause mortality at year 5 by baseline FGF23, PTH and 25(OH)vit D as categorical variables. Unadjusted and adjusted hazard ratios by Cox proportional hazards models are also given by category of each baseline variable.**

| Variable | Number (% of total cohort) n = 1,664 | Died prior to end of year 5 follow-up (%) | Univariable Hazard ratios (95% CI) | Hazard Ratios for all-cause mortality at end of year 5 follow-up period (95% CI) | | |
|---|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 | Model 3 |
| FGF23 (pg/ml) | | | | | | |
| <25 | 100 (6.0) | 15 (15.0) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |
| 25-51 | 1089 (65.4) | 172 (15.8) | 1.08 (0.63-1.82) | 0.81 (0.48-1.38) | 0.90 (0.52-1.57) | 0.93 (0.54-1.62) |
| 52-70 | 319 (19.2) | 63 (19.7) | 1.40 (0.80-2.47) | 0.77 (0.44-1.37) | 0.89 (0.49-1.61) | 0.88 (0.49-1.60) |
| >70 | 156 (9.4) | 39 (25.0) | 1.86 (1.03-3.38)* | 0.90 (0.49-1.66) | 0.88 (0.47-1.67) | 0.88 (0.47-1.67) |
| PTH (pg/ml) | | | | | | |
| <35 | 436 (26.2) | 54 (12.4) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |
| 35-65 | 806 (48.4) | 115 (14.3) | 1.19 (0.86-1.64) | 0.89 (0.64-1.24) | 0.84 (0.61-1.17) | 0.84 (0.61-1.18) |
| 66-95 | 286 (17.2) | 75 (26.2) | 2.39 (1.69-3.40)* | 1.41 (0.99-2.02) | 1.30 (0.90-1.87) | 1.30 (0.89-1.89) |
| >95 | 136 (8.2) | 45 (33.1) | 3.15 (2.12-4.68)* | 1.59 (1.06-1.09)* | 1.38 (0.91-2.08) | 1.37 (0.89-2.12) |
| 25(OH)Vit D (nmol/l) | | | | | | |
| <25 | 104 (6.3) | 34 (32.7) | 2.76 (1.79-4.26)* | 2.17 (1.40-3.35)* | 1.96 (1.26-3.05)* | 1.62 (1.01 – 2.58)* |
| 25-50 | 648 (38.9) | 117 (18.1) | 1.35 (0.97-1.87) | 1.16 (0.84-1.61) | 1.03 (0.74-1.45) | 0.94 (0.67 – 1.33) |
| 51-75 | 553 (33.2) | 86 (15.6) | 1.09 (0.78-1.54) | 1.07 (0.76-1.51) | 1.04 (0.73-1.47) | 1.01 (0.71 – 1.43) |
| >75 | 359 (21.6) | 52 (14.5) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (Ref) |

Model 1 – adjusted hazard ratios for age, baseline eGFR , gender and uACR

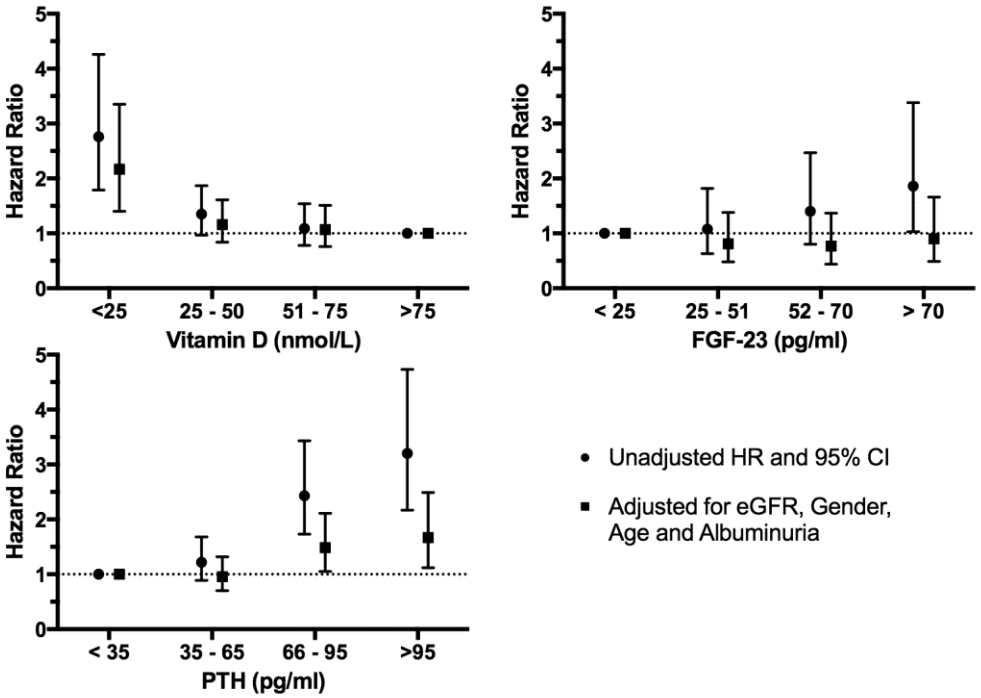
Model 2 –hazard ratios adjusted for variables in model 1, diabetes, previous cardiovascular disease, haemoglobin, bicarbonate and albumin

Model 3 – hazard ratios adjusted for variables included in model 2 plus vitamin D insufficiency (<50 nmol/l), elevated PTH (>65 pg/ml) and elevated FGF23 (>51 pg/ml) as appropriate

*$p < 0.05$

Univariable analysis revealed increasing hazard ratios for all-cause mortality across the strata for FGF23 and PTH. Vitamin D deficiency and insufficiency were associated with increased hazard ratios. Correcting these hazard ratios for baseline eGFR, age, uACR, and gender removed the association between increased FGF23 and all-cause mortality. However, the associations between both vitamin D deficiency and elevated PTH, and all-cause mortality remained (Figure 6.2).

**Figure 6.2 Unadjusted (●) and adjusted (■) hazard ratios by Cox proportional hazards models for all-cause mortality in five year follow-up period by categories of vitamin D, FGF23 and PTH. Error bars represent 95% confidence intervals.**



Multivariable Cox proportional hazards models showed that vitamin D deficiency was independently associated with all-cause mortality (HR 1.62, 95% CI 1.01 to 2.58) (Table 6.2). Elevated PTH (>65 pg/ml) also reached statistical significance in this model (HR 1.42, 95% CI 1.09 to 1.84). Elevated FGF23 levels did not enter significance in a comparable model.

Multivariable models were constructed in which vitamin D, PTH, and FGF23 were treated as continuous variables (Table 6.3). All were associated with all-cause mortality in univariate analysis. Whereas vitamin D (HR 0.81, 95% CI 0.72 to 0.91, per standard deviation of log transformed continuous variable) and PTH (HR 1.19, 95% CI 1.05 to 1.33) remained significant when adjusted for baseline eGFR, age gender and uACR, FGF23 was not independently associated with all-cause mortality in this model (HR 0.97, 95% CI 0.86 to 1.10). Vitamin D and PTH also remained significant when additionally adjusted for diabetes, previous cardiovascular disease, haemoglobin, albumin, and bicarbonate.

**Table 6.3 Hazard ratios for all-cause mortality treating vitamin D, PTH, and FGF23 as continuous variables following logarithmic transformation.**

| Variable | Univariate Hazard Ratio (95% CI) | Adjusted Hazard ratios (95% CI) | |
|---|---|---|---|
| | | Model 1 | Model 2 |
| Log25(OH)vit D | 0.75 (0.67 to 0.84)* | 0.81 (0.72 to 0.91)* | 0.86 (0.76 to 0.97)* |
| logPTH | 1.49 (1.33 to 1.67)* | 1.19 (1.05 to 1.33)* | 1.12 (1.00 to 1.27)* |
| LogFGF23 | 1.20 (1.08 to 1.34)* | 0.97 (0.86 to 1.10) | 0.95 (0.85 to 1.08) |

*p <0.005
All Hazard ratios expressed per standard deviation change in the independent variable
Model 1 – Hazard ratios adjusted for baseline age, eGFR,uACR and gender
Model 2 – Hazard ratios adjusted for all variables in model 1 and diabetes, previous cardiovascular disease, albumin, bicarbonate and haemoglobin

### 6.4.3 Sensitivity Analyses

Sensitivity analyses were performed to compare all-cause mortality in people with CKD 3a (eGFR ≥45 ml/min/1.73m$^2$) to those with CKD 3b (eGFR <45 ml/min/1.73m$^2$ at baseline visit). In both groups, FGF23 did not associate with all-cause mortality in univariable analysis or in multivariable models corrected for age, gender, baseline eGFR, and uACR. Vitamin D deficiency was associated with all-cause mortality in those with CKD 3a, but did not reach significance in the CKD 3b group. Additionally, in people aged less than 75 at baseline, vitamin D deficiency was strongly associated with all-cause mortality (Hazard Ratio 5.70, 95% CI 2.55 to 12.73) in multivariable analysis corrected for age, gender, baseline

eGFR and uACR. This relationship was not seen in participants aged 75 years or over (Hazard Ratio 1.41, 95% CI 0.81 to 2.43). There was no significant interaction between age ≥75 years and eGFR <45 ml/min/1.73m$^2$ when adjusted for gender and baseline uACR.

### 6.4.4 Cardiovascular Mortality

Both vitamin D deficiency and elevated PTH were associated with an increased risk of cardiovascular mortality in this cohort (Table 6.4). In contrast, there was no association with cardiovascular mortality across strata of baseline FGF23. When adjusted for age, gender, baseline eGFR and uACR, vitamin D deficiency and elevated PTH were associated with cardiovascular mortality. Again, there was no association between cardiovascular mortality and FGF23 levels in a similar model. When adjusted for other baseline variables (previous CVD, diabetes, haemoglobin, bicarbonate and albumin), only PTH elevated above 65 pg/mL remained an independent predictor of cardiovascular mortality. In a fully adjusted model, neither FGF23, PTH nor Vitamin D status remained significant.

**Table 6.4 Unadjusted and adjusted hazard ratios by FGF23, PTH, and 25(OH)vit D as categorical variables for cardiovascular mortality at 5 years.**

| Variable | Number of Cardiovascular Deaths (%) | Univariable Hazard Ratio (95% CI) | Adjusted Hazard Ratios (95% CI) | | |
|---|---|---|---|---|---|
| | | | Model 1 | Model 2 | Model 3 |
| FGF23 (pg/ml) | | | | | |
| <25 | 7 (7.0) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |
| 25-51 | 54 (5.0) | 0.71 (0.32-1.57) | 0.52 (0.24-1.15) | 0.61 (0.28-1.36) | 0.63 (0.28-1.41) |
| 52-70 | 21 (6.6) | 0.97 (0.41-2.27) | 0.47 (0.19-1.12) | 0.56 (0.23-1.35) | 0.57 (0.24-1.37) |
| >70 | 19 (12.2) | 1.85 (0.78-4.41) | 0.74 (0.30-1.84) | 0.78 (0.31-1.93) | 0.79 (0.32-1.97) |
| PTH (pg/ml) | | | | | |
| <35 | 13 (3.0) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |
| 35-65 | 48 (6.0) | 2.02 (1.09-3.72)* | 1.55 (0.84-2.87) | 1.49 (0.80-2.77) | 1.50 (0.80-2.79) |
| 66-95 | 20 (7.0) | 2.57 (1.28-5.16)* | 1.50 (0.74-3.04) | 1.36 (0.66-2.79) | 1.36 (0.66-2.83) |
| >95 | 20 (14.7) | 5.60 (2.79-11.26)* | 2.55 (1.25-5.21)* | 2.10 (1.02-4.30)* | 2.11 (1.00-4.47) |
| 25(OH)Vit D (nmol/l) | | | | | |
| <25 | 12 (11.5) | 2.67 (1.29-5.55)* | 2.13 (1.02-4.45)* | 1.83 (0.86-3.87) | 1.71 (0.77-3.78) |
| 25-50 | 41 (6.3) | 1.31 (0.75-2.28) | 1.15 (0.66-2.01) | 1.05 (0.60-1.85) | 1.02 (0.57-1.82) |
| 51-75 | 30 (5.4) | 1.09 (0.61-1.95) | 1.09 (0.66-1.95) | 1.07 (0.59-1.93) | 1.05 (0.58-1.91) |
| >75 | 18 (5.0) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |

Model 1 – adjusted hazard ratios for age, baseline eGFR , gender and uACR

Model 2 – adjusted hazard ratios for variables in model 1 plus diabetes, previous cardiovascular disease, haemoglobin, serum bicarbonate and serum albumin

Model 3 – hazard ratios adjusted for variables included in model 2 plus vitamin D insufficiency, elevated PTH and elevated FGF23 as appropriate

*$p < 0.05$

### 6.4.5 Progression of Kidney Disease

Progression of CKD was observed in 289 participants (17.4%) over 5 years. Mean eGFR decreased from 53.7 ± 11.9 to 53.3 ± 14.8 ml/min/1.73m$^2$ (p <0.001). Binomial logistic regression was used to assess the associations with CKD progression. In univariable analysis, risk of CKD progression increased across strata of both FGF23 and PTH. Vitamin D deficiency was also associated with progression (Table 6.5). However, in multivariable models correcting for baseline eGFR, age, uACR and gender, these associations were not preserved.

**Table 6.5 Odds ratios for CKD progression at year 5 study visit by baseline levels of FGF23, PTH, and 25(OH)vit D as categorical variables.**

| Variable | Univariable | Multivariable (Corrected for Age, eGFR, Gender and uACR) | |
|---|---|---|---|
| | OR (95% CI) | OR (95% CI) | P value |
| Vitamin D (nmol/l) | | | |
| <25 | 1.86 (1.01 – 3.41)* | 1.49 (0.79 – 2.82) | NS |
| 26-50 | 1.39 (0.97 – 1.98) | 1.26 (0.87 – 1.84) | NS |
| 51-75 | 1.17 (0.81 – 1.69) | 1.19 (0.81 – 1.75) | NS |
| >75 | 1 (Ref) | 1 (Ref) | |
| FGF23 (pg/ml) | | | |
| <25 | 1 (Ref) | 1 (Ref) | |
| 25 – 51 | 1.42 (0.77 – 2.45) | 1.22 (0.64 – 2.32) | NS |
| 52 - 70 | 2.07 (1.07 – 4.02)* | 1.38 (0.69 – 2.77) | NS |
| >70 | 2.27 (1.09 – 4.75)* | 1.27 (0.58 – 2.78) | NS |
| PTH (pg/ml) | | | |
| <35 | 1 (Ref) | 1 (Ref) | |
| 35-65 | 1.75 (1.24 – 2.45)* | 1.54 (1.08 – 2.20) | 0.016 |
| 66-95 | 2.14 (1.39 – 3.28)* | 1.58 (1.00 – 2.48) | 0.049 |
| >95 | 2.59 (1.48 – 4.53)* | 1.44 (0.79 – 2.62) | NS |

* $p<0.05$

Odds ratio (OR), Confidence Interval (CI), Reference (Ref), Fibroblast growth factor 23 (FGF23), Parathyroid hormone (PTH)

## 6.5 Discussion

In this cohort of people with CKD stage 3 with low risk of progression, managed predominantly in primary care, independent associations between all-cause mortality and both vitamin D deficiency and elevated PTH were observed. Conversely, there was no independent association between all-cause mortality and elevated FGF23. No association was found

between FGF23, Vitamin D status or PTH and progression of CKD.

Vitamin D deficiency has been shown to be associated with increased risk of all-cause mortality in the general population,[200] and a similar relationship is evidenced in this analysis. As well as its effects on calcium and bone homeostasis, vitamin D deficiency has been associated with the development of multiple markers of cardiovascular disease. Animal studies have shown links with atherosclerosis and atherosclerotic calcification,[205, 206] left ventricular hypertrophy, and renin angiotensin system activation.[206, 207] Additionally, vitamin D status impacts upon immune system function.[208] These mechanisms may underlie the association between vitamin D and all-cause mortality. Alternatively, vitamin D deficiency may associate with comorbidity and poor nutrition, which subsequently explain the observed links with mortality. Vitamin D deficiency is a potentially modifiable risk factor, but as yet there is no conclusive evidence that vitamin D supplementation provides a survival benefit in people with CKD, although most studies included participants with 25(OH)vit D levels above the range we have observed to be associated with increased risk.[209]

In addition vitamin D supplementation is associated with an increase in FGF23 levels that may counter some of the benefits.[210] Our data suggest that vitamin D deficiency is particularly associated with mortality in those aged less than 75 years. This suggests that other factors are more important as determinants of survival in the very elderly. Nonetheless, vitamin D deficiency in those aged less than 75 years represents a potentially modifiable risk factor, and is easily detectable and treatable within the primary care setting. Current consensus is that vitamin D deficiency should be treated but more prospective studies are required to evaluate the potential benefit of vitamin D replacement therapy in the context of CKD.[211]

This study found higher PTH was associated with increased all-cause mortality independently of eGFR. This result has been previously reported in people with CKD[212] and the general population.[213] Whilst elevated PTH may be secondary to vitamin D deficiency, PTH has also been shown to promote vascular calcification.[214] Additionally, PTH increases the intracellular calcium concentration, which has been hypothesised to promote myocardial necrosis and scar tissue formation[215] and this may explain observational

associations between elevated PTH and sudden cardiac death.[216] Further studies are required to investigate the mechanisms of this association and the impact of interventions to control PTH in early CKD.

In this cohort, FGF23 was not significantly associated with all-cause mortality or CKD progression in multivariable analysis. In contrast, results from the CRIC study showed associations between increased FGF23 and both a greater risk of all-cause mortality and progression of CKD to ESKD.[115] The same study subsequently showed association between cardiovascular events including congestive heart failure.[195] Elevated FGF23 levels have been associated with left ventricular hypertrophy, and animal models have suggested a causative role.[118] Important differences between the RRID and CRIC study cohorts may account for the different associations including relatively preserved renal function in the RRID study (mean eGFR 53 ml/min/1.73m$^2$ in RRID versus 43 ml/min/1.73m$^2$ in CRIC), older mean age (73 years versus 58 years in CRIC) and less severe albuminuria (median uACR 0.3 mg/mmol versus 5.9 mg/mmol). The CRIC study also contained a higher proportion of diabetics (48% versus 16.5% in RRID) and was more racially diverse (42% African-

American, versus 2.5% non-Caucasian in RRID). Additionally, we used an intact FGF23 assay, whereas the CRIC investigators used a C terminal assay, making direct comparison of the FGF23 measurements in these studies difficult. Nevertheless, the median FGF23 level reported in the CRIC study was greater than the reported normal range (median 145 RU/ml compared to upper limit of normal of 100 RU/ml)[196] whereas median FGF23 was below the upper limit of normal in the RRID cohort and only 29% evidenced elevated FGF23 levels. Thus FGF23 was relatively less elevated in our study population and this may account for the lack of association with all-cause mortality. FGF23 levels have also been reported to be associated with all-cause mortality in some but not all populations in the absence of CKD.[194, 217-221] The reasons for these variable results are not clear, but may relate to differences between the study populations and methodology.

### 6.5.1 Strengths and Limitations

Strengths of the RRID study include robust selection of participants meeting international criteria for CKD stage 3 at study recruitment. This cohort is representative of those with CKD stage 3 in the general United Kingdom population.[42]

Additionally, this analysis used gold standard assays for FGF23, PTH and 25(OH)vit D.

Limitations of the RRID study include a relative lack of ethnic diversity, which may impact upon the generalizability of these results to other populations. The RRID cohort is also predominantly elderly and evidenced a low rate of CKD progression over 5 years. These results may therefore not be applicable to a younger population, or those at higher risk of progressive disease. Finally, analyses of cardiovascular mortality and CKD progression were based upon a relatively low event rate and may therefore have lacked power to show weaker associations. Due to the very low incidence of ESKD, this analysis used a surrogate end-point of CKD progression as defined by KDIGO.

## 6.6 Conclusions

These results demonstrate that elevated PTH and vitamin D deficiency are independently associated with an increased risk of all-cause mortality in a cohort of people with CKD stage 3 recruited from primary care. In contrast to previous studies, FGF23 was not associated with mortality or progression of CKD in multivariable analysis. Whilst FGF23 may be useful as a risk

marker and potential therapeutic target in referred populations with more advanced CKD and a higher risk of progression to ESKD, our data do not support the use of FGF23 measurement as a risk marker in people with CKD stage 3 in primary care.[197] These findings suggest the hypothesis that detecting and treating vitamin D deficiency, a possible cause for elevated PTH, in those with CKD stage 3 may improve survival. However, this needs testing in a randomised controlled trial.

# 7. Results Chapter 4

# Skin Autofluorescence as a Non-Invasive Marker of Risk in Chronic Kidney Disease stage 3

## 7.1 Abstract

### 7.1.1 Introduction

Advanced glycation end products (AGEs) have been associated with adverse outcomes and complications in people with diabetes and all-cause mortality in those receiving haemodialysis. Accumulation of AGEs in the skin can be assessed quickly and non-invasively via measurements of skin autofluorescence (SAF) using an AGE reader. This chapter examines the role of SAF in risk prediction in people with CKD stage 3 as well as changes in SAF over 5 years.

### 7.1.2 Methods

Participants in the Renal Risk in Derby study were assessed at baseline, 1 year and 5 years. At each time point, participants had 3 measures of SAF taken from the forearm using an AGE reader (Diagnoptics), alongside biochemical and biometric data. Multivariable analysis was used to assess the impact of baseline SAF and the change in SAF over 1 year on outcomes in the study population.

### 7.1.3 Results

1,707 participants had measurements of SAF at baseline, and mean SAF was 2.7 arbitrary units (AU). 243 participants died prior to the end of the year 5 follow-up period. Cox-proportional hazards models showed that, when adjusted for predictors of outcomes included in models from results chapter 1, SAF at baseline was independently associated with all-cause mortality (HR 1.16 per standard deviation increase in SAF, 95% CI 1.02 to 1.32, p = 0.024). Additionally, the change in SAF over 1 year was associated with all-cause mortality. Neither baseline SAF, nor the change in SAF over 1 year was associated with CKD progression, or CKD remission in multivariable analysis.

### 7.1.4 Conclusions

This study evidenced an association in adjusted analyses between baseline SAF and all-cause mortality. Additionally, the change in SAF over the first year of the study was associated with all-cause mortality at 5 years in multivariable analysis. Neither SAF, nor its change over the first year of the study was associated with CKD progression. These findings support the hypothesis that interventions aimed at reducing AGE levels would be potentially beneficial in people with CKD, but this requires testing in a prospective randomised controlled trial.

These results have been presented in oral abstract form at UK Kidney week 2016, and in poster form at ASN Kidney week 2015, and EDTA-ERA 2016.

## 7.2 Introduction

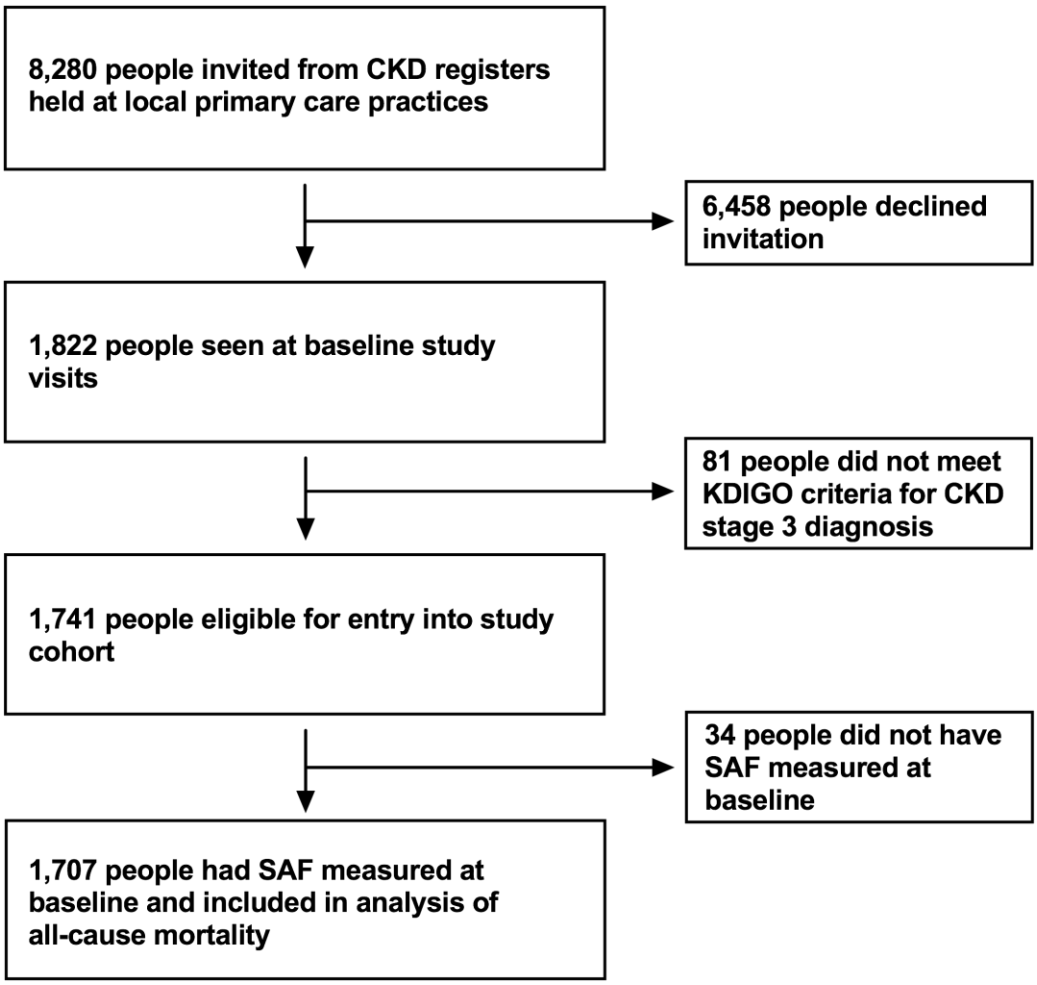
Advanced glycation end product (AGE) formation is the end result of a series of non-enzymatic reactions between peptides and sugars. These have been associated with adverse outcomes in several conditions, and have been described as a measure of cumulative oxidative stress.[222] Therefore, they are of interest as a potential risk marker. AGE levels depend upon many different factors including smoking status, diet, age, diabetes, and renal function.[223] Skin autofluorescence (SAF) has been used as a non-invasive marker of AGE accumulation in the skin, and higher levels have been associated with increased mortality in people receiving haemodialysis.[128] There is some evidence from CKD cohort studies that higher levels of SAF can also predict CKD progression.[132] Skin autofluorescence measurements can be carried out quickly and easily, and are non-invasive. Therefore, SAF is potentially useful as a tool to risk-stratify people with CKD, within the confines of a primary care consultation. Furthermore, AGE levels are potentially modifiable,[129] opening possibilities for novel intervention strategies in CKD.

This chapter seeks to evaluate the use of SAF as a non-invasive marker of tissue AGE accumulation to assess risk in people with CKD stage 3. Associations with progression of CKD, all-cause mortality and CKD remission will be described. Additionally, the change in SAF over time will be evaluated.

## 7.3 Methods

Patients were recruited and studied as described in the methods chapter. SAF was measured using an AGE reader (Diagnoptics Technologies BV, Groningen, The Netherlands). Three SAF measurements were taken from the forearm of each participant, avoiding any tattoos, or heavily pigmented areas of skin. Measurements were taken at baseline, year 1 and year 5. 1,707 participants had skin autofluorescence measured at baseline study visits (Figure 7.1).

**Figure 7.1 Flow chart showing the numbers of participants involved at each stage of the study.**




### 7.3.1 AGE Reader Mechanics

The AGE reader provides a non-invasive measure of skin AGE levels. It uses a light source emitting light at a wavelength of 320 to 400 nm. This excites fluorescent moieties in compounds in the skin. These fluoresce at a different wavelength (peak 440 nm) and are recorded by the AGE reader simultaneously. The recorded output (in arbitrary units, AU) represents the ratio between autofluorescence in the

range 420 to 600 nm and excitation light in the range 320 to 400 nm.[127]

### 7.3.2 Endpoint Definitions

CKD progression was defined based upon KDIGO criteria as previously described. CKD remission was defined as described in results chapter 1. Two participants died following developing ESKD. These individuals were described as having progressive CKD in the analysis of outcomes, and were censored at time of ESKD in survival analysis. Three participants underwent nephrectomy within the study period, and were therefore excluded from analysis of CKD progression.

### 7.3.3 Endpoint Data

Cause of death was obtained from death certificate data, received from the HSCIC (now NHS Digital) under a data sharing agreement. Cause of death was classified as cardiovascular, malignancy, infection or other, independently by AS and two RRID study co-investigators (RJF and MWT). Deaths were classified according to the primary disease leading to death. For example, an acute stroke leading to aspiration pneumonia would be classified as death due to a

cardiovascular cause. Differences between the assessors were resolved through discussion.

### 7.3.4 Statistical Methods

Study participants were split into tertiles on the basis of SAF at the baseline study visit. Normally distributed continuous variables were compared between tertiles using ANOVA. Non-normally distributed variables were compared using the Kruskal-Wallis test. Categorical variables were compared using Chi squared tests. Correlation between SAF measurements at different study visits was assessed using Pearson's correlation coefficient.

Multivariable models were constructed using Cox proportional hazards models for all-cause mortality and binomial logistic regression for CKD progression and CKD remission. All odds ratios and hazard ratios for continuous variables are expressed per standard deviation unless stated. Linear regression was used to evaluate the independent predictors of the change in SAF over the 5 years of study follow-up. Non-normally distributed continuous variables including uACR, were logarithmically transformed prior to inclusion in multivariable models.

## 7.4 Results

### 7.4.1 Baseline Characteristics

1,741 participants were studied at baseline. Baseline SAF readings were obtained in 1,707 people (98.0% of 1,741) and are included in this analysis. Mean SAF was 2.7 AU at the baseline study visit. Baseline characteristics are presented in Table 7.1 by tertile of baseline SAF. The tertile with the lowest SAF had a mean value of 2.1 AU, compared to the highest tertile, where the mean was 3.4 AU. Participants in the highest tertile of SAF were more likely to be male, diabetic and either a current or previous smoker. Additionally, univariable analysis revealed that greater age, lower baseline eGFR and greater levels of albuminuria also associated with higher tertiles of baseline SAF.

**Table 7.1: Cohort Baseline Characteristics by Tertile of Skin Autofluorescence**

| Variable | Lowest Tertile (n=560) | Middle Tertile (n=575) | Highest Tertile (n=572) | P value |
|---|---|---|---|---|
| eGFR (ml/min/1.73m$^2$) | 57.1 ± 11.2 | 53.6 ± 11.0 | 49.9 ± 12.2 | <0.001 |
| Baseline Age (years) | 70.7 ± 9.9 | 73.0 ± 8.6 | 74.9 ± 8.1 | <0.001 |
| Baseline SAF (AU) | 2.1 ± 0.2 | 2.7 ± 0.1 | 3.4 ± 0.4 | <0.001 |
| Female Gender | 360(64.3) | 359(62.4) | 317(55.4) | 0.005 |
| Haemoglobin (g/dl) | 13.5 ± 1.3 | 13.3 ± 1.4 | 12.9 ± 1.5 | <0.001 |
| Bicarbonate (mmol/l) | 25.6 ± 2.5 | 25.6 ± 2.7 | 25.3 ± 2.9 | 0.056 |
| Albumin (g/l) | 41.1 ± 2.9 | 41.0 ± 3.4 | 40.2 ± 3.1 | <0.001 |
| Phosphate (mmol/l) | 1. ± 0.2 | 1.1 ± 0.2 | 1.1 ± 0.2 | 0.90 |
| Corrected Calcium (mmol/l) | 2.38 ± 0.10 | 2.37 ± 0.10 | 2.38 ± 0.10 | 0.38 |
| Urate (μmol/l) | 373 ± 88 | 390 ± 96 | 387 ± 88 | 0.005 |
| Total Cholesterol (mmol/l) | 4.9 ± 1.2 | 4.8 ± 1.2 | 4.6 ± 1.1 | <0.001 |
| uACR (mg/mmol) | 0.2 (0.0 to 0.9) | 0.3 (0.0 to 1.3) | 0.6 (0.0 to 3.0) | <0.001 |
| BMI (kg/m$^2$) | 28.7 ± 4.9 | 29.3 ± 5.3 | 29.1 ± 5.2 | 0.15 |
| WHR | 0.89 ± 0.08 | 0.90 ± 0.09 | 0.92 ± 0.09 | <0.001 |
| SBP (mmHg) | 133 ± 18 | 133 ± 18 | 136 ± 19 | 0.006 |
| DBP (mmHg) | 74 ± 11 | 73 ± 11 | 71 ± 11 | <0.001 |
| PWV (m/s) | 9.6 ± 2.0 | 9.9 ± 2.0 | 10.2 ± 2.0 | <0.001 |
| Diabetes | 50 (8.9) | 82 (14.3) | 152 (26.6) | <0.001 |
| Previous CVD | 88 (15.7) | 127 (22.1) | 164 (28.7) | <0.001 |
| Smoking Status | | | | |
| *Current Smoker* | 18 (3.2) | 18 (3.1) | 43 (7.5) | <0.001 |
| *Previous Smoker* | 242 (43.2) | 283 (49.2) | 327 (57.2) | <0.001 |

Data are presented as mean ± standard deviation., number (percentage) or median (Interquartile range)

P values for trend across tertiles by ANOVA, Chi squared test or Kruskal-Wallis test

Estimated glomerular filtration rate (eGFR), Skin autofluorescence (SAF), Urine albumin to creatinine ratio (uACR), Body mass index (BMI), Waist to hip ratio (WHR), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Pulse wave velocity (PWV), Cardiovascular disease (CVD)

### 7.4.2 Change in Skin Autofluorescence Over Time

At 1 year follow-up, 1,545 participants (90.5% of 1,707) had a repeat measurement taken. There was moderate correlation

between baseline and year 1 values (Pearson's r = 0.67). Mean change in SAF was small (-0.1 AU), however, 310 participants (20.1% of 1,545) increased by more than 10% from baseline values. 98 participants (6.3% of 1,545) increased by more than 25% from baseline. Mean SAF at the year 5 visit was comparable to baseline visit levels. Table 7.4 shows changes in SAF and other variables at the year 1 and year 5 follow-up visits for the 953 participants who had SAF measured at year 5. The mean change over the 5-year follow up period was 0.02 AU.

**Table 7.2 Key variables at baseline, year 1 and year 5 study visits for participants with a skin autofluorescence measurement for year 5 (n = 953)**

| Variable | Baseline | Year 1 | Year 5 |
|---|---|---|---|
| SAF (AU) | 2.6 ± 0.6 | 2.5 ± 0.5 | 2.7 ± 0.6 |
| eGFR (ml/min/1.73m$^2$) | 55.4 ± 11.8 | 54.6 ± 12.9 | 54.1 ± 15.3 |
| Age (years) | 70.5 ± 8.7 | 71.6 ± 8.7 | 75.7 ± 8.7 |
| Male Gender | 361 (37.9) | - | - |
| Diabetes | 138 (14.5) | - | - |
| Haemoglobin (g/dl) | 13.4 ± 1.4 | 13.4 ± 1.3 | 13.1 ± 1.4 |
| Bicarbonate (mmol/l) | 25.5 ± 2.6 | 25.3 ± 2.5 | 25.1 ±2.6 |
| Albumin (g/l) | 40.8 ± 3.2 | 40.0 ± 3.1 | 37.0 ± 2.8 |
| Phosphate (mmol/l) | 1.1 ± 0.2 | 1.1 ± 0.2 | 1.1 ± 0.2 |
| uACR (mg/mmol) | 0.3 (0.0-1.1) | 0.5 (0.2-1.6) | 0.7 (0.0-3.2) |
| SBP (mmHg) | 133 ± 17 | 130 ± 16 | 139 ± 20 |
| DBP (mmHg) | 73 ± 11 | 71 ± 10 | 75 ± 10 |
| Smoking | | | |
| *Current Smoker* | 45 (4.7) | - | - |
| *Previous Smoker* | 450 (47.2) | | |

All data presented as mean ± standard deviation, median (interquartile range) or number (percentage)

Skin autofluorescence (SAF), Arbitrary units (AU), Estimated glomerular filtration rate (eGFR), Urine albumin to creatinine ration (uACR), Systolic blood pressure (SBP, Diastolic blood pressure (DBP)

Multivariable linear regression was used to assess the independent predictors of the change in SAF over 5 years. Univariable and multivariable models are given in Table 7.3. In a fully adjusted multivariable model, independent predictors of the change in SAF over 5 years were baseline eGFR, baseline SAF, baseline BMI (higher BMI associated with greater increase in SAF), diabetes, baseline ACR (higher uACR associated with greater increase in SAF), baseline cholesterol, baseline phosphate, and baseline bicarbonate (lower bicarbonate associated with greater increase in SAF).

**Table 7.3 Univariable and multivariable predictors of the change in skin autofluorescence over 5 years**

| Variable | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | B (95% CI) | p | B (95% CI) | p |
| Baseline eGFR | -0.004 (-0.007 to -0.001) | 0.008 | -0.004 (-0.007 to -0.001) | 0.017 |
| Baseline SAF | -0.36 (-0.42 to -0.30) | <0.001 | -0.46 (-0.52 to -0.40) | <0.001 |
| Age | -0.001 (-0.005 to 0.003) | 0.79 | 0.003 (0.00 to 007) | 0.08 |
| Log(uACR) | 0.05 (0.02 to 0.07) | <0.001 | 0.03 (0.01 to 0.06) | 0.001 |
| Diabetes at baseline | 0.13 (0.03 to 0.23) | 0.008 | 0.14 (0.04 to 0.23) | 0.005 |
| BMI at baseline | 0.009 (0.002 to 0.015) | 0.012 | 0.011 (0.005 to 0.018) | 0.001 |
| Total Cholesterol | -0.05 (-0.08 to -0.02) | <0.001 | -0.03 (-0.06 to 0.00) | 0.027 |
| Baseline Haemoglobin | 0.03 (0.01 to 0.06) | 0.016 | -0.01 (-0.03 to 0.02) | 0.64 |
| Baseline Phosphate | -0.24 (-0.43 to -0.05) | 0.013 | -0.19 (-0.37 to -0.01) | 0.035 |
| Baseline Albumin | 0.00 (-0.01 to 0.01) | 0.98 | 0.14 (-0.02 to 0.00) | 0.14 |
| Baseline Bicarbonate | -0.03 (-0.04 to 0.01) | <0.001 | -0.02 (-0.03 to -0.01) | 0.004 |
| Smoking | | | | |
| *Previous smoker* | 0.02 (-0.05 to 0.09) | 0.6 | 0.05 (-0.01 to 0.12) | 0.11 |
| *Current Smoker* | -0.05 (-0.22 to 0.11) | 0.53 | 0.10 (-0.05 to 0.25) | 0.21 |
| Log(HsCRP) | -0.001 (-0.08 to 0.07) | 0.98 | -0.034 (-0.11 to 0.04) | 0.34 |

All parameter estimates given per unit change in the dependent variable
uACR and HsCRP were logarithmically transformed (base 10) prior to regression analysis
Confidence interval (CI), Estimated glomerular filtration rate (eGFR), Skin autofluorescence (SAF), Urine albumin to creatinine ration (uACR), Body mass index (BMI), High sensitivity C-reactive protein (HsCRP)

### 7.4.3 All-cause Mortality at Five Years

243 participants (14.2% of 1,707) died prior to year 5 follow-up. Of these, 2 participants developed ESKD prior to death,

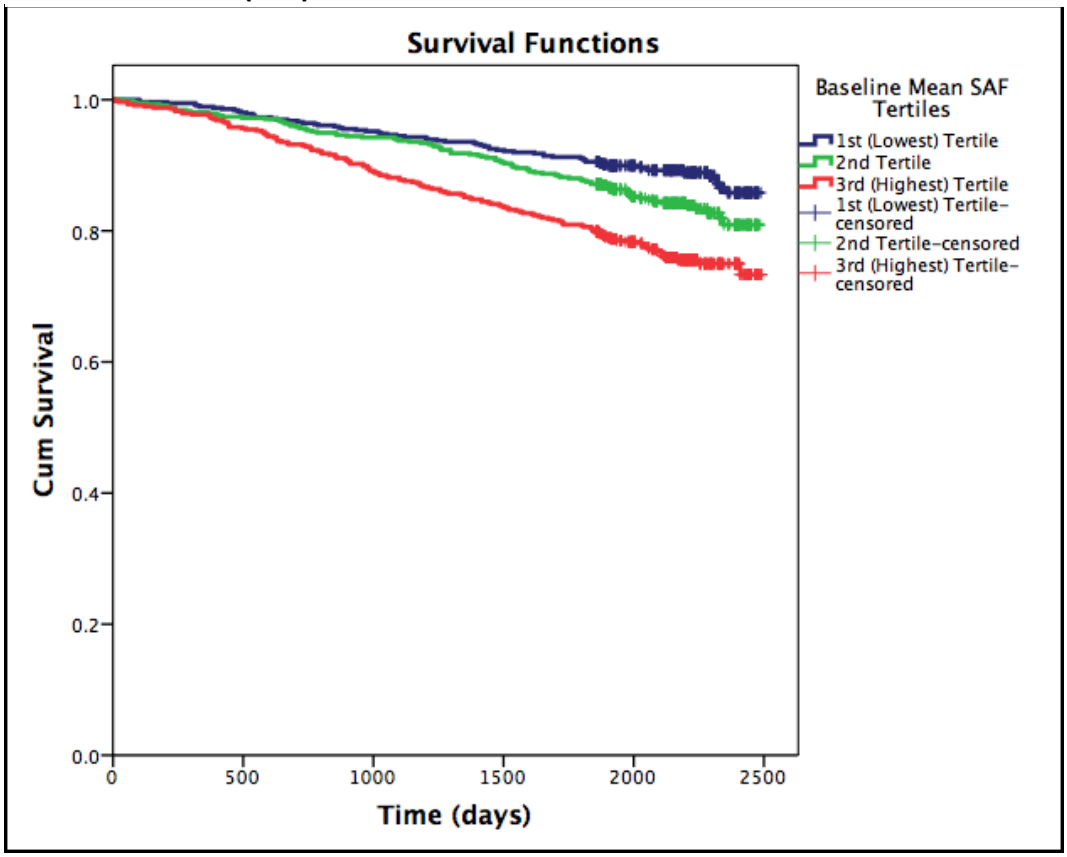
and were censored at time of ESKD in all-cause mortality analyses. Causes of death were classified as cardiovascular for 91 participants (37.3% of 243), malignancy for 63 (25.9%), infective for 48 (19.8%) and other causes for 35 people (14.4%). Cause of death data was not known for 6 participants (2.5%). The proportion of deaths increased across tertiles of baseline SAF (Table 7.2). Kaplan Meier plots of survival by tertile of baseline SAF are given in Figure 7.2. There was a statistically significant difference in survival between tertiles (log-rank test, $p<0.001$).

**Table 7.4 Outcomes at year 5 follow up by baseline tertile of skin autofluorescence (SAF)**

| Outcome | Lowest Baseline SAF tertile (n=560) | Middle Baseline SAF tertile (n=575) | Highest Baseline SAF tertile (n=572) | Whole Cohort (n=1707) |
|---|---|---|---|---|
| Stable CKD | 196 (35.0%) | 200 (34.8%) | 183 (32.0%) | 579 (33.9%) |
| CKD Remission | 154 (27.5%) | 117 (20.3%) | 61 (10.7%) | 332 (19.4%) |
| CKD Progression | 87 (15.5%) | 101 (17.6%) | 118 (20.6%) | 306 (17.9%) |
| Died prior to year 5 follow-up | 55 (9.8%) | 72 (12.5%) | 114 (19.9%) | 241 (14.1%) |
| Lost to follow-up | 68 (12.1%) | 85(14.8%) | 96 (16.8%) | 249 (14.5%) |

Data presented are number (percentage of tertile) with each given outcome
Skin autofluorescence (SAF), Chronic kidney disease (CKD)

**Figure 7.2 Kaplan-Meier plot of survival by tertile of baseline skin autofluorescence (SAF)**




Univariable Cox proportional hazards analysis showed baseline SAF (as a continuous variable) to be associated with all-cause mortality at 5 years (HR 1.53, 95% CI 1.37 to 1.72, p <0.001, Table 7.3). After adjusting for other determinants of all-cause mortality from multivariable models described in results Chapter 1 (age, sex, eGFR, uACR, previous CVD, haemoglobin, serum albumin and bicarbonate), baseline SAF remained a significant predictor of all-cause mortality at 5 years (HR 1.16, 95% CI 1.02 to 1.32, p = 0.024, Table 7.5).

**Table 7.5 Associations of baseline skin autofluorescence (SAF) with all-cause mortality over 5 years in univariable and multivariable Cox proportional hazards models**

| Variable | Univariable HR (95% CI) | Multivariable Models HR (95% CI) | |
|---|---|---|---|
| | | Model 1 | Model 2 |
| Baseline SAF | 1.53 (1.37-1.72)* | 1.24 (1.09-1.40)* | 1.16 (1.02-1.32)* |
| Baseline eGFR | 0.50 (0.44-0.57)* | 0.73 (0.63-0.85)* | 0.74 (0.63-0.87)* |
| Baseline uACR | 1.57 (1.37-1.80)* | 1.26 (1.10-1.46)* | 1.22 (1.05-1.41)* |
| Baseline age | 2.44 (2.08-2.86)* | 2.02 (1.70-2.40)* | 1.92 (1.61-2.28)* |
| Male gender | 2.21 (1.72-2.85)* | 1.55 (1.19-2.02)* | 1.81 (1.37-2.40)* |
| Previous CVD | 2.61 (2.02-3.37)* | - | 1.70 (1.30-2.22)* |
| Baseline haemoglobin | 0.71 (0.63-0.80)* | - | 0.87 (0.76-1.00)* |
| Baseline bicarbonate | 0.97 (0.85-1.10) | - | 1.17 (1.03-1.33)* |
| Baseline albumin | 0.74 (0.67-0.82)* | - | 0.83 (0.73-0.94)* |

* $p<0.05$

Hazard ratio (HR), Confidence interval (CI), Skin autofluorescence (SAF), Estimated glomerular filtration rate (eGFR), Urine albumin to creatinine ratio (uACR), Cardiovascular disease (CVD)

Baseline uACR was logarithmically transformed (base 10) prior to survival analysis

HRs are given per standard deviation for continuous variables

Change in SAF over 1 year was not associated with all-cause mortality in univariable analysis. When adjusted for baseline SAF however, both the change in SAF over 1 year, and baseline SAF were significantly associated with all-cause mortality. When adjusted for baseline age, eGFR, uACR and gender, both SAF and the change in SAF remained significantly associated with all-cause mortality. When both were included

in a model including all factors identified to be associated with all-cause mortality in results chapter 1, the change in SAF over 1 year remained significant (HR 1.16, 95% CI 1.001 to 1.344, p = 0.049). Results of multivariable analyses are given in Table 7.6.

**Table 7.6 Associations of both baseline skin autofluorescence (SAF) and the change in SAF over 1 year with all-cause mortality over 5 years in univariable and multivariable Cox proportional hazards models**

| Variable | Univariable HR (95% CI) | Multivariable Models HR (95% CI) | | |
|---|---|---|---|---|
| | | Model 1 | Model 2 | Model 3 |
| Baseline SAF | 1.53 (1.37-1.72)* | 1.64 (1.41-1.90)* | 1.27 (1.07-1.49)* | 1.19 (1.01-1.41)* |
| Change in SAF at Y1 | 1.08 (0.93-1.26) | 1.27 (1.10-1.45)* | 1.17 (1.01-1.35)* | 1.16 (1.00-1.34)* |
| Baseline eGFR | 0.50 (0.44-0.57)* | - | 0.73 (0.61-0.88)* | 0.74 (0.61-0.90)* |
| Baseline uACR | 1.57 (1.37-1.80)* | - | 1.23 (1.04-1.46)* | 1.19 (1.00-1.42) |
| Baseline age | 2.44 (2.08-2.86)* | - | 2.16 (1.76-2.65)* | 2.06 (1.67-2.55)* |
| Male gender | 2.21 (1.72-2.85)* | - | 1.67 (1.22-2.28)* | 1.88 (1.35-2.61)* |
| Previous CVD | 2.61 (2.02-3.37)* | - | - | 1.50 (1.09-2.06)* |
| Baseline haemoglobin | 0.71 (0.63-0.80)* | - | - | 0.85 (0.72-1.00)* |
| Baseline bicarbonate | 0.97 (0.85-1.10) | - | - | 1.11 (0.96-1.29) |
| Baseline albumin | 0.74 (0.67-0.82)* | - | - | 0.91 (0.78-1.07) |

* p<0.05

Skin autofluorescence (SAF), Hazard ratio (HR), Confidence interval (CI), Estimated glomerular filtration rate (eGFR), Urine albumin to creatinine ratio (uACR), Cardiovascular disease (CVD)

Baseline uACR was logarithmically transformed (base 10) prior to survival analysis

HRs are given per standard deviation for continuous variables

### 7.4.4 CKD Progression

942 participants (55% of 1,707) had measurements of both SAF and eGFR at the year 5 follow-up visit. There was no significant correlation between the change in eGFR and the change in SAF over this time (Pearson's r = -0.12, p = 0.7). 306 participants (17.9% of 1,707) of those with a baseline SAF measurement demonstrated CKD progression at year 5. Four people reached ESKD (0.2% of 1,707). In univariable analysis, baseline SAF was associated with CKD progression using binomial logistic regression (HR 1.36, 95%, CI 1.19 to 1.56, p <0.001). Baseline SAF remained significantly associated with progression when adjusted for age, uACR, eGFR (all measured at baseline), and gender (HR 1.18, 95% CI 1.02 to 1.37, p = 0.027). However, this association was lost (HR 1.09, 95% CI 0.94 to 1.27, p = 0.26) when baseline skin autofluorescence was additionally adjusted for baseline haemoglobin, bicarbonate and diabetes status, which were identified to be independent predictors of progression in results chapter 1.

### 7.4.5 CKD Remission

332 participants (19.7% of 1,707) met criteria for CKD remission at the year 5 visit. Lower SAF at baseline was

associated with CKD remission at year 5 (HR 0.68, 95% CI 0.58 to 0.78, p <0.001). This association did not persist, however, when baseline SAF was adjusted for key baseline variables (age, gender, eGFR and uACR)

### 7.4.6 Sensitivity Analysis

Sensitivity analyses were undertaken using only the non-diabetic population to ensure that associations seen in the whole population were not skewed by those with diabetes. These showed similar associations between baseline SAF and all-cause mortality (data not shown).

## 7.5 Discussion

These results show an independent association between baseline SAF and all-cause mortality in a population with predominantly early CKD stage 3. Additionally, the change in SAF over the first year of follow-up was associated with higher risk of mortality. However, whilst univariable analysis revealed associations between baseline SAF and CKD progression and remission, these did not remain when adjusted for clinical variables known to predict progression.

SAF has previously been shown to associate with an increased risk of all-cause mortality in the haemodialysis population.[128] This analysis found that baseline SAF and change in SAF at 1 year were independently associated with all-cause mortality in a cohort with only mildly reduced GFR. This suggests that SAF may be a useful biomarker in early as well as advanced CKD.

Several mechanisms may account for the association between higher SAF and mortality. AGEs are implicated in the formation of collagen cross-links resulting in increasing arterial stiffness that may contribute to the pathogenesis of cardiovascular disease. This process has been demonstrated to be reversible in dogs, resulting in reduced left ventricular stiffness.[224] Additionally, AGE may have a role in promoting inflammation through a specific receptor, RAGE and this may in turn contribute to the pathogenesis of atherosclerosis. In a murine model, atherosclerosis was prevented by blockade of RAGE,[225] suggesting that lower AGE levels may reduce this process, and subsequent cardiovascular risk.

This study found associations between SAF at baseline and CKD progression in univariable and limited multivariable

analyses but this association was lost in full multivariable models. An association between SAF and CKD progression has previously been reported in a Japanese cohort of 449 people with CKD stages 1 to 5.[132] The measured values for SAF in this study (2.1 AU, compared to 2.7 AU in the RRID study at baseline) do suggest some differences between the cohorts. Tanaka et al. included a much greater range of renal function in their study with participants with CKD stage 1 to 5. Thus, whilst the mean eGFR values at baseline in these studies are similar (55.5 ml/min/1.73m$^2$ versus 53.5 ml/min/1.73m$^2$ in the RRID study) the majority of those who demonstrated progression in the study by Tanaka et al. had CKD stage 4 or 5. Other clear differences between these study populations included age (mean age 64 years versus 73 years in the RRID cohort), ethnicity and smoking history (33% ever smoked versus 54% in the RRID cohort). Dietary differences between these populations may also have impacted upon AGE accumulation. It is possible that SAF is less useful to predict CKD progression in CKD stage 3. Alternatively, longer follow-up may be required to observe associations in this population, due to the relatively low rate of progressive CKD.

Few studies have described longitudinal changes in skin autofluorescence over time. This study found no significant changes in mean SAF over 1 and 5 year follow-up periods. However, as higher SAF associates with mortality, those with higher baseline levels, or a greater increase over time would be less likely to survive to year 5 follow-up. In a small population of people with type 1 diabetes, SAF was found to increase significantly over a four year period, and increasing SAF was associated with diabetic microvascular complications, including albuminuria, and reduced eGFR.[226] Other authors, and earlier analyses from the RRID cohort have reported associations between age and increased SAF in cross-sectional studies, suggesting that there is an increase in SAF over time.[223, 227]

This analysis evidenced independent determinants for the change in skin autofluorescence over 5 years of the study period, including baseline SAF, eGFR, uACR, diabetes, phosphate, bicarbonate, total cholesterol and BMI. Baseline SAF was negatively correlated with the change in SAF over five years. This is consistent with a degree of regression to the mean in the observations taken. Lower eGFR was associated with greater increases in SAF over 5 years. This may represent

reduction in the renal excretion of AGEs from the plasma, promoting tissue deposition. People receiving a renal transplant have been shown to have a lower SAF level than those with ESKD receiving with haemodialysis or peritoneal dialysis, again suggesting a role for reduced renal clearance in the development of increasing AGE levels.[129] Diabetes and higher HbA1c levels have been shown to associate with higher SAF and AGE levels in other populations.[228]

Smoking has been shown to be associated with increased levels of tissue AGE in other studies,[229] but was not associated with increased changes over a five year period. Rates of current smoking at recruitment in this cohort were low (4.5%), which may impact upon the power of this analysis to detect an impact. Other factors, such as dietary habits and cooking methods have been associated with changes in AGE levels, but were not captured in this population. Further study of these factors is worthwhile as a potential mechanism to reduce tissue AGE levels and thereby reduce cardiovascular risk.

SAF is of particular interest as a risk marker, as it is potentially modifiable. A cross sectional analysis showed lower SAF levels

in renal transplant patients compared to those on either peritoneal or haemodialysis, implying that SAF decreases with improved GFR after transplantation. This was confirmed by observation of a decrease in SAF levels in a small number of renal transplant recipients, compared with SAF values recorded while they were on dialysis.[129] AGEs may also enter the body from exogenous sources including smoking and diet, particularly foods cooked at high temperatures.[223] Dietary changes may therefore also reduce tissue AGE accumulation and SAF measurements. This has been shown in several small studies in a variety of populations including some with CKD.[230-232] Higher levels of dietary AGE intake have been associated with an increased incidence of CKD after 3 years of follow-up in an Iranian cohort.[233] The hypothesis that dietary AGE restriction is an effective intervention to reduce cardiovascular events and improve survival in people with early CKD requires testing in a prospective randomised controlled trial.

### 7.5.1 Strengths and Limitations

As with previous analyses from the Renal Risk in Derby cohort, this represents a well-characterised population, all of whom met strict criteria for CKD stage 3 diagnosis prior to

recruitment. Data were collected prospectively at protocol driven study visits. Few studies previously have collected longitudinal SAF data. Limitations of this analysis include a predominantly Caucasian population. Additionally, the AGE reader is limited in its applicability to people of African and African-Caribbean ethnicity due to reduced levels of reflected light from darker skin. Our findings may therefore not be applicable to more ethnically diverse populations.

## 7.6 Conclusions

This analysis showed that higher SAF was independently associated with all-cause mortality in a cohort of people with CKD stage 3, when adjusted for common clinical variables, and previously determined independent predictors. Additionally, the change in SAF over 1 year was associated with all-cause mortality, which has not previously been described. Neither SAF, nor its change over the first year of the study was associated with CKD progression. These findings support the hypothesis that interventions aimed at reducing AGE levels would be potentially beneficial in improving some outcomes in people with CKD but this should now be tested in a prospective randomised controlled trial.

# 8. Discussion

## 8.1 Outcomes

Results from this work have shown that, in a predominantly elderly population in primary care, risk of progression at 5 years from CKD stage 3 to ESKD was low (0.2%; 4 of 1,741 participants). CKD progression, based upon changes in eGFR or uACR, was seen in a minority of participants and the most common outcome was stable CKD. Criteria for CKD remission were met in a few individuals at each study time point. Rates of progression to ESKD were significantly lower than other cohort studies in people with CKD, highlighting a key difference between CKD managed in primary compared to secondary care. Additionally, risk of death was much greater than the risk of progression to ESKD, suggesting that the clinical focus for this group should be on minimising modifiable risks for premature death, rather than on preparation for renal replacement therapy.

## 8.2 CKD diagnosis and KDIGO guidelines

As people with CKD stage 3 often do not have symptoms as a direct result of their reduced renal function, diagnosis aims to detect asymptomatic individuals at risk of adverse outcomes.[234] Defining diagnostic criteria can be considered

to be a balance between sensitivity and specificity: overdiagnosis and medicalising otherwise healthy people on one hand, compared to missing those with significant modifiable risks on the other. Whilst guidelines in their current state are easy to apply, they raise several issues.

As illustrated in this study, many people identified as having CKD are at low risk of progressive renal disease. Some authors have stated the importance of identifying everyone with reduced eGFR, highlighting the increased risk that is associated with even small reductions in eGFR.[6] Others have favoured a change in the diagnostic criteria to avoid diagnosing CKD in those above 65 years without proteinuria and an eGFR greater than 45 ml/min/1.73m$^2$.[5, 172] Recently, following similar logic, Pottel et al. have proposed using age- and sex-normalised values of creatinine to diagnose abnormal renal function.[53] CKD is clearly a heterogeneous spectrum of different disease severities due to a range of underlying conditions. This is complicated by changes in renal function associated with ageing.[235] Risk, however can increasingly be assessed across different populations, as highlighted by the KFRE,[86, 157] allowing accurate information to be given to patients. This work

supports the continued monitoring of individuals managed in primary care with CKD 3 by current criteria, as progression was observed, though in a minority of participants.

Individualized discussion of risks associated with a diagnosis of CKD may be a beneficial management strategy. Data from the RRID cohort and other studies, have shown that people at high and low risk may be identified using common clinical parameters (age, eGFR, uACR and gender). Those at low risk may therefore be monitored and reassured that there is only a small probability of adverse renal outcomes. Medicines management, and education about risks of AKI may prove to be particularly important, although evidence around the temporary discontinuation of ACE inhibitors and diuretics, for example, at times of acute illness is currently lacking.[236]

## 8.3 CKD Remission

This study has described CKD remission in a substantial minority of participants, suggesting that individuals who meet criteria for CKD diagnosis may not necessarily always continue to do this. It is uncertain if this group truly has pathology, or is at higher risk than those people in the general population. Additionally, the concept of CKD remission raises further

questions about the diagnosis of CKD. One aspect to consider would be the timing of blood tests to make a diagnosis of CKD. Many people have blood tests done after a diagnosis of hypertension and after the introduction of antihypertensive medication.[237] Drugs including ACE inhibitors, ARBs and diuretics all are commonly used to treat hypertension and may transiently lower eGFR. Such blood tests could potentially lead to a diagnosis of CKD. Additionally, creatinine measurements are variable, and can be affected by non-renal factors including diet.[161] The possibility of a diagnosis of CKD based on potentially transiently lowered eGFR measurements suggests the need to increase the number of abnormal tests needed to define disease, or the length of time over which GFR should be abnormal before CKD is confirmed. Like progression, CKD remission can be predicted using common clinical variables (age, baseline eGFR, and baseline uACR). Mechanisms to predict positive outcomes are important to reassure patients appropriately, and to avoid subjecting this group of people to unnecessary medical interventions, that are unlikely to affect their outcome or health status.

## 8.4 CKD Progression

CKD progression was relatively uncommon in this cohort, with only 0.2% of 1,741 participants reaching ESKD, and less than 20% meeting criteria for progression based on the 2012 KDIGO guidelines. Whilst not necessarily causing increasing symptoms, progression in this sense represents transition to a higher risk group, and may warrant a higher frequency of monitoring or referral to secondary care. This thesis, and other studies have shown that those at high risk of progressive renal disease can be identified using common clinical variables.[86] It would therefore be feasible to risk stratify individuals in primary care according to risk of CKD progression. This could form part of future guidelines and referrals, and additionally could be used to reassure those at low risk.

Defining CKD progression in a primary care population such as RRID, where hard endpoints such as ESKD are uncommon, is challenging. This analysis has used definitions based upon the KDIGO criteria, and included progression in both eGFR and albuminuria dimensions of CKD classification. Epidemiological work has suggested that percentage changes in eGFR over short time periods may highlight those who will continue to progress to ESKD,[29] however no such robust studies exist

when describing changes in albuminuria. Both changes included in the definition employed in this analysis suggest increase in an individual's risk of a range of adverse outcomes, and would warrant an increase in review and monitoring for some, and referral to secondary care for others.

## 8.5 Competing Risks

Adverse outcomes in CKD are often described as competing risks. However, many outcomes may interact with each other. For example, both AKI and cardiovascular events may increase an individual's risk of CKD progression and all-cause mortality. Whereas worsening renal function may in turn increase risks of AKI and cardiovascular events. It might be reasonable to consider that these risks are synergistic, rather than competing, as they are often linked.

## 8.6 Cystatin C and CKD Diagnosis

Cystatin C has been proposed to be of use to confirm a diagnosis of CKD based on serum creatinine derived eGFR.[4, 7] However, this study showed only a small benefit in reducing CKD diagnosis which was outweighed by a greater proportion of participants having a worse classification of CKD as a result of cystatin C testing. This would have significant cost

implications if these people were referred to secondary care.[173] Whilst many studies have demonstrated increased risk associated with increasing levels of cystatin C, the recommendations made have not been directly tested in the population in which they would be applied (Predominantly older people with CKD 3a in primary care). We were unable to show significant clinical benefit associated with cystatin C testing in this population. These data would therefore not support the widespread implementation of the current NICE recommendations on the use of cystatin C in primary care in the UK. As well as the cost of the assay, which is not currently available routinely in all biochemistry laboratories, implementation would also require education of general practitioners regarding the use and interpretation of the results. Cystatin C may, however, prove useful in other populations, for example the assessment of eGFR in those at extremes of body habitus.

## 8.7 Skin Autofluorescence as a marker of risk

There is growing evidence that SAF as a marker of tissue AGE accumulation may associate with a range of adverse outcomes in CKD. Additionally, AGE may represent a modifiable risk factor. It remains to be demonstrated, however, that

impacting upon dietary AGE intake, for example, results in lower SAF readings, and in subsequently lower cardiovascular mortality. In this analysis, SAF was associated with all-cause mortality in fully adjusted models, but not CKD progression. However, it is not yet clear whether SAF adds substantially to basic clinical variables to be of use for risk prediction in primary care. Additionally, significant investment in and equipment would be required to obtain measurements. If SAF or AGEs were found to be a modifiable risk factor with impacts on cardiovascular events or all-cause mortality, then measuring SAF in primary care may well be more practicable, however, these studies have yet to be done. In summary, these results do not provide sufficient evidence to recommend routine assessment of SAF in those with CKD stage 3 in primary care.

## 8.8 Use of Biomarkers in Risk Prediction

Many biomarkers, beyond typically measured clinical variables, have been used in the assessment of risk in CKD. This thesis has examined several (cystatin C, FGF23, Vitamin D, PTH and SAF) in the context of CKD stage 3 in primary care. Several of these have been shown to associate with adverse outcomes in this work. Transition to use in clinical practice would require

evidence that a biomarker provides information above what is currently available from other routine clinical measurements and blood tests. Additionally, it should enable the detection of people at high (or low) risk, who would have been misclassified using other models.

### 8.8.1 Fibroblast growth factor 23

In contrast to other studies, the data presented suggest that in CKD stage 3, FGF23 does not add to risk prediction, and therefore, there is no indication to test or intervene on this in primary care populations. This is important because the findings will help prevent expenditure on an unnecessary additional blood test.

### 8.8.2 Vitamin D

These data suggest that vitamin D deficiency is independently associated with all-cause mortality in people with CKD stage 3. Current NICE guidance suggests vitamin D supplementation for all adults in the UK,[238] rather than identification of those at high risk. Evidence regarding the effect of vitamin D replacement in CKD and general population cohorts is currently lacking.

### 8.8.3 Parathyroid Hormone

PTH is commonly measured in secondary care nephrology due to the prevalence of secondary hyperparathyroidism in referred populations with more advanced CKD. These data indicate that elevated PTH is an independent predictor of all-cause mortality even in CKD stage 3. However, it is not clear whether intervention to reduce PTH levels would be beneficial and therefore these results do not support the introduction of PTH testing in primary care.

### 8.8.4 Cystatin C

Cystatin C did not add significantly to models of risk prediction in this cohort. This is in contrast to other epidemiological studies, which have demonstrated improved risk prediction for both progression to ESKD and all-cause mortality with $eGFR_{cys}$ compared to $eGFR_{creat}$.[111] There are, however, several non-GFR determinants of cystatin C levels that are also risk factors for cardiovascular disease. The additional risk conveyed by cystatin C may be explained by these factors. Nevertheless, our data do not support the used of cystatin C for risk prediction in primary care.

## 8.9 AKI risk and ACE inhibitors

CKD does not exist in isolation, and many people with CKD are also affected by other illnesses and co-morbidities. Many patients with hypertension, heart disease and CKD are prescribed ACE inhibitors or angiotensin receptor antagonists (ARBs). These are well known to contribute to AKI risk. For many with CKD, AKI at the time of intercurrent illness may be more significant than gradual progression of CKD over time. Consideration of the risks and benefits of ACE inhibitors in older, multimorbid individuals should therefore include risk of AKI. This may impact upon prescribing decisions, particularly in the older, primary care population, where risk of progressive renal disease is low, as evidenced by this study. Evidence to guide clinical practice here is limited. Mansfield et al. found that AKI incidence was only mildly increased in those prescribed ACE inhibitors compared to other antihypertensives, and that this risk was largely in patient groups at overall low risk of acute kidney injury. This study was unable, however, to evaluate AKI severity, and relied on hospital episode statistics.[239]

## 8.10 Genetics and Renal Risk

Many of the risk markers described in this analysis represent markers of renal damage (eGFR, uACR), environmental factors (vitamin D deficiency) or potential consequences of renal disease (Haemoglobin, Bicarbonate, PTH and FGF23). Genetics forms an evolving area in renal risk prediction, and may underlie an individual's risk of developing CKD, or the rate of progression once CKD is established. Many populations are known to have higher risk for CKD or disease progression than others, and some genetic mechanisms for this are currently being elucidated. Future assessment of risk may include an assessment of an individual's genetic predisposition, either to a specific renal disease, or within the context of CKD, to a high risk of progression.

## 8.11 Multi hit hypothesis

Many factors and mechanisms are associated with the development and progression of CKD.[69] Risk prediction in CKD is therefore difficult, as different factors may apply in different individuals and at different times. Accurate risk prediction for ESKD has been best achieved through the KFRE, which has been validated in several international populations. As CKD is a variable disease, progression may represent

different processes in different conditions. Future strategies for improving risk prediction include the incorporation of genetic risk into risk prediction models and the evaluation of AKI as a risk factor for CKD progression.

## 8.12 Strengths and Limitations of this Study

Main strengths and limitations of the RRID study have been discussed previously in the results chapters. The RRID study collected data in a prospective, protocol-driven fashion, unlike many large CKD datasets, which are based upon retrospective data collection. General strengths of this study included the requirement for two eGFR values at least 90 days apart in to CKD 3 range, meaning that all participants met diagnostic criteria for CKD stage 3 prior to recruitment. In contrast, many large epidemiological studies rely on single measurements of eGFR. For recruitment, eGFR was measured using the MDRD equation, which was the most widely used estimating equation available at the time of study design. However, this was subsequently superseded by the CKD EPI equations and final analysis was therefore conducted using the CKD EPI equation. Finally, the RRID study has allowed guidelines to be tested in the population in which they are to be applied, as participants were recruited directly from primary care.

The RRID cohort is predominantly Caucasian and elderly. Results may therefore not be applicable outside this population. For example, younger individuals with glomerular causes of CKD may not behave in the same way. CKD remission in a younger population with glomerular disease would be very unlikely, and rates of progression much higher than seen in this cohort. However, the majority of those diagnosed with CKD in developed countries are elderly, and the findings are therefore relevant to are large group of people. Studying this population specifically, has allowed understanding of the natural history of CKD in older people. In some regards, the RRID cohort is limited because of the study population which, by virtue of being recruited from primary care, is at low risk for progressive disease. This resulted in a very low rate of progression to ESKD, and necessitated an alternative endpoint to allow multivariable analysis.

# 9. Conclusions

These results demonstrate that the majority of those with CKD stage 3 in primary care are at low risk of adverse outcomes related to progression of CKD. Risk Prediction can be achieved with a reasonable degree of accuracy with respect to CKD progression, CKD remission and all-cause mortality. Cystatin C does not lead to a substantial reduction in diagnosis of CKD stage 3 in a predominantly elderly primary care population but does reclassify a large proportion to a more advanced stage of CKD. Markers of mineral bone disease and AGE accumulation may add to risk prediction models, but may not improve risk prediction sufficiently to be clinically useful. FGF23, however, was not significantly associated with adverse outcomes in this cohort.

The natural history of people with CKD managed in primary care is different to that seen in referred populations but CKD guidelines are primarily based upon studies looking at progressive renal disease in high-risk referred populations. The data from these studies will hopefully inform the development of future guidelines to make specific recommendations for the care of patients in primary care.

## 9.1 Recommendations for Clinical Practice

### 9.1.1 CKD Diagnosis

Given the strengths of the current KDIGO criteria for the diagnosis of CKD in its simplicity and universality, it would be reasonable to maintain the current boundaries for CKD diagnosis in GFR and uACR for all individuals. However, given the significant proportion of this population evidencing CKD remission, suggestions such as increasing the length of time that an abnormal GFR must persist before CKD is diagnosed, perhaps from 3 months to 6 months, may be considered to differentiate more accurately between those with and without disease. Additionally, the number of eGFR measurements could be increased from 2 to 3 in a similar fashion. In addition it would be beneficial to consider risk as part of this diagnosis, and use this to tailor management to the individual. Risks considered should include progressive renal disease and ESKD, AKI, and cardiovascular risk. The length of time between blood tests, and the number of blood tests required to confirm a diagnosis of CKD, may be made more stringent.

### 9.1.2 CKD Remission

A significant minority of study participants demonstrated improvement in renal function. In some cases, people had no

evidence of CKD at the year 5 study visit, suggesting that long term remission does occur. Future guidance should incorporate definitions of CKD remission, allowing this to be evaluated consistently and investigated in other populations. Similarly to progression, 'risk' of remission can be calculated using common clinical variables. This information could be used to reassure those at low risk of adverse outcomes.

### 9.1.3 Monitoring Frequency

For the majority of participants in the RRID study, annual follow-up would be appropriate, given the low rate of change over this time period and the low rates of complications of CKD. Some individuals, however, do demonstrate progression, and therefore identification of those at high risk would be important, allowing targeting of resources at those most likely to develop problems. Additional factors, such as AKI episodes, may also be important in guiding the frequency of follow-up in primary care cohorts.

### 9.1.4 Referral to secondary care nephrology

NICE and KDIGO guidelines describe specific criteria to prompt referral to secondary care. These are primarily based upon proteinuria and eGFR levels, or rate of decline of GFR. Again,

assessing risk using common clinical criteria, rather than absolute eGFR values, may target those at highest risk. A shared care approach between primary and secondary care may allow more individuals to be appropriately managed in the community. Incorporation of risk scores/equations into assessment of people with CKD, either in primary or secondary care, may facilitate the identification of those at high risk of adverse outcomes.

### 9.1.5 Cystatin C

This analysis does not support the use of cystatin C in the diagnosis of CKD stage 3a in primary care, as recommended in the NICE and KDIGO guidelines. Cystatin C testing is not yet widely available in the UK, but may have a place in assessment of those with extremes of body habitus, perhaps through secondary care.

### 9.1.6 Use of Novel Biomarkers in Risk Prediction

There is not currently enough evidence to support the use of SAF as a non-invasive marker of risk in people with CKD in primary care. Similarly, FGF23 does not seem to add to risk prediction models in these analyses, and therefore should not be tested in primary care on the basis of this evidence.

## 9.2 Directions for Future Research

### 9.2.1 Further analysis of Renal Risk in Derby Study Data

Further analysis of data collected during the year-5 study visits is ongoing. It would be beyond the scope of this thesis to thoroughly analyse all the data collected. Potentially fruitful topics to investigate in these data would include analysis of the cardiovascular outcomes within this cohort, and the association between these and the biomarkers discussed in this thesis. Additionally, investigation of the association between pulse wave velocity and cardiovascular outcomes and all-cause mortallty is planned. Finally, Further data collection is planned at 10 year follow-up, however this may be limited by the increasing age of the participants.

### 9.2.2 Transient decline in estimated Glomerular Filtration Rate

One aspect of CKD remission that cannot be addressed in the RRID cohort would be the degree of risk conferred by a transient decline in eGFR. General population cohorts could be used to assess if individuals who meet criteria for CKD and subsequently improve have similar rates of adverse outcomes to those who do not meet criteria for CKD. This would be of use in advising patients in primary care who demonstrate

remission. For example, at present it is uncertain if this group is at higher risk of future CKD and renal function decline, or AKI, than those without a transient decline.

### 9.2.3 Chronic Kidney Disease and Multimorbidity

It is increasingly understood that CKD exists in a context of multimorbidity, and therefore these comorbidities may have bearing on CKD outcomes. Similarly, balancing medications for people with a range of health problems remains challenging. Further research is required to better understand the role of comorbidity and polypharmacy in influencing outcomes associated with CKD.

### 9.2.4 Acute Kidney Injury in the Renal Risk in Derby Study

The RRID study has collected Hospital Episode Statistics data from NHS Digital. This will allow investigation of the association between AKI episodes and CKD outcomes in the RRID cohort. The interaction between AKI and CKD progression has been described in other settings. Interventions to reduce risk or severity of AKI may therefore be of benefit in preventing CKD progression, particularly in older individuals with otherwise low risk CKD. “Sick day rules”

regarding ACE inhibitors, and their balance of risk and benefit in older people, and those with more advanced CKD, represent a potential area for intervention in this regard.

### 9.2.5 Comparison of Participants Exhibiting CKD Progression versus those Exhibiting Stable CKD

Within the RRID cohort, the majority of participants fell within CKD stage G3a A1 or G3b A1. Whilst the largest proportion of these participants demonstrated stable CKD, a minority did show progression of CKD. Understanding the differences between these two groups could be used to improve risk prediction between these two outcomes, potentially enabling early intervention and closer monitoring of those at highest risk. Future work could include metabolomic or proteomic analysis of baseline samples to try to demonstrate factors associated with progression within this population. Potentially, those demonstrating progression at 5 year follow-up could be matched against non-progressors in a case-control study design. Similarly, the use of cystatin C tended to produce lower eGFR values a therefore reclassified many participants from CKD stage G3a A1 to G3b A1. Further work to investigate the differences between those exhibiting a low $eGFR_{cys}$ compared to $eGFR_{creat}$ may elucidate the causes for such a

difference, potentially defining a patient population in which $eGFR_{cys}$ may be used beneficially.

### 9.2.6 Genetics and Renal Risk

Genetic techniques are becoming more affordable and easier to access in a clinical setting. Several genetic polymorphisms have been shown to associate with risk of CKD progression, and future studies should aim to identify the range of genetic variation that may underlie renal risk. As well as benefitting risk prediction, this may lead to greater understanding of the molecular mechanisms driving renal disease, and provide potential targets for intervention.

### 9.2.7 Integration between primary and secondary care

The distinction between primary and secondary care is to some extent unhelpful because the two sectors should integrate fully to provide the best possible patient care. Further research and quality improvement work is therefore required to develop integrated systems to manage and monitor large populations with CKD across primary and secondary care. Risk prediction in this setting is important to enable targeting of interventions to those people who are at greatest risk of progressive CKD and other adverse outcomes.

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# Appendix 1

## Case Report Form

!

**Renal Risk In Derby Study ($R^2$ID)**

**Participant Clinical Visit Record**

Participant ID ! Date of Clinic Visit !

Participant GP Practice Name

Consent form signed? Y/N

Visit Year **5**

Researcher gone through background information form? Y/N

Current medication assessed? Y/N

Has participant eaten cooked meat in the last 12 hours? Y/N

Participant last eGFR result ! ml/min Date !

Bloods taken? 2 x EDTA (Purple) Y/N
3 x Clotted (Yellow) Y/N

!
3 x Urine Sent to Laboratory? Y/N

Urine specimen for storage? Y/N

Urine dipstick findings Blood?
Protein?
! ! ! ! ! Other?

Height ! cm

Weight ! kg

BMI ! kg/m$^2$

Mid Upper Arm Circumference ! cm

Waist Girth ! cm

Hip Girth ! cm

Waist:Hip !

Hand Grip Strength !

Blood Pressure 1) Systolic ! mmHg Diastolic ! mmHg
2) Systolic ! mmHg Diastolic ! mmHg
3) Systolic ! mmHg Diastolic ! mmHg

Karnofsky Score !

EQ5D Linear score ! Questionnaire completed Y/N

Clinic Visit Sheet 5.8 15th November 2013!
!

!

Sit to Stand 5 ! s

4 metre timed walk 1) ! s

2) ! s

Mean ! s

Gait speed ! m/s

Vegetarian diet? Y/N

Length of time vegetarian ! years

Herbal remedies? Y/N

If yes, which? -

-

Recreational physical activity ? Y/N

Number of minutes recreational physical activity per week ! mins

Pulse Wave Velocity

Carotid/Femoral ! m/s

Distance from suprasternal notch to mid-point of femoral cuff ! cm

AGE Analyser

Skin AF Readings 1) !

2) !

3) !

Mean !

IMD Score after confirming postcode !

EM Quintile ! National Quintile !

Completed by……..

Signature of Researcher…….....Date.....

Clinic Visit Sheet 5.8 15th November 2013!

!